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Routine Biomarkers for the Severity of COVID-19 Pneumonia May Present Differently in Kidney Transplant Recipients

María Molina*, Elena Burgos, Judit Cacho, Javier Juega, Laura Cañas, Omar Taco, Ines Perezpaya, Marina Urrutia-Jou, Javier Paul-Martinez, Fredzzia Graterol, and Ricardo Lauzurica

Department of Nephrology, Hospital Universitari Germans Trias i Pujol, Badalona, Barcelona, Spain

ABSTRACT

Background. The treatment of coronavirus disease 2019 (COVID-19) is based on the patient’s clinical status and levels of inflammatory biomarkers. The comparative activity of these biomarkers in kidney transplant (KT) patients with COVID-19 pneumonia from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and non–SARS-CoV-2 etiologies is unknown. The aim of this study was to compare the clinical presentation and inflammatory parameters at admission of KT patients with COVID-19 pneumonia and those with non–COVID-19 pneumonia over the same period.

Methods. Biomarkers were measured and compared between KT patients with COVID-19 pneumonia (n = 57) and non-COVID-19 pneumonia (n = 20) from March 2020 to March 2021.

Results. Both groups showed comparable demographics. The KT patients with COVID-19 had fewer neutrophils (6824 ± 5000 vs 8969 ± 4206; P = .09) than the non-COVID group, although there was no significant difference in the lymphocyte count. Non–COVID-19 pneumonia was associated with higher D-dimer (median, 921 [interquartile range (IQR), 495-1680] vs median, 2215 [IQR, 879-3934]; P = 0.09) and interleukin-6 (median, 35 [IQR, 20-128] vs median, 222 [IQR, 38-500]; P = 0.006) levels. The ferritin level was higher in the COVID-19 group (median, 809 [IQR, 442-1,330] vs median, 377 [IQR, 276-885]; P = 0.008). In multivariable analysis, only D-dimer (hazard ratio [HR], 1; 95% confidence interval [CI], 1-1.002; P = .02) and ferritin (HR, 1; 95% CI, 0.9-0.9; P = .02) increase the statistic significance.

Conclusion. COVID-19 pneumonia in KT patients shows a different presentation of inflammatory biomarkers than other non-COVID pneumonias. It could be useful to identify KT patients with COVID-19. More detailed studies are necessary to understand the presentation of biomarkers in KT with COVID-19.

The pathogenesis of coronavirus disease 2019 (COVID-19) includes 2 different aspects: the tissue damage induced by the virus and the indirect effects caused by an amplified immune response and inflammation mediated by a cytokine storm. This second part is associated with the elevation of some inflammatory parameters such as ferritin, D-dimer, and C-reactive protein (CRP) as well as proinflammatory cytokines, such as interleukin (IL)-6, in the general population [1,2]. These parameters have been used as early and severity markers of COVID-19. Additionally, treatment with drugs such as tocilizumab, an IL-6-receptor blocker, is based on clinical progression and the measurement of these inflammatory markers in the general population.

The same management protocol has been applied to patients who have undergone kidney transplant (KT). Unfortunately, some of these molecules have not been routinely measured after non–COVID-19 infection in KT patients. Thus, their comparative values in severe acute respiratory syndrome coronavirus 2 infection are unknown.

Elena Burgos and Judit Cacho contributed equally to this work.

*Address correspondence to María Molina, PhD, Department of Nephrology, Hospital Universitari Germans Trias i Pujol, Carretera de Canyet S/N, Badalona, Barcelona 08916, Spain. E-mail: mmgmolina@hotmail.com

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(SARS-CoV-2) and non–SARS-CoV-2 pneumonias are unknown. Therefore, extrapolation of the same behavior or cut-off point as that of the immunocompetent population for starting treatment in such patients is debatable [3].

This study describes the clinical presentation and inflammatory parameters at admission in a cohort of KT patients with COVID-19 pneumonia and non–COVID-19 pneumonia over the same period.

