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Prognostic role of Interleukin-6/lymphocytes ratio in SARS-CoV2 related pneumonia

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ABSTRACT

Introduction and aim: Interleukin-6 to lymphocyte (IL-6/Lym) ratio has been identified as a potential prognostic tool in patients with SARS-CoV2 related pneumonia. The aim of our study was to compare the prognostic power of IL-6/Lym ratio with other biomarkers in patients initially admitted in a non intensive unit and suffering for respiratory failure associated with SARS-CoV2 related pneumonia.

Materials and methods: IL-6/Lym ratio, IL-6, D-Dimer, D-Dimer/fibrinogen ratio, fibrinogen, C-reactive protein (CRP), lymphocytes count and neutrophil/lymphocyte (N/L) ratio collected at hospital admission were tested as prognosticators of negative outcome, defined as combined endpoint in-hospital mortality and/or Intensive Care Unit (ICU) admission requiring oro-tracheal intubation (OTI).

Results: Study population encompassed two hundreds and twenty-three patients (46% females) with mean age \pm DS 69.4 \pm 13.3 years. Eighty-nine patients (39.9%) suffered for severe respiratory failure and required non invasive ventilation, helmets and/or high flow nasal cannula. Forty-one patients (18.3%) died during hospital stay and/or required OTI. In these patients mean values of IL-6/Lym ratio, IL-6, CRP and N/L were significantly higher and lymphocytes count was significantly lower compared with patients discharged alive and/or not requiring OTI, while no difference was found in mean values of D-Dimer, D-Dimer/Fibrinogen ratio and fibrinogen. AUC (0.797, 95% CI: 0.738–0.848) of IL-6/Lym ratio was the highest compared with those of all the other analyzed biomarkers and the difference was significant with the exception of IL-6. At multivariate logistic regression IL-6/Lym ratio $>$ 66.5 resulted the only independent biomarker associated with mortality and/or OTI (OR 5.65; 95% 1.63–19.54).

Conclusion: IL-6/Lym ratio seems to be an optimal prognosticator in SARS-CoV2 related pneumonia. Its routine use in COVID-19 patients could be warranted.

1. Introduction

Cytokine storm represents one of the main determinant of progression and deterioration in SARS-CoV2 related pneumonia [1]. Lymphopenia, thrombocytopenia, and elevated levels of interleukin-6 (IL-6),

ferritin, D-dimer, aspartate aminotransferase, C-Reactive-Protein (CRP), procalcitonin, creatinine, neutrophils and leucocytes were associated with severe and fatal COVID-19 cases [2]. Recently, Yang B. et al reported on the role of interleukin-6 to lymphocytes (IL-6/Lym) ratio as prognosticator in severe COVID-19 patients [3]. In their paper the

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Table 1
Comparison of immune, inflammatory and coagulative biomarkers between groups.

| Biomarkers | Died and/or ICU admission with oro-tracheal intubation 41 patients | Alive and/or not requiring ICU with oro-tracheal intubation 182 patients | p |
|---------------------------------------|--|--|--------|
| IL-6/Lym ratio | 811 ± 2878 | 79 ± 161 | 0.0007 |
| IL-6 (pg/ml) | 396 ± 1085 | 54 ± 91 | 0.0001 |
| CRP (ng/mL) | 10.4 ± 5.7 | 7.1 ± 5.6 | 0.0008 |
| D-Dimer (microg/L) | 2167 ± 3590 | 2664 ± 9517 | 0.7427 |
| D-Dimer/Fibrinogen ratio | 3.61 ± 5.70 | 5.2 ± 22.4 | 0.6528 |
| Fibrinogen (mg/dL) | 663.3 ± 179.2 | 668.1 ± 165.8 | 0.8632 |
| Lymphocytes count 10 ³ /μL | 0.73 ± 0.35 | 0.95 ± 0.44 | 0.0031 |
| Neu/Lym ratio | 11.6 ± 8.9 | 7.7 ± 6.9 | 0.0023 |
| Platelets/Lym ratio | 319.7 ± 188.7 | 290.2 ± 204.8 | 0.4053 |
| Lym/CRP ratio | 0.11 ± 0.14 | 0.71 ± 2.32 | 0.0999 |

Legend: Lym = lymphocytes count; CRP = C reactive protein; IL-6 = interleukin 6.

