ABSTRACT BOOK

9th ADVANCES AGAINST ASPERGILLOSIS AND MUCORMYCOSIS

27 - 29 February 2020

Lugano, Switzerland

www.AAAM2020.org

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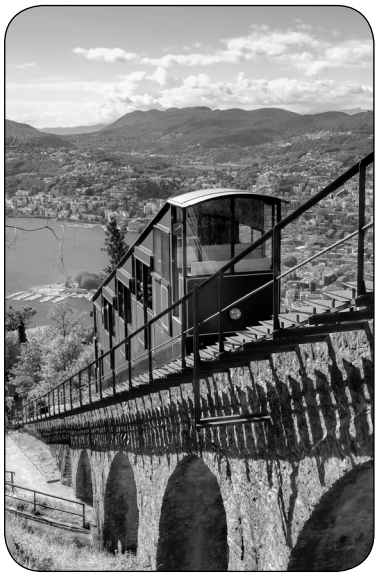


9th ADVANCES AGAINST ASPERGILLOSIS AND MUCORMYCOSIS

Lugano, Switzerland

27 - 29 February 2020

Palazzo dei Congressi Lugano

www.AAAM2020.org

9th ADVANCES AGAINST ASPERGILLOSIS AND MUCORMYCOSIS

27 - 29 February 2020 - Lugano, Switzerland

Dear Advances Against Aspergillosis and Mucormycosis Colleague,

This *9th Advances Against Aspergillosis and Mucormycosis* conference continues to grow and change with the field. The previous eight meetings were overwhelmingly successful, including the first meeting in 2004 (San Francisco) where we had 364 attendees from 28 countries, the second meeting in 2006 (Athens) with 464 attendees from 44 countries, the third meeting in 2008 (Miami) with 351 attendees from 48 countries, the fourth meeting in 2010 (Rome) with 533 attendees from 41 countries, the fifth meeting in 2012 (Istanbul) with 375 attendees from 39 countries, the sixth meeting in 2014 (Madrid) with 342 attendees from 33 countries, the seventh meeting in 2016 (Manchester) with 363 attendees from 38 countries, and the eighth meeting in 2018 (Lisbon) with 334 attendees from 38 countries and the first meeting where we added molecular and clinical mucormycosis research. When our 2020 meeting convenes, it will represent the 18th year of *Advances Against Aspergillosis* activity, beginning with the initial supplement in **Clinical Infectious Diseases**. **Because of all of you, this conference has now established itself as the premier forum for discussion of all aspects of *Aspergillus* and now mucormycosis infection and research.**

The *Aspergillus* field continues in a state of rapid advancement, including the publication of numerous post-genomic papers and substantial advances in translational, immunologic, and diagnostic research. We have seen the launch of another effective antifungal for invasive aspergillosis (isavuconazole) and ongoing or anticipated clinical trials of numerous newer compounds is an exciting time for mycology. Itraconazole, pan-azole, and echinocandin resistance has emerged, and combination therapy and targeted immune exploration remains an important area of interest. Greatly increased awareness of allergic aspergillosis has opened new market opportunities for both antifungal agents and immunotherapies. There is a continuing high death toll from invasive aspergillosis, particularly among patient groups not usually associated with this opportunistic infection.

This meeting is another chance to gather the world's aspergillosis and mucormycosis experts in one venue. A fundamental tenet of this colloquium continues to be to engender collaborative relationships amongst clinicians, scientists, and industry to further advance the field. Because of the international success of the *Advances Against Aspergillosis* meetings in raising awareness of filamentous fungal infections, in 2018 we expanded our program to also provide a home and venue for presentations on the related science and devastating clinical entities of mucormycosis. Due to the success of that venture, the 2020 program also includes an exciting new session and many posters for that discipline.

We thank the many corporate and foundation sponsors, listed in this program; without their support, this conference would not have been possible. We also thank the Scientific Committee, especially our Scientific Committee Chair Prof. Cornelia Lass-Flörl, for helping to assemble a truly international speaker list from the top centers in the world, with a focus on contemporary topics. By our design, much of the newest published literature and hypotheses in the field have originated from the speakers of this conference. In the program, we have introduced many speakers who did not speak at the previous *Advances Against Aspergillosis* meetings, including some young scientists and clinicians - a pattern we would like to repeat in future years. This year we have again increased the number of oral presentations from submitted abstracts to represent the wider community.

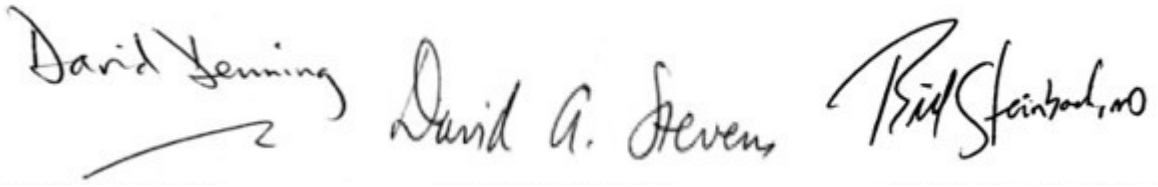
We also thank all the speakers and poster presenters for contributing to the success of this effort. Please also join us at the welcome reception, the symposia, the tour and dinner, and the poster sessions. An essential part of this conference is the new friendships we expect will result, and the support of young

scientists entering the field. To engage younger scientists in our field, over 9 meetings we have offered a total of 269 scholarships to trainees, largely from less developed countries, where the conference paid for travel and hotel costs to attend the meeting.

The proceedings of this 9th meeting will be published in *Journal of Fungi*, creating what we hope will be highlights of the newer insights from the many disciplines that encompass *Aspergillus* research and care. As *Advances Against Aspergillosis* has become the leading global meeting for basic and clinical science regarding *Aspergillus*, its efforts form one of the foundations of the repository of knowledge about this pathogen; 267 papers have been published in 9 Supplements, comprising 2,026 pages, as well as 1,429 abstracts from the meetings (not including this meeting).

Our plan is to continue this conference every other year, and you will notice that there is a special open planning session for the next conference at the end of this meeting. We invite you to come and offer any suggestions for new sessions or topics or locations you would like to see in the future.

Yours sincerely,



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ACKNOWLEDGEMENTS

We would like to offer very special thanks to the following organizations for their generous educational grants. Their financial support makes this conference possible.

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Teresa Zelante, PhD / *University of Perugia, Italy*

Li-Ping Zhu, MD PhD / *Huashan Hospital, Fudan University, China*

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Russia

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NAME	Paid Speaking Engagements	Research Contracts	Consultancy	Travel and Accommodation
ANDES, David	None	Amplyx, Matinas, Cidara	Amplyx, Matinas, Cidara, sFunga	None
BACHER, Petra	None	None	Miltenyi Biotec	None
BOCHUD, Pierre-Yves	None	None	Pfizer	Pfizer, Gilead, BMS
BOTGROS, Radu	None	None	None	None
BOWYER, Paul	Gilead	None	None	None
BROWN, Gordon	None	None	None	None
BUSH, Andrew	None	None	None	None
CHAKRABARTI, Arunaloke	None	None	None	ISHAM
CORNELY, Oliver	Astellas, Basilea, Gilead, Grupo Biotoscana, Pfizer, Merck	Actelion, Amplyx, Astellas, Basilea, Cidara, F2G, Da Volterra, Gilead, Janssen, Medicines Company, Pfizer, MedPace, Melinta, Merck, Scynexis	Actelion, Allecra, Amplyx, Astellas, Basilea, Biosys UK, Cidara, Da Volterra, Entasis, F2G, Gilead, Matinas, MedPace, Merck, Menarini Richerche, Octa-pharma, Paratek, Pfizer, PSI, Vical, Rempex, Scynexis, Seres, Tetraphase,	None
CORRY, David	None	None	None	None
DENNING, David	Dynamiker, Hikma, Gilead, Merck, Mylan, Pfizer	None	Scynexis, Zambon, Pulmatrix, iCo Therapeutics, Roivant, Fujifilm, Biosergen	None
DRUEY, Kirk	None	None	None	None
GARCIA-HERMOSO, Dea	None	None	None	None
GARRE, Victoriano	None	None	None	None
GUINEA ORTEGA, Jesús	Astellas, Gilead, MSD, Scynexis, Biotoscana-United Medical	FIS, Gilead, Scynexis, Cidara	None	None
HAAS, Hubertus	None	Vical	Vical	None
HAVLÍČEK, Vladimir	None	None	None	None
HÖNIGL, Martin	Gilead, Pfizer, Merck, Basilea, Astellas	Gilead, Pfizer, Merck, Scynexis	Amplyx, Cidara, Gilead, Merck	None
KHANNA, Nina	Pfizer, Interlaken	None	Pfizer, MSD, Basilea, Gilead	Pfizer

NAME	Paid Speaking Engagements	Research Contracts	Consultancy	Travel and Accommodation
KRAPPMANN, Sven	None	None	None	Gilead
KULLBERG, Bart-Jan	Cidara, Pfizer	None	Amplyx, Cidara, Scynexis	None
LAMOTH, Frederic	None	None	MSD, Gilead, Basilea	Gilead, MSD
LASS-FLÖRL, Cornelia	Astellas, Pfizer, Merck, Gilead, Basilea	Astellas, Gilead	Astellas, Pfizer, Merck, Gilead, Basilea	Astellas, Pfizer, Merck, Gilead, Basilea
LOEFFERT-FRÉMIOT, Sophie	None	None	None	None
MIYAZAKI, Yoshitsugu	Astellas, Pfizer, Sumitomo Dainippon	Riken, Medical & Biological Laboratories	None	None
MOHSENA, Tarik	None	None	None	None
MOSS, Richard	None	None	Pulmatrix, Mayne, Zambon, Regeneron	None
NIFANTIEV, Nikolay	None	None	XEMA Medical	None
PATTERSON, Thomas	Basilea, Gilead, United Medical	Cidara	Basilea, Gilead, Mayne, Merck, Pfizer, Sfunga, Scynexis, Toyama	Basilea, Gilead, Mayne, Merck, Pfizer, Sfunga, United Medical
PINHEIRO, Dolores	None	None	None	None
RANQUE, Stéphane	None	None	None	Pfizer
SHEPPARD, Donald	Merck, Pfizer, Astellas	Merck	Merck	Merck
STEINBACH, William	None	Astellas, Merck	Sfunga, Astellas	None
STEVENS, David	None	Astellas, Aridis	Pulmatrix, Mayne	Pfizer
WALSH, Thomas	None	Grants from: Allergan, Amplyx, Astellas, Lediant, Medicines Company, Merck, Scynexis, Tetraphase	Amplyx, Astellas, Allergan, Gilead, ContraFect, Lediant, Medicines Company, Merck	None
ZELANTE, Teresa	None	None	None	None
ZHU, Li-Ping	None	None	None	None

POSTER ABSTRACT INDEX

SCHOLARSHIP AWARDS

Sarah Ahmed, Sudan
Marielle Camargo dos Santos, Brazil
Paulami Chatterjee, India
Youngho Choi, Republic of Korea
Kambiz Diba, Iran
Alexis Garcia, USA
Wajiha Gohir, Canada
Irene Gonzalez, Spain
Lovely Gupta, India
Emily Hammond, Luxembourg & UK
Shanu Hoda, India
Ravil Huseynov, Azerbaijan
Hyun-Jun Kim, Republic of Korea
Matthew Mead, USA
Aryse Melo, Portugal & Brazil
Toine Mercier, Belgium
Alfred O. Mitema, Kenya & South Africa
Rita Oladele, Nigeria
Mragnayani Pandey, India
Agustin Resendiz Sharpe, Mexico
Mark Roundtree, USA
Taylor Schoen, USA
Pooja Sen, India
Keats Shwab, USA
Ashutosh Singh, India
Gagandeep Singh, India
Lingyue Sun, China
Jessie Uehling, USA
Norman Van Rhijn, Holland & UK
Sandeep Vellanki, India
Jose Lucio Vicente, Spain
Ling-Hong Zhu, China

We thank several sponsors for their generosity in providing funds for the scholarships, and the speakers for forgoing honoraria in order to supplement the scholarships fund.

Please congratulate these scholarship winners on their recognition, at their posters and when you see them at the sessions.

PROGRAMME

THURSDAY 27 FEBRUARY

All sessions will take place in the main lecture theatre (Room A) unless otherwise stated

Meet the Professor

08.00 - 08.55 **ECMM and ISHAM joint session: Excellence centres, fellows, guidelines, educational initiatives, working groups and publishing** *Room A*
Martin Hönigl, MD
Arunaloke Chakrabarti, MD

08.00 - 08.55 **Kids, difficult asthma and fungus** *Room C*
Andrew Bush, MD

09.00 - 09.15 **Introduction and summary of new antifungal drugs**
David W. Denning, MBBS

Aspergillus: Modelling the Host and Beyond

Moderators: David B. Corry, MD, Richard B. Moss, MD & David S. Perlin, PhD

09.20 - 09.45 **IL-22 and tryptophan metabolism in antifungal host defense**
Teresa Zelante, PhD

09.45 - 10.00 **Abstract #49: Uncoupling of cytokine signaling and LC3 associated phagocytosis (LAP) drives the development of invasive aspergillosis in patients with sepsis**
Tonia Akoumianaki, PhD

10.00 - 10.25 **Respiratory epithelial interaction**
Kirk M. Druey, MD

10.25 - 10.40 **Abstract #45: "Invasive aspergillosis on-a-chip" – a novel disease model to study *Aspergillus fumigatus* infection in the human lung**
Susann Hartung, PhD

10.40 - 11.05 **IL-17 immunity: from *Aspergillus* to *Candida***
Petra Bacher, PhD

11.05 - 11.35 ***Coffee Break*** *Room B*

Management of the Next Decade

Moderators: Arunaloke Chakrabarti, MD, Thomas F. Patterson, MD & Bart-Jan Kullberg, MD PhD

11.35 - 12.00	**Genomic predisposition and adaptation ito virulence in aspergillosis** Paul Bowyer, PhD	
12.00 - 12.15	**Abstract #51: Vaccine-induced immunogenicity and protection in a murine model of invasive pulmonary aspergillosis** Emily Rayens	
12.15 - 12.30	**Abstract #37: Modulation of TREM1 signalling in macrophages infected with *Aspergillus fumigatus*** Irene González Jiménez, PhD	
12.30 - 12.55	**Individualized genetic-based approach for IA prophylaxis** Pierre-Yves Bochud, MD	
12.55 - 14.30	***Lunch and Poster Session 1***	*Room B + Foyer*

Airways and Diseases

Moderators: Teresa Zelante, PhD, Martin Hönigl, MD & Sven Krappmann, PhD

14.30 - 14.55	**Regulation of Th2-immunity** David B. Corry, MD	
14.55 - 15.10	**Abstract #46: Single cell approaches reveal the key dendritic cell subsets that coordinate allergic airway inflammation against the fungal allergen *Aspergillus fumigatus*** Peter Cook, PhD	
15.10 - 15.25	**Abstract #74: Interdependency of host and pathogen protein persulfidation governs disease severity in experimental and human aspergilloses** Jorge Amich, PhD	
15.25 - 15.50	**The structure and immunogenicity of galactomannan** Nikolay Nifantiev, PhD	
15.50 - 16.20	***Coffee Break***	*Room B*

Aspergillus: Entering the Host and Beyond

Moderators: Elaine Bignell, PhD, Yoshitsugu Miyazaki, MD PhD & Gabriele Sass, PhD

16.20 - 16.45 **Siderophores and fungal pathogenesis**
Hubertus Haas, PhD

16.45 - 17.00 **Abstract #79: Iron overload decreases macrophage lysosomal acidification, impairing the clearance of *Aspergillus fumigatus* conidia**
Mikio Kaji

17.00 - 17.15 **Abstract #73: ROS-dependent and independent host-induced fungal regulated cell death in defence against invasive aspergillosis**
Neta Shlezinger, PhD

17.15 - 17.40 **Modelling IA – how close are predicted antifungal targets?**
Thomas Walsh, MD

17.40 - 18.45 ***Welcome Reception*** *Room B*

FRIDAY 28 FEBRUARY

All sessions will take place in the main lecture theatre (Room A) unless otherwise stated

Meet the Professor

08.00 - 08.55	**How to make a fast and accurate diagnosis – what can the clinician and lab do?** Dea Garcia-Hermoso, PhD Stéphane Ranque, MD PhD	*Room A*
08.00 - 08.55	**Difficult cases in fungal sinusitis** Tarik Mohsena, MD FRCPC FACP DTM&H Arunaloke Chakrabarti, MD	*Room C*

Emerging Aspergilli

Moderators: Jesús V. Guinea Ortega, PharmD PhD & Stéphane Ranque, MD PhD

09.00 - 09.25	**Current epidemiology of invasive fungal infections** Nina Khanna, MD	
09.25 - 09.40	**Abstract #82: Mucoricin is a mucorales ricin-like toxin critical for mucormycosis pathogenesis** Ashraf Ibrahim, PhD	
09.40 - 10.05	**Epidemiological surveillance for *Aspergillus* – is it of any value?** Sophie Loeffert-Frémiot, PhD	
10.05 - 10.20	**Abstract #92: Virus infection of *Aspergillus fumigatus* (Af) compromises Af in intermicrobial competition** Ioly Kotta-Loizou	
10.20 - 10.45	***Aspergillus calidoustus* – epidemiology and outcome** Frederic Lamoth, MD	
10.45 - 11.00	**New support initiatives in aspergillosis and mucormycosis research** Dona Love, PhD Sonia Sanchez, MD	
11.00 - 11.45	***Coffee Break and Poster Viewing***	*Room B + Foyer*

Pfizer Satellite Symposium

Moderator: Robert Krause, MD DTMP

Dealing with the threat of mucormycosis when there is differential diagnostic doubt

11.45 - 11.55 **Introduction and scene setting**
Robert Krause, MD DTMP

11.55 - 12.05 **Interactive case study part one – setting the scene of a difficult diagnosis**
Dionysios Neofytos, MD PhD MPH

12.05 - 12.30 **Risk factors for invasive aspergillosis and mucormycosis, and how to pick apart a diagnosis, supported by case studies**
Carolina Garcia-Vidal, MD PhD

12.30 - 12.55 **Treating invasive mould disease in the case of confounding factors**
Malgorzata Mikulska, MD PhD

12.55 - 13.05 **Interactive case study part two – treatment approaches to a complex patient with a complicated infection**
Dionysios Neofytos, MD PhD MPH

13.05 - 13.15 **Discussion, Q&A and close**

13.15 - 14.00 ***Lunch*** *(Sponsored by Pfizer)* *Room B*

SATURDAY 29 FEBRUARY

All sessions will take place in the main lecture theatre (Room A) unless otherwise stated

Meet the Professor

07.45 - 08.55 **Regulatory endpoints for invasive and chronic pulmonary aspergillosis – what is needed?** *Room A*
Oliver Cornely, MD
Thomas F. Patterson, MD
Radu Botgros, MD
Sumathi Nambiar, MD

08.00 - 08.55 **CLSI/EUCAST: AFST for breakpoints - where we are going?** *Room C*
David R. Andes, MD
Jesús V. Guinea Ortega, PharmD PhD

Resistance in *Aspergillus*

Moderators: Nina Khanna, MD, Thomas Walsh, MD & Don Sheppard, MD

09.00 - 09.25 **Acquiring azole resistant Aspergilli – where from?**
Dolores Pinheiro, MD

09.25 - 09.40 **Abstract #59: The negative cofactor 2 complex is a master regulator of drug resistance in *Aspergillus fumigatus***
Takanori Furukawa, PhD

09.40 - 09.55 **Abstract #114: The incidence of pulmonary aspergillosis in patients with cystic fibrosis in Russian Federation**
Yana Kozlova, MD

09.55 - 10.10 **Abstract #64: CRISPR-Cas9 mutation and characterization of the most overexpressed transcription factor of swollen state of *Aspergillus fumigatus***
Uxue Perez-Cuesta

10.10 - 10.35 **Management of aspergillosis due to azole-resistant *Aspergillus fumigatus***
Bart-Jan Kullberg, MD PhD

10.35 - 11.05 ***Coffee Break*** *Room B*

Molecular Mechanisms

Moderators: Gordon D. Brown, PhD, Hubertus Haas, PhD & Praveen Juvvadi, PhD

11.05 - 11.30	**Recognition of DHN-melanin by C–type lectin receptor** Gordon D. Brown, PhD	
11.30 - 11.45	**Abstract #93: *Aspergillus fumigatus* KnrA is an intrinsically disordered protein and functions as a potential substrate of calcineurin and protein kinase A in the cell wall integrity pathway** Praveen Juvvadi, PhD	
11.45 - 12.00	**Abstract #41: Epithelial uptake of *Aspergillus fumigatus* spores drives efficient fungal clearance *in vivo* and is aberrant in chronic obstructive pulmonary disease (COPD) patients** Margherita Bertuzzi, PhD	
12.00 - 12.15	**Abstract #80: Phagosomal removal of fungal melanin reprograms macrophage metabolism to promote antifungal immunity** Samuel Gonçalves	
12.15 - 12.40	**Antimicrobial peptides secreted by *Aspergillus fumigatus*** Sven Krappmann, PhD	
12.40 - 14.20	***Lunch and Poster Session 2***	*Room B + Foyer*

Mucormycosis Update

Moderators: Oliver Cornely, MD, Frederic Lamoth, MD & Dea Garcia-Hermoso, PhD

14.20 - 14.45	**One world - one guideline: mucormycosis** Oliver Cornely, MD	
14.45 - 15.10	**Distinguishing invasive mucormycosis from aspergillosis non-invasively** Vladimir Havlíček, PhD	
15.10 - 15.25	**Abstract #89: Ubiquity, diversity, and functional genomics of Mucoromycota and their Betaproteobacterial endosymbionts** Jessie Uehling, PhD	
15.25 - 15.40	**Abstract #55: Why are mucormycetes resistant against voriconazole?** Michaela Lackner, PhD	
15.40 - 16.05	**Mucor species & macrophages** Victoriano Garre, PhD	
16.05 - 16.35	***Coffee Break***	*Room B*

Risks for *Aspergillus*-Related Diseases: Clinical Hot Spots

Moderators: Chris Kosmidis, MD PhD & Sophie Loeffert-Frémiot, PhD

16.35 - 17.00 **Challenge for chronic pulmonary aspergillosis - a common but formidable disease**
Yoshitsugu Miyazaki, MD PhD

17.00 - 17.15 **Abstract #117: Prospective evaluation of quality of life and *Aspergillus* IgG in tuberculosis (TB) patients in Lagos, Nigeria**
Rita Oladele, PhD

17.15 - 17.40 **Biofilm formation by *Aspergillus fumigatus* – implications for treatment**
Donald Sheppard, MD

17.40 - 17.55 **Abstract #34: Altered *A. fumigatus* cell wall integrity by PC945, a novel inhaled azole**
Darius Armstrong-James, MD PhD

17.55 - 18.20 **The new world of inhaled antifungals & biologics – where might it take us?**
Richard B. Moss, MD

18.20 - 18.25 **Closing**
William J. Steinbach, MD
David A. Stevens, MD

18.30 ***Snacks and Discussion about AAA 2022*** *Room C*

ABSTRACTS OF INVITED FACULTY

ECMM AND ISHAM JOINT SESSION: EXCELLENCE CENTRES, FELLOWS, GUIDELINES, EDUCATIONAL INITIATIVES, WORKING GROUPS AND PUBLISHING

THURSDAY 27 FEBRUARY 2020 (08.00 - 08.55)

Arunaloke Chakrabarti, MD
Postgraduate Institute of Medical Education & Research, India

Martin Hönigl, MD
Medical University of Graz - Austria
University of California–San Diego - USA

Invasive mycoses present a global challenge with expansion into new hosts, emergence of new pathogens, and development of multidrug resistance. In response to these evolving challenges, the European Confederation of Medical Mycology (ECMM) is committed to providing international expertise, guidance, and leadership with the key objectives of improving diagnosis, treatment, outcome, and survival of persons with invasive fungal diseases. Representing 27 affiliated National Medical Mycology Societies, the ECMM has developed several major ways to achieving these critical objectives: [1] tasking 12 specific medical mycology working groups; [2] the ECMM Academy and Fellow program (FECMM), which is open to leading mycologists from around the world and accepted its 100th fellow in November 2019; [3] implementing the worldwide ECMM Excellence Centre Initiative; and [4] the ECMM Global Guideline Initiative "One world one guideline" focusing on mucormycosis (published in Lancet ID 2019), rare mold diseases, rare yeast diseases, endemic mycoses, and – in collaboration with ISHAM - cryptocccosis. We believe that these important initiatives and other strategies of the ECMM will advance the field of medical mycology and improve the outcome of patients with invasive mycoses worldwide.

KIDS, DIFFICULT ASTHMA AND FUNGUS

THURSDAY 27 FEBRUARY 2020 (08.00 - 08.55)

Andrew Bush, MD
Imperial College and Royal Brompton Hospital - London - UK

The child presenting with asthma-like symptoms, non-responsive to asthma therapy, should be the subject of a detailed, protocolised investigation [Lancet 2010; 376: 814-825]. Diagnoses mimicking asthma are excluded. A multi-disciplinary assessment (Respiratory Nurse, specialist physiotherapist, clinical psychologist in particular [Journal of Asthma and Allergy 2017; 10: 123-30]) next places the child into one of three categories, which may overlap [Respirology. 2017; 22: 886-897]:

- Severe therapy resistant asthma (proceeds to invasive assessment)
- Asthma plus (co-morbidities such as obesity, exercise induced laryngeal obstruction [Eur Respir J. 2017; 50. pii: 1602221])
- Difficult asthma (need to get the basics right – adherence, environment, psychosocial [Arch Dis Child 2009; 94: 780-4])

For the latter two categories, a bespoke multi-disciplinary intervention is provided, and if this is successful, no further action is needed [Eur Respir J 2012; 40: 264-267]. If despite intervention, the problem remains, the child is described as having 'refractory asthma plus' or 'refractory difficult asthma'.

Severe asthma with fungal sensitisation is defined in adults [Am J Respir Crit Care Med 2009; 179: 11-8] as high dose treatment (500 mcg FP/day, or continuous oral CS, or 4/6 Pred bursts in 12/24 months), IgE < 1000, -ve IgG precipitins to *Aspergillus fumigatus* and evidence of sensitization (SPT ≥ 3mm, RAST ≥ 0.4) to any of *Aspergillus fumigatus, Cladosporium herbarum, Penicillium chrysogenum* (*notatum*), *Candida albicans, Trichophyton mentagrophytes, Alternaria alternate* and *Botrytis cinerea*. There is no generally accepted paediatric definition; most use the adult definition, but without the IgE and IgG criteria, because allergic bronchopulmonary aspergillosis is rarely if ever seen in children with asthma. We have shown that these children have more severe inflammation than non-sensitized children, and that this appears to be driven by the alarmin IL-33, through a steroid resistant pathway [J Allergy Clin Immunol. 2015; 136: 312-322]; unfortunately, there is no commercially available anti-IL33 monoclonal to test this. Management in the first instance is to minimise mould exposure in the environment, including ensuring any nebulizers are not contaminated. The role of antifungals is unclear, with small conflicting trials only [Am J Respir Crit Care Med. 2009; 179: 11-8; J Allergy Clin Immunol. 2014; 134: 33-9]. Anecdotally, some individuals seem to benefit dramatically from antifungals, but if itraconazole is prescribed, it should be remembered there is an interaction with budesonide at the cytochrome p450 level, causing iatrogenic Cushing's syndrome unless the steroid dose is reduced [Endocr Pract. 2013; 19: e138-41].

IL-22 AND TRYPTOPHAN METABOLISM IN ANTIFUNGAL HOST DEFENSE

THURSDAY 27 FEBRUARY 2020 (09.20 - 09.45)

Teresa Zelante, PhD
University of Perugia - Italy

The evolutionary conservation of the tryptophan metabolism may be linked to the significance of the *de novo* synthesis of nicotinamide adenine dinucleotide (NAD+), to which it ultimately leads. Interestingly, tryptophan-derivative metabolites bearing the indole ring, are diffused in different ecosystems. Indole is considered a type of *'archetypical hormone'* able to regulate the relation between the host and microbes in plants but also in the animal kingdom. The mechanisms of action in the host by indole and indole derivatives are not well characterized yet, although a very large part of research has focused the mechanistic function on their capacity to bid the Xenobiotic Receptors. Interestingly, animals can't synthesize indole, while many bacteria and also fungi produce indole and indole-derivatives with some pathogens such as *A. fumigatus* synthesizing toxic indole alkaloids. However, the indole-derivative 3-IAld is a Xenobiotic Receptor ligand that promotes IL-22 production and restores antifungal resistance via gut NKp46+ cells. More recently, 3-IAld has been proved also changing together with a tryptophan-rich diet, the program of intraepithelial CD4+ T cells into immunoregulatory T cells in mice.

Thus, the scientific proof for a contribution of the Xenobiotic Receptors in the control of the barrier function and immune regulation would serve as a basis towards improvement of non-toxic probes and ligands as drugs. This is also put forward by the fact that several of those microbial metabolites are found in human blood at levels comparable to host metabolites, suggesting that systemic responses may be easily activated by targeted Xenobiotic Receptor -based therapy. These systems are thought to provide an additional level of interchange between the microbes and the host at the edge of their co-metabolism.

RESPIRATORY EPITHELIAL INTERACTION

THURSDAY 27 FEBRUARY 2020 (10.00 - 10.25)

Kirk M. Druey, MD
National Institute of Allergy and Infectious Diseases/
National Institutes of Health (NIAID/NIH) - Bethesda - USA

Type 2 inflammation associated with chronic allergic asthma leads to sloughing and hyper-permeability of respiratory epithelium. In this context, we detected a serine protease allergen of *Aspergillus fumigatus (Af*) (*Aspf13* or alkaline protease 1 [Alp1]) within the bronchial submucosa of lung biopsies from subjects with asthma but not healthy controls by immunohistochemistry. Alp1 immunoreactivity co-localized with airway smooth muscle (ASM) bundles, and within the asthma subgroup, the extent of Alp1 expression correlated strongly with clinical disease severity and with measures of lung functional impairment.

In mouse models of experimental asthma, administration of purified Alp1 to the lower respiratory tract was sufficient to induce airway hyper-responsiveness (AHR) in wild-type (Balb/c or C57Bl/6) mice or even in strains devoid of eosinophilic inflammation (DdblGATA). Likewise, proteinase activated receptor 2 (PAR2), which is expressed on respiratory epithelial cells and has been implicated in the inflammatory response to other protease allergens, was not required for Alp1-induced AHR. Alp1 provoked AHR in PAR2-deficient mice despite eliciting minimal increases in type 2 cytokines including IL-5 and IL-13 in the airways. Alp1 augmented airway contraction in response to bronchoconstrictors in precision cut lung slices (PCLS) from naïve mice and healthy non-asthmatic human donors, without the requirement for pre-existing allergen challenge. These results suggest that Alp1 promotes AHR through mechanisms that are in part independent of allergic inflammation.

In a biophysical ex vivo assay of contractile force, Alp1 directly induced spontaneous contraction of ASM cells. Alp1 degraded several components of extracellular matrix (ECM), including collagen and fibronectin, which in turn induced clustering of cultured human or mouse ASM cells. On an ultrastructural level, Alp1 reduced the number of integrin-mediated ECM attachments of ASM cells as well as quantities of focal adhesions, which are multiprotein complexes that transmit force from cell cytoskeleton to ECM substrates and thereby regulate cell contraction. In addition, Alp1 augmented contraction-related signaling pathways in ASM cells including Ca^{2+} flux and RhoA activation induced by G protein-coupled receptor (GPCR) agonists such as histamine or bradykinin.

In severe asthma, ASM hypercontraction contributes to prolonged and recurrent episodes of broncho-constriction, in part owing to resistance to bronchodilators. Targeting the ASM cytoskeleton is an alternative means of overcoming ASM contraction, and Alp1 may represent a unique and direct mediator of broncho-constriction.

IL-17 IMMUNITY: FROM *ASPERGILLUS* TO *CANDIDA*

THURSDAY 27 FEBRUARY 2020 (10.40 - 11.05)

Petra Bacher, PhD
University of Kiel & UKSH Kiel - Germany

Th17 cells are in general considered important mediators of protection against fungi. However, patients with impaired Th17 responses have an increased risk for mucocutaneous Candidiasis, but rarely for infections with airborne molds, such as *A. fumigatus*, one major cause of lethal invasive mycoses in immunocompromised patients. In contrast, in pulmonary diseases such as COPD, asthma or cystic fibrosis, increased Th17 responses are often considered as pathologic rather than being protective. However, which fungi actually induce Th17 responses in humans and whether fungus-specific Th17 responses may contribute to pulmonary diseases is not very well understood.

We used antigen-reactive T cell enrichment (ARTE) for the ex vivo analysis of human T helper cell responses against 30 common members of the human mycobiome. We identified the mucocutaneous pathobiont *Candida albicans* as the major direct inducer of human anti-fungal Th17 cells. Th17 cells directed against other fungi are induced by T cell cross-reactivity to *C. albicans*. Strikingly, Th17 cells cross-reactive to the airborne fungus *Aspergillus fumigatus* are selectively activated and expanded in patients with airway inflammation, such as asthma, COPD and cystic fibrosis and especially during acute allergic bronchopulmonary aspergillosis (ABPA), suggesting their specific contribution to lung pathology. This indicates a direct link between protective intestinal Th17 responses against *C. albicans* and lung inflammation caused by airborne fungi. In summary, we identify heterologous immunity to a single, ubiquitous member of the mycobiota as a central mechanism for systemic induction of human anti-fungal Th17 responses and as a risk factor for pulmonary inflammatory diseases.

GENOMIC PREDISPOSITION AND ADAPTATION ITO VIRULENCE IN ASPERGILLOSIS

THURSDAY 27 FEBRUARY 2020 (11.35 - 12.00)

Paul Bowyer, PhD
University of Manchester - UK

The genomes of isolates of *Aspergillus fumigatus* are complex and display a high degree of heterogeneity. Few studies have addressed pathogenicity in chronic pulmonary aspergillosis (CPA) and allergic pulmonary aspergillosis (ABPA) and little is known regarding genome variants that could predispose isolates to pathogenesis. This study aims to discover whether particular genomic features predispose isolates to pathogenicity or to particular types of aspergillosis and whether genome plasticity plays a role in virulence.

Genome sequences from 171 clinical and environmental isolates were obtained. Isolates were obtained from public datasets and through sequencing of isolates from the Manchester Mycology Lab, including isolates from ABPA, CPA and CPA -aspergillomas. Isolates were phenotyped for drug resistance, disease and growth morphology. Genomes were analysed to determine gene copy number, single nucleotide polymorphisms and deletions using GATK, PLINK, R packages for genotype and phenotype association and bespoke scripts. Phylogenies were estimated using RaxML of whole genome vcf files and ROADTRIPS to assess lineage specific contributions. Genes and regions identified from bioinformatic analysis were tested by gene knockout and phenotyped for secondary metabolite production (HPLC), pathogenicity, epithelial cell damage and detachment, induction of cytokines, phagocytic survival and ability to form *in-vitro* aspergilloma.

A wide range of genetic variants were discovered including 1.2m SNPs, 7 major chromosome duplications, 7 deletions (>10KB) and a range of probable translocation events. Large scale genomic changes were observed primarily in CPA and CPA-aspergilloma isolates. These included large scale genome duplications related to drug resistance and small scale deletions related to pathogenicity and epithelial damage phenotypes. SNPs leading to cryptic peroxisomal targeting via translational readthrough were strongly associated with disease. Finally clusters of genes that displayed heterogeneity across *A. fumigatus* populations were found to predispose such isolates to pathogenicity for certain aspergillosis disease types, particularly CPA.

Genome analysis has revealed important predisposing factors in *A. fumigatus* genomes that condition such isolates for particular forms of aspergillosis. Additionally analysis of genome changes statistically related to drug resistance and disease reveals several new mechanisms for drug resistance and pathogenicity in this fungus.

INDIVIDUALIZED GENETIC-BASED APPROACH FOR IA PROPHYLAXIS

THURSDAY 27 FEBRUARY 2020 (12.30 - 12.55)

Pierre-Yves Bochud, MD
Vaud University Hospital Center (CHUV) - Lausanne - Switzerland

Invasive mold infections (IMIs) represent a major concern in onco-haematological patients, with a morbidity and mortality ranging both 5-15%. While mold-active azoles are approved for primary prophylaxis in these patients, their systematic use is challenged by important costs, toxicities and emergence of resistance. Over the last decade, numerous studies reported associations between single nucleotide polymorphisms (SNPs) in immune genes and susceptibility to IMIs. While initial studies were limited by lack of reproducibility, small frequency of reported polymorphism(s) and absence of functional evidence supporting genetic association, certain SNPs recently emerged as more convincing predictors of susceptibility to IMIs. Particularly relevant are the associations observed between two frequent SNPs in pentraxin 3 (PTX3) and IMIs among hematopoietic stem cells transplant (HSCT), as they were replicated in other cohorts of HSCT patients and subsequently confirmed in solid organ transplant (SOT) recipients and patients undergoing myelosuppressive chemotherapy for acute leukemia. Altogether, these data make such SNPs good candidates for intervention strategies in which they can be used to stratify the infectious risk and guide the choice of antifungal prophylaxis.

REGULATION OF TH2-IMMUNITY

THURSDAY 27 FEBRUARY 2020 (14.30 - 14.55)

David B. Corry, MD
Baylor College of Medicine - Houston - USA

Fungi, especially *Aspergillus spp*., produce chronic, progressive infections of mucosal sites that pose unique diagnostic and therapeutic challenges. A key feature of chronic airway fungal infections is their characteristic airway immune signature of type 2 or allergic inflammation that includes adaptive immune T helper type 2 (TH2) and TH17 cells and IgE and IgG-secreting B cells, and innate inflammatory cells that include innate lymphoid cells (ILC) 2 and ILC3, eosinophils, and basophils. Paradoxically, whereas such inflammation is the fundamental intrinsic cause of airway diseases such as asthma, chronic rhinosinusitis, and allergic bronchopulmonary aspergillosis (ABPA) and likely contributes to other serious disorders such as cystic fibrosis (CF), allergic inflammation is host-protective, promoting the killing or expulsion of fungal parasites and preventing the often-lethal complication of systemic dissemination.

Despite the long-recognized association between fungal mucosal infections and allergic inflammation, the molecular mechanisms by which the mammalian host recognizes fungi and directs its multipotent immune system into distinctly type 2 effector pathways has until relatively recently remained unknown. Here I describe key insights developed over the past several years into fungal-driven type 2 immune pathways. From the fungal perspective, secreted proteinases are by far the most important virulence factors yet described that drive type 2 immunity. Innately, fungal proteinases cleave many host proteins, but notably the clotting factor fibrinogen, fragments of which, termed fibrinogen cleavage products (FCPs) signal innately through Toll like receptor 4 (TLR4) to drive ILC and other innate type 2 antifungal immune responses, but intriguingly not adaptive TH2 and IgE responses.

Recent studies now indicate that proteinases and potentially other fungal virulence factors activate other aspects of the hemostatic system, especially platelets, to drive adaptive type 2 immunity. Together, these findings suggest that the immune and hemostatic systems coordinately control fungal growth *in vivo*, suggesting new opportunities, but also extraordinary challenges, to subvert serious airway fungal disease.

THE STRUCTURE AND IMMUNOGENICITY OF GALACTOMANNAN

THURSDAY 27 FEBRUARY 2020 (15.25 - 15.50)

Nikolay Nifantiev, PhD
N.D. Zelinsky Institute of Organic Chemistry - Moscow - Russia

Fungi are the only eukaryotes protected by a polysaccharide shell with an ambivalent function among pathogens: a protective role against environmental stress and a negative role in the induction of an antifungal immune response (1). The carbohydrate fragments of the cell wall polysaccharide responsible for the induction of the immune response have often been poorly defined due to the structural complexity and heterogeneity of such biopolymers. This is the case for the galactomannan (GM) of *Aspergillus fumigatus*, which is composed of tetraose repeats with α-(1→2) and α-(1→6) linked mannose units bound to short side chains comprising β-(1→5)- and β-(1→6)-linked galactofuranose units of varying length (2-4). The isolation of pure water-insoluble polysaccharide from the cell wall and the assessment of its immunoreactive fragments is difficult without structural modifications resulting from the harsh chemical extraction procedure required to solubilize the cell wall oligosaccharides.

To fill these gaps the systematic investigation (in collaboration with Professor J.-P. Latgé and his colleagues) of structurally distinct oligosaccharides representing specified GM fragments was initiated. Specially developed chemical methods (4,5) open the way towards the preparation of oligosaccharides related to GM characteristic structural elements. Galf-containing synthetic oligosaccharides and a variety of glycoconjugates thereof was shown to be indispensable models and tools for further structural analysis of GM samples (4), monitoring the repertoire of anti-GM mAbs (6,7) and serum antibodies (8), raising specific mAbs (6) and investigation of immune-modulating properties of specific GM fragments in cell-based experiments (8,9). The use of glycoarray built up from synthetic oligosaccharide ligands permitted to re-investigate (10) the carbohydrate specificity of EB-A2 mAbs currently used in EIA sandwich diagnostic kits detecting GM as the marker of IA and suggested the explanation of false positive signals which were protocolled (11,12) in the cases of application of this mAb.

Obtained results to be communicated in this talk demonstrate that chemically synthesized glycoconjugates are the indispensable tools to investigate precisely the immune response not only against GM but also against other components of insoluble polysaccharide shells of *A. fumigatus* and other fungi (13). This was demonstrated with the use of oligosaccharides related to α- (14,15) and β-glucans (16), chitin, galactosaminoglactan (17), α- and β-mannans (18) etc.

This work was supported by the Russian Science Foundation (grant 19-73-30017).

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SIDEROPHORES AND FUNGAL PATHOGENESIS

THURSDAY 27 FEBRUARY 2020 (16.20 - 16.45)

Hubertus Haas, PhD
Medical University of Innsbruck - Austria

Iron is an essential but in excess toxic metal. Therefore, fungi evolved fine-tuned mechanisms for controlled uptake and storage of iron. The most common mold pathogen of humans, *Aspergillus fumigatus*, employs siderophores (low molecular mass ferric iron-specific chelators) for uptake, intracellular transport and storage of iron. Siderophores biosynthesis proved to be crucial not only for adaptation to iron starvation conditions but also for microbial competition as well as virulence in murine infection models. In agreement, biosynthesis of co-factors for siderophore biosynthesis, riboflavin and pantothenic acid, were recently shown to be virulence determinants of *A. fumigatus*. Siderophore biosynthesis is regulated by two iron-sensing transcription factors, SreA and HapX. SreA is important for adaptation to iron excess via repression of iron acquisition, while HapX is crucial for both adaptation to iron starvation and iron excess. during iron starvation, HapX activates iron acquisition and represses iron consumption to spare iron during iron starvation and activates iron detoxification during iron excess. In agreement with *A. fumigatus* facing iron limitation during infection, HapX, but not SreA, was found to be crucial for virulence of *A. fumigatus* in a murine model of aspergillosis. Moreover, the transcription factors SrbA and LeuB link regulation of biosynthesis of siderophores, sterols and leucine. Remarkably, a siderophore transporter was found to be essential for uptake of the novel antifungal drug VL-2397, a cyclic hexapeptide with a ferrichrome-type siderophores structure chelating aluminum, which successfully passed clinical phase I. Recent studies indicated a high potential of siderophores in diagnosis of fungal infections: Replacing iron in siderophores by the radionuclide Gallium 68 allows *in vivo* imaging of fungal infections in animal models via positron emission tomography and the siderophore triacetylfusarinine C is a promising biomarker for *A. fumigatus* infections allowing non-invasive testing in urine.

MODELLING IA – HOW CLOSE ARE PREDICTED ANTIFUNGAL TARGETS?

THURSDAY 27 FEBRUARY 2020 (17.15 - 17.40)

Thomas Walsh, MD
Weill Cornell Medicine of Cornell University and New York Presbyterian Hospital - USA

Animal model systems are a critical component of the process of discovery and development of new antifungal agents for treatment and prevention of invasive aspergillosis. The persistently neutropenic rabbit model of invasive pulmonary aspergillosis (IPA) has been a highly predictive system in identifying new antifungal agents for treatment and prevention of this frequently lethal infection.

Since its initial development, the persistently neutropenic rabbit model of IPA has established a strong preclinical foundation for dosages, drug disposition, pharmacokinetics, safety, tolerability, and efficacy for deoxycholate amphotericin B, liposomal amphotericin B, amphotericin B lipid complex, amphotericin B colloidal dispersion, caspofungin, micafungin, anidulafungin, voriconazole, posaconazole, isavuconazole, and ibrexafungerp in treatment of patients with invasive aspergillosis. The findings of combination therapy with a mould active triazole and an echinocandin in this rabbit model also predicted the outcome of the clinical trial for voriconazole plus anidulafungin for treatment of IPA. The plasma pharmacokinetic parameters and tissue disposition for most antifungal agents approximate those of humans in persistently neutropenic rabbits. Safety, particularly nephrotoxicity, also has been highly predictive in the rabbit model, as exemplified by the differential glomerular filtration rates observed in animals treated with deoxycholate amphotericin B, liposomal amphotericin B, amphotericin B lipid complex, and amphotericin B colloidal dispersion.

Therapeutic outcome in the rabbit model is measured by a panel of validated outcome variables: including residual fungal burden, markers of organism-mediated pulmonary injury (lung weights and infarct scores), survival, and serum biomarkers. In selected antifungal studies, thoracic computerized tomography (CT) also is used with diagnostic imaging algorithms to measure therapeutic response of pulmonary infiltrates, which exhibit characteristic radiographic patterns, including halo signs. Dosage-dependent models with humanized dosing are then developed that correlate residual fungal burden, lung weights, infarct scores, and survival with outcome for design of clinical trials for treatment, as well as for prophylaxis. Demonstration of a multivariate response in safely achievable humanized dosages in the challenging immunosuppression of profound neutropenia of the persistently neutropenic rabbit model informs and de-risks the subsequent clinical protocol, while increasing the probability of fulfilling study endpoints in immunocompromised patients with IPA.

Further strengthening the predictive properties of the model, therapeutic response to successfully developed antifungal agents in treatment of IPA has been demonstrated over the past two decades by biomarkers of serum galactomannan and (1→3)-β-D-glucan. The pattern of resolution of serum galactomannan and (1→3)-β-D-glucan in the persistently neutropenic rabbit model of IPA closely mirrors those documented in patients with IPA in clinical trials.

The decision to move from laboratory to clinical trials should be predicated upon a portfolio of complementary and mutually validating preclinical laboratory animal models studies. Other model systems, including those in mice, rats, and guinea pigs, are also valuable tools in developing clinical protocols. Thus, meticulous PRECLINICAL investigation of a candidate antifungal compound in a robust series of predictive laboratory animal models will optimize study design, de-risk clinical trials, and ensure tangible benefit to our most vulnerable immunocompromised patients with IPA.

HOW TO MAKE A FAST AND ACCURATE DIAGNOSIS – WHAT CAN THE CLINICIAN AND LAB DO?

FRIDAY 28 FEBRUARY 2020 (08.00 - 08.55)

Dea Garcia-Hermoso, PhD
Institut Pasteur - Paris - France

Stéphane Ranque, MD PhD
Timone Hospital - Marseille - France

The epidemiology of invasive fungal infections (IFIs) has been remarkably modified over the past few years, particularly regarding incidence, evolution and prognosis. This is characterized largely by the increased incidence involving new risk populations, the early diagnosis due to improved tools or the novel therapeutic strategies based on new drugs. These infections remain the major cause of high morbidity and mortality in high-risk groups of individuals. Several studies indicate that IFIs' fatality rate is correlated with a delayed treatment, and timely treatment, at least in part, depends on timely diagnosis. Local epidemiology with high IFIs incidence rates, lead clinicians to implement either prophylactic or preemptive treatment strategies. One of the main reasons for choosing these strategies, is the relatively poor diagnostic indices of the IFIs assays currently available at the laboratory. In a perfect world, an early diagnosis of IFIs would allow an optimal therapeutic management and certainly will improve patient outcomes. In this lecture, we will review the state of the art of laboratory diagnosis in IFIs, and more specifically in invasive aspergillosis and mucormycosis. In addition we will discuss the areas for improvement for these two life threatening diseases, from the bedside to the bench and from the bench to the bedside.

DIFFICULT CASES IN FUNGAL SINUSITIS

FRIDAY 28 FEBRUARY 2020 (08.00 - 08.55)

Tarik Mohsena, MD FRCPC FACP DTM&H
Aseer Central Hospital, King Khalid University - Abha - Saudi Arabia

Allergic fungal sinusitis (AFS) is noninvasive form of fungal rhinosinusitis. Regional variation in incidence has been reported in different parts of the world. Patient with allergic fungal rhinosinusitis commonly presented with chronic rhinosinusitis with nasal polyps, allergic manifestation in the form of atopy, and elevated total serum immunoglobulin E. In the presentation we will discuss epidemiology of AFS worldwide, pathophysiology and clinical presentations. Medical treatment of AFS has been molded to target immune systems including steroid and aggressive anti allergic therapy. Surgical intervention may be needed in some cases.

Li-Ping Zhu, MD PhD
Huashan Hospital, Fudan University - Shanghai - China

Compared with the acute invasive form, chronic invasive fungal rhinosinusitis (CIFRS) and granulomatous invasive fungal rhinosinusitis (GIFRS) are easily misdiagnosed. Long and indolent clinical courses are typically reported, usually lasting for more than 12 weeks and even decades. Severe complications are usually found as initial manifestations, including blindness, cranial infections, and even death. Earlier diagnosis and appropriate treatment are crucial for better outcome. Therefore, increased attention should be paid to CIFRS and GIFRS. We would like to discuss in detail the management of CIFRS and GIFRS in this topic.

CURRENT EPIDEMIOLOGY OF INVASIVE FUNGAL INFECTIONS

FRIDAY 28 FEBRUARY 2020 (09.00 - 09.25)

Nina Khanna, MD
University Hospital of Basel - Switzerland

Invasive fungal infections (IFIs) have emerged in the last three decades as an important cause of human disease and are associated with high morbidity, mortality, and economic burden. Many factors have likely contributed to the emergence of IFI, including the HIV epidemic, a rise in the number of patients receiving a wide spectrum of immunosuppressive therapies, and increasing populations with frequent nosocomial exposure and interventions. However, accurate estimation of the true burden of IFI is difficult due to variation in definitions and limitations inherent to available case-finding methodologies. In this presentation, I summarize the current numbers and trends including the distribution of organisms, risk factors and anti-fungal consumption.

EPIDEMIOLOGICAL SURVEILLANCE FOR *ASPERGILLUS* – IS IT OF ANY VALUE?

FRIDAY 28 FEBRUARY 2020 (09.40 - 10.05)

Sophie Loeffert-Frémiot, PhD
Laboratoires ANIOS - ECOLAB - Sainghin en Melantois - France

Invasive aspergillosis (IA) due to *Aspergillus* has been associated with building construction, which may increase spore's emission nearby immunocompromised patients. Specific guidelines of protective measures against fungal contamination must be applied during all construction activity in healthcare establishment to prevent severe invasive aspergillosis (IA) in immunocompromised patients. A study was conducted to evaluate the efficacy of protective measures in reducing *Aspergillus* contamination in the indoor environment of hospital units during demolition works and also to determine whether internal factors or outdoor factors could influence the indoor contamination at *Aspergillus* and may help to propose some improvements in actuals methods and practices.

A daily surveillance of fungal contamination was implemented during 11-months. Environmental survey was realized by air samplings, outdoor and indoor, with an automatic agar sampler. In parallel, surveillance of IA infection cases was conducted by epidemiological investigation. A total of 3885 air samples (1744 outdoor samples and 2141 indoor samples) were collected, allowing calculation of ratios (outdoor vs indoor) to confirm efficacy of preventives measures applied to reduce indoor aerocontamination. Outdoor continuous sampling of *Aspergillaceae* spores (spore/m3/day) was also realized by a Hirst collector. This collector was useful as alarm system for detection of contamination peaks. Similarly, monitoring of meteorological parameters seems to be an interesting tool, to prevent *Aspergillus* peaks. Finally, 394 isolates of AF, susceptible to antifungals (383 environmental and 11 clinical isolates) were genotyped using MLVA. Analysis of genotypes showed 7 similar genotypes shared by environmental and clinical isolates, suggesting that clinical colonization and/or infection may originate from the hospital environment.

This study draws attention to the efficiency of some protective measures implemented to significantly reduce indoor contamination. *Aspergillus* presence seems to be largely influenced by meteorological parameters and by the type and localisation of demolition works.

ASPERGILLUS CALIDOUSTUS – EPIDEMIOLOGY AND OUTCOME

FRIDAY 28 FEBRUARY 2020 (10.20 - 10.45)

Frederic Lamoth, MD
Lausanne University Hospital and University of Lausanne - Switzerland

Aspergillus of section *Usti* (group *ustus*) include about 21 species. Only *A. calidoustus* (and its closely related species *A. pseudodeflectus*) are themotolerant at 37°C and recognized as human pathogens, while the pathogenic role of other species remains uncertain. Contrarily to most *Aspergillus* spp., all species of section *Usti* exhibit some level of intrinsic resistance to triazoles and this particularity raises the question of their role as emerging causes of breakthrough invasive aspergillosis (IA) among patients receiving mold active antifungal prophylaxis. IA due to *A. calidoustus* are rare events, but have been increasingly reported over the last decade.

We conducted a retrospective multicenter analysis of IA due *Aspergillus* section *Usti*. Twenty-seven cases of proven/probable IA were identified from 8 European countries. These 27 cases were pooled with the 45 cases from our systematic review of previously published case series or case reports for epidemiological analyses. *A. calidoustus* represented the most frequent pathogen, with rare exceptions including *A. pseudodeflectus*, *A. granulosus* and *A. ustus*. Most patients were non-neutropenic hematopoietic stem cell or solid-organ transplant recipients receiving long-term immunosuppressive therapy. Ongoing mold active prophylaxis (mainly azoles) was present in about half of them. Extra-pulmonary sites of infection were observed in 54% cases, including mainly skin/soft tissues (28%) and brain (14%). Treatment consisted of multiple antifungal drugs (used consecutively or in combination) in 67% of cases and 24-week mortality was 58%.

In conclusion, *A. calidoustus* seems to affect mainly non-neutropenic transplant patients receiving mold active prophylaxis, with a propensity to affect skin and brain, and a high mortality rate.

REGULATORY ENDPOINTS FOR INVASIVE AND CHRONIC PULMONARY ASPERGILLOSIS – WHAT IS NEEDED?

SATURDAY 29 FEBRUARY 2020 (07.45 - 08.55)

Oliver Cornely, MD
University of Cologne - Germany

Thomas F. Patterson, MD
UT Health San Antonio - Texas - USA

Radu Botgros, MD
European Medicines Agency - Amsterdam - The Netherlands

Sumathi Nambiar, MD
US Food & Drug Administration - Maryland - Switzerland

CLSI: AFST FOR BREAKPOINTS - WHERE WE ARE GOING?

SATURDAY 29 FEBRUARY 2020 (08.00 - 08.55)

Jesús V. Guinea Ortega, PharmD, PhD
General University Hospital Gregorio Marañón - Madrid - Spain

David Andes, MD
University of Wisconsin - Madison - USA

Aspergillosis outcomes are suboptimal despite therapy with approved antifungal drugs. Emerging resistance to triazole antifungals further imperils patient outcome. Development of relevant susceptibility breakpoints for the triazoles and *Aspergillus fumigatus* can provide a tool to guide and optimize treatment. The Clinical Laboratory Standards Institute (CLSI) recently set breakpoints for voriconazole and *A. fumigatus* as a first step in this process. The committee utilized a combination of MIC distribution (including epidemiologic cut of assessment), preclinical pharmacokinetics and pharmacodynamics (PK/PD) study results, clinical outcome analysis for therapeutic drug monitoring (TDM) studies, analysis of outcome relative to MIC, and clinical PK/PD analysis of voriconazole clinical trials. The results of these complementary approaches identified voriconazole MIC values linked to treatment failure (≥2 µg/ml) and further identified susceptibility at concentrations ≤0.5 µg/ml. An intermediate category was also included to encompass isolates with an MIC of 1 µg/ml with clarifying comments regarding voriconazole dosing and optimal trough concentrations (>1 µg/ml). The individual datasets utilized in this decision process will be reviewed in this session.

ACQUIRING AZOLE RESISTANT ASPERGILLI – WHERE FROM?

SATURDAY 29 FEBRUARY 2020 (09.00 - 09.25)

Dolores Pinheiro, MD
Centro Hospitalar Universitário de S. João - Porto - Portugal

Aspergilli are the leading agents of human mold infection. They affect immunocompetent and immunocompromised patients with a broad-spectrum of diseases. In a variety of recommendations, azole treatment is considered the cornerstone in the management of human aspergillosis.

Beyond the intrinsic antifungal drug resistance, well recognized in certain *Aspergillus* species, reports of acquired azole resistance became more frequent, particulary in *A. fumigatus*-complex. They have world-wide distribution, but also exhibit differences, according to geography and hospital categories. They seem to be the result of prolonged treatment schedules or the exposure to resistant environmental strains.

MANAGEMENT OF ASPERGILLOSIS DUE TO AZOLE-RESISTANT *ASPERGILLUS FUMIGATUS*

SATURDAY 29 FEBRUARY 2020 (10.10 - 10.35)

Bart-Jan Kullberg, MD PhD
Radboud University Medical Center - Nijmegen - The Netherlands

Resistance to antifungal agents is an increasing problem in *Aspergillus* diseases. Acquired resistance to azoles is mainly found in *Aspergillus fumigatus,* and is reported globally. *Aspergillus* species can be intrinsically resistant to azoles, or may acquire resistance following exposure to azole compounds during therapy. Another route of resistance selection is through exposure of *A. fumigatus* to azole fungicides in the environment. As resistant spores are present in ambient air, patients may present with azole-resistant *Aspergillus* disease without previous azole therapy. Patients may inhale both azole-susceptible and azole-resistant spores, and mixed infection has been reported in patients with invasive pulmonary aspergillosis.

1. Treatment of acute invasive aspergillosis with proven susceptibility to azoles

In a pivotal randomized study, voriconazole was superior to amphotericin B deoxycholate for the treatment of invasive aspergillosis; in patients recategorized according to the 2008 EORTC/MSG definitions for invasive fungal infections, survival in probable/proven cases was 70% with voriconazole and 55% with AmB (Herbrecht 2002/2015). In a more recent randomized trial, survival was 81% with isavuconazole versus 80% with voriconazole (Maertens 2016). In a large cohort study on patients fulfilling EORTC/MSG criteria in France, survival associated with L-AmB initial therapy was 47%, versus 69% with voriconazole (Nivoix 2008). Probable/proven cases from the randomized study of L-AmB at 3 mg/kg/d compared with 10 mg/kg/d, recategorized according to the 2008 EORTC/MSG criteria, had similar survival rates (58% vs. 50%) (Cornely 2007/2011). Small trials on primary treatment with caspofungin have yielded survival rates of 50% to 53% (Viscoli 2009; Herbrecht 2010). Based on these data, voriconazole or isavuconazole are considered the primary treatment for invasive aspergillosis with proven azole susceptibility. Importantly, these data constitute the template for prediction models on treatment of azole-resistant aspergillosis.

2. Antifungal therapy of documented azole-resistant invasive aspergillosis

In line with animal models, case series now have shown high azole failure rates, ranging from 50% to 100%, in patients with azole-resistant acute or chronic pulmonary aspergillosis. In a recent cohort study of 196 culture-positive cases receiving voriconazole, the 6 weeks' survival was 23% lower in those with voriconazole-resistant invasive aspergillosis compared with voriconazole-susceptible cases, confirming that azoles should be avoided in those cases (Lestrade 2018). Thus, L-AmB is considered the treatment of choice in case of proven azole-resistant invasive aspergillosis.

3. Antifungal therapy of acute invasive aspergillosis with unknown azole resistance

Whereas frequency and distribution of environmental azole resistance varies widely, prevalence of azole-resistant aspergillosis has been increasing to >10% in specific regions or countries, e.g., in the Netherlands, with local resistance frequencies exceeding 25%. There are no known risk factors that enable identification of patients at high risk of acquiring environmental azole-resistant invasive aspergillosis. For initial antifungal therapy in cases with unknown azole resistance, both de-escalation strategies (step-down once azole-susceptibility has been confirmed), or escalation strategies (step-up from voriconazole to L-AmB in case of subsequent identification of resistance) may be considered. In a recent cohort study, such escalation strategy was associated with reduced survival (53%) compared with patients who received appropriate initial therapy (76%) (Lestrade 2018). Thus, escalation strategy as currently recommended by IDSA Guidelines is not appropriate in settings with high prevalence of environmental azole resistance. Initial broad-spectrum

antifungal therapy, followed by de-escalation guided by subsequent susceptibility results, is expected to be associated with better outcomes.

Most randomized studies of invasive aspergillosis have been conducted before the emergence of azole resistance, and the study results are limited to patients with an *Aspergillus* isolate that has confirmed susceptibility to azoles. Hence, recommendations on treatment of resistant strains are predominantly based on preclinical data and on modelling of clinical trial data obtained from patients azole-susceptible invasive aspergillosis. For azole-susceptible aspergillosis, the available data have suggested a 10% to 15% improved survival at 12 weeks with voriconazole, isavuconazole or voriconazole-echinocandin combination treatment, compared with therapies such as L-AmB or echinocandin monotherapy. Modelling suggests that combination therapy with an azole and either an echinocandin or L-AmB is associated with highest survival rates in patients with unknown azole susceptibility, combining the superior efficacy of the azoles against susceptible isolates with a second agent active against azole-resistant strains. The azole-echinocandin and azole-L-AmB combinations are predicted to be equally effective at prevalence rates for azole resistance of 5% to approximately 50%. At very high (≥50%) resistance rates, the azole-L-AmB combination is predicted to be more effective, in view of the limited effectiveness of *de facto* echinocandin monotherapy in azole-resistant cases treated with an azole-echinocandin combination. Even in settings with population environmental resistance rates of 10% to 30%, still the majority of patients (90% to 70%) will be infected by an azole-susceptible *Aspergillus* strain. Therefore, survival rates with L-AmB monotherapy in a setting up to 60% azole resistance are predicted to be less favourable than with either combination therapy.

Thus, the collective data favours a de-escalation strategy for patients with acute invasive aspergillosis, applying a combination therapy with either azole and echinocandin or azole and L-AmB in cases with unknown azole susceptibility, against a wide range of background environmental resistance rates of 5% to 60%. These combination strategies are predicted to lead to higher survival rates than monotherapy with either an azole or L-AmB.

RECOGNITION OF DHN-MELANIN BY C–TYPE LECTIN RECEPTOR

SATURDAY 29 FEBRUARY 2020 (11.05 - 11.30)

Gordon D. Brown, PhD
University of Exeter - UK

The last few decades has seen a tremendous increase in our understanding of the mechanisms underlying the development of protective anti-microbial immunity. Key among these discoveries is the identification of pattern recognition receptors (or PRRs) expressed by immune cells, which recognise conserved microbial components. Recognition of these structures by PRRs, such as members of the C-type lectin receptor (CLR) family, triggers intracellular signalling cascades that initiate a variety of cellular and inflammatory responses, and induce the development of pathogen-specific adaptive immunity. We now understand that innate recognition by CLRs is essential for the development of protective antimicrobial immunity, and particularly for the control of fungal infections. In this presentation, I will cover the recent developments in our understanding of the functions of these receptors, highlighting ongoing work from my laboratory.

ANTIMICROBIAL PEPTIDES SECRETED BY *ASPERGILLUS FUMIGATUS*

SATURDAY 29 FEBRUARY 2020 (12.15 - 12.40)

Sven Krappmann, PhD
University Hospital Erlangen - Germany

Opportunistic infections by fungal pathogens pose a global and prevalent threat for a variety of patients that commonly suffer from specific risk factors. To some extent, fungal virulence results from the combination of various determinants that act on growth and invasion and that have evolved at the primary ecological niche, serving as dual-use factors to support ready-made pathogenicity. Moreover, toxic compounds and antimicrobial activities may be produced by a fungus in the wild that gain advantage over competitors but that may also be of relevance in the context of infection. *Aspergillus (A.) fumigatus* as main causative agent of aspergillosis produces a plethora of secondary metabolites, some of them being mycotoxins. Its asexual spores, the conidia, are formed on specialized structures to become dissipated by air flows or water currents but serve also as infectious propagules to colonize and invade a susceptible host. We demonstrate that this mould expresses antimicrobial peptides resembling cysteine-stabilized (CS)-αβ-defensins that are produced in a highly specific spatial and temporal manner. Both peptides, AfusinN and AfusinC, are encoded by the *defX* locus, transcription of which is significantly increased in the course of asexual sporulation. Deletion of *defX*, however, does not result in any prominent phenotype. Localization studies using GFP as proxy and tagged defensing-like peptides indicate that the *defX* gene products are secreted onto the conidial surface to coat the asexual spores. Strikingly, co-cultivations of fungal spore extracts with bacteria revealed an antimicrobial function of the *defX* gene products, which might have implications for the infectious process resulting in aspergillosis when considering competition with the host microbiome or interaction with immune cells.