MATERIALS AND METHODS

Patients

In this retrospective series of cases with non-aleatory randomization, we included all consecutive KT patients who presented with pneumonias and underwent at least 2 determinations of polymerase chain reaction (PCR) assay with nasal and/or pharyngeal swab specimens for SARS-CoV-2 between March 2020 and March 2021 at the Hospital Germans Trias i Pujol. PCR tests for SARS-CoV-2 are considered the gold standard for acute infection, and the patients were classified as COVID-19 positive or COVID-19 negative according to the PCR results. The demographic, clinical, laboratory, and radiologic variables were collected retrospectively from the patients’ medical records. All patients were followed up until death or discharge. This study was approved by the ethics committee of the hospital.

Statistical Analysis

Quantitative data were expressed as the mean (SD) and median with (interquartile range [IQR]); qualitative variables were expressed as absolute and relative frequencies. The quantitative and qualitative variables were compared using the nonparametric Mann-Whitney U test and χ² test, respectively. P < .05 was considered statistically significant. Uni- and multivariable logistic regression was made.

RESULTS

Patient Characteristics

This study included 77 KT patients with pneumonia who were tested for SARS-CoV-2 using PCR from March 2020 to March 2021. Of the 77 patients, 57 tested positive for COVID-19. The patient characteristics and clinical presentation are shown in Table 1. There were no differences in the baseline characteristics of the 2 groups, except that the COVID-19 group received a higher percentage of angiotensin-converting enzyme inhibitors or angiotensin receptor blockers (P = .05), and therefore showed lower proteinuria. There were no differences in immunosuppression regimens or renal function.

Patients With and Without COVID-19

The first PCR test confirmed COVID-19 in 53 of the 57 positive cases, since a negative test was initially obtained in 4 cases that tested positive after 72 hours. Among the 20 patients who did not have COVID-19, the PCR test was performed twice in 10 patients and 3 more times in 10 patients. Two patients tested positive for pneumococcal antigens in urine, 1 patient showed Enterococcus faecium in the bronchoalveolar lavage (BAL) fluid, and another showed PCR-positive findings in the influenza virus test using nasopharyngeal swabs.

Inflammatory Parameters

Table 2 shows the biomarker findings in all patients at admission. Lymphocyte count < 1,200/mL were observed in 78% (60) of the patients. The d-dimer level was higher than 500 mg/mL in 61 patients(79%), while the IL-6 level was greater than 6.4 pg/mL (normal range) in all of the patients. Table 2 shows the distribution of biomarkers in the COVID-19 and non-COVID-19 groups. In multivariable analysis only d-dimer and ferritin rise the statistic significance (Table 2).

Evolution

In terms of mortality, 17 of the 57 patients with COVID-19 (29.8%) and 1 patient without COVID-19 (5%) died (P = .03). Admission at an intensive care unit and mechanical intubation were required for 19.3% of the SARS-CoV-2 infections vs 0% of pneumonias attributable to other etiologies (P = .06). However, the proportions of patients receiving high-flux cannulas and noninvasive mechanical ventilation were higher in the COVID-19 group (32.1 vs 5%; P = .01 and 24.5 vs 0%; P = .01, respectively).

DISCUSSION

SARS-CoV-2 infection causes an overwhelming inflammatory reaction with synthesis of inflammatory markers (e.g., CRP, ferritin, and d-dimer) and proinflammatory cytokines (e.g., IL-6, tumor necrosis factor-α, and IL-8) [1,2]. The group with COVID-19 had fewer neutrophils than the non–COVID-19 group, although there was no difference in the lymphocyte count. Non–COVID-19 pneumonia was associated with higher red cell distribution width (RDW) values and d-dimer and IL-6 levels than positive SARS-CoV-2 infection. In contrast, the ferritin level was higher in the COVID-19 pneumonia group. The other biomarkers showed no intergroup differences. These biomarkers have not been previously used for the evaluation of the outcome of COVID-19 infection in immunosuppressed patients, and some studies suggested that they may not necessarily work in renal transplant patients in the same way as they do in the general population [3]. However, the levels of these molecules are not routinely measured in patients who have undergone KT presenting with an infection, and the levels of proinflammatory biomarkers could be increased by infectious agents other than SARS-CoV-2 as well [1].

The immunosuppressive therapy in both groups was similar, and the biomarker data for KT patients with COVID-19 in the present study (Table 2) were consistent with the published literature. The d-dimer, CRP, IL-6, and ferritin levels also showed substantial variations, which could be explained by the heterogeneity of the population with respect to the degree and severity of the infection and the period of evolution.

