Monocyte absolute count as a preliminary tool to distinguish between SARS-CoV-2 and influenza A/B infections in patients requiring hospitalization

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SUMMARY

Since the most frequent symptoms of novel coronavirus 2019 disease (COVID-19) are common in influenza A/B (FLU), predictive models to distinguish between COVID-19 and FLU using standardized non-specific laboratory indicators are needed. The aim of our study was to evaluate whether a recently dynamic nomogram, established in the Chinese population and based on age, lymphocyte percentage and monocyte absolute count, might apply to a different context. We collected data from 299 patients (243 with COVID-19 and 56 with FLU) at Policlinico Umberto I, Sapienza University of Rome. The nomogram included age, lymphocyte percentage and monocyte absolute count to differentiate COVID-19 from FLU. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated for all associations. Multivariate logistic regression models were used to adjust for potential confounding. A p-value of less than 0.05 was considered statistically significant. Patients with COVID-19 had higher age, lymphocyte percentage and monocyte absolute count than patients with FLU. Although univariate analysis confirmed that age, lymphocyte percentage and monocyte absolute count were associated with COVID-19, only at multivariate analysis was monocyte count statistically significant as a predictive factor of COVID-19. Using receiver operating characteristic (ROC) curves, we found that a monocyte count >0.35x10^3/mL showed an AUC of 0.680 (sensitivity 0.992, specificity 0.368). A dynamic nomogram including age, lymphocyte percentage and monocyte absolute count cannot be applied to our context, probably due to differences in demographic characteristics between Italian and Chinese populations. However, our data showed that monocyte absolute count is highly predictive of COVID-19, suggesting its potential role above all in settings where prompt PCR nasopharyngeal testing is lacking.

Keywords: monocytes, lymphocytes, nomogram, SARS-CoV-2, influenza.

INTRODUCTION

Since the 2019 coronavirus disease (COVID19) was first reported in Wuhan province, China, in late December 2019, it has become one of the main health problems worldwide [1, 2]. As of September 1st, new outbreaks occur in the European area [3]. Thus, more people every day turn to health care facilities due to respiratory tract infections.

COVID19 and Influenza A/B (FLU) usually present with similar symptoms, therefore, with the upcoming FLU season, distinguishing between these two conditions is important and a rapid differential diagnosis might represent a crucial issue for patient management [4, 5].

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Even though the gold standard diagnostic test for SARS-CoV2 detection is the real time polymerase chain reaction (RT-PCR), molecular analysis is expensive and not available everywhere. Furthermore, a lot of primary hospitals do not have equipped laboratory nor trained staff to perform a RT-PCR [6, 7].

In this context, the study of Wang and colleagues about establishing a dynamic nomogram fits to differentiate COVID19 from influenza A/B (FLU) infection [8]. They conducted a retrospective single centre study on 448 Chinese outpatients (181 with standard RT-PCR confirmed COVID19 and 276 with influenza A or B infection). Using the receiver operating characteristic (ROC) curves, the authors identified age, monocyte absolute count and lymphocyte percentage as markers highly predictive for COVID19. The association between the aforementioned non-specific laboratory markers was more sensible and specific than single markers. Thus, a dynamic nomogram was established and it is now available online (https://bjdth.shinyapps.io/COVID-19/). The authors asserted that the model could have a role in the clinical practice to distinguish COVID19 from FLU.

Aim of this study was to validate the performance of the aforementioned nomogram in the context of non-Chinese patients with laboratory confirmed SARS-CoV2 or FLU infection and requiring hospitalization.

