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LETTER TO THE EDITOR

# Reduced activity of B lymphocytes, recognised by Sysmex XN-2000™ haematology analyser, predicts mortality in patients with coronavirus disease 2019

Dear Editors,

Coronavirus disease 2019 (COVID-19) has severely tested healthcare systems around the world. There is an urgent need not only to identify effective treatments and to develop a vaccine but also to identify clinical and laboratory predictors of disease progression towards serious and fatal forms. Currently, recent evidence outlines the role played by the immune system in combating severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and its clinical manifestations.[1]

In this study, we evaluated the discriminatory ability in predicting mortality of a series of clinical, haematological and biochemical parameters at hospital admission in patients who tested positive for SARS-CoV-2 at nasopharyngeal swab and developed COVID-19 that required hospitalisation. We included 183 COVID-19 consecutive cases, admitted to an Academic hospital during the first month of the epidemic in Northern Italy, from 6 to 31 March. Patients were observed until death or hospital discharge and the laboratory parameters were evaluated at different timing during hospitalisation, but only the data at admission, of *"naive" patients*, before the starting of any therapy, are reported.

In-hospital mortality rate was 17%. Demographic, clinical, haematological and biochemical parameters at admission are described in Table 1, and predictors of in-hospital mortality are presented in Table 2. Among demographic and clinical characteristics, in-hospital mortality was associated with age (OR 1.09; 95% CI 1.05-1.13, $P < .001$), pre-existing diabetes (OR 2.80; 95% CI 1.22-6.42, $P = .015$), arterial hypertension (OR 3.03; 95% CI 1.35-6.78, $P = .007$), cardiovascular disease (OR 5.29; 95% CI 2.34-11.96, $P < .001$) and kidney disease (OR 5.42; 95% CI 2.16-13.58, $P < .001$) at univariate analysis (Table 2).

Regarding biochemical parameters, C-reactive protein and creatinine values were significantly associated with a fatal form of COVID-19 at univariate analysis (OR 1.10; 95% 1.03-1.17, $P = .004$; OR 5.70; 95% 2.53-12.84, $P < .001$, respectively) (Table 2).[2]

At admission, not all haematological parameters were significantly different between nonsurvivors and survivors. In particular, median peripheral total blood leucocyte and lymphocyte counts were slightly but not significantly different (Table 2), confirming that during the early phase of the disease, when nonspecific symptoms are present, peripheral blood leucocyte and lymphocyte counts cannot be considered predictors of an unfavourable outcome.[3,4] Nevertheless, nonsurvivors, as compared with survivors, presented significantly lower absolute platelet counts (148 *vs.* 186 x $10^3$/μl; $P = .005$) (Table 2). Furthermore, RDW-CV (OR 1.21; 95% CI 1.06-1.38, $P = .005$), mean platelet volume (OR 2.74; 95% CI 1.76-4.26, $P < .001$) and platelet large cell ratio (OR 1.14; 95% CI 1.07-1.20, $P < .001$) resulted associated with mortality at univariate analysis (Table 1).[3-5]

The analyser used for the blood count test in the present study, Sysmex XN-2000™ Haematology System (Sysmex), is also able to determine a series of parameters that provide quantitative and qualitative information on the inflammatory response of the patient's immune system, specifically regarding the activation status of neutrophils (neutrophil reactivity intensity: NEUT-RI; neutrophil granularity intensity: NEUT-GI) and of lymphocytes (reactive lymphocytes: RE-LYMP; antibody-synthesising lymphocytes: AS-LYMP). These "extended inflammation parameters" are measured in the WBC differential (WDF) channel according to their cell membrane composition and cytoplasmic activity (RNA content) and become diagnostic reportable parameters with the XN software version 21.12.

