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BRIEF REPORT

Prognostic Significance of NLR and PLR in COVID-19: A Multi-Cohort Validation Study

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ABSTRACT

Introduction: Recent studies have highlighted the prognostic value of easily accessible inflammatory markers, neutrophil-to-lymphocyte ratio (NLR), and platelet-to-lymphocyte ratio (PLR) for predicting severe outcomes in patients affected by Coronavirus disease 2019 (COVID-19). Our study validates NLR and PLR cut-off values from a prior cohort at IRCCS Policlinico San Matteo (OSM) of Pavia, Italy, across two new cohorts from different hospitals. This aims to enhance the generalizability of these prognostic indicators.

Methods: In this retrospective cohort study, conducted at Milan's Ospedale Luigi Sacco (OLS) and IRCCS Ospedale Maggiore Policlinico (OMP) hospitals, we assess the predictive capacity of NLR and PLR for three main

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outcomes—non-invasive ventilation (NIV) or continuous positive airway pressure (CPAP) usage, invasive ventilation (IV), and death—in patients with COVID-19 at admission. For each outcome, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were computed separately for male and female cohorts. Distinct NLR and PLR cut-off values were used for men (7.00, 7.29, 7.00 for NLR; 239.22, 248.00, 250.39 for PLR) and women (6.36, 7.00, 6.28 for NLR; 233.00, 246.45, 241.54 for PLR), retrieved from the first cohort at OSM.

***Results*:** A total of 3599 patients were included in our study, 1842 from OLS and 1757 from OMP. OLS and OMP sensitivity values for both NLR and PLR (NLR: 24–67%, PLR: 40–64%) were inferior to specificity values (NLR: 64–76%, PLR: 55–72%). Additionally, PPVs generally remained lower (< 63%), while NPVs consistently surpassed 68% for PLR and 72% for NLR. Finally, both PLR and NLR exhibited consistently higher NPVs for more severe outcomes (> 82%) compared to NPVs for CPAP/NIV.

***Conclusions*:** Consistent findings across diverse patient populations validate the reliability and applicability of NLR and PLR cut-off values. High NPVs emphasize their role in identifying individuals less likely to experience severe outcomes. These markers not only aid in risk stratification but also guide resource allocation in emergencies or limited-resource situations.

Keywords: COVID-19; Neutrophil-to-lymphocyte ratio; Platelet-to-lymphocyte ratio; Prognostic indicators; Validation study

Key Summary Points

The neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) are easily accessible markers that can be used for predicting severe outcomes in patients with COVID-19.

Our study aimed at validating the applicability of previously established NLR and PLR cut-off values on different cohorts.

Despite the differences in timeframes and patient cohorts, the performance of NLR and PLR exhibited remarkable consistency across the board.

Our results confirm that the NLR and PLR might have a role in identifying individuals less likely to experience severe outcomes.

INTRODUCTION

Systemic inflammatory response is marked by dramatic immunologic alterations involving both the innate and adaptive immune systems [1]. The neutrophil-to-lymphocyte ratio (NLR) and the platelet-to-lymphocyte ratio (PLR) are two easily accessible inflammatory markers recognized for their potential prognostic value across various conditions. Specifically, the NLR is widely employed in predicting mortality in the case of sepsis, stroke, myocardial infarction, cancer, and trauma [2]. On the other hand, the PLR serves as a known marker for the hyperinflammatory state associated with rheumatologic diseases [3], and an elevated PLR is considered predictive of mortality in patients with cancer or acute pulmonary embolism [4, 5].

Severe Coronavirus disease 2019 (COVID-19) is associated with a sustained and amplified inflammatory response altering the leukocyte count, which is characterized by neutrophilia, lymphopenia, and thrombocytopenia [6]. Therefore, a similar rationale could be applied in this context. Indeed, the potential prognostic role of NLR and PLR has been of interest since the early stages of the SARS-CoV-2 pandemic [7].