MATERIALS AND METHODS

We conducted a retrospective single-centre study. The data were collected from 299 hospitalized patients (243 with COVID19 and 56 with FLU) admitted to Policlinico Umberto I, Sapienza University of Rome (Italy). The data of patients with FLU infection were collected from January to April 2019, whereas data of COVID19 patients were collected from January to May 2020. Inclusion criteria were age >18 years, positive RT-PCR for SARS-CoV2 or positive PCR for influenza A/B performed on nasopharyngeal or nasal swab, respectively. Nasopharyngeal swab samples were collected and SARS-CoV-2 or influenza A/B RNA were detected by using RT-PCR. As for SARS-CoV-2 infection, the definition of pneumonia or severe pneumonia was based on the WHO interim guidance and it included clinical signs of pneumonia (fever, cough, dyspnoea, fast breathing) with or without signs of severe pneumonia such as respiratory rate >30 breaths/min, severe respiratory distress, or SpO2 <90% on room air [9]. According to the published nomogram, we collected age, lymphocyte percentage and monocyte absolute count at the hospital admission and recorded them anonymously in an electronic database. The study was approved by the local Ethics Committee (ID Prot. 109/2020). All data were analysed using Statistical Package for Social Science (SPSS) version 20 or Microsoft Excel (Office 2018). Description of median with interquartile range (IQR 25%-75%), simple frequencies (n), proportions (or percentages) and rates of the given data on each variable has been calculated. The univariate analysis was used to compare patients divided in two groups: FLU, COVID19. Mann-Whitney test was conducted for continuous variables and chi-square for categorical variables. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated for all associations. Multivariate logistic regression models were used to adjust for potential confounding. P-value <0.05 was considered statistically significant.

RESULTS

A total of 299 inpatients were included in the study (243, 81% with COVID19 and 56, 19% with FLU). Most of our cohort had radiological signs

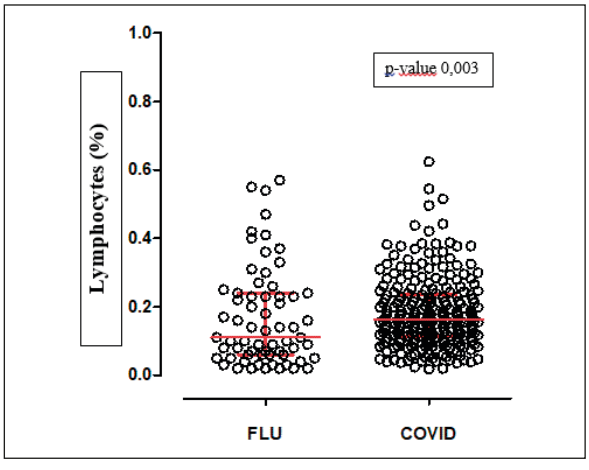

Figure 1 - Graphic distribution of lymphocyte percentage in COVID19 and FLU groups with median and IQR range 25%-75% (red lines). FLU: influenza A or B; COVID: COVID19.

of pneumonia (FLU 69.5% and COVID19 93%). Median age was 60 years (IQR 39-75) with median value for monocyte of 0.1x10^3/mL/mmc (IQR 40-200) and lymphocyte percentage of 12% (IQR 7-23). Patients with COVID19 were older than FLU ones (median age 62, (IQR 52-76) vs 57 (IQR 35-71), p=0.015) (Figure 1). The lymphocyte percentage was lower in COVID19 group than in FLU patients (median 16% (IQR 11-23) vs 10% (IQR 5-23) in COVID19 and FLU respectively, p

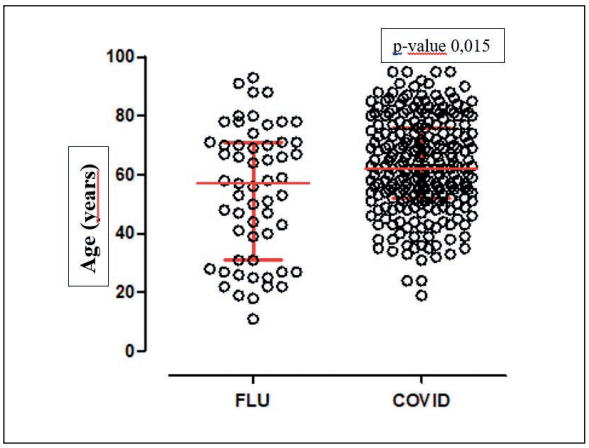

Figure 2 - Graphic distribution of age in COVID19 and FLU groups with median and IQR range 25%-75% (red lines). FLU: influenza A or B; COVID: COVID19.