Authors found that IL-6/Lym ratio was associated with an increased risk of in-hospital mortality and multiple organ dysfunction syndrome (MODS) with a hazard ratio on multivariate analysis of 3.4 (95% CI: 1.0–10.6) and 4.1 (95% CI:1.3–12.9) respectively [3]. Areas under receiving operating curves (AUCs) for IL-6/Lym ratio as predictive factor for in-hospital mortality and MODS were 0.919 (95% CI: 0.887–0.951) and 0.900 (95% CI: 0.863–0.937) respectively. Moreover, the Authors found a cut off of 2.50 in IL-6/Lym ratio as discriminant for defining low (<2.50) and high (≥2.50) risk patients. Interestingly, in the paper of Yang B. et al the IL-6/Lym ratio seems to be the best prognosticator of poor prognosis between demographic and co-morbidity data, and a lot of blood routine, biochemical, coagulation and immune-inflammatory indices [3]. To date, no external validation and/

or other literature evidence about the predictive power of IL-6/Lym ratio in COVID-19 patients has been reported, therefore the aim of this study was to report our experience about the prognostic role of IL-6/Lym ratio in patients initially admitted in a non intensive unit and suffering for respiratory failure associated with SARS-CoV2 related pneumonia.

2. Materials and methods

We retrospectively analyzed clinical and laboratory data of consecutive patients admitted in a non intensive unit for respiratory failure associated with SARS-CoV2 related pneumonia during the first and second wave of pandemic in Italy (first wave March-April 2020, second wave October-November 2020). We tested the predictive role of IL-6/Lym ratio as risk factor for negative outcome compared with IL-6, D-Dimer, D-Dimer/fibrinogen ratio, fibrinogen, CRP, lymphocytes count and neutrophil/lymphocyte (Neu/Lym) ratio, platelets/Lym ratio and Lym/CRP ratio collected at hospital admission. Negative outcome was defined as combined endpoint of in-hospital mortality and/or Intensive Care Unit (ICU) admission requiring oro-tracheal intubation (OTI). Quantitative determination of IL-6 levels was performed by using an immunoenzymatic chemiluminescent assay (Access Immunoassay System, Beckman Coulter, USA, lowest limit of detection 0.5 pg/mL).

For statistical analysis continuous variables were reported as mean ± standard deviation (SD). Categorical variables were analyzed by using the Fisher exact test. Multivariate logistic regression analysis was used to estimate Odds Ratios of biomarkers resulted significantly different when compared between patients died and/or required OTI and not; ORs and their 95th percentile confidence intervals (CI) were reported. ORs were calculated at biomarkers values associated with the best sensitivity and specificity according to Youden index. To evaluate the predictive power of biomarkers, the areas under the curve (AUC) of the Receiver Operating Characteristic (ROC) curve was calculated. Difference between AUCs was reported. A p value of < 0.05 was considered statistically significant. All analyses were performed using MEDCALC statistical software (MedCalc Software Ltd, Acacialaan 22, B-8400 Ostend, Belgium).

