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Aberrant innate immune profile associated with COVID-19 mortality in Pretoria, South Africa

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ABSTRACT

The African continent reported the least number of COVID-19 cases and deaths of all the continents, although the exact reasons for this are still unclear. In addition, little is known about the immunological profiles associated with COVID-19 mortality in Africa. The present study compared clinical and immunological parameters, as well as treatment outcomes in patients admitted with COVID-19 in Pretoria, South Africa, to determine if these parameters correlated with mortality in this population. The in-hospital mortality rate for the cohort was 15.79%. The mortality rate in people living with HIV (PLWH) was 10.81% and 17.16% in people without HIV ($p = 0.395$). No differences in age ($p = 0.099$), gender ($p = 0.127$) or comorbidities were found between deceased patients and those who survived. All four of the PLWH who died had a CD4+ T-cell count <200 cells/mm^3, a significantly higher HIV viral load than those who survived ($p = 0.009$), and none were receiving antiretroviral therapy. Seven of 174 (4%) patients had evidence of auto-antibodies neutralizing Type 1 interferons (IFNs). Two of the them died, and their presence was significantly associated with mortality ($p = 0.042$). In the adjusted model, the only clinical parameters associated with mortality were: higher fraction of inspired oxygen (FiO2) (OR: 3.308, $p = 0.011$) indicating a greater need for oxygen, high creatinine (OR: 4.424, $p = 0.001$) and lower platelet counts (OR: 0.203, $p = 0.009$), possibly secondary to immunothrombosis. Overall, expression of the co-receptor CD86 ($p = 0.021$) on monocytes and percentages of CD8+ effector memory 2 T-cells (OR: 0.45, $p = 0.027$) was lower in deceased patients. Decreased CD86 expression impairs the development and survival of effector memory T-cells. Deceased patients had higher concentrations of RANTES ($p = 0.003$), eotaxin (p = 0.003) and interleukin (IL)-8 ($p < 0.001$), all involved in the activation and recruitment of innate immune cells. They also had lower concentrations of transforming growth factor (TGF)-β1 ($p = 0.40$), indicating an impaired anti-inflammatory response. The immunological profile associated with COVID-19 mortality in South Africa points to the role of aberrate innate immune responses.

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1. Introduction

Of all the continents, Africa has reported the lowest number of COVID-19 cases and deaths [1]. A meta-analysis found an overall mortality rate of 4.8% and in-hospital mortality rate to be 4–29% in sub-Saharan Africa and 1.5% in South Africa [2]. Although this could partly be due to underreporting (for instance, South Africa's official death toll secondary to COVID-19 is 2.5–3 times lower than the excess natural deaths reported during the COVID-19 waves [3]), the reasons for the lower mortality in Africa remain elusive. In addition, there is comparatively less immunological data from the African continent, and little is therefore known about the immunological profiles associated with COVID-19 mortality in the African population [4].

A meta-analysis, based on 145 studies (seemingly none of African origin), identified an immune profile associated with COVID-19 mortality that consisted of impaired innate and adaptive immune responses, hypercytokinemia, inflammation, dysfunctional coagulation, and non-pulmonary organ injury [5]. Important hematological and immunological markers included decreased numbers of lymphocytes (B- and T-cells and natural killer cells), monocytes and eosinophils, together with elevated levels of the following cytokines and chemokines: interleukin (IL)-1β, IL-1 receptor antagonist (Ra), IL-2 receptor (R), IL-4, IL-6, IL-8, IL-10, IL-18, tumor necrosis factor (TNF)-α, and interferon gamma (IFN)-γ.

Clinical factors most commonly associated with COVID-19 mortality are older age, assigned male sex at birth, and chronic conditions such as hypertension, diabetes mellitus, and chronic kidney disease [2,6] and can be explained in terms of associated immunological dysfunction. By far the greatest epidemiological risk factor is age, with a risk of hospitalization or death doubling every five years from childhood onward, accounting for a 10,000 greater risk at 85 than 5 years of life (refs). Older individuals are known to have impaired CD4+ and CD8+ T-cell function and clonal diversity, which lead to decreased protection of the host against viral pathogens [2]. In addition, reduced type-2 cytokine responses are found in older individuals which predispose them to have a lasting pro-inflammatory response and, ultimately, worse disease outcomes [2]. Another major risk factor of hypoxemic COVID-19 pneumonia and death is the presence of auto-antibodies neutralizing type I IFNs, at all ages, but particularly in elderly patients. [7–13] It has been proposed that males may be at higher risk of mortality due to having lower CD4+ and CD8+ T-cell counts and lower expression of immunoregulatory genes that are mainly located on the X chromosome [2]. People with chronic kidney disease, as a result of increased oxidative stress, have higher levels of pro-inflammatory cytokines compared to healthy individuals, which could predispose these patients to develop more severe disease [2].

The exact underlying mechanisms of the association of diabetes mellitus with increased mortality in COVID-19 have not been clearly defined, but are postulated to be due to chronic inflammation [14]. Hyperglycemia is associated with impaired lymphocyte proliferation, as well as suboptimal innate immune responses, characterized by defective monocyte/macrophage and neutrophil function [15]. These associations may however, be heavily confounded by the fact that people living with diabetes mellitus are frequently overweight or obese, known risk factors for defective innate and adaptive immunity and hence worse COVID-19 outcomes [16]. Whether hypertension is an independent risk factor of COVID-19 is still controversial [17,18]. Hypertension is most frequently seen in older patients with other comorbidities such as chronic obstructive pulmonary disease, chronic kidney disease, diabetes mellitus, coronary artery disease, and cancer [17]. On the other hand, the chronic use of antihypertensive medications is associated with an increase in the expression of angiotensin converting enzyme 2 (ACE2), which facilitates the entry of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) into cells [18].

The impact of HIV infection on COVID-19 mortality seems to be contextual. Initially, case series and cohort studies reported no increased risk for PLWH co-infected with SARS-CoV-2 [19]. A meta-analysis that evaluated 36 studies from developed and low- and middle-income (LMIC) countries indicated that all PLWH with COVID-19 completely recovered from SARS-CoV-2 infection [20–25]. Conversely, however, a systematic review that included 25 studies from LMIC countries demonstrated an increased risk of SARS-CoV-2 infection and mortality in PLWH [4], while a meta-analysis undertaken by the World Health Organization (WHO) found that HIV had an adjusted hazard ratio of 1.29 (95% CI 1.23–1.35) compared to uninfected populations [26]. More recently, a review of all recorded hospital deaths in PLWH in South Africa showed higher mortality in PLWH across all SARS-CoV-2 variants compared to the population not living with HIV [27]. Since both SARS-CoV-2 and HIV can cause a decrease in CD4+ T-cell counts, it is plausible that COVID-19 progression might be accelerated in PLWH with compromised immunity [28,29]. This question is especially important in South Africa, which is home to one of the world's largest population of PLWH, estimated at 13% and 18.7% in the general and adult populations, respectively [30].