Lymphocytopenia is considered one of the most remarkable markers of COVID-19 and has been proposed as a criterion of...
However, lymphopenia is not specific and has been associated with respiratory infections caused by other viruses in KT patients [4]. We did not find any differences in the number of lymphocytes between COVID-19 and non−COVID-19 pneumonia. In immunosuppressed patients who received drugs with hematologic toxicity and experience infections by opportunistic pathogens, lymphopenia cannot be a good marker of SARS-CoV-2 infection. Neutrophil count and the neutrophil-lymphocyte ratio are reported to be increased in the general population with COVID-19 [1,2]. In the present study, the neutrophils and neutrophil-lymphocyte ratio were higher in the patients without COVID-19, but in the multivariable analysis they were not statistically different. On the other hand, RDW was higher in the non−COVID-19 group. In non−SARS-CoV-2 pneumonia, a higher RDW has been related to mortality [4]. Nevertheless, the role of RDW in COVID-19 is unknown.

Events such as infections or rejection activate the coagulation cascades and increase the D-dimer levels in KT. The higher D-dimer levels in non-COVID patients in the present study, even after excluding pulmonary oedema or thromboembolism, was a surprising finding. However, an explanation for this finding is beyond the scope of the present study. Nevertheless, we could not find studies that quantified the D-dimer levels in KT patients with infection, and the existing literature appears to have ignored the kinetics of this molecule in immunosuppressed patients with an infection. The behavior of D-dimer was different in general population. A metanalysis in the general population showed higher elevations of D-dimer in patients infected with SARS-CoV-2 than in patients with sepsis, especially critical COVID-19 [5].

IL-6 performs many different functions, including collaboration in inflammatory responses and initiation of the formation of acute-phase proteins [6]. Elevated serum IL-6 levels after KT are related to rejection, systemic bacterial infection, sepsis, cytomegalovirus, and the choice between cyclosporine vs tacrolimus treatment [1,6]. Thus, different triggers could influence the concentration of this IL [6]. In the present study, all patients showed increased levels of IL-6, but the elevation was higher in patients with non−COVID-19 pneumonia. Considering the