ONE WORLD - ONE GUIDELINE: MUCORMYCOSIS

SATURDAY 29 FEBRUARY 2020 (14.20 - 14.45)

Oliver Cornely, MD
University of Cologne - Germany

Mucormycosis is a difficult to diagnose rare disease with high morbidity and mortality. Diagnosis is often delayed, and disease tends to progress rapidly. Urgent surgical and medical intervention is lifesaving. Guidance on the complex multidisciplinary management has potential to improve prognosis, but approaches differ between health care settings.

From January 2018, authors from 33 countries in all United Nations regions analysed the published evidence on mucormycosis management and provided consensus recommendations addressing differences between the regions of the world as part of the "One World One Guideline" initiative of the European Confederation of Medical Mycology (ECMM). The author group based in 17 time zones, relied on electronic media including video tutorial on methodology, and central document repository with several daily updates.

Signs and symptoms of mucormycosis depend on organ patterns and underlying conditions. Diagnostic management does not differ greatly between world regions. Upon suspicion of mucormycosis appropriate imaging is strongly recommended to document extent of disease and is followed by strongly recommended surgical intervention. First-line treatment with high-dose liposomal amphotericin B is strongly recommended, while intravenous isavuconazole and intravenous or delayed release tablet posaconazole are recommended with moderate strength. Both triazoles are strongly recommended salvage treatments. Amphotericin B deoxycholate is recommended against, because of substantial toxicity, but may be the only option in resource limited settings.

Management of mucormycosis depends on recognising disease patterns and on early diagnosis. Limited availability of contemporary treatments burdens patients in low and middle income settings. Areas of uncertainty were identified and future research directions specified.

DISTINGUISHING INVASIVE MUCORMYCOSIS FROM ASPERGILLOSIS NON-INVASIVELY

SATURDAY 29 FEBRUARY 2020 (14.45 - 15.10)

Vladimír Havlíček, PhD
Institute of Microbiology of the Czech Academy of Sciences - Prague - Czech Republic

Under metal-restricted conditions, all microbes activate their sophisticated biosynthetic machinery to better compensate for their demands and to acquire free essential metals needed for microorganism survival. Microbial metallophores with high stability constants represent one of the many pleiotropic virulence factors by which a pathogen combats with other microbial players or an invaded host. The efficiency of non-ribosomal synthesis of various metallophores is tremendous [1], and their molecular structures are far different from those of their mammalian host. The specificity and sensitivity of microbial metallophore application in the early and non-invasive diagnosis of siderophores have been documented recently [2].

In a retrospective study, a literature-based database of seven hundred microbial siderophores was used to screen patients with invasive microbial infections. The database also covered the genera *Aspergillus, Rhizopus, Cunninghamella, Mucor,* and *Lichtheimia*. For the diagnosis of invasive zygomycosis, we used zygomycete-secreted rhizoferrin in a cohort of seven patients with zygomycosis. The other fourteen patients suffered from invasive pulmonary *A. fumigatus* infections (IPA), and another fourteen patients represented the negative controls (critically ill patients suffering from non-IPA diseases and non-zygomycosis). Human urine samples were creatinine-standardized and examined by liquid chromatography (LC) and Fourier transform ion cyclotron resonance (FTICR) mass spectrometry (MS). The biomarkers were quantified against matrix-matched standards and our in-house tool called CycloBranch (https://ms.biomed.cas.cz/cyclobranch) was used for compound dereplication.

In all patients infected with zygomycetes, either imido-rhizoferrin, rhizoferrin glycosylated analogue or their degradation products were detected in the patients' urine. Serum and bronchoalveolar lavage fluids were also examined. No zygomycete biomarkers were observed in a set of fourteen patients suffering from IPA in which urinal triacetylfusarinine C (uTAFC), ferricrocine (uFC), and gliotoxin (uGTX) were quantified along with their hydrolytic and metabolic products. The limit of quantitation of uTAFC was 0.3 ng/mL, and the maximum observed concentration was 1.6 μg/mL, i.e., beyond the current patented serology tools based on anti-siderophores. In this set of critically ill patients, which consisted of mostly non-neutropenic subjects suffering from IPA, the uTAFC sensitivity was 92.9% compared to serum galactomannan (sGM, 35.7%), less-specific (1,3)-β-D-glucan (85.7%) and *A. fumigatus* specific IgA and IgG (sIgAG, 20%) in serum. Notably, the sensitivity levels of uFC and uGTX were higher than those of sGM. In the same cohort, the specificity of uTAFC was 100% compared to sGM (73%) and sIgAG (66.6%).

Urinal samples are analytically attractive due to a lower chemical background compared to serum or bronchoalveolar lavage fluid samples and are clinically attractive due to non-invasive applications. Contrary to other molecular tools, no amplification (PCR), culture (Biotyper), or extensive sample preparation (serology) are needed for LC-FTICR analysis of urine, in which siderophores are concentrated.

Acknowledgements: Support from the Ministry of Education, Youth and Sports of the Czech Republic (LO1509), and the Czech Science Foundation (19-10907S).

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MUCOR SPECIES & MACROPHAGES

SATURDAY 29 FEBRUARY 2020 (15.40 - 16.05)

Victoriano Garre, PhD
University of Murcia - Spain

Mucormycosis is an emerging neglected infection caused by Mucorales, a group of early-diverging fungi barely studied. Despite the progress in knowledge of the molecular processes underlying the establishment and progression of this disease, several aspects are largely unknown. The innate immune response is the first barrier at the site of the infection, where phagocytic cells are recruited to internalize spores, but the molecular and cellular responses of Mucorales to host phagocytic cells are not well defined. Characterization of genes differentially regulated at this infection stage by transcriptomic analysis has been postulated as a straightforward strategy to elucidate key molecular aspects of the interaction. We have selected *Mucor circinelloides* to conduct a transcriptomic analysis in spores phagocytosed by macrophage because this Mucoral has a wide repertoire of molecular tools to manipulate its genome allowing the subsequent study of gene function. The transcriptomic analysis revealed the presence of an intricate gene network that enhances crucial functions to survive and germinate inside the phagosome, such as metabolic adaptation and response to stresses. Functional characterization of this response was carried out by deleting genes highly upregulated in response to macrophages. Deletion mutants generated in genes encoding putative transcription factors (*atf1*, *atf2*, and *gcn4*), extracellular proteins (*chi1* and *pps1*), and an aquaporin (*aqp1*) revealed that these genes perform important roles in survival following phagocytosis, germination inside the phagosome, and virulence in mice. Moreover, *atf1* and *atf2* play a key role in these pathogenic processes since their mutants showed the strongest phenotypes and both genes control a complex ATF-mediated germination pathway, which includes *chi1* and *aqp1*.

We reasoned that the wide genetic response showed by the spores after phagocytosis could be mediated by some mechanism that may controlled hundreds of genes at once. Three RNAi pathway has been described in *M. circinelloides* that can regulate the expression of high number of genes. One of these pathways, known as non-canonical because it is independent of key RNAi enzymes such as Dicer and Argonaute, is able to specifically repress gene expression by degradation of the corresponding mRNAs. Transcriptomic analysis of mutants in essential genes of this non-canonical RNAi pathway, *r3b2* and *rdrp1*, showed a constitutive activation of the response to macrophages even in their absence, suggesting that this RNAi pathway is repressed during phagocytosis allowing well-organized genetic response of the spores. This dramatic alteration of the fine genetic program to survive phagocytosis is expected to affect host infection, which is further supported by the reduced virulence of mutants in *r3b2* and *rdrp1* in mouse infection. The presentation will review and discuss the most recent works related to the gene regulation mechanisms operating in the spore after phagocytosis by macrophages, which could serve as pharmacological targets to control mucormycosis.

CHALLENGE FOR CHRONIC PULMONARY ASPERGILLOSIS - A COMMON BUT FORMIDABLE DISEASE

SATURDAY 29 FEBRUARY 2020 (16.35 - 17.00)

Yoshitsugu Miyazaki, MD PhD
National Institute of Infectious Diseases - Tokyo - Japan

Chronic pulmonary aspergillosis (CPA) shows a low 5-year survival rate of approximately 50%–60%, and its management remains still challenging due to limited number of antifungal agents available for treatment of this disease. Although we observed efficacy of intravenous echinocandin as induction therapy for those showed acute or subacute exacerbation, oral azole therapy is considered the mainstay for long-term treatment in outpatients. Currently, 4 oral anti-*Aspergillus* agents; itraconazole (ITCZ), voriconazole (VRCZ), posaconazole, and isavuconazole are available, and some clinical trials and retrospective observational studies for the treatment of CPA have been conducted, mostly for ITCZ and VRCZ. We recently reported that VRCZ showed better effectiveness than oral ITCZ as in clinical symptoms and signs, although multivariable Cox regression analysis showed no significant difference in overall mortality as well as CPA-associated mortality in between ITCZ and VRCZ. Further studies with newer azoles would be required to evaluate efficacies against CPA.

Further, azole antifungal resistance has been rising and some CPA patient is refractory to azole maintenance. Since there are several genetic mutations in the azole target Cyp51Ap reported to be associated with low azole susceptibility as a phenotype, we evaluated putative amino acid change if each change could really cause azole resistance in the strain itself in which the change had been found. CRISPR/Cas9 genome editing technique enabled such analysis, and would be useful to define appropriate target molecules for both diagnosis and treatments in mold infectious diseases.

BIOFILM FORMATION BY *ASPERGILLUS FUMIGATUS* – IMPLICATIONS FOR TREATMENT

SATURDAY 29 FEBRUARY 2020 (17.15 - 17.40)

Donald Sheppard, MD
McGill University - Montreal - Canada

Aspergillus fumigatus forms biofilms during both acute invasive and chronic pulmonary infection. Biofilms play an important role in the pathogenesis of infection, and help mediate adhesion to host tissues, immune evasion and resistance to antifungals. *A. fumigatus* biofilm formation is dependent on the production of the cell wall and secreted exopolysaccharide galactosaminogalactan (GAG), a heteropolysaccharide composed of α-1,4-linked galactose and partially deacetylated N-acetylgalactosamine. GAG plays a role in a wide range of biologic functions during infection. Cell wall-associated GAG mediates adhesion of hyphae to host cells and abiotic surfaces, and cloaks β-glucans from recognition by dectin-1. Soluble fractions of GAG induce neutrophil apoptosis and the production of the immunosuppressive cytokine IL-1 receptor antagonist. GAG also induces platelet aggregation and activation, suggesting that GAG may play a role in vascular thrombosis during pulmonary *Aspergillus* infection. GAG composition and production varies between *Aspergillus* species, and may contribute to species-specific differences in virulence among *Aspergilli*. Recent studies have indicated that GAG synthesis is more complex than previously appreciated, and requires the coordinated activity of at least 9 carbohydrate-modifying enzymes.

This presentation will review new insights into the molecular mechanisms governing GAG biosynthesis, the role of GAG in virulence, and the potential for the development of therapeutic anti-biofilm strategies that target the synthesis or function of this polysaccharide virulence factor.

THE NEW WORLD OF INHALED ANTIFUNGALS & BIOLOGICS – WHERE MIGHT IT TAKE US?

SATURDAY 29 FEBRUARY 2020 (17.55 - 18.20)

Richard B. Moss, MD
Stanford University - Palo Alto - USA

For well over half a century nebulization of intravenous formulations of amphotericin B have been employed clinically to treat various manifestations of pulmonary aspergillosis, but no antifungals specifically designed for inhalation have been developed and licensed despite substantial preclinical evidence suggesting benefits of enhanced safety margin (with high intrapulmonary and low systemic drug exposure) and efficacy. That situation is now changing with development of novel inhalational formulations of polyene and triazole antifungal agents[1-4]. While earlier attention focused on the potential of inhaled antifungals to prevent or treat invasive pulmonary aspergillosis in high-risk immunocompromised hosts, recent progress in understanding the prominent role of chronic endobronchial infection with *Aspergillus fumigatus* or other fungi in the T2-high pathogenesis of severe allergic asthma has led to concept of adjunctive antifungal therapy in this large immunocompetent population. Trials of systemic triazole therapy in mold-sensitized patients with severe asthma have produced mixed results, possibly related to issues with attainment of sufficient and durable airway drug concentration. Consequently there is current hope that specifically designed inhalational antifungals, possibly in combination with systemic antifungals[6], may have utility in allergic as well as invasive aspergillosis. Phase 2 clinical trials of at least two such novel inhalational antifungals active against *Aspergillus fumigatus*, PC945 (Pulmocide Ltd, London UK) and PUR1900 (Pulmatrix, Lexington MA, USA), are underway in patients with asthma (ClinTrials.gov NCT03745196) and allergic bronchopulmonary aspergillosis (NCT03960606) as well as cystic fibrosis (NCT03870841) and lung transplant (NCT03905447). On the host side of the equation, T2-targeted biological agents (omalizumab, mepolizumab, reslizumab, benralizumab, dupilumab) have proven safe and highly effective in treating T2-high severe asthma[6]; the oldest and most vetted agent, omalizumab, is effective in ABPA[7-9]. Newer licensed biologics targeting IL-5 or IL-4Rα also show promise and at least one clinical trial in ABPA has been initiated (NCT04108962). Prospects for safer and more effective agents than systemic steroids and triazoles against allergic aspergillosis appear bright, with possible anti-fungal/immunomodulatory combination therapy on the horizon as well.

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POSTER ABSTRACTS

1 SURVEILLANCE OF MUCORALES RESISTANCE TO AZOLES IN THE WASTE SORTING INDUSTRY: ASSESSMENT OF FILTERING RESPIRATORY PROTECTIVE DEVICES AND GLOVES

LA Caetano[1,2]*, M Dias[1], B Almeida[1], C Viegas[1,3,4]

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[2]Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, University of Lisbon, Portugal
[3]Public Health Research Centre, NOVA National School of Public Health, Universidade NOVA de Lisboa, Lisbon, Portugal
[4]Comprehensive Health Research Center (CHRC), NOVA Medical School, Universidade NOVA de Lisboa, Lisbon, Portugal

Purpose:
The use of filtering respiratory protective devices (FRPD) and gloves is mandatory in waste-sorting industry in Portugal as preventive of workers' exposure to bioaerosols, including fungi. Mucorales order includes a large number of ubiquitous saprophytes species that can cause severe infections, such as mucormycosis, which is associated with a great deal of morbidity. The emergence of resistance to azoles among fungal species, including Mucorales, in the environment challenges the management of severe fungal infections. The aim of this study was to assess the Mucorales burden retained by filtering respiratory protective devices (FRPD) (interior layer and exhalation valves) and present in gloves used in the waste sorting industry, and to screen the resistance of Mucorales to azole-drugs in this occupational environment.

Methods:
The sample consisted of 120 FRPD and corresponding interior layer and exhalation valves, and 67 gloves. All matrixes were extracted and streaked onto MEA and DG18. After incubation at 27ºC for 5 to 7 days, the Mucorales densities (CFU/m^2) were calculated, and the genera were identified through macro and microscopic characteristics. The prevalence of azole-resistance was determined in screening agar plates containing Sabouraud media supplemented with 4 mg/L itraconazole (ITRA), 1 mg/L voriconazole (VORI), and 0.5 mg/L posaconazole (POSA), incubated at 27°C for 5 days, adapted from EUCAST guidelines.

Results:
Mucorales was detected in FRPD both on interior layers (12.35% MEA; 0.03% DG18) and on exhalation valves (0.21% DG18), and in gloves (39.00% MEA; 4.03% DG18). Mucorales was also detected in azole-supplemented media with a relevant burden, as follows: in gloves ($7.90x10^6$ CFU.m^{-2} ITRA; $1.15x10^7$ CFU.m^{-2} VORI; $1.46x10^6$ CFU.m^{-2} POSA); in FRPD ($1.00x10^3$ CFU.m^{-2} ITRA; $5.01x10^5$ CFU.m^{-2} VORI; $2.50x10^5$ CFU.m^{-2} POSA - interior layer; $2.00x10^3$ CFU.m^{-2} ITRA; $7.52x10^5$ CFU.m^{-2} VORI; $1.00x10^3$ CFU.m^{-2} POSA - exhalation valves). *Rhizopus* sp.presented reduced susceptibility to posaconazole, besides voriconazole, at the tested concentration.

Conclusion:
The contamination of FRPD and gloves with Mucorales was observed at the waste sorting industry. Future trials to test protective efficacy of FRPD and gloves should be performed as fungal contamination was detected in both. Since posaconazole is one of the few effective azole drugs currently used for the treatment of mucormycosis, azole-resistance profile should be further determined to assess the risk of exposure to azole-resistant Mucorales in the waste sorting industry.

Acknowledgments:
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2 *ASPERGILLUS* SPP. AND AZOLE-RESISTANCE CHARACTERIZATION ON FILTERING RESPIRATORY PROTECTIVE DEVICES FROM WASTE SORTING INDUSTRY

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Purpose:
Studies performed on waste management industry have reported *Aspergillus* as the most frequent genera on waste-sorting, incineration and composting. Filtering respiratory protective devices (FRPD) are disposable after one-day use (workshift) and their use is mandatory in Portuguese waste-sorting industries. During FRPD use, humidity and temperature conditions provide a favorable environment for *Aspergillus* growth retained. The aim of this study was to characterize *Aspergillus* spp. presence in FRPD interior layer and exhalation valves, as well as to detect possible azole-resistant isolates in this complex indoor environment.

Methods:
The analyzed samples consisted of 120 FRPD (interior layer and exhalation valves). Fungal load was extracted from both matrixes with 10 mL of 0.1% Tween™ 80 saline solution (NaCl 0.9%) for 30 min at 250 rpm, and 150 µL of those extracts were streaked onto malt extract agar (MEA) supplemented with chloramphenicol (0.05%) and dichloran glycerol agar (DG18). After incubation at 27ºC for 5 to 7 days *Aspergillus* spp. densities (CFU/m^2) were calculated, and *Aspergillus* sections were identified through macro and microscopic characteristics. The frequency of azole-resistance was determined by inoculation of the extracts onto screening agar plates containing Sabouraud dextrose agar media supplemented with 4 mg/L itraconazole (ITRA), 1 mg/L voriconazole (VORI), and 0.5 mg/L posaconazole (POSA), incubated at 27°C for 5 days.

Results:
Aspergillus spp. was detected in both interior layers (77 out of 120; 64.17%) and exhalation valves (63 out of 120; 52.5%). Among the *Aspergillus* genera, section *Fumigati* presented the highest frequency, both in exhalation valves (76.57% MEA; 87.24% DG18) and in interior layers (75.81% MEA; 51.22% DG18). *Fumigati* and *Nigri* were the *Aspergillus* sections isolated more frequently on MEA. In addition, *Flavi*, *Circumdati* and *Candidi* sections were also frequently isolated on DG18. *Restricti* and *Aspergilli* sections were observed occasionally. DG18 allowed the detection of a more diversified set of *Aspergillus* species than MEA (in both FRPD matrixes). In azole-supplemented media, *Aspergillus* spp. was the most frequently found genus on exhalation valves (75.0% of the isolates that grew onto ITRA), suggesting that resistant isolates to ITRA at the tested concentration might be present in this occupational environment.

Conclusion:
This study reports *Aspergillus* contamination of FRPD used by workers at waste industry and *Aspergillus* isolates exhibiting reduced susceptibility to azoles. Future trials should be performed to test FRPD protective efficacy and establishment of times for FRPD replacement, and to continue monitoring the establishment of azole-resistant strains, as to reduce the risk of developing fungal infection in this work environment and failures in consequent therapeutic schemes.

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3 ANTIFUNGAL SUSCEPTIBILITY TESTING OF CLINICAL AND HOSPITAL *ASPERGILLUS* ISOLATES AT THE NEPHROLOGY WARD OF AN IRANIAN TRAINING HOSPITAL

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Purpose:
Recently, the rate of invasive infections caused by filamentous fungi like *Aspergillus* genus is growing in immunocompromised persons particularly in transplant recipients. Unfortunately, laboratory diagnostics and drug resistance of invasive aspergillosis remains real problems. The aim of this work was to introduce a reproducible molecular identification method and practically use the method for rapid identification of *Aspergillus* species isolated from clinical sources.

Methods:
We used PCR-restriction fragment length polymorphism with single restriction enzyme, confirmed by sequencing of *Aspergillus* rDNA gene. The minimum inhibitory concentrations (MICs) of amphotericin B, fluconazole, itraconazole, voriconazole, posaconazole, and caspofungin were determined based on the guidelines of Clinical and Laboratory Standards Institute.

Results:
Clinical isolates of *Aspergillus species* identified by PCR-RFLP included *A. fumigatus* (18.5%), *A. group nigri* (11.1%), *A. group flavi* (66.7%) and *A. terreus* (3.7%). All clinical *Aspergillus* were sensitive to Voriconazole and Itraconazole but one cases of *A. fumigatus* which showed resistance to Itraconazole. Also hospital indoor isolates of *Aspergillus* identified as *A. fumigates* (2 colonies per swab*), A. group nigri* (11 colonies) and *A. group flavi* (3 from 16 cases).

Conclusion:
Our results show a similar pattern of *Aspergillus* distribution at hospital indoor and cases were inpatients.

4 AZOLE- AND AMPHOTERICIN B-RESISTANT *ASPERGILLUS FUMIGATUS* STRAINS ISOLATED FROM CLINICAL SPECIMENS AND ENVIRONMENT IN AZERBAIJAN

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Purpose:
Aspergillus fumigatus is ubiquitous saprophytic mold associated with variety of pathological conditions in human. Small diameter of conidia facilitates their passage to lower respiratory tract airways making *A. fumigatus* the commonest etiological agent of aspergillosis. Azoles act on enzyme 14α-demethylase coded by cyp51 gene and block synthesis of ergosterol – the fungal cell wall component. It results in accumulation of toxic methylated sterols in cell and its damage.

Recently, intensive use of azoles in treatment and agriculture has resulted in emergence of azole-resistant strains. The aim of current investigation was to evaluate prevalence of azole resistant *A. fumigatus* strains in environment and clinical samples of patients applied to hospitals of Azerbaijan Republic.

Methods:
Both clinical and environmental samples were gathered during 2017-2019 period. Environmental samples were collected from 8 regions of Azerbaijan Republic. Clinical specimens were obtained from patients applied to Scientific-Research Clinical Microbiological Laboratory, Educational-Therapeutic clinic of Azerbaijan Medical University and Scientific Research Institute of Lung Diseases of Azerbaijan Republic. Identification of strains was performed on basis of cultural, morphological features with subsequent molecular genetic analysis of internal transcribing spacer regions 1 and 4 (ITS1 and ITS4). All strains were tested for susceptibility to voriconazole (VOR), posaconazole (POS) and amphotericin B (AMB) in accordance to European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines.

Results:
44 *A. fumigatus* strains were isolated and identified. Results of antifungal susceptibility of these strains are represented in table 1. Among 44 isolates 3 were resistant to both azoles, 38 – to POS, 20 – to AMB.

Table 1. Antifungal susceptibility of 44 *A. fumigatus* strains to 3 antifungal drugs

Source (n)	Antifungal agent	MIC (mg/l)							
		≤0.12	0.25	0.5	1	2	4	8	≥16
Clinical isolates (10)	VOR	-	1	4	1	2	2	-	-
	POS	-	-	-	2	2	6	-	-
	AMB	-	-	-	2	5	3	-	-
Environmental isolates (34)	VOR	-	12	14	7	1	1	-	-
	POS	2	3	5	3	6	10	5	-
	AMB	-	-	-	6	13	11	1	4

Among 34 environmental isolates 28 were resistant to POS, 15 – to AMB. 3 POS resistant strains were also resistant to AMB. 1 isolate was resistant to both azoles and was isolated from public garden in southern part of Azerbaijan Republic. 9 strains out of 10 clinical isolates were resistant to POS, 2 of which were also resistant to VOR. 4 strains were resistant to POS and AMB.

The results of investigation have shown high overall prevalence of azole resistant *A. fumigatus* isolates in the environment (12.2%) and unexpectedly high resistance rate (90%) in clinical samples. The main reason for this high resistance rate in clinical strains is low number (10 strains) of investigated isolates. However, all strains were resistant at least to one of azole drugs and 8 had MIC values for AMB ≥2 mg/l. Taking into account high environmental prevalence of resistant strains and possible linkage between clinical and environmental isolate we consider that resistance rate in clinical samples is high in Azerbaijan Republic.

Conclusion:
We revealed high resistance rates both in environmental and clinical isolates. Thus, studies should be continued in higher number of clinical strains with subsequent genetic analysis in order to obtain information about genes responsible for resistance and phylogenetic relevance between environmental and clinical strains.

5 *HMG1* GENE MUTATION IN TRIAZOLE-RESISTANT *ASPERGILLUS FUMIGATUS* CLINICAL ISOLATES WITHOUT *CYP51A* GENE MUTATIONS

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Purpose:
Reports of resistance in *Aspergillus fumigatus* to triazole antifungals, the recommended first-line therapy for prophylaxis and treatment of *Aspergillus* related-diseases, are increasing worldwide. Triazole-resistance most commonly is due to alternations in the *cyp51A* gene and its promoter region. Recently, mutations in the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase-encoding gene (*hmg1*), an upstream gene involved in the ergosterol production pathway, were described and associated with triazole-resistance in a small number of clinical isolates. The majority of these mutations were located in the sterol-sensing domain region, which is involved in the negative regulation of its enzymatic effects by direct interaction with sterols. In this study, we determined the prevalence of mutations in the *hmg1* gene in triazole-resistant *A. fumigatus* isolates lacking *cyp51A* gene associated triazole-resistant mutations in our collection.

Methods:
Clinical *A. fumigatus* isolates collected between March 2016 and March 2019 with a confirmed triazole-resistant phenotype and no *cyp51A* gene associated-resistance mutation were selected for analysis. Additionally, ten triazole-susceptible isolates were included as controls. DNA was extracted from homogenized overnight liquid cultures. After PCR amplification, we performed sequencing of the *hmg1 g*ene using 16 designed primers based on the *hmg1* gene genomic reference sequence (Fungi DB accession number AFUB_020770, A1163). Isolates *hmg1* sequences were assembled to create a sequence consensus and subsequently aligned to the *hmg1* reference sequence to determine genetic variances.

Results:
We analyzed 13 triazole-resistant *A. fumigatus* clinical isolates without *cyp51A* gene mutations and 10 triazole-susceptible *A. fumigatus* clinical isolates (Table 1). In the triazole-resistant group, sequencing of the *hmg1* gene revealed 3 isolates (23%) with mutations conferring amino acid substitutions. The mutations in the *hmg1* gene were E105K, W273S, S541G and H564Y (one isolate with two mutations, W273S and S541G). Mutation W273S is located in the sterol sensing domain. All the isolates with a *hmg1* mutation had an MIC value of 4 mg/L for voriconazole, MIC values ranging from 0.125 to 8 mg/L for itraconazole and from 0.125-0.5 mg/L for posaconazole. Among the triazole-susceptible group, no *hmg1* amino acid substitutions were observed.

Conclusion:
The prevalence of *hmg1* gene mutations in our collection of triazole-resistant clinical *A. fumigatus* isolates without c*yp51A* gene resistant-associated mutations was 23% (3/13). Two previously described *hmg1* gene mutations associated with elevated MIC values to triazoles were detected (E105K and S541G), as well as two novel mutations, W273S and H564Y. Further investigation is required to determine the precise role of these mutations. Triazole-resistant isolates with *cyp51A* gene mutations will be analyzed in the near future.

Table 1.- *Hmg1* gene mutations in triazole susceptible and resistant *Aspergillus fumigatus* clinical isolates without *cyp51A* gene associated-resistance mutations

Sample	***hmg1* gene mutation** (amino acid substitution)	**EUCAST (MIC =mg/L)***		
		Voriconazole	**Posaconazole**	**Itraconazole**
Triazole-resistant isolates				
CYP-15-18	-**	8	0.5	1
CYP-15-27	E105K	4	0.5	8
CYP-15-33	H564Y	4	0.125	0.125
CYP-15-41	W273S, S541G	4	0.5	1
CYP-15-75	-	4	1	8
CYP-15-93	-	4	1	>16
CYP-15-106	-	4	0.5	>16
CYP-15-108	-	8	1	>16
CYP-15-109	-	>8	1	2
CYP-15-115	-	4	0.5	>16
CYP-15-117	-	0.25	0.5	>16
CYP-15-146	-	4	0.5	>16
CYP-15-147	-	4	1	>16
Triazole-susceptible isolates (Wild type)				
ASFU 4058	-	0.5	0.06	0.125
ASFU 4361	-	0.25	0.025	0.125
ASFU 4415	-	0.25	0.025	0.06
ASFU-4701	-	0.25	0.025	0.06
ASFU-5291	-	0.06	0.25	0.125
ASFU-5458	-	0.25	0.06	0.25
ASFU-5496	-	0.25	0.06	0.125
ASFU-5771	-	0.5	0.06	0.125
ASFU-5774	-	0.5	0.125	.025
ASFU-5779	-	0.25	0.06	0.125

* EUCAST broth microdilution reference method for filamentous fungi.
Triazole-resistance was established based on the EUCAST clinical breakpoints for *A. fumigatus* (voriconazole >2, itraconazole >2, posaconazole >0.25)

** " - " = not detected

6 EMERGENCE OF AZOLE RESISTANT *ASPERGILLUS* SPECIES: A CONSEQUENCE OF ENVIRONMENTAL EXPOSURE TO AZOLE PESTICIDES IN AGRICULTURAL FIELDS OF NORTH INDIA

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Purpose:
Agricultural industry in India uses pesticides, mainly azole fungicides to protect crop from phyto-pathogens. The extensive usage of azole pesticide has resulted in emergence of resistance among *Aspergillus* species. *Aspergilli* are ubiquitous fungal pathogen associated with several life-threatening infections in immunocompromised patients. Azoles are the first line of treatment against these *Aspergillus* infections. Therefore, infections due to resistant *Aspergillus* leads to inefficacy of azole antifungal drugs even in azole-naïve patients, leading to high fatality rates. It is important for regular screening of environmental azole resistant *Aspergillus* to analyse development of resistance in human pathogenic fungi. Hence, the aim of the study was to analyze environmental isolates of *Aspergillus* species for multi azole resistance.

Methods:
Soil samples were collected from various agriculture fields of North India. *Aspergillus* colonies were isolated and identified using microscopic analysis. The colonies were screened for azole resistance using agar plate assay with ketoconazole itraconazole, voriconazole, and posaconazole. Azole resistant isolates were further processed via disc diffusion, E-strip and MIC assay. For molecular identification of these resistant isolates PCR amplification of 18S rRNA region of genomic DNA was conducted.

Results:
Total 80 soil samples were analyzed of which 180 isolates were identified as *Aspergillus* species. Out of them, 50 were found to be resistant to at least one azole drug. Resistance pattern were as follow: *A. fumigatus* (15), *A. niger* (32), *A. flavus* (2) and *A. nidulance* (1). All the resistant isolates have identified as *Aspergillus* species via ITS sequencing.

Conclusion:
There is significant azole resistance among environmental isolates of *Aspergillus* species probably due to use of fungicide in agriculture. The data from this study would be a value addition to the limited antifungal multidrug resistant data available from India and will help clinicians for future antifungal therapies.

7 ISOEUGENOL AS A POTENTIAL ANTIFUNGAL MOLECULE AGAINST AZOLE RESISTANT ENVIRONMENTAL ISOLATES OF *ASPERGILLUS FUMIGATUS* AND THEIR BIOFILM

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Purpose:
Invasive aspergillosis (IA) remains a serious opportunistic fungal infection, particularly in patients with reduced immune defense, such as hematological malignancy or transplant recipients. *Aspergillus* spp. are responsible for over 200,000 cases of IA annually. At a global level, most of the infections are being caused by *Aspergillus fumigatus* in millions of cases of chronic pulmonary aspergillosis and allergic bronchopulmonary aspergillosis. In the present scenario, acquired azole resistance in *A. fumigatus* is an emerging problem that compromises the clinical efficacy of azole antifungals. There are several mutations reported in the *cyp51A* gene of *A. fumigatus* that affects the activity of azole drugs. *A. fumigatus* strain also forms hydrophobic biofilm comprising of numerous intertwined hyphae covered with extracellular matrix (ECM). It forms a protective shield against antifungals, thereby reducing their efficacy drastically. The treatment of biofilm related *Aspergillus* infection is also a serious clinical challenge, emphasising the need for new therapeutic agents.

Methods:
A total of 12 azole resistant strains of *A. fumigatus* were isolated from 80 soil samples collected from the rice fields across North India. Minimum inhibitory concentrations (MIC_{100}) as well as minimum biofilm-eradicating concentration 80 ($MBEC_{80}$) of isoeugenol against azole resistant *A. fumigatus* with reference ATCC strain of *A. fumigatus* was calculated using micro-broth dilution method of CLSI. Scanning Electron Microscopic (SEM) studies were performed to evaluate the effect of the molecule on extracellular matrix formation in azole resistant *A. fumigatus*. Cell cytotoxicity of molecule was assessed on normal human lung epithelial cell line L-132. *In-silico* ADME/Tox study was conducted to determine the isoeugenol drug-likeness and health effect predictions profile.

Results:
The MIC_{100} of isoeugenol against azole resistant *A. fumigatus* strains was calculated and varied from 0.625-0.312 mg/ml. The effect of isoeugenol on pre-formed biofilm was analysed through 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and SEM. In MTT assay, $MBEC_{80}$ of molecule was calculated in the range of 1.25-0.625 mg/ml. SEM showed absence of extracellular matrix and tangled hyphae in treated biofilm whereas these structures were present in both untreated and amphotericin-B treated biofilm. In cell cytotoxicity study, molecule resulted non-toxic to normal human lung epithelial cell line L-132. It also showed drug-likeliness properties with no side effects on cardiovascular, lungs, liver, gastrointestinal systems through *in-silico* ADME/Tox study.

Conclusion:
The antifungal activity of isoeugenol against azole resistant strains of *A. fumigatus* was seen to be more effective and non-toxic as compared to amphotericin-B and can be explored further. However, the results of present *in-vitro* drug testing study need to be mutually substantiated with *in-vivo* therapeutic aspects.

8 POINT MUTATIONS IN THE 14A-STEROL DEMETHYLASE CYP51A OR CYP51C COULD CONTRIBUTE TO AZOLE RESISTANCE IN *ASPERGILLUS FLAVUS*

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Purpose:
Aspergillus species, as human pathogens, have become increasingly important because immunosuppressed patients are very susceptible to develop infection by these fungi. Currently *Aspergillus flavu*s is the second most common *Aspergillus* spp. causing invasive infections in humans. Recent reports of *A. flavus* isolates with *in vitro* azole resistance have been described and their azole resistance mechanisms are being analyzed. One particular characteristic of *A. flavus* is the existence of three *cyp*51-related genes (*cyp*51A, *cyp*51B and *cyp*51C) encoding 14-α sterol demethylase-like enzymes, the target of azole drugs. In this study we describe the analysis, characterization and phylogeny of *A. flavus cyp*51 genes, the presence of potentially relevant mutations and their potential role in *A. flavus* azole resistance.

Methods:
To carry out this study we selected 20 *A. flavus* clinical isolates, 3 of them showed reduced *in vitro* susceptibility to azole drugs and 17 azole-susceptible strains. Susceptibility testing was performed according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) standard methodology. Besides clinical azole drugs, 14α-demethylation inhibitors (DMIs) fungicides were tested. The *cyp*51A, *cyp*51B and *cyp*51C genes, including their promoter region, were amplified and sequenced for the detection of specific mutations that could be associated with azole resistance. Phylogenetic trees were obtained by CLUSTAL analysis. The deduce protein sequence of *A. flavus* Cyp51A, Cyp51B and Cyp51C, were compared with paralogs from different yeasts, basidiomycetes and filamentous fungi including other *Aspergillus* species, *Penicillium* spp., *Fusarium* and Mucorales.

Results:
Comparison of the deduced Cyp51A, Cyp51B and Cyp51C proteins with other fungal Cyp51 resulted in a protein homology that ranged from 40% to 74%. Cyp51A and Cyp51C presented several synonymous and non-synonymous point mutations among both susceptible and non-susceptible strains. However, two amino acid mutations were present in two resistant isolates: one strain harbored P220L substitution in Cyp51A, and another carried a H349R in Cyp51C. The isolates that showed reduced *in vitro* susceptibility to clinical azoles also showed a resistant phenotype to DMIs.

Conclusion:
Two different point mutations at *A. flavus* azole target Cyp51A and Cyp51C seem to be linked to the development of an azole resistance phenotype, although how these substitutions could affect the antifungal-enzyme interaction remains unclear. The fact that reduced *in vitro* susceptibility to clinical azoles shows cross-resistance to DMIs may suggest the involvement of both in *A. flavus* azole resistance development.

9 ANTIFUNGAL ACTIVITY OF NOVEL TRIAZOLE, EFINACONAZOLE, AND NINE COMPARATORS AGAINST 354 MOLECULARLY IDENTIFIED *ASPERGILLUS* ISOLATES, *IN VITRO*

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Purpose:
Management of superficial aspergillosis is a major challenge owing to the frequent relapses and treatment failure, which may pose a potential risk, thereby gradually developing resistant species. Therefore, necessitating the development of new antifungals with higher potency should be considered as alternative strategies for efficient management of infections. Thus, this study aimed to investigate the susceptibility of clinical (n = 218) and environmental (n = 137) *Aspergillus* isolates toward a novel triazole, efinaconazole, in comparison with various classes of antifungal drugs.

Methods:
Antifungal susceptibility testing was performed using 96-well microtiter plates, according to the Clinical and Laboratory Standards Institute (CLSI) M38-A2 guidelines against a large collection of *Aspergillus* isolates.

Results:
Efinaconazole exhibited poor activity against mutant *A. fumigatus* strains, *A. niger sensu stricto,* and *A. tubingensis* with GM MIC values of 3.62, 1.62, and 2 μg/ml, respectively; however, surprisingly, it efficiently inhibited the growth of *A. terreus sensu stricto*, followed by wild-type *A. fumigatus* and *A. flavus* with GM MIC values of 0.29, 0.42, and 0.52 μg/ml, respectively. Notably, the widest MIC ranges were recorded for efinaconazole against azole-resistant *A. fumigatus, A. niger sensu stricto,* and *A. tubingensis* (0.25–16, 0.5–4, and 0.5–16 μg/ml, respectively); however, efinaconazole presented higher potency than itraconazole, and had a similar effect as voriconazole against the azole-resistant *A. fumigatus*.

Conclusion:
Presumably, efinaconazole is inefficient in aspergillosis treatment due to the low susceptibility of *A. niger sensu stricto, A. tubingensis,* and mutant *A. fumigatus*; however, it may be effective in treating superficial aspergillosis caused by wild-type *A. fumigatus, A. terreus sensu stricto,* and *A. flavus*. Nevertheless, the clinical effectiveness of efinaconazole in treating superficial and cutaneous aspergillosis remains unclear.

10 SUSCEPTIBILITY PROFILE OF MUCORALEAN FUNGI ISOLATED FROM THE UNITED STATES TO CURRENT ANTIFUNGAL DRUGS

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Purpose:
Mucormycosis is an aggressive infection associated with significant morbidity and mortality, especially in immunocompromised individuals and those with poorly controlled diabetes. Reported cases per 1,000,000 individuals in the U.S. and Canada were approximately 3 and 1.2, respectively. However, the incidence has increased in recent years, owing to higher numbers of at individuals at risk for these infections. Although, amphotericin B, posaconazole, and isavuconazole are generally active against members of the order Mucorales, the causative agents of mucormycosis, some isolates may have reduced susceptibility to these antifungals. The purpose of this study was to evaluate the species distribution of Mucorales isolates in the U.S. over a 3.5-year period and antifungal susceptibility profiles against these fungi.

Methods:
Mucorales isolates received by the Fungus Testing Laboratory at the UT Health Science Center San Antonio for molecular identification and antifungal susceptibility testing between September 2015 and March 2019 were included. Species identification was performed by combined phenotypic characteristics and DNA sequence analysis of the ITS and D1/D2 rDNA regions. Minimum inhibitory concentrations (MICs) for amphotericin B, itraconazole, posaconazole, and isavuconazole were determined by broth microdilution susceptibility testing according to the methods in the CLSI M38 reference standard. MIC ranges, MIC_{50}/MIC_{90} values/ and geometric mean (GM) MICs were determined.

Results:
During this 3.5-year period, 942 Mucorales isolates were received and tested. The predominant genus was *Rhizopus* (56.6%) followed by Mucor (23.8%). Other genera, including *Lichtheimia, Rhizomucor, Scyncephalastrum,* and *Cunninghamella* comprised less than 10% of isolates. The most predominant species was *Rhizopus arrhizus* (39%), of which 64.8% were *R. arrhizus* var. *arrhizus* and 30.1% *R. arrhizus* var. *delemar.* Amphotericin B demonstrated the most potent *in vitro* activity with GM MICs of ≤0.25 mg/L against all genera with the exception of *Cunninghamella* sp. (GM MIC 1.21 mg/L). For the azoles, the most potent agent was posaconazole, followed by itraconazole and isavuconazole. Interestingly, *R. arrhizus* var. *arrhizus* and *Rhizopus microsporus* were more susceptible to isavuconazole compared to other genera, and the GM MIC for this azole against *R. arrhizus* var. *delemar* (3.70 mg/L) was markedly higher compared to that against *R. arrhizus* var. *arrhizus* (0.87 mg/L). Similar differences in isavuconazole GM MICs were observed between *M. circinelloides* f. *circinelloides* and *M. circinelloides* f. *janssenii* (7.62 mg/L vs. 3.44 mg/L, respectively).

Conclusions:
The majority of Mucoralesisolates included in this surveillance study were *Rhizopus* species, followed by *Mucor* and *Lichtheimia* species. Differences in azole and amphotericin B susceptibility patterns were observed between the genera with the greatest variability observed with isavuconazole. Clinical microbiology laboratories should be aware of these species distributions and differences in antifungal susceptibility patterns. Further studies are warranted to determine the clinical implications of these findings.

Table. MIC ranges, MIC_{50}/MIC_{90} values, GM MICs of amphotericin B, isavuconazole, itraconazole and posaconazole against genera within the order *Mucorales*. Values are expressed in mg/L.

Genus	Antifungal	MIC Range	MIC_{50}	MIC_{90}	GM MIC
Rhizopus	Amphotericin B	≤0.03-2	0.25	1	0.19
	Isavuconazole	≤0.125->16	1	4	1.24
	Itraconazole	≤0.125->16	1	4	0.66
	Posaconazole	≤0.125->16	0.25	1	0.28
Mucor	Amphotericin B	≤0.03-8	0.125	0.5	0.12
	Isavuconazole	2->16	8	>16	7.14
	Itraconazole	0.5->16	4	>16	4.33
	Posaconazole	0.125-8	1	2	1.02
Lichtheimia	Amphotericin B	≤0.03-0.5	0.125	0.5	0.15
	Isavuconazole	0.25-8	2	4	1.83
	Itraconazole	0.06-1	---	---	---
	Posaconazole	0.06-1	0.25	0.5	0.27
Syncephalastrum	Amphotericin B	≤0.03-1	0.06	0.5	0.11
	Isavuconazole	0.5->16	16	>16	8.79
	Itraconazole	0.125-8	---	---	---
	Posaconazole	0.06-4	0.5	2	0.68
Cunninghamella	Amphotericin B	0.5-1	1	2	3.32
	Isavuconazole	4->16	16	>16	18.32
	Itraconazole	0.5-2	---	---	---
	Posaconazole	0.25-1	0.5	1	2.35
Apophysomyces	Amphotericin B	≤0.03-0.125	---	---	---
	Isavuconazole	0.5-4	---	---	---
	Itraconazole	0.25-1	---	---	---
	Posaconazole	0.125-0.25	---	---	---
Saksenaea	Amphotericin B	≤0.03	---	---	---
	Isavuconazole	0.25-2	---	---	---
	Itraconazole	0.125-0.25	---	---	---
	Posaconazole	0.06-0.125	---	---	---

11 CLINICAL *ASPERGILLUS* ISOLATES CAUSING ASPERGILLOSIS IN THE LAST 20 YEARS: AN OVERVIEW OF AETIOLOGY AND ANTIFUNGAL RESISTANCE TO AZOLES AND AMPHOTERICIN B

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Purpose:
While the number of reported azole-resistant *Aspergillus* spp isolates is on the rise in different European countries, data from South Europe is very limited. We assessed the azoles and amphotericin B antifungal susceptibility and species identification of a collection of clinically significant *Aspergillus* spp. isolates from patients cared at a large tertiary hospital located in Madrid, Spain, in the last twenty years.

Methods:
A total of 356 *Aspergillus* spp isolates from 264 patients with proven (n=30) or probable invasive aspergillosis according to EORTC criteria and admitted to Gregorio Marañon Hospital (Madrid, Spain) from January 1999 to December 2018 were studied. Isolates sourced from samples from the respiratory tract (n=306) or other sources (n=24). Selected isolates – one isolate per species and patient – were molecularly identified and antifungal susceptibility testing was performed according to EUCAST 9.3.1 methodology. Resistant isolates were defined based on available EUCAST breakpoints, and c*yp51A* gen was sequenced in *A. fumigatus* sensu stricto isolates showing an MIC of itraconazole and/or voriconazole ≥ 4 mg/L.

Results:
Molecular identification yielded the following species distribution: section *Fumigati* (n=246; *A. fumigatus* sensu stricto, n=229; *A. lentulus*, n=11; *N. udagawae*, n=4; *A. novofumigatus*, n=1; *A. felis*, n=1), section *Flavi* (n=39; *A. flavus*, n=37; *A. tamari*, n=1; *A. alliaceus*, n=1); section *Terrei* (n=31; *A. terreus*, n=21; *A. citrinoterreus*, n=9; *A. hortai*, n=1), section *Nigri* (n=22; *A. tubingensis*, n=10; *A. awamori*, n=7; *A. niger*, n=5), section *Nidulantes* (*E. nidulans*, n=8), section *Usti* (*A. caleidoustus*, n=6), and section *Versicolores* (n=4; *A. sydowii*, n=3; *A. amoenus*, n=1). Patients were infected by species of the following sections: *Fumigati* (n=169, 64%), *Flavi* (n=6, 2%), *Terrei* (n=8, 3%), *Usti* (n=1, 0.2%), *Nidulantes* (n=2, 0.5%), *Nigri* (n=7, 2.5%), *Versicolores* (n=2, 0.5%), or the co-existence of ≥ 2 sections (n=69). In the latter, at least one of the species belonged to the section *Fumigati* in 65 patients. Cryptic species were found in 14 out of the 234 patients infected by *Fumigati* species, commonly (n=9/14) in co-existence with *A. fumigatus* sensu stricto, and sporadically found over the years (Figure).

In non-*Fumigati* sections, azole resistance was found in *Terrei* (2/31; *A. terreus* sensu stricto, n=1; *A. citrinoterreus*, n=1) and *Nidulantes* (1/8; *Emericella nidulans*). Isolates of *Fumigati* section showed the highest rate of antifungal resistance (Table), with the cryptic species as the mostly affected ones. Two *A. fumigatus* sensu stricto isolates showing resistance to azoles harboured the G448S and TR_{34}L98H *cyp51A* substitutions and were isolated in 2011 (proven brain aspergillosis) and 2012 (probable pulmonary aspergillosis), respectively. Most of isavuconazole resistant isolates (n=9) showed an MIC = 2 mg/L but were both susceptible to the remaining azoles and *cyp51A* wild type and probably should be considered susceptible to isavuconazole.

Conclusion:
Azole resistance in *A. fumigatus* sensu stricto isolates infecting patients admitted to our hospital was lower than 1%, and has not shown any sign of increase over the years. The overall rate of resistance to azoles and amphotericin B in *A. fumigatus* complex was highly impacted by the presence of cryptic species.

Figure:

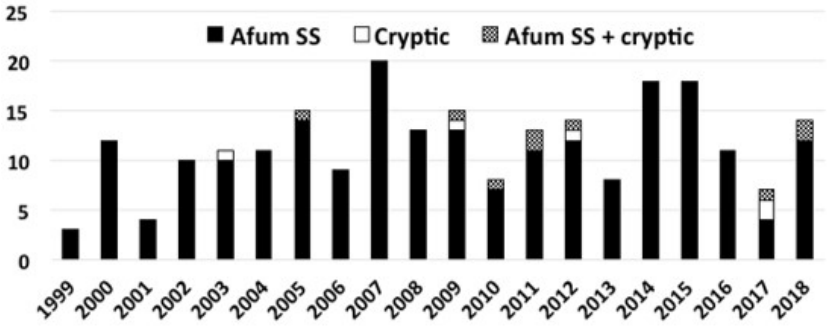

Table:

	Percentage of resistance (No. of isolates)									% of Resistance/ non wild type
	0.03	0.06	0.125	0.25	0.5	1	2	4	≥8	
***A. fumigatus* sensu stricto (n=229)**										
Amphotericin B	0	1	2	96	117	11	2	**0**	**0**	0 / 0.9
Itraconazole	0	0	8	85	125	9	1	**0**	**1**	0.45 / 0.9
Voriconazole	0	0	0	10	97	108	12	**1**	**1**	0.9 / 6
Posaconazole	7	104	91	25	**2**	**0**	**0**	**0**	**0**	0.9 / 0.9
Isavuconazole	0	0	0	14	122	82	**9**	**0**	**2**	5 / 0.9
Cryptic species (n=17)										
Amphotericin B	0	0	0	0	1	1	7	**5**	**3**	47.5 / 88.2
Itraconazole	0	0	0	1	5	4	5	**0**	**2**	12 / 41
Voriconazole	0	0	0	0	0	1	11	**3**	**2**	29 / 94
Posaconazole	0	0	5	9	**2**	**1**	**0**	**0**	**0**	18 / 17.6
Isavuconazole	0	0	0	0	0	9	**6**	**2**	**0**	47 / 11.7

Numbers in bold indicate resistance; underlined numbers indicate non wild type isolates based on ECOFFs.

12 SUSCEPTIBILITY PATTERNS OF CONTEMPORARY *ASPERGILLUS FUMIGATUS* ISOLATES FROM THE UNITED STATES TO AZOLE ANTIFUNGALS

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Purpose:
Invasive aspergillosis remains a significant cause of morbidity and mortality in highly immunocompromised patients. *Aspergillus fumigatus* is the most prevalent species of this genus to cause invasive disease. The mainstays of antifungal prophylaxis and treatment include the azole antifungals voriconazole, posaconazole, and isavuconazole due to both the availability of oral and intravenous formulations and results from randomized clinical trials demonstrating clinical efficacy. However, there is growing concern regarding azole resistance in *A. fumigatus*, which has been documented following both clinical and environmental exposure to azoles. Our objective was to evaluate the *in vitro* susceptibility of clinical isolates of *A. fumigatus* from the United States to voriconazole, posaconazole, and isavuconazole.

Methods:
Clinical isolates of *Aspergillus* cultured from humans and sent to the Fungus Testing Laboratory at the UT Health Science Center San Antonio for testing between September 2015 and March 2019 were included. Isolates from superficial sites (e.g., ears, nails, and skin) and those without information regarding culture site were excluded. Species identification was confirmed by combined phenotypic characteristics and DNA sequence analysis of the partial b-tubulin and calmodulin genes. Minimum inhibitory concentrations (MICs) against well-characterized *A. fumigatus* isolates were determined by broth microdilution susceptibility testing according to the methods in the CLSI M38 standard. MIC ranges, MIC_{50} and MIC_{90} values, and geometric mean (GM) MIC values were determined.

Results:
Species identifications were performed on 2620 *Aspergillus* isolates, of which 1533 (58.1%) were *A. fumigatus* sensu stricto. 1391 voriconazole, 773 posaconazole, and 1533 isavuconazole MIC values against *A. fumigatus* isolates were available for analysis. MIC ranges were ≤0.03 - >16 mg/L for each triazole, and the GM MIC was lowest for posaconazole (0.12 mg/L) followed by voriconazole (0.37 mg/L) and isavuconazole (0.66 mg/L). Similar trends were observed for MIC_{50} and MIC_{90} values (Table). Per published epidemiological cut-off values established using the CLSI M38 broth microdilution method, 3.49% - 5.15% of *A. fumigatus* isolates would be considered non-wild type to at least one of these triazoles. Per the proposed CLSI voriconazole *A. fumigatus* clinical breakpoints, 92.38% of isolates were susceptible to voriconazole (MIC ≤0.5 mg/L), 4.10% were intermediate (MIC 1 mg/L), and 3.52% were resistant (MIC ≥2 mg/L).

Conclusions:
Aspergillus fumigatus remains the predominant *Aspergillus* species cultured from patients in the U.S. Overall, the majority of *A. fumigatus* isolates received by our reference laboratory during this 3.5-year period are wild type based on epidemiological cut-off values established per CLSI methods. However, non-wild type isolates and those considered resistant to voriconazole based on proposed CLSI clinical breakpoints were identified throughout this surveillance period. Clinicians and microbiology laboratories in the U.S. need to be aware of azole resistance in *A. fumigatus* and continued vigilance is needed

Table. MIC ranges, MIC_{50}/MIC_{90} values, GM MICs, and percent wild type/non-wild type of clinical *A. fumigatus* isolates to voriconazole, posaconazole, and isavuconazole

MIC Parameter	Voriconazole	Posaconazole	Isavuconazole
MIC Range	≤0.03 - >16	≤0.03 - >16	≤0.03 - >16
MIC50	0.25	0.125	0.5
MIC90	0.5	0.25	1
GM MIC	0.37	0.12	0.66
ECV % Wild Type	96.48%	96.51%	94.85%
ECV % Non-Wild Type	3.52%	3.49%	5.15%
Voriconazole CLSI CBP	Susceptible	Intermediate	Resistant
Percent	92.38%	4.10%	3.52%

13 ELUCIDATING ECHINOCANDIN RESISTANCE MECHANISMS IN *MUCOR CIRCINELLOIDES*

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Purpose:
Mucormycosis is an emerging infection caused by fungi in the order Mucorales. Mucormycosis results in life-threatening risks to immunocompromised patients globally. The mortality resulting from mucormycosis remains unacceptably high reaching up to 90-100% among disseminated infections. Due to resistance of Mucorales fungi to antifungal drugs, the treatment options are limited. However, there is little or no knowledge regarding the mechanisms underlying this antifungal drug resistance, thus we propose experiments designed to elucidate the genetic mechanisms underlying the intrinsic resistance of Mucorales to the antifungal drug class echinocandins. Echinocandins are the newest antifungal drug class and act as non-competitive inhibitors of the enzyme β-(1,3)-D-Glucan synthase. Mucorales exhibit a resistance to this class of antifungal drugs despite harboring the *fks* genes that encode a β-(1,3)-D-Glucan synthase.

Methods:
Our preliminary study found that the model Mucorales species, *Mucor circinelloides* (denoted *Mucor*), carries three members of the echinocandin drug target gene family (*fksA, fksB,* and *fksC*). We achieved gene deletions in our study by using a pyrimidine recyclable marker system. Gene expression was quantified via quantitative real time PCR.

Results:
When *Mucor* was challenged with echinocandins we found *fksA* and *fksB* were overexpressed. In addition, we found that the *fksC* gene encodes an intrinsic amino acid alteration in a hotspot region which is known to confer acquired echinocandin resistance in other pathogenic fungi such as the *Candida* species. Our preliminary study also found that the serine/threonine phosphatase, calcineurin, regulates the expression of the *fksA* and *fksB* genes. Calcineurin is a phosphatase conserved across eukaryotes that orchestrates stress pathways in fungi.

Conclusion:
We hypothesize that through the calcineurin signaling pathway *Mucor* overexpresses the *fks* genes which encode the drug target, β-(1,3)-D-Glucan synthase, to compensate for the inhibiting nature of echinocandins. Furthermore, we also hypothesize that the naturally occurring amino acid change seen in the FksC hot spot 3 region contributes the intrinsic resistance to echinocandins.

14 THE ROLE OF PENTRAXIN-3 IN THE IMMUNOMETABOLIC REGULATION OF ANTIFUNGAL IMMUNITY

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Purpose:
The reprogramming of cellular metabolism is a fundamental mechanism through which innate immune cells meet the energetic and anabolic needs during host defense against invading pathogens. The long pentraxin-3 (PTX3) plays a pivotal role in the immune response to *Aspergillus fumigatus* as the result of its opsonic activity facilitating immune recognition and phagocytosis. However, whether PTX3 exerts its functions by regulating immunometabolic responses to the fungus remains unknown.

Methods:
In vitro and *in vivo* models of infection with *A. fumigatus* as well as macrophages from healthy individuals with single-nucleotide polymorphisms (SNPs) in the PTX3 gene were used to dissect the metabolic pathways involved in the PTX3-mediated regulation of anti-fungal immunity.

Results:
We demonstrate that PTX3-deficient bone marrow-derived (BMDMs) and human monocyte-derived macrophages (MDMs) harboring loss-of-function SNPs in PTX3 displayed an impaired glucose homeostasis, as revealed by the decreased levels of glucose consumption and lactate secretion upon infection. This effect on macrophage metabolism was accompanied by an impaired fungicidal activity and secretion of proinflammatory cytokines, including IL-6 and TNF. In accordance, PTX3-deficient mice subjected to experimental aspergillosis displayed a marked susceptibility characterized by an impaired expression of several enzymes involved in glucose homeostasis in the lungs. Ongoing studies are being performed to further dissect the mechanisms through which PTX3 coordinates host cell metabolism in response to fungal infection.

Conclusion:
We propose a novel PTX3-regulated mechanism contributing to anti-fungal immunity, namely by regulating adequate immunometabolic responses. These results may contribute towards the design of novel therapeutic approaches or metabolic adjuncts to reorient host cells towards immune protection against IPA.

15 GENOME WIDE ASSOCIATION STUDY (GWAS) FOR ANTIFUNGAL SENSITIVITY IN THE OPPORTUNISTIC PATHOGEN *ASPERGILLUS FUMIGATUS*

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Purpose:
Aspergillus fumigatus is a filamentous fungus that is typically found in soil, compost and other decaying organic matters. This species is also an opportunistic human pathogen that kills an estimated 100,000 people annually. Unfortunately, the frequency of *A. fumigatus* antifungal resistance has rapidly risen making treatment particularly challenging. Candidate gene approaches have yielded insight into a number of different antifungal resistance mechanisms including: target overexpression, target alteration, efflux overexpression and intake control. However, candidate gene approaches are inherently biased because they can overlook genes with minor but additive effects, or genes with functions that are unknown or seemingly unrelated.

Methods:
To avoid this bias, we applied Genome-Wide Association (GWA) to identify genetic variants associated with Itraconazole (ITCZ) sensitivity in *A. fumigatus*. Three different statistical approaches (RoadTrips, plink_permutation and plink_PCA) were performed to conduct GWA using 43,967 single nucleotide polymorphism (SNPs) in 69 isolates with matched minimal inhibitory concentrations (MIC) of ITCZ. We identified 26 SNPs, overlapping 14 genes, associated with ITCZ sensitivity. We used CRISPR to knockout the top seven candidate genes then tested the growth rate of knockouts in the presence of ITCZ.

Results:
Three of the seven genes (Afu2g02010, Afu2g02020 and Afu3g13670) showed significant effects on growth in the presence of ITCZ.

Conclusion:
Our results suggest that ITCZ sensitivity is regulated by a collection of genes, all of which had not been implicated previously. In addition, this study demonstrates the power of GWA paired with molecular genetics to better understand pathogenicity related traits in *A. fumigatus*.

16 CRISPR / CAS9 GENOME EDITING TECHNOLOGY TO VERIFY THE CONTRIBUTION OF KNOWN MUTATIONS IN THE *CYP51A* GENE AND ITS PROMOTER TO AZOLE RESISTANCE IN *ASPERGILLUS FUMIGATUS*

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Purpose:
Azole resistance in *Aspergillus fumigatus*is mainly associated with single nucleotide polymorphisms (SNPs) in the gene *cyp51A*encoding lanosterol 14α-demethylase, the target enzyme of azole antifungal agents, or increased expression of Cyp51A by tandem repeats (TRs) in the promoter region. Although several SNPs and TRs possibly linked to low susceptibility have been reported so far in azole resistant isolates, only a few studies have been carried out to prove that the SNPs and TRs could contribute to decreased azole susceptibility. Here, we investigated the known alteration in the Cyp51A on the same genetic background using CRISPR / Cas9 genome editing technology.

Methods:
To evaluate the contribution of known mutations and TRs of Cyp51A to azole susceptibility, PCR-amplified DNA fragments containing *cyp51A*with or without the mutation of interest and hygromycin marker were introduced simultaneously with Cas9 protein and *in vitro*-synthesized single guide RNA into protoplasts of the azole susceptible Ku-deficient strain AfS35. Nucleotide sequencing of the *cyp51A*gene and promoter region of recombinant strains were performed to verify mutations. Antifungal susceptibility testing was performed by CLSI M38-A2 broth microdilution method. mRNA expression level of *cyp51A*were examined by realtime RT-PCR.

Results:
Recombinant strains, each of which has a single amino acid substitution on six residues (G54E, L98H, P216L, M220I, T289A, G448S) or five TRs series (TR34, TR34 / L98H, TR46, TR46 / Y121F / T289A, TR53), were created by CRISPR / Cas9-mediated homologous recombination with PCR fragment containing hygromycin marker. Most strains constructed in this study demonstrated similar azole susceptibility as previously reported. As expected, increased mRNA expression of *cyp51A*was observed in TRs series recombinant strains, which showed itraconazole resistance.

Conclusion:
We proved by CRISPR / Cas9-genomic recombination that some of hot spots mutations in *cyp51A* are associated with azole antifungal resistance. Site-directed mutagenesis on a specific genomic locus, which has been a time-consuming and inefficient procedure so far in *A. fumigatus*, can be now achieved easily using one-step transformation method by CRISPR / Cas9 genome editing. This genome engineering technique could be useful to investigate azole resistant mechanism by genetic factors such as SNPs or genetic alteration.

17 HMG1 MUTATION CONFERRING MULTI-AZOLE RESISTANCE IN *ASPERGILLUS FUMIGATUS*

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Purpose:
The recent increase in azole-resistant *A. fumigatus* is a global concern. The mutations in *cyp51A* gene have been mostly studied as the causes of azole-resistance in the fungus, but uncovering the unknown (non-*cyp51A*) mutations responsible for azole resistance should be essential for developing novel methods for prompt diagnosis and effective drug treatment. In our recent study, we reported results that mutation of *hmg1*, which encodes HMG-CoA reductase, the rate-limiting enzyme in ergosterol biosynthesis, would be mechanism conferring azole-drugs resistance.[1] The aim of this study is to delineate the relationship between *hmg1* mutation and multi azole-resistance.

Methods:
We used clinical azole-resistant *A. fumigatus* strains collected in Japan and investigated the sequences of *hmg1* gene. To delineate the association between the *hmg1* mutation and triazole resistance, the mutant *hmg1* allele in two clinical multi-azole resistant strains (mutation; $Hmg1^{S269F}$ alone and, $Hmg1^{S305P}$ and $Cyp51A^{M220I}$ combination) were replaced with the wild-type Hmg1 allele by CRISPR-Cas9 system. Antifungal susceptibility testings were performed according to the CLSI-M38.

Results:
Among azole-resistant strains with mutations in the 220th methionine of Cyp51A, the strains both with and without Hmg1 mutation were found. Multi azole-resistance was found only in the strains possessing mutations in Hmg1. Resistant strains with $Hmg1^{S269F}$ restored sensitivity to ITCZ and VRCZ by a genetic complementation test of Hmg1. On the other hand, resistant strain both with $Hmg1^{S305P}$ and $Cyp51A^{M220I}$ only restored sensitivity to VRCZ. Currently, genetic complementation test of other SNPs in *hmg1* is underway.

Conclusion:
In this study, we confirmed the novel genetic changes related to azole resistance. We proposed that multi azole-resistance of the strains without the *cyp51* mutations are partly imparted by a mutation of Hmg1. Future elucidation of the molecular mechanism of the Hmg1 mutation would lead to a better understanding of the azole-resistant mechanism in *A. fumigatus*.

Reference:
1 Emerg Infect Dis. 2018, 24(10), 1889-1897

18 CLSI VERSUS EUCAST FOR AZOLES SUSCEPTIBILITY - THE NECESSITY TO UNIFY

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Purpose:
Nowadays, standard antifungal susceptibility testing for molds is made employing two protocols: CLSI and EUCAST. They differ in technical details and in the quantitative criteria for the Clinical Break Points (CBP). As azole treatment is the cornerstone in the management of human aspergillosis, the differences in CBP according to the adopted protocol are likely to result in different therapeutical decisions. The purpose of this work was to evaluate MICs by CLSI for azoles, in clinical isolates of *Aspergillus*, and compare these results with those previously obtained using EUCAST (1).

Methods:
Between January 2010 and March 2016, 227 *Aspergillus* were isolated from biological samples of 207 patients with proven or probable infections, and with colonization, who were admitted to Centro Hospitalar do Porto (CHUP),Centro Hospitalar Universitário S. João and Instituto Português de Oncologiado Porto (IPO). The susceptibilities were evaluated for itraconazole (ITZ), voriconazole (VCZ), posaconazole (PCZ) and isavuconazole (ICZ) following the CLSI M38-A2 protocol. The results were compared with those obtained with EUCAST (1).