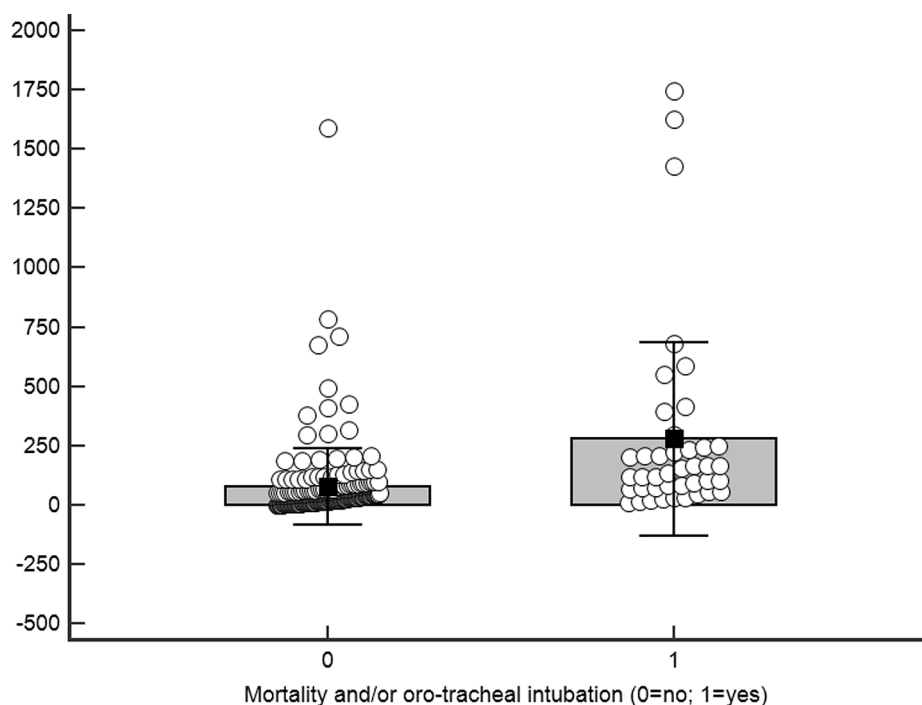


Fig. 1. Scattering graph showing the difference between IL and 6/Lym values in patients died and/or undergone to oro-tracheal intubation and discharged alive and/or not undergone to orto-tracheal intubation.

Table 2
Predictive power of biomarkers as prognosticator of death and/or ICU admission with oro-tracheal intubation.

| Variable | AUC | 95% CI | Criterion associated with best sensitivity* and specificity** at Youden index | Sensitivity* | Specificity** |
|---|-------|----------------|---|--------------|---------------|
| IL-6/Lym ratio | 0,797 | 0,738 to 0,848 | > 66.5 | 78.05% | 73.08% |
| IL-6 (pg/mL) | 0,772 | 0,711 to 0,826 | > 42 | 80.4% | 62.6% |
| Lym/CRP ratio | 0.700 | 0.635 to 0.759 | <0.11 | 78.05% | 62.09% |
| Neu/Lym ratio | 0,671 | 0,605 to 0,732 | >6.8 | 65.8% | 62.09% |
| CRP (ng/mL) | 0,669 | 0,602 to 0,730 | >7.6 | 68.2% | 62.09% |
| Lymphocytes count (10 ³ /μL) | 0.642 | 0.575 to 0.705 | <1.05 | 85.3% | 37.9% |
| D-Dimer/Fibrinogen ratio | 0,607 | 0,540 to 0,672 | >1.2 | 73.1% | 49.4% |
| D-Dimer (microg/L) | 0,586 | 0,518 to 0,652 | >726 | 73.1% | 48.9% |
| Platelets/Lym ratio | 0.559 | 0.491 to 0.625 | >328 | 40.0% | 72.5% |
| Fibrinogen (mg/dL) | 0.518 | 0.450 to 0.585 | >496 | 92.6% | 20.0% |

Legend: Lym = lymphocytes count; CRP = C reactive protein; IL-6 = interleukin 6.

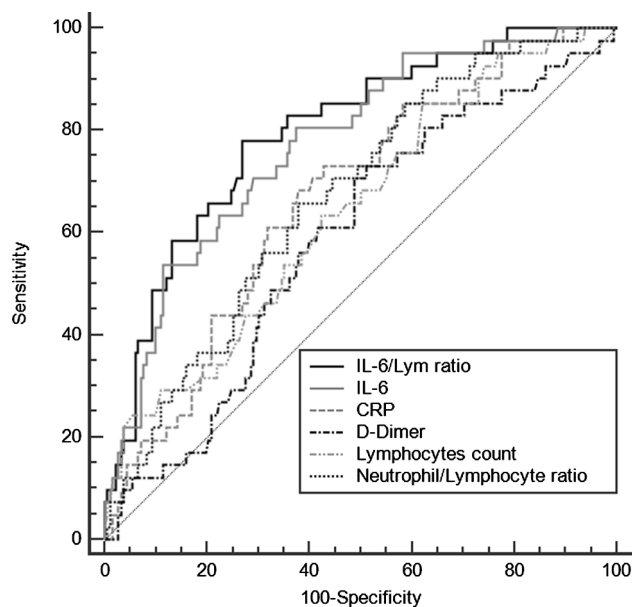


Fig. 2. Area under the receiving operating curves of biomarkers as prognosticators of in-hospital mortality and/or oro-tracheal intubation.