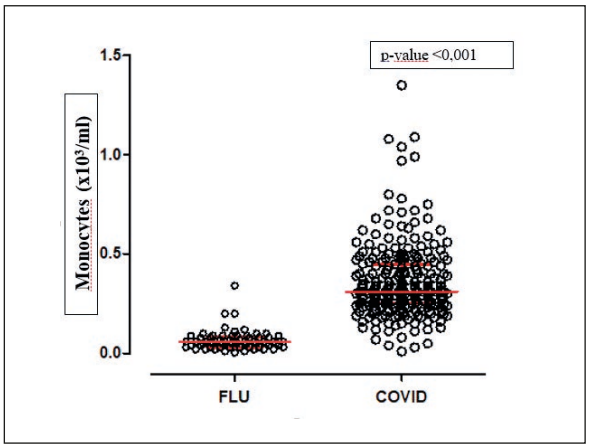

Figure 3 - Graphic distribution of monocyte absolute count in COVID19 and FLU groups with median and IQR range 25%-75% (red lines). FLU: influenza A or B; COVID: COVID19.

Table 1 - Demographics and laboratory parameters (according to the nomogram by Wang and colleagues) in patients with COVID-19 or influenza (FLU) (n=299). IQR: inter-quartile range.

Parameters	*FLU (n=56)*	*COVID19 (n=243)*	*p-value*
	Median, IQR (25%-75%)	*Median, IQR (25%-75%)*	
Age (years)	57 (35-71)	62 (52-76)	0.015
Lymphocytes (%)	0.1 (0.05-0.23)	0.16 (0.11-0.23)	0.003
Monocytes count (x10^3/ml)	0.06 (0.03-0.08)	0.31 (0.25-0.45)	<0.001

Table 2 - Univariate and multivariate analyses of predictive parameters associated with COVID19 (OR>1) or influenza A or B (FLU, OR<1).

Parameters	*FLU No. (%)*	*COVID-19 No. (%)*	*p-value*	*OR (95% CI)*	*Z*	*p-value*
Age >65 years	28 (50)	110 (45)	0.499	0.8 (0.5-1.47)	0.64	0.522
Lymphocytes >30%	11 (20)	32 (13)	0.178	0.6 (0.3-1.32)	1.23	0.216
Monocytes >350/ml	0 (0)	241 (99)	<0.001	337 (46-244)	8.1	<0.001

Table 3 - Area under the receiver operating characteristic (AUROC) curves of the nomogram model, lymphocyte count, monocyte count, and age.

Marker	*AUC*	*Sensitivity*	*Specificity*	*SE.AUC*	*Lower limit*	*Upper limit*	*Z*	*p-value*
Age >65 years	0.544	0.457	0.632	0.032	0.48	0.607	1.37	<0.001
Lymphocytes >30%	0.554	0.224	0.868	0.026	0.40	0.605	-1.74	<0.001
Monocytes >350/ml	0.680	0.992	0.368	0.028	0.62	0.734	6.43	<0.001

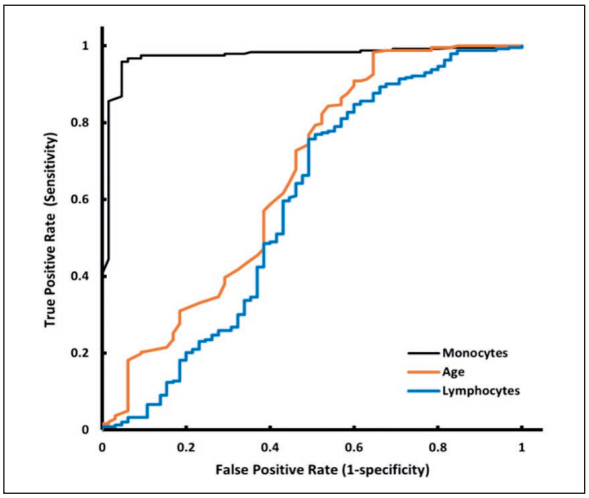

Figure 4 - Graphic representation of AUROC curves of monocyte count, age and lymphocytes percentage

value 0.003) (Figure 2). Of note, monocyte absolute count was higher in COVID19 than in FLU patients (median 310 vs 60 cell/ml, p<0.0001) (Figure 3). A summary of univariate analysis of age, lymphocyte percentage and monocyte absolute count in COVID19 and FLU patients is provided in Table 1.