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In a recent meta-analysis involving over 12,000 patients, it was revealed that individuals with severe COVID-19, notably those who were critically ill or deceased, exhibited elevated baseline NLR compared with milder counterparts [8]. At the same time, NLR emerged as a predictor for the necessity of intensive care treatment in patients with COVID-19 [9]. Several studies and meta-analyses have proposed different cut-offs for NLR and reported good sensitivity and specificity of NLR in predicting both disease severity and mortality [10, 11]. Conversely, there is a paucity of more varied data regarding the prognostic role of PLR in COVID-19. A systematic review with meta-analysis by Sarkar and colleagues retrieved PLR values specific for COVID-19 mortality and disease severity outcomes in 2768 and 3262 patients, respectively [12]. The authors observed higher values of PLR in critically ill or deceased patients compared to survivors and those with mild illness. While a higher PLR was undoubtedly found predictive of severity and longer length of stay (LOS) [13], multiple proposed cut-offs displayed varying degrees of sensitivity and specificity [13, 14].

The attractiveness of NLR and PLR lies in their cost-effectiveness, widespread availability, and reproducibility, making them convenient options for use in emergency scenarios or settings with limited resources.

Asperges et al. recently identified distinct NLR and PLR cut-off values to anticipate more severe outcomes in a cohort of patients spanning the first three waves of the COVID-19 pandemic at IRCCS Policlinico San Matteo of Pavia (OSM) in Northern Italy [15]. These cut-offs were used to predict severity indicators such as the use of continuous positive airway pressure (CPAP), intensive care unit (ICU) admission, invasive ventilation (IV), and mortality in patients with COVID-19. With all this in mind, our study aims to validate these NLR and PLR cut-offs on two new cohorts, collected from different time periods and specifically sourced from two additional hospitals. By extending our analyses, we aim to enhance the generalizability and robustness of these indicators, providing valuable insights into the predictability of COVID-19 outcomes across varied patient populations.

METHODS

Study Design and Setting

This retrospective cohort study, building upon a previous investigation [15], aimed to further explore the predictive capabilities of NLR and PLR at the time of admission in patients with COVID-19. Specifically, we aimed to assess their predictive value for three primary outcomes: the requirement for non-invasive ventilation (NIV) or CPAP, the need for IV, and mortality.

The previous study was conducted at IRCCS Policlinico San Matteo of Pavia, Italy (OSM) [15]. The current study spans two prominent teaching hospitals in Milan, Italy: Ospedale Luigi Sacco (OLS) and Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico of Milan (OMP). Despite both being key referral centers, they differ in patient loads; OLS, smaller and without hematology and solid organ transplant units, handles fewer immunosuppressed patients compared to OMP.

All adult patients (18 years or older) with a positive RT-PCR for SARS-CoV-2 on nasopharyngeal swabs who were admitted to either OLS or OMP were included in the study. The cases from OLS and OMP [16] were recruited from the onset of the pandemic (February 2020) until April 2021 and November 2022, respectively. Patients without at least one computable value of NLR or PLR were excluded.

Data were collected from OMP and OLS COVID-19 registries and included epidemiological data, LOS, comorbidities, laboratory data at admission, and outcomes (NIV/CPAP, IV, or death).

The patient cohorts from this study were collected during different time intervals than those from the initial hospital, providing a diverse temporal perspective on the impact of NLR and PLR on the specified outcomes.

Ethics Committee's Approval

All participants signed informed written consent and the cohorts were approved by the two hospitals' Institutional Review Boards. The study at OMP was approved by the Medical Ethics

Committee of the Fondazione IRCCS Ca′ Granda Ospedale Maggiore Policlinico (EC approval 241_2020, March 17, 2020), while the study at OLS was approved by the Ethics Committee of the Comitato Etico Interaziendale Area 1, Milan, Italy (Protocol No. 16088). The previous study conducted at OSM was approved by the Fondazione IRCCS Policlinico San Matteo's ethics committee with protocol number 20200046877 [15].