The present study compared clinical and immunological parameters, as well as treatment outcomes, in patients hospitalized with COVID-19 in Pretoria, South Africa, with the purpose of determining the parameters associated with mortality in this population.

2. Materials and methods

2.1. Study population and sample collection

In the current study, patients admitted with COVID-19 were consecutively recruited from May 2020 until December 2021 from Steve Biko Academic and Tshwane District Hospitals in Pretoria, South Africa. For each patient, approximately 20 mL of blood were collected in tubes containing ethylenediaminetetraacetic acid (EDTA) as anticoagulant on the first day of admission, before treatment was commenced. The inclusion criteria were as follows: SARS-CoV-2 polymerase chain reaction (PCR)-positive; 18 years or older; and willing and able to provide informed consent to participate. Samples were processed and flow cytometry performed, and relevant samples were stored on the day of venipuncture. Plasma was stored in sterile tubes at −80 °C until assayed. Results of routine pathology tests were extracted from the National Health Laboratory Service (NHLS) of South Africa Trakcare database. The study was approved by the Faculty of Health Sciences' Research Ethics Committee of the University of Pretoria (ref. 247/2020).

2.2. Monocyte flow cytometry

Fifty microliters of whole blood were added to a flow tube and the red cells lysed by adding 2 mL of lysis buffer (Versalyse: Beckman Coulter Inc., Brea, CA, USA). The sample was incubated, protected from light, for 15 min (min), then centrifuged at 500 xg for 5 min at room temperature. Afterwards the cells were stained with the following extracellular antibodies: CD14-ECD (Beckman Coulter Inc., Brea, CA, USA), CD16-KrO (Beckman Coulter Inc.), CCR2-FITC (Miltenyi Biotec, Bergisch Gladbach, North Rhin, Germany), CD80-AF-750 (Beckman Coulter Inc.), CD86-PC5.5 (Beckman Coulter Inc.), and programmed cell death-ligand 1 (PD-L1) - PC7 (Beckman Coulter Inc.). The excess antibodies were washed off by adding 2 mL of phosphate buffered saline (PBS) containing 2% bovine serum albumin and repeating the centrifugation step. The cells were resuspended in 500 μL PBS containing 0.1% formaldehyde (Beckman Coulter Inc.) and analyzed using a CytoFlex flow cytometer (Model number: A00-1-1102) (Beckman Coulter Inc.).

2.3. T-cell flow cytometry

T-cell phenotypes were investigated using the DuraClone T-cell subset kit (Beckman Coulter Inc.). The dry antibody reagents consisted of the following markers: CD45KO, CD45RA, CD3, CD4, CD8, CD28,

CD27, C—C chemokine receptor type 7 (CCR7), programmed cell death protein 1 (PD-1) and CD57. To the DuraClone T-cell subset tube, 50 μL of whole blood were added and the protocol was followed as per the manufacturer's instructions. After staining, the cells were resuspended in 500 μL of PBS containing 0.1% formaldehyde (Beckman Coulter Inc.) and analyzed using a CytoFlex flow cytometer (Beckman Coulter Inc.).

2.4. Flow cytometry data analysis

Data were cleaned and scaled using Kaluza software and uploaded to the Cytobank platform via the Kaluza Cytobank plugin. Within the Cytobank platform, the dimensionality reduction algorithm, UMAP, was used to provide a more comprehensive visualization of complex multi-parameter data by creating a two-dimensional map of the data. The unsupervised clustering algorithm, CITRUS, was used to identify significant differences in biological signatures between PLWH and people without HIV hospitalized with COVID-19. A detailed description of the flow cytometric analysis and algorithm settings is provided in the supplementary material section 1.1–1.3.

2.5. Evaluation of cytokines, chemokines, and growth factors

Circulating levels of cytokines were determined in plasma samples using a Bio-Plex Human Cytokine/Chemokine Magnetic Bead Panel Kit (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The plasma concentrations of the following cytokines, chemokines, and growth factors were determined: eotaxin, fibroblast growth factor (FGF), granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), IFN-γ, IL-1β, IL-1Ra, IL-2, IL-4, IL-5 IL-6, IL-7, IL-8, IL-9, IL-10, IL-12p70, IL-13, IL-15, IL-17, interferon gamma-induced protein 10 (IP-10), monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory protein (MIP)-1α, MIP-1β, platelet-derived growth factor-BB (PDGF-BB), TNF-α, and vascular endothelial growth factor (VEGF). Plasma samples were diluted four-fold and the experimental procedure was followed as per the manufacturer's instructions. Briefly, magnetic beads (50 μL) were added to each well of the microplate followed by washing of the beads using an automated magnetic microplate wash station (Bio-Rad Laboratories, Inc.). The diluted samples, blanks, range of standards of known concentration, and controls (50 μL) were added to appropriately designated wells of the microplate. The microplate was sealed and incubated in the dark at room temperature for 45 min with gentle agitation (800 rpm) on an orbital plate shaker (Thomas Scientific, Swedesboro, NJ, USA). Following the incubation step, the plate was washed three times using the automated magnetic wash station. Detection antibody (25 μL) was added to each well, the microplate was sealed and incubated for an additional 30 min at room temperature with gentle agitation. Following incubation, the microplate was washed three times and streptavidin-phycoerythrin (50 μL) was added to each well. The microplate was sealed and incubated for a further 15 min at room temperature as described above. The plate was then washed a final three times using the automated magnetic wash station. The beads were resuspended in 125 μL assay buffer and shaken vigorously for two minutes on a Cooke AM69 microplate shaker (Dynatech AG, Bleichestrasse, ZUG, CH). The well contents were assayed using a Bio-Plex Suspension Array platform (Bio-Rad Laboratories, Inc.). Bio-Plex Manager Software 6.0 was used for bead acquisition and analysis of median fluorescence intensity. Results are presented as picograms per milliliter (pg/mL).

