Prognostic Role of Neutrophil to Lymphocyte Ratio in COVID-19 Patients: Still Valid in Patients That Had Started Therapy?

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COVID-19 may appear with a widely heterogeneous clinical expression. Thus, predictive markers of the outcome/progression are of paramount relevance. The neutrophil/lymphocyte ratio (NLR) has been suggested as a good predictive marker of disease severity and mortality. Accordingly, we found that NLR significantly increased in parallel with the WHO severity stage in COVID-19 patients during the I\textsuperscript{st} wave (March-May 2020; \( n = 49 \)), due to the significant reduction of lymphocyte and the significant increase of neutrophil in severe COVID-19 patients. While, we did not observe significant differences of NLR between the WHO severity stage among COVID-19 patients of the II\textsuperscript{nd} wave (September 2020-April 2021; \( n = 242 \)). In these patients, the number of lymphocytes and neutrophils did not change significantly between patients of different severity subgroups. This difference likely depends on the steroids therapy that the patients of the II\textsuperscript{nd} wave performed before hospitalization while most patients of the I\textsuperscript{st} wave were hospitalized soon after diagnosis. This is also confirmed by serum interleukin (IL)-6 and myeloperoxidase (MPO) that gradually increased with the disease stage in patients of the I\textsuperscript{st} wave, while such biomarkers (whose production is inhibited by steroids) did not show differences among patients of the II\textsuperscript{nd} wave in different stages. Thus, the NLR could be tested at diagnosis in naïve patients before starting therapies.

Keywords: COVID-19, neutrophil to lymphocyte ratio, interleukin-6, myeloperoxidase, corticosteroid therapy

INTRODUCTION

The Coronavirus disease 2019 (COVID-19) may appear with a heterogeneous clinical expression, i.e., from asymptomatic or mild to severe forms causing a significant loss of lives. Therefore, it is fundamental to early identify COVID-19 patients with a higher risk of a poor clinical outcome and predictive markers are of paramount relevance (1, 2).

Various studies concluded that the neutrophil/lymphocyte ratio (NLR) has a good predictive value on disease severity and mortality in patients with COVID-19 infection (3). On the other
hand, the NLR ratio is an easy and rapid prognostic marker in
a myriad of clinical conditions that include solid tumors (4),
chronic obstructive pulmonary disease (5), liver cirrhosis (6),
rheumatoid arthritis (7), acute pancreatitis (8), sepsis (9) and
psoriasis (10).

COVID-19 pandemic had a 1st wave in Italy between March
and May 2020. During this period, we studied 49 patients,
including all consecutive patients admitted to our hospitals with
a diagnosis of COVID-19 (11). After a lockdown during summer
2020, since September 2020 the pandemic in Italy had a IInd
wave (12) and we studied 242 further patients. Herein, we report
a comparison of some laboratory parameters including NLR in
COVID-19 between the patients of the Ist and IInd waves.

METHODS

We enrolled adult patients with a diagnosis of COVID-19 (SARS-
CoV-2 infection) admitted from March to May 2020 (Ist wave)
or from September 2020 to April 2021 (IInd wave) at one of
the following hospitals: Department of Clinical Medicine and
Surgery - Section of Infectious Diseases, University Hospital
Federico II, Naples; Department of Infectious Disease and
Infectious Urgencies, Cotugno Hospital, AORN dei Colli, Naples.
The study was approved by the Ethical Committee of the
University Federico II of Naples; the lone exclusion criterion was
the refusal or the impossibility to obtain the informed consent.
No patient admitted to our hospitals was excluded.

The 49 patients of the Ist wave included 19 females (38.8%),
and had a mean age of 59 years (range: 24–92 years). The frequencies of comorbidities in Ist wave patients were the following: hypertension, 40%; cardiovascular disease, 35%;
chronic obstructive pulmonary disease, 25%; diabetes, 15%;
chronic renal failure, 5%.

The 242 patients of the IInd wave included 121 females
(50.0%), and had a mean age of 52.9 years (range: 17–94
years). The frequencies of comorbidities in IInd wave patients
were the following: hypertension, 43%; cardiovascular disease,
20%; chronic obstructive pulmonary disease, 22%; diabetes, 13%;
chronic renal failure, 6%.

