2023 IEEE 7th Portuguese Meeting on Bioengineering (ENBENG)
22-23 June, 2023. Porto, Portugal

Laboratory biomarkers associated to death in the first three COVID-19 waves in Portugal

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2023 IEEE 7th Portuguese Meeting on Bioengineering (ENBENG) | 979-8-3503-2257-6/23/$31.00 ©2023 IEEE | DOI: 10.1109/ENBENG58165.2023.10175345

Abstract— Besides the pandemic being over, new SARS-CoV-2 lineages, and sub-lineages, still pose risks to global health. Thus, in this preliminary study, to better understand the characteristics of COVID-19 patients and the effect of certain hematologic biomarkers on their outcome, we analyzed data from 337 patients admitted to the ICU of a single-center hospital in Lisbon, Portugal, in the first three waves of the pandemic. Most patients belonged to the second (40.4%) and third (41.2%) waves. The ones from the first wave were significantly older and relied more on respiratory techniques like invasive mechanic ventilation and extracorporeal membrane oxygenation. There were no significant differences between waves regarding mortality in the ICU. In general, non-survivors had worse laboratory results. Biomarkers significantly associated with death changed depending on the waves. Increased high-sensitivity cardiac troponin I results, and lower eosinophil counts were associated to death in all waves. In the second and third waves, the international normalized ratio, lymphocyte counts, and neutrophil counts were also associated to mortality. A higher risk of death was linked to increased myoglobin results in the first two waves, as well as increased creatine kinase results, and lower platelet counts in the third wave.

Keywords—COVID-19, Waves, Biomarkers, ICU, Risk of death

I. Introduction

In January 2020, the WHO declared a Public Health Emergency of International Concern as several cases of infection by the severe acute respiratory syndrome coronavirus (SARS-CoV-2) were identified in China. Following that, the virus spread to a variety of other countries, and on March 11 of the same year the Coronavirus Disease 2019 (COVID-19) pandemic was announced [1]. The first COVID-19 cases in Portugal were detected at this time, and in April 2021 the Alpha variant (B.1.1.7 lineage), which was first documented in the United Kingdom in September 2020, reached its peak frequency in the country [2].

The WHO focused on public health measures (isolation, quarantine, and social distancing) so as to slow down the transmission rate of the virus, protect health care systems and accurately diagnose, and treat COVID-19 patients. Collaborative work and research to find new and effective solutions for the global crisis being faced was also crucial, leading to new diagnostic tools, therapeutics, and vaccines [3].

In order to better understand the characteristics of COVID-19 patients and the primary risk factors for worse outcomes, several studies also tried to offer high quality data from diverse populations around the world [4]–[7]. Nevertheless, the critical roles of a number of hematologic biomarkers in the outcome of COVID-19 have not been examined when evaluating the several waves of the pandemic, more so in Portugal. Given the significant impact of the pandemic and the threats posed by SARS-CoV-2 new lineages and sub-lineages in the present, the identification of potential laboratory biomarkers for COVID-19 prognosis remains essential. Therefore, our major purpose in this preliminary study was to identify predictive biomarkers for this disease's outcome in critically ill patients of a single-center hospital in Lisbon, Portugal, in the first three COVID-19 waves.

II. Methods

A. Study population and data assembly

COVID-19 patients hospitalized to the intensive care unit (ICU) of *Centro Hospitalar Universitário de Lisboa Central* between March 2020 and March 2021, with a written informed consent regarding the availability of their data, were included in this study. Those under 18 years of age, without ICU admission/ discharge dates or hospital discharge dates (due to lack of data or because the individuals were still admitted), or COVID-19 diagnosis dates (by real-time polymerase chain reaction tests) were excluded. This led to differences between the waves' sample sizes. All demographic, clinical, and laboratory data was gathered from the hospital's electronic medical record system. This study was approved by the Institutional CHULC Ethics Committee (1043/2021, 20/05/2020), and informed consent was obtained from all subjects involved. All data was anonymized.