2.5.1. Preparation and dilution of: regulated-on activation, normal T-cell expressed and secreted, intracellular-adhesion molecule-1, and transforming growth factor-β1

The regulated-on activation, normal T-cell expressed and secreted (RANTES) concentrations were determined using the Human RANTES enzyme-linked immunosorbent assay (ELISA) kit (*E*-EL-H6006, Elabscience Biotechnology, Inc., Houston, TX, USA). Intercellular adhesion molecule 1 (ICAM-1) levels were measured using the Human ICAM-1/CD54 ELISA kit (*E*-EL-H6114, Elabscience, Biotechnology, Inc.). Samples for both assays were thawed at room temperature and diluted 20-fold before being assayed.

Transforming growth factor-β1 (TGF-β1) levels were determined using the Human TGF-β1 ELISA kit (E-EL-0162, Elabscience Biotechnology, Inc.). Latent TGF-β1 was activated to the immunoreactive form by adding 40 μL 1 N hydrochloric acid to 240 μL plasma (diluted eight-fold). The samples were vortex mixed and incubated for 10 min at room temperature followed by the addition of 40 μL 1.2N sodium hydroxide to neutralize the samples. The samples were mixed well and the assay was performed immediately.

2.5.2. Procedure for sandwich enzyme-linked immunosorbent assays

Levels of RANTES, ICAM-1 and TGF-β1 were determined as per the manufacturer's instructions described briefly below.

The standards and appropriately diluted plasma samples (100 μL) were added to the assigned wells of a 96-well microplate. The microplate was sealed and incubated at 37 °C for 90 min. Following the incubation period, the plate contents were discarded and 100 μL biotinylated detection antibody was added to each well. The microplate was incubated at 37 °C for a further 60 min. The microplate was then washed three times using an automated plate washer (BioTek Instruments, Inc., Winooski, VT, USA) followed by the addition of 100 μL horseradish peroxidase conjugate to each well. The microplate was incubated at 37 °C for 30 min followed by an additional five washes as described above. Substrate reagent, 3,3′,5,5′-tetramethylbenzidine (100 μL), was then added to each well and the microplate was incubated, protected from light, at 37 °C for a further 15 min. The reaction was terminated by the addition of 50 μL stop solution and the optical density read at a wavelength of 450 nm using a PowerWaveX microplate spectrophotometer (BioTek Instruments, Inc.). The concentration of the analyte present in each sample was determined from the generated standard curve and the results are presented as nanograms (ng)/mL.

2.6. Analysis of anti-interferon auto-antibodies

Auto-antibodies neutralizing type-I IFN. Auto-antibody determinations were performed as described by Bastard et al. [8] The blocking activity of anti-IFN-α2, anti-IFN-ω and anti-IFN-β auto-antibodies was determined on the basis of reporter luciferase activity. Briefly, HEK293T cells were transfected with a plasmid containing the firefly luciferase gene under the control of the human ISRE promoter in the pGL4.45 backbone and a plasmid constitutively expressing the Renilla luciferase for normalization (pRL-SV40). Cells were either left unstimulated or were stimulated with IFN-α2 and IFN-ω at a concentration of 10 ng/mL ('high' i.e. supraphysiological concentration) or 100 pg/mL ('low', i.e. physiological concentration), or with IFN-β at a concentration of 10 ng/mL ('high concentration') for 16 h at 37 °C. Neutralizing samples showed an induction lower than 15% of that of controls.

2.7. Data management and statistical analysis

Clinical information was captured from patient files and entered into an excel spreadsheet. Results of routine laboratory tests were obtained from the NHLS. Data were exported to IBM SPSS Statistics version 28 and Stata 17 for analysis. Data were assessed for distribution and appropriate tests applied. The student's *t*-test and Mann-Whitney test were used to compare continuous variables between groups, while Pearson's chi-square and Fisher's exact test were used for univariate comparison of categorical variables. Stepwise, backward, multivariable logistic regression analysis was used to examine associations with outcome after appropriate transformation of predictor variables. Spearman's correlation test, with Bonferroni correction for multiple comparisons, was used to assess correlations between continuous

variables.

3. Results

3.1. Clinical characteristics

A total of 178 patients were recruited into the study, of whom four were excluded due to the absence of a positive SARS-CoV-2 PCR test. The mean age of patients in this cohort was 52 (SD ±14) years and just over half (53.5%) identified as male. The majority (121/174–69.5%) identified as Black African, followed by White (44/174–25.3%), Colored (3/174–1.7%), Indian (3/174–1.7%) and Asian (1/174–0.6%), and data had not been recorded in two patients. Comorbidities were common: hypertension (42.8%), diabetes mellitus (34%), heart disease (14.5%), kidney disease (9.9%), lung disease (8.9%), and cancer (3.1%). Of the group, 37 (21%) were PLWH. Of those with disease severity recorded, three-quarters (112/150) had moderate to severe disease. A total of 27/171 (15.8%) patients died and the outcome of three patients is unknown.

No differences were found in age ($p = 0.099$), gender ($p = 0.127$), or the prevalence of hypertension ($p = 0.949$), diabetes mellitus ($p = 0.851$), or heart disease ($p = 0.100$) between those who survived and those who demised (Supplementary Table 1). Due to limited access to specialized scans, only ten cases of clinically detectable thrombotic events were reported (8/10 were in people without HIV, 2/10 were in PLWH). A total of 16 patients had thrombocytopenia: 4 PLWH with and 12 without HIV ($p = 0.714$). Differences in relevant clinical markers and routine laboratory test results between those who died and survived are presented in Table 1 and the full list of results is shown in Supplementary Table 2.

The mortality rate in the PLWH group was 10.81% (4/37) compared to 17.16% (23/134) in people not living with HIV, however, the difference was not statistically significant ($p = 0.668$). The median CD4+ T-cell count of PLWH who died was 29 (IQR 20–44) cells/mm^3 compared with 258 (IQR 119–396) cells/mm^3 in PLWH who survived ($p = 0.015$). All four of the PLWH who died had a CD4+ T-cell count <200 cells/mm^3 while none of those who survived had such severe immunodeficiency (4/37 [10.81%] versus 0/37 [0%]; $p = 0.0450$). PLWH who died also had a significantly higher HIV viral load (VL) (64,116 [IQR 13,139–64,841] vs 20 [IQR 20–1168] copies/mL; $p = 0.009$). None of the four PLWH who demised were receiving antiretroviral therapy.