Statistics

Categorical data are presented as counts and proportions while continuous variables are presented as mean with standard deviation. The distribution of continuous variables has been checked through graphical representation. The comparisons between the three populations have been carried out respectively through Chi-square test – reporting the relative value of Cohen's w – and ANOVA test – reporting the relative value of η^2; the post hoc comparison between OLS and OMP required Student's t test and Cohen's d estimations for continuous variables, while the Chi-square test and Cohen's w estimation for categorical ones; p values have been corrected with Bonferroni's method. For each outcome (NIV/CPAP, IV, or death), sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were computed separately for male and female cohorts. Distinct NLR and PLR cut-off values were used for men (7.00, 7.29, 7.00 for NLR; 239.22, 248.00, 250.39 for PLR for NIV/CPAP, IV and death outcomes, respectively) and women (6.36, 7.00, 6.28 for NLR; 233.00, 246.45, 241.54 for PLR for NIV/CPAP, IV and death outcomes, respectively). This approach ensures a gender-specific assessment. Cut-offs were retrieved from a first cohort study by Asperges et al. [15]. To assess their performance, the above-mentioned cut-offs were applied to the cohorts from OMP and OLS. Analyses were performed using the "cutpointr" package [17] in RStudio (R version 4.2.0) [18].

RESULTS

A total of 3599 patients were included in our study, 1842 from OLS and 1757 from OMP. The general characteristics of the two cohorts, alongside the data from the OSM cohort reported by Asperges et al. [14], are presented in Table 1. Regarding the two newly examined populations, the demographic characteristics were almost similar (64.2 vs. 63.6 years old: Cohen's $d=0.04$ – p adjusted $=0.570$. 64.9 vs. 65.1% men: Cohen's $w=0.001$ – p adjusted >0.90). Concerning underlying comorbidities, the prevalences were comparable between OLS and OMP (diabetes Cohen's $w=0.04$ – p adjusted $=0.039$; lung disease Cohen's $w=0.01$ – p adjusted >0.90), with more than half of included patients having any kind of heart disease (55% for OLS and 51% for OMP; heart disease Cohen's $w=0.04$ – p adjusted $=0.054$).

Differently, the prevalence of each outcome was dissimilar among the three hospitals (Table 2). Specifically, CPAP and IV were required in 41% and 18% of men hospitalized at OLS and in 33% and 14% of those hospitalized at OSM, compared with only 11% and 7% of men hospitalized at OMP. A lower mortality was also observed in the OMP cohort with respect to the OLS and OSM cohorts. In all three cohorts, the prevalence of CPAP/NIV and IV was higher in men compared with women, mortality instead was comparable between the two sexes.

Despite the variances in timeframes and patient cohorts, the performance of NLR and PLR exhibited remarkable consistency across the board (Tables 3 and 4). Specifically, the sensitivity for NLR ranged from 24 to 67%, with the highest values observed for the mortality outcome (54–67%). NLR performed better in terms of specificity, ranging from 64 to 76%, particularly for the CPAP/NIV outcome. Comparable findings were observed for PLR (sensitivity: 40–64%, specificity: 55–72%). Additionally, PPVs, both for NLR and PLR, generally remained lower (<63%), particularly for the OMP cohort, and tended to decrease for more severe outcomes (e.g., IV and death). In contrast, NPVs consistently surpassed 68% for PLR and 72% for NLR. Furthermore, PLR and NLR exhibited

Table 1 General characteristics of the cohorts of the three hospitals

Hospital	OSM of Pavia ($n = 2169$)	OLS of Milan ($n = 1842$)	OMP of Milan ($n = 1757$)	Effect size	p value
Period					
Range	Feb 2020–May 2021	Feb 2020–Apr 2021	Feb 2020–Nov 2022		
Population					
Males (*N*, %)	1317 (60.7%)	1196 (64.9%)	1144 (65.1%)	Cohen's $w = 0.04$	0.005
Females (*N*, %)	852 (39.3%)	646 (35.1%)	613 (34.9%)		
Age					
Mean ± SD	68 ± 16	64.2 ± 16.0	63.6 ± 16.0	$\eta^2 = 0.02$	< 0.001
Diabetes					
(*N*, %)	364 (16.8%)	286 (15.5%)	321 (18.7%)*	Cohen's $w = 0.03$	0.039
Lung disease					
(*N*, %)	151 (6.9%)†	277 (15.0%)	251 (14.6%)*	Cohen's $w = 0.12$	< 0.001
Heart disease					
(*N*, %)	727 (33.5%)††	1016 (55.2%)	877 (51.1%)*	Cohen's $w = 0.19$	< 0.001

The statistical test estimates refer to the comparison between the three populations

OSM Ospedale San Matteo, *OLS* Ospedale Luigi Sacco, *OMP* Ospedale Maggiore Policlinico

*Recorded for 1715 patients

†This count refers only to chronic obstructive pulmonary disease (COPD)

††This count refers only to hypertension

consistently higher NPVs for more severe outcomes (>82%) compared to NPVs for CPAP/NIV. Such trends were observed also in the previous cohort.