B. Study variables

Demographic variables included age, sex, and body mass index (BMI, Kg/m^2). Other clinical characteristics included the presence/ absence of the following comorbidities: arterial hypertension, diabetes mellitus, dyslipidemia, chronic

obstructive pulmonary disease (COPD), myocardial ischemia, and chronic renal disease. Invasive mechanical ventilation (IMV), extracorporeal membrane oxygenation (ECMO), and high-flow oxygen therapy (HFO) were considered respiratory support techniques, being used at least one time during the entire ICU length of stay. The patients' outcome relied on whether they died in the ICU or were discharged from the ICU. Disease onset was considered the first between COVID-19 diagnostic or symptom onset dates.

Depending on the laboratory result, to obtain a single daily measure, daily maximum or minimum findings were selected. For high-sensitivity cardiac troponin I (hs-cTn I), myoglobin, creatine kinase (CK), neutrophil counts, and the International Normalized Ratio (INR), maximum daily results were obtained. For eosinophil, lymphocyte, and platelet counts, minimum daily values were selected. Categorical variables were created for each biomarker considering the hospital's laboratory reference values.

C. *Statistical analysis*

Categorical data was presented as absolute frequencies and percentages. To assess the normality of the continuous variables, Kolmogorov-Smirnov and Shapiro-Wilk tests were used, as appropriate. Continuous variables with deviations from normality and asymmetric distribution were expressed by their medians and interquartile range (IQR). For nominal variables, comparisons between independent groups were performed with chi-squared or fisher's exact tests, as appropriate. Kruskal-Wallis One-Way ANOVA test (with significance values adjusted by the Bonferroni correction, for pairwise comparisons) was applied for continuous variables. Taking into account the entirety of the longitudinal data collected throughout the full ICU admission period, associations between the biomarkers and death were examined using Univariate Generalized Estimating Equations models (GEEs), logit link function, robust standard errors and an exchangeable working correlation structure. Descriptive and inferential statistics were obtained by IBM SPSS Statistics software, version 26 (IBM Corp., New York, United States), and STATA software, version 12 (StataCorp. LLC, Texas, United States). Statistical significance was set for two-sided p values of less than 0.05.

III. RESULTS AND DISCUSSION

Patients' characteristics were acquired individually for each wave (Table I). Most COVID-19 patients belonged to the second and third waves (n=136 and 139) (Fig. 1). This trend matches to the one from the general situation of the country in the first three waves [8]. The age distribution was significantly different between waves (p=0.021). Pairwise comparisons demonstrated that these differences were mostly because patients from the first wave were significantly older than those from the third wave (p=0.022). In all the waves, the percentage of men was higher (72.6%, 70.6%, and 61.9%, respectively). Concerning comorbidities, no significant differences were found but, in all waves, 80 to 90% of patients had one or more comorbidities. Between waves, the need for all kinds of respiratory support was significantly different (all $p \leqslant 0.05$). Both IMV and ECMO were more required in the first wave, and HFO in the second wave.

TABLE I. CHARACTERISTICS OF EACH WAVES' PATIENTS.

