admission days were, respectively, 1.5±1.3 and 5.9±7.1 days at baseline, and reduced to 0.5±0.7 and 1.6±3.1 days when weighting cases by follow-up duration (p=0.021 and p=0.134, respectively). Table 1 summarizes mean changes in different endpoint parameters of scheduled visits in the FDEP versus baseline. Hb level significantly increased, accompanied by a decrease in markers of hemolysis (absolute reticulocyte count, total bilirubin, and lactate dehydrogenase). Mean PoS decreased, and p50, 2,3-DPG level and ATP/2,3-DPG ratio significantly improved.
Conclusion: Treatment with mitapivat for up to 60 weeks in subjects with SCD showed no treatment related TEAEs grade ≥3. Improvements in anemia, markers of hemolysis, oxygen affinity, 2,3-DPG level and ATP/2,3-DPG ratio were seen. Follow-up data will be collected.

Table 1. Mean changes in Hb, parameters of hemolysis, sickling tendency, and biochemical parameters in the FDEP compared to baseline of SCD patients treated with mitapivat (n=8)

Parameter (unit)	Baseline	FDEP	p-value*
Hb (g/dL)	9.4 ± 1.0	10.5 ± 1.0	0.001
ARC (10^9/L)	246 ± 88	150 ± 54	0.001
Total bilirubin (mg/dL)	2.5 ± 1.4	1.2 ± 0.6	0.010
LDH (U/L)	404 ± 114	320 ± 67	0.017
PoS (mmHg)	40.2 ± 8.8	35.7 ± 7.5	0.065
p50 (mmHg)	23.4 ± 1.8	21.6 ± 1.0	0.002
2,3-DPG (mg/gHb)	11.3 ± 1.0	8.6 ± 1.3	<0.001
ATP (mg/gHb)	2.9 ± 0.7	3.4 ± 0.3	0.161
ATP/2,3-DPG ratio	0.25 ± 0.05	0.41 ± 0.05	0.002

Data are presented as mean ± standard deviation. *Paired t-tests or Wilcoxon signed-rank tests were used when appropriate. FDEP, fixed dose extension period; SCD, sickle cell disease; Hb, hemoglobin; ARC, absolute reticulocyte count; LDH, lactate dehydrogenase; PoS, Point of Sickling; p50, oxygen pressure at which Hb is 50% saturated with oxygen; 2,3-DPG, 2,3-diphosphyglycerate; ATP, adenosine triphosphate.

Table 1.
The authors do not declare any conflict of interest

PI-03 THE EFFECT OF HYDROXYUREA IN THE GUT MICROBIOME OF ANGOLAN CHILDREN WITH SICKLE CELL DISEASE

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Purpose: Sickle cell disease (SCD) is one of the most prevalent genetic disorders, affecting around 20 to 25 million individuals throughout the world. In Sub-Saharan Africa, where it is more prevalent, it can contribute up to 80% of under-5 mortality. Clinical manifestations of SCD are very heterogeneous and the intestinal microbiome has recently been reported to be crucial in the modulation of inflammation, cell adhesion and induction of aged neutrophils, which are key interveners of recurrent vaso-occlusive crises. Since gut bacteria can regulate aged neutrophils, defects in either the integrity of the intestinal walls or a chronic disequilibrium of the microbiota are very likely to emerge in SCD patients. Moreover, it has been suggested that Hydroxyurea (HU), the most common treatment for SCD, shows a multimodal action and may reduce microbiome dysbiosis and aged neutrophils. In this context, we aimed to understand how SCD and HU treatment modulates the microbiome and if these changes could be related with disease severity.
Materials and methods: In order to characterize the gut microbiome of an SCD pediatric population, which consisted of Angolan children before and after 6 months of continuous HU treatment, a total of 66 stool samples were collected in tubes with a preservative solution. Then, the metagenomic DNA was extracted, quantified and the bacterial 16SRNA gene for the V3-V4 regions was sequenced by NGS. Microbiome taxonomic profiling analysis was performed with the EzBioCloud pipeline and differences between the two groups were assessed with the Statistical Analysis of Metagenomic Profiles (STAMP) software, using Welch's t-test.
Results: Significant associations were observed in alpha-diversity between the two groups, with higher values for the children naïve for HU in several parameters, namely in OTU species count (p<0.001), phylogenetic diversity (p=0.004) and microbial richness (p<0.001), which was calculated by the ACE, Chao1 and Jackknife indices. We also noticed that children after HU treatment had higher proportions of some bacteria associated with health, including Blautia luti, Roseburia inulinivorans, Lactobacillus rogosae and Faecalibacterium, when compared to the beginning of the study. Additionally, the proportion of Firmicutes phylum was significantly lower before HU.
Conclusion: This was the first study to report gut microbiome changes before and after HU treatment in SCD children. Overall, our findings provide a rationale for further research about gut microbiota dysbiosis in this population. Determining the effect of specific bacteria will give evidence for the correction of microbiota composition and supplementation with beneficial strains in order to reduce gut microbiota-driven inflammation, which could ultimately mitigate disease severity. This work was supported by FCT/Aga Khan (project nº330842553) and FCT/MCTES (UIDB/05608/2020 and UIDP/05608/2020) –H&TRC.
The authors do not declare any conflict of interest