Results:
The 227 tested *Aspergillus* isolates included the following strains: *Aspergillus fumigatus sensu stricto* (190), *A. flavus/oryzae* (14), *A. welwitschiae* (7), *A. lentulus* (5), *A. terreus* (4), *A. pseudodeflectus* (2), *A. thermomutatus* (1), *A. felis* (1), *A. niger* (1), *A. nidulans* (1) and *A. sydowii* (1).

For *A. fumigatus sensu stricto* the MICs ranges (mg/L) by CLSI/EUCAST were: ITZ=0.12-16/0.25-16, VCZ=0.12-16/0.25-16, PCZ=0.03-2/0.03-16, ICZ=0.25-16/0.5-8 and the geometric means (mg/L) were: ITZ=0.81/1.49, VCZ=0.48/0.77, PCZ=0.18/0.34, ICZ=0.86/2.32. In general, the values were lower using CLSI and the difference was remarkable for isavuconazole. For the other

Aspergillus spp. a similar difference was observed. For all species with more than one isolate, except *A. lentulus,* and for all tested azoles according both protocols, the Essential Agreement, at two dilutions, were between 85.7 and 100%.

The number of *A. fumigatus sensu stricto* isolates resistant by CLSI/EUCAST were: ITZ=4/8, VCZ=1/5, PCZ=0/29, ICZ=not applicable/149; and the non-wild type isolates were: ITZ=4/44, VCZ=5/6, PCZ=12/not applicable, ICZ=9/34.

Conclusion:
In agreement with previous reports (2,3,4), the findings for the present population highlight the lower MICs by CLSI compared to EUCAST. In addition, they emphasize the need to unify both protocols, aiming to improve epidemiological comparisons and patient management.

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19 ANTIFUNGAL SUSCEPTIBILITY TESTING OF CLINICAL AND COMMUNITY ENVIRONMENT ISOLATES OF *ASPERGILLUS* SPECIES

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Purpose:
Aspergillus species are ubiquitous saprophytes causing various fungal infections. Most commonly encountered species are: *Aspergillus flavus, A.fumigatus* and *A.niger.* These primarily cause pulmonary infection with involvement of other body sites like paranasal sinuses and cutaneous tissue. Aspergillosis is a systemic infection, one of the most common causes of invasive mold disease in immunocompromised patients, after candidiasis, with an estimated every year 300,000 cases worldwide. Azoles are the mainstay of treatment; however, excessive use of these antifungals clinically as well as in agriculture has influenced the susceptible species of saprophytic flora, leading to the genetic changes, which is further contributing to the emergence of resistance among some *Aspergillus* species thereby challenging management. As such azole resistance rate of *A. fumigatus* is globally varying from 2-31%. Hence to determine the resistance, antifungal susceptibility testing (AFST) among clinical and community environment isolates of *Aspergillus* species against amphotericin B, itraconazole, voriconazole and caspofungin, was performed.

Methods:
Clinical as well as soil samples from community environment were processed as per Standard Mycological Techniques. Growth of *Aspergillus* species was identified by phenotypic as well as genotypic methods and AFST was performed by micro broth dilution as per CLSI M38-A2 protocol.

Results:
Among clinical samples, 30 (75%) were *A. flavus* and 9 (22.5%) *A. fumigatus* and one (2.5%) was *A. candidus*. All 30 (75%) isolates of *A. flavus* were sensitive to itraconazole while one strain of *A .flavus* was found resistant to voriconazole with MIC value of ≥2 µg/ml. Another strain was found to be resistant to amphotericin B with MIC value of 8 µg/ml and also had high MEC value of 0.12 µg/ml to caspofungin. All 9 (100%) isolates of *A. fumigatus* were found to be sensitive to itraconazole and voriconazole while one (11.11%) isolate showed elevated MIC value of 4 µg/ml for amphotericin B and MEC value of 0.12 µg/ml for caspofungin and another isolate showed elevated MEC value of 0.12 µg/ml to caspofungin. MIC value of *A. candidus* isolate (2.5%) for itraconazole, voriconazole, amphotericin B was 0.25 µg/ml, 0.06 µg/ml and 1 µg/ml, respectively. MEC value of *A. candidus* for caspofungin was 0.12 µg/ml. Outcome was not favorable in two resistant isolates of invasive aspergillosis such as *A .flavus* voriconazole resistant isolate and *A. fumigatus* amphotericin B resistant isolate with elevated MEC value for caspofungin. For community environment, soil samples were collected from different areas of Chandigarh such as various gardens, flower pots, soil admixed with birds droppings adjoining different gates of the medical campus. Among 22 *Aspergillus* isolates of community environment, 15 (68.18%) isolates were of *A. niger*, 4 (18.18%) isolates were of *A. flavus* and 3 (13.63%) isolates were of *A. fumigatus*. All species of *Aspergillus* from community environment were found to be sensitive for itraconazole and voriconazole while 2 (13.33%) isolates of *A. niger*, 3 (75%) isolates of *A. flavus* and one (33.33%) of *A. fumigatus* had elevated MEC value of 0.12 µg/ml for caspofungin. One (25%) strain of *A. flavus* was also found to be resistant to amphotericin B with MIC value of 4 µg/ml.

Conclusions:
These results suggest that resistance is emerging for salvage drugs such as amphotericin B and caspofungin while first line drugs such as azoles are comparatively sensitive for *Aspergillus* isolates**.** Considering the importance of emerging resistance, surveillance studies should be performed routinely and Antifungal Stewardship Programme should be followed so that considerable improvement in the outcome of the patients can be achieved at an early stage.

20 *IN VITRO* EVALUATION OF COMBINATION OF IBREXAFUNGERP AND AZOLES AGAINST *ASPERGILLUS* SPP. ISOLATED FROM LUNG TRANSPLANT RECIPIENTS

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Purpose:
Aspergillosis is the most common opportunistic mould infection. Over the past 2 decades, there has been a surge in non-*Aspergillus fumigatus* (non-Af) spp causing infections. This change in epidemiology might be partially attributable to increased use of broad-spectrum antifungal agents. Indeed, breakthrough infections while on azole prophylaxis or treatment have been attributed to azole-resistant non-Af species, and mortality associated with these infections is high. Ibrexafungerp (IBX) is a novel class of glucan synthase inhibitor that has broad activity against *Candida*, *Aspergillus* and *Pneumocysitis*. We evaluated the *in vitro* activity of IBX, either singly or in combination with isavuconazole (ISA), posaconazole (POSA) or voriconazole (VOR) against 51 *Aspergillus* isolates recovered from lung transplant recipients.

Methods:
MICs of antifungals were determined according to CLSI M38-A2 susceptibility standard. Concentrations tested were from 0.015 to 16 µg/mL. For combination testing, we used the checkerboard method. Fractional inhibitory concentration index (FICi = MICIBX(single)/ MICIBX(combo) + MICAZOLE(single)/MICAZOLE(combo)) was used to classify the interaction between 2 drugs as synergy (FICi<0.5), antagonism (FICi>4) or indifference (FICi 0.5-4).

Results:
Fifty-one isolates were tested (*A. calidoustus*, 5; *A. flavus*, 11; *Af*, 20; *A. glaucus*, 1; *A. niger*, 7; and *A. terreus*, 7). IBX, ISA, POSA and VOR MIC_{50} were 0.06 (range ≤0.015->16), 0.5 (0.03->16), 0.125 (≤0.015->16), and 0.125 (0.06->16) µg/mL, respectively. Azole MIC≥2 µg/mL was observed in 80% and 14% of A. calidoustus and A. terreus isolates, respectively. Median MIC of IBX was higher for *A. flavus* (0.125 µg/mL) than other species (0.06 µg/mL; p=0.002). *A. calidoustus* exhibited significantly higher MICs to all 3 azoles (median MIC 4 µg/mL, range: 0.25 to 16) versus other species (<0.0001). 14, 1 and 5 isolates exhibited IBX, ISA and POSA MIC ≤0.015 µg/mL, respectively, thus FICi cannot be determined. Synergy was observed in 63% between IBX and ISA, 56% between IBX and POSA, and 54% between IBX and VORI. Antagonism was not observed with any combination tested. Among isolates exhibiting either IBX or azole MICs ≤0.015µg/mL, the beneficial effect of the combination was still seen: the MIC of the 2nd drug was reduced by at-least 4-fold in 100% of ISA+IBX, 75% of POSA+IBX and 94% of VORI+IBX. For the remaining cases, in which synergy was not achieved, there was still a decrease, although not as dramatic, in MICs of one or both drugs when used in combination. Remarkably, for azole-resistant *A. calidoustus* and *A. terreus*, ISA, POSA and ISA MICs in combination with IBX decreased from the range of 2 to 16 µg/mL to 0.06-0.2 5µg/mL (ISA) and 0.03-0.125 µg/mL (POSA and VORI).

Conclusion:
The *in vitro* results with combinations of IBX and azoles against *Aspergillus* spp. are encouraging. Antagonism was not observed for this combination, and synergy was achieved against 54 to 63% of isolates. The effect of IBX on reducing azole MICs to low range for azole-resistant *A. calidoustus* and *A. terreus* is particularly noteworthy. Animal model and clinical studies are warranted to further elucidate the potential utility of IBX-azole combination therapy.

21 D430G MUTATION OF CYP51A IN *ASPERGILLUS FUMIGATUS* CAUSES AZOLE-RESISTANCE

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Purpose:
The increase of azole-resistant *Aspergillus fumigatus* is a global healthcare concern. The most common causative mutations of azole resistance in the fungus are TR34/L98H and TR46/Y121F/T289A, particularly in European countries. In Japan, however, such point mutations in the ORF region of *cyp51A* as G54, M220 or G448 are also important causes of azole resistance of *A. fumigatus*.

Methods:
A clinical isolate of *A. fumigatus,* which was taken from the patient with a history of receiving voriconazole for more than one year, was employed. Antifungal susceptibility test was performed according to CLSI M38. The *cyp51A* gene sequence was analyzed, and a mutated *cyp51A* gene was transfected to Δ*cyp51A* strain (Hagiwara D et al., PLoS Pathog 13: e1006096) using the shuttle vector pCB1004.

Results:
The analysis of sequence revealed D430G mutation in CYP51A. This mutation had not reported previously as the cause of azole-resistance, and we tried to check to see if this mutation is responsible for azole-resistance. We transferred the D430G *cyp51A* gene to Δ*cyp51A* strain of *A. fumigatus* using pCB1004-*cyp51A*^{D430G}, and the transgenic strain showed a higher MIC of VRCZ (>8).

Conclusion:
D430G mutation in CYP51A has confirmed as one of the responsible mutations for azole resistance of *A. fumigatus*.

22 ANTIMICROBIAL SUSCEPTIBILITY OF *ASPERGILLUS FUMIGATUS* AND *STENOTROPHOMONAS MALTOPHILIA* BIOFILMS: DID THEY FIND STRENGTH IN UNITY?

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Purpose:
Aspergillus fumigatus and *Stenotrophomonas maltophilia* (a gram-negative bacillus) are able to form biofilms and are commonly co-isolated in the respiratory tract of immunocompromised patients or during chronic respiratory diseases. Complexity of biofilms constitutes a therapeutic challenge and antimicrobial susceptibility of polymicrobial filamentous fungi-bacteria biofilms remains poorly studied. Antagonistic effects of *S. maltophilia* on *A. fumigatus* have already been described (1, 2) with fungal phenotype modifications which could affect the fungal susceptibility to antifungals. The aim of the present study was to investigate the effect of microbial interactions on the *in vitro* susceptibilities of both pathogens to two antimicrobial agents (alone and in two-drug combinations).

Methods:
Clinical reference strains of *A. fumigatus* (ATCC 13073) and *S. maltophilia* (ATCC 13637) and two clinical strains obtained from sputum of cystic fibrosis patients were tested in this study. The antagonistic effects of *S. maltophilia*on *A. fumigatus* have been studied via microscopic analyses and qPCR method. Amphotericin B (AMB) and levofloxacin (LVX) were chosen to explore their *in vitro* activities on mono and polymicrobial biofilms via qPCR analyses. The bacterial survival in polymicrobial biofilm was performed by CFU counts. A proteinase K treatment has been used to evaluate the role of fungal extracellular matrix (ECM) on *S. maltophilia* susceptibility in polymicrobial biofilm.

Results:
The presence of *S. maltophilia* in a polymicrobial biofilm increased the susceptibility of *A. fumigatus* to AMB. This increase of susceptibility was correlated with the fungal phenotype modification (thickened cell wall). In contrast, the susceptibility of *S. maltophilia* to LVX is decreased in presence of *A. fumigatus* according to the fungal biomass in polymicrobial biofilm. After proteinase K treatment, the susceptibility of *S. maltophilia* in polymicrobial biofilm is increased compared to bacterial biofilm suggesting that the fungal ECM may behave as a protective barrier for the bacteria. In addition, the combination of AMB and LVX significantly reduced the survival of *S. maltophilia* in polymicrobial biofilm.

Conclusion:
The polymicrobial biofilm may be benefit for *S. maltophilia* to fight antimicrobial therapy but not for *A. fumigatus*. *A. fumigatus* biofilm, especially the ECM, may protect *S. maltophilia* from the activity of LVX. But in contrast, the increase of the fungal phenotype induced by *S. maltophilia* could increase the susceptibility of *A. fumigatus*to AMB. Finally AMB with LVX could be an interesting antimicrobial combination to eradicate *A. fumigatus* and *S. maltophilia* in polymicrobial biofilms. In further studies, the mechanisms responsible for the increase of *A. fumigatus* susceptibility to AMB in polymicrobial biofilm could be better studied.

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23 EVALUATION OF DRUG SUSCEPTIBILITY OF *ASPERGILLUS* SPECIES ISOLATED FROM ICU OF HOSPITALS *IN VITRO*

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Purpose:
Invasive aspergillosis is the most threatening disease in immunocompromised patients that it has the highest morbidity and mortality rate amongst invasive fungal infections in the hospitals. The aim of present study was to assess antifungal susceptibility testing versus *Aspergillus* spp isolated from the hospital environment**.**

Methods:
After collecting 160 plates containing Sabouraud dextrose agar from the air and the environment of hospital's ICUs (intensive care units), the phenotypic and molecular identification of the colonies was performed in order to identification of *Aspergillus* spp. After DNA extraction, the molecular identification was carried out using universal fungal primers (ITS gene) and DNA sequencing. Antifungal susceptibility testing was performed with using the CLSI broth microdilution (M38-A2) method for *Aspergillus* isolates**.**

Results:
Out of 160 hospital environmental samples, 11 *Aspergillus* species were obtained. The eleven *Aspergillus* spp. were identified by sequencing as: 5 *A. flavus*, 3 *A. sydowii*, 1 *A. fumigatus* and 2 *A. oryzae*. Our antifungal susceptibility testing results indicated that *A. sydowii* and *A. fumigatus* were sensitive to amphotericin and voriconazole and were resistant to itraconazole. *A. sydowii* was resistant to caspofungin while *A. fumigatus* was sensitive to this drug. *A. flavus* was susceptible to all of the drugs.

Conclusion:
There were a number of reasons including delayed diagnosis, lack of appropriate curing, and the existence of various diseases and neutropenia which could lead to the high mortality rate of patients with aspergillosis, especially in patients of hospital's ICUs.

Keywords*:*
Drug susceptibility, *Aspergillus* SP. ICU

24 MUTATIONS IN *A. FUMIGATUS HMG1* WHICH CONFER TRIAZOLE RESISTANCE ALSO ALTER STEROL COMPOSITION AND INCREASE MULTI-DRUG EFFLUX PUMP GENE EXPRESSION

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Purpose:
The triazole antifungals, which inhibit ergosterol biosynthesis, are relied upon as both front-line and salvage therapies for the treatment of infections caused by *Aspergillus fumigatus*, the predominant pathogen of invasive aspergillosis. Recently, we have demonstrated that mutations in the *A. fumigatus* HMG-CoA reductase encoding gene, *hmg1*, represent a novel genetic determinant of clinical triazole resistance, conferring increased resistance to the entire class of clinically essential agents. In this work, we employ RNAseq-derived transcriptional profiling in combination with comprehensive sterol profiling to determine the effects of *hmg1* mutations with or without voriconazole treatment.

Methods:
Three previously characterized *hmg1* mutant strains with increased voriconazole resistance, constructed in the *akuB*$^{\Delta KU80}$ background and possessing mutations encoding the amino acid substitutions F262del, S305P, and I412S, as well as a *hmg1*^{WT} control strain, were included in this study. All strains were subjected to RNAseq-derived transcriptional profiling in biological triplicate following 48 hours of growth in RPMI media with and without voriconazole supplementation at half the minimum inhibitory concentration (MIC) (0.5mg/L for hmg1 mutant strains and 0.125mg/L for the wildtype control). Comprehensive sterol profiling was performed following the same growth conditions in biological triplicate.

Results:
Transcriptional profiling revealed *hmg1* mutant strains to overexpress genes encoding both multi-drug efflux pumps and enzymes involved in ergosterol biosynthesis. Following growth in RPMI media without voriconazole, all three *hmg1* mutant strains were observed to express both *abcA* and *mdrA* at levels 2 to 5-fold higher than the *hmg1*^{WT} control strain. Additionally, there was a 3 to 5-fold increase in the expression of *erg3B, erg24, erg24B,* and *erg25* among all three *hmg1* mutant strains relative to the *hmg1*^{WT} control. A corresponding increase in sterol intermediates downstream of these genes was also observed by sterol profiling under the same conditions. Following growth in voriconazole-supplemented RPMI media, all three *hmg1* mutant strains were observed to express a multitude of efflux pump encoding genes, including *abcA, abcC, abcD, atrI, mdr1,* and *mdrA* at levels 2 to 4-fold higher than the *hmg1*^{WT} control strain. Additionally, all three *hmg1* mutant strains exhibited a 2 to 3-fold increase in the expression of *erg6*, relative to the *hmg1*^{WT} control. No change in the expression of other sterol biosynthesis genes was observed following voriconazole treatment. Among all *hmg1* mutant strains, sterol profiling revealed a 2-fold decrease in ergosterol content relative to the *hmg1*^{WT} control strain, and a corresponding increase in C24 and C28 methylated sterols following voriconazole treatment. Five of these methylated sterols were not observed in the *hmg1*^{WT} control strain under any condition.

Conclusions:
The findings of this this study demonstrate that mutations in *hmg1* are associated with increased constitutive expression of both multi-drug efflux pump encoding genes and ergosterol biosynthesis genes. Additionally, following treatment with voriconazole, *hmg1* mutant strains exhibit increased expression of *erg6* and significantly altered sterol profiles consistent with increased sterol-methyltransferase activity. Further research is needed to delineate the direct contributions of these changes in efflux pump expression and sterol profiles towards clinical triazole resistance conferred by mutations in *hmg1*.

25 ACTIVITY OF DIPHENYL DISELENIDE AGAINST *ASPERGILLUS* ISOLATES

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Purpose:
Organoselenium compounds show promising antimicrobial activity against bacteria and some fungal species. Among these compounds, diphenyl diselenide $(PhSe)_2$ is a simple and chemically stable molecule with proven low toxicity to animal hosts. Although the mechanisms of action of this molecule are not totally clear, it has been reported that it has a pro-oxidative activity for microorganisms, owing to its effect of glutathione depletion. Since azoles are the most commonly used drugs available for treating aspergillosis, and the emergence of azole resistance *Aspergillus* sp. isolates is a global and rising concern, research towards the development of new molecules with antifungal potential are necessary. The aim of this study was to evaluate the *in vitro* susceptibility of *Aspergillus* spp. clinical isolates to $(PhSe)_2$.

Methods:
Thirty-three clinical *Aspergillus* spp. isolates from human or animal aspergillosis cases maintained in the fungal collection of the Mycology Laboratory from the Federal University of Rio Grande (FAMED-FURG) were included in the study. According to their macro- and micro-morphological characteristics they were identified as *Aspergillus* section *Fumigati*(n=20), *Flavi* (n=5) and *Nigri* (n=8). The broth microdilution method was performed according to the M38-A2 protocol of Clinical and Laboratory Standards Institute (CLSI), in duplicate, and a *Sporothrix brasiliensis* strain with a knowing Minimal Inhibitory Concentration (MIC) to $(PhSe)_2$ was used as a quality control to validate the test results. $(PhSe)_2$ was tested in concentrations ranged from 1 to 512 µg/ml, and the MIC was defined as the lowest concentration that completely inhibits the fungal growth (100% inhibition) after 48 hours of incubation at 37ºC. MIC_{50} and MIC_{90} were calculated, and MIC values were compared among the *Aspergillus* sections and analyzed by Kruskall-Wallis test with the SPSS 22.0 statistic program.

Results:
All the tested *Aspergillus* spp. isolates were inhibited by the $(PhSe)_2$ in concentrations within the tested range. The MIC geometric mean value, MIC_{50} and MIC_{90} were 165 µg/ml, 128 µg/ml and 512 µg/ml, respectively. The highest MIC geometric mean of $(PhSe)_2$ was against *Aspergillus* section *Fumigati* (274 µg/ml) (range 64 to 512 µg/ml), and the lowest against section *Nigri* (59 µg/ml) (range 32 to 128 µg/ml. Section *Flavi* showed a geometric mean of 111 µg/ml (range 64 to 128 µg/ml). The MIC_{50} and MIC_{90} of $(PhSe)_2$ were 265 and 512µg/ml, respectively, against section *Fumigati,* both128 µg/ml against section *Flavi,* and both 64 µg/ml against section *Nigri.* These MIC differences between *Aspergillus* sections were significant ($p<0.001$).

Conclusion:
$(PhSe)_2$ inhibits the growth of *Aspergillus* sections *Fumigati*, *Flavi* and *Nigri*. The relevance of the MICs reported here must be investigated in animal models. Its potential in combination with classical antifungals should be also investigated. $(PhSe)_2$ shows mechanisms of action different from those of azole drugs, which may make this molecule useful, given rising trends of resistance to other antifungals. Taking into account the higher MICs to azoles described to *Aspergillus* section *Nigri* in comparison to sections *Fumigati* and *Favi*, $(PhSe)_2$ may represent a possible choice in case of infection with isolates belonging to section *Nigri.*

26 MOLECULAR IDENTIFICATION OF *ASPERGILLUS* ISOLATES FROM MAGELLANIC PENGUINS

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Purpose:
Aspergillosis is an important disease in marine birds and has a mortality rate of 50% in Magellanic penguins in captivity (*Spheniscus magellanicus*). Currently, the precise identification of *Aspergillus* to species level is only possible by molecular methods. It is important to identify cryptic species since they show virulence and azole susceptibility differences, with epidemiological and clinical implications. Although there are many studies of the description, prevention, diagnostics and epidemiology of the disease in these birds, no data about the molecular identification of the isolates from Magellanic penguins is available.The aim of this work was to perform the molecular identification and the itraconazole susceptibility profile of *Aspergillus* isolates from penguins with proven aspergillosis.

Methods:
Aspergillus fungi isolated from penguins in captivity between 2011 and 2018 with proven aspergillosis were recovered from the fungal collection of the Mycology Laboratory of Federal University of Rio Grande (FURG- Brazil). The classification of isolates to section level was based on classic macro- and micromorphology characteristics. To perform the molecular identification, DNA was extracted, and the amplification of a partial *benA* gene was performed using the primers Btub1 and Btub2. Resulting sequences were analyzed using the program Mega 7.0 and then compared to sequences deposited in BLAST database. Identification to species level was accepted when the homology was >99%. To test itraconazole susceptibility, Minimal Inhibition Concentration (MIC) was determined according Clinical and Laboratory Standards Institute (CLSI- M-38A2), in duplicate, and concentrations ranged from 0.313 µg/ml to 16 µg/ml.

Results:
Nineteen *Aspergillus* sp. clinical isolates from penguins that died due to aspergillosis were recovered from the collection. All *Aspergillus* isolates from Magellanic penguins recovered in this study were morphologically identified as *Aspergillus* section *Fumigati*. Molecular identification showed that *Aspergillus fumigatus sensu stricto* was the etiological agent in the 19 proven cases of aspergillosis. The geometric mean of itraconazole MIC was 0.53 µg/ml, the $MIC_{50}$was 0.5 µg/ml and the MIC_{90}1 µg/ml. Two isolates had MIC=8 µg/ml, which were classified as itraconazole resistant isolates.

Conclusion:
Aspergillosis is a limiting factor during the recovery of captive penguins in rehabilitation centers and causes considerable mortality in penguins in zoos. *Aspergillus* section *Fumigati* are especially related to this disease, with a frequency of isolation of up to 99% in captive Magellanic penguins. In this work, we report *A. fumigatus sensu stricto* as the etiologic agent identified in all aspergillosis cases from the analyzed penguins. These findings are relevant to epidemiological considerations: clinical manifestation of aspergillosis in penguins includes the formation of fungal colonies in air sacs, which can result in release of conidia to their environment, and secondary infections. The data is also relevant to emergence of azole resistance in *A. fumigatus*, since itraconazole prophylaxis is used as preventive control of aspergillosis in penguins. The two resistant *A. fumigatus* were isolated from penguins that received itraconazole prophylaxis, which confirms the concern about resistance in this group of birds. Study of the mechanisms of resistance present in these isolates is being performed, and the results will also be presented.

27 *IN VITRO* AND *IN VIVO* ACTIVITY OF MANOGEPIX/FOSMANOGEPIX, A NOVEL ANTIFUNGAL WITH ACTIVITY AGAINST *ASPERGILLUS* AND RARE MOLDS

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Purpose:
The increasing global emergence of resistance to available classes of antifungal therapies has major clinical implications for the treatment of *Aspergillus* spp. and rare molds. Fosmanogepix (FMGX, APX001), and its active moiety manogepix (MGX, APX001A), is a novel, first-in-class antifungal agent, with broad spectrum of activity, including *Aspergillus* spp. and rare molds. The *in vitro* activity against a recent collection of mold isolates and *in vivo* efficacy were evaluated to support a Phase 2 clinical trial in invasive mold infections.

Methods:
The following data were evaluated: a) *in vitro* activity of MGX and comparator agents against 530 global mold isolates collected between 2017-2018 including *Aspergillus*, *Fusarium*, and *Scedosporium* species; b) *in vivo* efficacy from highly immunocompromised mouse models of IFI; c) evaluation of PK/PD.

Results:
MGX demonstrated potent *in vitro* activity ($MEC_{50/90}$, 0.03/0.06 mg/L) against all *Aspergillus* spp. isolates including infrequently encountered and azole-nonsusceptible isolates (Table). MGX activity was also notable against *Scedosporium* spp. ($MEC_{50/90}$, 0.015/0.03 mg/L) and *Fusarium* spp. (MEC_{50}, 0.03 mg/L) where treatment options are limited. FMGX *in vivo* efficacy was evaluated in immunocompromised mouse models of IFI with *Scedosporium*, *Fusarium* and *Rhizopus* where survival advantages and reductions in conidial equivalents/g tissue (CE) were demonstrated. Evaluation of FMGX PK/PD using 6 strains of *A. fumigatus* demonstrated that net stasis was achieved against all strains, including those that harbor Cyp51 mutations conferring triazole resistance, and 1 log reduction in CE was achieved for 5 of 6 strains. AUC/MEC was the best PK/PD index predictive of efficacy based on dose-fractionation analysis, similar to what was previously observed for *Candida* spp. Median 24 h free drug AUC/MEC targets for stasis and 1-log kill were 48 and 89, respectively.

Conclusions:
MGX, and prodrug FMGX demonstrated potent *in vitro* activity and *in vivo* efficacy, respectively. The current data supports the clinical evaluation of the novel antifungal agent FMGX in the treatment of invasive aspergillosis and rare molds infections.

	MIC-MEC$_{50/90}$ (mg/L)			
Organism (no. tested)	**Manogepix**	**Anidulafungin**	**Voriconazole**	**Amphotericin B**
Aspergillus spp. (497)	0.015/0.03	0.015/0.03	0.5/1	1/2
Aspergillus spp. (10)[a] (voriconazole non-susceptible)	0.015/0.015	0.008/0.015	2/4	1/2
A. flavus species complex (52)	0.015/0.03	≤0.008/0.015	0.5/1	2/2
A. fumigatus (350)	0.015/0.03	0.015/0.03	0.5/0.5	1/2
A. niger species complex (49)	≤0.008/0.015	≤0.008/0.015	1/2	0.5/1
A. terreus species complex (20)	0.015/0.03	0.015/0.03	0.5/0.5	2/2
Other *Aspergillus* spp. (26)[b]	0.015/0.03	0.03/0.12	0.5/4	2/2
Fusarium spp. (9)[c]	0.03/-	>4/-	4/-	2/-
Scedosporium spp. (24)[d]	0.03/0.06	4/>4	1/8	>2/>2

[a] *Aspergillus fumigatus* (9)*, A. niger species complex* (1)*.*
[b] *Aspergillus lentulus* (4)*, A. nidulans* species complex (9)*, A. nomius* (1)*, A. parasiticus* (1)*, A. sclerotiorum* (1)*, A. tamarii* (1)*, A. tubigenesis* (2)*, A. thermomutatus* (1)*, A. ustus species complex* (3)*, A. versicolor* (3).
[c] *Fusarium incarnatum-equiseti* species complex (3), *F. oxysporum* species complex (2), *F. solani* species complex (4).
[d] *Scedosporium apiospermum* (2), *S. apiospermum/boydii* (13), *S. aurantiacum* (2), *S. boydii* (3), *S dehoogii* (2), *S. prolificans* (2).

28 STUDY AND CHARACTERIZATION OF AZOLE RESISTANCE IN *ASPERGILLUS* SECTION *NIGRI*

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Purpose:
Invasive aspergillosis is a severe condition which mostly affects immunocompromised patients. It is mainly caused by *Aspergillus fumigatus,* followed by species belonging to other sections of the genus, such as sections *Nigri, Terrei* and *Flavi,* which represent the second leading etiological case of the disease.

As voriconazole (VRC) is the first-choice treatment against this condition, the progressive increase of azole-resistant isolates has limited therapeutic options in the clinical setting. Because of this, azole resistance mechanisms in *A. fumigatus* have been the focus of intense study. This contrasts with what occurs with other species of the genus.

Therefore, the aim of this work was to characterize azole resistance in *Aspergillus* section *Nigri* (species *A. niger* and *A. tubingensis*) by means of molecular analysis of the *cyp51* genes, which constitute the target genes for azole drugs.

Methods:
Using CRISPR/Cas9 methods coupled with microhomology HygR repair templates, single gene deletions of *cyp51A* and *cyp51B* were generated in *A. niger* and *A. tubingensis* strains with different VRC susceptibilities.

To characterize the role of the genes of interest in azole resistance, changes in the *in vitro* VRC susceptibility were assessed following the M38 protocol of the Clinical and Laboratory Standards Institute (CLSI). For gene expression analysis, total RNA was reverse transcribed into first-strand complementary DNA (cDNA) and reverse transcription-PCRs were carried out. Finally, the ergosterol content of the strains was spectrophotometrically determined.

Results:
Regarding the *in vitro* susceptibility results of the *cyp51A* and *cyp51B* single-gene mutants (*A. niger* and *A. tubingensis*), deletion of *cyp51A* seemed to increase VRC susceptibility, while the *cyp51B* deletion showed less impact on VRC MIC values.

Similarly, gene expression of *cyp51A* in the *cyp51B* defective strains slightly increased when compared to that of the wild-type strains. In contrast, in the absence of *cyp51A, cyp51B* expression was minimally altered.

Finally, although ergosterol quantification revealed differences in ergosterol content among the strains tested, we could not stablish a direct correlation with VRC Minimal Inhibitory Concentrations (MICs).

Conclusion:
Similar to findings in *A. fumigatus*, loss of *A. niger* or *A. tubingensis cyp51A* might impact azole susceptibility to a greater extent than *cyp51B*. Modulation of *cyp51A* gene expression also seems more responsive to pathway perturbations than that of *cyp51B* in both species. Our data contribute new evidence to the understanding of azole resistance in non-*fumigatus* species of *Aspergillus*. Due to the molecular complexity of azole resistance, future studies will focus on the deep analysis of the ergosterol biosynthesis pathway as well as other potential resistance mechanisms to characterize azole response in *Aspergillus.*

29 DOES MONITORING *CYP51A*-MEDIATED TRIAZOLE RESISTANCE IN *ASPERGILLUS FUMIGATUS* BY PYROSEQUENCING LEAD TO PATIENT BENEFIT?

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Purpose:
The global trend of rising triazole resistance rates in *Aspergillus fumigatus* requires close monitoring. The high volume culture (HVC) procedure developed at the NHS Mycology Reference Centre Manchester (MRCM) has improved culture positivity, thereby allowing EUCAST susceptibility testing to monitor resistance. However, as the failure rate is still significant, a molecular approach is warranted. Commercial assays testing for the environmental mutations (e.g. TR34/L98H) do not detect *cyp51A* mutations resulting from the pressure of long courses of azole treatment experienced by patients with progressive, chronic conditions. As a result of this limited coverage of polymorphisms, we adopted a pyrosequencing approach to monitor patients with chronic pulmonary aspergillosis (CPA) and/or allergic bronchopulmonary aspergillosis (ABPA), who demonstrated signs of clinical failure: those who demonstrated good drug levels but were persistently positive by *Aspergillus* spp. qPCR and remained symptomatic. Following over two years of testing, we assessed the impact of monitoring triazole resistance on patient outcome.

Methods:
To assess whether this molecular approach benefited patient care, we performed a retrospective audit over a 26-month period, between July 2017 and September 2019, at MRCM and the UK National Aspergillosis Centre (NAC). We reviewed the records of confirmed CPA and ABPA patients to assess the impact of susceptibility and/or pyrosequencing results on patient care. Hospital and laboratory databases were searched for patient history and well-being scores, laboratory findings and if/when the susceptibility or pyrosequencing results triggered a change in antifungal drug therapy.

Results:
Two hundred patients, who had at least one sample analysed by pyrosequencing, were considered for review alongside two hundred patients who did not have the test; these patients were selected randomly but matched as closely as possible to the latter cohort. For patients whose samples underwent pyrosequencing, there was resistance data available at twice the frequency of those patients for whom only culture was available. Moreover, resistance was identified by pyrosequencing in up to a quarter of culture negative samples. If pyrosequencing had not been used in these patients, 54 resistant cases would have been missed. Identification of the environmental TR34/L98H mutation was similar in both cohorts. Importantly, resistance was recognised by pyrosequencing before HVC results were available in up to one fifth of cases.

Conclusion:
Pyrosequencing had a higher sensitivity for detecting triazole resistance than culture-based susceptibility testing. The technology allowed for prompt recognition of resistant organisms and the selection of appropriate antifungal treatment. The detailed impact on improved patient outcomes and better antifungal stewardship will be discussed.

30 AZOLE RESISTANT *ASPERGILLUS FUMIGATUS* FROM AGRICULTURAL SETTINGS

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Purpose:
Azoles are widely used in agriculture to combat fungal pathogens of plants and in the clinic to combat fungal pathogens of people. Resistance to clinical antifungals has become a major problem in India and Europe in the last decade. Recently there has been an increase in azole-resistant *Aspergillus fumigatus* isolated in clinics in the US, but the prevalence of azole-resistant *A. fumigatus* in the environment is not well-understood.

Methods:
We sampled 56 agricultural sites in Georgia and Florida, including many with known past azole exposure. MICs for clinical azoles were determined. Microsatellites and whole genome sequencing were used to analyze strains.

Results:
We isolated over 100 strains that grew on low levels of tebuconazole, including several that were resistant to high levels of clinical azoles in microbroth dilution tests.

Conclusion:
Microsatellite and whole genome sequencing suggest that clinical azole resistance has an agricultural origin.

31 THE ROLE OF PTX3 IN INNATE REGULATION OF ANTIFUNGAL IMMUNITY IN CHRONIC PULMONARY ASPERGILLOSIS

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Purpose:
Chronic pulmonary aspergillosis (CPA) is an infection with devastating consequences to the lungs, especially for the expanding population of chronic obstructive pulmonary disease (COPD) patients. Susceptibility to CPA differs significantly even among patients with similar predisposing conditions, but the mechanisms that influence the efficacy of individual antifungal immune responses remain almost unknown. Among the many molecules endowed with regulatory properties of antifungal immune responses, the soluble pattern recognition receptor pentraxin-3 (PTX3) has been demonstrated to play a non-redundant role during infection with the fungal pathogen *Aspergillus fumigatus*. In immunocompromised hosts, genetic variants impairing the expression of PTX3 renders carriers highly susceptible to invasive aspergillosis. Whether PTX3 also influences susceptibly to chronic forms of aspergillosis in otherwise immunocompetent individuals remains unknown.

Methods:
Here, we have analyzed the contribution PTX3 to antifungal immunity, using a mouse model of CPA based on agar beads containing fungal conidia that trigger the formation of fungal granulomas in the lungs.

Results:
Using this model, we found that PTX3 is readily induced upon infection in the lungs and that its expression is required for the control of disease. Indeed, contrary to wild-type mice, Ptx3-deficient animals rapidly succumb to infection, a finding in line with the higher fungal burden detected in the lungs. Mechanistically, Ptx3-deficient macrophages were found to display impaired antifungal effector functions

Conclusion:
Collectively, these results highlight the requisite role of PTX3 to antifungal immunity and establish PTX3 as an attractive target for immunotherapeutic strategies to prevent or treat CPA.

32 ANTIFUNGAL SUSCEPTIBILITY PROFILES OF OLOROFIM (FORMERLY F901318), AND CURRENTLY AVAILABLE SYSTEMIC ANTIFUNGALS AGAINST MOLD AND YEAST PHASES OF *TALAROMYCES MARNEFFEI*

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Purpose:
Talaromyces marneffei is a thermal dimorphic fungus and is the etiologic agent of talaromycosis, a life-threatening disease which affects immunocompromised host especially those with HIV infection. The fungus is endemic in Southeast Asia and is known to be associated with bamboo rats. Talaromycosis is initially treated with amphotericin B but its use is limited due to toxic side effects. Therefore, the need for new antifungals to treat talaromycosis is urgent. Olorofim is a novel fungicidal drug which targets dihydroorotate dehydrogenase in the de novo pyrimidine biosynthesis pathway. It is highly active against *Aspergillus* and other filamentous *Ascomycetes*. However, the *in vitro* efficacy of olorofim against *T. marneffei* has yet to be reported. We therefore aimed to evaluate the susceptibility of *T. marneffei* to olorofim and other currently available systemic antifungals in its yeast as well as in mold phases.

Methods:
We tested 32 clinical and environmental *T. marneffei* strains recovered from southern China against 8 different antifungals according to the Clinical and Laboratory Standards Institute M38-A2 and M27-A3 guidelines.

Results:
The geometric means of the minimum inhibitory concentrations/minimum effective concentrations (MICs/MECs) of the antifungals against mold phase of all *T. marneffei* strains were (in increasing order): olorofim (0.0005 mg/mL), itraconazole and posaconazole (0.016 ug/mL), voriconazole (0.05 ug/mL), 5-flucytosine (0.08 ug/mL), terbinafine (0.1 ug/mL), caspofungin (0.4 ug/mL) and amphotericin B (2 ug/mL). The geometric means MICs/MECs against the yeast phase were, as follows: olorofim (0.0007 ug/mL), posaconazole (0.016 ug/mL), itraconazole (0.016 ug/mL), voriconazole (0.017 ug/mL), terbinafine (0.12 ug/mL), amphotericin B (0.13 ug/mL), 5-flucytosine (0.25 ug/mL), and caspofungin (4.5 ug/mL). Olorofim was the most active antifungal agent against both mold and yeast phases of all tested *Talaromyces marneffei* isolates, exhibiting an MIC range, MIC_{50}, and MIC_{90} of 0.0005-0.002 ug/mL, 0.0005 ug/mL, and 0.0005 ug/mL, respectively.

Conclusion:
In summary, olorofim demonstrated potent and consistent activity against all *T. marneffei* strains *in vitro,* and its activity was maintained in two different growth phases. Further studies are warranted to evaluate the *in vivo* efficacy of olorofim against this fungus.

33 SYNERGIC ANTI-FUNGAL ACTIVITY OF TOPICAL PC945, A NOVEL INHALED AZOLE, WITH SYSTEMIC ECHINOCANDIN ON *ASPERGILLUS FUMIGATUS IN VITRO* HUMAN ALVEOLI MODEL

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Purpose:
PC945 is a novel antifungal agent being developed as an inhalation therapy for the treatment of aspergillosis. We previously reported that topical PC945 and systemic azoles showed synergic antifungal effects *in vitro* and *in vivo* (Sci Rep, 2019, 9482). This study examined the anti-fungal activity of PC945 on growth of *Aspergillus fumigatus* (*A. fumigatus*) as a combination therapy with echinocandin *in vitro* using a model of human alveoli.

Methods:
A model of human alveoli was constructed in transwells consisting of a bilayer of human alveolar epithelial and endothelial cells. PC945 (0.1 μg/mL) was either added as monotherapy once daily on days 0, 1 and 2 to the epithelial compartment (upper chamber) mimicking inhalation treatment or was combined with caspofungin or micafungin (1 μg/mL) added to the endothelial compartment (bottom chamber) mimicking oral treatment. Transwells were infected with azole sensitive (NCPF2010) *A. fumigatus* at a concentration of 1 x 10^4 spores mL^{-1}, one hour after treatment on day 0. Subsequently, levels of galactomannan in the bottom chamber, which was collected Day 0 to Day 5 post inoculation, were quantified by ELISA (Platelia™, Biorad). Azole-resistant *A. fumigatus* harbouring TR34/L98H mutation was also infected in the presence of PC945 (1 μg/mL, upper) with/without caspofungin (0.1 μg/mL, bottom).

Results:
In the *in vitro* model of human alveoli, caspofungin or micafungin (1 μg/mL; bottom chamber) or PC945 (0.1 μg/mL; upper chamber) alone had only mild inhibitory effects on azole sensitive *A. fumigatus* invasion Day 1 post inoculation, whereas a combination of PC945 and caspofungin/micafungin achieved marked inhibition of fungal invasion up to Day 5 post inoculation. A similar pattern was observed for the azole resistant strain TR34-L98H, with caspofungin (1 μg/mL, bottom) or PC945 (1 μg/mL, upper) monotherapy weakly inhibiting fungus invasion. In contrast, treatment with a combination of PC945 and caspofungin provided much greater protection than monotherapy.

Conclusion:
In this study, combination therapy of PC945 and echinofungin was shown to inhibit azole-sensitive and azole-resistant *A. fumigatus* growth to a greater extent than monotherapy using either compound. PC945 therefore has the potential to be used in combination with established antifungal drugs for the treatment of azole sensitive and resistant *A. fumigatus* infection in humans.

34 ALTERED *A. FUMIGATUS* CELL WALL INTEGRITY BY PC945, A NOVEL INHALED AZOLE

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Purpose:
PC945 is a novel antifungal agent being developed as an inhalation therapy for the treatment of aspergillosis. We previously found that PC945 treatment was more effective in *in vivo A. fumigatus* infected mice than posaconazole although both compounds showed similar anti-fungal effects in MIC assay by broth microdilution *in vitro*. Therefore, our hypothesis is that PC945 alters *A. fumigatus* cell wall integrity, leading to antigen being exposed to immune cells and consequently more efficient fungal clearance.

Methods:
Congo red (CR) and calcofluor white (CFW) are cell wall perturbing agents that influence cell wall chitin and β-glucan deposition, respectively. *A. fumigatus* conidia (strain AF293)[10 – $1x10^5$ conidia] was spotted onto Saboraud agar containing CR (10 mg/ml) or CFW (10 mg/ml) with/ without DMSO (vehicle) or PC945 (0.008 μg/ml), and the plates were incubated at 37°C, 5% CO_2 for 24 h. In addition, to examine the presentation of β-glucan and chitin expression on the cell wall of *A. fumigatus,* the fungus was stained with fc-dectin-1a (human dectin-1a fused to human IgG1 fc domain) (1μg/ml; Invivogen) with AlexaFluor-647 conjugated anti-human antibody and FITC-conjugated wheat germ agglutinin (WGA-FITC; 1μg/ml; Sigma) after 3hour incubation with PC945. The level of fluorescent was detected by flow cytometer. In addition, PBMCs obtained from healthy volunteers were exposed to UV-inactivated PC945 loaded *A. fumigatus*, and IL-1β in the supernatant was measured by ELISA.

Results:
It was observed that PC945 enhanced susceptibility of *A. fumigatus to* CR and CFW, suggesting that PC945 compromised cell wall integrity. Furthermore, treatment of *A. fumigatus* with PC945 resulted in greater detection of β-glucan and chitin on the fungal body surface, detected by fc-dectin-1a and WGA-FITC, respectively. Furthermore, PC945 (0.008 μg/ml) treated *A. fumigatus* triggered increased expression of IL-1β from human PBMCs. The induction of IL-1β was attenuated by the presence of neutralising antibodies against Dectin-1. This data suggests that the enhanced inflammatory response generated against PC945 treated *A. fumigatus* is dependent on Dectin-1 mediated signalling pathways via activation of the inflammasome. Those effects were also confirmed in parconazole treated *A. fumigatus*, but PC945 was 2-fold more potent than posaconazole.

Conclusion:
This data suggests that β-glucan and chitin on *A. fumigatus* are exposed after PC945 treatment due to compromised cell wall integrity, and it is possible that PC945 induces increased recognition of *A. fumigatus* to immune cells to accelerate clearance of fungal body.

NOTE: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.

35 NEUTROPHIL ROS CONTROLS FUNGAL GROWTH AND INFLAMMATION IN PHAGOCYTE NADPH OXIDASE-DEFICIENT ZEBRAFISH

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Purpose:
Chronic granulomatous disease (CGD) is an inherited immunodeficiency caused by deficient phagocyte NADPH oxidase (PHOX) function and subsequent failure to generate phagocytic ROS. A unique characteristic of CGD is patient vulnerability to invasive infections by the typically non-pathogenic fungus *Aspergillus nidulans*. Neutrophils are phagocytes of the innate immune system that generate ROS and mediate host defense during fungal infection. However, the role of neutrophil ROS in fungal clearance and control of inflammation during *A. nidulans* infection is not well understood. It is also not clear how specific immunodeficiencies such as CGD allow for differential virulence of fungal pathogens. A major challenge in understanding this specific host-pathogen interaction has been direct *in vivo* visualization of inflammation and fungal growth over the course of multi-day infections. Here, we use real time imaging in a PHOX-deficient zebrafish model to reveal the underlying interactions between *A. nidulans* and the innate immune response.

Methods:
We exploited the optical transparency of zebrafish to image the effects of neutrophil ROS on fungal growth and neutrophil behavior during *A. nidulans* (FGSC-A4) infection. We developed a zebrafish model of CGD by utilizing zebrafish with a mutation in the p22 subunit (*p22*$^{-/-}$) of PHOX and generated *p22*$^{-/-}$ lines with fluorescently labeled phagocytes. To determine the neutrophil-specific role of PHOX, we expressed functional p22^{phox} exclusively in neutrophils under a cell-specific promoter.

Results:
We demonstrate that PHOX-deficient larvae are susceptible to *A. nidulans* infection, similar to human disease. In a wild-type host, *A. nidulans* germinates quickly, evokes a strong host response, and is cleared soon after infection. Global PHOX activity does not inhibit fungal germination but reduces invasive hyphal growth and excessive neutrophil recruitment. Neutrophil-specific expression of PHOX limits hyphal growth, neutrophil infiltration, and rescues host survival.

Conclusion:
Aspergillus nidulans elicits a strong immune response that leads to excessive inflammation and hyphal-induced tissue damage in a PHOX-deficient host. Neutrophil ROS limits invasive fungal growth and has immunomodulatory activities that contribute to the specific susceptibility of PHOX-deficient hosts to *A. nidulans* infection.

36 *IN VIVO* AND *IN VITRO* IMPAIRMENT OF TH CELL AND NEUTROPHIL RESPONSES AGAINST *MUCOR IRREGULARIS* IN CARD9 KNOCKOUT MICE

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Purpose:
Card9 is an essential signaling adaptor protein downstream of many pattern recognition receptors (PRRs), including Dectin-1, Dectin-2, Dectin-3 and Mincle. Via forming the Card9-BCL10-MALT1 complex, it mediates downstream activation and exerts a significant role in antifungal immunity. It has been reported that CARD9 deficiency is relevant to the increased susceptibility to many fungal infections, e.g. those caused by *Candida albicans, Aspergillus fumigatus, Cryptococcus neoformans, Rhizopus arrhizus* and some dematiaceous fungi, in different animal models. Regarding *Mucor irregularis*, a fungus frequently found in Southeast Asia, especially in China, its relationship with Card9 has never been studied. Our study is the first to establish a mucormycosis murine model with *Mucor irregularis* using wild-type (WT) mice and Card9 knockout (Card9$^{-/-}$) mice, aiming to investigate the antifungal effect of Card9 against *M. irregularis* infection both *in vivo* and *in vitro*.

Methods:
The clinical strain *Mucor irregularis* (BMU09468) used in our study was isolated from the first Card9 deficiency case of cutaneous mucormycosis caused by *Mucor irregularis*. We used this strain to perform all the experiments. For *in vivo* experiments, we assessed skin lesions of footpads, histopathology, fungal burdens, survival rates, cytokine and chemokine profiles. For *in vitro* experiments, we used bone-marrow-derived macrophages (BMDMs), bone-marrow-derived dendritic cells (BMDCs) and naïve CD4^{+}T cells to determine NF-κB pathway activation, Th cell responses and neutrophil responses.

Results:
For *Card9*$^{-/-}$mice, severe necrosis was found in the footpads and disfigurement at the end of the observation. Numerous nonseptate, wide and irregularly branched hyphae were found in the histopathological examination of footpads. The fungal burdens in the footpads and inguinal lymph nodes were higher than that of WT mice ($P<0.05$). TNF-α, IL-6, Th1-cytokine IFN-γ and Th17-cytokine IL-17A were lower while Th2-cytokine IL-4 and IL-10 were higher than that of WT mice ($P<0.05$). There was also impaired chemokine production, including CXCL1 and CXCL2, in *Card9*$^{-/-}$mice. Marked decrease was found in the NF-κB (p65) activation of BMDMs of *Card9*$^{-/-}$ mice compared to WT mice, illustrating a weaker NF-κB pathway activation. Reduced levels of IL-1β, IL-23 and IL-12 p70 secreted by dendritic cells and lower proportions of Th1/17 differentiation were found in *Card9*$^{-/-}$mice. Neutrophil-dependent antifungal immunity, including secretion of IL-6 and TNF-α and production of neutrophil extracellular traps, was also decreased in *Card9*$^{-/-}$ neutrophils.

For WT mice, there was no ulceration in the footpads and the fungal elements were completely cleared in histopathological examination.

Conclusion:
Card9$^{-/-}$mice were more susceptible to *M. irregularis* infection than WT mice, which could be linked to the impaired NF-κB pathway activation, local cytokine and chemokine production, Th cell responses and neutrophil responses in *Card9*$^{-/-}$mice. This might add another risk factor-gene deficiency, for developing mucormycosis, in addition to known factors: diabetes mellitus, immunosuppressed status, disruption of cutaneous barriers, iron overload and use of deferoxamine.

37 MODULATION OF TREM1 SIGNALING IN MACROPHAGES INFECTED WITH *ASPERGILLUS FUMIGATUS*

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Purpose:
Pattern recognition receptors (PRR) play a central role recognizing *A. fumigatus* components and initiating immune responses in the lungs. In the last few years, a new family of PRRs, called TREM, has been identified, of which TREM1 was the first to be characterized. TREM1 is constitutively expressed in myeloid cells such as neutrophils and macrophages. TREM1 potently synergizes with Toll-like receptors (TLRs) for a substantial amplification of the immune response and production of pro-inflammatory mediators such as TNF, IL1β, IL-6, IL-8 and MCP-1. Besides its role in the innate immune cell activity, the TREM1 pathway has been suggested as a potential target for the treatment of different inflammatory disorders.

Methods:
To accomplish this study, we used peritoneal macrophages isolated from *Trem*$1^{+/+}$ and *Trem*$1^{-/-}$ mice and the RAW 264.7 cell line. Cells were infected with *A. fumigatus* conidia and incubated for 2h or 6h depending on the experiment. After *A. fumigatus* challenge, cellular RNA was extracted and expression levels of cytokines (IL6, CXCL1, TNFα, MCP1, IL1β) and receptors (TLR2, TLR4, MyD88) involved in the TREM1 immune response were analyzed by quantitative PCR. The immune response against *A. fumigatus* was also analyzed in different *in vitro* conditions that included antifungals (voriconazole and caspofungin) and TREM1 inhibitors (LP17 and LR12).

Results:
Results show a significant decrease of expression levels of cytokines IL6, TNFα and CXCL1 in the TREM$1^{-/-}$ macrophages compared to wild type. Expression of TL2 and TLR4 was also greatly attenuated in TREM$1^{-/-}$ macrophages. Expression levels of TREM1, TLR4 and MyD88 were reduced when voriconazol was added to the infected cells while the expression of IL6, CXCL1, TNFα, and IL1β increased in the same conditions. The treatment with LP17 and LR12 peptides inhibited the expression of TREM1 although we did not observe statistical differences in the expression pattern of inflammatory cytokines.

Conclusion:
These data indicate that expression of TREM1 modulates the TLR signaling in macrophages by altering the expression of both receptor and effector proteins that are critical to the response against *A. fumigatus*. The possibility of modulating the inflammatory response in a favorable direction thought the TREM1 pathway may represent a new approach for the development of novel immunotherapeutic antifungals to treat patients with invasive aspergillosis.

***NOTE*: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.**

38 *ASPERGILLUS FUMIGATUS* AND *PSEUDOMONAS AERUGINOSA* PULMONARY CO-EXPOSURE RESULTS IN INCREASED IL-17A, EOSINOPHILIA, AND ACUTE LUNG INJURY IN ALLERGIC ANIMALS

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Purpose:
The lung consists of a complex microenvironment made up of microorganisms that interact with one another and the host cells via direct and indirect interactions. *Aspergillus fumigatus* is a respiratory allergen that has been associated with fungal-bacterial interactions in respiratory diseases like cystic fibrosis. In clinical pulmonary research, respiratory co-infections by *A. fumigatus* and *Pseudomonas aeruginosa* lead to more severe disease outcomes including decreased lung function and increases in hospitalizations. *In vitro* co-cultures show that *P. aeruginosa* and *A. fumigatus* can affect one another's growth and pathogenicity, but very few studies have attempted to model interactions of these microorganisms *in vivo*.

Methods:
To study a pulmonary dual assault of *A. fumigatus* and *P. aeruginosa* under allergic and non-allergic disease states, we utilized our murine nose-only inhalational model for exposure of *A. fumigatus* in immunocompetent animals non-allergic or allergic to the fungus. This was followed by an intranasal exposure to *P. aeruginosa.* Animals were monitored for 24h and removed from the study due to increased morbidity and mortality.

Results:
While both non-allergic and allergic co-exposed animals had significant neutrophilia and production of acute phase response cytokines and chemokines, the allergic co-exposed group had a greater mortality with 34.8% of the animals expiring by 24h in comparison to 12.5% for the non-allergic co-exposed animals and 100% survival in the controls. Histological assessment of lungs showed septal thickening and interstitial inflammation that was further confounded by edema and pulmonary hemorrhage characteristic of acute lung injury. More severe disease outcomes coincided with an increase in eosinophilic inflammation and IL-17A production, which was observed only when animals were co-exposed to a combination of live bacterial and live fungal microorganisms.

Conclusion:
Spatial and temporal co-localization of *A. fumigatus* and *P. aeruginosa* in the lung results in severe disease outcomes, especially during allergic exacerbations. Further characterization of these microbial interactions *in vivo* is intended to provide evidence-based insight for clinical practices and will provide a platform to study other pulmonary microbial interactions.

39 PHAGOCYTOSIS OF MUCORALES SPORES BY LUNG EPITHELIAL CELLS AND MACROPHAGES

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Purpose:
Opportunistic infections represent a serious health threat to immunocompromised patients nowadays. One group of relevant fungal pathogens are the Mucorales. Members of this group play an increasing role in the clinic, but still only little is known about their biology, disease establishment, progression, and immune response. As for other filamentous fungi, the infectious agents are inhaled spores. Therefore, we study the interaction of clinically relevant Mucorales with bronchial and alveolar epithelial cells that are most likely the first cells of contact.

Methods:
In order to enhance throughput and facilitate comparison of different Mucorales species, we adapted a flow cytometric method to study phagocytosis by lung epithelial cells and macrophages *in vitro*. With this method we can distinguish between internalized and attached spores by differential staining using FITC and uvitex. As representatives of alveloar epithelial cells we used A549 cell line and NHBE cells as an example for bronchial epithelial cells. Furthermore, we tested our method also with ThP1- macrophages.

Results:
Our results so far show that significant difference in phagocytosis rates are obvious within the various Mucorales species by lung epithelial cells, whereas in macrophages phagocytosis rates of different Mucorales species were similar.

Conclusion:
Our results so far indicate that mechanisms for recognition, binding and uptake of Mucorales spores is not only species dependent, but also is variable in different cell types. Future work will aim to elucidate the parameters involved in recognition and uptake and shed light on the fate of internalized Mucorales spores.

40 EVALUATING THE ROLE OF STAT3 IN CD4+ T CELLS IN SUSCEPTIBILITY TO INVASIVE ASPERGILLOSIS

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Purpose:
Th17 cells play a critical role in host defense against *Aspergillus* infection by activating transcription factor STAT3 to induce production of IL17 and IL22 which promote local inflammatory responses. Our aim was to determine whether inhibition of STAT3 in CD4+ T cells promotes susceptibility to invasive aspergillosis in CD4+(STAT3-/-) mice.

Methods:
To investigate the role of STAT3 in CD4+ T cells, we generated CD4+(STAT3-/-) mice. C57BL/6 wildtype (n=10) and CD4+(STAT3-/-) mice (n=10) were immunosuppressed to induce neutropenia and infected with *Aspergillus fumigatus* (ATCC strain: MYA-4609) using an inhalation chamber. Non-immunosuppressed wildtype (n=10) and CD4+(STAT3-/-) mice (n=10) were also infected and served as controls. Mice were monitored daily for 21 days post infection to evaluate their survival. At endpoints, lung bronchoalveolar lavages (BAL), lung tissue, and serum were collected. *Aspergillus* infection was confirmed by lung fungal culture counts, histology and galactomannan testing.

Results:
Immunosuppressed CD4+(STAT3-/-) mice began succumbing to infection on day 4; by day 7 only 30% of mice survived. Immunosuppressed wildtype mice started to succumb to infection on day 5, and 40% of mice remained alive by day 7. Histologic results showed multiple distinct nodules of conidia and hyphae within lungs of the infected mice. All mice that succumb to infection tested positive for BAL galactomannan (index >2). The non-immunosuppressed control wildtype and CD4+(STAT3-/-) mice maintained their weight over the course of the study, did not test positive for galactomannan and histology results confirmed that they were not infected with *A. fumigatus*. In the BAL, TNFa, IL6, IFNy, IL17A and IL22 levels were elevated in wildtype immunosuppressed mice compared to immunosuppressed CD4+(STAT3-/-) mice 3 days post infection. Plasmatic TNFa, IL6, IL17A, and IL22 were also increased in wildtype immunosuppressed mice.

Conclusion:
Our results suggest that inhibition of STAT3 in CD4+ T cells results in reduced production of inflammatory cytokines in immunosuppressed mice; however it does not increase susceptibility to *Aspergillus* infection.

41 EPITHELIAL UPTAKE OF *ASPERGILLUS FUMIGATUS* SPORES DRIVES EFFICIENT FUNGAL CLEARANCE *IN VIVO* AND IS ABERRANT IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD) PATIENTS

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Purpose:
Airway epithelial cells (AECs) are able to internalise *Aspergillus fumigatus* (*Af*) spores *in vitro* thereby limiting spore germination. This led us to hypothesise that spore uptake and killing by AECs is important for driving efficient fungal clearance *in vivo* and that defective AEC-mediated spore uptake and killing would represent major risk factors for *Aspergillus*-related diseases. In order to test these hypothesis, we measured spore uptake, and outcomes, in *ex vivo* (from primary AECs obtained from infected murine lungs) and *in vitro*, from primary AECs obtained from healthy human donors and COPD patients.

Methods:
We constructed a fluorescent *Af* reporter strain to directly trace microbial viability within AECs and developed an Imaging Flow Cytometric (IFC) multiplex platform and a microfluidic model for live-cell analysis of *Af*-AECs interactions and outcomes thereof. These single-cell technologies allowed us to identify, quantify, isolate and analyse individual host pathogen complexes from immortalised and primary AECs obtained from infected murine lungs and human lung resections. A protocol for isolation and maintenance of primary human AECs from healthy human donors and patients with COPD was devised combining tissue digestion and filtration, followed by fibroblast, macrophage and endothelial cell depletion.

Results:
In vitro, uptake of *Af* spores by immortalised AECs increased proportionately with duration of *Af*-AEC co-incubation and single AECs were able to internalise multiple *Af* spores. While viability of internalising AECs was largely unaffected, AECs were able to curtail the growth of the internalised spores efficiently, whereby only ~1% of internalised spores remained viable after 8 hours. Applying our approach directly to infected mouse lungs we demonstrated, for the first time, that *Af* spores are internalised and killed by AECs during whole animal infection. *Ex vivo*, only ~3% of internalised spores remained viable after 8 hours of co-incubation with murine primary AECs. Importantly, *in vitro* comparison of *Af*-AECs interactions of spore uptake by *Af*-challenged primary human AECs from healthy and at-risk donors revealed significantly alterations in the uptake and consequent outcomes in COPD patients.

Conclusion:
We have demonstrated that AECs efficiently kill *Af* spores upon uptake during infection in the whole animal and that this process is altered in COPD, a well-known risk factor for debilitating fungal lung disease, thereby suggesting that AECs critically contribute to the efficient clearance of inhaled *Af* spores and that dysregulation of curative AEC responses represents a potent driver of *Aspergillus*-related diseases.

***NOTE*: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.**

42 *ASPERGILLUS FUMIGATUS* CELL WALL PROMOTES APICAL AIRWAY EPITHELIAL RECRUITMENT OF HUMAN NEUTROPHILS

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Purpose:
Aspergillus fumigatus is a ubiquitous fungal pathogen capable of causing multiple pulmonary diseases including invasive aspergillosis, chronic necrotizing aspergillosis, fungal colonization, and allergic bronchopulmonary aspergillosis. Intact mucociliary barrier function and early airway neutrophil responses are critical for clearing fungal conidia from the host airways prior to establishing disease. Following inhalation, *Aspergillus* conidia deposit in the small airways where they are likely to make their initial host encounter with epithelial cells. Challenges in airway infection models have limited the ability to explore early steps in the interactions between *A. fumigatus* and human airway epithelium.

Methods:
Here, we use inverted air-liquid interface cultures to demonstrate that human airway epithelium responds to apical stimulation by *A. fumigatus* to promote transepithelial migration of neutrophils from the basolateral membrane surface to the apical "airway" surface.

Results:
Promoting epithelial transmigration with *Aspergillus* required prolonged exposure with live resting conidia. Swollen conidia did not expedite epithelial transmigration. Using *A. fumigatus* strains containing genetic deletions of cell wall components, we identified that deletion of the hydrophobic rodlet layer or DHN-melanin in the conidial cell wall amplified epithelial transmigration of neutrophils, using primary human airway epithelium.

Conclusion:
Ultimately, we show that an as-yet-unidentified non-secreted cell wall protein is required to promote early epithelial transmigration of human neutrophils into the airspace in response to *A. fumigatus*. Together, these data provide critical insight into the initial epithelial host response to *Aspergillus*.

43 THE ANTIGENS ENOLASE, TRIOSEPHOSPHATE ISOMERASE AND HEAT SHOCK PROTEIN HSS1 OF *MUCOR CIRCINELLOIDES* ARE RECOGNIZED BY SERA FROM IMMUNOCOMPROMISED INFECTED MICE

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Purpose:
The genera *Mucor* is the second most common causal agent of mucormycosis, only exceeded by *Rhizopus*. Among them, *Mucor circinelloides* is the most frequently isolated species of the genera. The mucormycosis is highly aggressive, affecting, above all, immunocompromised individuals, and its incidence has increased in the last years. In addition, its diagnosis is usually carried out at a late stage of infection, and the treatment strategies are not clear. Therefore, more research into this disease is of paramount importance.

In this context, the aim of this work was the identification of the most immunogenic antigens of *M. circinelloides*. An innovative approach using immunosuppressed mice was employed to detect them so that only the most immunoreactive were selected.

Methods:
The strain *M. circinelloides* CBS 125721 was grown into PDB for 24 h at 37ºC with 120 rpm. For secretome obtaining, the fungal material was separated by filtration and incubated for 20 h in PBS 2% glucose. Then, the supernatant was filtered, sterilized and dialyzed. For the total extract, the fungus was centrifuged, washed, and lysed using glass beads at 30 Hz for 20 min. Finally, proteins were precipitated and resuspended in 2-DE buffer.

