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Hematological abnormalities in immunosuppressed patients with COVID-19: Evidence from a single center. A cross sectional study

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ABSTRACT

Background: Changes in hematological parameters in patients with COVID-19 are emerging as important features of the disease in the general population. In the present study we aimed to explore the hematological characteristics and its prevalence proportion ratio in patients with immunosuppression with COVID-19.
Aim: To explore the differences between immunosuppressed and non-immunosuppressed patients, with and without COVID-19 from a hematological perspective.
Methods: This cross-sectional study reports on the baseline complete blood count in patients attending the HHA Hospital, in Chile. The study reports descriptive characteristics of the population, including sex, age, ethnicity, corticoids and biological therapy scheme and a complete report of blood test results. A total of 476 patients were enrolled in this study from October of 2020 to April 2021.
Results: Findings revels a significant increment (p value $\leq$ 0.001) on the median of total neutrophils and leucocytes, and in platelet-lymphocyte ratio (PLR), neutrophil- lymphocyte ratio (NLR) and monocyte-lymphocyte ratio (MLR) in immunosuppressed patients with COVID-19 (IS(+)) and immunocompetent patients with COVID-19 (IC(+)) compared with their respective controls. By contrast, a significant reduction on the median of lymphocytes, and eosinophiles was observed in IS(+) individuals compared with its controls. Also, the red blood cell count, hemoglobin, hematocrit, and mean corpuscular hemoglobin concentration were significantly reduced in IS(+) patients, whereas red blood cell, distribution width and mean corpuscular volume, were significantly higher in patients with COVID-19.
Conclusion: Rapid blood tests, including, neutrophil, lymphocytes count and PLR, NLR can be used for early assessment and management of patients with immunosuppression.

Abbreviations: IS(+), immunosuppressed with COVID-19; IS(-), immunosuppressed without COVID-19; IC(+), immunocompetent with COVID-19; IC(-), immunocompetent without COVID-19; PLR, platelets-to-lymphocyte ratio; NLR, neutrophils-to-lymphocyte; MLR, monocytes-to-lymphocyte; HCT, hematocrit; Hb, Hemoglobin; WBC, White count cells; RDW, red blood cell distribution width.

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1. Introduction

According to the Centers for Disease Control and Prevention (CDC) primary immunodeficiencies are inherited and are defined as absence or quantitative deficiency of cellular, humoral, or both components that provide immunity. While secondary immunodeficiency is defined as a loss or qualitative deficiency in cellular or humoral immune components that occurs because of a disease process or its therapy. Examples of secondary immunodeficiency include HIV infection, active treatment for solid tumor, hematopoietic malignancies, receipt of solid- organ transplant, treatment with radiation, asplenia, chronic renal disease and treatment with immunosuppressive drugs. The degree to which immunosuppressive drugs cause clinically significant immunodeficiency generally is dose related and varies by drug. High-dose corticosteroids (i.e., $\geq$ 20 mg prednisone or equivalent per day), alkylating agents, antimetabolites, transplant-related immunosuppressive drugs, cancer chemotherapeutic agents classified as severely immunosuppressive, tumor-necrosis (TNF) blockers, and other biologic agents that are immunosuppressive drugs [1] Immunosuppressive conditions may increase the risk of severe infection and result in an increased predisposition to transmit SARSCoV-2 virus [2].

COVID-19 clinical manifestation may range from asymptomatic to more severe forms of the disease characterized by acute respiratory syndrome (ARDS), including cardiac, neurological, renal, and hematological abnormalities. Among the hematological abnormalities, changes in platelet, white blood cell and hemoglobin [3], and coagulation/fibrinolytic alterations have been described [4].

In general, hematological parameters play an important role in early diagnosis and provide useful information to the physician regarding the inflammatory process. These are easily performed and inexpensive. For instance, red blood cell distribution width (RDW) has been reported as an important inflammatory marker in many other diseases such as cancer, cardiovascular diseases, and autoimmune inflammatory diseases. Among the platelets parameters, mean platelet volume (MPV), and platelet-lymphocyte ratio (PLR) have emerged as good indicators of subclinical systemic inflammation [5]. Among those hematological abnormalities, leukopenia, lymphopenia are reported to be the most commonly associated with COVID-19 infections in the general population [2].

Immunosuppressed patients may have suboptimal compensatory mechanisms to deal with hematological changes during the progression of COVID-19 disease and this may explain the severe presentation associated with those conditions [2].

As of yet, there is still limited information about hematological parameters in immunosuppressed patients. Therefore, it is necessary to identify the relationship between hematological parameters and immunosuppression. In addition, the differences between immunocompetent and immunosuppressed patients with COVID-19 could be used as predictors of clinical presentation. This information could allow for better informed clinical decisions and a stratification of risk in patients with COVID-19.

The present research aimed to explore the relationship between hematological parameters among immunosuppressed patients with COVID-19. It also aimed to assess the magnitude of those parameters between immunocompetent and immunosuppressed individuals with and without COVID-19. Hematologic marker analysis included neutrophils, lymphocytes, monocytes, platelets, neutrophil-to-lymphocyte ratio (NLR), monocyte-to-lymphocyte ratio (MLR), and platelet-to-lymphocyte ratio (PLR). All these parameters have been previously identified to contribute to clinical decision making in the general population with COVID- 19 [4,6].