A multivariable regression model, with mortality as binary outcome variable, revealed that the only clinical parameters remaining significantly associated with mortality were: FiO2, creatinine, and platelet count (Table 2). FiO2 and creatinine were higher, and platelet counts lower, in the deceased patients.

3.2. Anti-interferon auto-antibodies

Of the 174 patients tested, seven (4%), including two patients who demised, had evidence of auto-antibodies neutralizing Type 1 IFNs (Table 3). Two patients harbored auto-antibodies against IFN-α2, while two patients had auto-antibodies against IFN-ω only, one against IFN-α2 and IFN-ω, and two against IFN-α2, IFN-β, and IFN-ω. Three patients each had severe (8.8% of those with severe disease and 1.72% of the total number of 174 patients) or moderate disease (3.8% of those with moderate disease and 1.72% of the total number of 174 patients), and one patient had mild disease (2.6% of those with mild disease and 0.57% of the total number of 174 patients). There were no differences between patients with and without auto-antibodies in terms of age (52.3 ± 15 versus 51.8 ± 14 years; $p = 0.7652$), gender (4/92 [4.35%] men and 3/82 [3.66%] women; $p = 0.564$), population affinity ($p = 0.103$), disease severity ($p = 0.512$), or being HIV positive (2/37 [5.4%] versus 5/137 [3.65%]; $p = 0.641$). Even though the numbers were small, patients who had demised were significantly more likely to have auto-antibodies than patients who survived: 2/25 (8%) versus 4/140 (2.9%); $p = 0.042$. The risk of dying was more than double in patients with anti-IFN auto-antibodies when compared with patients without antibodies: 2/6 (33.3%) versus 25/167 (15%).

Table 1
Clinical markers and routine laboratory tests by outcome in patients with COVID-19.

Variable	Deceased ($n = 27$)	Survived ($n = 134$)	p-value
AST (U/L)	54 (28–89)	39 (29–61)	**0.038**
Creatinine (μmol/L)	95.5 (73–135)	72 (63–98)	**0.003**
D-dimer (mg/L)	1.08 (0.59–4.46)	0.64 (0.42–1.48)	**0.043**
eGFR (mL/min/1.73m^2)	68 (37–98)	93 (66–107.5)	**0.006**
Eosinophil (%)	0 (0–0.05)	0.1 (0–0.45)	**0.008**
Eosinophil: lymphocyte ratio	0 (0–0.002)	0.008 (0–0.037)	**0.015**
Ferritin (ug/L)	2729 (684–3928)	594 (236–1123)	**0.004**
FiO2(%)	0.7 (0.28–0.95)	0.4 (0.21–0.7)	**0.011**
HCO_3 (mmol/L)	16 (15–19)	19 (18–22)	**<0.001**
INR	1.18 (1.11–1.45)	1.13 (1.04–1.19)	**0.026**
Lactate (mmol/L)	2.25 (1.6–3.2)	1.35 (0.9–1.7)	**<0.001**
Lymphocyte count (x10^9/L)	0.75 (0.68–0.93)	1.22 (0.81–1.84)	**0.001**
Monocyte: lymphocyte ratio	0.55 (0.32–0.82)	0.37 (0.26–0.50)	0.073
Neutrophil: lymphocyte ratio	7.89 (4.7–12.78)	5.77 (2.89–8.77)	**0.035**
Neutrophil: platelet ratio	3.76 (2.53–4.33)	2.35 (1.65–3.25)	**0.014**
PaO2/FiO2	108.5 (79.05–207.25)	128.75 (84.84–234.29)	0.545
PCT (ug/L)	0.25 (0.08–2.45)	0.11 (0.05–0.3)	**0.010**
Phosphate (mmol/L)	1.2 (0.97–1.51)	0.89 (0.71–1.08)	**<0.001**
Platelets (x10^9/L)	232 (188–277)	273 (207–343)	**0.013**
SaO2/FiO2	115.5 (71.78–254.64)	192.98 (100.63–334.82)	0.193
Saturation on oxygen (%)	91.5 (90–95)	95 (92–98)	**0.015**
Saturation on room air (%)	84 (75–88)	90 (83.5–96)	**0.038**
Sodium (mmol/L)	133.5 (132–137)	136 (134–139)	**0.018**
Troponin I (ng/L)	65 (14–220)	10 (10–24)	**<0.001**
Urea (mmol/L)	8.25 (5.6–18.3)	5.1 (3.6–8.0)	**<0.001**

All variables are shown as median (interquartile range) except for eGFR category which is shown as numerator/denominator (percentage). Not all variables were available for all participants.
Abbreviations: aspartate aminotransferase (AST), estimated glomerular filtration rate (eGFR), fraction of inspired oxygen (FiO2), bicarbonate (HCO_3), international normalized ratio (INR), Partial pressure of oxygen, (PaO2), procalcitonin (PCT), arterial oxygen saturation (SaO2).
Values in bold were statistically significant.

Table 2
Multivariable logistic regression model of clinical characteristics associated with mortality in patients with COVID-19.

Outcome	Odds ratio	Standard error	z	$P > z$	95% Confidence interval	
FiO2	3.308	1.554	2.55	**0.011**	1.317	8.308
Platelets	0.203	0.124	−2.61	**0.009**	0.061	0.674
Creatinine	4.424	1.992	3.30	**0.001**	1.830	10.69
Constant	3.731	12.878	0.38	0.703	0.004	3233.154

Abbreviations: Fraction of inspired oxygen (FiO2).
Model characteristics: $n = 124$, LR chi2(3) =21.59, Prob<0.001, Pseudo R2 (0.191), Log likelihood = −45.61, goodness of fit $p = 0.251$.

3.3. Monocyte and T-cell profiles by outcome

The only significant difference, in terms of the monocyte populations between deceased patients and those who survived, was the overall expression of the co-receptor, CD86, which was lower in deceased

Table 3
Characteristics and outcomes of patients with anti-interferon auto-antibodies.