Protein separation was carried out by 2-DE and antigen detection was performed by WB against a pool of sera obtained from 2 groups of 5 mice infected intravenously with 10^5 conidia and PBS, respectively. These mice had been previously immunosuppressed using 200 mg/kg body weight of cyclophosphamide and once every 5 days thereafter. Mice were sacrificed at day 20.

To finish, the most immunoreactive proteins detected specifically by infected mice were identified by LC-MS/MS.

Results:
From the secretome, seven antigens were identified, these corresponding to three spots of enolase and four of triosephosphate isomerase (TPI). They are almost certainly different isoforms of each protein, as they have the same molecular weights and very similar isoelectric points. Regarding the total protein extract, only the two most immunoreactive spots were identified. These proteins were enolase and heat shock protein HSS1.

The TPI has been already described in literature as a fungal antigen but not in *Mucor*. Regarding HSS1, this belongs to the same family as the Hsp70, which has been widely described as immunoreactive in several fungi. Enolase deserves a special mention as it was identified in both analyzed extracts and was also previously identified as an important antigen for many pathogenic fungi, which could make it into a panfungal diagnosis target or to design new treatments or vaccines.

Conclusion:
In this work, the most immunoreactive antigens of the secretome and the total extract of *M. circinelloides* were identified. The proteins identified were HSS1, Enolase, and TPI. These proteins might be useful in the future for the development of a vaccine, antifungal treatments and/or for diagnosis, allowing the rapid detection and treatment of the disease and so lower the unacceptable mortality rates.

Financial support:
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44 AN IMMUNOPROTEOMICS APPROACH TO IDENTIFY *ASPERGILLUS FUMIGATUS* PROTEIN ANTIGENS IN CYSTIC FIBROSIS PATIENTS WITH ALLERGIC *BRONCHOPULMONARY* ASPERGILLOSIS (ABPA)

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Purpose:
Allergic bronchopulmonary aspergillosis (ABPA) is a hypersensitivity response to the colonisation of the airways with *Aspergillus fumigatus,* in particular in patients with cystic fibrosis or asthma. Despite some progress, the diagnosis of ABPA is difficult due to a lack of a unique biomarker and standardised diagnostic criteria. ABPA correlates with an increased concentration of total serum IgE and raised specific anti-*A. fumigatus* IgE and/or IgG titers. The commonly used crude allergen extracts for serological diagnosis show batch to batch variation, insufficient standardisation and lack of reproducibility. For this reason, panels of recombinant allergens of *A. fumigatus* (Asp f) have been tested for diagnosis of ABPA. However, known *A. fumigatus* allergens often lack specificity. For this reason, we aimed at screening for new *A. fumigatus* protein antigens with better diagnostic value.

Methods:
To characterise the antibody response to *A. fumigatus* in ABPA patients, we applied serological proteome analyses (SERPA) for the identification of protein antigens recognized by sera from CF patients with and without *A. fumigatus* colonisation, patients with probably invasive aspergillosis, and healthy controls. We separated extracellular proteins of *A. fumigatus* by 2D gel electrophoresis and antigenic proteins were subsequently identified using a Western blot approach with patient sera and secondary IgG-antibodies.

Results:
In total, 44 protein antigens, that gave a positive IgG antibody signal, were identified. Six proteins were only recognized by sera of CF patients. The remaining 38 proteins were also recognized by control sera and sera from patients with probable invasive aspergillosis. In general, CF patient sera recognized a higher number of different protein antigens compared to control sera.

Conclusion:
In summary, our comprehensive analysis detected new *A. fumigatus* antigens and provide new targets for diagnosis of *A. fumigatus* infections in cystic fibrosis patients.

45 "INVASIVE ASPERGILLOSIS ON-A-CHIP" – A NOVEL DISEASE MODEL TO STUDY *ASPERGILLUS FUMIGATUS* INFECTION IN THE HUMAN LUNG

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Purpose:
Invasive pulmonary aspergillosis is a great threat to immunocompromised patients as treatment options are limited and only successful upon early diagnosis which leads to high mortality rates. Infectious agents are conidia of the mould *Aspergillus fumigatus* that enter the lung alveoli but, in immunocompetent humans, are cleared by innate immune cells such as macrophages and neutrophils. In immunocompromised patients, however, conidia can germinate and grow into filamentous bodies (hyphae) leading to tissue destruction and invasion of blood vessels. To date, complex human cell-based models to investigate invasive aspergillosis are rare and often include either a limited number of involved cell types or insufficient mimicry of the human alveolar structures. Here, we established an "invasive aspergillosis on a chip" model to investigate *A. fumigatus* infection by conidia in different clinical situations.

Methods:
The microfluidic "lung-on-a-chip" includes human alveolar epithelial cells at an air-liquid-interface separated from human endothelial cells by a porous membrane to study the growth and the invasive behaviour of *A. fumigatus* hyphae. Based on this novel disease model, the advanced automated image analysis of 3-dimensional confocal microscopy data allowed us to visualize and quantify numerous parameters of hyphal growth, including length and branching levels. Such a detailed study has not been conducted anywhere else before.

Results:
The addition of human macrophages to the biochip partially inhibited the growth of the fungus (indicated by reduced hyphal length and branching) and facilitated the production of proinflammatory cytokines and chemokines (IL-6, IL-8, and MCP-1) compared to the model without macrophages. The perfusion of isolated healthy human leukocytes reduced the hyphal growth even further, indicating (in accordance with previous studies) the important contribution of infiltrating white blood cells to fungal clearance.

Conclusion:
The development of this versatile "invasive aspergillosis on-a-chip" system is very promising due to its potential contribution to the understanding of pathogenicity and pathophysiology in invasive aspergillosis. The model can be modified to mimic the physiological conditions in immuno-compromised patients, whilst these chips may also provide a much-needed tool for animal-free drug screening.

***NOTE*: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.**

46 SINGLE CELL APPROACHES REVEAL THE KEY DENDRITIC CELL SUBSETS THAT COORDINATE ALLERGIC AIRWAY INFLAMMATION AGAINST THE FUNGAL ALLERGEN *ASPERGILLUS FUMIGATUS*

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Purpose:
A. fumigatus (*Af*) spores are abundant in the environment and are a major cause of asthma, but the role of dendritic cells (DCs) in mediating allergic inflammation following fungal exposure is poorly understood. Furthermore, it has become clear that asthma is not just a type-2 eosinophil dominated disorder but is a broad disease with involvement of type-17 related neutrophilia. However, the specific DCs that dictate the character of the anti-Af response, and the mechanisms they utilise, are currently unknown.

Methods/Results:
We have found that repeated exposure of mice to live Af spores induces simultaneous eosinophilic/type-2 and neutrophilic/type-17 allergic inflammation in the airways, in which $CD4^+$ T cells and γδ T cells, not innate lymphoid cells, are the dominant cytokine source. Additionally, targeted depletion revealed this response was completely dependent on the action of DC induction of $CD4^+$ T cells. To identify the DC populations that mediate type-2 vs type-17 anti-*Af* allergic inflammation we performed single cell RNA sequencing of DCs from the lungs of mice exposed to *Af* spores, which revealed putative subsets essential for the induction of these responses. Ongoing work, including the use of mass cytometry, is establishing the mechanism(s) these identified DC subsets employ to direct anti-*Af* allergic responses. Furthermore, to confirm whether these murine results translate to human disease, we are profiling DC subsets and their activation status in the sputum of fungal asthma patients.

Conclusion:
Together, this work will provide a new level of understanding of the cellular networks that promote allergic responses in the airway while also defining key pathways in, and promising therapeutic targets for, pulmonary fungal disease.

NOTE: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.

47 MYCOBACTERIAL MODULATION OF MACROPHAGES RESPONSE TO *ASPERGILLUS FUMIGATUS*

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Purpose:
Chronic pulmonary aspergillosis (CPA) is a severe invasive pulmonary fungal infection with a high 5-year mortality rate. Susceptibility to CPA is strongly linked to a history of bullius emphysema, steroid usage, sarcoidosis and cytokine deficiencies . Additionally, history of pulmonary tuberculosis is recognised as a risk factor for developing CPA; however, the immunological basis for this has not been studied. We aimed to investigate the effect of the main mycobacterial virulent factor, lipoarabinomannan (LAM), on the macrophages' performance against *Aspergillus fumigatus* (AF).

Method:
Macrophage murine cell line J774A.1 were cultured and activated with IFN-γ the day before the experimental treatment. Macrophages were treated for two hours with purified LAM from *Mycobacterium smegmatis* prior infection with *Aspergillus fumigatus*. Fungal conidia from CEA-10 and eGFP strains were pre-swollen for 3 hours at 37 C. Cell death was assessed through propidium iodide (necrosis) and DEVD-amc (apoptosis) fluorescence in sequential readings using a TECAN M200 plate reader over 9 hours. TNF-α and IL-1β were measured by ELISA at 6, 12 and 18hpi. CFUs were performed to determine conidia killing capacity. J774A.1 macrophages were infected for 3 hours and lysed. Culture plates with Saboraud agar were seeded with lysed cells in serial dilutions and incubated at 37 C for 24 hours. Phagolysosome activity was assessed with fluorescence microscopy.

Results:
Cells treated with 500ng/mL of LAM showed 9.9% more cell death than untreated controls at 9hpi (34.76% vs 24.79%, $p<0.01$). This difference was not seen in macrophages treated with 100ng/mL. Uninfected cells treated with LAM, did not show statistical differences with uninfected untreated controls. Apoptosis, measured as DEVD-amc fluorescence, did not show significant differences between conditions. No statistical differences were appreciated in secretion of TNF-α, but macrophages treated with LAM had a significant increase of IL-1β at 12hpi, 73.05ng/mL in untreated cells against 211.4ng/mL for LAM at 100ng/mL ($p=0.015$) and 524.6ng/mL for LAM at 500ng/mL ($p<0.01$). Differences persisted at 18hpi, with 60.79ng/mL for untreated cells against 116ng/mL for LAM at 100ng/mL ($p=0.014$) and 374.2ng/mL for LAM at 500ng/mL ($p<0.01$) . Macrophages treated with 500ng/mL of LAM also showed significant higher number CFU (147×10^3 CFU/mL) against untreated cells (129×10^3 CFU/mL) ($p<0.01$) than untreated cells. Finally, lysotracker fluorescence was persistently lower in LAM treated cells for 6hpi.

Conclusion:
J774A.1 macrophages treated with LAM from *Mycobacterium smegmatis*, show an abnormal response against *Aspergillus fumigatus*, characterised by increased necrotic cell death and increased IL-1β secretion.

48 RECOGNITION OF *ASPERGILLUS FUMIGATUS* GALACTOMANNAN BY THE C-TYPE LECTIN RECEPTOR DECTIN-2

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Purpose:
The fungal cell wall is a complex and dynamic structure that can change in composition and structure based upon the morphological stage of fungi, growth conditions, and upon binding and engagement with immune cells. Dissecting the role of individual carbohydrates and identification of interacting proteins is challenging due to the complex nature of the fungal cell wall and limitations of soluble carbohydrates and fungal genetic mutants. Galactomannan is a carbohydrate found in the cell wall of *Aspergillus fumigatus* and also shed into the extracellular milieu where it is can be identified in tissues of infected patients. Galactomannan is a branched carbohydrate consisting of a linear backbone of α-linked mannose with branches of 4-5 β-linked galactofuranose residues. The mechanism by which galactomannan is recognized by macrophages is incompletely understood. Our aim was to identify galactomannan binding partners in macrophages.

Methods:
To identify potential galactomannan binding partners, we generated fungal-like particles (FLP) that consist of polystyrene beads coated with purified *A. fumigatus* galactomannan. To identify C-type Lectin Receptors (CLRs) capable of recognizing galactomannan, we screened a reporter cell library expressing CLRs and identified Dectin-2 as a potential binding partner for galactomannan.

Results:
We identified Dectin-2 as a potential binding partners for *A. fumigatus* galactomannan. We further confirmed binding of Dectin-2 to galactomannan using soluble Dectin-2 protein. Furthermore, we demonstrated that galactomannan promotes TNFα production in a Dectin-2 dependent manner and stimulated Syk phosphorylation.

Conclusions:
Our results demonstrate that Dectin-2 is a receptor for *A. fumigatus* galactomannan.

49 UNCOUPLING OF CYTOKINE SIGNALING AND LC3 ASSOCIATED PHAGOCYTOSIS (LAP) DRIVES THE DEVELOPMENT OF INVASIVE ASPERGILLOSIS IN PATIENTS WITH SEPSIS

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Purpose:
Aspergillus fumigatus is an opportunistic pathogen in a broad range of immunocompromised patients with defects in number or function of phagocytes. In particular, Invasive Aspergillosis (IA) is an emerging infection in critically ill patients recovering from sepsis in the ICU. Defects in cytokine production (e.g., IL-6) by professional phagocytes have been associated with sepsis induced immunoparalysis and increased susceptibility to opportunistic pathogens, including *Aspergillus*. However, the underlying molecular mechanisms that result in compromised microbicidal activity of phagocytes during sepsis remain uncharacterized. A non-canonical autophagy pathway termed LC3 associated phagocytosis regulates phagolysosomal fusion and confers protective immunity against *Aspergillus*. In view of the master regulatory role of LAP in phagocyte antifungal immune effector functions, we hypothesized that defective activation of LAP is implicated in sepsis immunosuppression and related susceptibility to IA. Furthermore, we explored molecular mechanisms of cytokine signaling cross talk with LAP that are inhibited in sepsis.

Methods:
In a prospective study we evaluated LAP in 38 consecutive adult patients admitted in the ICU of two University Hospitals with community-acquired septic shock. Monocytes isolated on the day of patient admission (day 1) and upon recovery from the infectious episode (day 7) were stimulated with conidia of *Aspergillus fumigatus.* Cytokines production was measured in culture supernatants by enzyme immunoassay (ELISA). LAP activation and other phagosome responses during fungal infection of monocytes were assessed by confocal microscopy. Functional studies were also performed in a physiologically relevant mouse model of peritonitis (CLP) with different degree of sepsis severity upon re-infection with *Aspergillus* by using GFP-LC3 mice, and in Atg5 knockout and IL-6 knockout mice.

Results:
We identified a distinct group of patients (n=11, 29%) with defective activation of LAP in monocytes upon sepsis recovery. Defective activation of LAP was associated with impaired phagolysosomal fusion and killing of *Aspergillus* conidia, cytokine hypo-responsiveness (suppressed IL-6 production), and all hallmark clinical features of sepsis-induced immune deactivation. Studies in the CLP model of polymicrobial sepsis mirrored the findings of human studies by demonstrating selective inhibition of LAP in macrophages of mice recovering from severe sepsis and resulting increased susceptibility to *Aspergillus* infection. Because of the striking correlation of LAP defects with impaired IL-6 production we explored a direct regulatory role of IL-6 signaling on LAP. Importantly, studies in macrophages from IL-6 KO mice demonstrated LAP blockade, broad phagosome biogenesis defects and defective killing of *Aspergillus*. Of interest, IL-6 supplementation restored LAP defects and immune deactivation in sepsis monocytes/macrophages.

Conclusion:
Our study uncovers a molecular mechanism of sepsis-induced immunosuppression leading to increased susceptibility for IA. In addition, our results provide a novel mechanistic link between defects in molecular pathways regulating IL-6 signaling and phagosome biogenesis in sepsis immunoparalysis.

<u>NOTE</u>: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.

50 HUMAN MAIT CELLS AS A NEW PLAYER IN THE IMMUNE RESPONSE AGAINST FILAMENTOUS FUNGI

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Purpose:
Mucosal associated invariant T cells (MAIT cells) are innate-like T cells activated by riboflavin metabolites, which are presented by the MHC class I-related gene protein (MR1) on the cell surface of antigen presenting cells. MAIT cells respond to a broad spectrum of microbes including bacteria and viruses. However, activation of MAIT cells by pathogenic moulds is, yet, only rarely described.

Methods:
Peripheral blood mononuclear cells (PBMC) were stimulated by conidia of *Aspergillus* species, including *A. fumigatus*, *A. flavus*, *A. nidulans* and *A. terreus* or by sporangiospores of *Mucorales* species, including *Lichtheimia corymbifera*, *Mucor circinelloides*, *Rhizopus arrhizus* or *Rhizopus microsporus*. MAIT cell activation was measured by flow cytometry.

Results:
After 4 h of coincubation, the amount of $CD69^+$MAIT cells was increased, Degranulation of MAIT cells was indicated by higher number of $CD107a^+$cells and a decreased percentage of MAIT cells storing granzyme A and perforin intracellularly. After 24 h, the amount of MAIT cells expressing CD25 and granzyme B was higher in the stimulated samples than in the resting control. By blocking MR1, the antifungal MAIT cell response was abolished indicating a T cell receptor (TCR) dependent activation pathway. Interestingly, TCR down-regulation was only observed after a 4 h stimulation by *Aspergillus* species, but not by *Mucorales*. The extent of MAIT cell activation differed between the tested species. By analyzing the phagocytosis rate of fungal spores, a correlation between the number of engulfing monocytes and activated MAIT cells was demonstrated for *Aspergillus*, but not *Mucorales* species.

Conclusion:
Taking together, MAIT cells are activated in a MR1-dependenent manner by spores of different *Aspergillus* and *Mucorales* species.These findings reveal MAIT cells as an interesting new player in antifungal immunity especially in hematological patients with highly reduced numbers of granulocytes and monocytes, the first line defense against moulds.

51 VACCINE-INDUCED IMMUNOGENICITY AND PROTECTION IN A MURINE MODEL OF INVASIVE PULMONARY ASPERGILLOSIS

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Purpose:
Infection with the opportunistic fungal pathogen *Aspergillus fumigatus* causes severe invasive pulmonary disease in immunocompromised individuals. Those at risk of *Aspergillus* infection on immunosuppressive therapies due to transplantation, cancer, or autoimmune disease and the rise in use of chemotherapy and immunosuppressive agents have increased the incidence of invasive pulmonary aspergillosis (IPA). Antifungal treatment is not always successful as the mortality rate of IPA continues to exceed 50% in neutropenic patients. Furthermore, treatment does not prevent future infection in continually susceptible patients, and there are no clinically-approved vaccines to prevent opportunistic fungal infections. Therefore, it is imperative to explore vaccine candidates that are more effective against IPA. Our purpose was to evaluate the recombinant protein AF.KEX1 as a potential vaccine candidate to prevent IPA.

Methods:
We have previously reported that a recombinant protein KEX1 from another opportunistic fungal pathogen *Pneumocystis jirovecii* induces protective immunity against the development of *Pneumocystis* pneumonia in a non-human primate model of HIV-induced immunosuppression. This KEX1 region is present and highly conserved in *Aspergillus fumigatus* and we then generated a 90 amino acid recombinant protein AF.KEX1. In this study, we tested the immunogenicity of an AF.KEX1 and TiterMax immunization in healthy CF-1 mice, as well as the protective nature of these responses after cortisone acetate-induced immunosuppression and *A. fumigatus* challenge.

Results:
We observed that vaccination with KEX1 generated a robust antibody response that significantly reduced *A. fumigatus*-induced mortality and lung fungal burden in a murine model of IPA. Furthermore, the lung fungal burden was inversely correlated with the peak antibody titer achieved following vaccination.

Conclusion:
These results present AF.KEX1 as a new vaccine candidate to prevent IPA in drug-induced immunosuppression.

***NOTE*: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.**

52 IMMUNOMODULATORY PROPERTIES OF ANTIFUNGALS ON HUMAN MONOCYTES: AN EXPLORATORY STUDY

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Purpose:
Across the various classes of antifungals used against *Aspergillus fumigatus*, differences regarding *in vitro* and *in vivo* activities have been observed, leading to varied clinical responses in patients. Aside from direct antifungal effects, immunomodulatory properties of antifungals may account for these variations in clinical responses. We therefore sought to explore the effect of different antifungals on the gene expression of major components of innate and adoptive immunity in human phagocytes.

Methods:
Human THP1 monocytic cells (ATCC® TIB-202™) were cultivated in liquid medium. *Aspergillus fumigatus* (strain ATCC® 13073TM; NIH 5233) was cultivated, conidia were retrieved, then incubated in RPMI/Fetal Bovine Serum 10%, at 37°C to obtain germed conidia. Co-incubation of THP1 with *A. fumigatus* was performed at a ratio of 10 germed conidia/1 monocyte, 10^6 monocytes per well, in duplicates, for 6 hours at 37°C. At the end of co-incubation, total cellular RNA was extracted then converted into cDNA. Multiplex RT PCR was performed using the RT² Profiler™ PCR Array, according to manufacturer's instructions. This array evaluates the transcription of 84 genes involved in human antifungal immunity, notably pattern recognition receptors, Dectin-1, NF-κB, TLR, complement, and NLR signaling pathways, as well as major cytokines, chemokines and phagocytosis. Tested conditions were THP1 cells and THP1 stimulated with *A. fumigatus*.

Results:
All samples passed the quality checks regarding PCR array reproducibility, reverse transcription efficiency, and genomic DNA contamination. Differential analysis of transcriptomic landscape revealed that a majority of immune-related genes were down regulated upon 6 hours of *Aspergillus* infection. Using a threshold of 2-fold, transcription was increased, stable and decreased for 12, 14, and 58 genes, respectively. The five most up-regulated genes were CCL20 (125.84 fold), CXCL8 (35.43 fold), thromboplastin (9.22 fold), IL1B (8.17 fold), CXCL1 (7.7 fold). The five most down-regulated genes were IL12A (-246.82 fold), CARD9 (-35.23 fold), PYCARD (-25.85 fold), TIRAP (-24.78 fold), and CLEC7A (-24.78 fold). A graphical representation of the differential expression of the evaluated genes is provided in figure 1 and table 1.

Conclusion:
These preliminary results confirm the technical feasibility of this *in vitro* evaluation. After 6 hours of co-incubation, the majority of up-regulated genes belonged to pro inflammatory chemokines and cytokines. Interestingly, the majority of innate immune pathway related to anti-*Aspergillus* defense (TLR, dectin 1 signaling, NOD receptors) appeared down-regulated, owing to their earlier activation during antifungal response. Ongoing experiments are evaluating, through an identical methodology, the monocytic transcriptional response under the influence of antifungals (amphotericin B deoxycholate, liposomal amphotericin B, voriconazole, and caspofungin) at clinically relevant concentrations. This will pave the way towards a better understanding of the variation in the efficacy of antifungals.

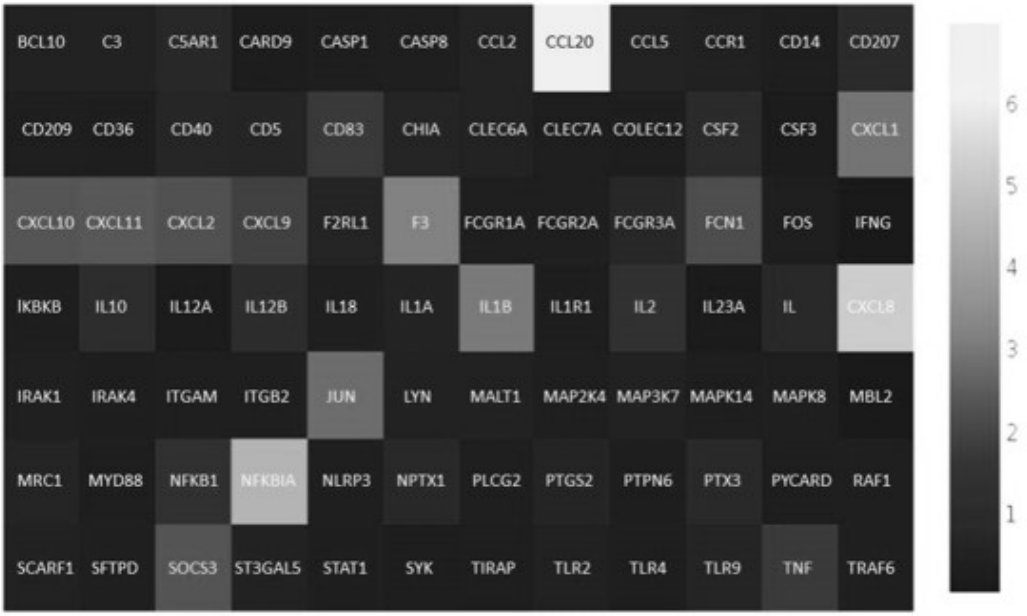

Figure 1: Graphical representation (heatmap) of the transcriptional intensity of the 84 evaluated genes in THP1 cells stimulated with *A. fumigatus*, in comparison with unstimulated cells

53 MODELLING EPITHELIAL CELL – MACROPHAGE COMMUNICATION IN FUNGAL ALLERGY

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Purpose:
Bronchial colonisation by *Aspergillus fumigatus* in patients with asthma and cystic fibrosis drives the development of allergic bronchopulmonary aspergillosis (ABPA). Although the disease is not usually fatal, the global burden might exceed 10 million people. In a study aiming to identify genetic susceptibility factors to ABPA amongst asthmatic individuals, we discovered patients with ABPA have a hotspot for polymorphisms in the IL17RA. The aim of to define the impacts of IL17RA deficiency in the early concerted response of epithelial cells and macrophages against *A. fumigatus*.

Methods:
16HBE bronchial epithelial cells and THP1 monocytes deficient in IL17RA were generated using the all-in-one CRISPR/Cas9 system from Santa Cruz. To study the interplay between epithelial cells and macrophages, a co-culture system was developed. This system allows cell types to be self-contained but permits transmission of secreted signals between cells. Matched pairs of 16HBE/THP1 or $16HBE^{IL17RA-/-}$/$THP1^{IL17RA-/-}$ were challenged with *A. fumigatus* spores for 16 h. Differentially regulated genes were identified for individual cell-type responses and for interactions using RNA-seq. Validation of the hits was performed using data from a previous transcriptomic experiment in our laboratory using macrophages samples from patients with ABPA and healthy controls.

Results:
Transcriptional analyses of the concerted response of epithelial cells and macrophages in response to *A. fumigatus* was unidirectional. Therefore, epithelial cell signalling increases the transcription of genes involved in controlling the inflammatory response of macrophages against *A. fumigatus* while macrophage signalling did not alter the transcriptomic response of epithelial cells. Remarkably, IL17RA deficiency led to an upregulation of cytokine expression by IL17RA while inhibiting interferon signalling in macrophages co-cultured with epithelial cells ($P < 10^{-12}$). Additionally, the transcriptomic changes associated with the anti-*Aspergillus* response in macrophages deficient in IL17RA significantly correlated with that seen in patients with ABPA ($P < 0.0001$).

Conclusion:
(i) Epithelia signalling is required for interferon pathway activation in macrophages; (ii) IL17RA deficiency increases the expression of cytokine encoding genes by IL17A and IL17F; (iii) Transcriptional response of primary cells from patients with ABPA strongly correlates with the observed phenotypes in the co-culture system of diseased epithelial cells and macrophages.

54 ANTIFUNGAL LIPOSOMES TARGETED TO FUNGAL CELLS HAVE DRAMATICALLY INCREASED EFFICACY

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Purpose:
Candida albicans, Cryptococcus neoformans, and Aspergillus fumigatus cause life-threatening candidiasis, cryptococcosis and aspergillosis, resulting in several hundred thousand deaths annually and several billions of dollars in medical costs annually. Patients at the greatest risk of developing these life-threatening invasive fungal infections have weakened immune systems. The vulnerable population is increasing due to rising numbers of immunocompromised individuals. While patients are treated with antifungals such as Amphotericin B (AmB), all antifungals have serious limitations due to lack of sufficient fungicidal effect and host toxicity. Even with treatment, one-year survival rates for patients with these diseases are low. Very few new antifungal drugs have gained acceptance in the last two decades. **The purpose or our research team's effort was to develop a pan-antifungal drug delivery system that would target drugs specifically to the surface of pathogenic fungal cells and increase the efficacy of any existing or new antifungal agent.**

Methods:
First, we designed new simplified protein chemical methods that allowed us to easily and inexpensively manipulate the relatively insoluble carbohydrate recognition domains of Dectins. Dectins are a subclass of C-type lectin receptors expressed on dendritic cells that signal fungal infection. Second, we developed methods to rapidly construct small batches of drug loaded liposomes and coating them with different Dectins proteins that enabled reiterative testing of novel liposomal constructs. Third, we constructed AmB-loaded 100-nanometer diameter liposomes coated with 1,500 Dectin monomers and 3,000 rhodamine molecules. The Dectin monomers were engineered to float in the liposome membrane such that they were conformationally free to form the dimers necessary for tight binding to their target polysaccharides. The rhodamine tag allowed us to monitor binding to the fungal cell wall and exopolysaccharide matrix microscopically and to quantify the relative levels of binding.

Results:
Dectin-coated AmB-loaded liposomes, DEC-AmB-LLs, bound to the surface of *C. albicans, C. neoformans, and A. fumigatus* cells and their exopolysaccharide matrices more than 100-fold more efficiently than an untargeted AmBisome® equivalent, AmB-LLs. Binding was specific for two different classes of target polysaccharides. Binding was rapid and essentially irreversible, perhaps due to the high avidity provided by multiple Dectins on the surface of each liposome binding cooperatively to fungal cells. **DEC-AmB-LLs liposomes inhibited or killed *C. albicans, C. neoformans, and A. fumigatus* 10-fold to 100-fold more efficiently than AmBisome®-likeAmB-LLs delivering the same concentration of Amphotericin B, and when delivering AmB concentrations near the minimum inhibitory concentrations for AmBisome® reported for these three species.** In short, we have reduced the effective dose for inhibition and killing of all three species more than 10-fold.

Conclusion:
By decreasing the effective dose of an antifungal drug, we should reduce the fungal burden in various host organs at drug concentrations that have reduced host toxicity. This targeting technology has the potential to increase the efficacy of all antifungal drugs against nearly all fungal pathogens and produce a paradigm shift in antifungal therapies. The technology should also work against protozoan parasites. Our immediate future efforts focus on examining pan-antifungal targeted liposomal drugs in mouse models of candidiasis, cryptococcosis, and aspergillosis.

55 WHY ARE MUCORMYCETES RESISTANT AGAINST VORICONAZOLE?

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Purpose:
Mucormycetes are intrinsically resistant to the short-tailed azole voriconazole (VCZ) but susceptible to the long-tailed azole posaconazole (PCZ). We used the high resolution X-ray crystal structure of *Saccharomyces cerevisiae* lanosterol 14α-demethylase (LDM) in complex with VCZ to postulate that the amino acid substitutions Y129F and V291A in the *Rhizopus arrhizus* LDM F5 isoform (RaLDMF5) are associated with the intrinsic resistance. Our aim was to experimentally test this hypothesis.

Methods:
Full-length recombinant C-terminal hexahistidine-tagged RaLDMF1 and RaLDMF5 were overexpressed from the *PDR5* locus in a *S. cerevisiae* host lacking 7 drug efflux pumps. Their cognate NADPH-cytochrome P450 reductase (RaNCP) was overexpressed from the *PDR15* locus. Phenotypic and biochemical analysis were used to determine structural and functional features of RaLDMF1 and RaLDMF5, with and without co-expression of RaNCP. Revertant strains were generated in the RaNCP1 background by substituting with RaLDMF5 F129Y or A291V individually and in combination.

Results:
RaLDMF1 and F5 were functionally overexpressed in *S. cerevisiae*. RaLDMF5 appeared to be the major contributor to VCZ resistance, with its expression resulting in a 32x higher VCZ minimal inhibitory concentration (MIC) than the recipient strain. Co-expression of RaLDMF5 with RaNCP increased the VCZ MIC 4-fold compared with RaLDMF5 expression alone. MICs for long-tailed azoles such as PCZ were essentially unaffected by the RaLDMF1 or RaLDMF5 overexpression. *S cerevisiae* co-expressing RaLDMF1 with NCP had MIC values for VCZ and PCZ similar to the recipient strain. The azole-susceptibility patterns of *S. cerevisiae* co-expressing RaLDMF5 and RaNCP reflected those of *R. arrhizus*. Experiments using revertants of RaLDMF5 showed that both the Y129F and V291A substitutions are required for full conferral of VCZ resistance.

Conclusion:
The *R. arrhizus* LDMF5 isoform dominates the conferral of resistance to short-tailed azoles. The combination of Y129F and V219A substitutions is needed to fully confer VCZ resistance. Biochemical and structural analysis of the purified protein are expected to provide deeper understanding of the observed phenotypes.

***NOTE*: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.**

56 TRANSCRIPTION FACTOR HSF1 DECREASES THE EXPRESSION OF SURFACTANT PROTEIN-D IN CELLS INFECTED WITH *ASPERGILLUS FUMIGATUS*

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Purpose:
Aspergillosis is a life-threatening disease in patients with compromised immune systems. Fungal invasion processes are a highly critical step during host cellular infection. Previously, we suggested that surfactant protein-D (SP-D) is a protein suppressed by *A. fumigatus* conidia directly. We investigate what transcription factor and where the promoter region of SP-D is involved in the infection process of conidia-treated cells.

Methods:
To investigate the promoter activity of SP-D in fungal infected cells, we cloned the different lengths of the promoter region (-1000 to +1) of SP-D and examined promoter activity in *A. fumigatus* conidia-treated A549 cells. Then, we assayed the activity of the promoter region of surfactant protein-D shows a response to conidia treatment. And then, we predicted what transcription factor is binding and what binding sites in the promoter regions of SP-D was involved using AliBaba 2.1 software. Using decoy assay, we confirmed the transcription factor is bind to the selected region directly.

Results:
Previously, we showed that the promoter activity of SP-D is down-regulated to the conidia treatment. Promoter region from -200 to -100 bp of SP-D is the important region to response in the treatment of conidia. We refine the map to elucidate the core region (-150/-126) involved in mediating the conidia-induced SP-D promoter activity. Using AliBaba2.1 software, we select some candidate transcription factors in the promoter region of SP-D that responds to infection of conidia. And, we know the transcription factor HSF1 is located in the response region (-142 to -134 bp). The decoy assay result shows that transcription factor HSF1 is sufficient to decrease the expression of the SP-D gene.

Conclusion:
We show that HSF1 as a negative regulator binds the promoter region of SP-D in conidia treated cells. We confirm that HSF1 suppresses the expression of SP-D in conidia-infected cells using decoy assay. We suggest that inhibition of HSF1 binding to the promoter region of SP-D is an important step to inhibit the infection of conidia.

57 *ASPERGILLUS* SECTION *FUMIGATI* MOLDS: A MODEL LINEAGE FOR STUDYING THE REPEATED EVOLUTION OF FUNGAL PATHOGENICITY

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Purpose:
Approximately 70% of all *Aspergillus* infections are caused by *A. fumigatus*, whereas the remaining 30% stem from other species in the genus. Some of these other pathogens are closely related to *A. fumigatus* and also belong in the taxonomic section *Fumigati*. However, the majority of species in section *Fumigati* are not pathogenic, and we know surprisingly little about the evolution of pathogenicity in section *Fumigati.*

Methods:
To study the evolution of pathogenicity in section *Fumigati*, we have taken a multi-pronged approach that utilizes comparative genomics, *in vitro* phenotyping, chemistry, and multiple disease models of aspergillosis.

Results:
Our examination of the distribution of pathogenic species in our newly-constructed, genome-scale phylogeny of section *Fumigati* revealed that *A. fumigatus* pathogenicity likely evolved after the species diverged from *A. oerlinghausenensis* and *A. fischeri*, the two non-pathogenic species that are most closely related to *A. fumigatus*. Interestingly, we found that this was also the case for other pathogenic species (ex. *A. udagawae* and *A. thermomutatus*), suggesting that pathogenicity independently arose multiple times in section *Fumigati*.

To further investigate one of these pathogenicity events, we compared *A. fischeri* and *A. fumigatus* for traits important for human disease. We discovered that *A. fischeri* was less virulent than *A. fumigatus* in a mouse model of disease, less tolerant than *A. fumigatus* to human body temperature and oxidative stress, and produced both unique and shared secondary metabolites. Genomic analyses of *A. fischeri*, *A. fumigatus*, and other section *Fumigati* species showed that most of the *A. fumigatus* genes known to be associated with virulence are highly conserved, including in non-pathogens like *A. fischeri*.

To determine if a genetic determinant of virulence in *A. fumigatus* that is conserved throughout section *Fumigati* functions similarly in *A. fischeri*, we deleted *laeA*, a master regulator of secondary metabolism. The wild type strain of *A. fischeri* was less pathogenic than *A. fumigatus* in a *Galleria mellonella* larval model of disease, but the pathogenicity of the D*laeA A. fischeri* strain was indistinguishable from wild type *A. fischeri*. This is a striking finding considering the conserved role we discovered *laeA* plays in the regulation of secondary metabolism in *A. fischeri* and the published evidence connecting secondary metabolism and *laeA* to pathogenicity in *A. fumigatus*.

Finally, we examined the evolutionary rates of genes like *laeA* that are found both in pathogenic and non-pathogenic species. We discovered that genes in highly pathogenic species exhibited higher rates of evolution than their homologs in species with medium levels of pathogenicity, which in turn evolved faster than their copies in lowly (or non-) pathogenic species. We hypothesize that the faster evolutionary rates in pathogenic species have facilitated their ability to adapt to the challenging environment of the human host.

Conclusion:
Our results show that pathogenicity in section *Fumigati* has independently evolved multiple times, reveal that traits (but few genes) associated with virulence in *A. fumigatus* differ across species in section *Fumigati*, and establish a broad framework for studying how major fungal pathogens evolve from historically innocuous organisms.

58 MONITORING OF MUCORMYCOSIS AND DRUG EFFICACY TESTING BY THE USE OF *M. CIRCINELLOIDES* REPORTER STRAINS

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Purpose:
Invasive infections caused by mucormycetes are increasingly seen in the clinics and are still associated with unacceptable high mortality rates. Still, little is known about the biology of the pathogens, the establishment and progression of the infection, antifungal resistance mechanisms and successful therapy. Therefore, we aimed to generate a tool for (1) alternative methods of drug testing *in vitro*, (2) non-invasive monitoring of the infection in different model hosts, and (3) visualization of antifungal efficacy.

Methods:
Firefly luciferase, both mammalian or codon-optimized without the peroxisomal target sequence was cloned in the pMAT1477 vector under the control of different promoters. Linear plasmid was used to transfect *M. circinelloides* protoplasts of auxotrophic strains. Positive transformants were checked for gene integration and then light emission measured under various conditions. Selected strains were used to determine antifungal susceptibility, virulence potential and *in vivo* monitoring of mucormycosis in *Galleria mellonella*.

Results:
Firefly luciferase was successfully expressed in *M. circinelloides* with a single integration and light emission detected by imaging and luminometer. Codon optimization was critical to enhance light emission, making these strains usable for allow real-time, non-invasive infection monitoring in insect and murine models, and the testing of antifungal efficacy by means other than survival. Phenotype, virulence potential in *G. mellonella* and antifungal susceptibility are indifferent to the wild-type strains.

Conclusion:
The construction and further optimization of bioluminescent *Mucor* strains allows for the visualization of temporal and spatial progression of infection by a non-invasive method in insect and murine models, and the testing of antifungal efficacy by other means than survival only. This will give valuable new insights in the pathogenesis of Mucorales infections.

59 THE NEGATIVE COFACTOR 2 COMPLEX IS A MASTER REGULATOR OF DRUG RESISTANCE IN *ASPERGILLUS FUMIGATUS*

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Purpose:
Over 3 million people suffer annually from invasive or chronic infections caused by *Aspergillus fumigatus* leading to more than 600,000 deaths every year. Very few drugs are available to treat the various forms of aspergillosis and therapy relies predominantly upon the azole class of agents. The escalating frequency of azole resistance is therefore a critical concern. However, our understanding of the factors governing azole resistance in *A. fumigatus* is not fully defined. In the present study we have investigated a whole transcription factor knockout library of *A. fumigatus* with the aim of identifying transcriptional regulators, which have the potential to drive clinically relevant drug resistance phenotype.

Methods:
A genome-wide screen of the transcription factor null library was carried out in the presence of itraconazole to identify key regulators associated with azole resistance and sensitivity. Detailed molecular biological, immunological, and pathlogical analyses were conducted to reveal the role of two CBF/NF-Y family transcriptional regulators AFUB_029870 (NctA) and AFUB_045980 (NctB).

Results:
Our screening of the transcription factor null mutant library identified a cohort of 12 transcription factors that govern azole resistance and sensitivity in *A. fumigatus*. Here we report in detail the role of two CBF/NF-y family transcription regulators NctA and NctB in azole resistance in *A. fumigatus*. We revealed that NctA and NctB are the orthologues of BUR6 and NctB in *Saccharomyces cerevisiae* that form a heterodimeric regulatory complex called as the Negative Cofactor 2 (NC2). We demonstrated that loss of the NCT complex leads to a multi-drug resistance phenotype including the azoles (itraconazole, voriconazole and posaconazole) as well as the salvage therapeutic amphotericin B and terbinafine. To characterise the molecular basis of the drug resistance mechanisms mediated by the NCT complex, we performed functional genomics analyses using RNA-seq and ChIP-seq. Through these analyses, we revealed that the NCT complex act as a key regulator of ergosterol biosynthesis and the azole exporter CDR1B. Furthermore, loss of this complex results in a notable increase in the immunogenic properties of *A. fumigatus* but does not result in loss of virulence.

Conclusion:
Using the transcription factor null mutant library of *A. fumigatus*, we have identified the network of regulators governing azole resistance. The results of our study has highlighted that loss of function of a single gene can drive both high-level pan-azole resistance and cross-resistance to the salvage therapeutic amphotericin B and terbinafine without significantly impacting virulence.

***NOTE*: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.**

60 THE PROTEIN KINASE A-DEPENDENT PHOSPHOPROTEOME OF *ASPERGILLUS FUMIGATUS* REVEALS NOVEL KINASE TARGETS ASSOCIATED WITH DIVERSE AND ESSENTIAL CELLULAR PATHWAYS

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Purpose:
Invasive aspergillosis, caused by the filamentous fungus *Aspergillus fumigatus,* is a leading infectious cause of death in immunocompromised patients. Despite this, the specific molecular effectors responsible for virulence are poorly understood, underscoring a significant barrier to the development of better therapeutic strategies. Protein kinase A (PKA) signaling is critical for the growth and virulence of *A. fumigatus*. Elucidation of the myriad processes regulated by the PKA signaling network will significantly advance a mechanistic understanding of overall fungal pathogenesis. Although PKA signaling has been well studied in the model and pathogenic yeasts with respect to nutrient sensing, its role in the mechanics of filamentous fungal growth, tissue invasion, and pathogenesis are largely disparate. Even in well studied systems, very few direct PKA targets have been identified. Therefore, there is a compelling need to define the yet unknown underlying fungal-specific PKA-dependent effectors regulating growth and virulence in *A. fumigatus*.

Methods:
We utilized liquid chromatography-tandem mass spectroscopy (LC-MS/MS) to interrogate the PKA-dependent phosphoproteome of *A. fumigatus* in order to identify essential components of the PKA signaling network. Comparison of open protein levels between a wild-type and PKA deletion strain of the fungus revealed overall proteomic shifts, while titanium oxide (TiO_2)-enriched LC-MS/MS analysis of total protein extracts led to the identification of more than 1,000 phosphorylated proteins, and enrichment with PKA target motif-specific antibodies was used to detect potential direct PKA targets. Several of the most promising direct target candidate proteins were selected for further investigation into their roles in fungal physiology via gene deletion and site-directed mutagenesis in association with phenotypic testing, murine virulence assays, and *in silico* structural modeling.

Results:
Comparative quantification of the differential total proteomes of wild-type and PKA-deficient *A. fumigatus* strains revealed significant PKA-dependent shifts in the levels of more than 1,500 proteins enriched for functional categories of protein synthesis, autophagy, transcription, aerobic respiration, and other essential cellular pathways. Analysis of TiO_2 and PKA target motif antibody-enriched phosphoproteins enabled identification of 127 strong PKA target protein candidates with roles in diverse cellular processes. Genetic manipulation of prioritized candidates identified three potential direct PKA targets, Atg24, HapB and Not4, that each play essential roles in the growth and development of *A. fumigatu*s. The putative mitophagy-associated sorting nexin Atg24 was found to be required for both oxidative and cell wall stress tolerance, and PKA-dependent phosphorylation of Atg24 was demonstrated to regulate the response to the cell wall-targeting antifungal agent caspofungin. HapB, a predicted member of the CCAAT-binding transcriptional regulatory complex, was found to play important roles in multiple alternative carbon source utilization pathways. Finally Not4, a putative component of the CCR4-NOT global regulatory complex was identified as a key factor in translational and heat stress tolerance.

Conclusion:
Our phosphoproteomic analysis reveals PKA to be a master regulator of many diverse and essential cellular pathways in *A. fumigatus*, and provides the most comprehensive identification of probable direct PKA targets in a fungal human pathogen to date. Furthermore, we have identified and characterized three novel PKA-regulated proteins with major roles in the growth, development, and stress responses of this important fungus.

61 IDENTIFICATION OF AZOLE RESISTANCE MECHANISMS IN *ASPERGILLUS LENTULUS*

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Purpose:
The number of cases of invasive aspergillosis due to cryptic *Aspergillus spp.* has increased in previous years. However, the true prevalence of these species is unknown due to misidentification or lack of species-level accurate identification. *Aspergillus lentulus* is an emerging pathogen that is morphologically indistinguishable from *Aspergillus fumigatus* and, unlike the latter, displays intrinsic resistance to amphotericin B and reduced susceptibility to triazoles and caspofungin. Consequently, the mortality rates associated with this opportunistic pathogen are high, despite antifungal treatment. The objective of this study was to investigate molecular mechanisms responsible of azole resistance in a collection of clinical isolates from the USA.

Methods:
Antifungal susceptibility to itraconazole, isavuconazole, posaconazole and voriconazole was determined for 26 *A. lentulus* clinical isolates after 48 hours at 35 °C in RPMI, according to CLSI standards. For all isolates, the full coding sequence of *cyp51B* and *hmg1*, and the coding sequence plus 700 bp of the promoter region of *cyp51A* were amplified, sequenced and compared to *A. lentulus* strain IFM 54703, the genome of which has been recently sequenced, as well as to the *A. fumigatus* Af293 laboratory strain. In addition, we employed RT-qPCR to quantify the relative expression of *cyp51A* and *cyp51B*, as well as of the ABC transporter *cdr4*, homologous to the AbcC efflux pump in *A. fumigatus*, in each of the *A. lentulus* isolates. The expression of each gene was compared to that of *A. lentulus* isolate displaying the lowest MICs.

Results:
Voriconazole MICs were lower or equal to the established ECV, i.e. 1 μg/ml, in 5 out of 26 isolates, while all other strains had higher voriconazole MICs. All the strains that showed low susceptibility to voriconazole displayed cross-resistance to the other azoles tested. Sequencing analyses showed high homology between *cyp51A*, *cyp51B* and *hmg1* between *A. lentulus* and *A. fumigatus*. However, several SNPs were observed. Interestingly, the most susceptible *A. lentulus* isolate contained a SNP that was not observed in any other *A. lentulus* isolate, but was present in a consensus *A. fumigatus cyp51A* sequence. In addition, one of the isolates displaying the highest degree of azole cross-resistance contained a mutation in the predicted sterol sensing domain of *hmg1*. The expression of *cyp51A* among the triazole-resistant isolates ranged from 1.4- to 3.6-fold that of the susceptible comparator isolate. Only two isolates displayed *cyp51A* expression levels statistically higher than those observed in the susceptible control. For *cyp51B* and *cdr4*, no statistical differences were observed between the expression in the triazole resistant isolates and the susceptible control.

Conclusion:
Azole resistance in *A. lentulus* does not appear to be underpinned by overexpression of the triazole target or of the efflux pump *cdr4*. However, *cyp51A* and *hmg1* SNPs might play an important role, causing a reduced affinity to the azole drug or by creating an alternative sterol profile in the *A. lentulus* plasma membrane. Future allele swap experiments between genes from resistant and susceptible strains will be performed to demonstrate the importance of these SNPs in *A. lentulus* triazole resistance.

62 INVESTIGATION OF THE MOLECULAR MECHANISMS OF COPPER HOMEOSTASIS IN *ASPERGILLUS FUMIGATUS*

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Purpose:
Copper (Cu) is an essential metal for all living organisms, although it is toxic in excess. Recently, antimicrobial activities of Cu, e.g., Cu alloy surfaces, are utilized in a hospital to reduce nosocomial infections. Mechanisms of antimicrobial effects caused by Cu have not been fully understood. *Aspergillus fumigatus* is the most important pathogenic fungus among *Aspergillus* species associated with aspergillosis. Despite the importance of Cu as an innate antifungal agent as well as a crucial virulence factor for *A. fumigatus* pathogenesis, the molecular mechanisms of copper-acquisition and -detoxification have not been fully studied yet. To gain more insights into the copper homeostasis, we investigate the roles of copper-dependent transcription factors and adaptations of *A. fumigatus* to high copper levels.

Methods:
We constructed the deletion mutants of three copper-dependent transcription factors, Δ*Afmac1,* ΔaceA, and Δ*cufA* in *A. fumigatus*. Growth assays of Af293 and three deletion mutants were performed in PDA and AMM. We conducted transcriptome analyses under high and low Cu conditions. We generated the laboratory-evolved strains resistant to environmental copper through passaging for 10 generations, and performed growth assays and transcriptome analyses.

Results:
Growth assays suggested that AfMac1 and AceA rather than CufA play important roles as copper-dependent transcription factor in copper homeostasis. The Δ*Afmac1* mutant exhibited not only significantly slower growth but also produced shorter conidia chain and less-pigmented conidia. Transcriptome analyses showed that AfMac1 could activate the expressions of Ctr copper transporter (CtrA1, CtrA2, and CtrC), metalloreductase (Afu8g1310 and Fre7), superoxide dismutase (Sod3) , and copper-exporting ATPase (CrpA) in low copper levels. While, in high copper levels, AceA exerts not only increasing *crpA* expression but also suppressing the gene expressions regulated by AfMac1, such as CtrA1, CtrC, Afu8g1310, and Sod3. These results suggest that AfMac1 and AceA could coordinately regulate the copper-acquisition and -detoxification mechanisms under the copper-deficient and -replete conditions, respectively. We successfully generated the laboratory-evolved strains. By the transcriptome analyses, we identified more than 100 genes whose expressions were significantly increased in all laboratory-evolved strains compared with those in original strains.

Conclusion:
These results suggested that the copper-dependent transcription factors AfMac1 and AceA play a major role in copper homeostasis. Analyses of laboratory-evolved strains also indicate that the fungus was able to evolutionarily adapt to the high-copper condition via remodeling intracellular molecular machinery.

63 *IN VIVO* COMPETITIVE FITNESS PROFILING REVEALS PROTEIN KINASES REQUIRED FOR DRUG TOLERANCE AND ADAPTATION OF *ASPERGILLUS FUMIGATUS* TO THE MURINE HOST ENVIRONMENT

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Purpose:
Our understandings of the factors that drive pathogenicity in *Aspergillus fumigatus* are limited. In this study we provide a functional genomic analysis to describe the role of protein kinases in the pathobiology of *A. fumigatus*.

Methods:
As part of the *A. fumigatus* genome-wide knockout program (COFUN), we have generated a library of 90 genetically barcoded protein kinase null mutants. Using a competitive fitness profiling approach, we assessed the relative fitness of each mutant under 10 *in vitro* growth conditions and two infection models (*Galleria mellonella* larvae and neutropenic mouse).

Results:
By comparing the null mutants using their fitness scores, clusters of kinases forming known signalling pathways were identified alongside other clusters of kinases that may represent functional partners. Although several mutants had fitness defects in *in vitro* this did not always correlate with loss of virulence and *vice versa.*

Conclusion:
We identified an intreguging overlap between those factors required for pathogenicity and those required for tolerance to the antifungal itraconazole. Although some protein kinases are required for growth *in vitro* and *in vivo*, others are specifically required for adaptation to the host environment. Little correlation was observed between those kinases required for infection in *Galleria mellonella* and mouse.

64 CRISPR-CAS9 MUTATION AND CHARACTERIZATION OF THE MOST OVEREXPRESSED TRANSCRIPTION FACTOR OF SWOLLEN STATE OF *ASPERGILLUS FUMIGATUS*

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Purpose:
The germination is the first adaptive process that the fungus must undergone to colonize the environment or infect animals. *Aspergillus fumigatus* germination process involves a first step known as swollen and the subsequent hyphae elongation. This study focuses on the transcriptomic analysis of the genes differently expressed between swelling conidia and early hyphae output to detect new molecular targets overexpressed in swollen state and to generate a disruption mutant of this by CRISPR-Cas9 technique. With the use of this mutant, we study its implication in fungal biology, virulence and adaptive process of *A. fumigatus*.

Methods:
The *A. fumigatus* reference strain Af293 was incubated at 37°C without agitation in RPMI supplemented with 5% fetal bovine serum (FBS) for 4.5 and 6 hours to obtain swelling conidia or early hyphae respectively. Total RNA was extracted and maintained in RNA later until use. RNA samples were studied using the AWAFUGE microarray (Agilent Whole *A. fumigatus* Genome Expression 44K v.1) designed by our research group. The disruption mutant strain was generated by CRISPR-Cas9 technique. The gene was disrupted in the initial methionine to suppress its expression and breaking the open reading frame with the hygromycin resistance gene. Mutants were validated by PCR. For characterization was used glucose minimum medium alone or supplemented with 80 µg/ml Congo red, 80 µg/ml Calcofluor white, 0'0125% SDS, 1M KCl, 1M NaCl or 1.2M Sorbitol. In addition, three different pH were assayed: 6.5, 5 and 8.The strains were incubated in broth YG with and without 5% FBS to measure fungal density with spectrophotometry. All the experiments were done at least in triplicate.

Results:
The transcriptomic analysis detected 158 and 256 genes overexpressed in the swelling and early hyphal stages, respectively. The Afu3g08050 was the most overexpressed transcription factor in swollen state and was selected to obtain the disruption mutant strain by CRISPR-Cas9. Phenotypical characterization shown that the mutant was viable but with low growth rate in YG. In contrast, the supplementation with serum supposes larger exponential growth stage. Furthermore, the conidiation of the mutant was remarkably lower than the wild type strain and the emergence of the germ tube was affected by the disruption. The absence of the transcription factor activity supposes higher sensitivity to pH, osmotic and membrane stresses assayed indicating a possible role of this gene in the exchange of ions and sugars across the membrane.

Conclusion:
The results obtained in this work indicate that AFUA_3g08050 transcription factor has an important role in the adaptation process of *A. fumigatus* to different stresses, in conidiation process and in conidial germination. In addition, highlights its involvement in membrane homeostasis.

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65 BIOSYNTHESIS OF ALPHA-AMINO-B-HYDROXYLBUTANOYL-GLYCYLURIDINE: A KEY COMPONENT IN ANTIFUNGAL NUCLEOSIDE ANTIBIOTICS

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Purpose:
Structural elucidation and enzymatic transformation for non-heme hydroxylase enzyme in lipo-nucleoside antifungal and antibiotics pathway with the aim of designing novel inhibitors.

Methods:
Metabolite Analysis: Streptomyces and the mutant strains were cultured and incubated for 3 days. For metabolite analysis, small amount of culture was applied by LC-MS.

General experimental methods: NMR data was recorded at 800 MHz for H1 and 13C. Analytic HPLC was performed with Agilent. LC-MS analyses were performed using thermos scientific LTQ XL. Protein purification was done by AKTA protein purification system.

Chemically synthesis: synthesis of designed compounds followed a previously described procedures in collaboration with Prof. Ichikawa from Hokkaido University.

Results:
First of all, BAC (bacterial artificial chromosome) system for homologous expression was successfully constructed and transformed. All knock out strains were cultured and intermediates molecules in this pathway were purified and analyzed by LC-MS and NMR. Following, targets genes in this pathway extracted and were expressed in *E. coli* Bl21 system. Purified proteins as enzymes were tested in *in-vitro* conditions. Results confirmed the predicted pathway details including some corrections. Small molecules as intermediates in the pathway were successfully synthesized. Fortunately, in this study we could to crystalize two out of five enzymes. One enzyme's function is alpha KG dependent hydroxylase and another one is an amide ligase. Crystal structure of hydroxylase enzyme shows the locations of ferro iron amino acids and alpha KG amino acid. Moreover, we can see the complex structure of two others inhibitors in the active site.

Conclusion:
Veni, vidi, vici is the best explanation for antifungal nucleosides (Tunicamycin, Nikkomycin, liposodomycin, polyoxin, blasticidin, arginomycin and mildimycin). Most of them are potent competitive inhibitors of fungal chitin synthases. Based on our results, in this pathway only two alpha KG enzymes are located that both accept specific substrates. Furthermore, hydroxylase enzyme also accept methionine as a nonspecific substrate to producing methionine sulfoxide that can be as a donor in other metabolic pathways or as a moiety of natural products. Our data clarifying unknown enzymes in this pathway as a key component in antifungal nucleoside antibiotics. These achievements not only elucidate the pathway of this class of natural product but also guide future studies to designing novel antifungal drugs.

66 EFFICACY AND SAFETY OF HIGH-DOSE CASPOFUNGIN IN THE SALVAGE THERAPY OF PULMONARY ASPERGILLOSIS

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Purpose:
To investigate the clinical efficacy and safety of high-dose caspofungin (70 mg/d) as salvage therapy for both invasive and chronic pulmonary aspergillosis.

Methods:
Twenty-three patients with proven or probable pulmonary aspergillosis, who were refractory to or intolerant of prior antifungal therapies and received at least 7 days of caspofungin (70 mg/d) as salvage therapy were eligible for enrollment. Pulmonary aspergillosis was defined as either "acute", "subacute" or "chronic" types as described by the guideline for diagnosis and management of Chronic pulmonary aspergillosis. The study was conducted from June 2014 to October 2018 in Huashan Hospital, Fudan University (a tertiary health care center in Shanghai), and characteristics, clinical efficacy, adverse reactions and outcomes were evaluated.

Results:
Three proven and 20 probable pulmonary aspergillosis cases were enrolled, of whom 4 were acute invasive pulmonary aspergillosis, 7 were subacute and 12 were chronic pulmonary aspergillosis. The main predisposing conditions included pulmonary tuberculosis and chronic obstructive pulmonary disease (30% each), diabetes mellitus (26%), steroids or immunosuppressant use (22%), solid organ tumors (22%), solid organ transplantation (13%), autoimmune diseases (9%) and hematological malignancy (9%). Predisposing factors co-existed in 16 patients (70%). Most patients received high-dose caspofungin monotherapy (17/23, 74%) and 6 received caspofungin-based antifungal combination therapy. The mean duration of high-dose caspofungin treatment was 80 days (range 27-210 days). At week 12, the overall response rate was 73% (16/22) with 1 complete response and 16 partial responses, whereas 4 patients (18%) had a stable disease, 1 (5%) had progressive infection and 1 (5%) patient died. In 4 patients with acute invasive pulmonary aspergillosis, 2 had a partial response, and 6 and 8 cases in subacute and chronic types achieved favorable responses, respectively. Patients receiving caspofungin monotherapy and combination therapy had response rates of 69% (11/16) and 83% (5/6), respectively. Response rates did not significantly differ by age, gender, or weight. However, favorable response rates were significantly higher in patients with hypoproteinemia than with normal serum albumin levels (93% vs 50%; $P = 0.04$). After a median follow-up of 13 months (range, 12 to 33 months), 19 patients (86%) were alive with 1 patient lost to follow-up. The all-cause mortality was 3 (14%) and no patients died of pulmonary aspergillosis. In all 22 patients included in the safety analysis, no patients discontinued caspofungin therapy due to drug interactions. Six (27%) patients experienced hepatotoxicity, of whom only 1 liver transplantation case had severe hepatotoxicity (grade 3 on CTCAE), and an immune rejection rather than drug toxicity was consequently confirmed by liver biopsy. Additionally, we evaluated the incidence of hepatotoxicity in the same population after adjustment of Child-Pugh scoring. The number of patients who had hepatotoxicity was noted in 27% (4/15) and 29% (2/7) in Child A and Child B class, respectively, and no statistical difference was found between 2 classes ($P = 1.0$).

Conclusion:
High-dose caspofungin showed efficacy and safety for both invasive and chronic pulmonary aspergillosis.

67 INCIDENCE OF CUTANEOUS SQUAMOUS CELL CARCINOMA IN PATIENTS RECEIVING VORICONAZOLE THERAPY FOR CHRONIC PULMONARY ASPERGILLOSIS

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Purpose:
Voriconazole has been associated with cutaneous squamous cell carcinoma (cSCC) in the immunocompromised such as those with haematopoietic or solid organ transplant, but less is known about the risk in the immunocompetent. Patients with chronic pulmonary aspergillosis (CPA) usually have a background of chronic lung disease without significant immunosuppression and are often treated with azoles including voriconazole for prolonged periods. Our aim was to define the risk and clinical presentations of cSCC in patients with CPA treated with voriconazole.

Methods:
All patients with the diagnosis of CPA referred to the National Aspergillosis Centre, Manchester, UK from 2009 to March 2019 were included. Clinical information was collected from all clinic visits until March 2019 unless the patient was discharged, lost to follow up or died, in which case the last observation point was the last clinic visit. Voriconazole use was recorded; patients receiving voriconazole for less than 28 days were considered non-exposed. The crude incidence rate and age-specific incidence rate of cSCC were calculated. Clinical features, treatment and outcome of cSCC were recorded.

Results:
A total of 1199 patients with CPA were identified. Fifty-five had only one attendance, 32 had no clinical information and one patient had cSCC diagnosed before referral to the NAC and were excluded. Of the 1111 remaining patients, 681 (61%) received voriconazole. Mean age was 66.6 (range: 21-96). 477 (42.9%) were female. There were 12 cases of cSCC; nine in patients who received more than 28 days of voriconazole prior to cancer diagnosis. All patients developed photosensitivity prior to diagnosis. The mean duration of voriconazole therapy was 36.7 months and the mean time from the start of therapy to cancer diagnosis was 47.2 months. Two patients had COPD, one had bronchiectasis and one had prior TB. Five were considered immunocompromised: two had sarcoidosis, one had previous lung cancer, one had active liver cancer and one had interstitial lung disease. All patients had successful treatment, but one necessitated plastic reconstruction. Three patients had relapse of lesions after resection. In three cases, cSCC affected the ear, in two cases the scalp, in one case the forehead and in one case the face. In two cases, there was no clinical information on the site of the lesion. The crude incidence rate was 4.74 per 1000 person/year in patients who received voriconazole and 2.81 per 1000 person/year in patients who did not receive voriconazole. In those treated with voriconazole, age-adjusted incidence rates were 9.8 per 1000 person/years in those aged >74, 4.5 per 1000 person/years in those aged 65-74 and 1.1 per 1000 person/years in those younger than 65.

Conclusion:
Long-term voriconazole was associated with an increased rate of cSCC in patients with CPA, although the relative risk appears lower than that reported for transplant patients. Mean duration of voriconazole use was around 3 years. Photosensitivity preceded cSCC in all cases. Caution should be used when using long-term voriconazole in patients with CPA and treatment stopped or converted to alternative azole if possible, especially if photosensitivity is an issue.

68 ACCUMULATION OF A NOVEL INHALED AZOLE, PC945, IN ALVEOLAR CELLS IN TEMPORALLY NEUTROPENIC IMMUNOCOMPROMISED MICE INFECTED WITH *ASPERGILLUS FUMIGATUS*

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Purpose:
PC945 is a novel inhaled antifungal agent, whose profile maximises the likelihood of clearing a pulmonary *Aspergillus* infection, while minimising the risk of either direct toxicity or toxicity/medical complications arising from unwanted drug-drug interactions. Since the target of PC945 is pulmonary *Aspergillus* infections, attempts to develop a useful pharmacokinetic (PK): pharmacodynamic (PD) model to aid clinical development have focused on exploring the relationship between local concentrations of PC945 in the lung and effect versus the fungus.

Methods:
A/J mice (males, 5 weeks old) were dosed with hydrocortisone (125 mg/kg, sc,) on days 3, 2 and 1 before infection, and with cyclophosphamide (250 mg/kg, ip) 2 days before infection to induce temporary neutropenia. On day 0, animals were infected intranasally with 35 µL of the spore suspension of *Aspergillus fumigatus* (ATCC 13073) at a concentration of 1.67×10^8 spores mL^{-1} of physiological saline. PC945 (0.016 mg/mL) was treated prophylactically and intranasally once daily on days -7 to 0 (8 days) or Day -1 to 0 (2 days), and animals were culled on day 3 post *Aspergillus fumigatus* intranasal inoculation. Bronchoalveolar lavage fluid (BALF), lung and plasma were collected on 24 or 72 hrs post the last dose (post infection) for biomarker and PK analysis. BAL cell pellet (alveolar cells) and BALF supernatant were collected separately after centrifugation of BALF.

Results:
Intranasally dosed PC945 showed much stronger antifungal effects (CFU in lung, galactomannan (GM) in BALF and plasma) by 8 days prophylaxis than 2 days prophylaxis. PC945 was not detectable in plasma on either 24hrs or 72hrs post the last dose although a much higher level of PC945 was detected in the lung at 24hr post the last dose for 8 days prophylaxis (709±126 ng/g lung) than 2 days prophylaxis (301±56 ng/g lung). Interestingly, no or little PC945 was detected in BAL supernatant (<10 ng/mL) but significantly higher level of PC945 was detected in BAL cell pellets. The concentrations in alveolar cell pellets show an impressive correlation with anti-fungal activity in the lung.