2. Materials and methods

A cross-sectional study was conducted on a total of 474 patients from October 2020 to April 2021. The enrolled patients were from the public Hospital Hernán Henríquez Aravena, La Araucania, Chile. The protocol was approved by two different Ethics Committees, belonging to Universidad de La Frontera and Servicio de Salud Araucania Sur, and it adhered to the Declaration of Helsinki. The patients were classified into immunosuppressed and immunocompetent individuals.

Those groups were then sub-classified according to PCR and serology results in 113 immunocompetent without COVID-19 (IC(-)) and 151 immunocompetent with COVID-19 (IC(+)). One hundred twenty-five immunosuppressed patients without COVID-19 ((IS (-)) and 85 immunosuppressed patients with COVID-19 ((IS (+)).

Polymerase chain reaction (PCR) and hematological tests were carried out the same day and in some cases with a delay of 7 days between diagnosis with PCR and hematological blood test. The first symptoms of COVID-19 appeared around 4 days before the diagnosis by PCR or serology with a standard deviation of 3 days in all individuals enrolled in this study.

2.1. Inclusion and exclusion criteria

This study included individuals who agreed to participate through written informed consent. Those on medication and with other immunosuppressive conditions that can alter the immune system were not excluded. Only patients on antiplatelet drugs were excluded (n = 1). No pediatric patients (<18 years) were included.

2.2. CBC measurement

Peripheral venous blood samples (5 mL) were collected into EDTA tubes and processed using the flow cytometry technique. A CAL 6000 hematological counter (Mindray; Nanshan, Shenzhen, P.R. China) was used to analyze the blood samples following manufacture instructions. Reference intervals were calculated for hematological parameters according to local laboratory criteria. In terms of red cell parameters, the clinical diagnosis for anemia was defined by a hemoglobin concentration at $\leq$ 12 mg/dL (see appendix 1).

2.3. Blood test variables

Complete blood count included total leucocytes, neutrophils, lymphocytes, monocytes, platelets, basophiles, eosinophiles, platelets-to-lymphocyte ratio (PLR), neutrophils-to-lymphocyte (NLR), monocytes-to-lymphocyte (MLR), hematocrit (%) (HCT), hemoglobin (Hb), MCHC, MCV and RDW.

All variables were treated as continuous variables. The normal ranges for the blood test variables were determined according to the criteria of the local laboratory that performed the tests. Lymphopenia was defined as $< 1.0 \times 10^9$/L of the absolute count, thrombocytopenia as $< 150 \times 10^9$/L, leukopenia $< 3 \times 10^9$/L, neutrocytophenia $< 1{,}5 \times 10^9$/L and monocytopenia as $< 0{,}2 \times 10^9$/L.

2.4. Statistics analysis

Statistical analyses were performed using STATA/IC 16.1 version (Stata Corp, College Station, TX, USA). The continuous data were described by medians and categorical data were described as percentages (%). A normality test of continuous variables was performed using the Shapiro Wilk method. The correlation between variables was determined using Spearman's analysis. The bivariate analysis was carried out using demographical and hematological parameters, in accordance with the Kruskal-Wallis method.

To compare the blood results of the 4 groups (IC(+), IC(-), IS(+), and IS(-)) based on immunocompetency and infection with COVID-19, we used a Nominal logistic regression. The variables that present a correlation over 0.7 between hematological parameters were rejected from the statistical model. $p < 0{,}05$ was considered statistically significant.

Table 1
Demographic characteristics of 474 patients by immunocompetence and COVID-19 status.

	Immunocompetent		Immunosuppressed		Total (N:474) (100%)
	COVID-19 (-) (N:113) (24%)	*COVID-19 (+) (N:151) (32%)*	*COVID-19 (-) (N:125) (26%)*	*COVID-19 (+) (N:85) (18%)*	
n Male, (%)	36 (32%)	81 (54%)	24 (19%)	48 (56%)	189 (40%)
Age (median) [range]	33 [20–71]	44 [18–83]	51[21–74]	61 [26–80]	47 [18–83]
n Mapuche ethnicity, (%)	33 (28%)	54 (35%)	34 (27%)	21 (25%)	142 (30%)
ICU n (%)	0 (0%)	74 (49%)	0 (0%)	63 (74%)	137 (29%)
Deaths n (%)	0 (0%)	17 (15%)	0 (0%)	21 (25%)	38 (8%)
Corticoids n, (%)	0 (0%)	0 (0%)	32 (25%)	5 (5%)	37 (8%)
Biologic treatment n, (%)	0 (0%)	0 (0%)	58 (46%)	2 (2%)	60 (13%)

3. Results

3.1. Demographic and characteristics

A total of 474 patients were included in the study, 113 (24%) were immunocompetent without COVID-19 (IC(-)) and 151 (32%) were immunocompetent with COVID-19 (IC(+)). 125 patients (26%) were immunosuppressed without COVID-19 ((IS(-)) and 85 patients (18%) were immunosuppressed with COVID-19 ((IS(+)) (refer to Table 1).

In the 113 (24%) immunocompetent patients without COVID-19 ((IC (-)), the median age was 33 years old [20–71], 36 (32%) were men and 33 (28%) were mapuche. Whereas, in the 125 (26%) immunosuppressed patients without COVID-19 (IS(-)), the median age was 51 years old, [21–74], 24 (19%) were men, and 34 (27%) were mapuche.

Regarding individuals with COVID-19 ((IC(+)), in the 151 immunocompetent patients, the median age was 44 years old [18–83], 81 (54%) were men and 54 (35%) were mapuche.

While, of the 85 immunosuppressed patients with COVID-19 ((IS (+)), the median age was 61 years old [26–80], 48 (56%) were men, and 21 (25%) were mapuche (Table 1).