The diagnosis of COVID-19 was confirmed by molecular
analysis (RT-PCR) of the nasopharyngeal swab (11). All the
enrolled patients were classified on the basis of the seven
ordinal scale made by the World Health Organization (WHO)-
Research and Development Blueprint expert group and used
in previous influenza studies. According to this scale, patients
with COVID-19 can be classified as: (1) not hospitalized with
remission of normal activities; (2) not hospitalized, but unable
to resume normal activities; (3) hospitalized, not requiring
supplemental oxygen; (4) hospitalized, requiring supplemental
oxygen; (5) hospitalized, requiring nasal high-flow oxygen
therapy, non-invasive mechanical ventilation, or both; (6)
hospitalized, requiring extra corporeal membrane oxygenation,
invasive mechanical ventilation, or both; and (7) death. For
each patient we considered the worst WHO stage during the
infection (13, 14). We divided our population study in three
subgroups: WHO 3, WHO 4, and WHO 5–7 (including COVID-
19 patients with WHO stage from 5 to 7). Whole blood
samples were collected at admission in tubes containing EDTA
and then immediately analyzed for neutrophil and lymphocyte
count. Serum samples were separated from blood cells after
the collection in tubes without anticoagulant and stored at
−80°C until interleukin (IL)-6 and myeloperoxidase (MPO)
measurements by Human Magnetic Luminex Assay on Biorad
Bio-Plex 100 system (Labospace s.r.l., Milan, Italy).

Continuous data were reported as mean and standard error
(SE). Comparisons between two groups were performed by
Mann-Whitney U-test. Statistical differences between three
groups were assessed by ANOVA test and Bonferroni test as
d-post-hoc test. Categorical data were reported as frequency
and percentage. The chi-square test was used to compare
the frequency of categorical variables between groups. To test the
association of neutrophils, lymphocytes and NLR values vs.
age, steroids and azithromycin therapies, a linear regression
analysis with neutrophils, lymphocytes and NLR as dependent
variables was performed using a stepwise approach. Statistical
analyses have been evaluated by SPSS (version 26, IBM SPSS
Statistics). Graphics have been performed by KaleidaGraph
software (version 4.5.4, Synergy, Reading, PA, USA). P-values <
0.05 were considered as significant.

RESULTS

Figure 1 shows the data of NLR in the 49 COVID-19 patients
of the Ist wave and in the 242 COVID-19 patients of the IInd
wave, classified on the basis of the WHO score. Table 1 reports
either the comparison of the data between the WHO subgroups
of patients of the same wave, and the comparison of patients
of the two waves bearing to the same WHO subgroup. Among
the patients of the Ist wave, the NLR was significantly higher in
### TABLE 1 | Comparison of age and laboratory parameters in 49 COVID-19 patients of I\textsuperscript{st} wave and 242 patients of II\textsuperscript{nd} wave at admission with different severity according to worst WHO stage for each patient.

| Wave   | WHO 3 | WHO 4 | WHO 5–7 | ANOVA |
|--------|-------|-------|---------|-------|
| \(N\)  | I\textsuperscript{st} | 14    | 19      | 16    | –     |
|        | II\textsuperscript{nd} | 60    | 127     | 55    | –     |
| \(\text{Age (years)}\) I\textsuperscript{st} | 48.8 (3.5) | 60.4 (3.5)\textsuperscript{a} | 66.3 (3.6)\textsuperscript{b} | *     |
|        | II\textsuperscript{nd} | 38.9 (1.9) | 56.7 (1.4)\textsuperscript{a} | 58.8 (2.0)\textsuperscript{a} | **    |
| \(\text{IL-6 (pg/mL)}\) I\textsuperscript{st} | 168 (55) | 399 (179) | 703 (401) | n.s.  |
|        | II\textsuperscript{nd} | 38 (5) | 60 (24) | 41 (7) | n.s.  |
| \(\text{MPO (ng/mL)}\) I\textsuperscript{st} | 624 (118) | 785 (71) | 917 (83)\textsuperscript{b} | n.s.  |
|        | II\textsuperscript{nd} | 389 (19) | 322 (23) | 350 (15) | n.s.  |
| \(\text{Neutrophils (N/mmc)}\) I\textsuperscript{st} | 4514 (560) | 5255 (887) | 9130 (1523)\textsuperscript{b} | *     |
|        | II\textsuperscript{nd} | 5790 (324) | 6332 (247) | 7685 (426)\textsuperscript{b} | n.s.  |
| \(\text{Lymphocytes (N/mmc)}\) I\textsuperscript{st} | 2082 (284) | 1118 (98)\textsuperscript{a} | 770 (169)\textsuperscript{a} | **    |
|        | II\textsuperscript{nd} | 1425 (97) | 1246 (63) | 1229 (117) | n.s.  |
| \(\text{NLR}\) I\textsuperscript{st} | 2.9 (0.6) | 5.6 (0.9)\textsuperscript{a} | 17.8 (5.5)\textsuperscript{a} | **    |
|        | II\textsuperscript{nd} | 5.0 (0.4) | 7.1 (0.6) | 8.5 (1.0) | n.s.  |