Conclusion:
Intranasally dosed PC945 accumulated in alveolar cells. These observations will be pursued, and it is intended that BAL cell concentrations of PC945 be measured in the future clinical study rather than standard BALF measurement.

69 EFFECTS OF INHALED PC945 ON FUNGAL LOAD IN MOUTH WASH COLLECTED FROM HEALTHY SUBJECTS IN THE FIRST-IN-HUMAN STUDY (NCT02715570)

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Purpose:
A first in human (FIH) study of PC945, a novel inhaled antifungal triazole, was conducted to evaluate the safety and pharmacokinetics of PC945 delivered as a suspension formulation by nebulisation (NCT02715570). During this study, mouth wash and pharyngeal swabs were collected for exploratory investigation of the impact of PC945 treatment on observed fungal loads.

Methods:
Healthy volunteers (HVs) received Placebo or PC945 as either single ascending doses (0.5-10 mg) (SAD Cohort) or 5 mg once daily for 7 days (repeat dose [RD] cohort). Mild asthmatics received a single dose of PC945, 5 mg or Placebo (asthma cohort). Mouth wash and pharyngeal swabs were collected pre- and 48hrs post dose. Mouth wash was cultured in Potato dextrose agar (PGC) and CHROMAgar, and the presence of *Candida albicans* assessed using polymerase chain reaction (PCR). Yeasts were identified using CHROMAgar, PCR and Maldi-Tof MS analysis

Results:
Fungal detection rates and fungal burden in both cohorts of healthy volunteers were very low. In addition, fungal burden distribution was poorly randomised. In the SAD and asthma cohorts, no yeasts or moulds were detected in culture in the Placebo groups pre-dose. The commonest yeast species observed was *Candida albicans* and, for mould species, *Aspergillus fumigatus*, *Penicillium rubens* and *Penicillium chrysogenum* were detected.

In the SAD cohort, PC945 dose-dependently inhibited yeast load detected in PGC agar and CHROMagar up to 5mg dose. Overall, treatment with PC945 statistically significantly inhibited *Candida* culture load ($p=0.023$) and PCR signal ($p=0.009$) (paired comparison pre- versus post-dose) in samples yeast positive pre-dose. In the RD and asthma cohorts, no yeast was detected in the Placebo treated group, but the trend of PC945 inhibition of yeasts post-dose was observed in the asthma cohort compared with pre-dose. In all cohorts, mould detection was limited, and no conclusive data were obtained. In addition, almost no or little yeast and mould PCR signal was detected in pharyngeal swabs.

Conclusion:
Samples taken during this FIH study allowed a first exploratory investigation of PC945's antifungal activity in humans, although the study was neither powered nor appropriately randomised for this purpose. The results do, however, suggest a trend of PC945 treatment-dependent inhibition of oral yeasts which may warrant further clinical study.

70 TOPICAL LIPOSOMAL AMPHOTERICIN B (AMBISOME®, GILEAD SCIENCES LTD, ABINGTON, UNITED KINGDOM) AS AN ADJUNCTIVE THERAPY IN THE MANAGEMENT OF POST-TRAUMATIC INVASIVE FUNGAL INFECTION (IFI)

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Purpose:
Topical antifungal preparations, such as Dakin's solution (sodium hypochlorite 0.4-0.5%), have been described in the management of IFIs in military trauma patients. To our knowledge, we describe the first successful utilisation of an AmBisome® preparation to irrigate invasive infections with *Aspergillus fumigatus* and the mucoraceous mould, *Rhizopus arrhizus*, alongside aggressive surgical debridement and intravenous AmBisome® therapy.

Methods:
We describe the case of a 32-year-old male with type one diabetes mellitus admitted to our centre with severe polytrauma following a crush injury between a train and platform edge. The patient had a prolonged extrication and was transfused several units of packed red blood cells on scene. Initial assessment revealed complex pelvic injury with bilateral open femoral fractures, retroperitoneal haematoma, bladder rupture, perineal injury and bilateral lower limb soft tissue injuries. He required multiple debridements of his complex lower limb injuries and had fixation of his femoral and pelvic fractures. The right leg was deemed unsalvageable and amputated due to the extent of tissue loss and contamination.

At day 26 of admission, marked tissue necrosis and an appearance reminiscent of sub-dermal mycelial growth were noted at dressing removal on the left lower limb (Figs. 1 and 2). An infectious diseases consult was obtained and empiric intravenous AmBisome was initiated. The patient was taken to theatre for aggressive surgical debridement of the devitalised muscle tissue (Figs. 3 and 4). In theatre, a solution of AmBisome® and 5% dextrose was used to irrigate infected areas of tissue post-debridement. Tissue samples were sent for microbiology (including prolonged mycological cultures) and histology.

Results:
Aspergillus fumigatus and *Rhizopus arrhizus* were cultured from tissue samples at time of debridement. Serum galactomannan was negative while serum (1, 3)-b-D glucan (BDG) was raised. After aggressive surgical debridement with topical therapy and intravenous Ambisome® for 2 weeks, the patient's tissue samples from left leg were culture negative for fungi with a healthier appearance.

Conclusion:
Post-traumatic IFI is a rare and devastating clinical entity with mortality approaching 41% in the civilian population. In our patient, diabetes mellitus is a secondary risk factor for the development of invasive mucormycosis. While there is consensus that early, aggressive surgical debridement and systemic antifungals are vital, the optimal therapeutic approach is unknown and there is a role for further research on adjunctive therapies to preserve tissue and reduce mortality.

It has been suggested that tissue penetration of systemic antifungals is suboptimal in IFI due to thrombosis and tissue necrosis secondary to angio-invasive mould species. Thus, topical antifungal therapies may provide anti-mycotic activity at the site of disease and aid in ensuring the resolution of IFI and preventing its recurrence.

To our knowledge, this case report describes the first use of AmBisome® in liquid preparation as a topical therapy during debridement for IFI. We suggest that the direct application of a broad-

spectrum, mould-active antifungal at the site of infection may assist in reducing disease burden alongside aggressive surgical management and systemic therapy. Randomised trials are necessary to determine whether such therapy adds benefit in similar patient cohorts.

Figure 1

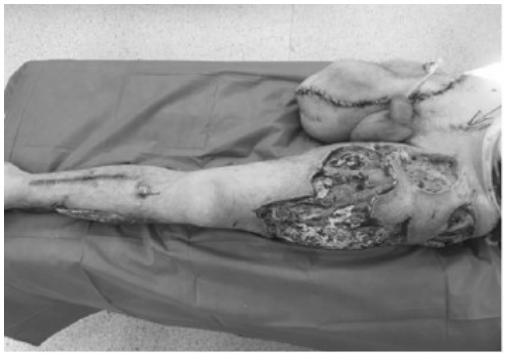


Figure 2

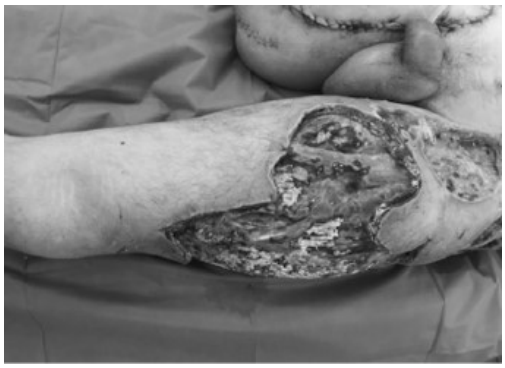


Figure 3

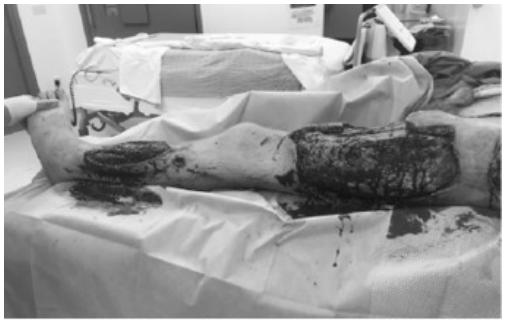
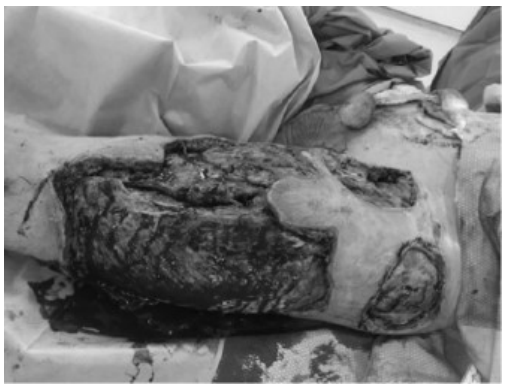


Figure 4



71 GALLIUM AS AN ANTI-*ASPERGILLUS* ANTIFUNGAL

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Purpose:
Iron is an essential element in biological processes, including for *Aspergillus*. Because of similarity of gallium's oxidation state to Fe^{+3} in atomic radius, electron configuration, charge and ionic bonding, it can function as an Fe^{+3} mimic. As gallium cannot be similarly reduced, it can disrupt redox-driven biological processes. It can also interfere with iron uptake mechanisms. Inhibitory activity *in vitro* for Gram negative bacteria has been shown. As gallium has been used therapeutically or diagnostically in human neoplastic, inflammatory or electrolyte imbalance disorders, it is known to be safe. Gallium citrate has the biologically most desirable salt characteristics, the product AR-501 was used in our studies.

Methods:
Macrobroth dilution was studied *in vitro* per previously published methodology. Ranges of gallium, amphotericin B, voriconazole, caspofungin tested were 0.0625-256, 0.03-8, 0.5-8, 0.02-50 mcg/ml, respectively. Endpoints were 50% inhibition (MEC), 100% inhibition (MIC, clear tube), and MFC (≥96% killing of inoculum); in checkerboard assays, fractional inhibitory or killing (FIC, FFC) endpoints were also noted and indices (sums; FICi/FFCi) calculated. Indices <0.5, 0.5-1, and >1 were termed synergy, additive or antagonistic, respectively; no interaction=indifference.

Results:
For 11 *A. fumigatus* isolates, the gallium MEC/MIC/MFC50 (i.e., concentration to affect half the isolates) were 1, 32, >256 mcg/ml, respectively; for 10 *A. flavus* 1, >256, >256. The gallium results were the same for 6 voriconazole-resistant isolates. One *A. niger* and *terreus* had same results as *fumigatus.*

Gallium *fumigatus* checkerboards (3 susceptible and 2 resistant *fumigatus*, 3 drug checkerboards each, 3 endpoints/checkerboard): antagonism rarely seen (4/45 endpoints). Synergy or additive interaction noted in 12/15 with amphotericin, 10/15 voriconazole; indifference 10/15 with caspofungin. By assay type, synergy or additive 8/15 for MEC, 11/15 MIC, 7/15 MFC.

Conclusion:
Aspergilli are inhibited *in vitro*. MECs are lower than MICs, indicating *in vitro*, gallium alone is fungistatic. Gallium appears similar in this activity to echinocandins vs. filamentous fungi. The profile of gallium in drug interactions is generally favorable, and contributed to inhibition or killing. Notably, majority of interactions with amphotericin and voriconazole were synergistic or additive. It would be of interest to know whether gallium resistance can develop, and test antifungal activity *in vitro* in the presence of host phagocytes, against *Aspergillus* biofilms, and in animal models. Susceptibility of *Candida* species (also susceptible to iron deprivation) are also of interest, and of other species where are no highly effective clinical therapy options. Gallium shows potential for clinical antifungal therapy.

72 THE UK NATIONAL ASPERGILLOSIS CENTRE – TEN YEARS' SERVICE

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Purpose:
The National Aspergillosis Centre (NAC) is commissioned as a Highly Specialised Service within the NHS and has completed its 10th year. The NAC was commissioned on 1st May 2009 by the NHS Highly Specialised Commissioning Group to provide long term care in both out-patient and inpatient setting for patients with chronic pulmonary aspergillosis (CPA) for England and Scotland. The centre provides high level expertise in the clinical management of CPA, diagnostic testing and monitoring with access to high cost drugs for these patients. It is supported by the Mycology Reference Centre Manchester, an ECMM Centre of Mycological Excellence which provides an essential role in the management of these patients (50, 000 tests per annum): Azole antifungal monitoring, sputum fungal culture, *Aspergillus* PCR, susceptibility testing, galactomannan and pyrosequencing for azole resistant mutations

Methods:
Patient attendance and disease severity data are submitted to the Commissioners on a monthly basis. These data were collated at year end with input from the laboratory and communications team and put into the annual report which is available for the public. https://aspergillosis.org/nac-reports/

Results:
Since commencement of the service 2764 patients have been assessed with some form of *Aspergillus* disease. 1185 patients had confirmed CPA, with 130-140 new referrals annually. Some of these patients died (n = 422). The majority of patients have significant co-morbidities and their management is complex. Around 184 patients have been discharged. We have saved >1000 inpatient bed days following the setup of OPAT services. A total of 117 bronchial artery embolisation procedures and 33 surgical resections have been undertaken.

Antifungal and gamma interferon expenditure – the budget for drugs is directly supported by the NHS commissioners who allow high cost drugs to be used for CPA patients. Voriconazole has now come off patent and the cost has fallen. Posaconazole and isavuconazole are used on an n-of-1 trial basis and Ambisome (~3mg/Kg) and/or micafungin (150mg/d) for failure, intolerance and azole resistance. Patients on oral medication are put on Homecare delivery where appropriate so they can receive antifungals at home. The service set up a postal blood service for monitoring azole levels between visits.

In 2009, there were 3 consultants, 2 specialist nurses, 1 manager and 2 communications/Aspergillus Website staff. The team has now grown to: 7 consultants, 6 clinical fellows, 6 specialist nurses, 5 OPAT nurses, 1 nursing assistant and 2 specialist physiotherapists. In addition we have 4 communications staff and 5 admin staff.

Conclusion:
The National Aspergillosis Centre in Manchester has overseen a dramatic increase in the number of CPA cases over its first 10 years, with a 9-fold increase in patient numbers. Chronic Obstructive Pulmonary Disease (COPD) is the most common comorbidity. Many CPA patients live in the North West of England but other 'hot-spots' are likely to be in the North East, East and West Midlands, London, Home Counties and South Wales, that do not refer patients as readily. Given the increasing numbers a hub and spoke service model is being discussed to support patients in other parts of the country.

73 ROS-DEPENDENT AND INDEPENDENT HOST-INDUCED FUNGAL REGULATED CELL DEATH IN DEFENSE AGAINST INVASIVE ASPERGILLOSIS

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Introduction:
Human fungal pathogens are major drivers of devastating morbidity and mortality accounting for a staggering 1.6 million deaths annually. *Aspergillus fumigatus* is a leading cause of infectious mortality in immune compromised individuals. Oxidative burst, which generates reactive oxidative species (ROS) via NADPH oxidase plays a pivotal role in the defense against fungal pathogens and was long thought of as the primary effector system against *A. fumigatus*. However, we observed that NADPH-deficient leukocytes have significant residual conidiacidal activity *in vivo*. Mitochondria are major sites of ROS production in most cells; however, mitochondrial ROS (mROS) have traditionally been regarded as byproducts of oxidative respiration.

Methods and Results:
To determine whether mROS was required for host defense against *A. fumigatus* infection, we infected transgenic mice that express a mitochondrial-targeted catalase (mCAT) in absence and presence of NADPH-oxidase. Using functional reporters of fungal regulated cell death, we show that mROS regulates macrophage conidiacidal activity independent of NADPH oxidase-derived ROS. Nonetheless, mROS presence did not affect infectious outcomes, arguing for the presence of alternate non-oxidative antifungal effector mechanisms. To explore this hypothesis, we employed genome-wide dual omic approaches to simultaneously profile transcriptional changes of host leukocytes and fungal cells in response to host-fungal encounters in order to identify additional host inducers of fungal RCD and the corresponding fungal pathways. Using mixed chimeric mice that contain both WT (p91phox +/+) and -deficient (p91phox -/-) neutrophils we identified infection-specific alterations in several murine and fungal metabolic pathways upon fungal internalization.

Conclusions:
Our study illustrates the efficacy of dual RNA sequencing in unraveling the virulence and defense mechanisms of neglected, genetically intractable fungal pathogens. Thus, our findings provide molecular insights into RCD that may extend beyond the *A. fumigatus* pathosystem and will lay the groundwork for the development of innovative, fungal-selective, pan-antifungal drugs.

***NOTE*: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.**

74 INTERDEPENDENCY OF HOST AND PATHOGEN PROTEIN PERSULFIDATION GOVERNS DISEASE SEVERITY IN EXPERIMENTAL AND HUMAN ASPERGILLOSES

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Purpose:
The ability to adapt to the harsh conditions imposed by a host is fundamental for pathogens' infective capacity and, concomitantly, the host cellular response must be finely tuned to mount an efficient response against the pathogen and clear infection. Post-translational modifications (PTMs) are important for adaptation to stress and therefore are expectedly crucial for both pathogen and host. Here we investigated the relevance of the PTM persulfidation for *Aspergillus fumigatus* and for antifungal host defence in mammalian lung cells.

Methods:
We have genetically engineered *A. fumigatus* and the A549 human alveolar epithelial cell line to delete the gene encoding the enzyme primarily responsible for persulfidation, as confirmed by fluorescence proteomic and microscopic analyses. We have then used enzymatic assays, killing experiments and a mouse survival experiment to determine the relevance of persulfidation for *A. fumigatus* virulence. We employed a cumulative incidence method to investigate the genetic association of a Single Nucleotide Polymorphism (SNP) in the human gene with the probability of suffering Invasive Pulmonary Aspergillosis in stem cell transplant recipients. We then investigated the killing capacity of host cells using our engineered A549 cell line and also alveolar macrophages and neutrophils derived from a knock-out mouse line. Finally, we investigated the interdependency of fungal and human persulfidation levels using Western-blot and fluorescence microscopy.

Results:
Persulfidation seems to be essential in *A. fumigatus*, as a double deletant of the two enzymes primarily responsible for this PMT (cystathionine-γ-lyase, MecB, and cystathionine-β-synthase, MecA) could not be constructed. We show that low levels of persulfidation altered the activity of at least two proteins related with virulence, Aspf3 and AlcC. The weakly persulfidating *A. fumigatus ΔmecB* mutant is more susceptible to host-mediated killing and displays reduced virulence in a murine model of infection. Besides, we found that a single nucleotide polymorphism (SNP) in the human gene encoding cystathionine-γ-lyase, the main enzyme responsible for protein persulfidation in the lungs, predisposes to Invasive Pulmonary Aspergillosis in hematopoietic stem cell transplant recipients. This is likely due to an imbalanced cytokine environment and a decreased antifungal activity of lung-resident host cells (alveolar macrophages and epithelial cells), but not neutrophils. Interestingly, levels of host protein persulfidation determine the levels of fungal persulfidation, reflecting a direct host-pathogen cross-talk.

Conclusion:
We show that correct protein persulfidation is important for both *A. fumigatus* pathogenic potential and host antifungal defence and also that host persulfidation determine the level of persulfidation in the fungal pathogen. Furthermore, we propose that persulfidation is an essential cellular process. Therefore, persulfidation must be considered as a relevant post-translational modification for infection, where its modulation may be a promising and novel strategy to target both pathogens and immune responses.

NOTE: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.

75 IN HIGH IRON ENVIRONMENT, PYOCYANIN IS A MAJOR ANTI-*ASPERGILLUS* MOLECULE

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Purpose:
Airways of immunocompromised patients, or individuals with cystic fibrosis, are common ground for *Pseudomonas aeruginosa* and *Aspergillus fumigatus* infections. Hence, in such a microenvironment both pathogens compete for resources. In recent studies we showed that the siderophore pyoverdine is the most anti-fungal *P. aeruginosa* product under low iron conditions. We now investigated activity of *P. aeruginosa* against *A. fumigatus* biofilms under high iron conditions.

Methods:
P. aeruginosa wildtype strains PA14 and PAO1, as well as PA14 mutants *pvdD-, pchE-, pvdD-pchE-, pqsA-, pqsH-, rhlR-, lasR-, lasR-rhlR-,* and *lasI-* were cultivated in RPMI 1640 minimal medium with or without addition of ferric iron (iron), or blood at 37°C and 100 rpm for 24 hours. Bacterial supernatants were tested for bacterial growth (A600 nm), and filter sterilized (0.22 µm). Pyoverdine (A405 nm, and UV test), pyocyanin (A360 nm, or A540 nm following chloroform extraction) in supernatants were measured. Supernatants, or purified molecules alone, were assayed for effects on *A. fumigatus* biofilm formation, as well as *A. fumigatus* preformed biofilms.

Results:
Under high iron conditions *P. aeruginosa* supernatants no longer contained pyoverdine, but still possessed considerable anti-fungal activity. Spectrometric analysis of *P. aeruginosa* PA14 wildtype supernatant revealed the presence of the toxin pyocyanin. In response to iron, pyocyanin was not found in supernatants of PA14 mutants defective in phenazine production. Supernatants without pyocyanin under high iron conditions were significantly less anti-fungal towards *A. fumigatus* biofilms. Pure pyocyanin, at concentrations measured in PA14 supernatants, interfered with *A. fumigatus* biofilm metabolism. When blood (as a natural source of iron), as low as 0.5%, was added during *P aeruginosa* supernatant production, pyoverdine disappeared from supernatants, and pyocyanin appeared. Although pyoverdine was no longer present in those supernatants, strong anti-fungal activity against *A. fumigatus* biofilms was observed. Neither iron, nor blood, induced pyocyanin production by PAO1, indicating important differences between standard reference *P. aeruginosa* strains. Under high iron conditions PAO1 supernatants showed significantly lower anti-fungal activity than PA14 supernatants.

Conclusion:
While under low iron conditions the major anti-fungal product of *P. aeruginosa* is pyoverdine, under high iron conditions, pyoverdine is no longer present, giving rise to the expectation of lower anti-fungal activity of *P. aeruginosa* supernatants. In fact, anti-fungal activity of supernatants under high iron conditions was only lower when strains were not able to deliver the *P. aeruginosa* toxin pyocyanin in response to iron. Under high iron conditions pyocyanin therefore appears to replace the anti-fungal activity of pyoverdine observed under low iron conditions. In conclusion, *P. aeruginosa* has mechanisms to compete with *Aspergillus fumigatus* under low or high iron conditions, and can switch from iron-denial-based (pyoverdine-based) to toxin-based (pyocyanin-based) anti-fungal activity.

76 PALMITOLEALDEHYDE TARGETING CONIDIAL PIGMENTATION AND SURFACE MORPHOLOGY IN *ASPERGILLUS FUMIGATUS*

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Purpose:
Aspergillus fumigatus is a major fungal pathogen associated with varied invasive fungal infections all over the globe. The rapid emergence of antifungal drug resistance in *A. fumigatus* since past few years, has led to increased morbidity and mortality mainly among immunocompromised patients. Conidial pigment DHN-melanin is a major virulence factor that is well associated with other cell surface virulence determinants. It protects the fungus during unfavourable conditions and enhances their survival *in-vitro* and *in-vivo*. Therefore, the present study aims to investigate the antifungal efficacy of the phytocompound palmitolealdehyde (C9H) targeting conidial pigmentation and surface morphology in *A. fumigatus.* C9H is a naturally occurring fatty aldehydic compound that is present in various medicinal plants but its melanin inhibiting property against *A. fumigatus* is still unexplored.

Methods:
Minimum effective concentration (MEC) of the phytocompound leading to formation of white demelanised *A. fumigatus* colony was determined by broth micro-dilution assay. Reduction in conidial pigmentation and other surface virulence traits such as hydrophobins, polysaccharide and glycoproteins were estimated via biochemical assays. The transcript analysis of polyketide synthase gene *pksP/alb1* (the 1st gene activated during conidial pigment biosynthesis)was done using RT-qPCR. Total proteome profiling was performed using LC-MS/MS. MTT assay was performed for cytotoxicity determination. Fractional inhibitory concentration (FIC) was determined using checkerboard assay.

Results:
A. fumigatus formed white demelanised colony at 0.07 mg/mL concentration of C9H. There was 96% inhibition of melanin content in C9H treated *A. fumigatus*. The melanin reduction resulted in altered conidial surface with reduced surface hydrophobicity (55%) and cell wall polysaccharides such as glucans (53%), chitin (46%) and glycoproteins (40%). The phytocompound treatment enhanced the expression of *pksP/alb1* gene (3.5 fold) in comparison to wild type *A. fumigatus*. The total proteome analysis showed that from a total of 1809 proteins, 309 proteins were differentially expressed. The gene ontology study, further revealed 36% membrane proteins and 7% extracellular proteins, were differentially expressed. On the basis of biological function, it was found that 3% cell wall integrity proteins, 9% secondary metabolites and 5% cell stress proteins were differentially expressed between the total protein isolated from C9H treated and WT *A. fumigatus*. Polyketide synthase protein (PKS) was up-regulated; however no further down-stream proteins formed during DHN-melanin biosynthesis were observed. The phytocompound C9H has been found to be non-cytotoxic for human lung epithelial normal cell line L-132 up to 0.625 mg/mL. FIC indexing revealed that C9H had an additive effect when used in combination with antifungal drug Amphotericin B (AmpB) and the therapeutic efficacy of both AmpB (4-fold) and C9H (2-fold) was enhanced.

Conclusion:
The outcome of *in vitro* studies indicated that the phytocompound C9H has potential role in inhibition of conidial pigmentation leading to reduction in surface proteins and polysaccharides responsible for adherence and spreading infection in host body. The combinatorial approach may also help in overcoming the severe side-effects due the high dosage of the available antifungal drugs. Hence, it can be explored further as a lead for future therapeutic agent.

77 PHENOTYPIC ANALYSIS OF *ASPERGILLUS FUMIGATUS* MUTANT LACKING THE P-TYPE NA^+-ATPASE ENCODING GENE *ENAA*

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Introduction:
Aspergillus fumigatus is a hyaline septate mold, commonly found in the in-door environment and is important cause of opportunistic infection in immuno-compromised individuals. During infection in the host, the fungus is exposed to alkaline pH and toxic cations. Thus, an efficient detoxification system is crucial for survival. In most fungi, sodium ATPases play important role in pumping the exceed Na^+ out of the cells and are also important for growth under alkaline condition. In the pathogenic yeast, *Cryptococcus neoformans* the gene *ENA1*, encoding the P-type Na^+-ATPase has been implicated in response to alkaline stress(1). However, data about how pathogenic molds are coping with alkaline environment are still limited. In a recent study, a homologue of *ENA1* in *A. fumigatus* was investigated and the results showed that *ENA1* homologue in *A. fumigatus* was rapidly upregulated when exposed to alkaline condition(2). Nevertheless, the role of *A. fumigatus ENA1* homologue in adaptation to cationic stress has not been studied. The aim of this research is therefore to explore the involvement of *ENA1* homologue in *A. fumigatus*, termed in this study as *enaA*, in response to cationic stress and alkaline pH.

Methods:
To observed the phenotype of *A. fumigatus* mutant strain lacking the *enaA* gene, 10^6 conidia/ml of both mutant and wild-type strain were inoculated on GMM medium under different kind of stress conditions, including cationic stress (NaCl, $CaCl_2$, $MnCl_2$, $MgCl_2$, KCl), alkaline, acidic pH and osmotic stress. The colony diameters were measured after 72 hours.

Results:
The results showed statistically significant reduction of hyphal growth of Δ*enaA* strain under high concentration of Ca^{2+} and Mn^{2+} and alkaline pH, when compared to the wild type strain.

Conclusion:
Aspergillus fumigatus gene *enaA* may play important role in response to certain cationic stresses and alkaline environment.

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78 REGULATORY CONTROL OF EPITHELIAL DAMAGE DURING *ASPERGILLUS FUMIGATUS* INFECTION

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Purpose:
A unifying feature of *Aspergillus fumigatus* lung disease is epithelial damage, the regulatory control and the mechanistic basis of which is poorly characterised. Previous studies have identified a number of fungal attributes such as epithelial adhesion, spore uptake, germination, hyphal penetration and release of secreted factors as critical contributors to epithelial damage during *A. fumigatus* invasive infection. This study aims to identify, characterise and mechanistically understand the *A. fumigatus* transcription factors driving those activities during epithelial infection.

Methods:
A549 epithelial monolayers were challenged with 479 *A. fumigatus* transcription factor mutant strains and epithelial damage was assessed by i) epithelial cell detachment via quantitative imaging of infected monolayers, ii) quantitation of epithelial cell lysis via lactate dehydrogenase activity. To ascertain the mechanistic basis of damage, transcription factor mutants exhibiting reduced epithelial damage were analysed for adhesion capacity, germination efficiency, hyphal extension rates, efficiency of spore uptake as well as capacity to secrete factors causing damage to epithelial cells. Cell wall defects were identified by checking for susceptibility to cell wall destabilising agents-calcofluor white, congo red and sodium dodecyl sulphate.

Results:
A cohort of 28 transcription factors were identified as regulating epithelial cell detachment and 24 transcription factors as regulating epithelial cell lysis during *A. fumigatus* infection. Of these, only one transcription factor regulated both cell detachment and cell lysis. Each of the transcription factor mutants exhibited a unique phenotypic profile with respect to epithelial adhesion, cell wall defects, and uptake of spores by epithelial cells. No significant difference in germination was observed compared to the parental strain; however, all mutants deficient in causing cell detachment also exhibited reduced rates of hyphal extension. Remarkably, the loss of secreted fungal factors was observed to, almost universally, account for reductions in cytotoxicity of the transcription factors regulating epithelial cell lysis.

Conclusion:
This study for the first time has identified and characterised the transcription factors regulating epithelial damage during *A. fumigatus* infection. Future work will involve systems biology approaches to map the architecture of the regulatory network driving epithelial damage during *A. fumigatus* infection.

79 IRON OVERLOAD DECREASES MACROPHAGE LYSOSOMAL ACIDIFICATION, IMPAIRING THE CLEARANCE OF *ASPERGILLUS FUMIGATUS* CONIDIA

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Purpose:
One-in-three lung transplant patients suffers from an *Aspergillus fumigatus* (*Af*)-related pulmonary disease. Macrophages play a key role in *Af* conidia clearance by recognizing, phagocytosing and killing conidia with enzymes that are active in low pH. However, studies of macrophages in *Af* infection have differed with some suggesting that macrophage depletion increases infection and other suggesting that it does not impact *Af* infection. Using a murine orthotopic tracheal transplant (OTT) model, we have previously shown that alloimmune-mediated microhemorrhage leads to iron overload, inducing *Af* invasion. In this study, we investigated the role that iron-induced macrophages dysregulation plays in *Af* transplant invasion.

Methods:
Macrophage phagocytosis and killing of *Af* conidia was measured by flow cytometry and confocal microscopy. Iron concentrations were chosen based on the iron content of hemoglobin in blood with a murine hematocrit of 30% containing 0.3mg/mL of free iron. Primary murine lung macrophages were exposed to increasing iron concentrations and co-cultured with DsRed-expressing (Af-FLARE)/AF633 double fluorescently labeled conidia. To determine cellular pH under increasing concentrations of iron, we used a LysoSensor Yellow/Blue dextran probe. Macrophage iron-induced lysosomal leakage was determined using a FITC-dextran/TRITC-dextran probe. The OTT model was used to evaluate the role of iron-laden macrophages in *Af* invasion. Transplanted mice were infected with GFP-expressing *Af* conidia. *Ex vivo* tracheas were sectioned and stained for iron (ferritin) and macrophages (F4/80). The phenotype of these iron-laden macrophages was studied by flow cytometry. To determine the role of macrophages in *Af* invasion we depleted macrophages in transplanted mice, using clodronate liposomes and measured *Af* invasion histologically.

Results:
Iron overload impacted the ability of macrophages to kill conidia *in vitro* at iron concentrations >0.1mg/ml. Because macrophage conidial killing is governed by lysosomal function, we investigated if iron overload could induce changes in the cellular pH and lysosomal leakage. Macrophage cellular pH increased significantly in an iron-dependent fashion. Lysosomal leakage also increased in macrophages treated with iron concentrations > 0.1mg/mL. In the OTT model, immunofluorescent staining of *Af* infected tracheas showed a co-localization among macrophages, ferritin, and *Af*, suggesting that these macrophages were not able to clear *Af* conidia. Immunophenotyping of macrophages isolated from transplanted tracheas showed a significant increase in the M1/M2 ratio with iron overload. To clarify the role of macrophages in *Af* infection after airway transplantation, we depleted macrophages using clodronate liposomes. Flow cytometry confirmed that clodronate significantly decreased macrophages but did not affect the populations of other professional phagocytes (e.g., neutrophils). To our surprise, macrophage depletion did not favor *Af* invasion.

Conclusion:
Our results suggest that macrophage iron-overload impairs *Af* conidia clearance by lysosomal alkalization, as a result of lysosomal leakage, leading to dysfunction in the late phagosomes. Results from the OTT model suggest that macrophage dysregulation may play a central role in *Af* transplant invasion. Further studies are needed to clarify the exact cellular mechanisms that are being impaired due to iron-overload and the role that iron-laden macrophages play in *Af* infections.

NOTE: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.

80 PHAGOSOMAL REMOVAL OF FUNGAL MELANIN REPROGRAMS MACROPHAGE METABOLISM TO PROMOTE ANTIFUNGAL IMMUNITY

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Purpose:
The opportunistic fungal pathogen *Aspergillus fumigatus* can cause a wide spectrum of diseases with clinical manifestations that range from colonization, to allergic syndromes, to invasive forms of infection. Because there are no licensed vaccines and the currently available tests for the diagnosis of invasive pulmonary aspergillosis (IPA) lack accuracy, mortality rates after infection remain unacceptably high. Therefore, an improved understanding of host defense mechanisms allowing the development of more effective treatment or control measures for IPA remains a pressing demand. Among the cells from the innate immune system, macrophages are considered critical in preventing fungal germination and tissue invasion early after infection. In response to infection, macrophages rapidly adapt their metabolic programs whereby enhanced glycolysis fuels specialized antimicrobial effector functions.

Methods:
By resorting to different pharmacological and genetic tools manipulating both the host and the fungus, we dissected the signals and pathways that regulated the link between the metabolic reprogramming of macrophages and the activation of antifungal effector functions.

Results:
Here, we establish fungal melanin as an essential molecule required for the metabolic rewiring of macrophages during infection with *A. fumigatus*. We reveal a molecular link between calcium sequestering by fungal melanin inside the phagosome and the induction of macrophage glycolysis required for efficient innate immune responses. By remodeling the intracellular calcium machinery and impairing signaling via calmodulin, fungal melanin drives an immunometabolic signaling axis towards glycolysis. The contribution of glucose homeostasis to antifungal immunity was further supported by the identification of genetic variants in the glycolytic enzyme PFKFB3 that act as cytokine quantitative trait loci and predispose recipients of stem-cell transplantation to IPA as the result of a deregulated reprogramming of cellular metabolism.

Conclusion:
These data demonstrate a pivotal mechanism in the immunometabolic regulation during fungal infection and highlight the metabolic repurposing of immune cells as a potential strategy to prevent or treat IPA.

NOTE: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.

81 HUMAN LUNG COLONIZATION ENABLES PARASEXUAL RECOMBINATION IN *ASPERGILLUS FUMIGATUS*

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Purpose:
Aspergillus fumigatus is a saprobic fungus that causes a range of pulmonary diseases in humans, some of which are characterized by fungal persistence. Creation of genetic variation is critical for *A. fumigatus* to adapt to the lung environment, but in chronically colonized patients *Aspergillus* survives strictly as hyphae in biofilms. We hypothesized that in chronic colonization genetic variation may be created through parasexual recombination and therefore investigated a large selection of *A. fumigatus* isolates.

Methods:
As diploids are the hallmark of parasex, we screened 799 *A. fumigatus* isolates obtained from patients with cystic fibrosis (CF), chronic pulmonary lung disease, acute invasive aspergillosis and from the environment for spore size. Benomyl sensitivity, nuclear content measurements through fluorescence activated cell sorting and scanning electron microscopy were used to confirm the diploid state of large size spores.

Results:
We identified 11 diploids in our culture collection, exclusively in isolates recovered from six of 11 (55%) CF-patients and from one of 24 (4%) chronic aspergillosis patients, but not in 368 isolates from acute infection and the environment. In addition by performing whole genome sequencing we showed that diploid formation was associated with accumulation of mutations and variable haploid offspring including a voriconazole-resistant isolate.

Conclusion:
The ability of *A. fumigatus* to engage in parasexual reproduction as detected in this study is highly significant for understanding the genetics and biology of *A. fumigatus,* for adaptation and persistence in the human host, as well as for antifungal drug resistance development and management.

82 MUCORICIN IS A MUCORALES RICIN-LIKE TOXIN CRITICAL FOR MUCORMYCOSIS PATHOGENESIS

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Purpose:
Angioinvasion and extensive tissue necrosis are hallmarks of mucormycosis. We previously reported on the ability of dead *Rhizopus* (the most common cause of mucormycosis) to cause damage to host cells *in vitro.* Therefore, we hypothesized that *Rhizopus* harbors a toxin-like substance(s) that is responsible for tissue necrosis often seen with mucormycosis.

Methods:
Purification of toxin-like substances was conducted by *Rhizopus* hyphae extraction followed by fractionation with column chromatography, thin-layer chromatography, HPLC and LC-MS. The ^{51}Cr-release assay was used to evaluate the ability of the fractions to cause damage to alveolar epithelial cells (A549). Cross-reactivity studies were conducted with dot plot, ELISA, and Western blotting techniques using commercially available castor bean holotoxin and generated anti-*Rhizopus* toxin antibodies. The toxicity of the purified toxin was evaluated in mice. Finally, the contribution of *R. delemar* toxin to mucormycosis pathogenesis was determined by generating gene knockdown strains of *R. delemar* and comparing their virulence to the wild-type strain in mice (n=20/group), and by evaluating the role of anti-toxin antibodies in protecting against *R. delemar*-induced murine mucormycosis (n=20/group). Statistical analysis was conducted by the non-parametric Log Rank test for survival studies and by Wilcoxon Rank Sum test for host cell damage comparisons. A P <0.05 was considered significant.

Results:
We identify a ricin-like toxin (RLT) from the order Mucorales which is responsible for the lethal fungal infection, mucormycosis. The hyphae-associated toxin, named mucoricin, has features of ricin including ribotoxic A- and lectin B-chains, and a conserved motif for vascular leak. Anti-RLT antibodies cross-reacts to ricin holotoxin. The purified mucoricin damages human cells *in vitro*, causes hypovolemic shock of intravenously-injected mice, organ necrosis and ultimate death. Further, the anti-mucoricin antibodies prevent host cell damage by ~50% (P=0.002 vs. isotype-matched antibody) and protect mice from mucormycosis (70% survival of infected mice and treated with anti-mucoricin antibody vs. 5% survival of infected mice treated with isotype-matched control antibody, P<0.0001). Finally, inhibition of mucoricin expression by RNAi, compromises the ability of the fungus to damage host cells by ~45% (P<0.0001 vs. wild-type strain) and severely attenuates virulence in mice (median survival time of 6 days and 10% survival for mice infected with *R. delemar* harboring the empty plasmid vs. a median survival time of 21 days and 70% survival of mice infected with mucoricin-attenuated expression strain, P=0.001).

Conclusion:
We identified a ricin-like toxin that is universally present in Mucorales and required for mucormycosis pathogenesis. Apart from the translational aspect of developing a novel treatment to block the function of mucoricin during mucormycosis, our results introduce evidence for the presence of RLT beyond the plant kingdom.

***NOTE*: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.**

83 *ASPERGILLUS FUMIGATUS – STENOTROPHOMONAS MALTOPHILIA* CO-INOCULATION MODEL ON BRONCHIAL EPITHELIAL CELLS: ANTIBIOSIS EFFECT AND INFLAMMATORY RESPONSE ACCORDING TO BACTERIAL STRAINS

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Purpose:
Aspergillus fumigatus (*Af*) and *Stenotrophomonas maltophilia* (*Sm*) are both opportunistic pathogens which are frequently described and positively associated in the pulmonary microbiota of cystic fibrosis (CF) patients. We have previously developed an *in vitro* model of mixed *Af-Sm* biofilm on polystyrene support with clinical reference strains (*Af*13073 and *Sm*13637). The aim of the present work was to develop an *Af-Sm* inoculation model on bronchial epithelium cells (BEAS 2B) with CF clinical strains, in order to study the dynamic of colonization between both pathogens, as well as the cell inflammatory responses to this mixed inoculation.

Methods:
Two *Sm* strains (Sm7 and Sm9) isolated from CF patients were used in association with a clinical *Af* strain (Af-CF). Bacterial strains presented different fitness (Sm7 grew slowly than Sm9). We performed simultaneous inoculations with one (*Af* or *Sm*) or both pathogens (*Af* & *Sm*). qPCR and scanning electron microscopy were used to describe the process of simultaneous co-inoculation. The cellular survival was evaluated by colorimetric lactate dehydrogenase assay and the cell inflammatory response via a dosage of interleukins (IL-6 and IL-8) by ELISA. Sequential microbial inoculations of *Af* and *Sm* were also performed to test the adhesion (first inoculation at 4 h or 15 h + second inoculation at 4 h) and the pathogens growth (6 h + 24 h) after a primo-inoculation of BEAS 2B cells. From 4 h to 6 h, only *Af* conidia or a low *Sm* concentration was observed on BEAS 2B cells, and in 15 h, *Af* hyphae or a high *Sm* concentration was observed.

Results:
Differences of microbial growth according to the microbial combination have been observed. Only Sm9 caused antibiosis effect on Af-CF (30 % decrease in Af-CF biomass and change of hyphae morphology) when there were simultaneous inoculated or after a primo-inoculation with bacteria. However, this fungal inhibition was reduced (10 %) when Af-CF was inoculated 6 h before Sm9. Both *Sm* strains adhered similarly on conidia, Af-CF hyphae or BEAS 2B but a less adhesion of Af-CF conidia on BEAS 2B was observed only when the bacterial concentration was very high (15 h of primo-inoculation). All *Af-Sm* co-inoculations caused a decrease in BEAS 2B lysis compared to monomicrobial inoculations. Both *Sm* strains, in mono and polymicrobial inoculations, induced different inflammatory responses (IL-6 and IL-8 profiles). Indeed, additive effect was only observed with Af CF-Sm9 co inoculation for IL-6 compared to monomicrobial inoculation.

Conclusion:
A mixed inoculation model on BEAS 2B epithelial cells has been developed. The fungal inhibition in presence of Sm9 was not due to a decrease of *Af* adhesion on BEAS 2B, but probably due to the secretion of secondary metabolite(s) by *Sm*. Both *Sm* CF strains do not induce the same cell inflammatory response of BEAS 2B cells in single and co-inoculation *Af-Sm*. Further analyses are needed to confirm these preliminary results, especially using transcriptomic assays to analyze the cell inflammatory response.

84 GENETIC POLYMORPHISM AND MATING TYPE OF *ASPERGILLUS FUMIGATUS* STRAINS ISOLATED FROM CYSTIC FIBROSIS PATIENTS

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Purpose:
Bronchial airways of cystic fibrosis patients may be chronically colonized by different *Aspergillus fumigatus* strains. Through the large genetic exchange, sexual and/or parasexual reproduction favour the adaptation to fungi to a specific environment. These modes of reproduction require the mating-type to be opposite or similar, respectively. This study aimed to analyse the mating type and genotype of *A. fumigatus* strains isolated from CF patients to test the existence of sexual/parasexual cycle in the bronchial airways of those patients.

Methods:
Six children chronically (≥ 4 isolations/year) colonized with *A. fumigatus* and having a positive anti-*Aspergillus* IgG serology were selected. Sixty-nine clinical isolates (6 to 19/child; mean 11± 5) collected during a follow-up from 10 to 33 months were available for testing. In this panel, there were 6 clusters of isolates (2 isolates) collected in a given sample. Also, 2 patients with occasional *A. fumigatus* colonization (≤2 isolations/year), and an alternate of *Aspergillus* species during the follow up were also collected, leading to the collection of 6 isolates. Finally, 10 environmental strains were also characterized. Genotyping was performed by the mean of cell-wall surface protein (CSP) genotyping. The mating-type was determined according to the size of a specific amplified fragment.

Results:
Concerning chronically colonized patients, 79% of their clinical isolates harboured the mating-type *mat1-2* vs 50 % for the strains from occasionally colonized patients and 55% for the environmental strains. For all chronically colonized patients, there was a majority (100% to 53.8%, mean 64.9%) of isolates with the *mat1-2* type. In 7 cases, we detected the presence of two mating-type in an isolate. We found 8 different CSP genotypes, 37 (50%) being of T01 type, followed by 24% of T02 type. Persistence of colonization with strains of similar CSP genotype was detected in the chronically infected patients. In those patients, a period of CSP and *mat* genotype variations can occur. In opposite, different genotypes over time were observed for the 2 occasionally infected patients. In 4 isolates, manual edition supported the superimposition of two chromatographs (T01 plus T02 type or T01 plus T04 type). We found 3 chronically infected patients (out of 6) colonized by *Aspergillus fumigatus* strains which presented the same CSP type but different mating-types during their follow up, suggesting recombination between the strains colonizing a patient.

Conclusion:
This study demonstrates a higher rate of *mat1-2* in *A. fumigatus* isolates collected from bronchial airways of chronically colonized CF patients. This suggests a better adaptation of this mating type to this specific environment. Presence, during the follow-up, of strains with the same CSP and different mating-type lead us to hypothesise that recombination can occur in the bronchial airway of the patients. Characterization of the isolates must be confirmed by microsatellite analysis. Comparative fitness of the *mat1-1* or *mat1-2* isolates must be tested on bronchial epithelial cells as well as the combination of strains with the same or the opposite mating-types compare to the strains alone.

85 THE INTEGRAL MEMBRANE PROTEIN STOMATIN PLAYS AN IMPORTANT ROLE IN PHAGOCYTOSIS OF *ASPERGILLUS FUMIGATUS* CONIDIA

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Purpose:
Aspergillus fumigatus can cause a wide spectrum of acute and chronic diseases. In lung alveoli, resident alveolar macrophages display the initial contact point of inhaled conidia with the innate immune system. The conidial pigment dihydroxynaphthalene (DHN)-melanin plays an important role in the interaction with macrophages. For example, in comparison to melanized wild-type conidia, recognition and phagocytosis of non-pigmented *pksP* mutant conidia by alveolar macrophages is enhanced. In this context the role of lipid microdomains in membranes of macrophages during antifungal defense was characterized. As a member of the SPFH (Stomatin/Prohibitin/Flotillin/HflK/C) protein family stomatin is a major component of lipid rafts and coexpressed with flotillins and caveolins. Proteomic analyses demonstrated an enhanced amount of stomatin in *pksP* conidia-containing phagolysosomes compared to wild-type conidia-containing phagolysosomes. Lipid microdomains are involved in receptor and ion channel regulation. Therefore we investigated the role of stomatin for receptor recruitment, phagocytosis of *A. fumigatus* conidia and lipid microdomain formation in phagosomes.

Methods:
To study the interplay between lipid microdomains and the phagocytosis of conidia, a knockout of stomatin in RAW264.7 macrophages was generated by CRISPR/Cas9 gene editing. The localization of receptors was investigated by fluorescence-activated cell sorting and immunofluorescence staining. Phagocytosis and intracellular processing of conidia was monitored by confocal laser scanning microscopy. Differences in protein levels of $STOM^{-/-}$ cells were determined by western blot analyses.

Results:
Our data indicate that the absence of stomatin in RAW264.7 macrophages decreased the phagocytosis rate of DHN-melanin-deficient *A. fumigatus* conidia. In addition to impaired phagocytosis, membrane localization of the β-glucan receptor dectin-1 was reduced in $STOM^{-/-}$ cells. In contrast, localization of other fungal pattern recognition receptors was not affected. Further analyses revealed that localization of the lipid raft marker protein flotillin depends on stomatin.

Conclusion:
These results provide evidence that stomatin plays a crucial role in lipid microdomain formation and presence of dectin-1 in the plasma membrane and phagolysosomal membrane, and thereby impacts phagocytosis of *A. fumigatus* conidia by macrophages.

86 INHIBITORY EFFECT OF A-BISABOLOL ON GLIOTOXIN PRODUCTION IN *ASPERGILLUS FUMIGATUS* AF293

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Purpose:
Gliotoxin (GLX) is one of the fungal secondary metabolites and an important virulence factor of *Aspergillus fumigatus*, causing invasive aspergillosis. In the present study, the effects of α-bisabolol on GLX production of *Aspergillus fumigatus* was studied in relation to the expression of two essential genes involved in toxin biosynthesis and secretion, *gliP* and *gliA*.

Methods:
A. fumigatus Af239 was grown (10^5 spores/ml) at 100 ml Erlenmeyer flasks including 30 ml Sabouraud Dextrose Broth medium in the presence of serial two-fold concentrations of (62.5-2000 µg/ml) of α-bisabolol at 35º with 180 rpm shaking for 72 h. The culture filtrate was separated from fungal mat by filtration and the toxin was extracted. GLX concentration in the culture medium was measured by high performance liquid chromatography (KNAUER D-14163 UV–VIS system, Berlin, Germany). The amounts of GLX in the unknown sample were measured at wavelength of 254 nm by comparison of the under-curved area with the authentic standards, treated in the same manner. The expressions of *gliP* and *gliA* genes were evaluated by real-time PCR.

Results:
Our data had demonstrated that α-bisabolol strongly suppressed gliotoxin production in the range of 27.26 % to 98.88 % at the 62.5 to 2000 µg/ml of α-bisabolol in comparison to the control (untreated). Moreover, the expression of *gliP* and *gliA* genes was significantly suppressed at different concentrations of α-bisabolol. In maximum concentration of α-bisabolol (2000 µg/ml) the expression of *gliP* and *gliA* genes was 11.43% and 7.17% respectively in comparison with the control.

Conclusion:
These results indicate that α-bisabolol may be considered as a potent candidate to eliminate gliotoxin production in *Aspergillus fumigatus* and α-bisabolol may potentially be considered as a lead compound in designing new antifungal drugs in the future.

Keywords:
Aspergillus fumigatus, gliotoxin, α-bisabolol

87 GENETICALLY DISTINCT TRANSCRIPTIONAL CIRCUITS DRIVE STRESS-ADAPTATION AND HOST CYTOTOXICITY IN THE MAJOR MOULD PATHOGEN OF THE HUMAN LUNG

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Purpose:
Aspergillus fumigatus causes 90% of all *Aspergillus*-related infections. It has been demonstrated that virulence of *A. fumigatus* is multifactorial and several virulence factors have been identified. In particular, the ability of the fungus to respond to various host-imposed stresses has repeatedly been proven to be essential for pathogenicity. Therefore, regulation of genes by transcription factors plays a critical role in establishing infection. However, we have a limited understanding of the molecular basis of *A. fumigatus* responses to environmental stress.

Methods:
Hypothesising that tolerance of host-imposed stresses might explain the dominance of *A. fumigatus* as a pathogen, we compared a compendium of >300 transcriptome datasets to transcriptomics derived from *in vivo* leukopenic and corticosteroid infections. This revealed involvement of iron limitation, hypoxia and adaptation to pH as dominant host-imposed stresses.

Results:
We carried out systematic phenotyping of a genome-wide transcription factor knockout (TFKO) collection of 484 *A. fumigatus* TFKO mutants to define and characterize adaptation to host imposed stresses. A high throughput analytical pipeline was developed in a liquid microculture 96-well plate format to analyze relative fitness under infection-related stress conditions. Fitness was assessed in the presence of iron starvation or hypoxia, at elevated temperature and in response to alkaline stress. This resulted in the identification of 11 previously uncharacterized transcription factors and new phenotypes for previously identified transcription factors. Furthermore, we carried out high-throughput parallel pooled infections in mice to assess pathogenicity. By quantifying relative fungal burdens we enumerated the abundance of individual mutants after several days of infection.

Conclusion:
A highly interconnected network for transcription factors regulating stress adaptation was identified, notably involving 16 of the 22 transcription factors previously reported to drive murine pathogenicity. This network is only linked to transcription factors driving host damage by several core transcription factors, alluding to the environmental origin and coincidental evolution of virulence of *A. fumigatus*.

88 *ASPERGILLUS FLAVUS* BIOMASS IN MAIZE AND USE OF A BIOCONTROL STRATEGY TO LIMIT AFLATOXIN PRODUCTION

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Purpose:
Aspergillus flavus colonisation of maize can produce mycotoxins that are detrimental to both human and animal health. Screening of maize lines resistant to *A. flavus* infection together with a biocontrol strategy could help minimize subsequent aflatoxin contamination.

Methods:
We developed a qPCR assay to measure *A. flavus* biomass and showed that two African maize lines, GAF4 and KDV1, had different fungal loads for the aflatoxigenic isolate (KSM014), fourteen days after infection.

Results:
The qPCR assay revealed no significant variation in *A. flavus* biomass between diseased and non-diseased maize tissues for GAF4 while KDV1 had significantly higher *A. flavus* biomass (P, 0.05) in infected shoots and roots compared to the control. The biocontrol strategy using an atoxigenic isolate (KSM012) against the toxigenic isolate (KSM014) showed aflatoxin production inhibition at the co-infection ratio, 50:50 for both maize lines (KDV1, 99.7% and GAF≥69.4%) as confirmed by bioanalytical techniques.

Conclusion:
As far as we are aware, this is the first report in Kenya where the biomass of *A. flavus* from maize tissue was detected and quantified using a qPCR assay. Our results suggest that maize lines that have adequate resistance to *A. flavus* together with the appropriate biocontrol strategy could limit outbreaks of aflatoxicoses.

89 UBIQUITY, DIVERSITY, AND FUNCTIONAL GENOMICS OF MUCOROMYCOTA AND THEIR BETAPROTEOBACTERIAL ENDOSYMBIONTS

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Introduction:
Zygomycetous fungi in the Mucoromycota, including both human fungal pathogens and environmental isolates, have ancient and intimate associations with bacterial endosymbionts. In particular, *Burkholderia* and *Mollicutes* related bacteria have been studied in plant-associated Mucoromycota fungi. Comparative genomics in select model systems have established that these interactions rely on trade of primary and secondary metabolites (SMs) and use of secretion systems. Endosymbiont presence in Mucoromycota fungi strongly impacts fungal transcriptional regulation, metabolism, cell wall composition, and the expression of transmembrane sensors. Pathogenicity roles for endobacterial secondary metabolites in fungal host interactions have been established and characterized in plant pathogen systems. While the majority of research has been focused on plant associated Mucoromycota, endosymbiont presence has recently been recognized in human fungal pathogenic species, although their role in pathogenesis is yet to be established.

Purpose:
Key questions include: How frequently are endosymbionts present in Mucoromycota fungi? What are the implications of bacterial fungal interactions on the host fungi and their potential for interactions with other organisms? Can we harness knowledge of fungal bacterial interactions to better inform treatment of infections from Mucoromycota fungal species?

Methods:
We probed 638 public and private fungal Mucoromycota genome sequencing projects of both environmental and clinical isolates computationally, to evaluate the ubiquity and identity of endosymbiotic bacteria. We assembled and annotated partial genomes of the putative endosymbionts using the programs Kaiju, Prokka and antiSMASH to evaluate bacterial identity and the diversity of SM gene clusters in fungal endosymbionts in the Mucoromycota.

Results:
We found that 102 isolates, both environmental and clinical, regularly housed putative endosymbionts. The endosymbionts are largely Proteobacteria, however Actinobacteria, Mollicutes, Firmicutes, Bacteroidetes, and Tenericutes were also detected. Over half of the fungal isolates examined (72 of 102) had bacterial DNA belonging to families in the Burkholderiales, with 63 isolates belonging to the genus *Burkholderia*. While some putative endosymbiont genomes had no SM gene clusters predicted (33 of 102), we annotated multiple SM gene clusters in many bacterial endosymbiont genome assemblies, with each isolate averaging 5 SM gene clusters per genome. We found that the on average the *Burkholderia* isolates had more predicted SM gene clusters, averaging 7 per isolate, than bacteria from other lineages which had on average 3 per isolate. Within the SMs *Burkholderia* metabolites predicted, we observed an overabundance of Non-ribosomal Peptide Synthesis (NRPS) gene clusters.

Conclusions:
We found that Mucoromycota fungi from both clinical and environmental settings frequently house endosymbiotic bacteria, often belonging to the genus *Burkholderia* and relatives. We assembled and annotated the genomes of over 100 isolates and found the distribution of secondary metabolite gene clusters, especially NRPS gene clusters to be overrepresented in the Burkholderiaceae isolates.

Products of NRPS gene clusters have been documented to facilitate fungal host interactions in plant pathogenic Mucoromycota fungi, with known antimitotic functions in plants and animal cell lines. Evaluation of homology and conservation between the predicted NRPS gene clusters and those with known products will be conducted and results will be presented.

<u>NOTE</u>: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.

90 CHARACTERIZATION OF G-PROTEIN COUPLED RECEPTOR NOPA IN *ASPERGILLUS FUMIGATUS*

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Purpose:
Aspergillus fumigatus is an airborne fungal pathogen and a leading cause of invasive aspergillosis in immunocompromised patients. The G-protein coupled receptor (GPCR) is a cell surface receptor that receives external signals and activates them to transmit intracellular signals. NopA is assumed to be a bacterial rhodopsin family GPCR, but its exact function in fungi is unknown.

Methods:
To characterize the role of *nopA*, deletion mutants were constructed using the Double-joint PCR method. The radial growth rates of wild type (WT) and Δ*nopA* on solid MMG were measured on 3 days with three replications. To identify the roles of NopA in asexual development, quantitative real time PCR (qRT-PCR) analysis was performed for asexual developmental regulator genes. For determining conidial germ tube formation rate, both strains were inoculated in liquid MMG at 37°C in the presence or absence of glucose. The tolerance of conidia and hyphae against ultraviolet (UV) irradiation was also analyzed at 0, 50, 100, 150, and 200 J^{-2}, respectively.

Results:
Δ*nopA* strain of *A. fumigatus* showed in increased colony size and had lower hyphae density at the colony edge compared to WT strain. The germination rate and asexual sporulation rate were reduced by the loss of nopA. In addition, the mutant strain showed decreased tolerance to UV compared to the WT strain. The levels of mRNA encoding key asexual development (*brlA, abaA, wetA*,and *vosA*) were reduced in Δ*nopA* strain compared to WT.

Conclusion:
These results indicates that *nopA* of *A. fumigatus* is involved in hyphal growth and conidiation and has a positive regulatory role in germination of conidia. In addition, NopA may be involved in UV radiation stress response.

91 A NOVEL RESISTANCE MECHANISM TO CALCINEURIN INHIBITORS IN *MUCOR CIRCINELLOIDES*

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Purpose:
Mucormycosis is a fungal infection caused by Mucorales and recognized as an emerging infectious disease characterized by invasive hyphal growth. We study *Mucor circinelloides* (*Mucor*) as a model genetic system for Mucorales. Previously we have shown that inhibition of calcineurin, a serine/threonine phosphatase, in *Mucor* by using calcineurin inhibitors or deletion of the calcineurin regulatory subunit (*cnbR*) results in a yeast-locked growth. However, *Mucor* can become resistant to calcineurin inhibitors by amino acid mutations in calcineurin components, or by silencing the drug-receptor (FKBP12) gene. Here we identified a novel mechanism through which *Mucor* can also become resistant to calcineurin inhibitors.

Methods:
Whole-genome and targeted sequencing were used to analyze calcineurin bypass mutants. A homologous recombination approach was used to generate *bycA*Δ and *bycA*Δ *cnbR*Δ mutants. cAMP and PKA activity assays were performed to identify the "missing" link (see results) between calcineurin and protein kinase A (PKA). Human umbilical vein endothelial cells and J77A4.A1 macrophage cell lines were used to study *Mucor* – host interactions. *Galleria mellonella* was used as a host to study virulence of calcineurin mutants.

Results:
We analyzed thirty-one mutants that exhibit hyphal growth in the presence of calcineurin inhibitors or in the *cnbR*Δ mutant background with no known mutations in calcineurin components. With whole-genome and targeted sequencing analysis, we found that all the mutants had alteration in a common gene, denoted as *bycA* (bypassing of calcineurin A), which encodes for an amino acid permease. We verified that *bycA*Δ and *bycA*Δ *cnbR*Δ mutants are resistant to calcineurin inhibitor – FK506, thereby demonstrating a novel resistance mechanism. *bycA* expression was significantly elevated when calcineurin function is suppressed, suggesting *bycA* is a positive regulator of yeast growth, and calcineurin transcriptionally suppresses *bycA* expression to promote hyphal growth. PKA is positively associated with yeast growth. Previously we have shown that calcineurin and PKA are inversely related. Here, we found that in the absence of calcineurin, BycA elevated PKA activity in a cAMP-independent manner. We also found that *cnbR*Δ (yeast) and *bycA*Δ *cnbR*Δ (hyphae) mutants undergo phagosome maturation in macrophages, cause less damage to endothelial cells, and exhibit less virulence in a *Galleria mellonella* host, compared to wild type.

Conclusion:
These findings demonstrated that the novel resistance mechanism against calcineurin inhibitors is mediated by a link between calcineurin and PKA. BycA is involved in the *Mucor* dimorphic transition as our data demonstrates a positive correlation between *bycA* expression, PKA activity, and *Mucor* yeast-growth. Calcineurin promotes hyphal growth and suppresses the pathway that promotes yeast growth. In the absence of calcineurin function, BycA activates PKA via a cAMP-independent pathway yet to be elucidated. Our future studies will focus on elucidating how calcineurin regulates *bycA*, and how BycA activates PKA to promote yeast growth. We have also found that it is not morphology but calcineurin that plays a key role in *Mucor* host interactions that contribute to pathogenesis.

92 VIRUS INFECTION OF *ASPERGILLUS FUMIGATUS* (AF) COMPROMISES AF IN INTERMICROBIAL COMPETITION

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Purpose:
Af isolates may carry viruses. The consequences of virus infection have not been completely elucidated. We studied the effect of virus infection on intermicrobial competition, specifically that between *Pseudomonas aeruginosa* (Pa) and Af, the commonest bacterial and fungal pathogens of immunocompromised patients, and of individuals with Cystic Fibrosis.

Methods:
Many studies, over decades, have investigated this Pa-Af competition. We examined methods of interaction classically studied, and others, described below. Reference Pa strain PAO1 was used.

Results:
(i) Af293, a reference Af strain in many laboratories (the whole genome has been sequenced), was studied. One US and one UK Af293 laboratory strain were found, following double-stranded (ds) RNA extraction and virus purification, to be identically infected with Af polymycovirus (AfuPmV)-1. This previously described virus has four dsRNA segments as its genome, which are not enclosed in a protein capsid or lipid envelope. AfuPmV-1 can be transmitted both horizontally, from one Af strain to another, and vertically, from parent to offspring, and was the first virus shown to be infectious as dsRNA.

(ii) The UK strain was also cured of the virus by previously described methods, using the protein synthesis inhibitor cycloheximide in combination with single conidia isolation, as proven by Northern blotting and reverse transcription followed by polymerase chain reaction with virus-specific oligonucleotide primers, yielding virus-free (VF) Af.

(iii) VF was also re-infected using polyethylene glycol (PEG)-mediated protoplast transfection in 3 different experiments, producing 3 new, virus-infected, strains.

(iv) We found planktonic PAO1 supernatant was significantly less inhibitory to VF (a) during Af biofilm formation, or (b) on preformed Af biofilm in RPMI1640 medium (assayed by Af metabolism, XTT), or (c) in growth on agar, compared to the infected strains. Also, (d) PQS (Pseudomonas Quinolone Signal), or (e) Pa siderophore pyoverdin, were each less inhibitory to VF in RPMI1640. Finally, (f) PAO1 volatiles were also less inhibitory to VF on agar. The original UK and US infected Af strains gave identical results to each other. Pa reference strain PA14 was also studied, and gave the same results as with PAO1.

(v) Regarding other properties, iron alone was more stimulatory to VF in RPMI1640. On some agars (especially those with lower iron content), VF grew more robustly and produced fewer conidia. In RPMI1640, VF and infected strains grew equally well. A Pa siderophore mutant, disproportionately producing pyocyanin, produced more VF conidiation than that of infected strains. In contrast, virus infection had no effect on susceptibility to voriconazole, amphotericin, or caspofungin.

(vi) The 3 re-infected Af strains behaved as did the original infected Af strains.

Conclusion:
Virus infection alters Af phenotype. Our studies indicate virus infection weakens Af in intermicrobial competition. These novel observations might also have long-term implications for Af treatment, analogous to phage therapy of bacteria.