Variables	**COVID-19 Waves**			
	Wave 1 (n=62)	***Wave 2 (n=136)***	***Wave 3 (n=139)***	***p value***
Age (years), median (IQR)	69.0 (57.8-76.0)	65.5 (52.0-74.0)	60.0 (53.0-71.0)	0.021
Sex, n (%)				
Female	17 (27.4)	40 (29.4)	53 (38.1)	0.190
Male	45 (72.6)	96 (70.6)	86 (61.9)	
BMI (Kg/m^2), median (IQR)	27.7 (24.9-31.3)	26.1 (24.5-29.3)	28.1 (25.9-31.5)	0.013
Comorbidities, n (%)				
Arterial Hypertension	34 (54.8)	79 (58.1)	79 (56.8)	0.912
Diabetes Mellitus	22 (35.5)	53 (39.0)	50 (36.0)	0.840
Dyslipidemia	15 (24.2)	38 (27.9)	35 (25.2)	0.812
COPD	5 (8.1)	8 (5.9)	4 (2.9)	0.254
Myocardial Ischemia	3 (4.8)	6 (4.4)	14 (10.1)	0.140
Chronic Renal Disease	5 (8.1)	8 (5.9)	8 (5.8)	0.803
Respiratory Support, n (%)				
IMV	54 (87.1)	94 (69.1)	106 (76.3)	0.023
ECMO	14 (22.6)	18 (13.2)	5 (3.6)	<0.001
HFO	17 (27.4)	55 (40.4)	33 (23.7)	0.009
Days in the ICU, median (IQR)	9.0 (3.8-20.0)	8.0 (4.0-14.0)	9.0 (4.0-17.0)	0.544
Death in the ICU, median (IQR)	20 (32.3)	53 (39.0)	51 (36.7)	0.662

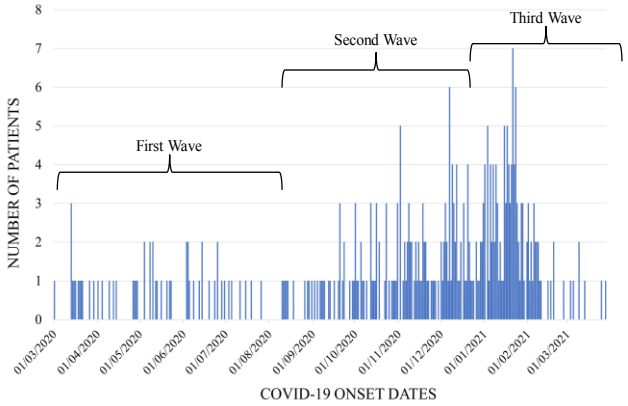

Fig. 1. Patients' disease onset dates, in the first three COVID-19 waves. Second wave considered from August 24, until December 20, 2020.

In Fig. 2 the absolute frequency of patients with biomarkers' results above/below the established normality cutoffs can be observed, for each day of ICU admission. It is also possible to observe the amount of data that was available for the longitudinal analysis. The only biomarkers for which the frequency of findings above the normalcy cutoff points was higher than those regarded as normal, in each day of ICU admission, were myoglobin and neutrophil counts. As time

went by, the number of patients with results above/below the established normality cutoffs decreased for all biomarkers.

Associations between the biomarkers and death were tested by means of univariate GEEs models (Table II). Patients with increased hs-cTn I levels had a higher risk of death, in all waves (p=0.019, p=0.002, and p=0.003, respectively). This risk was higher by 0.4% in the first wave, 1.7% in the second wave, and 2.1% in the third wave, in comparison to patients with normal hs-cTn I levels. The elevation of cardiac troponins can be related to cardiac myocyte injury and/or necrosis. It has been described that this kind of cardiac manifestation in COVID-19 patients is linked to a higher risk of mortality [9]. For patients with increased myoglobin, the risk of death was 2.4% higher in the first wave and 18.9% in the second one. In the third wave, increased CK levels resulted in a 2.3% greater risk of death. Myoglobin rises in circulation after myocyte damage, and it has been demonstrated that when paired with CK, it has an even higher predictive value for adverse outcomes and mortality [10].

Considering the INR, for patients with elevated results, the risk of death increased 1.0% in the second wave and, in the third wave, for every unit increase in the INR, the same risk raised in 1.4%. For patients with decreased platelet counts, the risk of death increased 3.9% in the third wave.
Low platelet counts can be related to dysfunctions in the coagulation process and, in other studies, they have been associated with an increased risk of death and worse outcomes [11], [12].

Regarding eosinophil counts, in the first wave, for every unit increase, the risk of death decreased by 0.3%. Also, patients with diminished eosinophil counts had an increased risk of death of 0.9% in the second wave, and of 1.4% in the third wave. Other authors reported that decreased eosinophil counts are related with both worse disease outcomes and death in COVID-19 [13]. Instead, eosinophilia has been associated with milder disease courses and better outcomes [14].