At multivariate analysis, only monocyte absolute count was significantly predictive of COVID19 rather than age and lymphocyte percentage (OR 337, CI 46-2444, p-value <0.001) (Table 2). As shown in Table 3, monocyte absolute count higher than $0.35x10^3$/mL showed an area under the ROC (AUROC) curve of 0.680 (sensitivity 0.992, specificity 0.368) as a tool differentiating COVID19 from FLU. A graphic presentation of AUROC curves is shown in Figure 4.

DISCUSSION

In the present report, we tried to apply the recently published nomogram with the aim of differentiating FLU from COVID19 by only using easy-to-perform laboratory analyses and we found that, as it has been conceived, it did not fit well for our population. In contrast, the value of monocytes count at hospital admission was able to discriminate patients with COVID19 from those with FLU. Despite molecular tests remain the gold standard for diagnosis of COVID19, they do not have the same availability in every hospital nor in every country [7]. Symptoms of FLU are very similar with those of COVID19, especially in the early phase [9, 10]. Thus, it is fundamental to discriminate between these two conditions by the use of an easy to calculate nomogram, possibly available in every setting.

However, the proposed dynamic nomogram including age, lymphocyte percentage and monocyte absolute count could not be applied to our context, probably due to differences in demographic characteristics. In fact, our cohort included only adults (age >18 years) whereas Wang and colleagues included also children and infants with an age range of 1-92 years and a median age of 38 years in COVID19 group and 29 in FLU group, significantly lower than our population. These differences might account for the absence of reproducibility of the proposed nomogram.

In fact, it is held in literature that the immune system, during COVID19, has different response in children compared with adults [11]. Thus, the yield of lymphocyte percentage and monocyte absolute count may also undergo age related changes, possibly explaining the observed differences.

Our cohort was composed by patients hospitalized after diagnosis of COVID19 or FLU, based on their clinical conditions. Conversely, Wang and colleagues did not accurately describe this point. In fact, they asserted that only outpatients had been tested but there was no mention if they required hospitalization in case the test was positive.

In our population, we found extremely low values of lymphocyte percentage both in COVID19 and FLU cohorts. Even if it is impossible to differentiate between COVID19 and FLU due to an overlapping of the values, low lymphocyte percentages might assume a negative prognostic role in the two groups. Additionally, low lymphocyte number has been recognized as risk factor for *Aspergillus* superinfection [12]. This seems to change the natural history of COVID19 and FLU with worse outcomes [13].

The most interesting finding of the present study is that monocyte count might be able to help in distinguishing between FLU and COVID19, with higher absolute value in COVID19 subjects. Our results are in accordance with other studies in literature that suggest a potential prognostic role of monocyte- macrophages activation [14]. The number and the activation status of monocyte can be

evaluated through differentiation and activation markers measurable at different stages of COVID19. This provides insights into the contribution of monocytes to pathogenesis and a possible guide to treatment. To this end, it has been suggested that serial determinations of monocytes counts during the course of infection might represent a tool for monitoring severity and potential complications of SARS-CoV2 infection [15].

Based on these considerations, we suggest a potential role of monocyte count as part of a population-specific dynamic nomogram for differential diagnosis between COVID19 and FLU. This could acquire a priority importance to peripheral hospitals with lack of performing rapidly RT-PCR.

Our study has several limitations. First, a low number of study population, especially FLU patients compared with COVID19 group. Although the main aim was to apply the nomogram by using only three parameters, an additional limitation of the study is represented by the lack of comorbidities or clinical information. Differently from Wang et al., we included in the study hospitalized patients mostly affected by pneumonia, thus at a more advanced stage of both diseases, which represent only a subset of infected patients, probably with distinct laboratory findings. Finally, the retrospective and single centre nature of the study might limit the conclusion to a specific setting of population, with obvious differences in different setting.

In conclusion, our findings indicate that lymphocyte percentage and age cannot be used in every settings to distinguish between COVID19 and FLU. Conversely, in our population the monocyte absolute count appeared predictive for COVID19 with high sensitivity. Further studies are needed in order to create specific nomograms including monocytes by which rapidly distinguish between COVID19 and other respiratory tract infections.

Conflict of interest

The authors declare no conflict of interest.

Funding

None

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