DISCUSSION

Our study aimed at validating NLR and PLR cut-off values established by Asperges et al. to predict severe COVID-19 [15]. Specifically, to enhance the generalizability and robustness of these two prognostic indicators, we applied the cut-offs on two different cohorts of patients sourced from two important COVID-19 hubs in Lombardy, Italy. Despite differences in patient populations and timeframes, NLR and PLR performed consistently, indicating their potential for broad applicability across various settings.

Asperges and colleagues provided NLR cut-offs ranging from 6.36 to 7.29, depending on sex and type of ventilation, along with mortality cut-offs of 6.28 for women and 7.00 for men. In terms of disease severity, the chosen NLR cut-off slightly exceeds those proposed by studies conducted in China, Iran, and Ethiopia, spanning from 4.5 to 6.5 [19–21], while the mortality cut-offs are notably lower than those reported in other studies, often surpassing 7.9 [19, 22, 23]. The discrepancies in cut-offs can be attributed to baseline differences among patients' cohorts, including ethnicity, and variations in the definition of severity among different studies. While numerous cut-offs exist for NLR, data for PLR remain relatively scarce. Values obtained in the previous cohort from OSM ranged from 233 to 250.39. Two small retrospective studies in China proposed PLR cut-offs of 126.7 and 274

Table 2 Prevalence of the different outcomes in the three hospitals

	Male			Female		
	CPAP/NIV	**IV**	**Death**	**CPAP/NIV**	**IV**	**Death**
OSM of Pavia						
Prevalence	0.33	0.14	0.26	0.21	0.05	0.23
95%CI	0.31, 0.36	0.12, 0.16	0.24, 0.29	0.18, 0.24	0.03, 0.06	0.21, 0.26
OLS of Milan						
Prevalence	0.41	0.18	0.20	0.27	0.08	0.19
95%CI	0.38, 0.44	0.16, 0.20	0.18, 0.22	0.23, 0.30	0.06, 0.10	0.16, 0.23
OMP of Milan						
Prevalence	0.11	0.07	0.15	0.12	0.04	0.14
95%CI	0.09, 0.13	0.05, 0.08	0.13, 0.17	0.10, 0.15	0.03, 0.06	0.11, 0.17

Prevalence of the outcomes by hospital and gender

95% CI 95% confidence interval, *OSM* Ospedale San Matteo, *OLS* Ospedale Luigi Sacco, *OMP* Ospedale Maggiore Policlinico, *CPAP/NIV* continuous positive airway pressure/non-invasive ventilation, *IV* invasive ventilation

for longer hospitalization and severe pneumonia [13, 14].

Notably, the two cohorts studied here differed in terms of collection timeframes and patient loads. First, data for the two patient cohorts from OLS and OMP were collected during different time intervals than those from OSM, which were recorded during the first three pandemic waves of COVID-19 (February 2020–May 2021). In contrast, the cases from our study were collected from the onset of the pandemic until November 2022, thus also including a comparatively quieter period in the pandemic characterized by a lower proportion of patients with severe disease [16]. This, in turn, would explain the lower prevalence of more severe outcomes such as IV requirement and death for the OMP cohort, which included patients until the end of 2022. Second, OLS and OMP differ dramatically in patient loads. OLS is smaller and lacks hematology and solid organ transplant units, therefore handling fewer immunocompromised patients compared to OMP and OSM, which are key referral transplant centers. Nevertheless, the demographic characteristics and basic comorbidities of the two examined populations were similar. These similarities could be explained by the magnitude and severity of the first waves of the pandemic, which affected not only immunocompromised patients but also often middle-aged men with other comorbidities, such as hypertension or diabetes [24].