| Characteristic                              | All Patients (N = 77) | COVID-19−Positive (n = 57) | COVID-19−Negative (n = 20) | P Value |
|--------------------------------------------|-----------------------|-----------------------------|-----------------------------|---------|
| Demographics                               |                       |                             |                             |         |
| Male, % (n)                                | 68.8 (53)             | 68.4 (39)                   | 70 (14)                     | > .99   |
| Age (y), mean ± SD                         | 64 ± 10               | 65 ± 10                     | 63 ± 10                     | .47     |
| Death donor, % (n)                         | 87 (67)               | 89.5 (51)                   | 80 (16)                     | .27     |
| Months after transplantation, HR (95% CI)  | 43 (18-99)            | 46 (18-104)                 | 35 (16-97)                  | .34     |
| Induction therapy with thymoglobulin, % (n)| 26 (20)               | 28.1 (16)                   | 20 (4)                      | .56     |
| Maintained immunosuppression, % (n)        |                       |                             |                             |         |
| Steroids                                   | 97.4 (75)             | 96.5 (65)                   | 100 (20)                    | > .99   |
| Calcineurin inhibitor                      | 100 (77)              | 100 (57)                    | 100 (20)                    | > .99   |
| Mycophenolate                              | 71.1 (54)             | 75.4 (43)                   | 57.9 (11)                   | .16     |
| iMTOR                                      | 9.1 (7)               | 7 (4)                       | 15 (3)                      | .37     |
| Serum creatinine (mmol/L), mean ± SD       | 155 ± 58              | 142 ± 48                    | 161 ± 84                    | .65     |
| Glomerular filtration rate (mL/min), HR (95% CI)| 40 (30-51)         | 40 (31-50)                  | 41 (21-59)                  | .83     |
| Proteinuria mg/g, HR (95% CI)              | 217 (94-520)          | 184 (87-433)                | 315 (217-1400)              | .006    |
| Hypertension, % (n)                        | 94.8 (73)             | 93 (53)                     | 100 (20)                    | .57     |
| Diabetes mellitus, % (n)                   | 58.4 (45)             | 56.1 (32)                   | 65 (13)                     | .60     |
| Chronic lung disease, % (n)                | 28.6 (22)             | 24.6 (14)                   | 40 (8)                      | .25     |
| Coronary heart disease, % (n)              | 20.8 (16)             | 14 (8)                      | 40 (8)                      | .02     |
| Body mass index >30 kg/m², % (n)           | 16.9 (13)             | 15.8 (9)                    | 20 (4)                      | .73     |
| ACEI/ARB, % (n)                            | 20.8 (16)             | 26.3 (15)                   | 5 (1)                       | .05     |
| Symptoms                                   |                       |                             |                             |         |
| Fever, % (n)                               | 88.3 (68)             | 87.7 (60)                   | 90 (18)                     | > .99   |
| Cough, % (n)                               | 70.1 (54)             | 73.7 (42)                   | 60 (12)                     | .27     |
| Dyspnea, % (n)                             | 70.1 (54)             | 71.9 (41)                   | 65 (13)                     | .58     |
| Diarrhea, % (n)                            | 23.4 (18)             | 29.8 (17)                   | 5 (1)                       | .03     |
| Myalgia, % (n)                             | 32.5 (25)             | 42.1 (24)                   | 5 (1)                       | .002    |
| Vital signs                                |                       |                             |                             |         |
| Hypotension, % (n)                         | 18.2 (14)             | 21.1 (12)                   | 10 (2)                      | .33     |
| Tachypnea, % (n)                           | 50.6 (39)             | 56.1 (32)                   | 35 (7)                      | .12     |
| PaO₂/FiO₂, mean ± SD                       | 238 ± 133             | 230 ± 123                   | 262 ±164                    | .41     |
| PaO₂/FiO₂ ratio <200, % (n)                | 42.9 (27)             | 47.9 (23)                   | 26.7 (4)                    | .14     |
| Radiology                                  |                       |                             |                             |         |
| Multifocal patchy opacities                | 55.8 (43)             | 71.9 (41)                   | 10 (2)                      | .001    |

ACEI/ARB, angiotensin-converting enzyme inhibitors/angiotensin II receptor blockers; CI, confidence interval; COVID-19, coronavirus disease 2019; HR, hazard ratio; ICU, intensive care unit; iMTOR, inhibitors of the mammalian target of rapamycin; PaO₂/FiO₂, arterial oxygen partial pressure (mm Hg)/fractional inspired oxygen; SD, standard deviation.
**Table 2. Biomarkers of Inflammation in Kidney Transplant Patients With Pneumonia at Admission**

| Normal Range | All Patients (N = 77) | COVID-19—Positive (n = 57) | COVID-19—Negative (n = 20) | P Value | Multivariable |
|--------------|-----------------------|-----------------------------|-----------------------------|---------|---------------|
|              | Value                 | Value                       | Value                       |         | Value         |
| Leukocytes/µL| 4.000-11.000          | 8.909 ± 5.197               | 8.344 ± 5.300               | .11     | 1.0002        |
| Neutrophils/µL| 1.500-6.500          | 7.381 ± 4.872               | 6.624 ± 5.000               | .03     | 1.002         |
| Lymphocytes/µL| 1.200-3.500          | 904 ± 510                   | 886 ± 494                   | .61     |               |
| Monocytes/µL  | 100-800               | 500 (300-760)               | 450 (300-700)               | .17     |               |
| Hemoglobin g/dL| 12-16                | 12.3 ± 2                    | 12.4 ± 2.1                  | .73     |               |
| RDW (w%     | 10-15                 | 14.8 (13.7-16.3)            | 14.3 (13.7-15.8)            | .002    | 1.4           |
| Platelets × 10^9/µL| 150-400             | 181 ± 76                    | 182 ± 75                    | .75     |               |
| NLR          | 7.7 (4-13.5)          | 6.6 (3-13)                  | 9.5 (6.7-13.9)              | .06     |               |
| MHR          | 0.5 (0.3-1)          | 0.5 (0.3-0.9)               | 0.8 (0.4-1.3)               | .49     |               |
| PLR          | 231 (129-312)        | 235 (134-361)               | 217 (118-307)               | .44     |               |
| D-dimer μg/mL| 0-500                 | 1061 (577-2.140)            | 921 (495-1.680)             | .09     | 1.002         |
| LDH U/L      | 135-247               | 256 (198-309)               | 264 (202-341)               | .06     | 0.9-1.01      |
| CK U/L       | 0-145                 | 64 (33-109)                 | 64 (33-108)                 | .23     |               |
| CRP mg/L     | 0-5                   | 72 (16-145)                 | 76 (19-145)                 | .41     |               |
| IL-6 pg/mL   | 0-6.4                 | 44 (21-142)                 | 35 (20-128)                 | .06     | 1.03          |
| Ferritin ng/mL| 30-400               | 662 (374-1.137)             | 809 (442-1.330)             | .08     | 0.9-0.9       |
| Procalcitonin ng/mL| 0.3 (0.1-1.2) | 0.3 (0.1-1.3)               | 0.2 (0.1-1.3)               | .52     |               |