PI-04 OXYGEN GRADIENT EKTACYTOMETRY-DERIVED BIOMARKERS ARE ASSOCIATED WITH THE OCCURRENCE OF ACUTE COMPLICATIONS IN SICKLE CELL DISEASE

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Purpose: Sickle cell disease (SCD) is a monogenetic disorder with a highly complex pathophysiology. There is an unmet need for robust reproducible biomarkers that can assess red blood cell (RBC) function and predict disease severity and complications.
Materials and methods: In this study, we investigated the association between oxygen gradient ektacytometry-derived biomarkers, and blood viscosity with incidence of major (acute) SCD-related complications. Oxygen gradient ektacytometry measures RBC deformability continuously while the sample is gradually deoxygenated and subsequently reoxygenated, and identifies the oxygen tension at which sickling occurs. We examined associations between the occurrence of acute chest syndrome, cerebral infarction and vaso-occlusive crisis (VOC) and known biomarkers such as fetal hemoglobin, blood viscosity as well as exploratory oxygen gradient ektacytometry-derived biomarkers in an adult cohort of 50 individuals with SCD (HbSS or HbS/βo-thalassemia) and a pediatric cohort consisting of 177 children with SCD (HbSS or HbS/βo-thalassemia). A substantial number of subjects were on hydroxyurea therapy (64% of adults and 89% of children). Subjects that received a blood transfusion less than three months prior to measurements were excluded from the study. A logistic regression analysis was performed; odds ratios were adjusted for age and hydroxyurea therapy.
Results: In the adult cohort, for every 10 mmHg increase in Point of Sickling (PoS, the pO2 tension where RBCs start to sickle, reflecting sickling tendency) the likelihood of >1 acute complication increased; the adjusted odds ratio (aOR) was 3.00 (p=0.015). For every 0.1 increase in EImax (reflecting RBC deformability at normoxia), the aOR was 0.33 (p=0.035, Table 1). In the pediatric cohort, for every 10 mmHg increase in PoS, the likelihood of >1 acute complication increased; the aOR was 1.65 (p=0.006). For every 0.1 increase in EImin (reflecting RBC deformability at hypoxia), the aOR was 0.50 (p=0.007). Fetal hemoglobin and blood viscosity levels were not associated with likelihood of multiple acute complications. However, fetal hemoglobin was associated with reduced likelihood of VOC in adults (aOR of 0.32 for every 10% increment, p=0.010) but not in children (aOR of 0.68, p=0.231, data not shown). In the adult cohort higher EImax was associated with reduced likelihood of VOC (aOR 0.31, p=0.029). There was a trend found for an association between higher PoS and greater likelihood of VOC (aOR 2.22, p=0.050), and no association for EImin. In the pediatric cohort only EImin was associated with VOC (aOR 0.68, p=0.036).
Conclusion: These findings indicate that oxygen gradient ektacytometry generates novel clinically relevant biomarkers and provide a rationale for further development of these biomarkers in the evaluation of novel therapies, as part of clinical care, or clinical trial endpoints. In particular, in assessment of treatment strategies that do not target HbF induction, such as pyruvate kinase activators, voxelotor and l-glutamin, oxygen gradient ektacytometry can generate relevant biomarkers.