	Age (years)	Gender	Population affinity	Co-morbidity	IFN antibody	Disease severity	Complications	Outcome
1	55	Male	Black	HT, DM	IFN-ω	Severe	Acute respiratory distress syndrome	Survived
2	63	Male	White	HT, heart disease, lung disease	IFN-α2, −β, −ω	Severe		Survived
3	69	Female	White	HT, DM (newly-diagnosed), heart disease	IFN-α2	Moderate		Unknown
4	39	Female	Black	HIV (not on ART)	IFN-ω	Moderate	Anemia	Deceased*
5	46	Female	Black	HIV (on ART)	IFN-α2, −ω	Mild		Survived
6	66	Male	Asian	HT	IFN-α2	Moderate	Right pulmonary artery thrombosis	Survived
7	28	Male	Black		IFN-α2, −β, −ω	Severe		Deceased

Abbreviations: antiretroviral therapy (ART), diabetes mellitus (DM), hypertension (HT), interferon (IFN).
* PLWH with uncontrolled HIV that died due to cryptococcal meningitis.

patients (99.74% [IQR: 95.62–99.93] vs 99.90% [IQR: 99.57–99.97], $p = 0.021$). The analysis comparing the monocyte populations by outcome is shown in Supplementary Table 3.

On univariate analysis, no significant associations were found between T-cell populations and mortality (Supplementary Table 4). However, stepwise backward logistic regression analysis revealed several T-cell populations of interest (Table 4). Patients who demised were more likely to have increased percentages of CD4+ effector memory 4 T-cells (EM4: CD45RA-CCR7-CD28 + CD27-) expressing PD-1, as well as decreased percentages of CD8+ effector memory 2 (EM: CD45RA-CCR7-CD27 + CD28-) and, albeit just missing significance, CD8+ end stage effector (E: CD45RA + CCD7-CD28-CD27-) terminally differentiated T-cells re-expressing CD45RA that expressed the exhaustion marker CD57.

3.4. Cytokine, chemokine, and growth factor profiles by outcome

Multiple differences were found between the cytokine, chemokine, and growth factor profiles of the outcome groups (Table 5). Deceased patients had significantly higher concentrations of RANTES, IL-1β, IL-1Ra, IL-6, IL-8, eotaxin, G-CSF, MCP-1/MCAF, MIP-1α, and TNF-α. The results of the full list of markers tested are shown in Supplementary Table 5.

In the multivariable logistic regression model (Table 6), higher concentrations of RANTES, eotaxin and IL-8 were associated with mortality. In addition, albeit just missing significance in the univariate analysis ($p = 0.051$), the pleiotropic cytokine, TGF-β1, was significant in the multivariate model with lower concentrations associated with mortality.

Table 4
Multivariable logistic regression model of T-cell populations associated with mortality in patients with COVID-19.

Outcome	Odds Ratio	Standard error	z	P > z	Confidence interval	
CD8 + EM2	0.348	0.128	−2.86	**0.004**	0.169	0.718
CD8+ TEMRA E CD57+	0.372	0.189	−1.94	0.052	0.137	1.008
CD4 + EM4 PD-1+	2.240	0.815	2.22	**0.027**	1.098	4.571
Constant	16.624	41.412	1.13	0.259	0.126	2193.623

Abbreviations: Effector memory (EM), programmed cell death protein 1 (PD-1), terminally differentiated T-cells re-expressing CD45RA (TEMRA).
Model characteristics: $n = 149$, LR chi2(4) =14.46, Prob = 0.002, Pseudo R2 (0.113), Log likelihood = −56.87, goodness of fit $p = 0.356$.

Table 5
Cytokines, chemokines and growth factors by COVID-19 outcome.

Biomarker	Deceased (n = 27)	Survived (n = 134)	*p*-value
Eotaxin	34.16 (24.37–42.57)	22.03 (15.68–30.6)	**<0.001**
G-CSF	200.93 (146.42–291.77)	147.73 (100.88–220.33)	**0.012**
IL-1β	2.86 (1.84–3.68)	1.95 (1.46–2.66)	**0.018**
IL-1Ra	1089.68 (707.11–1603.61)	823.25 (606.29–1046.00)	**0.008**
IL-4	4.7 (3.67–6.73)	4.14 (3.07–5.39)	0.064
IL-6	8.19 (2.86–31.69)	4.055 (1.36–9.51)	**0.003**
IL-8	27.4 (16.68–45.03)	17.335 (9.70–22.96)	**<0.001**
IP-10	2315.22 (952.33–4685.26)	733.19 (409.7–1512.16)	**<0.001**
MCP 1	50.91 (27.77–142.83)	25.57 (14.47–38.68)	**<0.001**
MIP-1α	4.41 (3.04–5.98)	3.265 (2.36–4.61)	**0.005**
RANTES	122.96 (66.31–269.04)	78.78 (41.91–150.50)	**0.014**
TGF-β1	6.44 (5.07–9.63)	8.54 (5.92–12.24)	0.051
TNF-α	95.47 (78.66–134.25)	83.87 (67.76–102.39)	**0.022**

All variables are shown as median (interquartile range) in pg/mL with the exceptions of RANTES and TGF-β1which are expressed in ng/mL.
Abbreviations: Granulocyte colony-stimulating factor (G-CSF), interleukin (IL), IL-1 receptor antagonist (IL-1Ra), interferon gamma-induced protein 10 (IP-10), macrophage inflammatory protein-1α (MIP-1α), monocyte chemoattractant protein 1 (MCP-1), regulated-on activation, normal T-cell expressed and secreted (RANTES), transforming growth factor-β1 (TGF-β1), tumor necrosis factor-α (TNF-α).

3.5. Correlations between clinical and immunological markers by outcome

Correlograms comparing the associations between clinical and immunological parameters overall, as well as within each group, are shown in Fig. 1. In deceased patients, strong negative correlations were found between IL-8 and platelet counts ($r = -0.72$, $p = 0.019$), CD86 and creatinine ($r = -0.78$, $p = 0.008$), monocyte to lymphocyte ratio (MLR) and estimated glomerular filtration rate (eGFR) ($r = -0.54$, $p = 0.029$) and between CD4 + EM4 PD-1+ and FiO2 ($r = -0.71$, $p = 0.021$), as well as MLR ($r = -0.65$, $p = 0.040$). Strong positive correlations were found between CD8 + EM and eotaxin ($r = 0.74$, $p = 0.014$), CD4 + EM4 PD-1+ and IL-8 ($r = 0.75$, $p = 0.007$), TGF-β1 and eosinophils ($r = 0.86$,

Table 6
Multivariable logistic regression model of cytokines associated with mortality in patients with COVID-19.