***NOTE*: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.**

93 *ASPERGILLUS FUMIGATUS* KNRA IS AN INTRINSICALLY DISORDERED PROTEIN AND FUNCTIONS AS A POTENTIAL SUBSTRATE OF CALCINEURIN AND PROTEIN KINASE A IN THE CELL WALL INTEGRITY PATHWAY

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Purpose:
Calcineurin phosphatase (CN) and protein kinase A (PKA) signaling pathways orchestrate growth and virulence in major clinically relevant pathogenic fungi including *Aspergillus fumigatus*, responsible for life-threatening infections worldwide. Key cellular functions of CN and PKA, particularly in the regulation of hyphal growth and cell wall integrity, make them attractive antifungal targets. Understanding the CN and PKA network of proteins in *A. fumigatus* and the mechanism of how this kinase-phosphatase module regulates critical fungal-specific effectors involved in cell wall biosynthesis and organization will lead to designing novel therapeutic approaches for the treatment of invasive aspergillosis. Here we characterize a cell wall-related protein, KnrA, predicted as a beta-glucan synthesis protein, which we identified as a potential substrate of CN and PKA using advanced whole phosphoproteomics.

Methods:
We utilized the *A. fumigatus* wild-type, calcineurin (Δ*cnaA*) and PKA (Δ*pkaC1*) deletion strains and performed comparative whole phosphoproteomics to identify effectors important for hyphal growth. We generated a Δ*knrA* strain and characterized its phenotype. KnrA tagged to GFP was expressed to investigate its localization pattern during growth and understand the mechanistic basis for its function. Phosphorylation of KnrA was assessed by GFP-Trap affinity purification in the wild-type, calcineurin (Δ*cnaA*) and PKA (Δ*pkaC1*) gene deletion backgrounds. KnrA co-localization studies were also performed with CnaA and the beta-glucan synthase FksA to investigate their potential interaction *in vivo*.

Results:
KnrA was identifed as a potential downstream effector of both calcineurin and PKA in the *A. fumigatus* whole phosphoproteome. Sequence analysis revealed KnrA as a highly disordered protein at its N- and C-terminus. KnrA was phosphorylated at 24 unique sites with several residues showing differential phosphorylation levels between the wild-type, calcineurin (Δ*cnaA*) and PKA (Δ*pkaC1*) gene deletion backgrounds indicating possible roles for PKA-dependent phosphorylation and CnaA-dependent dephosphorylation in KnrA function. Deletion of *knrA* caused radial growth and conidiation defects, and increased sensitivity to cell wall destabilizing agents. Microscopy of Δ*knrA* strain revealed irregular septation and hyphal tip lysis reminiscent of cell wall defects. In accordance with these morphological defects, KnrA-GFP was found localized at the hyphal tips, indicating its importance for hyphal growth.

Conclusion:
Our study provides the first characterization of a cell wall related protein, KnrA, as a downstream effector in calcineurin and PKA signaling in *A. fumigatus*.

***NOTE*: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.**

94 ANALYSIS OF *CYP51A* POLYMORPHISMS OF *ASPERGILLUS FUMIGATUS* IN JAPAN

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Purpose:
Aspergillus fumigatus is the most important *Aspergillus* species that causes chronic pulmonary aspergillosis (CPA) and invasive pulmonary aspergillosis (IPA). Azoles are first-line antifungal drug treatment for CPA and IPA. However, azole resistance in *A. fumigatus* isolates is increasingly recognized as a cause of treatment failure in the last decades. The resistance mechanisms are mostly related to *cyp51A* point mutations in Japan. On the other hand, *cyp51A* mutations were found in about 10% of the azole-susceptible isolates in Europe. These polymorphisms in Europe consist mainly of two combinations of mutations, i. e. combinations of five single nucleotide polymorphisms (SNPs) of *cyp51A* (F46Y, M172V, N248T, D255E, and E427K), referred to *cyp51A*-5SNPs, and combinations of three SNPs of *cyp51A* (F46Y, M172V, and D255E), referred to *cyp51A*-3SNPs. In Japan, little has been known about the prevalence of *cyp51A* polymorphisms of azole-susceptible *A. fumigatus* isolates. Here, we investigated *cyp51A* polymorphisms in *A. fumigatus* strains in Japan and their relation to the susceptibility to azoles.

Methods:
We analyzed 118 environmental isolates and 102 clinical azole-susceptible isolates stored at Chiba University Medical Mycology Research Center (Chiba City, Japan). Genotyping was performed by microsatellite for these isolates, and 20 environmental isolates and 3 clinical isolates were excluded due to identical combinations of short tandem repeats (STRs). We searched for point mutations in *cyp51A* using Surveyor Nuclease (SN) and tandem repeats (TRs), and sequenced *cyp51A* region of the isolates that were expected to have *cyp51A* mutations.

Results:
Among 98 environmental isolates, *cyp51A* polymorphisms were found in 8 isolates (8.2%). N248K was detected in 3 isolates and *cyp51A*-5SNPs was detected in 1 isolate. On the other hand, among 99 clinical isolates, *cyp51A* polymorphisms were found in 15 isolates (15.2%) . N248K was detected in 14 isolates and no *cyp51A*-5SNPs or *cyp51A*-3SNPs was found. The isolate which possesses *cyp51A*-5SNPs had higher MIC of azoles, as the previous reports showed.

Conclusion:
The most prevalent *cyp51A* polymorphism in Japan was N248K in both environmental and clinical isolates. *Cyp51A*-5SNPs, which was reported to be prevalent in Europe, was found in only one environmental isolate. Consequently, there may be differences between the *cyp51A* polymorphisms of *A. fumigatus* in Japan and those in Europe. Furthermore, N248K mutations were more prevalent in clinical isolates than in environmental isolates. Further study is required to clarify the virulence of the isolates with N248K mutations.

95 PATHOGENIC ALLODIPLOID HYBRIDS OF *ASPERGILLUS* FUNGI

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Purpose:
Aspergillosis, the ensemble of diseases caused by species in the genus *Aspergillus*, is responsible for hundreds of thousands of human infections and very high mortality rates. We discovered that clinical isolates originally identified as *Aspergillus nidulans* are in reality isolates of *Aspergillus latus*, a species that our analyses suggest is an allodiploid formed via hybridization of two closely related *Aspergillus* species. Our study suggests that accurate identification of clinical isolates is an important parameter in the design of disease management strategies and that allodiploid hybridization is a novel mode of evolution among filamentous fungal pathogens of humans.

Methods:
We use a combination of whole genome sequencing, evolutionary analyses, and extensive phenotyping (e.g., macrophage phytocytosis, killing of hyphae by neutrophils, antifungal susceptibility, and others) to determine the evolutionary history and phenotypic profiles of *A. latus, A. delacroxii,* and *A. nidulans.*

Results:
We discovered that 6 clinical isolates from patients with aspergillosis originally identified as *Aspergillus nidulans* (section *Nidulantes*) are in reality allodiploid hybrids that formed by the fusion of *Aspergillus spinulosporus* with an unknown close relative of *Aspergillus quadrilineatus*, both in section *Nidulantes.* Further examination revealed that these isolates belong to *Aspergillus latus,* an allodiploid hybrid species, and that they differ from *A. nidulans, A. spinulosporus,* or both in diverse clinically-relevant traits.

Conclusion:
Our study suggests that accurate identification of clinical isolates is an important parameter in the design of disease management strategies and that allodiploid hybridization is a novel mode of evolution among filamentous fungal pathogens of humans.

96 THE DELETION OF *IDOC* GENE IN *ASPERGILLUS FUMIGATUS* AFFECTS ITS NORMAL GROWTH AND RESISTANCE TO DIFFERENT STRESSES

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Purpose:
The development of *Aspergillus fumigatus* mutant strains deficient in a gene, either by complete deletion or by disruption of the sequence, is a classical approach to understand the function that genes fulfill. In this case, we selected the *idoC* gene, involved in tryptophan metabolism, to study its function by constructing a deletion mutant strain because it was detected overexpressed during an intranasal murine infection, and co-incubations with macrophages and lung epithelial cells.

Methods:
Firstly, we compared the transcriptomic results obtained from an *A. fumigatus* intranasal infection using immunosuppressed mice, a co-incubation of *A. fumigatus* and the murine macrophages (RAW 264.7) during 8 hours and the co-incubation of *A. fumigatus* and the human lung epithelium cell line A549 during 8.5 hours. Among all the genes differentially expressed in all abovementioned conditions, we selected the gene *idoC* (Afu7g02010) because of its putative involvement with tryptophan metabolism, an essential amino acid that *A. fumigatus* synthesizes through the shikimate-chorismatase pathway.

The loss of function mutant strain was built by CRISPR-Cas9 technology using the *A. fumigatus* genetic background Af293. The transformation method was carried out using a standard protocol. For the phenotypic spot characterization assays, we used Glucose minimal medium (GMM) agar plates supplemented with Congo red (80 µg/ml), Calcofluor white (80 µg/ml), NaCl (1 M), KCl (1 M), Sorbitol (1.2 M) and SDS (0.0125%). Response to oxidative stress was studied using GMM plates with 200 mM of H_2O_2 added in a central hole of the plate, and germination was determined microscopically after incubation in RPMI supplemented with 10% of fetal bovine serum during 8 hours.

Results:
The transcriptomic analysis showed 103 fungal genes differentially expressed after the intranasal infection, and 2137 and 5325 after the incubations with macrophages and lung epithelium, respectively. Among all of them, we only found nine genes differentially expressed in the three situations studied. Of them, we selected the *idoC* gene that codifies an Indolamine 2,3-dioxigenase to perform a fungal mutant strain.

The CRISPR-Cas9 technique provided a gene-targeting efficiency of 67% using the *A. fumigatu*s genetic background Af293. After spot dilution assays analysis, differences in the fungal growth between the wild type (WT) and the mutant strain (Δ*idoC*) were not found in the presence of Calcofluor white, NaCl, KCl, Sorbitol and SDS, but in contrast, Δ*idoC* was hyper susceptible to Congo red, indicating some misbalance in the fungal cell wall. Despite being a dioxygenase deficient strain, Δ*idoC* was as resistant as the WT to H_2O_2. Finally, after 6 and 8 hours of incubation, the germination ability of the Δ*idoC* strain was significantly lower than the WT strain.

Conclusion:
CRISPR-Cas9 is an accurate technique that allowed us to generate, with a great efficiency, the mutant strain Δ*idoC*. This mutant showed inability to grow in presence of Congo red as well as to germinate adequately. Future studies will explore in deep the role of *idoC* in animal models of IA.

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97 EPIGENETIC MECHANISMS OF AZOLE STRESS ADAPTATION IN *ASPERGILLUS FUMIGATUS*

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Background:
Azole drugs, such as voriconazole, represent the first-line treatment of invasive aspergillosis. Emergence of azole resistance among *Aspergillus fumigatus* is a concern and has been mainly attributed to mutations in genes of the ergosterol biosynthesis pathway (*cyp51A, hmg1*). Our objective was to investigate epigenetic (i.e. non mutation-related) mechanisms of azole stress adaptation in *A. fumigatus*.

Methods and results:
An azole-resistant *A. fumigatus* strain (Ku80R) was generated following consecutive exposure of the parental wild-type strain (Ku80) to sub-inhibitory concentrations of voriconazole. The minimal inhibitory concentration (MIC) of voriconazole was 4 µg/mL in Ku80R (compared to 0.25 µg/mL in Ku80). Cross-resistance to other azoles (posaconazole, isavuconazole) was observed. No mutations were found in Ku80R azole target genes (*cyp51A* and *hmg1*) and azole resistance was partially reversible suggesting epigenetic stress adaptation. Transcriptomic analyses revealed overexpression of drug-transporters of the ATP binding cassette (ABC: *abcB*, *abcC*, *abcD*, *atrI, mdr1*) and major facilitator superfamily (MFS: *mfsC, mdrA*), as well as genes of the ergosterol biosynthesis pathway (including *cyp51A*) in Ku80R.

Mass spectrometric analysis of sterol composition of the cell membrane did not show any difference between Ku80 and Ku80R. ABC transporters activity measured by rhodamine 6G was higher in the resistant strain compared to the wild-type strain and use of an ABC transporters inhibitor, milbemycine oxime (MLB), could decrease transporters activity in both Ku80 and Ku80R. The interaction of MLB with azoles was synergistic for posaconazole (fractional inhibitory concentration: 0.25), but not for voriconazole (0.75) and isavuconazole (0.625).

Conclusion:
Drug transporters play a role in adaptive stress response to azole drugs, which may lead to clinically relevant azole resistance in the absence of *cyp51A* mutations. Targeting drug transporters may represent a novel adjuvant therapeutic approach of invasive aspergillosis.

98 INVESTIGATION OF ADAPTATION TO HYPOXIA IN *ASPERGILLUS FUMIGATUS*

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Purpose:
Aspergillus fumigatus is a major cause of invasive fungal infection in human being. It is conceivable that the fitness in hypoxia is one of important traits for its virulence. However, the phenotypic and genomic changes during adaptation to hypoxia remain unknown. Here, we aim to investigate how *A. fumigatus* adapts to the hypoxia condition.

Methods:
Using six A. fumigatus strains including two laboratory-used strains (Af293 and KU) and four clinical strains with different fitness in hypoxia, we generated the hypoxia-adapted strains (EVOL20) by passing conidia for 20 generations, i.e., a total of 80 days under the hypoxia condition. The phenotypes and genomes were compared between the EVOL20 and original strains.

Results:
We observed that EVOL20 strains exhibited more resistant to hypoxia compared with the original strains. Besides, *A. fumigatus* gained the other traits like cell wall integrity and resistance to hydrogen peroxide along with the adaptation to hypoxia. Interestingly, we found that the adaptation trajectory among different strains is heterogeneous.

Conclusion:
We managed to illustrate the underlying genomic mechanism of hypoxia adaptation of *A. fumigatus,* and confirmed the relationships between hypoxic fitness and other properties during adaptation in the host environment.

99 STUDY ON GENE EXPRESSION OF NA^+-ATPASE ENCODING GENE *ENAA* DURING STRESS RESPONSE IN *ASPERGILLUS FUMIGATUS*

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Introduction:
Similar to other living organisms, the opportunistic fungal pathogen *Aspergillus fumigatus* requires certain cations as essential cofactors for many important signaling pathways. Nevertheless, high concentration of ions is toxic and can induce stress conditions, probably by interfering with functions of macromolecules in the cells. Therefore, a tight regulation of ion homeostasis is essential for survival and will allow fungal growth under a wide range of environmental conditions. In the yeast *Saccharomyces cerevisiae*, the P-type Na^+-ATPase Ena1p is crucial for response to salt-stress and growth under alkaline environment [1]. In *Aspergillus nidulans*, the homologous gene *enaA* has been identified as regulator of fungal tolerance to cationic and alkaline stress [2-3]. Unlike in *Saccharomyces cerevisiae* and *Aspergillus nidulans*, the physiological role of P-type Na^+-ATPase in *Aspergillus fumigatus* has not been well investigated. This study aims to investigate the involvement of *Aspergillus fumigatus* P-type Na^+-ATPase encoding gene *enaA* in response to cationic, osmotic and alkaline stress.

Methods:
To assess expression of the gene *enaA* under tested stress conditions, *Aspergillus fumigatus* isogenic strain AF293 was exposed to high concentration of Na^+, K^+, Ca^{2+}, Mn^{2+}, sorbitol and alkaline pH for 20 minutes prior to RNA extraction and quantitative real time PCR.

Results:
The results revealed that expression of *enaA* was significantly increased when *Aspergillus fumigatus* was exposed to high Na^+, Ca^{2+}, Mn^{2+} and alkaline pH,

Conclusion:
The gene *enaA* has probably multiple role in stress responses, including cationic and alkaline stress.

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100 NAVIGATING BARRIERS TO UNDERSTAND EARLY ENDOSOME TRAFFICKING IN *ASPERGILLUS FUMIGATUS*

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Purpose:
Filamentous fungi have long served as model systems to study the microtubule-based trafficking of early endosomes within polarized cell types. Though this trafficking has been implicated in a number of varying functions, the effects it has on fungal physiology remain poorly understood. We set out to understand the importance of early endosome trafficking for polarized growth and pathogenesis in the opportunistic human pathogen *Aspergillus fumigatus*.

Methods:
To generate mutants with perturbed endosome trafficking, we deleted the gene encoding for FtsA, a protein which links early endosomes to microtubule-based motor proteins. After confirming endosome perturbation via TIRF microscopy, we used microfluidics-based approaches to investigate how individual hyphae navigated obstacles. This allowed us to gauge the importance of early endosome motility for contact-induced hyphal branching. Finally, we used an invertebrate infection platform to investigate how perturbing early endosome motility affects virulence.

Results:
In the FtsA knockout, we found a near-complete perturbation of early endosome motility that led to a dramatic clustering of the endosomes at the hyphal tip. This disruption led to changes in colony morphology and altered growth behaviors as hyphae navigated microenvironments. In spite of these differences, the perturbation of early endosome motility did not exhibit an effect on pathogenicity in the invertebrate infection model.

Conclusion:
We have shown that losing the ability to transport early endosomes causes individual hyphae to have different growth patterns in response to obstacles presented in controlled microenvironments. Given the conserved nature of early endosome trafficking in polarized cells, understanding how it affects growth could have important implications for the study of both pathogenic fungi and neurons.

101 FUNGAL CONTAMINATION RATES OF WATER IN LAGOS UNIVERSITY TEACHING HOSPITAL WATER DISTRIBUTION SYSTEMS

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Purpose:
Reports of outbreaks of invasive fungal infections due to fungal contamination of hospital water have been documented. However, water safety and quality regulations commonly use bacterial parameters as indicators. Based on the foregoing, the water distribution systems of Lagos University Teaching Hospital, Nigeria and retailed/commercial brands of water procured for patients' consumption within the hospital were assessed for fungal contamination.

Methods:
105 water samples were collected from hospital units categorized into; (1) High risk (Theatres, ICUs, Renal Dialysis Centre, CSSD unit); (2) Medium risk (Acute Stroke Unit, Accidents and Emergency Unit) and (3) Low risk unit (Wards, Children Emergency Unit). Representative samples were collected from taps, showers heads, faucets and water storage tanks. 11 brands of retailed drinking water were also analyzed. The membrane filtration method for water analysis was used. 100 mL of water sample was filtered through 0.45µm pore size, 47mm diameter membrane filter. Filters were placed on SDA plates supplemented with gentamycin and chloramphenicol. Plates were incubated at room temperature and at 37°C. Pure colonies from subculture were identified using microscopic and colony morphology. Isolates that could not be identified were stored away for future molecular identification.

Results:
All (100%) water samples analyzed contained fungal contaminants. 230 isolates were documented. The predominant genus was *Aspergillus* with 60 isolates & 5 species identified including *Aspergillus fumigatus* (7.8%), *flavus* (7.0%), *niger* (7.0%), *terreus* (3.0%), and *versicolor* (0.9%). 31 fungi species of the Zygomycota and Ascomycota phyla were identified, while some were unidentified (4.8%: 11 isolates) due to non-sporulation and poor distinguishing features. 28 fungi species were identified in the hospital, while 7 were identified from retailed drinking water. 24 species were identified from the high risk units. Fungi of the order Mucorales identified include *Rhizopus stolonifer* (3.5%), *Mucor* spp (2.2%) and *Cunninghamella* spp (0.9%). *Aspergillus fumigatus* (7.8%) was the most abundant, while the least abundant were *Curvularia* spp (0.4%), *Cladophialophora* spp (0.4%) and *Nocardia* spp (0.4%). Other identified species include, *Rhizoctonia solani* (1.7%), *Candida albicans* (7.0%), *Candida tropicalis* (7.0%), *Penicilium* spp (6.1%), *Rhodotorula* spp (5.2%) and *Trichoderma viride* (0.9%).

Conclusion:
The absolute rates recorded in this index study points to water as a plausible source of infections in hospitals which in most cases are often neglected with regards to fungi, due to paucity of reports of outbreaks of waterborne fungal infections. The abundance of *Aspergillus* species highlights risk of aspergillosis, as a result exposure due to aerosolization of spores, while those of the order mucorales indicate a possible source of mucormycosis in the hospital. Thus, a standard protocol for monitoring and regulation of fungi in water needs to be developed particularly for hospitals as a means of infection prevention and control. Studies need be conducted to determine link between fungal water contamination and hospital acquired infections.

Keywords:
Fungi, *Aspergillus*, *Mucor*, water contamination, fungal contaminants, hospital water, aspergillosis, mucormycosis

102 PROBABLE HOSPITAL SOURCES OF *ASPERGILLUS* SPECIES ISOLATED FROM BEDFAST CASES

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Purpose:
Invasive aspergillosis has a mortality rate of 90%, among the *Aspergillus* infections. Incidence rate of invasive *Aspergillus* infections is about 17-26%, 5-24% and 5-15% in lung transplant, acute leukemia and allogenic bone marrow transplant patients respectively. The aim of this study was finding sources of nosocomial infections via identification of *Candida* and *Aspergillus* species and other fungi isolated from hospital, clinical and environmental specimens.

Methods:
Our subjects included clinical specimens of case with HAI which collected during 24 month from October 2014 to September 2016 at the UMSU educational hospitals, Urmia, Iran. Totally 256 hospital indoor specimens were collected from bed rooms, floor, walls, trolleys, sink, medical devices and air in adition of finger touch and body surface cases, personal and visitors, were collected. All samples were transported to the Medical Mycology Center, School of Medicine, UMSU. A primary diagnosis including, direct microscopic preparations of the clinical specimens performed for the detection of probable fungi causing HAIs. Morphologic identifications were conducted using sabouraud glucose agar (SGA 4%), chapek yeast extract agar (CYA). The molecular test PCR-RFLP was performed to confirm the identifications and molecular typing of environmental isolates. The restriction enzyme *Mwo*I was used for this digestion. Matching the findings of clinical and hospital *Aspergillus* isolations, a typing method of random amplified polymorphic DNA was used.

Results:
During 24 month, August 2014 to September 2016, totally 198 samples were obtained from cases with proven HAI. The results of experimental studies on the specimens showed 93(47%) positive for a fungal or bacterial infections from the above case, 54(58%) had a fungal infection.

Total of isolated fungi *Candida* 36(66.6%), *Aspergillus*, 17(31.4%) and *Alternaria* just one case was identified. The fungi isolated from environments included: *Candida* and *Aspergillus* species and other molds: *Alternaria, Saccharomyses, Penicillium, Cladosporidium* and fungi order Mucoral. Total of fungal isolates, 35(31.5%) were *Candida*, 48(43.2%) *Aspergillus* and the other isolated fungi 28(20.3%). Among all yeasts isolated from cases, personnel and visitors *Candida albicans* (60%), *C. krusei* (17%), *C. glabrata* (14.3%), *C. tropicalis* (0.7%) and *C. parapsillusis* (3%) were identified as well as *Aspergillus* isolated from hospital, indoor surfaces including: *A. niger* (43.7%), *A. flavus* (41.8%), *A. fumigatus* (14.7%). Some *Aspergillus* isolates of clinical specimens were commonly matched with those of hospital indoor.

Conclusion:
Although most of the yeast infections sourced by patients skin and mucosa, *Aspergillus* species causing hospital acquired infections probably sourced by hospital indoor places close to beds.

103 ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS, *ASPERGILLUS FUMIGATUS* CHRONIC COLONIZATION AND CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR GENOTYPE; A COMPLICATED RELATIONSHIP IN CYSTIC FIBROSIS PATIENTS

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Purpose:
The clinical spectrum of *Aspergillus fumigatus* diseases in cystic fibrosis (CF) patients, including allergic bronchopulmonary aspergillosis (ABPA) and *Aspergillus fumigatus* chronic colonization, has recently gained attention due to its association with the progression of lung disease. Moreover, several studies have evaluated the role of cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations on the colonization of respiratory system with bacteria. However, a possible correlation of *CFTR* gene mutations with ABPA and *A. fumigatus* chronic colonization has not been clarified yet.

Methods:
Greek CF children patients diagnosed with ABPA or/and *A. fumigatus* chronic colonization were grouped according to their *CFTR* genotype. Patients with "minimal" *CFTR* function were defined as carrying a combination of class I, II or III mutations, while patients with "residual" function as carrying at least one class IV, V or VI mutation. A number of parameters were also collected from patients' medical records.

Results:
Forty-nine CF children were included in the study. Twenty-nine patients were diagnosed with ABPA, 16 were chronically colonized by *A. fumigatus* and 4 patients had a history of both ABPA and *A. fumigatus* chronic colonization. Among the 98 *CFTR* alleles of the study sample, 12 different *CFTR* mutations were detected. P.Phe508del mutation was the most common (n=60, 61.2%), followed by c.489+1G>T (n=10, 10.2%) and G542X (n=4, 4.1%) mutations. When the type of class mutation was examined, 63 (64.3%) mutations belonged to class II, and 24 (24.5%) to class I. No significant differences were detected among patients who were diagnosed only with ABPA (n=29) and those who had only a positive history of *A. fumigatus* chronic colonization (n=16). The median age of ABPA diagnosis was significantly lower than the median age of *A. fumigatus* chronic colonization (P=0.011), while no significant difference was detected on median FEV_1 % predicted among two groups.

Conclusion:
This is the first study that evaluated the relationship between *CFTR* genotype, *A. fumigatus* chronic colonization and ABPA among Greek CF children patients. No significant differences were detected in the type of *CFTR* mutation among patients who were diagnosed only with ABPA and those who had only a positive history of *A. fumigatus* colonization.

104 THE CLINICAL SPECTRUM OF *ASPERGILLUS FUMIGATUS* DISEASES IN CYSTIC FIBROSIS

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Purpose:
Cystic fibrosis (CF) patients suffer mainly from recurrent respiratory infections. Recently, attention has been paid to the clinical significance of fungal isolation in patients' respiratory secretions, such as *Aspergillus* species. CF patients might a] be colonized by *A.fumigatus* for a short (transient carrier) or a long period (chronically colonized), b] suffer from bronchitis due to *A. fumigatus*, c] be sensitized to *A. fumigatus* and/or d] develop allergic bronchopulmonary aspergillosis (ABPA). Cases of aspergilloma and invasive aspergillosis have been rarely reported. Our aim was to study the spectrum of *Aspergillus fumigatus* diseases among CF children patients at CF center of "Aghia Sophia" Children's Hospital.

Methods:
Medical records of 207 CF patients were studied retrospectively for a 20-year period in order to identify cases that belong to the spectrum of *Aspergillus fumigatus* diseases.

Results:
Thirty-six 36 (17.3%) CF patients were colonized transiently by *A.fumigatus*, while25 (12.1%) CF patients were chronically colonized. Sixty-one (29.5%) patients were sensitized to *A. fumigatus*, while 34 (16.4%) patients were diagnosed with ABPA durning a 20-year period. Four (1.9%) patients experienced bronchitis due to *A. fumigatus* and 2 (0.9%) patients developed lung abscess due to *A.fumigatus*.

Conclusion:
To the best of our knowledge, our study revealed for the first time, the frequency of *A. fumigatus* isolation in respiratory samples as well as the frequency of sensitization to the fungi among Greek CF children patients. Further studies are needed in order to clarify the necessity of antifungal treatment in asymptomatic cases that belong to the spectrum of *Aspergillus fumigatus* diseases.

105 *ASPERGILLUS TERREUS* INFECTION IN A PATIENT WITH CARDIAC IMPLANTABLE ELECTRONIC DEVICE

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Purpose:
Rapid identification of *Aspergillus terreus* from infected tissue and pacemaker leads by MALDI-TOF.

Methods (Case report):
A 55 year old man presented in the emergency with fever for 7 days, redness and discharge from pacemaker pocket site. The patient had a history of essential hypertension for 12 years on multiple antihypertensive drugs. In 2001 the patient permanent pacemaker was placed in view of complete heart block. The patient was asymptomatic till 2010 when he presented with pocket site infection for which lead extraction was performed. Culture of the lead grew *Staphylococcus aureus* for which he was treated. In 2015, the patient presented with bacterial endocarditis, causative organism being *Staphylococcus aureus* for which he was treated with vancomycin.

Chest X-ray revealed cardiomegaly and transthoracic echocardiogram showed restrictive cardiomyopathy with pulmonary artery thrombus with multiple vegetations.

The patient underwent pulmonary artery thrombectomy, pacemaker removal and necrosed tissue removal. The necrosed tissue and the pacemaker leads were sent to the Microbiology Laboratory for bacterial and fungal culture, of which bacterial culture was negative. The sample was sent in wide mouth screw capped sterile container which contained black, necrosed, blood tinged tissue in saline. On performing direct microscopy with calcofluor white-Koh mount septate hyphae was visualized. Based on this finding clinicians started the patient on intravenous amphotericin-B. On culturing the sample on saboraud's dextrose agar, white downy growth with white border was seen within 5 days which turned to cinnamon brown later, on further incubation. Lactophenol cotton blue mount on initial growth didn't show any sporulation so the report was given as *Aspergillus species*. From this initial growth identification by MALDI-TOF was done, which turned out to be *Aspergillus terreus*. Serum galactomannan was 2.83.

IV amphotericin was immediately stopped and the patient was started on voriconazole 400 mg twice a day for 6 weeks after which the wound healed and cultures were found to be negative. After the infection subsided, new pacemaker implant was placed for the patient.

Conclusion:
CIED pocket infection must be suspected when there is continuous fever and signs of inflammation over the pocket skin (e.g. reddish discolouration, purulent discharge) is seen. The most common causative organism is *Staphylococcus aureus and coagulase negative staphylococcus*, but unusual organisms such as in our case *Aspergillus terreus* must not be ruled out.

The importance of identifying till the species level is important since treatment of the patient changes as in our case. Rapid diagnostic modalities as in our case, MALDI-TOF helped to identify the organism and change the treatment. Strict aseptic precautions must be followed in taking care of the pacemaker pocket to avoid reinfection. Pacemaker removal and appropriate antifungal therapy remains the mainstay of treatment.

106 MUCORMYCOSIS: EMERGING AND DEVASTATING FUNGAL INFECTION, CONTINUING FURTHER ONE-YEAR EXPERIENCE FROM TERTIARY CARE HOSPITAL, NORTHERN INDIA

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Purpose:
Mucormycosis is an acute angioinvasive emerging and devastating infection caused by fungi belonging to order Mucorales. This is the third most common opportunistic fungal infection after candidiasis and aspergillosis. It is categorized into six clinical types: rhino-orbito-cerebral, pulmonary, cutaneous, gastrointestinal, disseminated and isolated renal mucormycosis. The fast-growing aseptate hyphae being angioinvasive, invade blood vessels entailing infarction and necrosis. In developing countries like India, it is seen mainly in patients with uncontrolled diabetes mellitus, unlike developed countries where it is seen among patients with underlying hematological malignancies. The study was conducted over a period of one year from November 1, 2018 to October 31, 2019 to understand the extent of clinico-epidemiological profile of further continuing over the ongoing disease in a tertiary care north Indian hospital.

Methods:
Among the suspected mucormycosis patients, nasal crust in rhino-orbito-cerebral, sputum/BAL in pulmonary, necrotic tissue in cutaneous, anterior chamber aspirate in ocular and stomach biopsy in gastrointestinal infection were processed for mycological etiology. The direct demonstration of fungus was done by KOH/CFW wet mounts. Histopathology was done by H&E, PAS and GMS stainings. Fungal culture was done on SDA, blood agar, BHIA as per standard mycological protocol. Morphological identification was done by lactophenol cotton blue mount. Further identification of isolates was done by sequencing of ITS region, comparing with those of type strains. Antifungal susceptibility testing was performed for amphotericin B, posaconazole, itraconazole and terbinafine as per the CLSI M38-A2 document.

Results:
A total of 32 cases of mucormycosis were reported during this one-year period. Of these, 22 were males and 10 were females patients. Most common presentation was rhino-orbito-cerebral (12), followed by cutaneous (10), pulmonary (8), ocular (1) and gastric (1). Diabetes mellitus was the underlying risk factor in eleven cases of rhino-orbito-cerebral; while only DM (4), DM with trauma (1), bedridden with pelvic fracture (1) were the risk factors in cutaneous. In pulmonary disease the risk factor was diabetes mellitus (5). The sole gastric patient was also having diabetes mellitus. Isolates identified were *Rhizopus arrhizus* (6) and *Apophysomyces variabilis* (2) in rhino-orbito-cerebral infection; *A. variabilis* (4), *Rhizopus homothallicus* (1), *Mucor indicus* (1) in cutaneous infection and *R. homothallicus* (1), *R. arrhizus* (1), *Lichtheimia corymbifera* (1) in pulmonary mucormycosis. A solitary case of ocular mucormycosis was seen in diabetic patient. All patients were treated with liposomal amphotericin B with extensive surgical debridement. On AFST of total 17 isolates, amphotericin B was the most sensitive drug and posaconazole was judged as the next best agent for salvage therapy.

Conclusion:
Mucormycosis is a growing menace in tropical country like India, which provides potential environmental niche for the survival of these fungi. Now, India is being termed as the diabetic capital of the world with an exponential increase in susceptible population, mucormycosis is also being encountered at an alarmingly increasing level. It is a life-threatening condition invariably proves to be fatal in a short span of time. Hence an early diagnosis, reversal of underlying risk

factors, prompt institution of appropriate antifungal therapy and extensive surgical debridement are the key maneuvers for an apt management. The battle with this dreaded infection continues and should never be given up to save the population at risk.

107 CLINICAL SPECTRUM AND ANTIFUNGAL SUSCEPTIBILITY PROFILE OF *ASPERGILLUS TERREUS* SPECIES COMPLEX: A SINGLE CENTRE STUDY FROM INDIA

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Purpose:
The *Aspergillus terreus* species complex is found in a wide variety of habitats, and the spectrum of diseases caused covers allergic bronchopulmonary aspergillosis, *Aspergillus* bronchitis and/ or tracheobronchitis, and invasive and disseminated aspergillosis. Invasive infections are a significant cause of morbidity and mortality mainly in immunocompromised patients. Infections due to *Aspergillus terreus* appears to be an emerging cause of infection at our institution. Thus the epidemiology, susceptibility profile and outcome of *A. terreus* infections over a period of two years (April 2017 to March 2019) was assessed.

Methods:
This was a prospective study where all cases of proven and probable invasive aspergillosis (IA) due to *A. terreus,* according to the EORTC/MSG criteria, were included. Demographic details, radiological findings, clinical presentation, laboratory investigations were noted. Antifungal susceptibility testing was performed by the broth microdilution method as per CLSI M38-A2. Outcome was assessed.

Results:
A total of 30 patients had *A. terreus* infection. The median age was 40.5 years (range 5 months to 78 years). The male to female ratio was 3:2. Of the 30 patients, *A. terreus* was isolated from 28 respiratory samples, 1 each from pus and tissue biopsy. Cystic fibrosis (07/30, 23.3%) followed by non-cystic bronchiectasis (04/30, 13.3%), two cases each of silicosis and COPD were the most common underlying diseases. Fourteen patients (14/30, 46.6%) received high dose of steroids. Isolates were susceptible to all the antifungal drugs that were tested. Eleven patients received voriconazole as the sole antifungal agent, nine received only amphotericin B, voriconazole plus fluconazole in two patients, and voriconazole plus amphotericin B in one patient. A total of nine patients had a fatal outcome.

Conclusion:
Aspergillus terreus infections are being increasingly reported from our centre, Cystic fibrosis and other structural deformities of the lung are important underlying diseases. Patients on high dose steroids were predisposed to infections due to *A. terreus*. There was around one third mortality (9/30). As *A. terreus* is inherently resistant to amphotericin B, correct identification is a key factor in management of these patients.

108 PREVALENCE OF *ASPERGILLUS FUMIGATUS* AND *ASPERGILLUS FLAVUS* IN SOIL OF AGRICULTURAL FIELDS IN NEPAL

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Purpose:
Aspergillus species are found worldwide and present in soil and decaying organic matter. Conidia of pathogenic *Aspergillus* species can be dispersed to ambient air and inhalation eventually cause infection in a susceptible host particularly immunocompromised patients. *Aspergillus fumigatus* is the global leading agent of invasive aspergillosis (IA) as well as other forms of aspergillosis, including allergic bronchopulmonary aspergillosis (ABPA), chronic pulmonary aspergillosis (CPA) and aspergilloma. *A. flavus* is the second common agent of IA and also common cause of fungal rhinosinusitis and fungal keratitis. *Aspergillus* infections have been reported frequently in Nepal. In this study, different pathogenic *Aspergillus* species were isolated from soil of agricultural fields in Nepal and prevalence of *A. fumigatus* and *A. flavus* was determined in soil environment.

Methods:
A total of 168 soil samples from agricultural fields, including soil from paddy/ wheat/ buckwheat/ maize/ mustard fields, vegetable fields and tea gardens, of Jhapa (n=56), Sunsari (n=48), Chitwan (n=32) and Rupandehi (n=32), Nepal, were investigated during January 2016-February 2019. For isolation of *Aspergillus* species, two grams of soil sample was suspended in 8 mL of NaCl (0.85%), vortexed and allowed to settle for 30 seconds. Then, the suspension was diluted 1:10 and 100 µL of diluted suspension was plated in triplicate on Sabouraud dextrose agar (SDA) plates with chloramphenicol (50 mg/L) and incubated at 37°C for 48 hours. The growth of *Aspergillus* species isolates were identified to species complex level by macroscopic and microscopic culture morphology. *A. fumigatus* isolates were further identified by growth at 50°C (Snelders et al., 2009; Chowdhary et al., 2012).

Results:
Of 168 soil samples, 118 (70.2%) samples showed the presence of *Aspergillus* species and 365 colonies of *Aspergillus* species were isolated from soil of agricultural fields in Nepal. Among these *Aspergillus* species isolates, 16.2% of *A. fumigatus*, 24.6% of *A. flavus,* 31.3% of *A. niger* and 27.9% of *A. terreus* were obtained from soil samples. The highest rate of isolation of *A. fumigatus* and *A. flavus* was observed in soil from wheat fields followed by soil from paddy fields.

Conclusion:
A. flavus was more prevalent than *A. fumigatus* in soil of agricultural fields in Nepal. The predominance of *Aspergillus* species in soil has shown a high risk of *Aspergillus* infections on exposure to fungi in agricultural fields.

109 DIAGNOSIS OF FEMALE GENITAL TUBERCULOSIS USING XPERT MTB/ RIF

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Purpose:
A case report.

Material and Methods:
For microscopic examination of acid-fast bacilli (AFB), biopsy material was ground well using homogenizer and the concentrated mix was taken for smear and was stained with Ziehl-Neilsen stain. Xpert MTB/RIF was performed on homogenized tissue, briefly 1ml of sample was lysed with 2ml of reagent buffer (1:2 ratio).

Results:
ZN stained smears were negative were negative for AFB. Xpert MTB/RIF was positive for *M. tuberculosis* and rifampicin sensitive. Histopathology report showed presence of caseating granulomas surrounded by epitheloid cells, lymphocytes, plasma cells and giant cells of tuberculosis. Patient was started with anti-tubercular treatment (ATT).

Conclusion:
Endometrium tissue from suspected genital tuberculosis patients should be used for screening of tuberculosis using Xpert MTB/RIF in order to rule out tuberculosis for initiation of ATT.

110 CLINICAL EVALUATION OF CHRONIC PULMONARY ASPERGILLOSIS IN PATIENTS WITH NONTUBERCULOUS MYCOBACTERIAL LUNG DISEASE

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Purpose:
To clarify clinical features of chronic pulmonary aspergillosis(CPA) in patients with nontuberculous mycobacterial lung disease (NTM-LD).

Methods:
We retrospectively investigated the medical records of 64 patients with CPA treated with the antifungals in Tokyo National Hospital from October 2013 to September 2018, who had been previously or simultaneously diagnosed with NTM-LD.

Results:
The patients' median age was 70.5 years. The major underlying diseases other than NTM-LD and CPA were old pulmonary tuberculosis and chronic obstructive pulmonary disease. The causative NTM species included *Mycobacterium avium complex* (MAC;82%) and *Mycobacterium kansasii* (8%).Many of NTM-LD patients were severe cases, exhibiting cavitary lesions (90%) and both sides of the lung involved (90%) in the radiological findings, and presenting smear-positive in a quarter of cases at the time of diagnosis of NTM-LD combined with CPA. At the time of combined diagnosis of the diseases, fever and hemoptysis were common symptoms. The imaging demonstrated the thickened walls of cysts and cavities in half of the cases and infiltration also in half. Twenty-six cases have already died by April 2019; with median survival time of 12 months after the diagnosis of the combined diseases.

Conclusion:
Since many of CPA patients in association with NTM-LD were severe with poor prognosis, comorbid pulmonary aspergillosis should be noted as early as possible in severe cases of NTM-LD.

111 RE-ADMINISTRATION OF VORICONAZOLE AFTER HEPATIC TOXICITY

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Purpose:
Infections caused by *Aspergillus* species are often life-threatening. Drugs effective for *Aspergillus* infection are limited compared to other bacterial infections. As a result, we sometimes need to struggle through various adverse effects without switching to other agent. Voriconazole is one of the most important drugs for the treatment of *Aspergillus* infection. Around 16.9-50.1 % of patients are reported to experience abnormal liver function tests and 2.8-50.1 % of them had to stop the drug administration. The monitoring of serum concentration is the first thing to do in case of liver toxicity. However, we sometimes experience liver toxicity even though the serum concentration of voriconazole is within the normal range. In the treatment of tuberculosis, we regularly re-challenge same drugs after abnormal liver function test, and 70-90% of them are reported to succeed. We report cases successfully re-administered voriconazole after liver toxicity.

Methods:
We retrospectively evaluated three patients to whom voriconazole was re-administered after liver toxicity between April 2006 and March 2019.

Results:
All patients succeeded in the re-administration of voriconazole.

Conclusion:
Re-administration of voriconazole can be one of the options after the appearance of voriconazole-induced liver toxicity.

112 FUNGAL BURDEN IN A CLINICAL PATHOLOGY SERVICE OF A CENTRAL HOSPITAL IN LISBON

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Purpose:
The indoor environment of hospitals is complex and different from any other indoor environment. Mycobiota presence has been reported as a potential cause of hospital infections. This study aims at characterizing fungal burden in one Clinical Pathology Service applying different sampling methods and using both culture and molecular-based approaches.

Methods:
Sampling was based either on swabbing ventilation grids from 15 sampling locations or the use of 16 electrostatic dust collectors (EDCs), where dust was settled for 15 days. Both matrixes were extracted and streaked onto MEA and DG18. The frequency of azole-resistance was determined in EDC samples in plates containing Sabouraud media supplemented with 4 mg/L itraconazole (ITRA), 1 mg/L voriconazole (VORI), and 0.5 mg/L posaconazole (POSA). After incubation at 27ºC for 5 to 7 days, fungal densities (CFU/m^2) were calculated and identification was performed. Molecular identification of the different fungal sections/species was achieved in the same samples by quantitative PCR (qPCR) using the CFX-Connect PCR System (Bio-Rad).

Results:
Fungal contamination in the ventilation grids swabs ranged from 0 to 158.5 CFU/swab in MEA, and from 0 to 124.5 CFU/swab in DG18. Thirteen fungal species were found in MEA, while only eleven were observed in DG18, but the most abundant species were the same in both media: *Chrysonilia sitophila* (32.8% in MEA, 14.91% in DG18), *Penicillium* sp. (24.5% in MEA, 32.4% in DG18), and *Cladosporium* sp. (24.3% in MEA, 37.98% in DG18). In the EDC, the fungal contamination in MEA ranged from 0 to $5.41x10^4$ CFU.m^{-2}, and in DG18 from 0 to $1.8x10^3$ CFU.m^{-2} Eight fungal species were found in MEA, with the most common being *Mucor* sp. (68.61%), *C. sitophila* (17.15%) and *Cladosporium* sp. (11.39%); in DG18, five different species were found, *Cladosporium* sp. (54.62%), *Penicillium* sp. (24.57%), and *Aspergillus* sp. (11.27%). Fungal growth on azole-supplemented media was found in 75% (12/16) EDC samples, ranging from 0 to $9.55x10^2$ CFU.m^{-2}. Among the five fungal species identified, *Penicillium* sp. presented the highest frequency (13.6% ITRA, 12.0% VORI, 66.7% POSA), followed by *C. sitophila* (4.5% ITRA, 20.0% VORI) and *Cladosporium* sp. (4.5% ITRA, 64% VORI). In the MEA swab samples, four *Aspergillus* sections were found, being *Flavi* (3.69%), *Nigri* (2.86%) and *Versicolores* (0.21%) the most prominent) whereas within the DG18 six sections, *Nigri* (6.79%), *Flavi* (3.42%) and *Circumdati* (1.27%) were the most prevalent. In the EDC samples, only one *Aspergillus* section was found in MEA (*Versicolores* 0.17%), and three sections were found in DG18 (*Versicolores* 6.94%, *Circumdati* 2.31%, and *Candidi* 2.02%). qPCR analysis has detected the *Aspergillus* section *Fumigati* in ten of the fifteen areas assessed with the ventilation grid swabs (66.7%), and seven of the twelve areas studied with EDC (58.33%). *Aspergillus* section *Versicolores* was also detectedin the EDC samples collected (1 out of 12 areas, 8.33%). Neither species were detected by culture-based methods.

Conclusion:
The use of different methods in this study has enabled the detection of various potentially harmful fungal species and draws attention to the establishment of measures to eliminate or significantly reduce these contaminants in the hospital environment.

113 AN EXOTIC CASE OF PALATAL ENTOMOPHTHOROMYCOSIS DUE TO *CONIDIOBOLUS CORONATUS*

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Purpose:
To describe a case palatal entomophthoromycosis which may mimic chronic mucormycosis.

Methods:
Patient & microbiological Methods: History & clinical examination were performed at the time of admission to our hospital. Tissue biopsy was subjected to histopathology and fungal culture studies. The isolate was confirmed by sequencing the ITS region using ITS 5 & ITS 4 primers.

Results:
A 45-year-old male farmer, from Odisha, India was referred to us as a case of intractable zygomycosis. He had consulted a private practitioner 6 months back for progressive dysphagia, who found a granulomatous mass in the soft palate on magnetic resonance imaging (MRI) which was confirmed by video laryngoscopy and biopsied it for histopathological examination. Aseptate hyphae with splendore hoeppli material around the hyphae was observed on histology leading to the diagnosis of zygomycosis and he was treated with oral itraconazole for 6 months without any relief and worsening dysphagia. On admission at our hospital, he was pale, cachectic and weighed 40 kilograms. Oral cavity examination showed diffuse swelling over hard palate, soft palate and tonsils, which was firm in consistency with few small black macules on it. The nose was remarkably normal. A repeat MRI scan reconfirmed the presence of ill-defined isointense soft tissue with its epicentre in the soft palate which was biopsied. Histopathological examination of the biopsy showed broad aseptate fungal hyphae surrounded by granulomatous reaction and splendore-hoeppli phenomenon and culture yielded white to cream coloured waxy colonies with radial grooves and satellite colonies. The isolate showed broad aseptate hyphae and spherical conidia with the papillate base on lactophenol cotton blue preparation. It was identified morphologically as *Conidiobolus coronatus* and confirmed by sequencing the ITS region (Accession number: MN421921). After confirmation of entomophthoromycosis and due to inability to afford amphotericin B the patient was treated with saturated solution of potassium iodide (SSKI) 14 drops three times for 2 weeks along with oral itraconazole. The patient improved symptomatically with a reduction in dysphagia and started gaining weight within 2 weeks of therapy. He was discharged with advice to continue SSKI and itraconazole 200mg twice a day for 6 months. After 3 months the patient is currently relived of dysphagia and has regained his lost weight on follow up to date.

Zygomycosis is an obsolete term used to describe mucormycosis and entomophthoromycosis. It was discarded because both the entities were clinicopathologically distinct and etiologic agents didn't form a single group on phylogenetic studies1. Recently, chronic mucormycosis was described which occurred in immunocompetent individuals and also shows aseptate hyphae without vascular invasion on histopathology 2. Entomophthoromycosis appears similarly on histology but in addition, has eosinophilic material around hyphae. Moreover, the culture will help in differentiating this syndrome from chronic mucormycosis when it occurs in sites like palate.

Conclusion:
Although rare conidiobolomycosis should be considered in the differential diagnosis of young immunocompetent patients from tropical countries presenting with dysphagia and granulomatous lesion in the soft palate.

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2. Int Arch Otorhinolaryngol. 2019 Jan; 23(1): 92–100.

114 THE INCIDENCE OF PULMONARY ASPERGILLOSIS IN PATIENTS WITH CYSTIC FIBROSIS IN RUSSIAN FEDERATION

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Purpose:
To assess the incidence of various forms of pulmonary aspergillosis in patients with cystic fibrosis in Russian Federation.

Methods:
During 2014-2017 in the prospective study were included 190 patients with cystic fibrosis. The median age was 14 years (range 1 - 37), children – 68.5%, males – 50.5%. The serum total IgE (tIgE) and specific IgE(sIgE) to 6 fungal allergens (*Aspergillus fumigatus*, *Alternaria alternata*, *Cladosporium herbarum*, *Penicillium notatum*, *Rhizopus nigricans*, *Candida albicans*, Alkor Bio, Saint Petersburg, Russia) determination and mycology (microscopy and culture of sputum or bronchoalveolar lavage - BAL) examinations was performed in all patients. Chest computed tomography scans were performed according to the indications. For the allergic bronchopulmonary aspergillosis (ABPA), chronic pulmonary aspergillosis (CPA), and invasive aspergillosis (IA) diagnosis criteria of Stevens et al., 2003, Denning et al, 2016, and EORTC/MSG, 2008 were used respectively.

Results:
Aspergillus spp. from sputum or BAL were cultured in 36 (19%) CF patients: *A. fumigatus* – 18% (n=34), *A. flavus* – 4.7% (n=9), *A. niger* – 3.7% (n=7), *A. nidulans* – 0.5% (n=1), and *A. terreus* – 0.5% (n=1). Two or more *Aspergillus* spp. were detected in 8.7% of CF patients. The total IgE level varied from 1 to 3861 IU/ml in CF patients. In 10 (5.3%) patients it exceeded proposed by experts threshold level of 500 IU/ml. In CF patients the incidence of fungal sensitization was 57%, to *Aspergillus* spp. – 27%. ABPA was diagnosed in 11 CF patients (5.7%), children (n=4), adults (n=7). The minimum patient's age of ABPA diagnosis was 8 years, while the maximum age was 29 years. The incidence of CPA was 4.2%, and IA developed in one patient (0.5%) after liver transplantation and immunosuppressive therapy.

Conclusion:
In patients with cystic fibrosis the incidence of pulmonary aspergillosis was 10.4%: allergic bronchopulmonary aspergillosis - 5.7%, chronic pulmonary aspergillosis – 4.2%, and invasive aspergillosis – 0.5%. Mycological examination is indicated for patients with cystic fibrosis for timely detection of various forms of pulmonary aspergillosis.

<u>NOTE</u>: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.

115 A DIFFICULT-TO-TREAT CASE OF MUCORMYCOSIS

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Purpose:
Mucormycosis is a rare invasive fungal infection that is difficult to treat. Correction of underlying disease, aggressive surgical debridement and systemic amphotericin B are the cornerstones of treatment. Although treatment-resistant cases have been reported in the literature, there are not enough data in the treatment guidelines to support combined antifungal therapy. In this article, we aimed to present a case of chronic lymphocytic leukemia (CLL) and mucormycosis treated with recurrent debridement and combined antifungals.

Case:
79-year-old patient with CLL was followed-up for 3 years without treatment. Due to stage 3 symptomatic disease, he treated with bendamustine and rituximab. Three months after this treatment, the patient had hearing loss, redness of the outer ear, swelling, and tomography was taken. Imaging revealed right nasal cavity and lytic lesions suggesting mucormycosis in the ear. He was operated by otolaryngologist and histopathologic examination was in compatible with mucormycosis. Therefore, liposomal amphotericin B 5 mg / kg was started intravenously. The patient was operated on by the otolaryngologist for 7 times, each time the necrotic tissues in the middle-outer ear, ear canal and sinuses were removed. Despite the liposomal amphotericin B and recurrent debridement, there was not enough improvement. So that micafungin 1x150 mg was added to amphotericin B treatment considering clinically resistant fungal infection. One week after the addition of micafungin, the patient›s examination findings improved and fever control was achieved. After 45 days of treatment, the patient was discharged with oral posaconazole. The total duration of treatment with oral treatment was completed to three months. Antifungal treatment was discontinued when no pathology was detected in his controls.

Conclusion:
Mucormycosis is difficult to treat condition in many cases. Combined use of antifungals can be life-saving in cases that are unresponsive to standard treatments.

116 NEW PERSPECTIVE: EVALUATION OF MUCORMYCOSIS CASES WITH EUROPEAN QUALITY (EQUAL) SCORE

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Purpose:
Mucormycosis is a life threatening disease especially in patients with heamatologic malignancies. Excellence Centre for Medical Mycology developed a score to quantify guideline adherence, called EQUAL score for mucormycosis patients. We aimed to evaluate our patients according to this score for detection the adherence to current guideline recommendations.

Material-methods:
We evaluated our nine patients respectively using the EQUAL mucormycosis score developed by Koehler et al in 2018. The records of patients were reviewed retrospectively and 18 item for each patient were measured.

Results:
Nine patients from Cukurova University were included. Of those six patients had acute myeloid disease, two had acute lymphoblastic leukemia, chronic one patient had chronic lymphocytic leukemia. The mean age was 43 (18-78) years.

Four patients died and their mean score was 12.5 (9-18). The others were recovered and their mean score was 22.6(18-25). The EQUAL score was higher in patients with good prognosis.

Conclusion:
The EQUAL mucormycosis score may be a good predictor of prognosis. It may be useful for monitoring patients.

117 PROSPECTIVE EVALUATION OF QUALITY OF LIFE AND *ASPERGILLUS* IGG IN TUBERCULOSIS (TB) PATIENTS IN LAGOS, NIGERIA

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Purpose:
Globally tuberculosis (TB) is a major public health problem and remains one of the world's deadliest communicable diseases. Currently TB services and clinical research focuses on outcomes of mortality and microbiologic cure, and have often neglected patients' perceived health-related quality of life (HRQoL). Pulmonary tuberculosis (PTB) results in residual anatomical and functional changes despite therapeutic cure. HRQoL assessment tools, which are questionnaire based, allows physicians to determine the disease impact and to identify patients with pulmonary impairment after therapy. The aim of this study was to determine the HRQol of TB patients in a resource limited setting, to determine rates of pulmonary impairment after tuberculosis treatment and to identify how many patients will develop chronic pulmonary aspergillosis during or post-TB therapy and the link between this complication and HRQoL.

Methods:
We conducted a longitudinal study amongst TB patients attending two DOTS clinic in Lagos, Nigeria. 200 confirmed TB patients were recruited over a nine months period. They were smear/ and/or geneXpert/and/or culture positive for TB. Two QoL assessment tools: WHOQoL-BREF and SGRQ questionnaires were self-administered. They were completed at start of treatment and then subsequently at 3 months interval until 6 months post therapy (a total of 5 visits). Sera for *Aspergillus* IgG were collected at each visit and analysed using the Bordier Kit. The mean scores for the HRQoL of participants was calculated using the WHOQOL Bref – World Health Organization. CXR was done at beginning of treatment, at 3, 6, 9 and 12 months.

Results:
A total of 204 participants were recruited into this study. Most (70.6%) were aged age 18-39 years, only 3.9% were above 60 years. 66.7% were males. 189 (92.6%) participated in the 3 months assessment, 174 (85.3%) at 6 months, 139 (68.1%) at 9 months, 99 (48.5%) at 12 months. At baseline, only 42.9% felt 'good' in HRQoL score, which improved to 60.3% at 3 months, 48.2% at 6 months, 50.5% at 9 months and 55.7% at 12 months. At baseline, 10.4% had positive *Aspergillus* IgG levels, 15.1% at 3 months, 11.5% at 6 months, 16.7% at 9 months and 19.3% at 12 months. Those with a positive *Aspergillus* IgG at 6 months had worse physical health (P=0.001), psychological health (P=0.002), social relationships (P=0.006) and environment (P=0.001) domains of HRQoL scores. There was a statistically significant difference between mean overall score of participants (using the SGRQ) between months of assessment as determined by one-way ANOVA (F=15.058, P-value <0.001). A tukey Post hoc's test revealed that the mean baseline score was statistically significantly higher than all other months, and significantly higher at 3months than 12months. 38 (18.6%) relocated at end of 6 months treatment, 16 (7.8%) were lost to follow-up and 11 (5.4%) died.

Conclusion:
Our findings reveal a significant relationship between TB patients QoL and *Aspergillus* IgG levels. Further follow-up studies and additional imaging are required to determine when patients develop chronic pulmonary aspergillosis and its clinical impact.

***NOTE*: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.**

118 FATAL MUCORMYCOSIS AND ASPERGILLOSIS IN AN ATYPICAL HOST: WHAT DO WE KNOW ABOUT MIXED INVASIVE MOLD INFECTIONS?

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Purpose:
Mixed invasive mold infections (MIMI) are rarely diagnosed given lack of non-invasive diagnostic methods with adequate sensitivity. Previous studies, including one published case of fatal mucormycosis with positive *Aspergillus* galactomannan (GM) at our institution, implicate that corticosteroid administration is an important, potentially underestimated risk factor for (M)IMI. We present a case of fatal MIMI in a host with no classic risk factors for MIMI other than high-dose corticosteroids, and review the literature on MIMI.

Case Study:
A non-neutropenic 79-year-old man with history of hypertension and CLL presented with dysphasia after his first cycle of chlorambucil (alkylating agent) and obinutuzumab (CD20-directed cytolytic monoclonal antibody). He had worked for many years in construction and was still very active in his house and garden. Brain MRI showed three distinct abscesses in the left parietal and temporal lobes with significant edema and mass effect, for which he was started on dexamethasone. Chest CT showed one pulmonary nodule (Fig. 1, top). Initial b-D-glucan (bDg) and GM were negative. Stereotactic biopsy of one abscess failed to include its necrotic core and was non-diagnostic. Cell-free DNA next generation sequencing (cfDNA-NGS) of a plasma sample showed strong signal for *Nocardia abscessus*, and he was started on treatment with intravenous antibiotics. Repeat imaging showed decrease in the size of all brain abscesses and the pulmonary nodule, which demonstrated central cavitation (Fig. 1, bottom). However, brain edema worsened upon tapering the corticosteroids, therefore the patient remained on high-dose dexamethasone. Two months after his initial presentation, he presented with multiple new lung abscesses, heart failure and renal failure (Fig. 2A). Repeat bDg was negative, but GM was >8 (assay cut-off). His family opted for comfort measures only and kindly agreed to an autopsy.

Results:
Autopsy showed 1. filamentous, FITE stain-positive organisms consistent with *Nocardia* in the brain (Fig. 2A); 2. multiple septate, narrow-angled hyphae invading the vessels and lung parenchyma. Lung cultures were positive for *Aspergillus fumigatus* (Fig. 2C); 3. acute invasive mold infection of the heart, left kidney, thyroid, lymph nodes and brain, with numerous fungal hyphal forms of a second morphology (aseptate, wide-angled), consistent with Mucor, in the parenchyma and blood vessels (Fig. 2D). Molecular study (28s rRNA) was positive for *Lichtheimia corymbifera*.

Conclusions:
1. The incidence of MIMI is likely underestimated. 2. High-dose corticosteroid administration is a major risk factor for IMI, including mucormycoses and MIMI. 3. “Immunomodulating” factors, such as immunosenescence and immunosuppression not meeting classic host criteria for IMI (e.g. CLL) may be important contributors. 4. There is an urgent and unmet need for well-designed studies on: a) the “net state of immunosuppression” and environmental exposure as risk factors for (M)IMI; b) non-invasive fungal diagnostics, including serial cfDNA-NGS and development of new assays; c) efficacy, safety and cost-effectiveness of anti-mold prophylaxis in potentially susceptible hosts, other than transplant recipients and neutropenic patients.

Figure 1

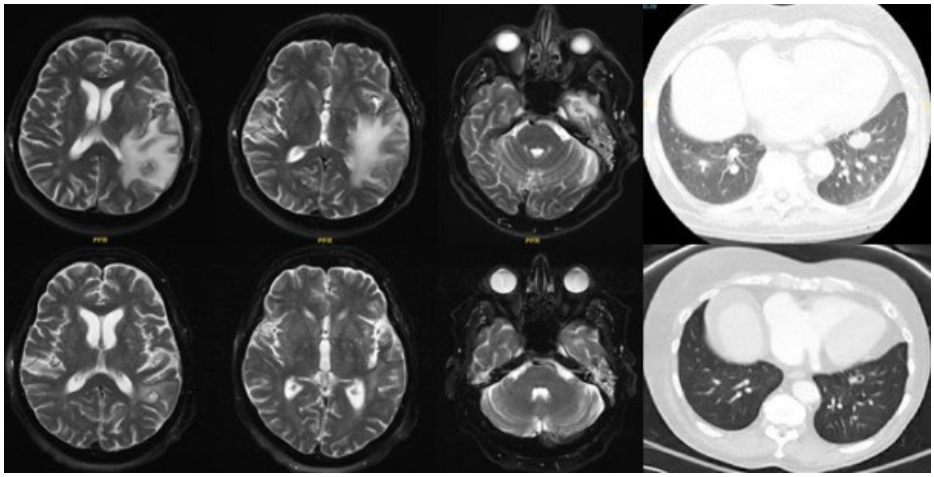
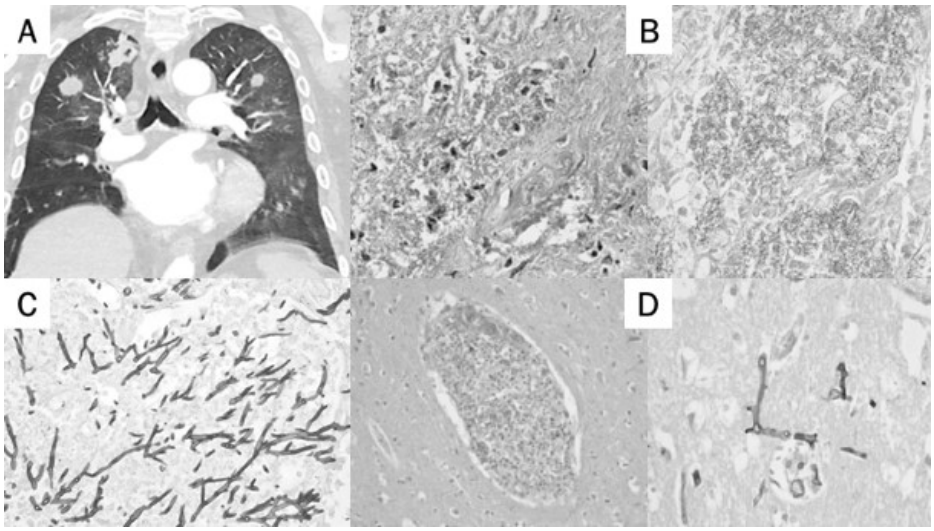


Figure 2



119 *ASPERGILLUS FUMIGATUS* RHINOCEREBRAL ABSCESS IN A DIABETIC PATIENT

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Purpose:
Rhinocerebral mucormycosis is a major cause of severe mold infection in diabetic patients; albeit unusual, it contrasts with the rare rhinocerebral abscess caused by *Aspergillus fumigatus* (1,2,3). Here, is reported the case of a diabetic patient with abscess of left frontal sinus and ethmoidal labyrinths, where the single infectious agent isolate was *Aspergillus fumigatus.*

Clinical Case:
A Caucasian 75 years-old man with hypertension, dyslipidemia and type 2 diabetes mellitus with renal and ocular involvement, and a generalized tonic-clonic seizure (GTCS) was observed at the emergency department (ED) of our hospital. His family referred a progressive mental deterioration over the past two weeks. Tests performed in the ED showed plasma glucose 372 mg/dL, creatinine 1.92 mg/dL, normal sodium and potassium and arterial pH7.38, with pCO2 37 mmHg and anion gap 13.1. A lumbar puncture was preformed and reveled 2 cells, glucose 219 mg/dL, proteins 1.24 g/L. The cerebral TC scan showed lesions of ancient trauma involving the left frontal sinus and the medial and lateral walls of left orbit, and tissue fill of the left frontal sinus and ethmoidal labyrinths. In the following five days he was afebrile, exhibiting periods with consciousness and others without. Additional GTCS episodes were studied by cerebral magnetic resonance, which favored left frontal abscess. Drainage was made, whose content was sent to microbiology cultures, and ceftriaxone, metronidazole and voriconazole were prescribed. Two days later, when *Aspergillus fumigatus* was identified, voriconazole was switched to amphotericin B. Despite the treatment, the metabolic disorder and the infectious process continued, and the patient died 39 days after admission to hospital and 22 days of therapy with amphotericin B.

Results:
Microbiology: Samples of the frontal abscess were cultured in blood and chocolate agar for bacteriological exam and in Sabouraud dextrose agar with gentamicin and chloranphenicol for mycological assessment. In all media colonies were grown, where *Aspergillus fumigatus* was identified by morphological and microscopic characteristics. The susceptibility testing was done following the microdilution CLSI protocol and the MICs (mg/L) were itraconazole (0.25), voriconazole (0.25), posaconazole (0.125), isavuconazole (0.25), amphotericin B (1.0), caspofungin (0.25) and anidulafungin (≤0.015).

Conclusion:
The case here reported, an unexpected presentation of mycological rhinocerebral abscess, highlights the importance to perform all microbiological exams, whenever the infectious cause is likely. Correct and fast laboratory diagnosis will help adequate patient management.