Seventy-four patients of the 151 (49%) immunocompetent with COVID-19 ((IC(+)) were hospitalized and required ventilation, while this ratio increased (63/85 (74.1%)) in immunosuppressed ones with COVID-19 ((IS(+)). Fifteen percent of patients from the IC(+) group and 25 % from the IS(+) group died.

Only immunosuppressed patients received corticoids and/or biological agent therapy. Of the 125 immunosuppressed patients without COVID-19, 32 (25%) and 58 (46%) received corticoids and biological treatment respectively. While of the 85 immunosuppressed patients with COVID-19, only 5 (5%) and 2 (2%) received corticoids and biological treatment.

3.2. Hematological findings of patients on admission

According to a bivariate analysis, a significant increment (p value $\leq$ 0.0001) on the median of total leucocytes, neutrophils (cellx10^9/L), platelets-to-lymphocyte ratio (PLR), neutrophils-to-lymphocyte (NLR) and monocytes-to-lymphocyte (MLR) was observed in patients with COVID-19 in both groups, immunocompetent and immunosuppressed patients, compared with its respective control without COVID-19 (Fig. 1 and Table 2 and Appendix 2).

By contrast, it was observed that lymphocytes, eosinophils levels (10^9/L) were significantly reduced (p value $\leq$ 0.0001) in patients with COVID-19 in both groups, immunocompetent and in immunosuppressed compared with its respective controls ((IC(+)) and IS(-). Also, a decreased median of platelet count in immunosuppressed patients with COVID-19 was observed, compared to the control ((IS(-)) and the immunocompetent groups. (p value: 0.019) (Table 2, Appendix 2).

The red blood cell parameters' evaluation (Fig. 2 and Table 3) shows that red blood cells (RBC), hemoglobin (Hb), the hematocrit (HCT) and mean corpuscular haemoglobin (MCH).

concentration (MCHC) were significantly reduced in immunosuppressed patients with COVID-19. The morphological parameters, such as red cell distribution width (RDW) and mean corpuscular volume (MCV),

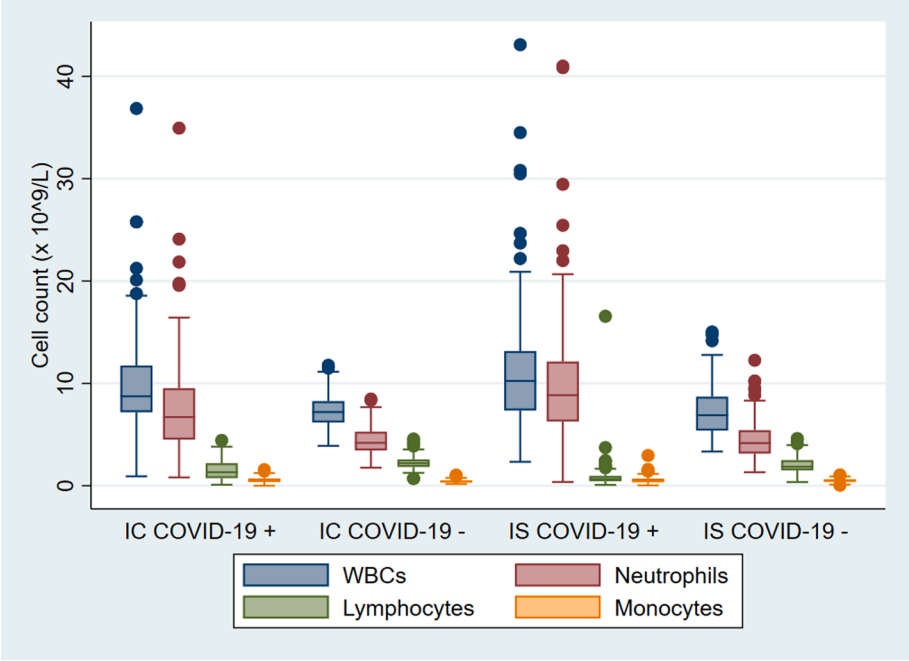

Fig. 1. Main white cells distribution by immunocompetence and COVID-19 status.

Table 2
White cells and platelets hematological parameters by immunocompetence and COVID-19 positivity.

White cells and platelets hematological parameters Mean (median) [range]	**Immunocompetent**		**Immunosuppressed**	
	COVID-19(-) (N:114)	*COVID-19 (+) (N:150)*	*COVID-19 (-) (N: 125)*	*COVID-19 (+) (N: 85)*
Total leucocytes (10^9/L)	7.34 (7.20) [3.9–11.8]	9.89 (8.74) [0.92–37]	7.25 (6.89) [3.3–15]	11.62 (10.24) [2.34–43.09]
Neutrophils (10^9/L)	4.42 (4.19) [1.8–8.5]	7.72 (6.71) [0.82–35]	4.51 (4.17) [1.3–12.2]	10.47 (8.85) [0.4–41]
Lymphocytes (10^9/L)	2.25 (2.20) [0.7–4.6]	1.42 (1.32) [0.1–4.4]	2.01 (1.86) [0.36–4.61]	1.00 (0.65) [0.1–16.56]
Monocytes (10^9/L)	0.44 (0.42) [0.17–1.0]	0.54 (0.50) [0.0–1.6]	0.52 (0.49) [0.1–1.1]	0.58 (0.48) [0–2.96]
Platelets (1000/mm3)	273 (269) [104–499]	299 (284) [41–622]	259 (252) [108–571]	232 (228) [4–533]
Basophiles (10^9/L)	0.04 (0.04) [0–0.1]	0.01 (0.01) [0–0.2]	0.04 (0.04) [0–0.1]	0.00 (0.00) [0–0.1]
Eosinophiles (10^9/L)	0.18 (0.13) [0.0–1.1]	0.01 (0.04) [0–0.84]	0.17 (0.14) [0–0.8]	0.05 (0.00) [0–0.3]
PL Index	129.65 (125.24) [52.8–363.9]	341.21 (228.66) [62–2950]	148.58 (129.24) [45.4–599]	404.34 (306.25) [5–2488]
NL Index	2.09 (1.88) [0.8–6.5]	10.95 (4.71) [0.93–164.8]	2.67 (2.04) [0.4–11.1]	19.67 (14.92) [0.5–170.8]
ML Index	0.20 (0.19) [0.1–0.6]	0.60 (0.39) [0.0–5.17]	0.299 (0.24) [0.0–1.47]	0.85 (0.74) [0–3.6]