Outcome	Odds Ratio	Standard error	Z	P > z	95% Confidence interval	
RANTES	2.86	1.021	2.95	**0.003**	1.423	5.759
IL-8	6.63	3.302	3.80	**<0.001**	2.499	17.598
TGF-β1	0.43	0.177	−2.05	**0.040**	1.683	13.933
Eotaxin	4.84	2.61	2.93	**0.003**	0.190	0.962
Constant	1.30e-07	4.24e-07	−4.87	**<0.0001**	2.20e-10	7.0e-5

Abbreviations: Interleukin (IL), regulated-on activation, normal T-cell expressed and secreted (RANTES), transforming growth factor-β1 (TGF-β1), tumor necrosis factor-α (TNF-α).
Model characteristics: $n = 170$, LR chi2(4) =47.98, Prob<0.001, Pseudo R2 (0.322), Log likelihood = −50.42, goodness of fit $p = 0.831$, 86.47% correctly classified.

$p = 0.001$) and between MLR and creatinine ($r = 0.69$, $p = 0.003$) and urea ($r = 0.72$, $p = 0.002$). These correlations were absent in patients who survived.

Within the group that survived, the only strong correlation was a negative correlation between CD86 and eosinophils ($r = -0.86$, $p < 0.001$). The weak correlations that were found within people who have survived are: TGF-β1 was negatively correlated with eotaxin ($r = -0.36$, $p = 0.026$) and CD8 + EM ($r = -0.33$, $p = 0.044$), CD86 was negatively correlated with CD8 + EM ($r = -0.32$, $p = 0.048$), CD4 + EM4 PD-1+ ($r = -0.35$, $p = 0.030$) and the MLR ($r = -0.38$, $p = 0.018$), IL-8 was positively correlated with eotaxin ($r = 0.33$, $p = 0.046$) and eosinophils ($r = 0.41$, $p = 0.010$), MLR was positively correlated with NLR ($r = 0.47$, $p = 0.003$) and TGF-β1 was positively correlated with RANTES ($r = 0.45$, $p = 0.004$) and FiO2 ($r = 0.32$, $p = 0.047$).

4. Discussion

The present study compared clinical and immunological parameters in patients admitted with COVID-19 in Pretoria, South Africa, to determine if these parameters were associated with mortality in this population. Patients' clinical profile was generally similar to what has been reported in South Africa, as well as globally, in settings with an overall high burden of comorbidities [19,29] However, in contradiction to several previous studies [31–33], people who identified as male were not associated with an increased risk of mortality. This could be secondary to the age of the patients; as in the aforementioned studies, male patients were considerably older than in the present study.

The mortality rate among PLWH in this study (11.1%) was slightly lower than that reported by other South African studies (15%–25%) [19,29] and also, albeit non-significant, lower than in people without HIV. Jassat et al. reported an increased risk of mortality among PLWH

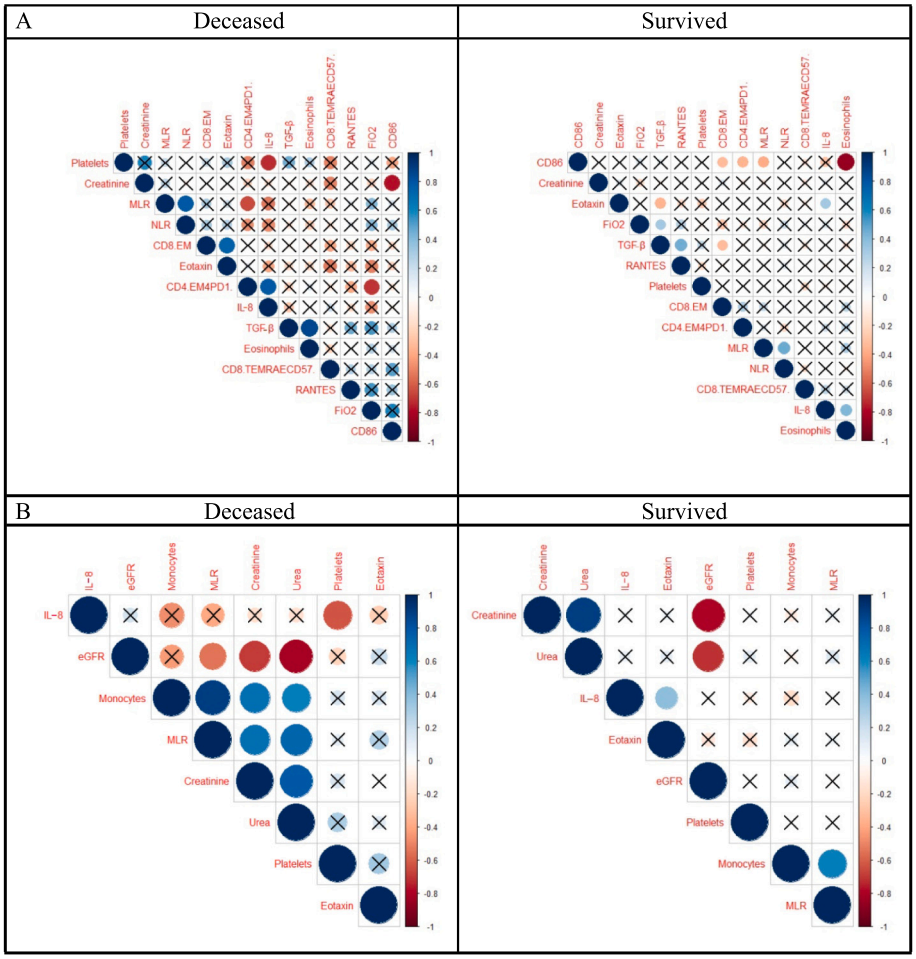


Fig. 1. Spearman correlations of significant clinical and immunological markers within the patients who survived and those that died from COVID-19. A: Correlations of significant immunological markers within each group (deceased and survived). B: Correlations of clinical markers and IL-8 within each group (deceased and survived).
Abbreviations: Effector memory (EM), estimated glomerular filtration rate (eGFR), (FiO2) fraction of inspired oxygen, interleukin (IL), programmed cell death 1 (PD-1), regulated-on activation, normal T-cell expressed and secreted (RANTES), terminally differentiated T-cells re-expressing CD45RA (TEMRA), transforming growth factor-β1 (TGF-β1), tumor necrosis factor-α (TNF-α).

with a CD4+ T-cell count of below 200 cell/μL, which is in line with the findings of the current study [27]. Improved survival in our study is likely due to the relatively higher CD4+ T-cell counts and low prevalence of *Mycobacterium tuberculosis* infection and diabetes mellitus, factors known to be associated with mortality [34–36]. A meta-analysis conducted by the WHO found that HIV was an independent risk factor for mortality, but only in the WHO African region and not in the WHO European or the WHO Region of the Americas, after adjusting for age, sex assigned at birth, underlying conditions, and clinical presentation at hospital admission. In addition, after excluding data from South Africa, the risk was no longer significantly higher. These differences highlight the important impact of the context and clinical profile of PLWH on treatment outcomes.