\(p < 0.01; \quad \text{\textsuperscript{a}}p < 0.001.\)

\(\text{\textsuperscript{a}}p < 0.01, \text{WHO 4 vs. WHO 3.}\)

\(\text{\textsuperscript{b}}p < 0.01, \text{WHO 5–7 vs. WHO 3.}\)

\(\text{\textsuperscript{c}}p < 0.01, \text{WHO 5–7 vs. both WHO 3 and WHO 4.}\)

NLR: neutrophil/lymphocyte ratio; n.s.: not significant. Mean and standard error (SE).

### TABLE 2 | Steroids and/or azithromycin therapies before hospitalization in II\textsuperscript{nd} wave patients.

| Subgroups       | Not treated | Only steroids | Only azithromycin | Steroids and azithromycin |
|-----------------|-------------|---------------|-------------------|---------------------------|
| WHO 3           | 48 (80)     | 5 (8)         | 1 (2)             | 6 (10)                    |
| WHO 4           | 27 (21)\textsuperscript{a} | 24 (19)       | 7 (6)             | 69 (54)\textsuperscript{a} |
| WHO 5–7         | 12 (22)\textsuperscript{a} | 10 (18)       | 4 (7)             | 29 (53)\textsuperscript{a} |

\(\text{\textsuperscript{a}}p < 0.0001, \text{vs. WHO 3, N \%}.\)

WHO 5–7 than both WHO 3 and WHO 4 subgroups due to: (i) the number of lymphocytes that was significantly lower and the number of neutrophils that was higher in WHO 5–7 than both WHO 3 and WHO 4 patients. While, in patients of the II\textsuperscript{nd} wave the NLR values were not statistically different among the WHO subgroups. This depends on the number of either neutrophils and lymphocytes that was not different among the WHO subgroups.

Furthermore, the values of IL-6 resulted higher in all 49 patients of the I\textsuperscript{st} wave, with an increase (although not significant) with the WHO stage, while in patients of the II\textsuperscript{nd} wave the values of MPO were significantly lower in each WHO subgroup as compared to the corresponding subgroup of patients of the I\textsuperscript{st} wave, with no differences between patients of different WHO stages.

Among the II\textsuperscript{nd} wave, 155/242 patients performed steroids and/or azithromycin therapies before hospitalization differently from all patients of the I\textsuperscript{st} wave that were hospitalized soon after diagnosis. As reported in Table 2, a significant higher percentage of patients treated with both steroids and azithromycin was observed in WHO 4 and WHO 5–7 subgroups as compared to WHO 3. Therefore, we evaluated the associations of neutrophils, lymphocytes and NLR vs. the age, steroids and azithromycin therapies in each WHO subgroup (Table 3). Linear regression analysis revealed that only lymphocyte number was independently related to the age in WHO 3 and WHO 5–7.
subgroups. In addition, we found that neutrophil number and NLR were positively related to steroids therapy in WHO 4 subgroup as well as lymphocytes in WHO 3. While, no significant relation was observed for azithromycin, as independent variable.

**DISCUSSION**

We found an increase of NLR values in 49 patients with COVID-19 of the I\textsuperscript{st} wave, with a significant increase of the index in parallel with the WHO disease severity, that depends on the gradual increase of neutrophils and on the gradual decrease of lymphocytes in WHO 5–7 patients (11). These evidences fully agree with previous studies (3), among which that of Qin et al. (15) which included more than 400 COVID patients. On the other hand, the cytokine storm that occurs in patients with severe COVID-19, confirmed by the high serum levels of IL-6 in our patients of the I\textsuperscript{st} wave, contributes to the lymphocyte exhaustion (11).