were significantly higher in the group of patients with COVID-19 and markedly higher among the immunosuppressed with COVID-19, compared with the control group (IS(-)). Also, it was observed that median hematocrit was reduced significantly in the IS(+) patients at 33% compared with IS(-) (hct 39.5%). In immunocompetent patients with COVID-19, a reduction in HCT (38.5%) compared with immunocompetent patients without COVID-19 (40.7%) was also observed.

Similar results were found in hemoglobin levels (gr/dL). In IS(+) the median hemoglobin decreased at 11 gr /dL compared with IS(-) 13.4. In immunocompetent individuals, a significant reduction in median Hb levels in IC(+) (12.9 g/L.) compared with IC(-) (13.7 g/L.) was also observed.

The reduction of platelets under 150 × 10^9/L is indicative of thrombocytopenia. The highest percentage of patients with thrombocytopenia was found in the IS(+) group, 18/85 (21%) (refer to Table 4). Lower percentages of thrombocytopenia were found in IS(-) group with 7/125 (6%), IC(+) 6/151 (4%) and in IC(-)1/113 (1%). Lymphopenia was defined as < 1.0 × 10^9/L of the absolute count and this was observed in 64/85 (75%) of IS(+) group, 10/125 (8%) in IS(-) group, 55/151 (36%) in IC(+) and 2/113 (2%) in IC(-).

In addition, monocytopenia was defined as < 1.0 × 10^9/L. The highest percentage of patients with monocytopenia was found in the IS (+) group, with 8/85 (9%). To a lesser extent, monocytopenia was found in 2/125 (2%) of the IS(-) group, 6/151 (4%) in IC(+), and 1/113 (1%) in IC(-) (refer to Table 4). Neutropenia and leukopenia were not frequently present in any of our groups of patients.

Additionally, the prevalence of patients with neutrophils > 8 × 10^9/L. and PLR < 180 in the IC(+) were 9 times (PR:9,72; p: 0,004; CI95% [2,06–45,88]) and 4 times higher (PR: 4,15; p: 0,005; CI95% [1,54–11,22]) than IC(-) group respectively. Similar results were observed in IS (+) patients with high count neutrophils (PR: 14,8; p:

Table 3
Red cells hematological parameters by immunocompetence and COVID-19 *positivity.*

Red cells parameters Mean (median) [range]	***Immunocompetent***		***Immunosuppressed***	
	COVID-19 (-)	*COVID-19 (+)*	*COVID-19(-)*	*COVID-19 (+)*
Hematocrit (%)	40.7 (40.5) [22.9–51.5]	38.7 (39.2) [22.4–49.2]	39.5 (39.5) [22.5–50.2]	33.5 (34.2) [19.5–48.6]
Hemoglobin (gr/dL)	13.7 (13.7) [7.5–17.4]	12.9 (13.2) [7.3–16.9]	13.4 (13.5) [7.4–16.8]	11.0 (11.3) [5.6–15.7]
MCHC (gr/dL)	33.6 (33.6) [30.6–36.3]	33.3 (33.3) [30.4–38.5]	33.9 (34.0) [30.8–37.2]	32.7 (32.7) [28.7–36.7]
MCV (fL.)	88.9 (89.8) [64.4–99.4]	90.9 (90.6) [9.5–112]	87.8 (88.0) [62.3–106.1]	90.8 (90.9) [63.3–105.5]
RDW (fL.)	12.9 (13.0) [10.9–19]	13.5 (13.2) [11.6–45.3]	13.5 (13.2) [11.1–22.5]	14.6 (13.9) [12.1–24.8]