Table 3
Difference between AUC* of IL-6/Lym ratio as prognosticator of mortality and/or oro-tracheal intubation and AUCs of other biomarkers.

| | Difference between AUCs | 95% CI | p |
|--------------------------|-------------------------|---------------------|---------------|
| IL-6 | 0.0235 | -0.0109–0.0579 | 0.1800 |
| Lym/CRP ratio | 0.094 | 0.0106–0.179 | 0.0273 |
| CRP | 0.129 | 0.0312–0.226 | 0.0097 |
| Lymphocytes count | 0.155 | 0.0641–0.247 | 0.0008 |
| D-Dimer | 0.208 | 0.100–0.316 | 0.0002 |
| D-Dimer/Fibrinogen ratio | 0.189 | 0.0711–0.306 | 0.0017 |
| Fibrinogen | 0.278 | 0.179–0.377 | <0.0001 |
| Neus/Lym ratio | 0.127 | 0.0333–0.220 | 0.0078 |
| Platelets/Lym ratio | 0.236 | 0.131–0.340 | <0.0001 |

*AUC IL-6/Lym ratio 0.797 (95% CI: 0.738–0.848).

Legend:Lym = lymphocytes count; CRP = C reactive protein; IL-6 = interleukin 6.

Table 4
Logistic regression multivariate analysis.

| Variable | Odds ratio | 95% CI |
|---|---------------|--------------------------|
| IL-6/Lym ratio (>66.5) | 5,6573 | 1,6378 to 19,5414 |
| CRP (>7.6 ng/mL) | 1,5974 | 0,6717 to 3,7990 |
| IL-6 (>42 pg/mL) | 1,4413 | 0,4316 to 4,8132 |
| Neu/Lym ratio (>6.8) | 1,0334 | 0,4076 to 2,6201 |
| Lymphocytes count (<1.05 10 ³ /μL) | 0,7571 | 0,2410 to 2,3781 |

Legend: Lym = lymphocytes count; CRP = C reactive protein; IL-6 = interleukin 6.

3. Results

Two hundreds and twenty-three patients (46% females) with mean age ± DS 69.4 ± 13.3 years composed the study population. Eighty-nine patients (39.9%) suffered for severe respiratory failure and required non invasive ventilation, helmets and/or high flow nasal cannula (HFNC). Forty-one patients (18.3%) died during hospital stay and/or required OTI (in-hospital mortality 15.2%, OTI 6.2%). In these patients mean values of IL-6/Lym ratio, IL-6, CRP and N/L were significantly higher and lymphocytes count was significantly lower compared with patients discharged alive and/or not requiring OTI, while no difference was found in mean values of D-Dimer, D-Dimer/Fibrinogen ratio, fibrinogen, platelets/Lym ratio and Lym/CRP ratio (Table 1, Fig. 1 for IL-6/Lym ratio). Predictive power for poor prognosis of IL-6/Lym ratio was the highest compared with those of all the other analyzed biomarkers with an AUC of 0.797 (95% CI: 0.738–0.848) (Table 2, Fig. 2). AUC for IL-6/Lym ratio as prognosticator of negative outcome was significantly higher compared with those of all the other biomarkers with the exception of IL-6 (Table 3). At multivariate logistic regression IL-6/Lym ratio > 66.5 resulted the only independent biomarker associated with mortality and/or OTI with an OR of 5.65 (95% 1.63–19.54) (Table 4).

4. Discussion

Assessing the prognosis of patients with respiratory failure associated with SARS-CoV2 related pneumonia is fundamental in clinical practice. Cytokine storm represents the cornerstone of SARs-CoV2 related pneumonia and maybe it's the major determinant of poor prognosis and a potential target of pharmacological treatment [4]. IL-6 plays a pivotal role on inflammation with a pleiotropic effect on liver, B cells, T cells, monocytes and platelets. In response to acute infection, IL-6 stimulates acute phase reactants, immune response and hematopoiesis [5]. Despite the exact pathophysiology mechanism remains unclear, much literature evidence shows that high levels of IL-6 and low lymphocytes count are biomarkers of cytokine storm and immune response in COVID-19 patients and their prognosis [6]. However the predictive

power of these biomarkers is evaluated singularly. Combining two biomarkers by using the ratio between them could increase their predictive power. In COVID-19 patients lymphocytes count has been included in a lot of ratios with other biomarkers for testing outcomes such as Neu/Lym ratio [7], platelets/Lym ratio [8], Lym/CRP ratio [9], lymphocyte to monocyte ratio [10], eosinophil to lymphocyte ratio [11]. As abovementioned, recently IL-6/Lym ratio has been proposed as an interesting prognosticator in patients with COVID-19. In the present study, we confirm the findings of Yang B. et al [3] showing that IL-6/Lym has high power to predict negative outcome in patients with respiratory failure associated with SARS-CoV2 infection. Of note, we found that compared with other biomarkers, IL-6/Lym has the best predictive power and that between the analyzed biomarkers it's the only independent risk factor for mortality and/or OTI increasing the risk of more than five-fold.

