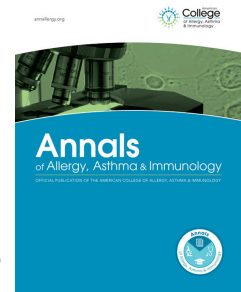


# Journal Pre-proof

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Monitoring Neutrophil to Lymphocyte Ratio in COVID-19 Patients  
Receiving Tocilizumab

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1 Abbreviations

2 CRP–C-reactive protein

3 NLR–neutrophil to lymphocyte ratio

4 NET–neutrophil extracellular traps

5

The novel coronavirus disease (COVID-19) is an infectious disease caused by SARS-CoV-2, an enveloped non-segmented positive-sense RNA  $\beta$ -coronavirus. The virus can trigger a cascade of biological events that can lead to critical illness. It is urgent to gain an in-depth understanding of the critical activators that foreshadow patients highest risk for mortality.(1) Patients who have received tocilizumab for COVID-19 related cytokine storm have had improvements in c-reactive protein (CRP), lymphocytosis, and chest CT findings.(2) There exist a lack of understanding on which patients would benefit from Tocilizumab administration. We aimed to characterize the group of patients that received Tocilizumab and failed to improve, needing mechanical ventilation, or suffering mortality. We studied clinical and laboratory variables in patients receiving Tocilizumab; comparing those that did not require mechanical ventilation to those needing mechanical ventilation including those that suffered mortality.

Adult patients with PCR-confirmed COVID-19 infection treated with tocilizumab at Spectrum Health System Hospitals from March 20-May 18 were studied. Laboratory indices analyzed were metabolic panel, blood count, CRP, ferritin, fibrinogen, d-dimer, lactate dehydrogenase, and triglycerides. Our Institutional protocol dictated that patients received tocilizumab when they required at least 4 liters of oxygen or

more respiratory support and <14 days of symptoms. Tocilizumab dosing was: 400 mg for 50-60 kg, 600 mg for 60-85 kg and 800 mg for >85 kg. Doses could be given every 8 hours, for a maximum of 3 doses. Day 0 was defined as the day tocilizumab was started. Cytokine panels were performed on day 0. Patients were followed for maximum of 30 days until they met the endpoint of hospital discharge or mortality.

Quantitative data was expressed as mean±SD, whereas nominal data as percentages. The groups studied (intubated vs. non-intubated or survivors vs. deceased) were analyzed using the unpaired two-tailed t-test, while difference for nominal data were determined using the chi-square test or the Fisher's Exact test. In addition, mixed effects general linear modeling was performed. The independent variables were days following tocilizumab administration (Day -1, 0, 1 and 3; reference value: Day 0), and either intubation status or mortality.

For all analyses, significance was assessed at  $p < 0.05$ . All analyses were performed using Stata v.16.1 (StataCorp, College Station, TX).

Baseline characteristics revealed more type 2 diabetes in intubated patients and more smokers in the deceased group (table 1). Neutrophil-to-lymphocyte ratio (NLR) increased from day -1 to 0 in intubated patients ( $p=0.057$ ) and NLR showed a decrease

in the non-intubated group from 6.9 to 3 compared to 6.8 to 6.7 from day 0 to 3 in the intubated group. Similarly, in survivors, there was a significant decrease in NLR from day 0 to day 3 from 7.1 to 3.3 (51.2% decrease) whereas in non survivors an increase from 5 to 6.8 (33.6% increase). In all groups, mean absolute neutrophil count was highest on day 0. In non-survivors day 0 ALC was 34.1% lower ( $p=0.046$ ). No significant interactions were seen in NLR from day -1 to day 0 in all groups.

Only IL-10 and IL-6 were higher in intubated patients compared to not-intubated patients (20.4 pg/mL versus 9.5,  $p=0.001$ ; 26.5 pg/mL versus 10.5,  $p=0.002$ ); there was no difference in sIL2r, IL-10, and IL-6 in patients who survived compared to deceased. A moderate correlation was observed between day 0 NLR and IL-6 ( $\rho=0.307$ ;  $p=0.021$ ) and between day 0 NLR and CRP ( $r=0.326$ ;  $p<0.001$ ). No correlation was observed between day 0 NLR and sIL2r, IL-10, or ferritin.

Studies have shown an increased NLR to be an independent risk factor for severity in patients with COVID-19 infections. This is congruent with mounting evidence on importance of neutrophil response in severe COVID-19. While non-specific, existing cohorts have shown onetime  $NLR>3.13$  and interquartile NLR range of 5.82-15.1 to be predictive of critical illness, and

76 persistent elevation correlated with death.(3, 4) The mean  
77 ratio dropped between day 0 and 3 across all groups except in  
78 the patients suffering mortality. Further, NLR correlated with  
79 IL-6 levels and may represent a proxy to monitor response to IL-  
80 6 blockade.

81 Beyond neutrophilia, neutrophil activation and degranulation are  
82 the most biologically active processes in severe COVID-19.(5)  
83 Increased activated neutrophil extracellular traps (NETs) is  
84 associated with severity of illness in patients with severe  
85 COVID-19.(6, 7) NET activation contributes to neutrophilia in  
86 COVID-19 simultaneously increasing IL-1 $\beta$  and IL-6, giving  
87 biologic plausibility to their respective blockade being  
88 therapeutic in severe COVID-19.(6, 7)

89 The patients that seem to have a more favorable clinical course  
90 after Tocilizumab are the same patients that showed a drop in  
91 neutrophil levels. This is true for other biological conditions  
92 in which patients receiving Tocilizumab have shown clinical  
93 improvement which correlated with a drop in neutrophil count  
94 during therapy.(8, 9). The elevated IL-6 and IL-10 and  
95 persistently elevated NLR in the deceased group indicate that  
96 further studies may be needed to determine if the elevated  
97 levels suggest an advanced disease state resistant to  
98 Tocilizumab therapy. We posit Immunophenotyping such differences

in prospective trials will be imperative to individualize treatment for these patients.