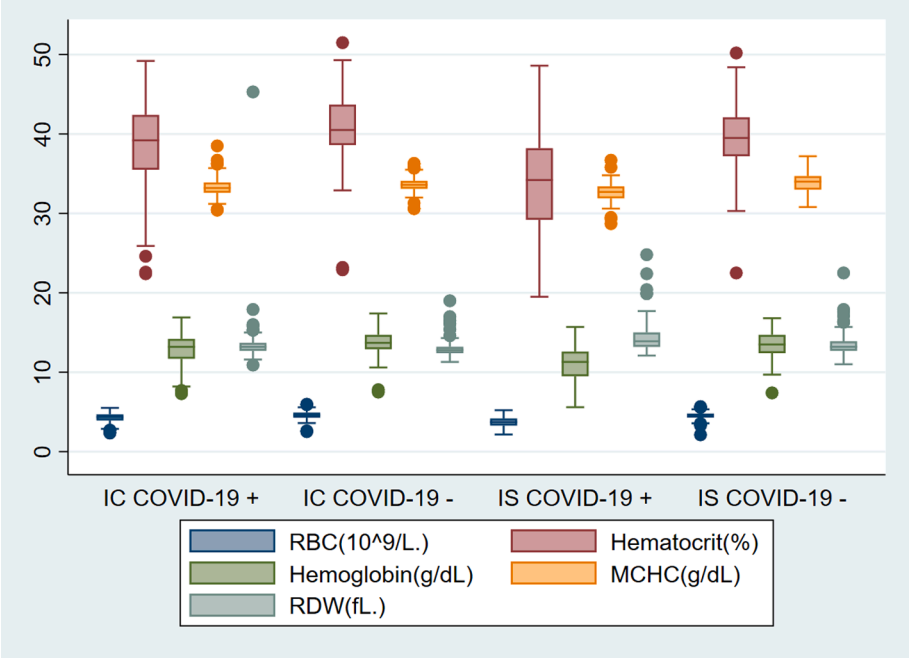

Fig. 2. Red cell parameters distribution by immunocompetence and COVID-19 status.

Table 4
Count and percentage of patients with thrombocytopenia, neutropenia, leucopenia, lymphopenia and monocytopenia by immunocompetence and COVID-19 status.

Hematological deficiencies founded n (%)	IC(-) (N: 113)	IC(+) (N:151)	IS(-) (N:125)	IS(+) (N:85)	Total (N:474)
Leukopenia (<3 X 10^9/L)	0	4	0	1	5
	0,00	2,65	0,00	1,18	1,05
Neutropenia (<1,5 X 10^9/L)	0	2	1	1	4
	0,00	1,32	0,80	1,18	0,84
Lymphopenia (<1,0 X 10^9/L)	2	55	10	64	131
	1,77	36,42	8,00	75,29	27,64
Monocytopenia (<0,2 X 10^9/L)	1	6	2	8	17
	1,13	9,06	2,5	6,8	80,58
Thrombocytopenia (<150 X 10^9/L)	1	6	7	18	32
	0,88	3,97	5,60	21,18	6,75
Anemia (Hemoglobin < 12 g/L.)	12	39	20	54	125
	10,62	25,83	16,00	63,53	26,37

0,001; [2,90–75,47]) and PLR (PR: 4,96; p: 0,014; CI95%: [1,38–17,71]) (refer to Table 5).

Finally, the multinomial logistic regression model reveals statistical differences between IC(-) and IS(-) in terms of males prevalence ratios (PR: 0,37; p: 0,006; CI95% [0,18–0,75]) (Table 5). Regarding the age, the IS(+) group presents a 5 times higher prevalence of individuals ranging ages between 40 and 60 years old (PR:5,24; p: 0,006; CI95% [1,61–16,99]), and almost 10 times higher prevalence of individuals over 60 years than IC(-) group (PR:9,73; p: 0,005; CI95% [2,00–47,32]). The IC(-) groups between 40 and 60 years (PR:5,13; $p < 0.001$; CI95% [2,71–9,71]); and over 60 years (PR:11,76; $p < 0.001$; CI95% [3,79–36,47]) presents higher prevalence (and statistically significant) in comparison with patients under 39 years (control group) (Table 5).

4. Discussion

In the general population, abnormalities in the hematological parameters have been reported in other acute respiratory syndromes such as SARS, Middle East respiratory Syndrome (MERS) and COVID-19 [7]. This study did not find a relationship between sex or ethnicity and COVID-19 positivity or immunocompetency. A systematic review pointed out that the relationship between ethnicity and COVID-19 is uncertain. Emerging reports suggest that Black, Asian, and Ethnic Minority individuals are at an increased risk of acquiring SARS-CoV-2 compared to White individuals and are likely to achieve worse clinical outcomes because of COVID-19 [8]. Chile has 17.076.076 inhabitants according to censuses in 2017, and of those, 2.185.792 inhabitants (12.8%) are indigenous people. In the area where this study was performed, 79.84% of these indigenous people are Mapuche [9]. Therefore, assessing the role of ethnicity in the current pandemic was considered and import public health concern. Our results did not find any correlation between ethnic background (belonging to the Mapuche ethnicity, in this case) and COVID-19 prevalence. This result is in line with previous reports that did not find a link between mortality, rates of hospitalization or intubation, risk of infection with SARS-CoV-2 and ethnicity [8].

In terms of age, the IS(+) group presents a 5 times higher prevalence of individuals aged between 40 and 60 years old, and almost 10 times higher prevalence of individuals over 60 years than IC(-) group. Lymphopenia has been reported in 54% −100% of patients with COVID-19 [4,7,10]. Those results agree with our findings that show that 50.4% of our COVID-19 positive patients presented with lymphopenia. In addition, we found that lymphopenia occurs more frequently in patients with immunosuppression (75%) with COVID-19, than in immunocompetent ones with COVID-19 and patients without COVID-19 infection (IS(-) and IC(-), which may explain why those IS(+) individuals progress to a more severe presentation of COVID-19, requiring mechanical ventilation assistance (63/85 patients of IS(+) were admitted to intensive care).

Table 5
Results of nominal logistic regression according to groups of immunocompetence an COVID-19 status.