ALT, alanine aminotransferase; CK, creatine kinase; CI, confidence interval; COVID-19, coronavirus disease 2019; CRP, C-reactive protein; HR, hazard ratio; IL, interleukin; LDH, lactate dehydrogenase; MLR, monocytes-to-lymphocyte rate; NLR, neutrophil-to-lymphocyte rate; PLR, platelet-to-lymphocyte ratio; RDW, red blood cell distribution width.

nonspecific role of IL-6, recent clinical trials with tocilizumab, an IL-6 receptor inhibitor, did not show a benefit in the general population with COVID-19. In the general population, the IL-6 level in patients with COVID-19 was lower than that in patients with sepsis and other acute respiratory distress syndromes unrelated to COVID-19 [5].

Although ferritin levels are known to increase in pneumonia associated with other infections in patients who have undergone KT, the ferritin levels associated with SARS-CoV-2 infection in the present study were greater than those associated with non-COVID pathogens. A meta-analysis in the general population yielded similar results as in the present study. The ferritin level was highly elevated in patients with COVID-19 [5].

We performed microbiological diagnosis in only 4 patients with non-COVID pneumonia. We recollected blood cultures from all patients. Some studies have proven that blood cultures have a suboptimal sensitivity . In contrast, BAL is an established method for detecting pulmonary infectious [7]. However, we encountered limitations in performing diagnostic tests such as BAL and other tests because the hospital resources were focused on COVID-19 patients during the pandemic.

The most novel aspect of the present study was the measurement of the same inflammatory parameters in the patients with COVID-19 pneumonia and non—COVID-19 pneumonia at the moment of admission. We found a single study that employed a similar methodology. Arenas compared hemodialysis and KT patients with confirmed and suspected COVID-19 at a Spanish hospital. In their non-COVID KT group, only 1 patient had pneumonia, and the laboratory data of this patient were not available [8].

This study had several limitations because it was a descriptive, single-center study with a small sample size. The control group was extremely heterogeneous, and microbiological diagnoses were not possible in many cases. However, this study questions the specificity of biomarkers in pneumonia caused by COVID-19, and the topic has not been described previously. Nevertheless, we performed biomarker assessments only once in the cohort of patients with SARS-CoV-2 infection, and the measurements were performed at different points of infection. Thus, more measurements will be required to determine the kinetics of the biomarkers.

**CONCLUSIONS**

COVID-19 infection in patients who have undergone KT presents with a distinct inflammatory pattern, namely lower neutrophil counts and D-dimer and IL-6 levels but higher ferritin levels than those observed in non—COVID-19 pneumonia. However, the small sample size of the present study may have limited the detection of other differences. To increase knowledge about biochemical presentation of SARS-CoV-2 infection in KT patients could be helpful to differentiate COVID from other diseases. An explanation of the enteropathogenesis of the differences in the infection biomarkers was also beyond the scope of this study. Larger studies with patients who have undergone KT, ideally using logistic regression and receiver operating characteristic curve methods, are required for a more robust evaluation of the ability of these inflammation biomarkers to discriminate the occurrence of COVID-19 pneumonia from pneumonia caused by other etiologies.

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