Typically, activated neutrophils (increased NEUT-RI, increased NEUT-GI), immature granulocytes (IG), reactive lymphocytes (RE-LYMP) and T cell-independent plasma cells (AS-LYMP) are found in the initial, nonspecific phase of infection.[5,6] These cells are quantified in the WDF channel according to their cell membrane composition and cytoplasmic activity (RNA content). In particular, total reactive lymphocytes (RE-LYMP) include activated B and T lymphocytes, recognised by an increased fluorescence intensity on XN-2000™ (Sysmex), compared to that of not activated lymphocytes, while the antibody-synthesising lymphocytes (AS-LYMP) are exclusively activated B lymphocytes, recognised by the markedly high increase of fluorescence intensity compared to that of not activated lymphocytes. Even though our data about AS-LYMP were not subsequently confirmed by immunophenotyping, the strong correlation of these high fluorescence lymphocytes determined by Sysmex analysers with peripheral plasma cells or activated B lymphocytes identified by immuno-flow cytometry method has been shown in previous studies.[7,8] Therefore, the combination of the RE-LYMP and AS-LYMP parameters provides additional information regarding the cellular activation of the innate and adaptive immune response.

In our series, RE-LYMP and AS-LYMP counts at hospital admission were significantly lower in nonsurvivors, as compared with

  

**TABLE 1** Demographic, clinical and laboratory data of patients included in the study

| Parameters | Alive (no. 152) | Dead (no. 31) | *P* value |
|---|---|---|---|
| Age (years) | 62 (51-73) | 80 (74-85) | <.001* |
| Male sex (no., %) | 86 (57) | 25 (81) | .022* |
| Diabetes (no., %) | 28 (18%) | 12 (39%) | .018* |
| Hypertension (no., %) | 57 (38%) | 20 (65%) | .009* |
| CV disease (no., %) | 35 (23%) | 19 (61%) | <.001* |
| Lung disease (no., %) | 19 (13%) | 8 (26%) | .09 |
| Renal disease (no., %) | 14 (9%) | 11 (36%) | .001* |
| Liver disease (no., %) | 5 (3%) | 2 (7%) | .338 |
| WBC ($\times 10^3$ cells/μL) | 5.75 (4.55-7.44) | 6.32 (4.70-11.15) | .138 |
| Hb (g/dL) | 13.50 (12.33-14.80) | 13.10 (10.80-14.10) | .064 |
| RDW-CV (%) | 13.20 (12.70-14.08) | 14.50 (12.20-17-30) | .001* |
| MPV (fL) | 10.50 (9.90-11.00) | 11.40 (10.70-12.10) | <.001* |
| P-LCR (%) | 28.5 (23.35-33.20) | 35.90 (31.20-42.40) | <.001* |
| PLT ($\times 10^3$ cells/μL) | 186.50 (152.25-226.50) | 148.00 (125.00-197.00) | .005* |
| Neutrophils ($\times 10^3$ cells/ML) | 4.06 (2.9-5.44) | 4.60 (3.45-8.02) | .058 |
| Lymphocytes ($\times 10^3$ cells/ML) | 1.08 (0.85-1.44) | 0.85 (0.69-1.24) | .093 |
| Monocytes ($\times 10^3$ cells/ML) | 0.45 (0.33-0.64) | 0.45 (0.25-0.71) | .602 |
| Q-flag Blasts?/Abn lympho?* | 40 (40-50) | 40 (40-50) | .817 |
| Q-flag Left shift?* | 0 (0-10) | 0 (0-10) | .678 |
| Q-flag Atypical lympho?* | 20 (10-20) | 10 (0-20) | .009* |
| Q-flag Fragments?* | 0 (0-0) | 0 (0-13) | .021* |
| IG ($\times 10^3$ cells/μL) | 0.02 (0.02-0.05) | 0.03 (0.02-0.07) | .234 |
| HFLC ($\times 10^3$ cells/μL) | 0.03 (0.01-0.05) | 0.02 (0.01-0.04) | .008* |
| AS-LYMP ($\times 10^3$ cells/μL) | 0.02 (0.00-0.05) | 0.00 (0.00-0.02) | .007* |
| RE-LYMP ($\times 10^3$ cells/μL) | 0.07 (0.04-0.10) | 0.04 (0.03-0.08) | .014* |
| NEUT-RI (FI) | 47.30 (45.20-49.70) | 46.90 (45.85-48.75) | .779 |
| NEUT-GI (SI) | 151.30 (148.25-155.80) | 152.00 (148.85-156.10) | .677 |
| CRP (mg/dL) | 3.80 (2.24-8.74) | 8.60 (5.71-15.06) | .002* |
| Creatinine (mg/dL) | 0.85 (0.69-1.02) | 1.20 (0.91-1.94) | <.001* |