In line with international research [9,10,13], patients in our study with evidence of auto-antibodies to type I IFNs (IFN-α, IFN-β, IFN-ω) were significantly more likely to demise during their admission with COVID-19. The prevalence of anti-IFN auto-antibodies (4% in the total group; 8.8% of those with severe disease; and 7.4% of those who demised) was lower than that reported by others. For instance, Manry et al. found that auto-antibodies were present in 20% of patients who succumbed to COVID-19 across all age groups [13]. In the general population, auto-antibodies are present in about 1% of those younger than 70 years of age and higher than 4% in those older than 70 years of age [13]. The proportion of male patients with auto-antibodies is reported to be greater than female patients [13]. Interestingly, both of the patients in the current study were younger than 40 years of age and only one patient was male. While some groups have found a very high prevalence of anti-IFN antibodies in PLWH with acute COVID-19 [37], we found no difference between patients with and without HIV infection.

In the current study, FiO2 was significantly higher in those individuals who succumbed to SARS-CoV-2. FiO2 is the estimation of the amount of oxygen that is inhaled, indicating the gaseous exchange at the alveolar level [38]. A normal FiO2 at room air is 0.21 [38]. A lower SaO2/FiO2 and PaO2/FiO2 ratio at admission has been shown to predict the development of acute respiratory distress syndrome (ARDS) and, ultimately, mortality [39]. In this cohort, patients who died had lower SaO2/FiO2 and PaO2/FiO2 ratios, but not significantly so when compared to patients who survived.

Yang et al. reported a U-shaped association between platelet count and mortality for patients hospitalized with COVID-19: mortality rates were 34.6%, 19.1%, 12.5% and 14.4% for those with platelet counts of $<100 \times 10^9$/L, 200–300 $\times 10^9$/L, 300–400 $\times 10^9$/L and $> 400 \times 10^9$/L, respectively [34]. These findings correlate well with the overall mortality rate for the current study, which was 15.8%, with a median platelet count of 232 $\times 10^9$/L (IQR 188–277) found in deceased patients.

In the current study, a strong negative correlation was found between platelet count and IL-8 in those individuals who died. Quah et al., investigating the relationship between the release of IL-8, activated platelets and leukocytes, found that IL-8 is mainly released by monocytes, but is potentiated in a thrombin-dependent manner by activated platelets [40]. These results suggest that IL-8 concentrations increase in the presence of activated platelets. Thrombocytopenia, which is associated with increased platelet consumption, has been linked to COVID-19 [41]. Generally, during viral infections, and also shown during SARS-CoV-2 infection, platelet consumption is caused by coagulation due to inflammation, phagocytosis, and hypersplenism that leads to sequestration, while platelet production is impaired due to flawed megakaryopoiesis or myelosuppression due to hypercytokinemia [42]. In addition, virus-induced platelet activation may lead to platelet exhaustion and clearance from the circulation [41,43] and is often associated with bleeding abnormalities [44–46]. This could explain the high levels of IL-8, lower platelet counts and hypercoagulation, as measured by D-dimer and the international normalized ratio (INR), seen in deceased patients in this study.

Routine laboratory markers that were increased in those individuals who succumbed to SARS-CoV-2 were urea and creatinine, while the estimated glomerular filtration rate (eGFR) decreased in tandem. Creatinine is a chemical waste product of creatine that results from muscle breakdown and protein metabolism in the body, and is excreted in the urine. Creatinine levels are known to increase when there is a decrease in creatinine clearance [47] and elevated levels are used to diagnose acute kidney injury. High creatinine levels at admission, and which increase over the period of hospitalization, have been associated with more severe COVID-19, as well as mortality [48–50]. Acute kidney injury is known to be a common complication in COVID-19 and it has been estimated to be present in 46% of hospitalized patients [50]. Other routine laboratory parameters that were reported to be elevated in deceased patients in China, along with creatinine, were aspartate aminotransferase (AST), D-dimer, procalcitonin and lactate, which is in line with the results presented here [48]. In addition, high creatinine levels were associated with higher leukocyte counts and lower lymphocyte counts at admission [48]. Deceased patients had a higher MLR compared to patients who survived. The median MLR for deceased patients was 0.55 and strongly correlated with eGFR, urea and creatinine in deceased patients [48]. Chen et al. reported that renal injury was significantly increased in patients with an MLR above 0.3 [48,51]. A higher MLR along with higher concentrations of creatinine and urea and lower eGFR in deceased patients could be indicative of the development of acute kidney injury in the patients who died.

The only monocyte marker that differed significantly between the groups was CD86, with lower levels seen in deceased patients. CD86, along with CD80, are co-stimulatory molecules on the surface of monocytes that enable monocytes to activate T-cells by engaging with CD28 on the surface of CD4+ and CD8+ T-cells [52]. CD86 is constitutively expressed on the surface of monocytes, whereas CD80 is only upregulated when an immune response is triggered, thus CD86 is present from the start of immune recognition [53]. Once antigen-presenting cells (APC) interact with T-cells, a second signal is transmitted between CD86 and CD28, which is facilitated by the formation of microdomains consisting of multiple kinases that associate with the T-cell receptor and mediate signal transduction [53]. Only later does the up-regulated CD80, which has a slower but sustained binding to ligands on CD4+ T-cells, enhance the co-stimulatory signal [53]. A co-stimulatory signal, along with engagement of the T-cell receptor, is essential for T-cell expansion, survival and the development of effector functions [52]. CD86, and not CD80, is believed to be the main co-stimulatory molecule for T-cell activation by dendritic cells in vitro [52]. Lower CD86 expression at the beginning of immune recognition causes reduced functioning of the microdomains that are essential for the activation of T-cells, which leads to an impaired effector response [52,53]. Another mechanism by which reduced CD86 expression affects effector function is the downregulation of the production of IL-2, which is essential for the development and survival of effector memory T-cells [54]. IL-2 and costimulatory molecules work in synergy, resulting in an optimal T-cell response to a pathogen [55]. The binding of CD86/CD80 to CD28 increases the production of IL-2 by activating the mitogen-activated protein kinase, calcium-calcineurin and nuclear factor-κB pathways [53,55,56]. T-cells stimulated with CD86-deficient APCs produce less IL-2, IL-4 and IFN-γ, leading to impaired T-cell cytotoxic function and proliferation [52,57]. Isotype class switching in germinal centers is also impaired after antigenic challenge, indicating an impaired humoral and cellular immune response in the absence of CD86 expression [52,53]. T-cells that are activated without a stimulation signal become anergic [56]. In the current study, taking all these effects into account, the lower percentages of CD8 + EM2 and CD8+ end stage TEMRA cells are therefore likely to be indicative of impaired effector response to SARS-CoV-2, and hence associated with mortality.