For every unit increase in neutrophil counts, the risk of death increased 0.2%, both in the second and third waves. Results above the established normality range were also linked to higher risks of death, namely of 1.8% and 1.3%, in the same waves, respectively. Besides the association to poorer outcomes in COVID-19 cases, neutrophilia is linked to the development of thrombosis, and pulmonary infiltrates. Severe cases of the disease are also associated with increased neutrophil-to-lymphocyte ratios, meaning that lymphocyte counts could be low [15]. In fact, for patients with decreased lymphocyte counts, the risk of death increased 1.4% in the second wave and 2.7% in the third wave. On the other hand, for every unit increase in lymphocyte counts the risk of death decreased 1.5% in the second wave and 1.6% in the third wave. In a meta-analytical study, lymphocyte count was considered the most powerful predictor of death between 13 other laboratory biomarkers from COVID-19 patients [16].

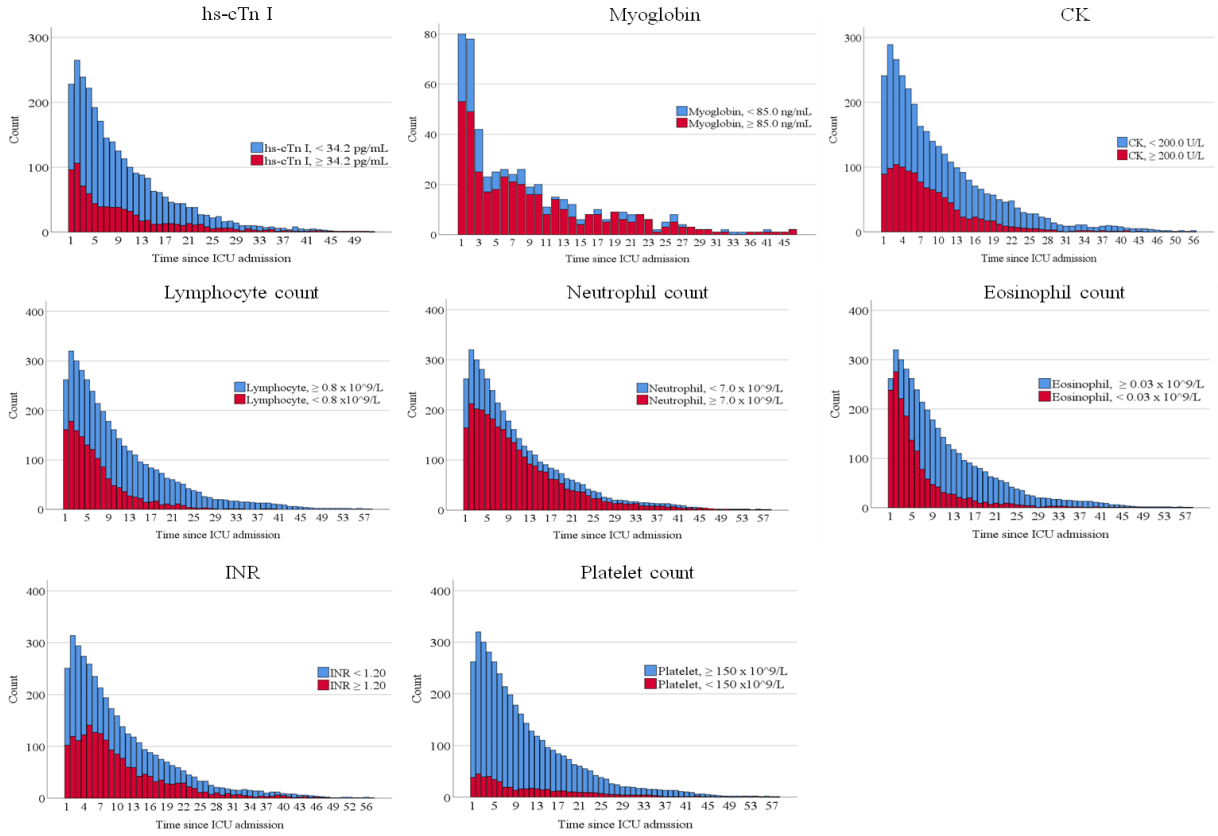

Fig. 2 - Frequency of patients with increased/ decreased depending on the cut-offs for each biomarker, in every day of ICU admission.