However, despite these underlying differences, NLR and PLR performed similarly in the two new cohorts, indicating the generalizability of these measurements and their potential to be used in different settings and different populations. Specifically, NLR and PLR sensitivity values (NLR: 24–67%, PLR: 40–64%) were inferior to specificity values (NLR: 64–76%, PLR: 55–72%). When compared with OSM, the cut-offs performed better in terms of sensitivity (NLR: 62–67%, PLR: 56–61%) with respect to specificity (NLR: 50–55%, PLR: 50–51%) in the first cohort. A recent meta-analysis aimed at finding predictive values of NLR on COVID-19 severity and mortality reported sensitivity and specificity of 78% for severity and 83% for mortality. However, the study included a wide range of different cut-offs, both for mortality and for

Table 3 Performance of neutrophil-to-lymphocyte ratio (NLR) in the three hospitals

Outcome	Male			Female		
	CPAP/NIV	IV	Death	CPAP/NIV	IV	Death
NLR (Neutrophil-to-lymphocyte ratio)						
Cutpoint	**7.00**	**7.29**	**7.00**	**6.36**	**7.00**	**6.28**
OSM of Pavia						
Sensitivity	0.65	0.67	0.66	0.62	0.67	0.66
Specificity	0.51	0.50	0.50	0.51	0.55	0.51
PPV	0.41	0.19	0.33	0.27	0.07	0.31
NPV	0.73	0.90	0.80	0.82	0.97	0.82
OLS of Milan						
Sensitivity	0.58	0.61	0.62	0.49	0.57	0.54
Specificity	0.76	0.69	0.67	0.73	0.74	0.70
PPV	0.63	0.30	0.32	0.40	0.16	0.30
NPV	0.72	0.89	0.88	0.80	0.95	0.86
OMP of Milan						
Sensitivity	0.61	0.53	0.65	0.47	0.24	0.67
Specificity	0.68	0.64	0.65	0.71	0.71	0.72
PPV	0.39	0.09	0.25	0.29	0.03	0.29
NPV	0.83	0.95	0.91	0.84	0.96	0.93

Cutpoints for NLR derive from the previous study conducted by Asperges et al. [15]. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) are reported for each outcome, divided by gender and hospital

OSM Ospedale San Matteo, *OLS* Ospedale Luigi Sacco, *OMP* Ospedale Maggiore Policlinico, *CPAP/NIV* continuous positive airway pressure/non-invasive ventilation, *IV* invasive ventilation

disease severity [25]. With regards to PLR, the cut-offs investigated here performed similarly to NLR in terms of sensitivity and specificity. Evidence on PLR use in predicting the severity and mortality of COVID-19 is more limited compared with NLR. Few retrospective studies conducted in Turkey and China obtained sensitivity and specificity values similar to those we retrieved, although each of the mentioned studies applied different cut-offs compared to ours [14, 26, 27].

Additionally, PPVs generally remained low both in the previous and in the novel cohorts. On the other hand, we observed high NPVs both for PLR and NLR, especially for IV and mortality outcomes. This underscores PLR and NLR's crucial role in reliably identifying individuals who are less likely to experience severe outcomes, emphasizing their potential not only for risk stratification but also for guiding resource allocation and clinical decision-making.

Moreover, given their low costs and high accessibility, NLR and PLR stand out as convenient tools during emergencies or in resource-limited situations.

Specifically, individuals with low PLR and NLR values are less prone to severe disease. Thus, patients presenting with COVID-19 symptoms but with negative PLR and NLR results might

Table 4 Performance of platelet-to-lymphocyte ratio (PLR) in the three hospitals

Outcome	Male			Female		
	CPAP/NIV	IV	Death	CPAP/NIV	IV	Death
PLR (platelet-to-lymphocyte ratio)						
Cutpoint	**239.22**	**248.00**	**250.39**	**233.00**	**246.45**	**241.54**
OSM of Pavia						
Sensitivity	0.61	0.61	0.55	0.65	0.56	0.56
Specificity	0.50	0.50	0.51	0.50	0.51	0.51
PPV	0.39	0.17	0.30	0.27	0.06	0.27
NPV	0.71	0.88	0.75	0.83	0.96	0.78
OLS of Milan						
Sensitivity	0.53	0.53	0.53	0.52	0.47	0.40
Specificity	0.72	0.66	0.68	0.68	0.67	0.65
PPV	0.57	0.26	0.29	0.37	0.11	0.22
NPV	0.68	0.87	0.85	0.80	0.93	0.82
OMP of Milan						
Sensitivity	0.64	0.51	0.57	0.62	0.48	0.63
Specificity	0.59	0.55	0.57	0.59	0.60	0.61
PPV	0.36	0.07	0.19	0.27	0.05	0.21
NPV	0.82	0.94	0.88	0.86	0.96	0.91