Limitations of our cohort include its retrospective nature and smaller number of patients. Because all patients in the cohort received tocilizumab due to the severe nature of their illness, we are unable to make a statement on clinical effectiveness of tocilizumab. We instead focused on biologic markers that could be indicative of disease severity and death. Our study period encompassed a time of varying hydroxychloroquine use, and dexamethasone was not widely used, however there was no baseline difference between the groups.

This report suggests that persistently elevated NLR is associated with unfavorable outcomes, but it is unclear whether COVID-19 disease, secondary infections, or other causes are driving elevated NLR in patients with more severe disease. We suggest that an elevated NLR is one potential marker. Further prospective studies are needed to show performance of NLR versus or in combination with other markers (CRP, complement components, cytokines, etc). NLR should be considered as a marker COVID-19 that is resistant to anti-inflammatory therapy with need for infection monitoring and need for aggressive life support measures.

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**Table 1**

|                             | Intubation       |                  |       | Mortality        |               |        |
|-----------------------------|------------------|------------------|-------|------------------|---------------|--------|
|                             | No<br>(n=34)     | Yes<br>(n=32)    | p     | No<br>(n=54)     | Yes<br>(n=12) | p      |
| Age (Mean)                  | 56.1±15.2        | 57.2±11.0        | 0.745 | 55.2±13.4        | 63.3±10.6     | 0.053  |
| Gender (%)                  |                  |                  | 0.569 |                  |               | 0.512  |
| Male                        | 20 (58.8%)       | 21 (65.6%)       |       | 32 (59.3%)       | 9 (75.0%)     |        |
| Female                      | 14 (41.2%)       | 11 (34.4%)       |       | 22 (40.7%)       | 3 (25.0%)     |        |
| Race/Ethnicity              |                  |                  | 0.414 |                  |               | 0.463  |
| Caucasian                   | 11 (32.4%)       | 13 (40.6%)       |       | 18 (33.3%)       | 6 (50.0%)     |        |
| African American            | 4 (11.8%)        | 3 (9.4%)         |       | 5 (9.3%)         | 2 (16.7%)     |        |
| Hispanic                    | 14 (41.2%)       | 15 (46.9%)       |       | 25 (46.3%)       | 4 (33.3%)     |        |
| Asian                       | 5 (14.7%)        | 1 (3.1%)         |       | 6 (11.1%)        | 0 (0%)        |        |
| BMI (Mean)                  | 29.7±5.0         | 31.4±7.2         | 0.259 | 30.1±5.4         | 32.2±9.2      | 0.304  |
| GFR > 60 (N)                | 28/31<br>(90.3%) | 26/31<br>(83.9%) | 0.707 | 45/50<br>(90.0%) | 9<br>(75.0%)  | 0.177  |
| Diabetic                    | 7 (20.6%)        | 18 (56.3%)       | 0.003 | 19 (35.2%)       | 6 (50.0%)     | 0.348  |
| Smoking History             | 9 (26.5%)        | 15 (46.9%)       | 0.085 | 13 (24.1%)       | 11<br>(91.7%) | <0.001 |
| Hypertension                | 15 (44.1%)       | 21 (65.6%)       | 0.079 | 26 (48.2%)       | 10<br>(83.3%) | 0.051  |
| Tocilizumab Dose (mg)       | 688±112          | 688±113          | 0.979 | 693±108          | 667±130       | 0.471  |
| Number of Tocilizumab Doses |                  |                  | 0.147 |                  |               | 0.659  |
| 1                           | 1 (2.9%)         | 0 (0%)           |       | 1 (1.9%)         | 0 (0%)        |        |
| 2                           | 5 (14.7%)        | 1 (3.1%)         |       | 6 (11.1%)        | 0 (0%)        |        |
| 3                           | 28 (82.4%)       | 31 (96.9%)       |       | 47 (87.0%)       | 12<br>(100%)  |        |
| Additional Therapies        |                  |                  |       |                  |               |        |
| Convalescent Plasma         | 11 (32.4%)       | 14 (43.8%)       | 0.340 | 20 (37%)         | 5 (41.7%)     | 0.754  |
| Remdesivir                  | 0                | 1 (3.1%)         | 0.485 | 0                | 1 (8.3%)      | 0.182  |
| Hydroxychloroquine          | 20 (58.8%)       | 18 (56.3%)       | 0.833 | 33 (61%)         | 5 (41.7%)     | 0.333  |
| Corticosteroids             | 5 (14.7%)        | 9 (28.1%)        | 0.183 | 11 (20.4%)       | 3 (25%)       | 0.708  |
| Infections                  |                  |                  |       |                  |               |        |
| Positive sputum cultures    |                  |                  |       | 11 (20.2%)       | 8 (66.7%)     | <0.001 |
| Candida in sputum           |                  |                  |       | 2 (3.7%)         | 5 (41.7%)     | <0.001 |