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120 ENVIRONMENTAL DISTRIBUTION OF *ASPERGILLUS TERREUS* SPECIES COMPLEX IN TYROL, AUSTRIA

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Purpose:
Invasive fungal infections due to *Aspergillus* species have become a major cause of morbidity and mortality among immunocompromised patients. Fungal infections due to *A. terreus* species complex occur worldwide, and the global prevalence of *A. terreus* species in fungal disease revealed an overall occurrence of 5.2%. This is of serious concern due to intrinsic resistance of *A. terreus* to amphotericin B. At the Medical University of Innsbruck, *A. terreus* infections are recognized as a frequent agent of invasive aspergillosis, noted since 1994. The reason for this epidemiological situation is unclear. The aim of this study is to investigate the ecological niche and epidemiology of *A. terreus* complex species in Tyrol, Austria.

Methods:
A large number of environmental samples, including soil, air, water, living and dead plant material, decaying material, dust, and excreta are collected at different locations in Tyrol, screed for the frequency of *A. terreus*, and classified according to environmental conditions. Positive *A. terreus* isolates will be characterized, including genotyping, morphology, amphotericin B susceptibility testing, and comparison to clinical isolates. Also, collected sample types will be characterized according to environmental features.

Results:
By now, a total of 1691 environmental samples were collected in Tyrol. Sampling locations covered urban and rural districts as well as mountain regions with altitudes ranging from 540 m to 3100 m. We can show that *A. terreus* species complex displays an overall environmental distribution of 3.9% in Tyrol. However, the frequency of positive *A. terreus* samples significantly differs within environmental clusters. Most frequently, *A. terreus* colonies were isolated from soil, followed by plant material, air, and decaying material. Overall, the highest density of *A. terreus* isolates was detected in agriculturally used fields.

Conclusion:
Our data describe the environmental distribution of *A. terreus* species complex in Tyrol, Austria, to gain a better understanding for the elevated density of *A. terreus* infections in this area.

121 ENVIRONMENTAL *ASPERGILLUS* AND MUCORALES SPECIES IN BEACH-SAND OF ISRAELI MEDITERRANEAN COAST: POSSIBLE IMPACT ON HUMAN HEALTH

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Purpose:
This study is part of a collaborative research in the frame of an ECMM-EFISG-ISHAM working group consisting of over 20 laboratories, which aims to explore fungal contamination in beach-sands around the Mediterranean Sea and other water bodies, in view of possible impact on human health. Fungal sand contamination and the possible impact on human health were explored regarding three aspects: 1. Fecal contamination, as judged by presence in sand of the human GI commensal – Candida albicans and other Candida species; 2. Contamination by fungi known for involvement in dermal infections, such as Dermatophyte species; 3. Presence in sand of various molds which may possibly be causes of respiratory allergies, such as *Aspergillus*, Penicillium, Mucorales species and/or other molds. The present report relates specifically to the presence of *Aspergillus* and Mucorales species in sands of beaches around the Mediterranean Sea in Israel.

Methods:
Six beaches from North to South (Haifa, Keisaria, Tel Aviv, Palmachim, Ashdod, Ashkelon) were examined. Based on a technique described previously by Brandao and colleagues, water- extracts of sand samples collected at the 6 sites were cultured on standard mycological media. The fungal load in sand was evaluated quantitatively by enumeration of colony forming units (CFU) of the fungi isolated from the sand samples. The fungi were identified phenotypically, spectrally by MALDI –TOFF mass spectroscopy using the Bruker system (with two data bases), and by ITS sequencing (not all isolates).

Results:
The 3 rounds of samplings of the sands from the 6 beaches during one year yielded 157 fungal isolates, consisting of 125 molds (79.6% of total isolates) and 32 yeasts (21.4%). The identified molds could be assigned to 20 fungal species, out of which were 9 *Aspergillus* species, including a total of 56 *Aspergillus* isolates (44.8% of mold species). The major 4 *Aspergillus* species found in sand were *A. fumigatus* (14 isolates), *A. niger* (11 isolates), *A. flavus* (11 isolates) and *A. welwitschiae* (10 isolates). It is of interest that no *A. terreus* isolates were found in our survey, although *A. terreus* was isolated previously in Israel from onychomycosis patients. We assume that the high temperatures of the sand in Israel beaches may be a factor limiting the growth of this species. Most of the *Aspergillus* isolates grew well at 280C and 370C. Mucorales species, including *Mucor circinelloides* and *Rhizomucor pusillus* were also found in the sands.

Conclusions:
All these species are known human opportunist pathogens and also known as potential allergens. Thus, these findings may have relevance as potential environmental source for infections in immunocompromised individuals and be of significance as exposure source for asthmatic individuals. Furthermore, while regulations for beaches are partially available regarding bacterial contamination, they are non-existent for fungal contamination. Hence, data on fungal contamination of beaches may possibly contribute to development of regulatory measures also for fungal contamination, contributing thereby to public health.

122 DESIGN OF A PHASE 2 STUDY TO EVALUATE FOSMANOGEPIX (APX001), A NOVEL ANTIFUNGAL, FOR THE TREATMENT OF PATIENTS WITH INVASIVE MOLD INFECTIONS (IMI) WITH LIMITED TREATMENT OPTIONS

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Purpose:
There is an urgent need for new and effective options to treat invasive fungal diseases in the ever growing number of immunocompromised patients who are at increased risk for developing these life-threatening infections. Although *Aspergillus* spp. is a major cause of IMI in these patients, infections caused by other fungi, such as rare molds (e.g., *Fusarium* spp., *Scedosporium* spp., and Mucorales) are clearly emerging. Fosmanogepix (FMGX, APX001), is a novel, first-in-class antifungal agent, with a broad-spectrum of activity, comprising *Aspergillus* spp. and rare molds, including those that are resistant to standard of care (SOC) therapies. Its unique mechanism of action targets the highly conserved fungal enzyme Gwt1, which catalyzes an early step in glycosylphosphatidyl inositol (GPI)-anchor biosynthesis. Fosmanogepix has the potential to provide broad-spectrum antifungal coverage, with low risk of hepatic, renal or other complications frequently associated with SOC therapies. This proof of concept study was designed to demonstrate efficacy for FMGX in this vulnerable patient population with limited treatment options due to antifungal resistance, adverse events, and/or drug-drug interactions that are difficult to manage in patients receiving SOC therapies

Methods:
The study design utilized previous IMI studies as a basis. It has been brought up-to-date in accordance with the latest regulatory guidance's, expert opinions and new approaches in the diagnosis of IMI.

Results:
This study will enroll 50 adults (age ≥18 years) with documented IMI caused by *Aspergillus* or rare molds, with limited treatment options due to documented/anticipated resistance, contraindication, intolerance, or lack of clinical response to SOC antifungal therapy. Patients will initially receive IV FMGX with step down to PO to complete six weeks of treatment. A modified version of the 2008 Revised EORTC/MSG criteria will be used for the diagnosis of IMI. Serum samples for galactomannan and β-D-glucan will be collected at baseline, and sequentially during the study. All fungal isolates will be sent to the central mycology reference laboratory for confirmation of identification and susceptibility testing. In addition, blood and other diagnostic specimens will be tested utilizing next-generation sequencing, PCR, and the AspUTest®. The primary endpoint will be all-cause mortality at 6 weeks. An external Data Review Committee will confirm the diagnosis of IMI at baseline, and will assess global response at end of study treatment, using a standardized approach to evaluating imaging findings. An independent Data Safety Monitoring Board will evaluate safety on an ongoing basis.

Conclusions:
This innovative phase 2 study will evaluate the safety and efficacy of fosmanogepix, a promising, novel antifungal therapy, in patients with IMI and limited treatment options.

123 CLINICAL AND MICROBIOLOGICAL CHARACTERIZATION OF INFLUENZA ASSOCIATED ASPERGILLOSIS IN SOUTHERN TAIWAN

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Purpose:
Invasive pulmonary aspergillosis (IPA) has recently been recognized as a pivotal complication following influenza infection. To gain more insight into influenza associated aspergillosis (IAA), this study investigated the clinical and microbiological characteristics of IPA in patients with influenza.

Methods:
Patients from whom *Aspergillus* isolates have been cultivated from respiratory samples during a laboratory confirmed influenza infection at a medical center in Southern Taiwan were included. *Aspergillus* isolates were identified based on morphology and ITS and calmodulin sequencing, subjected to susceptibility testing with the CLSI M38-A2 method, and determined for genetic relatedness using microsatellite genotyping. *cyp51A* gene of azole-resistant isolates was analyzed. The definition of IPA was modified from the AspICU algorithm as described by Alexander et al. (Lancet Respir Med 2018;6:782-92), and clinical data of patients was reviewed.

Results:
A total of 60 *Aspergillus* isolates from 29 patients were identified during 2016-2018, among whom 23 (79.3%) patients (influenza type A [17] and B [6]) developed IPA, ie. IAA. Of 23 IAA patients, two-third (15, 65%) were aged more than 65 years (mean: 66.8 yrs; range: 38-90 yrs). Nine (39%) and 14 (61%) patients acquired IPA in community and hospitals, respectively. The recovery of *Aspergillus* isolates was within 10 days after laboratory confirmation of influenza in all patients. Only a minority (4, 17%) of patients fulfilled the EORTC host factor definition, and the co-morbid conditions identified included diabetic mellitus (11 patients, 48%), chronic obstructive pulmonary disease (6, 26%), steroid use (5, 22%), chronic kidney diseases (4, 17%), and hemato-oncological malignancies (3, 13%); only one patient was previously healthy. *Aspergillus* species recovered from 23 IAA patients include *Aspergillus fumigatus* (10 patients, 44%), *Aspergillus flavus* (4, 17%), *A. terreus* (4, 17%), *A. fumigatus* plus *A. flavus* (3, 13%), and *A. fumigatus* plus *A. nomius* (2, 9%); overall, *A. fumigatus* was the mostly common species (65%), followed by *A. flavus* (30%), *A. terreus* (17%), and *A. nomius* (9%). Microsatellite genotypes of *A. fumigatus*, *A. flavus*, and *A. terreus* isolates all varied between individual patients, except that *A. flavus* isolates from two patients, who were not temporally or spatially related, shared an identical microsatellite genotype that was also widely found in Taiwan. Four patients carried *A. fumigatus* isolates of more than one microsatellite genotype simultaneously (ranged 2-4 genotypes per patient) and one patient carried *A. flavus* isolates belonging to two microsatellite genotypes. Mixed azole-susceptible and azole-resistant infections were identified in two patients: clinical condition of the patient carrying azole-resistant *A. fumigatus* (ARAF) with *cyp51A*-inpedenent resistance mechanisms deteriorated on voriconazole treatment, while the other patient carrying ARAF with TR34/L98H mutation was clinically improved under voriconazole treatment. All but one patients received mechanical ventilation and the overall IAA-attributable mortality rate was 30%. Multivariate analysis failed to identify independent risk factors for mortality.

Conclusions:
This study calls for increased awareness towards IPA which might develop early following influenza infection in patients without classical risk factors for IPA in both community and hospital settings.

NOTE: THIS ABSTRACT HAS BEEN SELECTED FOR ORAL PRESENTATION.

124 USING SOCIAL MEDIA TO EXPAND THE IMPACT OF LIFE WORLDWIDE IN FUNGAL MEDICAL EDUCATION

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Purpose:
LIFE Worldwide has been providing free online fungal education to clinicians for more than 6 years via our website and email newsletter. Since the last AAA meeting, we have updated this approach to make use of social media channels to disseminate the latest mycology news and research, as well as to promote resources such as Microfungi.net and Drug Interactions Pro. We also used the analytics generated during this process to inform a total redesign of the LIFE website (www.life-worldwide.org), which is now integrated with our social media content and much easier to navigate.

Methods:
We constructed profiles for LIFE Worldwide on a range of major social media platforms (Twitter, YouTube, LinkedIn, Pinterest, Facebook), then began to share content and grow our networks of followers. Success was judged using analytics such as number of subscribers, engagements (likes/shares/comments) and impressions (how many people saw our content in their feed).

Results:

- **Twitter** (@LIFEworldwide) is heavily used by the medical education (MedEd) community and is particularly well suited to targeting information to relevant specialties (e.g. transplants, diagnostics), professions (e.g. radiologists, nurses) and geographical regions. In 6 months of activity, LIFE Worldwide gained 407 followers (primarily clinicians), and regularly achieves >1500 impressions for original Tweets. Multiple choice questions are particularly popular and the results from these help us to better understand gaps in clinician knowledge. Shareable media (videos/infographics) are also popular, as are research highlights and information on jobs/funding.
- **YouTube** (Leading International Fungal Education Worldwide; UCMbjRZb4EofRTkKK9yHU8qQ) is a flexible and easy-to-use platform for hosting clinical lectures and video lab protocols, which integrates easily with the new website through a well-supported plugin. In the past year we have gained 360 subscribers and currently generate around 4,500 views per month. The most popular videos are clinical lectures on the major azole antifungals.
- **LinkedIn** (www.linkedin.com/company/life-worldwide/) is a useful platform for targeting academic, laboratory and pharmaceutical/diagnostics industrial professionals, particularly for disseminating key news and research stories that will be of broad interest.
- **Pinterest** (@fungaleducationworldwide) may be a useful platform for helping patients share infographics and peer support materials, but its use in clinician education (especially beyond undergraduate level) appears to be limited.
- **Facebook** is already used by our team for hosting patient peer support groups, but was unfortunately found not to be suitable for reaching out to medical professionals.

Conclusion:
Social media is a very effective tool for promoting static resources such as the free Microfungi.net course and the new LIFE website, as well as for disseminating the latest news and scientific research. Twitter is a particularly useful platform for sharing ‘clinical pearls’ with the potential to change clinical and laboratory practice.

125 DISSEMINATED INVASIVE ASPERGILLOSIS. RESULTS OF RETROSPECTIVE ANALYSIS OF THE LARGE REGISTER

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Purpose:
Analysis of demographic parameters, underlying diseases, risk factors, etiology, clinical features, treatment, and survival rates in patients with disseminated (≥2 organs involvement) invasive aspergillosis (IA).

Methods:
Retrospective analysis of patients with disseminated proven and probable IA in 1998-2019 yy. We used criteria EORTS/MSG, 2008 for the diagnosis of proven and probable IA.

Results:
In 1998-2019 yy we observed 803 patients with proven (12%) and probable (88%) IA. Disseminated IA was diagnosed in 75 (9%) patients, median age – 35 years (1 – 99), children and adolescents – 23%, males – 50%. The comparison group consisted 728 patients, median age – 42 years (1 – 86), children and adolescents – 15%, males – 56%. In both groups IA predominantly occurs in patients with hematologic malignancies (82% vs 87%). The disseminated IA more often developed in patients with acute leukemia (60% vs 42%, p< 0,05), significantly less often in patients with lymphomas (8% vs 28%, p <0.05) and multiple myeloma (1% vs 6%, p <0.05). Non-hematologic background diseases were 18% and 13%, respectively; there were no disseminated IA in patients with chronic obstructive pulmonary disease and tuberculosis.

The main risk factors for the IA development in both groups were persistent severe neutropenia (83% vs 73%) and lymphocytopenia (78% vs 60%, p <0.05), steroids use (67% vs 65%), long-term immunosuppressive therapy (38% vs 27%, p< 0,05), graft versus host disease (GVHD) after hematopoietic stem cell transplantation (allo-HSCT) (29% vs 19%,p< 0,05), and intensive care unit (ICU) stay (20% vs 14%, p<0,05).

The main sites of infection were lungs (92% vs 95%). Sinuses (43% vs 1,5%, p<0,05) and central nervous system (CNS) involvement (40% vs 1,5%, p<0,05) more often occurred in disseminated IA group. In patients with disseminated IA were more often observed respiratory failure (51% vs 36%, p <0.05), CNS symptoms (32% vs 1%, p<0,05), and concomitant bacterial infection (42% vs 34%, p <0.05)

The main causative agents were *A. fumigatus* (45% vs 51%), *A. niger* (23% vs 29%), and *A. flavus* (26% vs 14%). Disseminated IA was more often caused by ≥2 *Aspergillus* spp. (35% vs 9%, p <0.05).

Antifungal therapy was used in 96% vs 99% patients. The overall 12-week survival rate for disseminated IA was significantly lower (63% vs 82%, p <0.05).

Conclusion:
Disseminated IA developed in extremely immunocompromised patients with acute leukemia (60%), persistent severe lymphocytopenia (78%), long-term immunosuppressive therapy (38%), and GVHD after allo-HSCT (29%). The main pathogens were *A. fumigatus* (45%), and ≥2 *Aspergillus* spp. (35%). The main sites of disseminated IA were lungs – 92%, sinuses – 43%, and CNS – 40%. Respiratory failure (51%) and CNS symptoms (32%) were frequent in disseminated IA group. The overall 12-week survival rate of disseminated IA patients was significantly lower than the main cohort (63% vs 82%, p<0.05).

126 CLINICAL AND LABORATORY FEATURES OF INVASIVE ASPERGILLOSIS IN HIV-POSITIVE PATIENTS

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Purpose:
Analysis of underlying diseases, risk factors, etiology, clinical features, treatment and survival rates in HIV-positive patients with invasive aspergillosis (IA).

Methods:
Retrospective analysis of the register of patients with IAin 1998-2018 yy. For diagnosis IA we used criteria EORTS/MSG, 2008.

Results:
In group I we included 12 HIV-positive adult patients with IA from 25 to 52 years old, median – 34, males – 58%. The control group consisted of 545 adult patients with hematological malignancies, from 18 to 78 years old, median - 47, males – 58%. The study of risk factors showed significant differences between these groups. Lymphocytopenia was detected predominantly in HIV-positive patients (75% vs 56%), duration 35 vs 14,5 days, (p = 0.006), while agranulocytosis in this group was observed less frequently – 58% vs 81%, p = 0.008, duration 8 vs 13 days (p = 0.01). Among the HIV+ patients there were no recipients of allogeneic stem cell transplants (0% vs 19%, p = 0.001) and patients receiving immunosuppressive therapy (0% vs 29%, p = 0.02). The main sites of infection were lungs - 100% vs 98%, however hemoptysis and dissemination of infection more often registered in HIV+ patients – 17% vs 6% (p = 0.03) and 17% vs 8% (p = 0.04), respectively. Galactomannan test in BAL was positive in 42% vs 75% cases. *Aspergillus* spp. were isolated in 42% vs 44%, in all HIV+ patients the main etiological agent of IA was *A. fumigatus* - 100% vs 45% (p = 0.001). Mixed fungal infection was detected in 33% vs 11% (p = 0,001). «Proven» IA was diagnosed in 17% vs 7% (p=0.02). Antifungal therapy was used in 100% vs 99% of patients; the most commonly used drug was voriconazole (58% vs 77%). Twelve weeks overall survival rate was 80% vs 81%.

Conclusion:
The features of invasive aspergillosisin HIV-positive patients were: prolonged lymphocytopenia (75%), agranulocytosis was a rare risk factor (58%), more frequent mixed infection - 33%, and high frequency of dissemination of aspergillosis – 17%. The overall 12-week survival did not differ in the studied groups (82% vs 79%).

127 INVASIVE ASPERGILLOSIS IN ADULT NON-HEMATOLOGICAL PATIENTS

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Purpose:
Identification of features of invasive aspergillosis (IA) in non-hematological patients.

Methods:
Retrospective analysis of clinical data of adult non-hematologicpatients with IA. The EORTS/MSG 2008 criteria were used for IA diagnosis and assessment of response of therapy.

Results:
In the main group were included 87 adult non-hematological patients with IA, median age – 51 years (19 - 99), females - 53%. The control group included 591 hematological patients, median age – 46 years (18 - 79), females – 42%.

The underlying conditions in the main group were autoimmune diseases – 16%, oncology – 16%, viral-bacterial pneumonia – 13%, organ transplantation – 11%, COPD – 10%, chronic sinusitis – 10%, AIDS – 6%, severe infections – 6%, heart diseases – 4%, congenital lung abnormalities – 4%, and other – 4%. In the control group the underlying diseases were acute leukemia – 40%, lymphomas – 34%, chronic leukemia – 9%, and other – 17%.

In non-hematological patients systemic steroids were significantly less often used (46% vs 75%, $p<0.05$), lymphocytopenia (40% vs 64%, $p < 0.05$) and neutropenia (10% vs 83%, $p<0.05$) were also noted less often. Additional risk factors for IA development in non-hematological patients were precede surgical treatment – 31%, stay in ICU – 23%, renal or hepatic failure – 11%, and decompensated diabetes – 8%.

The main clinical symptoms were fever (87% vs 74%, p=0.04), cough (71% vs 65%), dyspnea (62% vs 44%, p=0.01), chest pain (30% vs 7%, p=0.001), and hemoptysis (26% vs 7%, p=0.02).

The main sites of infection were lungs (78% vs 98%). In non-hematologic patients, other organs involvement was more often noted: sinuses (13% vs 5%, p=0.01), heart (6% vs 0.001%, p=0.0001), digestive organs (6% vs 1%, p=0.006), and central nervous system 8% vs 4%. Disseminated (≥2 organs) infection was in 13% vs 8% patients. In non-hematological patients, destruction cavities in lungs were more often detected on CT scan (37% vs 9%, p=0.005).

The main etiological agents were *A. fumigatus* (62% vs 45%), *A. niger* (15% vs 34%), *A. flavus* (17% vs 15%), other species accounted for 6% in each group. «Proven» IA was diagnosed in 42% vs 7%, p=0.004.

Voriconazole was used in 56% vs 76% patients, surgical treatment – 11% vs 4%. The overall 12-weeks survival rate was 81% vs 78%.

Conclusion:
Invasive aspergillosis developed in non-hematological patients usually with autoimmune (16%) or oncology diseases (16%). The main risk factors were steroid therapy (46%), lymphocytopenia (40%), immunosuppressive therapy (22%), and additional - precede surgical treatment (31%), and ICU stay (23%). The main etiological agents were *A.fumigatus* (62%), *A.flavus* (17%), and *A.niger* (15%). The main site of invasive aspergillosis was lungs (78%), disseminated (≥2 organs) infection was in 13% patients. Overall 12-weeks survival rate was 81%.

128 CLINICAL AND LABORATORY FEATURES OF INVASIVE ASPERGILLOSIS IN PATIENTS WITH MULTIPLE MYELOMA

O Shadrivova[1], V Pivovarova[1], Y Chudinovskikh[2], T Shneyder[3], O Uspenskaya[3], M Popova[4], A Volkova[4], E Desyatik[5], Y Borzova[5], S Ignatyeva[5], T Bogomolova[5], L Zubarovskaya[4], B Afanasyev[4], N Vasilyeva[5], N Klimko[1]*

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Purpose:
Identification offeatures of invasive aspergillosis (IA) in patients with multiple myeloma (MM).

Methods:
Retrospective analysis of 337 adult hematological patients with IA. In the main group were included 39 patients with MM, median age – 56 years (41 - 79), females - 59%. The control group included 298 hematological patients (acute leukemia – 45%, lymphoma – 36%; chronic leukemia – 13%, myelodysplastic syndrome – 5%; other – 1%), median age – 53 years (40 - 78), females – 56%. The EORTS/MSG 2008 criteria were used for IA diagnosis and assessment of response of therapy.

Results:
The main risk factors for IA were steroid use (87.5% vs 59.5%, p = 0.03), severe neutropenia (51% vs 76%, p = 0.03; median 14 vs 18 days), lymphocytopenia (33% vs 53%, median 10 vs 12.5 days), auto-HSCT (28% vs 4%, p = 0.01), and allo-HSCT (8% vs 13%). The main sites of infection were lungs (97,4% vs 97,3%), usually bilateral (69% vs 77%). The main clinical symptoms were fever (80% vs 78%), cough (69% vs 61%), chest pain (16% vs 5%, p = 0.03), and hemoptysis (0% vs 6.4%, p = 0.001). *Aspergillus* spp. positive culture was received in 69% vs 46% patients. The etiology of IA in patients with MM: *A. niger*– 45%, *A. fumigatus*– 35%, *A. flavus*– 10%, *A. candidus*– 5%, *A. ochraceus*– 5%. Antifungal therapy (voriconazole – 68,2% vs 64%) was used in 100% MM and 98% control group patients. The overall 12-weeks survival rate was 96% vs 80%, p = 0.01.

Conclusion:
The typical risk factors for invasive aspergillosis in multiple myeloma patients were steroids use (87,5%), and auto-HSCT (28%). The main sites of infection were lungs (97,4%). The main etiological agents were *A. niger* (45%) and *A. fumigatus* (35%). In multiple myeloma patients the overall 12-weeks survival rate was significantly higher compared to the control group (96% vs 80%, p = 0.01).

129 THE COMBINATION OF INVASIVE ASPERGILLOSIS WITH OTHER MYCOSES IN ADULT HEMATOLOGIC PATIENTS

O Shadrivova[1], S Khostelidi[1], E Shagdileeva[1], Y Chudinovskikh[2], M Popova[3], A Volkova[3], E Desyatik[4], S Ignatyeva[4], T Bogomolova[4], L Zubarovskaya[3], B Afanasyev[3], N Vasilyeva[4], N Klimko[1]*

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Purpose:
To investigate the features of mixed invasive mycoses (IM) in adult hematologic patients.

Methods:
Retrospective analysis of the register data (1998-2019 yy.). The EORTS/MSG 2008 criteria were used for proven or probable IM diagnosis and assessment of therapy response.

Results:
In the study were included 72 adult hematologic patients with combination of proven or probable invasive aspergillosis (IA) and non-*Aspergillus* caused mycoses, median age – 41 years (18 - 75), males – 71%. In the comparison group were included 519 adult hematological patients with IA, median age – 46.5 years (18 – 79), males – 56%.

The predominant underlying diseases in both groups were acute leukemia (51% vs 44%) and lymphomas (35% vs 34%). Mixed IM less often developed in patients with chronic leukemia (6% vs 9%) and multiple myeloma (3% vs 8%). The main risk factors for the IM development in both groups were severe neutropenia (79% vs 73%), and steroid therapy (72% vs 69%). Mixed IM occurred significantly more frequently in patients with persistent lymphocytopenia (63% vs 52%, p = 0.01), long-term immunosuppressive therapy (33% vs 23%, p = 0.04), ICU stay (29% vs 7%, p = 0.0001), and after allo-HSCT (33% vs 23%, p = 0.04).

The main sites of infection were lungs (99% vs 98%). In patients with mixed IM ≥ 2 organs (29% vs 5%, p = 0.001), CNS (15% vs 2%, p = 0.03), and paranasal sinus involvement (10% vs 4%) were more often observed. Clinical signs of IM were fever ≥ 38,5^{0}C (75% vs 73%), cough (58% vs 60%). In mixed IM patients, respiratory failure (51% vs 34%, p = 0.0001) and hemoptysis (13% vs 5%, p = 0.005) were more often noted. CT signs were similar in both groups, but in the mixed IM group, hydrothorax developed more often (9% vs 3%). The main causative agent of IA in both groups was *A. fumigatus* (40% vs 43%); *A. niger* (34% vs 33%) and *A. flavus* (20% vs 14%) were detected less often. In mixed IM group, non-*Aspergillus* pathogens were: mucormycetes – 35%, *Pneumocystis jirovecii* – 25%, *Candida*spp. – 22%, hyalohyphomycetes - 9%, *Cryptococcus neoformans* – 4%, rare yeasts – 4%, and pheohyphomycetes – 1%. Overall 12-week survival in the mixed-infection group was significantly lower (52% vs 84%, p = 0.0001).

Conclusion:
Mixed invasive mycoses accounted for 12% of adult hematologic patients with invasive aspergillosis. Mixed invasive mycoses occurred significantly more frequently in patients with persistent lymphocytopenia (63% vs 52%, p = 0.01), long-term immunosuppressive therapy (33% vs 23%, p = 0.04), ICU stay (29% vs 7%, p = 0.0001), and after allo-HSCT (33% vs 23%, p = 0.04). The main non-*Aspergillus* etiological agents were mucormycetes – 35%, *Pneumocystis jirovecii* – 25%, and *Candida* spp. – 22%. In patients with mixed invasive mycoses, ≥ 2 organs (29% vs 5%, p = 0.001) and CNS involvement (15% vs 2%, p = 0.03) were more often observed. Overall 12-week survival in patients with mixed invasive mycoses was significantly lower (52% vs 84%, p = 0.0001).

130 INVASIVE ASPERGILLOSIS IN ELDERLY PATIENTS

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Purpose:
Analysis of underlying diseases, risk factors, etiology, clinical features, treatment and survival rates in elderly patients with IA.

Methods:
Retrospective analysis of the register data in 1998-2018 yy. We included 646 adult patients with proven and probable IA. For diagnosis of IA we used criteria EORTS/MSG, 2008.

Results:
In group I we included 137 patients with IA ≥ 60 years old, males – 60%. The control group consisted of 509 patients aged from 18 to 59 years, males – 53%. In elderly patients non-Hodgkin's lymphoma prevailed (35% vs 13%, $p < 0.05$) among underlying diseases, whereas other hematologic diseases were less common: acute lymphoblastic leukemia (6% vs 13%, $p < 0.05$), Hodgkin's lymphoma (6% vs 13%, $p < 0.05$). Non-hematological diseases were more frequent in elderly patients (23% vs 13%, $p = 0.002$). We identified differences in risk factors between the two groups: prolonged agranulocytosis was detected in 59% vs 75%, ($p = 0.0003$), lymphocytopenia - 45% vs 60%, ($p = 0.002$). Significantly smaller numbers of elderly patients were recipients of allogeneic stem cell transplants (3% vs 26%, $p = 0.00001$) and received immunosuppressive therapy (17% vs 31%, $p = 0.0002$). No significant differences were obtained in the localization of the fungal infection, as well as in clinical symptoms and results of galactomannan test. *Aspergillus* spp. were isolated more often in elderly patients (52% vs 40%, $p = 0{,}01$). The main etiological agents were *A. fumigatus* (59% vs 45%) and *A. niger* (25% vs 34%). Mixed fungal infection was found in 9% vs 11% patients. Antifungal therapy was used in most of patients, the main drug used was voriconazole (73% vs 72%). Twelve weeks overall survival rate was 82% vs 79%.

Conclusion:
Non-hematological diseases were more frequent in elderly patients – 23%. The features of risk factors in elderly patients was less severe immunosuppression: prolonged agranulocytosis – 59%, lymphocytopenia – 45%, allogeneic stem cell transplants – 3%, immunosuppressive therapy - 17%. The overall 12-week survival in elderly patients with IA was not significantly different (82% vs 79%).

131 CASES OF SUCCESSFUL TREATMENT OF CHRONIC PULMONARY ASPERGILLOSIS IN PATIENTS AFTER DESTRUCTIVE PNEUMONIA

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Purpose:
We present clinical cases of successful treatment of chronic pulmonary aspergillosis (CPA) in patients after destructive pneumonia.

Methods:
For the CPA diagnosis the criteria Denning, 2016 were used.

Results:
Clinical case No. 1. Patient P., 37 years old male, with community-acquired right-sided pneumonia, received therapy from November to December 2018. In December 2018, episodes of haemoptysis with red blood, and negative CT dynamics with the appearance of a lesion in the upper lobe of the right lung were noted. In January 2019, he was admitted in a TB hospital, and right-sided upper-lobe abscessed pneumonia was diagnosed. He received an antibacterial treatment with a positive clinical effect. Laboratory tests for tuberculosis were negative. From February to April 2019, a course of preventive treatment with anti-TB drugs was carried out. During therapy, the progression of the inflammatory process in the upper lobe of the right lung, and the appearance of foci in the left lung were revealed. In June 2019 the patient was examined at the mycological clinic. Based on the clinical manifestations and examination results (a cavity with a soft-tissue component was visualized on CT scan; a positive *Aspergillus* IgG titre - 1: 1600; *A. fumigatus* growth in BAL), CPA was diagnosed. While on antifungal therapy with voriconazole 400 mg/day, in August 2019, an upper lobectomy on the right, and a marginal resection of the middle lobe was performed. The postoperative material was examined with Grocott's stain and PAS-reactions. Histological examination revealed a chronic abscess in the lung tissue with necrotic masses, conidia, and branching at an angle of 45^0 mycelium of fungi, similar to *Aspergillus* spp. Antifungal therapy was continued until October 2019.Control examination revealed no clinical and laboratory signs of active CPA.

Clinical case No. 2. Patient S., 61 years old male, in July 2018 suffered a community-acquired right-sided upper lobe destructive pneumonia. Patient received antibiotic therapy for 3 weeks without a significant effect. Complaints of coughing, weakness and fatigue, and CT signs of pneumonia were still persisted. Tuberculosis was excluded.

In October 2018, patient was examined in a mycological clinic.CPA was diagnosed based on CT-signs (presence of a cavity with a soft tissue component), a positive galactomannan test in BAL, and positive BAL microscopy for mycelium. The patient received preoperative therapy with voriconazole 400 mg/day for 2 weeks. In November 2018, a resection of C2 of the right lung was performed. Septated mycelium was detected by microscopy of postoperative material and *A. fumigatus* growth was obtained. Antifungal therapy was continued for 2 more weeks. During control examination in the mycology clinic, there were no signs of active CPA, and antifungal therapy was stopped.

Conclusion:
Destructive pneumonia is a risk factor for the CPA development. For the successful treatment of CPA a combination of antifungal therapy and surgical treatment is necessary.

132 MUCORMYCOSIS IN LARGE COHORT OF PEDIATRIC AND ADULT PATIENTS AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION & CHEMOTHERAPY

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Purpose:
The number of publications on the invasive fungal disease (IFD) cause by rare pathogens *Mucorales* in singe-center large cohort of pediatric and adult patients after hematopoietic stem cell transplantation (HSCT) & chemotherapy is limited.

Methods:
The retrospective analysis included cases of IFD cause by *Mucorales* in CIC725 (EBMT) center with high transplant activity rate (nearly 3000 HSCT with 60% of allo-HSCT) for a 10-year period from 2009 to 2018.During the observation period 26 probable and proven mucormycosis (EORTC/MSG 2008 criteria) cases were diagnosed in children and adults with hematological malignances and non-malignant hematological diseases after allo-HSCT (n=19), auto-HSCT (n=1), and chemotherapy (CT) (n=6). The median age was 24 (2-59) y.o., males – 57% (n=15). The median follow up time for mucormycosis cases was 3 months; for survivors – 30 months.

Results:
In six patients, this complication developed after CT and four of them proceed to allo-HSCT. The most frequent underlying diseases were acute lymphoblastic leukemia (27%) and acute myeloid leukemia (30%). The median time of onset of IFD after allo-HSCT was 104 (21-1057) days, auto-HSCT – 138 (60-216), after start of CT – 161 (79-189). Etiology of infections caused by *Mucorales* was identified by culture in 26% cases: *Rhizopus* spp. – 66,8%, *Rhizomucor pusillus* – 8,3%, *Rhizomucor stolonifera – 8,3%, Rhizomucor microspores – 8,3%*, *Rizopus arhizus* – 8,3%. In 84,6% cases (mucormycosis) were diagnosed by microscopy. In 61% cases mucormycosis developed after or in combination with invasive aspergillosis. The main site of infection were lungs (88%), the main clinical symptom – febrile fever (95%). Antifungal therapy was used in all patients: lipid amphotericin B – 31%, lipid amphotericin B + caspofungin – 38,4%, voriconazole – 3,8%, posaconazole – 11,4%, lipid amphotericin B + posaconazole – 7,7%, and echinocandins – 7,7%. Surgery was used in 10% patients. Overall survival at 12 weeks from the diagnosis of mucormycosis was 53,8%. The 12-weeks overall survival was better in patients after CT and auto-HSCT (87,5%) than allo-HSCT (38,9%), p=0,028.

Conclusion:
Mucormycosis is mainly observed in acute leukemia patients and after allo-HSCT. The main etiology is *Rhizopus* spp. Mucormycosis is a late complication after chemotherapy and HSCT and usually developed after or in combination with invasive aspergillosis. The worst prognosis is observed in allo-HSCT recipients with IFD caused by *Mucorales*.

133 FEATURES OF INVASIVE ASPERGILLOSIS IN B-CELL LYMPHOMA PATIENTS: RISK FACTORS, DIAGNOSTICS, TREATMENT AND SURVIVAL

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Purpose:
To study invasive aspergillosis (IA) in B-cell lymphoma patients after cytostatic chemotherapy (CCT) and autologous stem cell transplantation (ASCT).

Methods:
The prospective study included 813 B-cell lymphoma patients: Hodgkin lymphoma (HL) – 363, 16-65 years (median – 33), and non-Hodgkin lymphoma (NHL) – 450, 19-74 years (median – 50). For the IA diagnosis criteria EORTS/MSG 2008 were used.

Results:
Frequency of IA in patients with B-cell lymphoma was 4,98% (HL – 5,6%; NHL – 4,5%, p=0,49), after ASCT – 2,85% (HL – 2,35%; NHL – 3,33%, p≥0,05), during induction and relapse therapy – 4,09% vs 7,14% (p=0,08) accordingly. In patients with relapse of NHL frequency of IA was 10,25%, during induction therapy – 2,88% (p=0,004). The main etiology agents were *Aspergillus fumigatus* (41%), *A.niger* (39%), and *A.flavus* (14%). Risk factors for IA were: relapse of lymphoma (p=0,005), B-symptoms (p=0,035) and radiation therapy in anamnesis (p=0,041), profound neutropenia (p=0,000), concurrent lung (p=0,007) and renal pathology (p=0,03). In patients with HL additional risk factor was viral infection (p=0,002), in patients with NHL – ≥2 lines of CCT in anamnesis (p=0,032). The lungs were involved in 100% cases, ≥2 organs involvement was in 4,5% NHL patients. Clinical symptoms of IA were nonspecific: fever 68%, cough 48%, dyspnea 32,5%, hemoptysis 4,7%, and chest pain 4%. Chest CT scan sings of IA were nonspecific: focal changes 63,5%, infiltrates 58,7% and "ground-glass opacity" 23%; bilateral lung damage – 62,7%. Galactomannan test was positive in BAL fluid and serum in 83,6% cases. The presence of septate mycelium in BAL was observed at microscopy in 15,5% patients. *Aspergillus* spp. culture was obtained in 34,7% of patients with B-cell lymphoma (HL – 20,4%; NHL – 46,3%, p=0,004). "Probable" IA was diagnosed in 92,9%, "proven" – in 7,1% of cases. The main antifungal drug was voriconazole – 79%. In patients with IA the 12-weeks overall survival (OS) was 84,9% (HL – 88,1%; NHL – 82,1%). The use of bronchoscopy and voriconazole improved 12-weeks OS (88,1% vs 64,7%, p=0,011; 92,6% vs 71,1%, p=0,004, accordingly) and 1-year OS (78,9% vs 52,9%, p=0,010; 82,7% vs 62,2%, p=0,010, accordingly). IA did not influence on 2- and 4-year OS, 1,2,3- year progressive-free survival (PFS) and 1-,2-,2,5- year relapse-free survival (RFS) in patients with induction chemotherapy. Furthermore IA did not influence on 2-year OS, 1-,2-year PFS and 1-,2-year RFS in patients with relapsed or refractory B-cell lymphoma (p>0,05).

Conclusions:
Frequency of IA in patients with B-cell lymphoma was 4,98% (HL – 5,6%; NHL – 4,5%), in patients with relapse of NHL - 10,25%. Risk factors for IA were relapse of lymphoma (p=0,005), B-symptoms and radiation therapy in anamnesis (p=0,035 and p=0,041), profound neutropenia (p=0,000), concurrent lung and renal pathology (p=0,007 and p=0,03). Etiology agents were *A.fumigatus* (41%), *A.niger* (39%), *A.flavus* (14%). Clinical and CT-signs were nonspecific. The main antifungal drug was voriconazole – 79%. Overall 12-weeks survival in patients with IA was 84,9%. IA did not influence on OS, PFS and RFS survival of patients with B-cell lymphoma, who receive induction and anti-relapse cytostatic chemotherapy.

134 CASE OF SUCCESSFUL TREATMENT OF INVASIVE ASPERGILLOSIS IN GIRL WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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Objectives:
Publications about invasive aspergillosis (IA) in children with systemic lupus erythematosus are limited.

Methods:
For diagnosis of IA we used criteria EORTS/MSG, 2008.

Results:
16-year-old girl was hospitalized in children's hospital with joints pain, and prolonged fever in February 2018. Systemic lupus erythematosus was diagnosed in 2015. She received prednisolon 1 mg/kg/day for 3 months. On the 20th day of hospitalization pulmonary symptoms appeared. She was in acute respiratory distress (respiratory rate 40 breaths/min) and required intubation and transfer to the intensive care unit. On examination, she was hypoxic with FiO2 - 55%, PO2 - 43%, presented with an episode of profuse epistaxis and oliguria. On the 23th day of hospitalization the patient developed ventricular fibrillation and had a sudden cardiac arrest. Resuscitation measures were successful. She was in a state of unconsciousness. The patient is diagnosed with acute kidney injury: increase of creatinine serum values up to 297g/l, urea - 45,2 g/l, anuria and anasarca occurred, and proteinuria to 3 g/daily. She received methylprednisolone pulse therapy 1 g/day for 3 days and prednisolone 10 mg/kg/day with gradual dose reduction. On the 23th day of hospitalization a chest X-ray showed bilateral interstitial infiltrates, infiltrative changes in S3 of the left lung, consolidation of the lower lobe of the left lung. Examination bronchoalveolar lavage (BAL) revealed numerous septate fungal hyphae with 45° branching compatible with *Aspergillus* spp. BAL culture yielded *A. niger*. BAL Monofluo *P. jirovecii* test and galactomannan test were negative. The patient received caspofungin 70 mg on day 1 and 50 mg/day from day 2. On the 12th and 23th days treatment repeated thorax CT revealed cavities in S6 of the right lung, and abscesses on the right lung. Her renal function and hematological parameters improved gradually as she became hemodialysis free, on day 19 of antifungal therapy caspofungin was replaced with voriconazole 400 mg/day. Voriconazole was discontinued on the 59th day of therapy. The patient's condition was good and chest CT scan improved.

Conclusions:
Patients with systemic lupus erythematosus often require glucocorticoids and other immuno-suppressive agents to induce remission or lower disease activity. In these circumstances, it is necessary to remember the possibility of developing invasive aspergillosis.

135 INVASIVE ASPERGILLOSIS IN PEDIATRIC PATIENTS WITH MALIGNANCIES: RETROSPECTIVE REGISTER REVIEW

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Background:
Invasive aspergillosis (IA) is an important cause of morbidity and mortality in patients with malignancies. There is limited number of publications about IA in pediatric patients with malignancies.

Aim:
To identify risk factors and clinical features of IA in pediatric patients with malignancies.

Materials/Methods:
Retrospective analysis of IA cases in children with pediatric patients with malignancies from 1997 to August 2019 was done. EORTC/MSG, 2008 diagnostic criteria were used.

Results:
We included 56 pediatric patients with malignancies. Median age was 9,5 years (1 - 18), males – 78,2%. The underlying hematological malignancies were in 84% patients, solid tumors – 16%. Equally frequent were myeloid leukemia and acute lymphoblastic leukemia (35,7%), among solid malignancies – central nervous system tumors (42,8%). IA was diagnosed in debut of cancer prior to anticancer treatment in 7% patients, after chemotherapy – 93%, among them after high-dose chemotherapy with hematopoietic stem cells transplantation (HSCT) – 17,8%. Risk factors of IA were long-term neutropenia ≥ 10 days (89,2%), corticosteroid therapy (70%), lymphocytopenia (60,7%) and cytomegalovirus (CMV) infection (17,8%). Clinical signs were non-specific. Main site of IA were lungs (87,5%). Typical «halo sign» on CT scan was in 39,2% patients, “air crescent” sing – 3,5%. Bronchoscopy was done in 62,5% patients with BAL positive galactomannan test in 86%. Positive galactomannan test in serum or cerebrospinal fluid was 33% patients.

Mycological examination of specimen was attempted in 23% patients, among them septated hyphae by microscopy was diagnosed in 17,8%. *Aspergillus spp*. were isolated in culture in 26% (*A.fumigatus* – 54%, *A.niger* – 29%, and *A.ustus* – 13%). Antifungal treatment received 100% patients, with voriconazole only – 55,3%. 12-week survival was 85,7%. Infection control in all cases was achieved before initiation or continuation of anticancer therapy was continued. Antifungal treatment with anticancer chemotherapy was used in 61% patients.

Conclusions:
Cases of IA in children with hematological malignancies were registered more frequently compared to solid tumors (84% vs 16%). Risk factors of IA were prolonged neutropenia (89,2%), steroid therapy (70%), and lymphocytopenia (60,7%). Voriconazole monotherapy was used in 55,3% patients. 12-weeks overall survival was 85,7%. Antifungal treatment with anticancer chemotherapy was used in 61% patients.

136 CHEST COMPUTED TOMOGRAPHY (CT) SCAN FEATURES OF CHRONIC PULMONARY ASPERGILLOSIS (CPA)

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Purpose:
The improvement of CT scan diagnostic of CPA.

Methods:
In the prospective study were included 62 patients with chronic pulmonary aspergillosis (CPA). In the control group were included 39 patients with suspected CPA without laboratory and serological confirmation of CPA diagnosis. CT scanning was performed by Toshiba Aquillion 64-slices CT with a cut-off thickness of 0.9 mm, pitch 1, voltage to the 120kV tube with field of view (FOV) and construction of multiplanar reconstructions, projections with maximum and minimum intensities (MIP, MinIP). The ECMM/ESCMID/ERS 2016 criteria were used for the diagnosis of CPA.

Results:
CPA was confirmed in 62 patients, median age – 60 years (17 – 81), males – 43%. In the control group 39 patients median age was 53 years (24 – 72), males – 34%.

Underlying diseases in CPA patients were a history of tuberculosis – 28%, CODP, bronchial asthma, bronchiectasis – 37%, emphysema – 12 %, sarcoidosis – 10%, destructive pneumonia – 8%, non-tuberculosis mycobacteriosis – 3%, idiopatic pulmonary fibrosis - 2%.

The main underlying diseases in control group patients were: chronic obstructive pulmonary disease, bronchial asthma, chronic bronchitis - 39%, bronchiectasis - 15%, bronchopneumonia post-inflammatory lesions – 12%, emphysema – 9%, post-tuberculous changes, non-tuberculous mycobacteriosis - 6%, sarcoidosis – 6%, cystic fibrosis -5% Wegener's granulomatosis - 4%, lung adenocarcinoma, secondary focal changes - 4%.

In 92 patients (90%) was performed CT dynamic, median number of CT examination was 2 (1 – 6). Focal lesions in lungs were identified in 24% vs 30 % patients, diffuse changes - 14% vs 17%. Bilateral lung lesions were revealed in 31 % vs 17% cases, and unilateral changes – 30% vs 20%. Bronchiectases were determined in 26% vs 14% patients.

The "air-crescent" sign was revealed in 61% vs 3% (p=0,00004) patients, pleural thickening - 53% vs 3% (p=0,0002). Clinical-radiological forms of CPA were single aspergilloma - 30%, *Aspergillus* nodule(s) - 33%, cavitary aspergillosis - 24%, and fibrosing *Aspergillosis* - 13%.

Conclusion:
The "air-crescent" sign was revealed in 61% of CPA patients, pleural thickening - 53%. Single aspergilloma was in 30% patients with CPA, *Aspergillus* nodule(s) - 33%, cavitary aspergillosis - 24%, and fibrosing aspergillosis - 13%.

137 THE GLOBAL IMPACT OF *ASPERGILLUS* INFECTION ON COPD

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Purpose:
Hospitalisation for exacerbation is common among those with more advanced chronic obstructive pulmonary disease (COPD). Two studies indicated that invasive aspergillosis (IA) was present in 1.3-3.9% of hospitalised patients with COPD. Other studies suggest that *Aspergillus* sensitisation worsens symptoms in COPD. We have performed a systematic meta-analysis of COPD prevalence (stages II-IV), annual hospitalisation rates, IA incidence and likely mortality.

Methods:
A complete systematic review of the literature using PRISMA guidelines was undertaken, and estimates derived by country and globally. Only published papers between January 2000 and May 2019 with >50 subjects were used. GOLD criteria for grade II, III or IV (FEV1/FVC <70% and FEV1 <80%) using spirometry were use for prevalence estimates. Studies estimating prevalence to be close to or 0% and studies conducted in specific patient groups; e.g., in acutely unwell patients only were excluded. Limited data were available for individual countries on their annual rates of COPD-related hospitalisations, so a conservative 10.5% estimate was assumed based on the data from studies in Algeria (Polatli, 2012). The proportion of patients with IA was taken Spain (1.3%) (Guinea, 2012) and China (3.9%) (Xu, 2014) and based primarily on positive cultures of *Aspergillus* and radiological findings. Mortality was estimated based on the diagnosis of IA abeing made and patients treated. A separate literature search was undertaken to assess the rate of *Aspergillus* sensitisation and its impact on severity of COPD (by FEV1).

Results:
The global prevalence of COPD GOLD stages II-IV is estimated at 551,800,000 people (7.12% of the population) with 339,206,893 (8.58%) in Asia, 84,770,538 (8.60%) in the America, 64,298,051 (5.37%) in Africa, 59,484,329 (7.77%) in Europe and 4,032,543 (10.86%) in Oceania. The prevalence of *Aspergillus* sensitisation in COPD was 7.0-18.3% (weighted mean 13.6%) and was not related to lower predicted FEV1% (P >0.05). An estimated 57,938,000 people with COPD are admitted to hospital annually and of these 760,000 - 2,272,000 develop IA. 545,000 – 976,000 deaths are predicted annually (43-71%), assuming the diagnosis made and treatment given.

Conclusion:
The number of patients with IA complicating COPD and resulting deaths annually is substantially larger than any other risk group for IA and would appear to be a major cause of death of COPD patients. *Aspergillus* sensitisation probably does not affect COPD symptoms or exacerbation rates. Improved rapid diagnosis of IA using culture and non-culture based techniques is required in COPD hospital admissions to reduce mortality.

138 SYSTEMIC ASPERGILLOSIS SECONDARY TO ACUTE ENTEROCOLITIS IN FOALS

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Purpose:
Aspergillus spp. can be part of the normal gastrointestinal microbiota of horses. Although it can cause opportunistic disease in horses with severe loss of intestinal mucosa integrity, systemic aspergillosis is rarely described in this specie. The aim of this work was to describe fatal cases of enterocolitis associated with systemic aspergillosis in foals.

Case reports:
In a period of six months (Jan-July 2019), one thoroughbred and one Criollo breed, 8 and 9 months old, respectively, from two different private farms in the same region of southern Rio Grande do Sul-Brazil, were sent to the veterinary hospital (HV-UFPel) with diarrhea, abdominal pain, fever, hemodynamic disturbances and other clinical signs characteristic of Systemic Inflammatory Response Syndrome (SIRS). Gastrointestinal physical examination and ultrasound abdominal scanning confirmed the diagnosis of enterocolitis. Leukocytosis with neutrophilia, hypoproteinemia and high serum fibrinogen were found in both foals. In addition to the signs of acute severe enterocolitis, from the second day of admission, the Criollo foal showed abnormal clinical signs associated with the respiratory tract. Both animals received dexamethasone for three days, and intensive supportive care with fluid therapy, and non-steroid anti-inflammatory for the entire treatment period. Aggressive antibiotic therapy with erythromycin and rifampicin or ceftriaxone and metronidazole was given, but both foals were unresponsive to treatment, dying ~two weeks (12 and 14 days) after admission. Salmonella spp. was isolated from faecal culture of the thoroughbred foal. In addition to necrosis identified in villi and intestinal crypts in the thoroughbred foal necropsy, there was dissemination of several whitish nodules with a dense and friable cystic wall in the myocardium, brain and particularly in the lungs. The Criollo foal necropsy showed dark disseminated nodules with yellowish center in the lungs, liver and intestines. Histopathology of the nodules from both foals showed hyaline septate branched fungal hyphae, which were later speciated to be *Aspergillus* section *Fumigati* infection, isolated in fungal culture from lung tissue.

Conclusion:
Immunosuppression is the main risk factor for systemic and invasive presentations of aspergillosis. Although none of our foals were neutropenic, both had SIRS associated with an acute and severe enterocolitis and received corticotherapy. While the pathogenesis of systemic aspergillosis in horses is poorly understood, studies suggest the loss of gastrointestinal mucosa integrity can predispose to fungal tissue invasion and dissemination. In fact, *Aspergillus* hyphae were found in the intestinal mucosa lesions of our cases. On the other hand, both animals showed highly infected lungs with *Aspergillus*, highlighting that the *Aspergillus* infections could also have occurred by conidia inhalation from the environment/hay. Of interest, at least four other cases of systemic aspergillosis associated with enterocolitis were described in the same region where our foals were housed and in the same period. Given that aspergillosis is a rare opportunistic disease in horses with enterocolitis, and it was never described in this frequency in our region, these cases require investigations regarding the fungal virulence, the host susceptibility and the risk factors. Veterinary clinicians must be aware of the occurrence of fungal diseases in horses with enterocolitis not responsive to antibiotic therapy.

139 RHINOCEREBRAL MUCORMYCOSIS IN A NEWLY DIAGNOSED TYPE I DIABETIC: A CASE REPORT AT A TERTIARY FACILITY IN NORTHERN NIGERIA

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Purpose:
The aim of this report is to increase awareness on mucormycosis and highlight challenges to diagnosis and management in a resource limited setting

Methods:
A case report in which we describe the clinical history, management and outcome of rhinocerebral mucormycosis in a newly diagnosed type I diabetic.

Results:
We describe the report of a 32 year old single man who was referred from a peripheral hospital with 1 month history of excessive urination, 4 day history of pain on micturition and I day history of altered sensorium. The patient noticed increase in urinary frequency from about 7 times to 15 times during the day and from 1 to 7 times at night. This was associated with increase in volume of urine from approximately 2 to 6 liters per day. There was no history of urethral discharge or trauma. There was associated weight loss despite increase in appetite. There was no history of vomiting, headache or convulsion however patient was not fully conscious at presentation

He was not a known diabetic or hypertensive with no history of kidney or liver disease and had no family history of diabetes or hypertension. He neither smoked cigarettes, ingested tobacco nor drank alcohol and had no previous hospital admissions prior to current illness.

On examination he was found to be acutely ill looking, not pale, anicteric, acyanosed, moderately dehydrated and febrile with bilateral pitting pedal oedema.

He had a GCS of 11, pulse rate of 101 beats/minute, BP 130/80mmHg and first and second heart sounds only. His respiratory rate was 24 cycles per min, with normal percussion notes and vesicular breath sounds. On abdominal examination right renal angle tenderness was elicited though no organomegaly, nor ascites was demonstrable.

Investigations at admission were random blood glucose 30.2mmol/l ,PCV 39.0%, urea 9.1,Na 139,K 6.5,Cl 106,HC03 23;urinalysis urine PH 6.0 with + blood and glucose but negative for ketones, leucocytes and proteins.

An impression of hyperglycaemic emergency Type 1 precipitated by pyelonephritis was made. The patient was commenced on IVF 5% dextrose saline; soluble insulin and intravenous Ceftriaxone. His condition improved clinically with a GCS of 15. However, after 3 days on admission he was noted to have swelling of the right side of the face.

Further examination revealed ptosis with periorbital swelling, subconjunctival protrusion and chemosis of the right eye, associated purulent eye discharge, bilateral nasal discharge which was mucoid and black stained. Examination of the buccal cavity revealed an ulcer on the right side of the hard palate measuring 3x5 cm with a necrotic base. Biopsy samples from nasal palate sent for mycology culture revealed broad aseptate hyphae on microscopy and *Rhizopus oryzae* on culture. Liposomal amphotericin B was prescribed at a dose of 3mg/kg commenced on day 6 of admission however he had only 4 doses for financial constraint and was off amphotericin B for 12 days, recommenced amphotericin B deoxycholate for 6 days before he developed profuse watery diarrhea and died on day 27 of admission.

Conclusion:
Rhinocerebral mucormycosis is a rare but severe invasive mycosis. A high index of suspicion, prompt diagnosis and availability of treatment is important in improving outcome.

140 A CASE OF INVASIVE PULMONARY ASPERGILLOSIS OCCURRING IN A NON-NEUTROPENIC PATIENT AT A TERTIARY FACILITY IN NORTH-WEST NIGERIA

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Purpose:
We report a case of invasive pulmonary aspergillosis (IPA) occurring in a non-neutropenic patient to increase awareness and index of suspicion for diagnosis of IPA in such patients

Methods:
A case review of IPA which occurred in a non-neutropenic patient reporting the history, clinical management and outcome

Results:
We describe the case of a 65 year old married male farmer being managed as a case of chronic myeloid leukaemia (CML) in chronic phase. The patient had been diagnosed with TB adenitis a year ago and had completed an 11 month course of anti-TB treatment. Three weeks later he presented with cough productive of scanty mucoid sputum, not associated with fever, dyspnea, chest pain, haemoptysis, night sweats though had weight loss. Patient is a known hypertensive though not diabetic

On clinical examination he was afebrile, not pale, anicteric, with cervical and inguinal lymphadenopathy, largest measuring 2x3 cm and no pedal oedema. His pulse rate was 88 beats per minute, blood pressure 120 /80mmHg. Heart sounds were SI and $II only. Respiratory system exam revealed a respiratory rate of 20 cycles per minute, vesicular breath sounds, no crepitation's or rhonki. The abdomen was full soft with the liver 2cm and spleen 6cm below right and left costal margins respectively. Musculoskeletal and central nervous system examination were not contributory. An impression of CML with lower respiratory tract infection was made.

The patient was referred to TB clinic and investigated for pulmonary TB with sputum AFB, sputum MCS, Mantoux and chest Xray. Results for sputum AFB were negative, sputum MCS yielded normal flora, Mantoux at 3mm was negative and chest Xray showed normal study. An impression of resolved TB adenitis on background CML was made. Plan was to do chest CT and patient was commenced on Benylin with codeine for 2 weeks and referred back to Hematologists.

Primary physicians reviewed his management and requested for FBC differentials, sputum MCS, for fungal cultures. He was treated empirically with oral albendazole 400mg daily for 3 days and erythromycin 500mg 6 hourly for 10 days.

Results showed PCV 37.2%, WBC count of 28.86 $x10^9$/L, neutrophils 60%, myelocytes 6%, metamyelocytes 2%, band forms 2%, eosinophils 4%, lymphocytes 24%, monocytes 2%. platelets 192$x10^9$/L. Sputum MCS yielded *Aspergillus fumigatus* and *Enterobacter gergoviae,* chest Xray showed bilateral hilar fullness. Chest CT scan not done for financial constraints. Medical Microbiology was asked to review. Additional finding was absence of digital clubbing other findings generally as documented previously. An impression of pulmonary TB on background CML and possible colonisation with aspergillosis was made.

Repeat sputum MCS and Gene Xpert for MTB was requested. Gene Xpert MTB was negative and repeat sputum cultures yielded *Aspergillus* species. An impression of IPA was made and patients serum for *Aspergillus* galactomannan assay sent to Lagos University Teaching Hospital. The patient was commenced on itraconazole 200mg 12 hourly while awaiting results of galactomannan assay which were obtained 2 months later and was positive. Patient is currently stable and symptom free after 8 months on itraconazole

Conclusion:
Diagnosis of IPA was delayed in this case and posed a challenge in the face of limited diagnostic facilities.

141 *ASPERGILLUS* KERATITIS IN THE CENTER OF TUNISIA: A 37-YEAR RETROSPECTIVE STUDY

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Purpose:
Aspergillus keratitis is a serious pathology that can engage the visual prognosis. The aim of this study is to specify the epidemiological and mycological characteristics of *Aspergillus* keratitis in the center of Tunisia.

Methods:
Retrospective study of 10 cases of *Aspergillus* keratitis cases diagnosed in the laboratory of Parasitology-Mycology at Farhat Hached Teaching hospital of Sousse, Tunisia, during a 37-year period (1982-2018). Laboratory diagnosis was made by direct microscopic examination and culture on Sabouraud-chloramphenicol medium of corneal scrapings, contact lenses and their maintenance fluid. The identification of fungi was based on macroscopic and microscopic characteristics of culture.

Results:
Aspergillus keratitis involved 8 men and 2 women (sex ratio: 4). The mean age of patients was 49.3 years. Presumed predisposing factors were identified only in 3 patients. One patient had underlying diseases (diabetes and herpes simplex keratitis), the second patient had undergoing ocular surgery and the third patient had been using contact lenses. Direct microscopic examination was positive in 3 cases (30%) and culture was positive in all cases. *Aspergillus* species identified were *Aspergillus (A) niger* in 40%, *A. flavus* in 30%, *A. fumigates* in 10%, *A. clavatonanicus* in 10% and *A.spp* in 10% of cases.

Conclusion:
Rapid and accurate identification of the causative agent of fungal keratitis can guide the prescription of antifungals and condition response to treatment. A proper understanding of agent, host factors and risk factors involved in the infection can improve the outcome and management of this condition.

142 EPIDEMIOLOGICAL TRENDS IN *ASPERGILLUS* SPECTRUM INVOLVED IN HUMAN ASPERGILLOSIS IN SOUSSE REGION, TUNISIA: A 33-YEAR RETROSPECTIVE STUDY (1986-2019)

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Purpose:
To study the spectrum of *Aspergillus* involved in human pathology in various clinical samples received in the laboratory of Parasitology-Mycology at Farhat Hached Teaching Hospital of Sousse, central Tunisia.

Methods:
Our work is a retrospective cross-sectional study covering a period of 33 years from January 1st, 1986 to October 31st, 2019. The study population included all *Aspergillus* strains isolated from human biological samples in the laboratory of Parasitology-Mycology at Farhat Hached Teaching Hospital of Sousse, Tunisia, during the study period. Mycological diagnosis was based on direct microscopy of biological specimens and species identification by culture on Sabouraud-Chloramphenicol and Czapec mediums.

Results:
Over the 33-year study period (1986-20119), 1040 *Aspergillus* isolates were recovered from 954 biological samples collected from 879 patients. Men accounted for 52,5% of the patients. The most frequently isolated species were *A. flavus* (40,6%) and *A. niger* (34,6%). A single *Aspergillus* specie was isolated in 725 samples (76%), two species in 65 samples (6,8%) and three species in 12 samples (1,26%). The *Aspergillus* isolates were recovered from the external auditory canal (48,74%), the lower respiratory tract (36,7%), toenails (8%) and other sites (6,56%). The direct examination was positive for 519 (54,1%) samples. It showed mycelial filaments for 431 (45,2%) specimens.

Conclusion:
This study highlights the remarkable diversity of manifestations caused by *Aspergillus* species in human and the prevalence of different species in our region. Antifungal susceptibility is not similar in all species. We found that *A. flavus* is the most common specie in our region. These findings are very useful to more accurate selection of empirical antifungal therapy in aspergillosis management.

143 PULMONARY MUCORMYCOSIS IN DIABETIC PATIENTS: A REPORT OF 4 CASES

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Purpose:
Pulmonary mucormycosis, a life-threatening fungal infection under diagnosed by clinicians due to unspecific clinical picture and lack of awareness. The aim of our work is to better characterize the population at risk, presenting symptoms, diagnostic methods, therapy, and outcome of pulmonary mucormycosis in diabetic patients.

Methods:
It is a retrospective study that was carried, from 2001 to 2019. It included 4 cases with pulmonary mucormycosis. The patients were referred to the Mycology-Parasitology laboratory of Farhat Hached hospital in Sousse for mycological examination.

The zygomycete infection was confirmed by either or both histologically and mycological examination of pulmonary specimens.

Results:
The average age of the patients was 48 years (ranged between 19 and 77 years) and the male/female ratio was 3. In addition to diabetes, one patient presented other risk factors as acute renal failure and neutropenia. The main symptoms were cough, fever, dyspnea and haematic tracheal secretions.

The Chest Computed tomography, performed in three cases, showed bilateral alveolar-interstitial infiltrate with alveolar consolidation in the right lower lobe in one case, an excavated lesion in the left lower lobe in another case and a proximal hilar lesion invading the middle lobar bronchus in the latter one.

Direct mycological examination showed large and non-septal hyphae in 3 cases and culture grew *R. arrhizus* in two cases and *lichtemia sp* in one case. The diagnosis of mucormycosis was confirmed by histological examination in 3 cases.

Antifungal treatment consisted in systemic amphotericin B in three patients. The latter one did not receive specific mucormycosis therapy for fulminant death. Combined treatment with systemic amphotericin B and surgery resulted in a favorable outcome in only one case. The others were unsuccessfully managed with medical therapy.

Conclusion:
Clinicians ‘awareness to early diagnosis, combined antifungal treatment and adjuvant surgery, offer a greatest chance of cure of a rapidly progressive disease with high mortality and morbidity.

144 RHINOCEREBRAL MUCORMYCOSIS IN CENTRAL TUNISIA: A STUDY OF 15 CASES

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Purpose:
Rhinocerebral mucormycosis, a rare fungal infection, is significant for its rapid progression and high mortality even after active management.

The aim of this study was to determine clinical, mycological, therapeutic and outcome characteristics of rhinocerebral mucormycosis in central Tunisia.

Methods:
It is a retrospective study that was carried out over a 25- year period from 1992 to 2017 in the Parasitology-Mycology laboratory of Farhat Hached Hospital in Sousse. It included 15 cases of rhinocerebral mucormycosis. The diagnosis was carried out with histopathologic and mycological examination of necrotic tissue.