TABLE II. STATISTICALLY SIGNIFICANT RESULTS FROM UNIVARIATE GEES MODELS IN EACH WAVE.

Wave	Laboratory Biomarkers	Univariate GEEs		
		Crude OR	*95% CI*	*p value*
1	hs-cTn I, ≥ 34.2 pg/mL	1.004	1.001-1.007	0.019
	Myoglobin, ≥ 85.0 ng/mL	1.024	1.009-1.038	0.002
	Eosinophil, x 10^9/L	0.997	0.993-1.000	0.043
2	hs-cTn I, ≥ 34.2 pg/mL	1.017	1.006-1.027	0.002
	Myoglobin, ≥ 85.0 ng/mL	1.189	1.034-1.367	0.015
	Lymphocyte, x 10^9/L	0.985	0.977-0.993	<0.001
	Lymphocyte, < 0.8 x 10^9/L	1.014	1.005-1.023	0.002
	Eosinophil, < 0.03 x 10^9/L	1.009	1.004-1.015	<0.001
	Neutrophil, x 10^9/L	1.002	1.000-1.002	<0.001
	Neutrophil, ≥ 7.0 x 10^9/L	1.018	1.009-1.027	<0.001
	INR ≥ 1.20	1.010	1.002-1018	0.010
3	hs-cTn I, ≥ 34.2 pg/mL	1.021	1.007-1.035	0.003
	CK, ≥ 200.0 U/L	1.023	1.012-1.034	<0.001
	Lymphocyte, x 10^9/L	0.984	0.973-0.995	0.004
	Lymphocyte, < 0.8 x 10^9/L	1.027	1.017-1.038	<0.001
	Eosinophil, x 10^9/L	0.968	0.954-0.982	<0.001
	Eosinophil, < 0.03 x 10^9/L	1.014	1.006-1.022	0.001
	Neutrophil, x 10^9/L	1.002	1.000-1.003	0.006
	Neutrophil, ≥ 7.0 x 10^9/L	1.013	1.001-1.024	0.021
	INR	1.014	1.006-1.023	0.001
	Platelet, < 150 x10^9/L	1.039	1.013-1.067	0.004

Depending on the wave, the number and kind of biomarkers that were linked to death varied. The first wave had the least biomarkers associated to death, probably because there weren't as many differences between the laboratory results of survivors and non-survivors (data not shown). Biomarkers like increased hs-cTn I and eosinophil counts were significantly associated to death in all waves. Besides being associated with worse outcomes in several studies, decreased platelet counts were only linked to death in the third wave. Thus, one can conclude that, it is crucial to take COVID-19 waves into account while examining patients' features and looking for biomarkers linked to mortality, since the outcomes might be significantly different. These differences may result from the distinct SARS-CoV-2 variants and their effects on disease progression, changes on the available therapeutic options (e.g., antivirals, immunomodulating medicines, vaccines), and changes in the health system's capacity to manage the disease. Besides increasing our study sample, we expect to also take this information into consideration in the future and use multivariate analysis and more complex prediction models in order to accurately predict patients' outcome.

ACKNOWLEDGEMENTS

This study is inserted in the project Predictive Models of COVID-19 Outcomes for Higher Risk Patients Towards a Precision Medicine (PREMO), supported by *Fundação para a Ciência e Tecnologia* (FCT), under the following grant: DSAIPA/DS/0117/2020.

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