We recognize that our study has limitations, main of these due to retrospective design, relative small sample size and single center. However our study could add information about the knowledge of prognosis of COVID-19 patients and important relapse for clinical practice.

In conclusion, in our study IL-6/Lym ratio seems to predict better than IL-6 and lymphocytes count alone and other inflammatory and coagulative biomarkers. Its routine use as prognosticator in COVID-19 patients could be warranted.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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infection.

References

- [1] P. Mehta, D.C. Fajgenbaum, Is severe COVID-19 a cytokine storm syndrome: a hyperinflammatory debate, *Curr. Opin. Rheumatol.* 33 (5) (2021) 419–430, <https://doi.org/10.1097/BOR.0000000000000822>.
- [2] A.K.G. Melo, K.M. Milby, A.L.M.A. Caparroz, et al., Biomarkers of cytokine storm as red flags for severe and fatal COVID-19 cases: A living systematic review and meta-analysis, *PLoS One* 16 (6) (2021), <https://doi.org/10.1371/journal.pone.0253894>.
- [3] B. Yang, X. Chang, J. Huang, W. Pan, Z. Si, C. Zhang, H. Li, The role of IL-6/lymphocyte ratio in the peripheral blood of severe patients with COVID-19, *Int. Immunopharmacol.* 97 (2021) 107569, <https://doi.org/10.1016/j.intimp.2021.107569>.
- [4] L. Tang, Z. Yin, Y. Hu, H. Mei, Controlling Cytokine Storm Is Vital in COVID-19, *Front. Immunol.* 11 (2020) 570993, <https://doi.org/10.3389/fimmu.2020.570993>.
- [5] L. Velazquez-Salinas, A. Verdugo-Rodriguez, L.L. Rodriguez, M.V. Borca, The Role of Interleukin 6 During Viral Infections, *Front. Microbiol.* 10 (2019) 1057, <https://doi.org/10.3389/fmicb.2019.01057>.
- [6] C. Pelaia, C. Tinello, A. Vatrella, G. De Sarro, G. Pelaia, Lung under attack by COVID-19-induced cytokine storm: pathogenic mechanisms and therapeutic implications, *1753466620933508*, *Ther. Adv. Respir. Dis.* 14 (2020), <https://doi.org/10.1177/1753466620933508>.
- [7] J.R. Ulloque-Badaracco, W. Ivan Salas-Tello, A. Al-kassab-Córdova, E.A. Alarcón-Braga, V.A. Benites-Zapata, J.L. Maguiña, A.V. Hernandez, Prognostic value of neutrophil-to-lymphocyte ratio in COVID-19 patients: A systematic review and meta-analysis, *Int. J. Clin. Pract.* 75 (11) (2021), <https://doi.org/10.1111/ijcp.14596>.
- [8] M.A. Man, R.M. Rajnoveanu, N.S. Motoc, et al., Neutrophil-to-lymphocyte ratio, platelets-to-lymphocyte ratio, and eosinophils correlation with high-resolution computer tomography severity score in COVID-19 patients, *PLoS One* 16 (6) (2021), <https://doi.org/10.1371/journal.pone.0252599>.
- [9] W. Ullah, B. Basyal, S. Tariq, T. Almas, R. Saeed, S. Roomi, S. Haq, J. Madara, M. Boigon, D.C. Haas, D.L. Fischman, Lymphocyte-to-C-Reactive Protein Ratio: A Novel Predictor of Adverse Outcomes in COVID-19, *J. Clin. Med. Res.* 12 (7) (2020) 415–422, <https://doi.org/10.14740/jocmr4227>.
- [10] M. Seyit, E. Avcı, R. Nar, H. Senol, A. Yılmaz, M. Ozen, A. Oskay, H. Aybek, Neutrophil to lymphocyte ratio, lymphocyte to monocyte ratio and platelet to lymphocyte ratio to predict the severity of COVID-19, *Am. J. Emerg. Med.* 40 (2021) 110–114, <https://doi.org/10.1016/j.ajem.2020.11.058>.
- [11] V.E. Georgakopoulou, N. Garpis, C. Damaskos, et al., The Impact of Peripheral Eosinophil Counts and Eosinophil to Lymphocyte Ratio (ELR) in the Clinical Course of COVID-19 Patients: A Retrospective Study, *In Vivo.* 35 (1) (2021) 641–648, <https://doi.org/10.21873/invivo.12303>.