Group	Prevalence Ratio	Standard error	z	P > z	CI 95% []
IC(-)	(Base outcome)				
IC(+)					
Sex					
Male	1,54	0,5	1,35	0,178	[0,81–2,91]
Ethnics					
Mapuche	1,4	0,47	1,12	0,264	[0,75–2,75]
Age (18–40 yrs.)					
40–60	1,07	0,36	0,22	0,829	[0,55–2,09]
> 60	1,54	0,98	0,69	0,49	[0,44–5,35]
Hematocrit (%)					
<35	1,3	0,74	0,56	0,577	[0,46–3,97]
MCV (fL.)					
<80	0,098	0,11	−1,92	0,055	[0,00–1,04]
MCHC (gr/L)					
<33	1,58	0,57	1,26	0,208	[0,77–3,23]
> 39	4,4	3,94	1,66	0,098	[0,76–25,51]
Neutrophiles (10^9/L.)					
<2	4,01	5,02	1,11	0,266	[0,34–46,71]
> 8	9,72	7,69	2,87	0,004	[2,06–45,88]
Lymphocytes (10^9/L.)					
<1	5,74	5,03	2	0,046	[1,03–32,00]
> 4	0,9	1,23	−0,07	0,943	[0,06–13,17]
Monocytes (10^9/L.)	2,05	1,16	1,26	0,208	[0,67–6,26]
Platelets (10^9/L.)					
< 150	0,73	0,96	−0,23	0,815	[0,057–9,45]
> 400	1,11	0,82	0,15	0,878	[0,26–4,70]
PLR					
> 180	4,15	2,10	2,81	0,005	[1,54–11,22]
Constant	0,072	0,08	−2,25	0,025	[0,00–0,71]
IS (-)					
Sex					
Male	0,37	0,13	−2,74	0,006	[0,18–0,75]
Ethnics					
Mapuche	1,09	0,36	0,27	0,79	[0,56–2,09]
Age (18–40 yrs.)					
40–60	5,13	1,66	5,04	0	[2,71–9,71]
> 60	11,76	6,79	4,27	0	[3,79–36,47]
Hematocrit (%)					
35	0,83	0,46	−0,33	0,741	[0,27–2,48]
MCV (fL.)					
< 80	1,29	0,93	0,36	0,72	[0,31–5,34]
MCHC (gr/L)					
< 33	1,02	0,38	0,07	0,942	[0,49–2,12]
> 39	4,34	3,96	1,61	0,107	[0,72–25,97]
Neutrophils (10^9/L)					
< 2	4,23	4,90	1,25	0,212	[0,43–40,93]
> 8	2,01	1,72	0,82	0,41	[0,37–10,74]
Lymphocytes (10^9/L)					
< 1	0,91	0,84	−0,11	0,915	[0,14–5,62]
> 4	1,91	2,10	0,58	0,56	[0,21–16,64]
Monocytes (10^9/L.)	1,97	1,15	1,16	0,247	[0,62–6,24]
Platelets (10^9/L.)					
< 150	3,35	4,11	0,98	0,325	[0,30–37,23]
> 400	0,28	0,24	−1,45	0,146	[0,05–1,55]
PLR					
> 180	2,94	1,54	2,05	0,04	[1,04–8,24]
Constant	0,096	0,11	−1,96	0,05	[0,00–1,00]
IS (+)					
Sex					
Male	1,43	0,62	0,84	0,403	[0,61–3,37]
Ethnics					
Mapuche	1,16	0,53	0,33	0,74	[0,47–2,85]
Age (18–40 yrs.)					
40–60	5,24	3,14	2,76	0,006	[1,61–16,99]
> 60	9,73	7,85	2,82	0,005	[2,00–47,32]
Hematocrit (%)					
< 35	3,67	2,17	2,2	0,028	[1,15–11,70]
MCV (fL.)					
< 80	1,5	1,65	0,37	0,708	[0,17–12,95]
MCHC (gr/L)					

(*continued on next page*)

Table 5 (*continued*)

Group	Prevalence Ratio	Standard error	z	P > z	CI 95% []
< 33	2,42	1,10	1,94	0,052	[0,99–5,92]
> 39	11,47	15,34	1,82	0,068	[0,83–157,78]
Neutrophils (10^9/L.)					
< 2	0,00	0,00	−0,02	0,983	[0–0]
> 8	14,8	12,30	3,24	0,001	[2,90–75,47]
Lymphocytes (10^9/L.)					
< 1	9,93	9,22	2,47	0,013	[1,60–61,29]
> 4	0,0	0,00	−0,01	0,991	[0–0]
Monocytes (10^9/L.)	2,56	1,63	1,47	0,141	[0,73–8,93]
Platelets (10^9/L.)					
< 150	3,15	4,09	0,89	0,375	[0,24–39,99]
> 400	0,6	0,53	−0,57	0,568	[0,10–3,45]
PLR					
> 180	4,96	3,22	2,47	0,014	[1,38–17,71]
Constant	0,0018829	0,00	−4,33	0	[0,00–0,03]

Even though there are no studies comparing immunocompetent and immunosuppressed individuals, Chen et al., 2020 and Wang et al., 2020 pointed out that lymphopenia occurs in severe presentation of COVID-19. Lymphopenia may be caused by SARSCoV-2 persistently invading more lymphocytes, causing their death or depletion when they reach the spleen and other immune organs [11].

In addition, thrombocytopenia was observed in 5–21% of patients with severe presentation of COVID-19 [4]. Usul and colleagues (2020) reveal statistical significance reduction in the count of thrombocytes in COVID-19 patients, when compared to individuals without COVID-19 (n:119). We found that the median of platelet count decreases significantly in IS(+) patients compared with IC(-) and IC(+) which is consistent with what was found by Usul and colleagues in the general population.