Surprisingly, when we analyzed the NLR in 242 patients with COVID-19 of the II\textsuperscript{nd} wave, we did not observe any difference of the ratio between patients of different WHO stage. Furthermore, we also lack to observe any difference in the number of lymphocytes and neutrophils between patients of different WHO stages. These differences between the patients of the two COVID-19 waves are not due to the younger age of the patients of the II\textsuperscript{nd} wave because the linear regression analysis showed that age did not significantly influence NLR and neutrophil within none of the three WHO subgroups although, a negative relation between lymphocytes number and age was observed in WHO 3 and WHO 5–7 subgroups. Thus, the differences between the two waves likely depend on the different therapies that the patients performed before hospitalization and thus before the sampling, as steroids therapy was the only independent variable related to NLR and neutrophils in the widest subgroup (WHO 4). In fact, all the 49 patients of the I\textsuperscript{st} wave were diagnosed as COVID-19 for symptoms followed by molecular analysis on nasopharyngeal swab (often completed 2 or 3 days after the sampling), and they were soon hospitalized after the result. Such patients were treated with antivirals that now we know to be less effective than hoped (12). While, most patients from the II\textsuperscript{nd} wave were diagnosed by molecular analysis performed when they were still asymptomatic, mostly because they had had a contact with a COVID patient and had been traced (12). In all cases, the result of the nasopharyngeal test was obtained within 1 day (thanks to the improvement of laboratory organization), and all the patients started to be treated several days before hospital admission. In our population study, 64% of patients of the II\textsuperscript{nd} wave assumed steroids (12) and/or azithromycin for several days before hospitalization despite the use of dexamethasone results in lower mortality only among COVID-19 patients who were receiving invasive mechanical ventilation (i.e., patients in advanced WHO stages) (16), while in patients not requiring respiratory support the immunosuppressive effects of glucocorticoids hamper antiviral responses (17).

However, the effects of such drugs observed in our patients of the II\textsuperscript{nd} wave, were: a slight reduction of neutrophil number, possibly due to a transient effect of steroids after a few days of therapy (18) combined to the same effect of azithromycin (19); a reduction of neutrophil activity due to steroids (20) demonstrated by the lower values of serum MPO; the inhibition of pro-inflammatory cytokines among which IL-6; and a consequent less severe lymphocyte exhaustion.

A study limitation is represented by the relatively small number of patients of I\textsuperscript{st} wave in comparison to II\textsuperscript{nd} wave. Further studies need to confirm our findings as a recent work documents NLR predictive value on both I\textsuperscript{st} and II\textsuperscript{nd} waves (21). However, at the state of the art, there is not a clear consensus on the role of such drugs, frequently used friendly without medical supervision (22) and on their impact on COVID-19 patients. In any case, the present data suggest that such therapies impair the use of NLR as a marker of outcome and disease severity.

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**TABLE 3 | Linear regression analysis in II\textsuperscript{nd} wave COVID-19 patients.**

|                | Age | Steroids | Azithromycin |
|----------------|-----|----------|--------------|
| Neutrophils    |     |          |              |
| WHO 3          | −0.209 | 0.055 | 0.180 | 0.086 |
| WHO 4          | 0.014 | 0.438 | 0.287 |
| WHO 5–7        | 0.105 | 0.219 | 0.475 |
| Lymphocytes    |     |          |              |
| WHO 3          | −0.225 | 0.042 | 0.213 | 0.051 |
| WHO 4          | −0.097 | 0.138 | 0.479 | −0.034 | 0.354 |
| WHO 5–7        | −0.313 | 0.010 | 0.126 | −0.163 | 0.117 |
| NLR            |     |          |              |
| WHO 3          | 0.097 | 0.231 | 0.025 | 0.424 |
| WHO 4          | 0.100 | 0.131 | 0.034 | 0.354 |
| WHO 5–7        | 0.164 | 0.115 | 0.058 | 0.336 |

Significant values are reported in bold.
in COVID-19 patients, and its use should be limited to naïve patients before starting potential interfering therapies.

**DATA AVAILABILITY STATEMENT**

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

**ETHICS STATEMENT**

The studies involving human participants were reviewed and approved by Ethical Committee of the University Federico II of Naples. The patients/participants provided their written informed consent to participate in this study.

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**AUTHOR CONTRIBUTIONS**

GCa, GF, IG, and RP: design of the work, manuscript writing, and validation. MG, SC, BP, AD, GCe, FS, and MM: methodology, investigation, and data analysis. All authors read and approved the final manuscript.

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