Results:
Patient ages ranged from 16 to 76 years (median age 47.1 years) and the female: male ratio was 1.1.

The main risk factors were diabetes mellitus (66.7%) and hematologic malignancies (20%).

Direct mycological examination showed large and non-septal hyphae in 13 cases (86.7%). Culture was performed in all cases and was found positive in 13 cases. *Rhizopus* species (*Rhizopus arrhizus*: 10 cases, *Rhizopus sp* and *rhizopodiformis*: 1 case respectively) were the most common pathogen (92.3%) among the identified Mucorales.

Histopathology was performed in all patients and revealed thick, non-septate hyphae branching at right angle.

Antifungal treatment consisted of Amphotericin B deoxycholate in 14 patients. The latter one did not receive specific mucormycosis therapy for fulminant death. Combination of antifungal treatment and surgical debridement was registered in nine cases and mucormycosis was considered responsible for death in nine patients.

Conclusion:
Mucormycosis is an angioinvasive mycosis with high morbidity and mortality. Rapid diagnosis and early aggressive therapy to prevent cerebral involvement by this severe infection provides the best chance for a good outcome.

145 VARIOUS CLINICAL FORMS OF EAR MUCORMYCOSIS: REPORT OF FOUR CASES

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Purpose:
Mucormycosis, a deadly invasive fungal infection, is seen more commonly in the nose and paranasal sinuses. Ear mucormycosis is a rare entity. Our objective was to describe four cases of ear mycormycosis with various clinical forms as otitis externa, otitis media and otocerebral localisation.

Methods:
This was a retrospective study, being performed in the Parasitology- Mycology laboratory of Farhat Hached Hospital in Sousse, over a period of 11 years from 2006 through 2018. The zygomycete infection was confirmed by either or both histologically and mycological examination of ear specimens.

Results:
Patient ages ranged from 3 to 78 years (median age: 58.75 years) and the male: female ratio was 3.

The predisposing factors were kidney failure in one patient, cell-mediated immunity defect in another one and diabetes mellitus was noted in one case. No underlying condition was registred in one case.

Auricular mucormycosis involved otocerebral (2 patients), chronic otitis media (1 patient) and malignant otitis externa (1 patient).

Direct mycological examination showed large and non-septal hyphae in two cases and the culture grews *Lichtemia corymbifere* in two cases, *Rhizopus arrhizus* in one case, and *Rhizopodiformis* in the latter case. The diagnosis of mucormycosis was confirmed by histological examination in 3 cases.

Treatment consisted of combination of antifungal treatment and surgical debridement in two cases. The quasi majority of antifungal-treated patients (3/4cases) received an amphotericin B formulation (amphotericin B deoxycholate in two cases and liposomal amphotericin B (L-AmB) in the other case. Two (2/3) of these patients received amphotericin B in combination with other antifungals (fluconazole in one case and itraconazole in the other case). Mucormycosis was considered responsible for death in one patient with otocerebral localisation.

Conclusion:
A prompt diagnosis, control of predisposing factors, early surgical debridement of infective tissue and administration of antifungals are keys to successful therapy.

146 PRIMARY CUTANEOUS ASPERGILLOSIS: REPORT OF TWO CASES

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Purpose:
Primary cutaneous aspergillosis is a rare condition, usually associated with immunodeficiency, secondary to hematologic disorders. The aim of our work is to better characterize clinical, diagnostic and therapeutic aspects of primary cutaneous aspergillosis in our hospital.

Methods:
It is a retrospective study that was carried, from 2010 to 2019. It included two cases with primary cutaneous aspergillosis. The diagnosis is carried out with histopathologic examination and culture of skin biopsy specimen.

Results:
Case 1: A32-year-old man with an anaplastic large T-cell lymphoma was admitted at the haematology department of Farhat-Hached Hospital (Sousse, Tunisia) on 2010 for a suspected lymphoma relaps. The lymphadenectomy was performed and confirmed the relapse of the malignancy. Salvage chemotherapy was initiated, and on day 1 of the neutropenic phase, the patient presented fever and the lymphadenectomy wound did not heal despite local care. Despite antibiotics treatment, the wound worsened and showed an extensive necrosis. Histopathologic examination of a skin biopsy specimen showed abundant hyphae consistent with *Aspergillus* species and *Aspergillus* galactomannan antigenemia was positive. Direct microscopic examination revealed abundant septate hyphae and cultures yielded *Aspergillus flavus*. Treatment consisted of combination of voriconazole and surgical debridement. Patient died of multiorgan failure caused by bacterial sepsis without secondary dissemination of the *Aspergillus* infection.

Case 2: A 22-year-old man diagnosed with idiopathic bone marrow aplasia was admitted to haematology department of Farhat-Hached Hospital (Sousse, Tunisia) on 2019 for therapeutic management. At day 15 of cyclosporine, the patient presented fever and folliculitis on his right leg. Despite antibiotics treatment, the patient developed leg necrotic fasciitis, requiring the use of surgical excision and modification of initial antibiotherapy. Mycological examination of the necrotic tissue detected many mycelial filaments on direct examination and the culture grews *A. flavus*. Anatomopathological examination revealed fibrino-necrotic material containing mycelial hyphae consistent with *Aspergillus* species and *Aspergillus* galactomannan antigenemia was positive. Systemic treatment with Voriconazole led to complete resolution.

Conclusion:
Aspergillus infection should be suspected in front of any cutaneous lesion in neutropenic patients, to avoid misdiagnosed cases and treatment delay.

147 GALACTOMANNAN DETECTION IN BRONCHOALVEOLAR LAVAGE FLUIDS: A DIAGNOSTIC APPROACH FOR FUNGUS BALL IN PATIENTS WITH PULMONARY TUBERCULOSIS?

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Purpose:
In this present study we aimed the evaluation of GM levels in the BAL samples for fungus ball diagnosis in patients with PTB.

Methods:
This retrospective study was conducted on the PTB patients referred to the Reference Center for TB and Pulmonary Diseases of Iran, during 2017-2019. The patients were evaluated in terms of radiological findings, histopathological results, and mycological findings of BAL samples including GM detection and culture.

Data were analyzed using SPSS® 22.0 (SPSS Inc., Chicago, IL, USA). The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for each possibility of the cut-off values of GM test were evaluated. The optimal cut-off for BAL GM testing was determined by receiver operating characteristic (ROC).

Results:
A total of 110 PTB patients (60 with active PTB and 50 with previous history of PTB) were included. The age range of patients was 16-87 years with a mean age of 56.5 years. Of 110 patients, 40 (36.3%) and 45 (40.9%) cases were positive for septate hyphae in direct microscopic examination and growth of *Aspergillus* species in culture of BAL samples, respectively. *A. flavus* complex (35, 38.9%) was the most common followed by, *A. fumigatus* complex (20, 22.2%) and *A. niger* complex (19, 21.1%).

Out of 110 patients with PTB, 9 (8.18%) patients showed fungus ball, all with old PTB. Histopathology demonstrated fungus hyphae in biopsied tissue from all 9 patients. The patients with positive results in histopathology and culture for *Aspergillus* in BAL samples were considered as aspergilloma (4, 44.4%) and others as fungus ball (5, 55.5%). The molecular approach on isolated *Aspergillus* species in aspergilloma cases confirmed the isolates as *A. flavus* (2, 50%), *A. fumigatus* in (1, 25%), and *A. ochraceus* (1, 25%).

GM index ≥ 0.5 of BAL samples were positive in 66.0% of old TB patients and in 100% of patients with fungus balls. The statistical analysis of the mean ± SEM of BAL GM level was demonstrated a higher levels of GM in patients with fungus ball and aspergilloma in comparison with active and old TB patients without fungus ball or aspergilloma with a statistically significant difference. The sensitivity and specificity for GM detection (index ≥ 0.5) in BAL samples from old TB patients with fungus ball were 100%, 41.5%, respectively. By ROC curve analysis, we found that the optimal cut-off value for BAL GM detection was 0.50.

Conclusion:
According to our results the detection of GM in BAL samples could be considered as an approach for diagnosis of fungus ball in patients with underlying condition including TB. Our data have

also supported that the optimal cut-off value for BAL GM detection is index ≥ 0.5 in PTB patients suspected to fungus ball. However further studies with a larger samples are needed in order to assess the possible use of GM detection for diagnosis of chronic pulmonary aspergillosis including fungus ball or aspergilloma in suspected patients.

148 PCR BASED METHODS FOR DIAGNOSIS OF MUCORMYCOSIS

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Purpose:
Invasive mucormycosis (IM) is a life-threatening infection caused by *Mucorales*. However, rapid progression of the disease and absence of early and reliable diagnostic assay lead to high mortality and morbidity. The sensitivity of conventional methods including direct microscopy and culture is around 50% and data utilizing molecular assays for diagnosis is very limited. Therefore the present study was conducted to assess the diagnostic utility of (panfungal PCR in combination with Mucorales specific PCR) among suspected cases of IM.

Methods:
This was a prospective study where clinically suspected cases of IM attending our tertiary care hospital from August 2015 – March 2018 were enrolled. All the cases were defined as proven/ probable and possible cases of mucormycosis based on EORTC/MSG guidelines. Conventional identification was performed using direct microscopy and culture. In addition, panfungal and Mucorales specific PCR assay were performed simultaneously on all the collected specimens using primers for ITS region of fungi. The amplified products were further subjected for sequencing to confirm species identification

Results:
A total of 297 clinical samples were collected from 239 clinically suspected cases of IM. Diabetes mellitus was the most common underlying predisposing condition documented in 90.79% (217/239) cases. Among 239 IM cases, six (2.51%) cases were classified as Proven, 134(56.06%) as probable and 99(41.42%) as possible cases of IM.

- **Comparison of direct microscopy and PCR**

Among 297 clinical specimens, 180 specimens (60.60%) showed the presence of broad aseptate hyphae on direct microscopy. Pan fungal PCR was positive in 215 specimen and Mucorales specific PCR was positive in 199 specimens among which 178 (178/215, 82.79 %,) were direct microcopy positive and remaining 37 (37/215, 17.29 %,) were negative. Overall the concordance rate documented between direct microscopy and PCR was 86.86%. The sensitivity and specificity of PCR was found to be 98.88% and 68.37% respectively taking direct microscopy as reference.

- **Comparison of culture and PCR:**

Mucorales grew on culture in 55 samples, and all of which panfungal PCR and Mucorales specific PCR positive too. Among culture negatives (n=242), PCR was positive in 160 (66.11%) cases. Concordance rate of 46.12% was recorded between culture and pan fungal PCR. Taking culture as reference technique, the sensitivity and specificity PCR was documented to be 100% and 33.88% respectively.

Conclusion:
In conclusion, as the sensitivity and specificity of conventional method is low, panfungal PCR in combination with Mucorales specific PCR followed by sequencing may play a significant role in diagnosis especially among those with negative direct microscopy and culture.

149 MUCORALES-SPECIFIC QUANTITATIVE PCR ON PERIPHERAL BLOOD IS A SENSITIVE AND EARLY DIAGNOSTIC MARKER FOR INVASIVE MUCORMYCOSIS

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Purpose:
Invasive mucormycosis is still a potentially lethal infection, requiring early and aggressive therapy. However, making a timely diagnosis is a challenge due to the lack of sensitive diagnostic tests. Recently, Mucorales-specific quantitative PCR (qPCR) assays were developed for the detection of Mucorales DNA in patient samples. These assays have already proven to be useful on biopsy specimens or on bronchial alveolar lavage fluid from infected sites. However, getting a biopsy or other sample from the affected body site is not always possible. Detection of circulating Mucorales DNA in blood could offer an attractive diagnostic tool in these patients. We therefore evaluated the sensitivity a commercial Mucorales-specific qPCR and kinetics of Mucorales DNA in serial blood samples from patients with culture-positive invasive mucormycosis.

Methods:
We retrospectively collected serial serum, plasma or whole blood samples from the biobanks at 2 hospitals in Belgium (University Hospitals Leuven and AZ St Jan Brugge) from patients with culture-positive invasive mucormycosis. Cases were classified according to the 2008 revised EORTC / MSG consensus definitions. We added a classification of "putative" mucormycosis for patients with well-recognized risk factors for mucormycosis (such as diabetic ketoacidosis or iron chelation therapy), but not fulfilling the EORTC/MSG-defined host criteria. The date on which the sample that resulted in a positive Mucorales culture was taken, was defined as the date of diagnosis (D+0). We collected all blood samples from our biobanks from 2 weeks before up to 2 weeks after the date of diagnosis (maximum 2 samples per week). We extended our search period until the samples became negative, or until there were no more stored samples available in the biobank, as applicable. All samples were tested using a Mucorales-specific qPCR (MucorGenius®, PathoNostics, The Netherlands).

Results:
We identified 16 patients with invasive mucormycosis between 2009 and 2019 and retrieved 102 blood samples for qPCR testing. The temporal evolution of each patient is shown in Figure 1. We found an overall sensitivity of 0.75 (95% CI 0.48 – 0.93). Serial testing of blood samples showed that DNA was present up to 106 days (median 10 days, inter-quartile range [IQR] 1.75 – 16.25) before diagnosis by culture, and up to 25 days (median 6 days, IQR 0.75 – 15.5) before the first signs of fungal infection on imaging.

qPCR was positive in all patients who died within six weeks, whereas qPCR was negative in 40% of patients who survived for more than six weeks (6/6 vs 6/10, p=0.234). All patients who succumbed before week 6 died of mucormycosis. Autopsy reports in the five patients in whom this was performed showed disseminated disease in all five cases, also involving organs that showed no clear signs of infection pre-mortem such as the liver and spleen.

Conclusion:
The MucorGenius® assay in blood proves to be a sensitive and early diagnostic tool for invasive mucormycosis. It allows a diagnosis to be made before cultures are positive and before typical signs are visible on imaging.

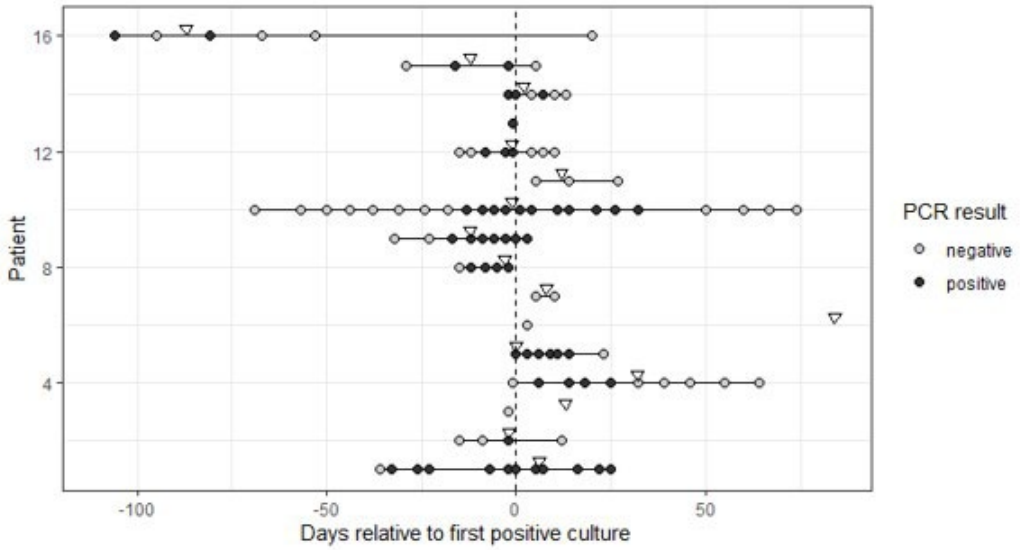

Figure 1. Temporal evolution of blood qPCR results. A white triangle denotes initiation of antifungal therapy.

150 DEFINING NEW RISK FACTORS FOR *ASPERGILLUS* BRONCHITIS

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Purpose:
Aspergillus bronchitis is a chronic non-invasive infection of the lower respiratory airways affecting immunocompetent patients, many with bronchiectasis. These patients present with breathlessness, repetitive bacterial infections and mucus plugging. Bronchoscopy examination reveals thick mucus often with ulceration and superficial hyphal growth can be detected in biopsies. However, little is known about the pathophysiology of the disease. Our objective was to define new pathophysiological aspects contributing to *Aspergillus* bronchitis.

Methods:
46 patients with *Aspergillus* bronchitis diagnosed at the UK National Aspergillosis Centre were included in the study. DNA from blood samples was used to determine the prevalence of the fungal-colonisation at risk allele rs35699176 in the transcription factor ZNF77. Associations between genotype and clinical characteristics were analysed. Additionally, we optimised a protocol to characterise the mycobiome composition on 51 DNA sputum samples from 24 patients with *Aspergillus* bronchitis. For that, a fragment ITS-1 region was amplified on 2 µl of sputum DNA. Libraries were constructed using the NextEra indexing kit and amplicons were resolved by Illumina Mi-seq. Negative controls, positive controls including known amounts of fungal DNA and technical replicates were included in the experiment.

Results:
Seventeen per cent of the patients with *Aspergillus* bronchitis carried the genetic variant rs35699176 we previously associated with fungal colonisation in the airways (Gago, NatComms 2018). The prevalence of this SNP in *Aspergillus* bronchitis was 8% higher than ABPA. Patients carrying the genetic variant rs35699176 were significantly more sensitised to *Alternaria alternata* and *Aspergillus fumigatus* and had increased serum levels of mannose-binding lectin ($P < 0.05$). Additionally, bacteria and/or fungi were more frequently detected in the airways from those patients than in patients with functional ZNF77 (50% vs 30% for fungi and 38% vs 16% for bacteria). Due to the nature of the respiratory samples used, the most common fungal species detected on sputum samples using Illumina sequencing belonged to *Candida* and *Saccharomyces* genera followed by environmental moulds such as *Cladosporium, Alternaria* and *Penicillium*. *Aspergillus* species were detected in 31% of the samples (> 10 reads) but the abundance was much lower than other taxonomical groups. Reproducibility of 10 of the samples tested in technical replicates was higher than 90%.

Conclusion:
The prevalence of the fungal-colonisation at risk allele rs35699176 in patients with *Aspergillus* bronchitis was significantly higher than the reported for patients with fungal allergy. Patients diagnosed with *Aspergillus* bronchitis were highly colonised by environmental fungi such as *Cladosporium* and *Alternaria*, raising the possibility that this clinical entity is actually 'fungal bronchitis' or 'airway mycosis' or 'fungal-associated airways disease'. Further research is needed to determine host factors contributing to the composition of the lung mycobiome in those patients.

151 A HIGH FREQUENCY OF AZOLE RESISTANCE DUE TO G54 MUTATIONS IN *ASPERGILLUS FUMIGATUS* FROM BRONCHOALVEOLAR LAVAGE OF CHRONIC RESPIRATORY DISEASES PATIENTS IN A REFERRAL CHEST HOSPITAL IN DELHI, INDIA USING THE ASPERGENIUS® RESISTANCE REAL-TIME PCR ASSAY AND A NEW G54/M220 ASSAY

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Purpose:
Azole therapy for invasive, chronic and allergic diseases of lungs caused by *Aspergillus fumigatus* reduce mortality and morbidity in aspergillosis patients. A low burden of azole resistance in aspergillosis could be attributed to limited culture yield of respiratory specimens, leading to uncertainty of true triazole resistance. The present study evaluated the diagnostic performance of AsperGenius®, a multiplex real-time PCR assay to detect *A. fumigatus* and *Cyp51A* gene mutations in BAL specimens as compared to direct microscopy and fungal culture.

Material and methods:
A total of 160 BAL samples received during 2017-18 in a referral chest hospital in Delhi, India were processed for microscopic examination (10% KOH-Blankophor), fungal culture and serum/ BAL galactomannan. In parallel BAL were tested with the AsperGenius® Resistance multiplex real-time PCR assay consisting of two multiplex real-time PCRs to detect *Aspergillus* DNA. This assay differentiates *A. fumigatus* and *A. terreu*s among *Aspergillus* species and detect four mutations in the *Cyp51A* gene (TR34/L98H, Y121F/T289A) of *A. fumigatus*. Additionally, a newly developed AsperGenius® G54/M220 multiplex real-time PCR assay, using melting-curve analyses was performed.

Results:
Overall, 83% of BAL were positive for *A. fumigatus* by AsperGenius®, out of which 33 (25%) had mutations associated with resistance. Notably, 70% of resistance mechanism was attributed to G54 mutations. The distribution of mutations included G54 (67%, n=22), Y121F/T289A (15%, n=5), TR34/L98H (12% n=4), and single each of M220 and TR34/L98H + G54. Of 83% of the *A. fumigatus* PCR positive specimens, 75% (n=100) also harboured *Cyp51A* WT *A. fumigatus*. In contrast to PCR only 23% of BAL specimens yielded *Aspergillus* species in culture and a very low positivity (2%) on direct microscopic examination. Furthermore, only 5% (n=2) of culture positive *A. fumigatus* had resistance mutations (G54 and TR34/L98H). Interestingly, 71% of BAL specimens were from chronic obstructive lung diseases and post tuberculosis patients. The remaining were from suspected cases of CPA, APBA and IPA.

Discussion:
A high proportion (n= 133,83%) of BAL samples harboured *A. fumigatus* by PCR which is linked to high burden of COPD, ABPA, CPA and post tuberculosis patients analysed. In previous reports the AsperGenius® commercial assay included most common environmentally mediated azole resistance mutations i.e., TR34/L98H. However, SNPs such as G54 in *Cyp51A* develops *in vivo* on long term azole therapy, although this mutation has been detected in environmental *A. fumigatus* isolates. The AsperGenius® G54/M220 multiplex real-time PCR is a newly developed assay which can detect G54 and M220 and differentiate from WT mutations in *Cyp51A*. A high burden of G54 mutations is probably attributed to the patient population with CPA and ABPA analysed in the present study who had previously received itraconazole. Considering that only 5% of culture positive *A. fumigatus* had resistance mutations whereas this new non-culture assay detected resistance mutations in 24% of samples suggests a higher sensitivity of this assay to detect azole resistance as compared to culture. In conclusion, we report for the first time G54 and M220 mutations using the new AsperGenius® G54/M220 real-time PCR assay in patients with chronic respiratory diseases.

152 MITOCHONDRIAL MARKERS A FUTURE DIRECTION FOR THE DETECTION, IDENTIFICATION AND QUANTIFICATION OF MUCORMYCETES?

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Purpose:
Mucormycosis infections are infrequent yet aggressive and serious fungal infections that are emerging particularly in countries with high rates of uncontrolled diabetes, such as India. Early diagnosis of mucormycosis and delimitation from other fungal disease such as candidiasis, aspergillosis, and cryptococcosis is required for targeted treatment. The majority of the molecular assays use either 18 S rDNA or 28 S rDNA.

The aim of the current study was to explore the potential of the mitochondrial rnl(encoding for large-subunit-ribosomal-RNA) gene as a novel molecular marker.

Methods:
Rnl was evaluated as a marker for: (1) detection (pan-Mucorales), (2) species identification, (3) growth stage, and (4) quantification. Sensitivity, specificity, discriminatory power, the limit of detection (LoD), and cross-reactivity were evaluated. Assays were tested using pure cultures, spiked clinical samples, murine organs, and human paraffin-embedded-tissue (FFPE) samples.

Results:
Mitochondrial markers were found to be superior to nuclear markers for degraded tissue samples of mucormycosis. Rnl outperformed the UMD universal® (Molyzm) marker in FFPE (71.5% positive samples versus 50%). Spiked blood samples highlighted the potential of rnl as a pan-*Mucorales* screening test. Fungal burden was reproducibly quantified in murine organs using standard curves. Identification of pure cultures gave a perfect (100%) correlation with the detected internal transcribed spacer (ITS) sequence.

Conclusion:
In conclusion, mitochondrial genes, such as rnl, provide an alternative to the nuclear 18 S rDNA and 28S rDNA genes and deserve further evaluation.

153 EVALUATION OF SERUM *ASPERGILLUS* IGG LEVEL IN PULMONARY TUBERCULOSIS PATIENTS IN INDONESIA

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Purpose:
Pulmonary tuberculosis (TB) is one of the two most common risk factors for chronic pulmonary aspergillosis (CPA). Moreover, these two diseases have the same clinical and radiological manifestations with a completely different management approach. Indonesia ranked as the second-largest country with tuberculosis burden in the world (845,000 in 2018). There has been no study about CPA in TB patients in Indonesia. These studies aim to estimate the optimal diagnostic cut-off for CPA among pulmonary TB in Indonesia, its incidence in pulmonary TB and assess serum *Aspergillus* IgG seroconversion during TB therapy.

Methods:
We conducted 2 (prospective and cross-sectional) studies in parallel in treated pulmonary TB patients in Indonesia. We recruited patients at the end (4-6 months after starting) TB therapy. *Aspergillus* IgG titre was measured using the Siemens Immulite 2000 system with a manufacturer cutoff of 10 mg/L. We analysed all chest x-ray (and CT scans if available) for cavities, pleural thickening adjacent to a cavity, area of consolidation, aspergilloma, nodules and/or bronchiectasis. To determine the optimum cutoff, we compared the *Aspergillus* IgG titre in only those patients with cavities and/or pleural thickening and persistent symptoms at the end of TB therapy with a) normal controls (101 people) and b) other patients seen at the end of TB therapy without persistent symptoms and cavities and/or pleural thickening. We plotted a ROC curve using the best Youden's J statistic (sensitivity + specificity – 1). We then applied that cut-off to patients seen in the first 2 months of TB therapy and compared *Aspergillus* IgG titre by time point.

Results:
204 (133 from prospective study and 71 from the cross-sectional study) patients were assessed at the end of TB therapy. Of these 204 patients, 23 (11.3%) had CPA based on positive *Aspergillus* IgG, symptoms and radiological features, using a cutoff 11.5 mg/L. Only 7 (30%) of these patients had a positive GeneXpert test at baseline (no sample analysed in 12 patients). At this cutoff, 11 (10.9%) normal controls and 72 (35.3%) patients completing TB therapy were positive for serum *Aspergillus* IgG (sensitivity 100% and 100% and specificity 88.1% and 72.9% respectively). At the beginning of TB therapy, 41 (30.8%) had a positive Aspergillus IgG and of these 20 (49%) were GeneXpert and/or acid-fast bacilli negative, suggesting that they did not have TB, but CPA. Using the same cut-off and ignoring any change of +/- 10%, an increase of *Aspergillus* IgG was observed in 59 (44.4%) patients while 43 (32.3%) patients showed decreased titre during 4-6 months of observation during TB therapy. Additionally, 15 (11.3%) patients developed positive *Aspergillus* IgG from initiation of TB therapy to end of TB treatment and 14 (10.5%) had a fall to negative level in *Aspergillus* IgG.

Conclusion:
CPA is common in presumed tuberculosis patients in Indonesia. The currently accepted Siemens Immulite cut-off of 10 mg/L for CPA diagnosis may require slight adjustment for the Indonesian population. There is a high percentage of positive *Aspergillus* IgG titre in TB patients during therapy raising concern about both mis-diagnosis of TB and the development of CPA during TB therapy.

154 MOLECULAR DETECTION OF *ASPERGILLUS* IN RESPIRATORY SAMPLES COLLECTED FROM PATIENTS WITH SUSPICION OF RESPIRATORY FUNGAL INFECTION

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Purpose:
HIV+ patients are frequently misdiagnosed with pulmonary tuberculosis when *Aspergillus* is, in fact, the etiological agent of infection. Thus, the aim of this study was to determine the potential of a real-time multiplex PCR to detect *Aspergillus* spp. DNA in respiratory samples from a cohort of patients with suspicion of respiratory fungal infection. The study focus were patients who have higher risk to develop chronic pulmonary aspergillosis: HIV+ patients and patients with active or previous *Mycobacterium* infection.

Methods:
The prevalence of *Aspergillus* in the respiratory samples (n=182) of a cohort of 141 patients was determined. *Aspergillus* detection was performed by the standardized laboratory methods used for diagnosis of aspergillosis - culture and/or galactomannan detection. The respiratory samples of a subgroup of 44 patients were amplified by real-time multiplex PCR with the AsperGenius® kit and the prevalence of *Aspergillus* in these samples was calculated. The instructions of the manufacturer were followed, except that we consider a sample as positive for *Aspergillus* spp. when a Ct<38 was obtained for the ROX probe. We compared the results obtained previously by the conventional methods with the results obtained by real-time multiplex PCR.

Results:
Despite the differences in the denominator, the prevalence of *Aspergillus* detected by the standardized diagnostic methods was 39.0%, while the prevalence of these fungi detected by real-time PCR was 77.3%. In the cohort of HIV+ patients, *Aspergillus* prevalence was calculated using similar denominators and it was 6.3% versus 64.3%, respectively (Table 1). Differences were also observed between the other groups of patients analyzed (Table 1). *Aspergillus* DNA was detected by real-time PCR in all the four samples analyzed by ELISA, although two of these samples had been negative for galactomannan. The results of the cultural methods were corroborated by real-time PCR for 56.4% of the samples. In the other cases, culture was negative for *Aspergillus*, but *Aspergillus*-directed PCR was positive (in 94.1% of the cases when discrepant results were obtained) (Figure 1).

Conclusion:
Real time PCR allowed a higher rate of positive results due to its higher sensitivity. From these positive results, we cannot exclude the existence of colonization. However, a high prevalence of *Aspergillus* spp. was found in the cohort of HIV+ patients suggesting that, when there is suspicion of mycobacterial infection in this group of patients, their respiratory samples should be tested for *Aspergillus* in order to avoid misdiagnosis.

155 VALIDATION OF DIAGNOSTIC CRITERIA IN PATHOLOGICAL OR PHYSICIAN-DIAGNOSED CASES WITH ALLERGIC BRONCHO-PULMONARY ASPERGILLOSIS/MYCOSIS

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Purpose:
There are several clinical diagnostic criteria for allergic bronchopulmonary aspergillosis (ABPA); however, no criteria for allergic bronchopulmonary mycosis (ABPM) are currently available. We previously proposed new diagnostic criteria for ABPA/ABPM consisting of 10 components in AAAM2018, and now validate the sensitivity and specificity of the existing and new criteria in the population with pathological or physician-diagnosed ABPA/ABPM.

Methods:
The new diagnostic criteria for ABPA/ABPM include 1) current or previous history of asthma or asthmatic symptoms; 2) peripheral blood eosinophilia (≥ 500 cells/mm^3); 3) elevated levels of total serum immunoglobulin E (IgE, ≥ 417 IU/mL); 4) immediate cutaneous hypersensitivity or presence of specific IgEs for filamentous fungi; 5) presence of precipitins or specific IgGs for filamentous fungi; 6) growth of filamentous fungi in cultures of sputum or bronchial lavage fluid; 7) presence of fungal hyphae in the mucus plugs of the bronchi; 8) central bronchiectasis on high-resolution computed tomography (HRCT); 9) presence of mucus plugs in the central bronchi, as determined by HRCT/bronchoscopy, or history of mucus plug expectoration; and 10) high attenuation mucus in the bronchi on HRCT. Patients that met 6 or more of these criteria were diagnosed with ABPA/ABPM and those who met 5 components were considered probable cases. The Rosenberg-Patterson criteria (1977), International Society for Human and Animal Mycology (ISHAM) criteria (2013), and the new criteria were applied to 79 cases of allergic mucin in the bronchi containing fungal hyphae that fulfilled the pathological criteria for ABPM proposed by Bosken et al. (1988), 37 cases with allergic mucin in the absence of hyphae, and 64 cases with chronic eosinophilic pneumonia, 26 cases with *Aspergillus*-sensitized severe asthma, and 24 cases with chronic pulmonary aspergillosis. Physician-diagnosed ABPA/ABPM was reported in a retrospective, cross-sectional survey of in 2013 from 132 clinical centers in Japan certificated by the Japanese Respiratory Society and/or the Japanese Society of Allergology. Among 499 cases with possible ABPA/ABPM reported in this survey, 179 cases with appropriate clinical, laboratory, and radiologic data available were also applied to validate the diagnostic criteria.

Results:
Among the 79 cases with pathological ABPA/ABPM, 20 (25.3%) and 61 (77.2%) cases fulfilled the Rosenberg-Patterson criteria and ISHAM criteria, respectively. Using the new diagnostic criteria, 75 cases (94.9%) were diagnosed as definite ABPA/ABPM, and other 2 cases (2.6%) as probable ABPA/ABPM. Among 179 cases with physician-diagnosed ABPA/ABPM, the sensitivity with the new criteria were 79.9% (definite ABPA/ABPM) and 94.4% (definite/probable ABPA/ABPM), whereas the sensitivity with Rosenberg-Patterson's criteria and ISHAM's criteria were 49.2% and 82.7%, respectively. The specificity was 98.4%, 86.8%, 96.7%, and 90.1% for Rosenberg-Patterson criteria, ISHAM criteria, and the new criteria for definite ABPA/ABPM and for definite/probable ABPA/ABPM, respectively.

Conclusion:
The new diagnostic criteria showed a better sensitivity and superior specificity for diagnosing ABPA/ABPM, when compared to existing criteria.

156 LOCAL EXPERIENCE FEEDBACK ON PERFORMING ROUTINELY THE PCR MUCORALES ON BLOOD SAMPLES IN HEMATOLOGICAL PATIENTS

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Purpose:
Our center has been applying a strategy of invasive fungal disease (IFD) screening on blood for at risk hematology patients (induction, GVHD, HSCT, feverish aplasia, compatible imagery), including systematically the Mucorales qPCR (in-house technique). The aim of this study was to describe the mucormycosis cases diagnosed thanks to this local strategy in the past five years.

Methods:
All the patients with at least two successive positive Mucorales qPCR between January 2015 and November 2019 were included. Clinical data were collected for all patients.

Results:
A total of 21 mucormycosis cases were obtained with 2 proven cases and 19 possible cases based on the EORTC criteria. Most of the patients were diagnosed with acute myeloid leukemia (AML) (76%), 81% of them were HSCT recipients. The positive Mucorales qPCR allowed immediate prescription of liposomal amphotericin B. Two thirds of the patients negativated their Mucorales qPCR in less than 5 days. In 38% of the cases, a mixed IFD was diagnosed (with concomitant positive galactomannan detection and/or positive *Aspergillus fumigatus* qPCR, and even once positive blood culture for *Paecilomyces variotti*) which would have led to voriconazole treatment alone (75% of the patients with mixed IFD were alive 6 months post IFD diagnosis). Broncho-alveolar lavage fluids were performed in 33 % of the cases despite compatible imagery and positive Mucorales qPCR results. Surgery was performed only for the proven cases (9.2%).

Conclusions:
The positive Mucorales qPCR is associated with earlier appropriate therapeutic management with liposomal amphotericin B. This local feedback experience showed that 90% of the possible mucormycosis cases (19/21) were diagnosed and treated appropriately thanks to the systematic screening.

157 DEVELOPMENT OF MAIN SPECTRAL PROFILES DATABASE FOR MALDI-IDENTIFICATION OF COMMON ASPERGILLOSIS CAUSATIVE AGENTS FROM THE COLONIES OBTAINED IN LIQUID MEDIUM

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Purpose:
To compose the MSP-database of *Aspergillus* spp. – common causative agents of invasive aspergillosis – to identify their cultures grown in liquid nutrient medium without mechanical agitation.

Methods:
Study was performed using the Laser *ToF* LT2Plus MALDI-TOF-mass-spectrometer (SAI, UK) and BactoSCREEN software (Lytech, Russia). In order to create the MSP-database strains of *A. fumigatus*, *A. flavus*, *A. niger*-complex (*A. niger* & *A. awamori*) and *A. nidulans* identified by the target DNA-sequencing of ITS and beta-tubulin loci according to the CLSI MM18 standard (2nd Ed.) were obtained from the Russian collection of pathogenic fungi. Only the most detailed mass-spectra with low noise levels were selected. The MSP-database design was carried out according to the instructions of the software manufacturer. 166 *Aspergillus* spp.of 5 species were processed for a positive identification control, 199 fungal cultures of 37 species and 117 bacterial cultures of 85 species were included in the trial for negative control (specificity control). All of the strains used in the study were isolated from human biomaterials. The processing of mycelial fungi cultures was carried out as described previously [Riabinin I.A. et al., AAA-2014], bacterial and yeast cultures were prepared by direct deposition and acid etching on MALDI-target. As a trail's resume the effectiveness parameters were calculated for the created MSP-database relative to the main commercial MSP-database of software manufacturer.

Results:
The «AMPSL» (**A**spergillosis **M**ain **P**athogens **S**pectral **L**ibrary) database was created, which included high-quality MSP of 47 *Aspergillus* spp. cultures. Compared to the manufacturer's main MSP-base, AMPSL was more sensitive (100% vs 79.8%), although slightly less specific (98.2% vs 100%). False positive identification using the AMPSL-database was detected for individual isolates of *Penicillium sp*., *P. digitatum*, *Purpureocillium lilacinum* and *Scopulariopsis brevicaulis*. These micromycetes are closely related to *Aspergillus* spp. and have very similar composition of MALDI-mass-spectra, which explains their inaccurate identification. However, the conventional microbiological methods (stereomicroscopy of colonies, microscopy of a preparation from a culture) such problem strains are easy to determine correctly. In general, the created «AMPSL» base showed greater diagnostic efficiency compared to the standard MSP-base of mass-spectrometer (98.5% vs 96.7%). A distinctive feature of the AMPSL utilization is the more successful identification of *A. flavus* and *A. terreus* isolates.

Conclusion:
MALDI-TOF-mass-spectrometry of the cell extract has been successfully used to identify clinical isolates of micromycetes, including *Aspergillus* spp. Modern databases of main spectral profiles (MSPs) are intended mainly for working with cultures in «pellets» that are grown from spores in a liquid medium on a rotator. Working with «pellets» is associated with some inconveniences, in this regard many microbiologists prefer to work with membranous cultures obtained on liquid medium

without rotation. The study allowed us to create the MSP-database suitable for the cultural diagnosis of aspergillosis using MALDI-TOF-mass-spectrometry. In the future, it is advisable to expand the range of species of the database, taking into account the more rare but actual causative agents of aspergillosis (e.g. *A. sydowii, A. ochraceus, A. candidus, A. calidoustus* and others).

158 RELATIONSHIP BETWEEN CLINICAL AND ENVIRONMENTAL *ASPERGILLUS* ISOLATES FROM THE HIGH-RISK AREAS OF A TERTIARY CARE HOSPITAL

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Purpose:
Invasive aspergillosis is airborne disease, patients usually acquire infections by inhalation of spores. Depends on the clinical status person either colonizes the spores initially, then gets infections. In recent years there has been an inexorable increase in number of highly immunocompromised patients in the hospital environment. There is also another group of patients without severe immunosuppression still they are at risk of invasive fungal infections. High concentration of *Aspergillus* spores in the hospital or renovation work adjacent to the hospital environment is major risk factors for acquiring the infections. Therefore, our main aim was to evaluate the presence of airborne *Aspergillus* spores within a hospital environment and its correlation with clinical isolates, if any.

Methods:
A total of 192 air samples from different high-risk areas wards and intensive care area were collected every month for a year (From May 2017 to May 2018). All samples were collected in potato dextrose agar plates. Sixty-six patients suspected to have of fungal infection during the same time and same wards were included for the study. All the 66 clinical isolates were identified by standard phenotypic methods as 38 *A. fumigatus* ,19 *A. flavus*, 5 unidentified *Aspergillus species*, 3 *A. niger*, and 1 *A. terreus*. Random amplified polymorphic DNA (RAPD-PCR) was performed for all the 192 environmental and 66 clinical isolates, isolated in the same time frame was done by using R108, R112, UBC90 primers to compare *Aspergillus* isolates of clinical cases with the relevant environmental sources.

Results:
In this study RAPD method resulted various differential patterns, some *Aspergillus* isolates from the clinical and hospital indoor were completely matched (matched pairs) and others did not matched. Only few isolates had similar RAPD pattern; 8 *Aspergillus fumigatus*. (4 clinical, 4 environmental), 4 *A flavus* (2 clinical, 2 environmental) isolates, 2 *A. niger* (1 clinical, 1 environmental) isolates. Indicating that every isolate present in the environment is potential pathogen if encountered with appropriate host.

Conclusion:
Although genetic analysis cannot discriminate absolutely between clinical and environmental isolates. Both the identity of fungal species in clinical and environmental samples indicates that every isolate present in the environment is potential pathogen if encountered with appropriate host. Routine environmental surveillance of high-risk areas for fungi may help to control the contamination. It will further help to reduce the incidence of fungal infections.

159 MULTIPLEX REAL TIME PCR FOR DETECTION AND IDENTIFICATION OF *ASPERGILLUS* AND *MUCORMYCETES* SPP. IN NATIVE AND FORMALIN-FIXED PARAFFIN-EMBEDDED TISSUE SAMPLES OF PATIENTS WITH MYCOSIS

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Purpose:
The aim of the study was to test a multiplex real time PCR with high resolution melt analysis (mHRM-RT-PCR) on clinical samples for simultaneous detection and identification of *Aspergillus* and *Mucormycetes* spp. in tissue samples.

Methods:
We tested 44 native and formalin-fixed paraffin-embedded tissue samples from 34 patients with aspergillosis and 12 clinical samples from 10 patients with mucormycosis in Saint-Petersburg between 2013 and 2019 yy. As controls, 21 tissue samples were collected from patients without mycoses. Fungal DNA was extracted from clinical samples by a chloroform-isoamyl extraction method. DNA amplification was performed using *Aspergillus* - and *Mucormycetes* - specific primers pairs separately and EvaGreen based mHRM-RT-PCR on Rotor-Gene 6000 cycler.

Results:
The mHRM-RT-PCR allows to identify the representatives of *Aspergillus* to the genus and mucormycetes to the species level:*Rhizopus arrhizus, Mucor racemosus, Rhizomucor pusillus, Rhizopus microsporus, Lichtheimia corymbifera.* The study included 56 biological specimen: tissue from sinuses - 13, lungs - 25 , central nervous system - 4, bowel - 4, heart - 1 , spleen - 1, liver -6, kidney - 1 and omentum -1. In patients with aspergillosis direct microscopy of 15 native tissue samples was positive in 80% cases. *Aspergillus fumigatus* was isolated in 80% and *Aspergillus flavus* - in 20% cases. Only in 1 of 3 native tissue samples of patients with mucormycosis and positive direct microscopy *Lichtheimia corymbifera* was isolated. PCR assay was positive in native and formalin-fixed paraffin-embedded tissue samples of 97, 0% patients with IA and 100% patients with mucormycosis. mHRM-RT-PCR allowed to identify the representatives of mucormycetes: *Lichtheimia corymbifera* in 6 and *Rhizomucor pusillus* in 3, Rhizopus microsporus in 1 from 12 samples. In biological specimens of 2 patients the PCR assay detected a mixed infection by *Aspergillus* and *Mucormycetes* spp.*: Aspergillus* spp.+ *Rhizopus microsporus* and *Aspergillus* spp. + *Rhizopus arrhizus* . The positive results of PCR assay in patients with aspergillosis in 95% and mucormycosis 100% of cases correlated with traditional methods. In 21 control tissue samples PCR test was negative.

Conclusion:
The multiplex RT-PCR has high sensitivity and specificity in tissue samples of patients with aspergillosis and mucormycosis. This study indicated that the mHRM-RT-PCR may be a useful tool for detection of etiologic agents of mycoses, particularly in the case of a mixed infection by *Aspergillus* and *Mucormycetes* spp.

160 ADAPTATION OF A QUANTITATIVE MUCORMYCOSIS PCR TO A FAST-PROTOCOL FOR A SAME-DAY RESULT

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Purpose:
Fungi of the *Mucorales* order might be human opportunistic pathogen causing infection in immunocompromised patients with a high mortality rate. Conventional diagnostic consist in a macro- and micro-scopy observation when a positive culture is obtained. However, culture is often fastidious, and identification to the genus level might be somehow problematic and required trained people. Targeted PCRs for the four main *Mucorales* genera responsible for human infections so called, *Rhizopus, Mucor*, *Lichteimia*, and *Rhizomucor,* were shown to exhibit a higher sensitivity and allow accurate identification and quantification[1]. Until now, no such PCR were available at the CHUV and such a diagnostic was outsourced at the University Hospital of Besançon, France[1]. This outsourcing was time, and cost consuming, and not convenient for the clinicians in our hospital. We, therefore, decided to import the 3 PCRs (*Rhizopus-Mucor*, *Lichteimia*, and *Rhizomucor*) performed in University Hospital of Besançon, developed by Millon et collaborators[1]. However, our platform has a TAT of 4 hours using a Fast qPCR program completed in 45 min[2]. We thus had to adapt and validate the previously developed *Mucorales* PCR for a fast protocol.

Methods:
Primers and probes were assessed for annealing and amplification temperature of at least 58°C and 69°C respectively using the Santa-Lucia algorithm. Two probes for *Rhizopus*-mucor, and for *Rhizomucor* detection, had to have their Tm increased by addition of Locked nucleotid acid (LNA) in the sequences. Several probe and primer concentrations were tested for each PCR on 10-fold serial dilutions of reference plasmids, which contained the targeted sequences. Efficacy of the PCRs were assessed essentially through the slope of the standard curved obtained with reference plasmids, and the Delta normalised reporter values (DRn). Specificities were tested on samples containing other fungal or eukaryotic pathogens. Sensibilities were assessed on plasmid dilutions and on a set of 20 positive and 20 negative retrospective samples.

Results:
The three *Mucorales* PCRs were efficiently transferred into our Fast qPCR platform. Sensibility was as below as 100 copies /ml. Specificity was excellent as no amplification was obtained from the 20 samples containing other fungi or parasites. The 20 negative samples tested were detected as negative. On the 20 positive samples, only two initially weakly positive for *Lichteimia* (Ct<38) were not detected.

Conclusion:
This study allow us to introduce the detection of *Rhizopus, Mucor, Lichteimia*, and *Rhizomucor* on our Fast qPCR platform with a possible one-day result.

1- Millon et al., Clin. Infect. Dis. (56) 2013
2- Greub et al. Future Microbiology (11), 2016

161 THE POTENTIAL OF EARLY LOW-DOSE CHEST COMPUTED TOMOGRAPHY (CT) PLUS PULMONARY ANGIOGRAPHY (CTPA) TO IMPROVE MANAGEMENT OF INVASIVE MOULD DISEASE (IMD) IN HIGH-RISK PATIENTS WITH HEMATOLOGICAL MALIGNANCIES: A PILOT STUDY

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Purpose:
Chest CT is an essential component for diagnosis of invasive mould disease (IMD) but is seldom performed during the initial 72 hours of fever. We examined whether earlier (< 72 hours) low-radiation dose CT (LD-CT) followed by CT pulmonary angiography (CTPA) could improve the early diagnosis of IMD.

Methods:
We prospectively enrolled 68 consecutive adult patients with hematological malignancies and a predicted 60-day baseline probability of IMD >5% determined with an institutionally validated risk score. All patients underwent a LD-CT exam within the first 72 hours of developing fever. A standard radiation dose chest CT (SD-CT) exam was then repeated between 7-10 days after the initial LD-CT. Patients with evaluable lesions on LD-CT or SD-CT immediately underwent CTPA if nodules > 10 mm were identified by the radiologist according to previously described criteria (Stanzani et al. Clin Infect Dis 2015). All patients underwent an extensive diagnostic and microbiologic workup to establish the cause of infection within 60 days of CT imaging.

Results:
Among the 68 enrolled patients, 5 (7.3%) and 9 (13.2%) patients eventually met EORTC/MSG definitions of proven/probable and possible IMD, respectively. At the time of first fever, 23.5% of patients were receiving mould-active triazole prophylaxis. Several CT imaging signs suggestive of IMD were detected at similar or higher frequencies at the time of LD-CT vs. SD-CT, including halo sign (17.6% vs. 8.8%, P=0.06); nodules < 10 mm (16.2% vs. 11.8%, P=0.06); nodules 10-15 mm (17.5% vs. 5.9%, P=0.74); and nodules >15 mm (17.6% vs. 5.9%, P=0.08); central lobar micronodules (20.6% vs. 11.8%, P=0.002); and the tree-in-bud sign (14.7% vs. 8.8%, P=0.01). CTPA was performed in 11/68 patients following LD-CT, with a positive vessel occlusion sign (VOS) in 5/11 (45%). All 5 of patients (100%) subsequently met EORTC/MSG criteria for proven/probable IMD. After the follow-up SD-CT, CTPA was performed in the remaining 16/57 patients with a positive VOS in 7/16 (43.8%) of cases. Of these CTPA positive patients (5/7) 71% subsequently were upgraded to proven/probable IMD, while 2/7 did not have further mycological criteria but no other etiologic agent was diagnosed. No patient with a non-mould etiologic diagnosis who underwent CTPA had a positive VOS.

Conclusion:
CT signs suggestive of IMD were detected more frequently with LD-CT performed in the first 72 hours versus SD-CT after 7 days of fever. Early screening with LD-CT combined with characterization of lesion angioinvasion characteristics by CTPA can improve the timely diagnosis of IMD and support early decisions regarding invasive diagnostic procedures and initiating or withholding antifungal therapy.

162 ESTABLISHMENT OF A WHOLE BLOOD ELISA TO QUANTIFY T-CELLULAR CYTOKINE RELEASE IN RESPONSE TO *ASPERGILLUS FUMIGATUS* ANTIGENS

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Purpose:
Depending on the host immune status, the ubiquitous mould *Aspergillus fumigatus* can cause a range of disease manifestation from invasive infection to severe hypersensitivity syndromes. Reliable bio-effect monitoring tools to efficiently track the multifaceted T-cell response to *Aspergillus* antigens are lacking. Flow cytometry or ELISPOT have been established to monitor *Aspergillus*-specific T-cells as a supportive biomarker for environmental exposure and invasive aspergillosis, yet are complicated by their time- and resource-intensive nature and pre-analytic difficulties. Cytokine release assays are successfully used as a T-cell-driven diagnostic marker for tuberculosis and cytomegalovirus (CMV) infections and are easy to use with minimal hands-on time. We therefore sought to develop a T-cell optimized whole blood-based interferon gamma (IFN-γ) and interleukin (IL)-17 release assay for *A. fumigatus* antigens.

Methods:
0.5 ml heparinized blood was injected into stimulation tubes containing RPMI medium, costimulatory molecules αCD28 and αCD49d plus CMV phosphoprotein (pp) 65, *A. fumigatus* mycelial lysate, or recombinant *A. fumigatus* antigens Aspf4, Aspf6 and Crf1. Phytohemagglutinin (PHA) was used as a positive control. After incubation for 8 to 48 h, plasma was obtained by centrifugation and analysed by IFN-γ and IL-17 ELISA.

Results:
In initial experiments to optimize T-cell stimulation, the combination of costimulatory factors αCD28 and αCD49d, 1:1 dilution of whole blood with RPMI medium, and 24 h incubation at 37 °C yielded the most robust detection of IFN-γ and IL-17 after stimulation with *A. fumigatus* mycelial lysate or PHA. We then validated the technical reliability of our stimulation protocol by comparing the IFN-γ response to pp65 in CMV-negative (Median: 0 pg/ml) and CMV-positive (Median: 378 pg/ml, $p<0.001$, $n=16$) patients after allogenic stem cell transplantation (60-180 days post-transplant), finding a sensitivity and specificity of 100 %. Testing the cytokine response to *A. fumigatus* antigens in mould-exposed organic farmers, we found higher IFN-γ ($p<0.05$, $n=15$) and IL-17 (Crf1: $p=0.05$, Aspf4: $p=0.07$) levels for Aspf4 and Crf1 stimulation than in non-occupationally-exposed subjects and minimal unspecific background secretion in unstimulated control tubes.

Conclusion:
We have developed and optimized a fast, simple, and resource-efficient whole blood ELISA assay to track T-cell responses to *A. fumigatus* antigens, providing a differentiated representation of individual T-cell subsets by their signature cytokines. Our proof-of-concept study in organic farmers demonstrated the potential of whole blood T-cell ELISA as a surrogate biomarker of *A. fumigatus* exposure that merits further evaluation in patients with mould-related hypersensitivity syndromes, mould exposure after geo-meteorological disasters, and invasive aspergillosis.

163 ETANERCEPT TREATMENT AND MONOCYTOPENIA INCREASE THE RISK FOR INVASIVE ASPERGILLOSIS IN PATIENTS AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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[6]*Institute for Hygiene and Microbiology, University of Wuerzburg, Germany*
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[9]*School of Science and Health, Western Sydney University, Campbelltown, Australia*

Purpose:
Diagnosis of invasive aspergillosis (IA), a life-threatening mold disease, remains challenging, due to poor conventional diagnosis and often non-specific clinical symptoms. The aim of this study was to identify additional risk factors that might, in combination with established diagnostic tests improve the diagnosis and management of IA in patients after allogeneic stem cell transplantation (alloSCT).

Methods:
In our longitudinal case-control study, alloSCT patients at the University Hospital of Würzburg were followed and categorized according to EORTC/MSG criteria[1]. Clinical information from 36 probable IA cases and 36 control patients (without fungal infections) was collected and significant associations among cases and controls were investigated by applying univariate and multivariable logistic regression. To evaluate our findings, RNA-sequencing and cytokine profiling of monocyte-derived macrophages (MDM), infected with *Aspergillus fumigatus* in the presence or absence of etanercept were performed. Additionally, CXCL10 release in patient sera was investigated by ELISA assay.

Results:
Our data show that low monocyte counts ($p = 4.8x10^{-06}$) and the administration of the TNF-alpha blocker etanercept ($p= 3.5x10^{-03}$) are significantly associated with IA in alloSCT patients. Our *in-vitro* data suggest that the presence of etanercept in MDM infected with *A. fumigatu*s significantly down-regulates genes involved in immune response and TNF-alpha signalling, as well as decreasing the secreted levels of the chemokine CXCL10. Moreover, we observed significantly lower CXCL10 serum concentrations ($p=0.017$) in IA patients with etanercept treatment in comparison to IA patients without treatment.

Conclusion:
Our study offers new insights regarding the individual risk for IA in alloSCT patients. Further validation of low monocyte counts and administration of etanercept in IA patients is necessary before such information is used for decision making regarding monitoring of patients at risk for IA or the use of antifungal prophylaxis.

Details of the study can be found in Zoran, T. *et al.* Treatment with etanercept and low monocyte concentration contribute to the risk of invasive aspergillosis in patients post allogeneic stem cell transplantation. Sci Rep, doi.org/10.1038/s41598-019-53504-8 (*in press*, 2019).

Literature

1. De Pauw, B. *et al.* Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. Clin Infect Dis 46, 1813-1821,

164 BURDEN OF SERIOUS FUNGAL INFECTIONS IN TOGO

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Purpose:
Over the years the focus of infectious diseases in many African countries has been mainly on HIV, malaria and tuberculosis. Fungal infections which are more frequent in these countries, and with comparable mortality rates, remain neglected due to lack of awareness. Our study aimed to estimate the prevalence and/or incidence of serious fungal infections in Togo and identify the need to change the current situation.

Methods:
To estimate incidence and prevalence of serious fungal infections, deterministic modelling was applied, using socio-demographics, health system's information, risk-groups data and fungal infection rates obtained from national and international studies.

Results:
Results showed that about 7.7% of the 7,265,286 Togolese population suffer from serious fungal infections annually. For the estimated 110, 000 HIV population, 1,342, 1,650 and 330 develop cryptococcal meningitis, Pneumocystis pneumonia and disseminated histoplasmosis respectively per year. Oral and oesophageal candidiasis annually affects 19,800 and 7,535 persons living with HIV respectively. Incidence of invasive aspergillosis (IA) was 265 cases. Prevalence of chronic pulmonary aspergillosis was estimated to be 745 cases including 128 cases following tuberculosis. The prevalence of allergic bronchopulmonary aspergillosis (ABPA) and severe asthma with fungal sensitisation (SAFS) was respectively 4,577 and 6,042 cases. Tinea capitis affects nearly 404,000 children while recurrent Candida vaginitis (>4 episodes per year) affects 108,979 women. Candidaemia prevalence was 5 cases per 100 000 inhabitants. Fungal keratitis in Togo may affect 2,071 persons annually.

Conclusions:
These results demonstrate significant prevalence and incidence rates of serious fungal infections in Togo and thus the need to increase awareness among healthcare professionals, enhance diagnostic and therapeutic capacities and intensify epidemiological studies, in all, leading to rapid diagnosis and effective management of fungal infections in Togo.

165 NIAID RESOURCES POSTER

Come by the poster sessions to meet with scientific program staff from the National Institute of Allergy and Infectious Diseases (NIAID) at the NIH. Learn about upcoming funding opportunities and resources to enhance your research. The National Institute of Allergy and Infectious Diseases (NIAID) funds one of the largest medical mycology research portfolios. This portfolio includes the major human fungal pathogens and covers basic fungal biology and the more translational areas of therapeutics, vaccines, and diagnostics. NIAID utilizes many granting mechanisms that are open to US and international researchers. These include both investigator-initiated mechanisms (R01, R21, and R03s) and targeted announcements to facilitate fungal research. Additionally, NIAID has a suite of preclinical services supporting therapeutic, diagnostic and vaccine development. These services are free and available to investigators in academia, not-for-profit organizations, industry, or governments worldwide. The NIAID granting mechanism can be complicated. Tips and tricks for navigating the NIAID application process and preclinical services will be discussed.

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The US Cystic Fibrosis Foundation is proud to be able to help in sponsoring this meeting which contributes to advances in *Aspergillus* science and therapy. The US Cystic Fibrosis Foundation is committed to adding tomorrows and improving todays for individuals with cystic fibrosis from all around the world.

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SCYNEXIS is proud to sponsor the 9th Advances Against Aspergillosis and Mucormycosis. We are committed to delivering innovative anti-infective therapies for difficult-to-treat and often life-threatening infections. We are developing Ibrexafungerp (formerly SCY-078), the first representative of a novel oral and intravenous glucan synthase inhibitor from the triterpenoid antifungal family, for the treatment of several serious fungal infections, including vulvovaginal candidiasis (VVC), invasive candidiasis, invasive aspergillosis (IA) and refractory invasive fungal infections (rIFI). It is currently in phase 3 development for VVC and rIFI; and in phase 2 for IA. For more information, email us at info@scynexis.com
NASDAQ: SCYX
www.scynexis.com

Amplyx Pharmaceuticals is focused on developing innovative therapies for immunocompromised patients, including cancer and transplant patients, and the critically ill. The company's two lead products are fosmanogepix (APX001) for the treatment of life-threatening fungal infections caused by pathogens such as *Candida*, *Aspergillus* and rare molds; and MAU868, a monoclonal antibody that potently neutralizes the BK virus which can cause significant morbidity and mortality in transplant patients. For more information, please visit **www.amplyx.com**

Bordier Affinity Products SA, a small biotech company located near Lausanne (Switzerland), has been developing and producing laboratory reagents and kits for the diagnosis of parasitic and mycologic infections since 1991. Collaborations with academic institutions combined with extensive expertise in biochemistry, mycology and parasitology allow the company to offer products of premium quality.

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The California Institute for Medical Research (CIMR) is an independent research facility on the campus of the Santa Clara Valley Medical Center, an affiliated hospital of Stanford University Medical School. Since its founding in 1963, CIMR has been a resource for researchers, regional hospitals and doctors. More than 300 research programs have been conducted at CIMR. This has resulted in nationally recognized discoveries in medicine and medical care. CIMR provides a convenient location in San Jose, California, for start-up biotechnology companies, local physicians and medical research scientists wanting to start new projects or expand current research. The Foundation for Research in Infectious Diseases supports the Infectious Disease Research laboratory at CIMR. For more information, see the website: **www.cimr.org**

Cipla Technologies is a clinical stage, specialty drug subsidiary of Cipla Ltd, a global pharmaceutical company established in 1935 with headquarters in Mumbai, India. Cipla Technologies focuses on bringing to market treatments that address unmet patient needs in various therapeutic areas such as respiratory diseases. The company currently has various products under development. Amongst there is the PUR1900, an inhaled itraconazole for the treatment of respiratory fungal infections, in conjunction with its partner Pulmatrix,. Cipla Technologies is headquartered in San Diego, California. For more, please visit **www.cipla.com**.

Dynamiker Biotechnology (Tianjin) Co., Ltd, a manufacturer focusing on serological tests for Medical Mycology, is a high-tech enterprise integrated with R&D, production and sales globally. It is focused on developing early, rapid and innovative *in vitro* diagnostics of invasive fungal diseases (IFD). Over 13 newly developed IFD products have recently been CE-IVD marked under ISO-13485. We offer a total solution for the diagnosis and monitoring of IFD with a comprehensive panel of ELISA and LFA assays to detect major fungal infections, such as Aspergillosis, Candidiasis, PJP, Cryptococcosis, etc.

Era Biology started business operation in 1997. As one manufacturer focusing on IFD diagnosis field, Era Biology offers clinical diagnostic products including (1-3)-β-D-Glucan test, rapid tests such as *Aspergillus* GM test, *Cryptococcal* antigen test and *Candida* Mannan test, and fully automatic instrument with exclusive patents. Era Biology has passed the authentications of CMD ISO9001, ISO13485, Korea GMP and North America MDSAP. It's the first company producing Full-Automatic Chemiluminescence Immunoassay System for IFD diagnosis. Our goal is innovation for better health.

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F2G is a world-leading UK- and Austria-based antifungal company (F2G Ltd and F2G Biotech GmbH) focused on the discovery and development of novel therapies to treat life-threatening invasive fungal infections. F2G has discovered and developed a completely new class of antifungal agents called the orotomides. The orotomides target dihydroorotate dehydrogenase (DHODH), a key enzyme in the de novo pyrimidine biosynthesis pathway. This is a completely different mechanism from that of the currently marketed antifungal agents and gives the orotomides fungicidal activity against a broad range of rare and resistant fungal mould infections. Olorofim (formerly F901318) is F2G's leading candidate from this class and is in a Phase 2b open-label study focussing on rare and resistant invasive fungal infections such as aspergillosis (including azole-resistant strains), scedosporiosis, and lomentosporiosis. Olorofim is being developed both as IV and oral formulations. Olorofim was recently granted FDA Breakthrough Therapy designation.

The Fungal Infection Trust is one of the world's foremost charities (foundations) that focusses exclusively on fungal disease research, education, awareness and patient support across the world.
www.fungalinfectiontrust.org

Journal of Fungi (ISSN 2309-608X, https://www.mdpi.com/journal/jof) is an international, peer-reviewed scientific open access journal that provides an advanced forum for studies related to pathogenic fungi, fungal biology, and all other aspects of fungal research. Original articles, reviews, communications, conference reports and short notes are all welcome. Journal of Fungi has been covered by leading indexing services, including PubMed, Science Citation Index Expanded (Web of Science), Scopus and other relevant databases.

IMMY focuses primarily on manufacturing rapid, high-quality fungal diagnostics. With products for Aspergillosis (Aspergillus GM LFA), Cryptococcosis (CrAg LFA), Histoplasmosis, Coccidioidomycosis (Coccidioides Ab LFA), and Blastomycosis, IMMY is setting the standard with accurate and affordable diagnostics. It has been our goal to bring diagnostics closer to the patient by developing simple, rapid tests that can be used in any laboratory setting, on any shift. IMMY is bridging the gap between fungal infections and proper treatment by Saving Lives One Diagnostic at a Time.

OLM Diagnostics is a medical diagnostics company, which develops and distributes innovations in fungal diagnostics to the healthcare sector. Our novel and reliable rapid-diagnostic tests fit seamlessly into current treatment pathways and can reduce the rate of drug resistant infections by promoting a new diagnostic- led approach. This is achieved, whilst delivering clear financial and clinical benefits to hospitals, clinicians and patient care.

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Pulmatrix is committed to the development of novel and transformational medicines to patients globally, with an initial focus on respiratory disease, using our proprietary iSPERSE dry powder inhalation technology to optimize pharmacokinetics and pharmacology. In partnership with Cipla Technologies, Pulmatrix is developing PUR1900 (Pulmazole) as potentially the 1st inhaled anti-infective to treat fungal infections in patients with Allergic Bronchopulmonary Aspergillosis (ABPA). Our pipeline is complemented by PUR1800, a narrow spectrum kinase inhibitor, currently in clinical development for the treatment of acute exacerbations in chronic obstructive pulmonary disease (AECOPD).

Pulmocide is a biotechnology company which is focussed on the discovery and development of novel antifungal agents which have been optimised for lung delivery by inhalation. Our lead compound PC945 is now undergoing clinical phase testing.

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Agenda

Friday 28 February, 2020
11:45–13:15

AAAM 2020 | Lugano, Switzerland
Room A

11:45–11:55
Introduction and scene setting
Professor Robert Krause (Chair)

11:55–12:05
Interactive case study part one – setting the scene of a difficult diagnosis
Professor Dionysios Neofytos

12:05–12:30
Risk factors for invasive aspergillosis and mucormycosis, and how to pick apart a diagnosis, supported by case studies
Professor Carolina Garcia-Vidal

12:30–12:55
Treating invasive mould disease in the case of confounding factors
Professor Malgorzata Mikulska

12:55–13:05
Interactive case study part two – treatment approaches to a complex patient with a complicated infection
Professor Dionysios Neofytos

13:05–13:15
Discussion, Q&A and close
All

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Dealing with the threat of mucormycosis when there is differential diagnostic doubt

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