In addition, our results reveal that thrombocytopenia was more common in patients with immunosuppression (IS(+)) than in the immunocompetent patients (IC(+)) affected by COVID-19. Thrombopenia was present in 21% of immunosuppressed patients (IS(+)) and in only 4% of the immunocompetent individuals (IC(+)). A similar trend was found when examining thrombocytopenia in patients without COVID-19 in both IC(-) and IS(-) groups. Thrombocytopenia affected 5.6% of individuals with immunosuppression, and 0.9% of the immunocompetent individuals. Thrombocytopenia (platelets $< 150 \times 10^9$/L) was correlated with a major risk of mortality [7]. Early reports of SARSCoV-2 show that the coronavirus can attack directly or indirectly platelets [12] or immune complexes which might lead to thrombocytopenia and an increase in the risk of thrombosis and death [12]. In fact, we report that 21/85 of IS(+) and 17/150 of IC(+) patients died during the course of the study.

Leucopenia was reported in 20–40% of COVID-19 patients [4], which is different to what was found in our study. We report leucopenia in 2,7% of IC(+) and 1.2% of IS(+), none of the patients without COVID-19 presented with leucopenia.

Also, eosinophils have been previously reported to decrease significantly in 53% of patients with COVID-19 [4,11] and the effect is more remarkable in patients that progress to more severe presentation [13]. In the results of this study, which are also consistent with Liao and Wang's reports, we show a significant decrease in eosinophils' median in IS(+) patients, reaching values of zero. In our study we report a significant increment in neutrophils as Liao and colleagues (2020) report. However, it seems controversial, because Usul and colleagues (2020) report a decrease in neutrophil counts in patients with COVID-19 infection compared with non-infected ones [5]. Others report morphological changes in neutrophils, in the shape of the nucleus and cytoplasmatic granulation. Apoptotic and immature granulocytes were observed in peripherical blood due to abnormalities in granulopoiesis [4]. Another important hematological parameter is NLR. The literature reports that the NLR is a marker for evaluation of the progression and prognosis in patients with infections such as COVID-19, and tumors, and an increment of NLR indicated a poor prognosis. Ding et al., (2020) and Wang et al. (2020) reported that NLR and PLR increased significantly in patients with COVID-19 with severe presentation. Both ratios show the best differential parameter for diagnosis efficacy in COVID-19 patients. Our findings show that NLR, PLR and MLR increased significantly in patients with COVID-19 and the effect is more remarkable in patients with immunosuppression, which is in agreement with Ulanowska & Olas (2021) and in disagreement with Usul et al. (2020), who report a drop in NLR and PLR in COVID-19 cases compared with non-infected individuals.

Previous reports indicated that the effect on red blood cells was not significant, but structural changes have been noticed in patients with COVID-19 [4]. Our study reveals that the red blood cell parameters such as RBC, Hb, HCT, MCHC were significantly reduced in IS(+) patients, whereas RDW, MCV, were significantly higher in the group of patients with COVID-19 and more significant in patients IS(+) compared with its respective controls. These coincided with the findings reported by Ulanowska & Olas (2021) and Santos-Lozano et al. (2020), who reported lower levels of hemoglobin, that could be one of the major predictors of mortality.

Red blood cells abnormalities in patients with COVID-19 may be explained by the direct attack of heme in hemoglobin by SARSCoV-2 virus, this leads to a bone marrow suppression, resulting in anemia [4]. And, as a compensatory mechanism, the system recurs to hyperplasia of red cell lines which leads to immature red cell release to the bloodstream resulting in the activation of apoptosis and phagocytosis [13]. In addition, the similarity between the spike protein of the virus and hepcidin causes dysregulation of iron metabolism, which may lead to a reduction of hemoglobin or haemoglobinopathy [14].

In our study, anemia, affected 63.5% of IS(+) patients, 25.8% of IC (+), compared with 16% in IS(-) and 10.6% IC(-).

5. Limitations

The greatest limitation of this study was the limited number of patients with immunosuppression with COVID-19. Studies conducted with a larger patient group will better reflect the importance of biomarkers from peripheral blood tests in the diagnosis of immunosuppressed patients with COVID-19.

6. Conclusions

Lymphopenia, thrombocytopenia, and NLR are indicative of COVID-19 disease in immunocompetent and immunosuppressed patients. However, in immunosuppressed patients with Covid-19 the reduction of lymphocytes and platelet count is more severe. The identification of hematological factors in patients with COVID-19 may be useful in the early diagnosis of high-risk individuals, such as immunosuppressed patients.

Table A1
General criteria of pathological parameters in hematological values in patients.

Hematological parameters	Reference values	
	Lower limit	Upper limit
Total Leukocytes (10^9/μL)	3	11
Neutrophils (10^9/μL)	1.5	8
Lymphocytes (10^9/μL)	1	4.8
Monocytes (10^9/μL)	0.2	1.1
Platelets (10^3/μL)	150	400
Hemoglobin (g/dL)	12	15.4
Hematocrit (%)	30	55

Table A2
p-values of Bivariate analysis by Kruskal-Wallis method.