*Note:* Quantitative variables are expressed as median (interquartile range), while qualitative variables as absolute frequency and percentages.

Abbreviations: AS-LYMP, antibody-synthesizing lymphocytes; CRP, C-reactive protein; CV, cardiovascular; FI, fluorescence intensity; Hb, haemoglobin; HFLC, highly fluorescent lymphocyte cells; IG, immature granulocytes; IQR, interquartile range; MPV, mean corpuscular volume; NEUT-GI, neutrophil granularity intensity; NEUT-RI, neutrophil reactivity intensity; P-LCR, platelet large cell ratio; PLT, platelets; RDW-CV, red blood cell distribution width (coefficient of variation); RE-LYMP, reactive lymphocytes; SI, scatter intensity; WBC, white blood cells.

*In XN-2000, the probability of abnormal findings is indicated by the Q value, which is based on a scale from 0 to 300, with increments of 10 arbitrary units. In our laboratory, the cut-off for flagging is set at 100.

survivors (Table 2). Moreover, the AS-LYMP count was significantly associated with mortality at univariate analysis (OR 0.005; 95% CI 0.00-0.01, $P = .017$) (Table 2).

Finally, at multivariate regression analysis, among demographic and clinical characteristics, the only independent predictors of in-hospital mortality due to COVID-19 were age (OR 1.04; 95% CI 1.00-1.09, $P = .030$), C-reactive protein (OR 1.10; 95% 1.01-1.20, $P = .022$), creatinine (OR 2.76; 95% 1.07-7.08, $P = .035$) and the AS-LYMP count (OR 0.005; 95% CI 0.00-0.01, $P = .021$) (Table 2).[9] Moreover, analysis of the receiver operating characteristic (ROC) curve showed that basal AS-LYMP displayed significantly high diagnostic accuracy for in-hospital mortality (AUC 0.662, 95% CI 0.562-0.763, $P = .009$). The statistical best cut-off for predicting in-hospital mortality, calculated by Youden's index, was 0.025, with sensitivity (Sn) = 0.81 and specificity (Sp) = 0.48.

In summary, older age, high C-reactive protein, impaired renal function and decreased count of activated B lymphocytes at hospital admission independently predicted risk of mortality in our series. As confirming the previously reported impact of age in determining the in-hospital mortality among patients with COVID-19,[9] we emphasized the role of simply evaluable parameter of impaired adaptive immune response in predicting an unfavourable outcome of SARS-CoV-2 infection.

It is well known that the counts of leucocytes, neutrophils and lymphocytes help to discriminate between inflammation and