Cutpoints for PLR derive from the previous study conducted by Asperges et al. [15]. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) are reported for each outcome, divided by gender and hospital

OSM Ospedale San Matteo, *OLS* Ospedale Luigi Sacco, *OMP* Ospedale Maggiore Policlinico, *CPAP/NIV* continuous positive airway pressure/non-invasive ventilation, *IV* invasive ventilation

potentially be managed through outpatient follow-up, allocating hospital care for those at higher risk of severe disease and contributing to more efficient resource allocation and personalized patient care pathways. This underscores the practical significance of these biomarkers beyond risk stratification, emphasizing their role in guiding clinical management during emergencies.

Finally, despite the insightful findings and contributions of this study, some limitations need to be acknowledged. Firstly, the retrospective nature of the study design may introduce inherent biases and limitations in data collection. Secondly, the study covers a period marked by different phases of the pandemic, including the initial waves and subsequent periods with varying infection rates, and the evolving nature of the pandemic might influence the prevalence and severity of COVID-19 cases. Similarly, the study spans different timeframes for data collection across the three cohorts, with the OSM and OLS cohorts spanning the first three pandemic waves (February 2020–May 2021) and the OMP cohort extending until November 2022. Variations in patient management, treatment protocols, and the prevalence of severe cases over time may impact the generalizability of the findings. Thirdly, the study did not incorporate external validation from another geographical region or

country, which could further confirm the generalizability of the identified cut-off values.

CONCLUSIONS

In conclusion, the consistent performance of NLR and PLR across diverse patient populations and temporal contexts validates their identified cut-off values, confirming their reliability and applicability. The high NPV underscores its efficacy in identifying patients less prone to developing severe diseases, emphasizing their valuable role in clinical decision-making.

Author Contribution. Marta Colaneri conceived the original idea; Marta Colaneri and Camilla Genovese wrote the first draft; Marta Colaneri, Camilla Genovese, Federico Fassio, and Marta Canuti wrote the manuscript. Data collection and analysis was performed by Federico Fassio and Marta Canuti. Andrea Giacomelli, Anna Lisa Ridolfo, Erika Asperges, Giuseppe Albi, Raffaele Bruno, Spinello Antinori, Antonio Muscatello, Bianca Mariani, Ciro Canetta, Francesco Blasi, Alessandra Bandera, and Andrea Gori reviewed the methodology and the results. All authors read and approved the final version of the manuscript.

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Data Availability. The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of Interest. Marta Colaneri, Camilla Genovese, Federico Fassio, Marta Canuti, Andrea Giacomelli, Anna Lisa Ridolfo, Erika Asperges, Giuseppe Albi, Raffaele Bruno, Spinello Antinori, Antonio Muscatello, Bianca Mariani, Ciro Canetta, Francesco Blasi, Alessandra Bandera and Andrea Gori declare that they have no competing interests. Dr. Marta Canuti was affiliated with the University of Milan and her current affiliation is Department of Veterinary and Animal Sciences, University of Copenhagen, Frederiksberg C, Denmark.

Ethical Approval. All participants signed informed written consent and the cohorts were approved by the two Hospitals' Institutional Review Board. The study at OMP was approved by the Medical Ethics Committee of the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico (EC approval 241_2020, 17 March 2020), while the study at OLS was approved by the Ethics Committee of the Comitato Etico Interaziendale Area 1, Milan, Italy (Protocol No. 16088). The previous study conducted at OMS was approved by the Fondazione IRCCS Policlinico San Matteo's ethics committee with protocol number 20200046877 [15].

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