	Total Leukocytes		
	IC COVID-19 (+)	IS COVID-19 (-)	IS COVID-19 (+)
IC COVID-19 (-)	0.0001	0.224	0.0001
IC COVID-19 (+)	–	0.0001	0.0914
IS COVID-19 (-)		–	0.0001
	Neutrophils		
	IC COVID-19 (+)	IS COVID-19 (-)	IS COVID-19 (+)
IC COVID-19 (-)	0.0001	0.7142	0.0001
IC COVID-19 (+)	–	0.0001	0.0002
IS COVID-19 (-)		–	0.0001
	Lymphocytes		
	IC COVID-19 (+)	IS COVID-19 (-)	IS COVID-19 (+)
IC COVID-19 (-)	0.0001	0.0006	0.0001
IC COVID-19 (+)	–	0.0001	0.0001
IS COVID-19 (-)		–	0.0001
	Monocytes		
	IC COVID-19 (+)	IS COVID-19 (-)	IS COVID-19 (+)
IC COVID-19 (-)	0.0018	0.0004	0.0436
IC COVID-19 (+)	–	0.8663	0.779
IS COVID-19 (-)		–	0.8229
	Platelets		
	IC COVID-19 (+)	IS COVID-19 (-)	IS COVID-19 (+)
IC COVID-19 (-)	0.0809	0.0137	0.0001
IC COVID-19 (+)	–	0.0002	0.0001
IS COVID-19 (-)		–	0.0190
	Eosinophiles		
	IC COVID-19 (+)	IS COVID-19 (-)	IS COVID-19 (+)
IC COVID-19 (-)	0.0001	0.8745	0.0001
IC COVID-19 (+)	–	0.0001	0.0002
IS COVID-19 (-)		–	0.0001
	Basophiles		
	IC COVID-19 (+)	IS COVID-19 (-)	IS COVID-19 (+)
IC COVID-19 (-)	0.0001	0.9805	0.0001
IC COVID-19 (+)	–	0.0001	0.0001
IS COVID-19 (-)		–	0.0001
	Index ML		
	IC COVID-19 (+)	IS COVID-19 (-)	IS COVID-19 (+)
IC COVID-19 (-)	0.0001	0.0001	0.0001
IC COVID-19 (+)	–	0.0001	0.0001
IS COVID-19 (-)		–	0.0001
	Index NL		
	IC COVID-19 (+)	IS COVID-19 (-)	IS COVID-19 (+)
IC COVID-19 (-)	0.0001	0.00686	0.0001
IC COVID-19 (+)	–	0.0001	0.0001
IS COVID-19 (-)		–	0.0001
	Index PL		
	IC COVID-19 (+)	IS COVID-19 (-)	IS COVID-19 (+)
IC COVID-19 (-)	0.0001	0.1178	0.0001
IC COVID-19 (+)	–	0.0001	0.0029
IS COVID-19 (-)		–	0.0001
	Erythrocytes		
	IC COVID-19 (+)	IS COVID-19 (-)	IS COVID-19 (+)
IC COVID-19 (-)	0.0001	0.3426	0.0001
IC COVID-19 (+)	–	0.0008	0.0001
IS COVID-19 (-)		–	0.0001
	Hematocrit		
	IC COVID-19 (+)	IS COVID-19 (-)	IS COVID-19 (+)
IC COVID-19 (-)	0.0028	0.0172	0.0001
IC COVID-19 (+)	–	0.4036	0.0001
IS COVID-19 (-)		–	0.0001
	Hemoglobin		
	IC COVID-19 (+)	IS COVID-19 (-)	IS COVID-19 (+)
IC COVID-19 (-)	0.0006	0.1622	0.0001
IC COVID-19 (+)	–	0.0367	0.0001
IS COVID-19 (-)		–	0.0001
	HCM		
	IC COVID-19 (+)	IS COVID-19 (-)	IS COVID-19 (+)
IC COVID-19 (-)	0.2779	0.6623	0.4999
IC COVID-19 (+)	–	0.1102	0.0957
IS COVID-19 (-)		–	0.798
	CHCM		
	IC COVID-19 (+)	IS COVID-19 (-)	IS COVID-19 (+)
IC COVID-19 (-)	0.0006	0.0846	0.0001
IC COVID-19 (+)	–	0.0001	0.0007
IS COVID-19 (-)		–	0.0001
	Erythrocytes		
	VCM		
	IC COVID-19 (+)	IS COVID-19 (-)	IS COVID-19 (+)
IC COVID-19 (-)	0.0025	0.0405	0.0079
IC COVID-19 (+)	–	0.0001	0.8589
IS COVID-19 (-)		–	0.0001

Table A3
Resume of p-values of bivariate analysis of demographics by Covid-19 status and Immunosuppression.

Demographic variables		**Covid Status (n)**		**OR (IC95%)**	**P value**
		Yes	No		
Sex	Male	129	60	3.36 [2.25–5.02]	> 0.001
	Female	107	178		
Ethics	Mapuche	75	67	1.23 [0.81–1.87]	0.283
	Other	162	178		
Age	< 40	67	107	0.48 [0.32–0.72]	0.0002
	40 or more	169	131		
Demographic variables		**Immunosuppression (n)**		**OR (IC95%)**	**P value**
		Yes	No		
Sex	Male	72	117	0.65 [0.44–0.96]	0.020
	Female	138	146		
Ethics	Mapuche	55	84	0.75 [0.49–1.15]	0.171
	Other	155	179		
Age	< 40	35	138	0.18 [0.11–0.28]	> 0.001
	40 or more	175	125		

Declaration of Competing Interest

This study was founded by the ANID, the Chilean National Research and Development Agency. The research project ID is COVID0605.

Appendix A

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