**TABLE 2** Univariate and multivariate regression for demographic, clinical and laboratory data

| Predictors of in-hospital mortality | Univariate Regression | | Multivariate Regression | |
|---|---|---|---|---|
| | OR (95% CI) | *P* value | OR (95% CI) | *P* value |
| Age | 1.09 (1.05-1.13) | **<.001*** | 1.04 (1.00-1.09) | **.030*** |
| Gender | 3.20 (1.24-8.24) | **.016*** | | .232 |
| Diabetes | 2.80 (1.22-6.42) | **.015*** | | .119 |
| Hypertension | 3.03 (1.35-6.78) | **.007*** | | .410 |
| CV disease | 5.29 (2.34-11.96) | **<.001*** | | .506 |
| Renal disease | 5.42 (2.16-13.58) | **<.001*** | | .744 |
| Lung disease | 2.44 (0.95-6.22) | .063 | | |
| Liver disease | 2.03 (0.38-10.96) | .412 | | |
| WBC | 1.05 (0.98-1.12) | .178 | | |
| Hb | 0.834 (0.699-0.996) | **.045*** | | .822 |
| RDW-CV | 1.21 (1.06-1.38) | **.005*** | | .562 |
| PLT | 1.00 (0.99-1.00) | .109 | | |
| P-LCR | 1.14 (1.07-1.20) | **<.001*** | | .082 |
| MPV | 2.74 (1.76-4.26) | **<.001*** | | .101 |
| Neutrophils | 1.15 (1.03-1.28) | .012* | | |
| Lymphocytes | 0.84 (0.44-1.61) | .592 | | |
| Monocytes | 1.69 (0.83-3.46) | .152 | | |
| CRP | 1.10 (1.03-1.17) | **.004*** | 1.10 (1.01-1.20) | **.022*** |
| Creatinine | 5.70 (2.53-12.84) | **<.001*** | 2.76 (1.07-7.08) | **.035*** |
| Q-flag Blasts/Abn Lympho? | 1.00 (1.00-1.01) | .348 | | |
| Q-flag Left shift? | 0.99 (0.94-1.03) | .534 | | |
| Q-flag Atypical lympho? | 0.97 (0.93-1.00) | **.046*** | | .392 |
| Q-flag Fragments? | 1.00 (0.99-1.02) | .735 | | |
| IG | 7.46 (0.16-348.21) | .306 | | |
| HFLC | 0.00 (0.00-4.62) | .076 | | |
| AS-LYMP | 0.00 (0.00-0.01) | **.017*** | 0.005 (0.00-0.01) | **.021*** |
| RE-LYMP | 0.02 (0.00-146.42) | .395 | | |
| NEUT-RI | 0.98 (0.88-1.08) | .640 | | |
| NEUT-GI | 1.01 (0.94-1.10) | .729 | | |

Abbreviations: AS-LYMP, antibody-synthesizing lymphocytes; CRP, C-reactive protein; CV, cardiovascular; FI, fluorescence intensity; Hb, haemoglobin; HFLC, highly fluorescent lymphocyte cells; IG, immature granulocytes; IQR, interquartile range; MPV, mean corpuscular volume; NEUT-GI, neutrophil granularity intensity; NEUT-RI, neutrophil reactivity intensity; P-LCR, platelet large cell ratio; PLT, platelets; RDW-CV, red blood cell distribution width (coefficient of variation); RE-LYMP, reactive lymphocytes; SI, scatter intensity; WBC, white blood cells.

To facilitate data interpretation, the values in bold and with an asterisk identify the statistically significant results (*P* value ≤.05).

infection, between the different pathogenic causes of infection (viral versus bacterial) and the different types of immune response (early innate, cellular or humoral). In particular, lymphocytosis and neutrophilia are normally associated with viral and bacterial infections, respectively, and, usually, the AS-LYMP of white blood cells (WBC) is higher in viral infections.[5,6]

In our COVID-19 nonsurvivors, the antibody-synthesising lymphocyte count was significantly lower, already at hospital admission, compared with survivors, suggesting an impaired B-cell humoral immune response as the cause of a fatal outcome in COVID-19-infected patients. Therefore, the reduction of AS-LYMP seems to be an important indicator of severity and an independent predictor of mortality in critically ill COVID-19-infected patients. If confirmed in a larger population, these data might be relevant to researchers for a better understanding of the altered immunologic response in severely affected patients and might become an important support to formulate

a tailored treatment approach and promptly provide intensive care to those who are at greater risk of a fatal outcome in SARS-CoV-2 infection.


## KEYWORDS

AS-LYMP, COVID-19, hospital mortality, humoral immune response, XN-2000


## CONFLICT OF INTEREST

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