Interestingly, CD86 was strongly and negatively correlated with creatinine in patients who died in the current study. It is known that monocyte counts correlate with renal dysfunction, but the exact interaction between CD86 expression and creatinine needs to be explored

further [58].

Three stages are involved in CD4+ and CD8+ T-cell differentiation upon antigen exposure [59]. The first step is clonal expansion (first 0–7 days of infection) during which activated antigen- specific T-cells differentiate into effector cells and ultimately result in viral clearance [59,60]. The next step is contraction (8–15 days after infection) [60]. At this stage, T-cells die by apoptosis [59]. Memory formation is the last stage, with a small fraction of T-cells surviving and becoming long-lived in order to protect against reinfection [59,60]. Various subsets of T-cells can be found in the blood, including: naïve T-cells that can respond to antigens from novel pathogens, as well as central memory (CM), EM, and TEMRA T-cells, which evolve from previous antigen exposure and can maintain long-term immunity as well as rapidly eliciting a protective response [59]. CM cells are highly proliferative with tissue homing characteristics [59]. Upon T-cell receptor activation, CM cells release high quantities of IL-2 and can differentiate into EM T-cells and effector T-cells [61]. On the other hand, EM cells respond immediately, expressing high levels of IFN-γ and perforin through the interaction of the Fas ligand, creating pores in the target cells, resulting in apoptosis [60,61]. A larger population of TEMRA cells are found within the CD8+ T-cell lineage [59]. TEMRA cells proliferate poorly, but exhibit strong cytotoxic capacity [59]. An exhausted T-cell phenotype has previously been described during SARS-CoV-2 infection [60]. These T-cells express cytotoxicity-related genes and have strong cytotoxic abilities [60].

CD4+ T-cells enhance the expression of the costimulatory CD86/CD80 on APCs through CD40/CD40L signaling, consequently aiding the activation of CD8+ T-cells [60]. In the current study, higher percentages of CD4 + EM4 cells expressing PD-1 were associated with mortality. PD-1 is an inhibitory immune checkpoint present on the surface of all activated T-cells [62]. The marker also has an important role in fine-tuning T-cell differentiation and effector functions and the development of immunological memory [63]. The immunoregulatory role of PD-1 comes into effect when the marker interacts with PD-L1 on the surface of monocytes, leading to the deactivation of T-cells, thus limiting immune-mediated tissue damage during infection [63]. Interestingly, Saris et al. found that increased activated CD4+ EM4 T-cells in peripheral blood were associated with mortality [64]. This may underscore the necessity to control inflammation during COVID–19-infection. In addition, deficient CD8+ T-cell activation has been linked to a worse outcome in SARS-CoV-2 infection [65]. This supports the association between higher percentages of activated CD4 + EM4 and lower percentages of CD8 + EM and CD8+ end stage TEMRA cells and mortality found within this cohort.

While the adaptive immune system plays an important role in COVID-19-related mortality, and hyperinflammation is strongly associated with increased concentrations of Th1 and Th2 cytokines and chemokines as well as acute phase inflammatory proteins [5,66], the immunological signature that was associated with mortality in patients with COVID-19 in the current study point to the importance of the innate immune system. RANTES attracts and activates basophils, eosinophils, natural killer cells, memory T-cells, dendritic cells and monocytes [67–70] RANTES is also known to play an important role during viral infections, particularly HIV, since it binds to CCR5 thus competing with the virus for binding [68,69]. During respiratory infections, RANTES plays a crucial role in the migration of dendritic cells to the draining lymph node, enhancing the speed of recruitment of T-cells to the lungs and the survival of alveolar macrophages [68]. In the present study, RANTES, IL-8 and eotaxin, which are chemokines that attract and activate innate immune cells, were all significantly associated with mortality. In contrast to this, however, Balnis et al. and Zhao et al. found that RANTES was negatively associated with mortality and suggested that it might have a protective effect in COVID-19 infection [69,71]. RANTES is known to be produced by CD8+ T-cells when stimulated with antigens and it has been reported that clonal expansion of CD8 + T-cells in broncho-alveolar fluid was associated with milder COVID-19 [69,72]. Zhao et al. proposed that an adequate T-cell response in mild cases of the disease could be essential to clear SARS-CoV-2 before lung inflammation could commence [69]. The RANTES results presented here could therefore be a consequence of lower CD8+ T-cell responses in our cohort.

Since the start of the pandemic, eosinopenia ($<0.01 \times 10^9$/L) has been reported in patients hospitalized with COVID-19, and shown to be correlated with markers of coagulation, as well as liver and kidney damage [73,74]. On the other hand, higher eosinophil counts have been associated with mild disease as well as a lower incidence of complications and mortality [74]. Eosinophils are known to infiltrate the lungs in response to pneumonia-related pathogens, impacting both viral clearance and the pathogenesis (exacerbation of tissue damage and inflammation) of the infection [73]. The mechanisms by which eosinophils participate in viral clearance include: the expression of various Toll-like receptors (TLR), such as TLR3, 7 and 9 that can recognize viruses, the secretion of IFN-β, IL-6, IP-10, interferon regulatory factor 7 (IRF7) and ribonucleases from granules and, lastly, by inhibiting viral replication through the production of oxidizing agents such as nitric oxide [74]. Eosinophils have also been discovered to have an immunomodulatory role by interacting with cellular and humoral mediators in the innate and adaptive immune systems [75]. These cells can act as APCs by travelling to draining lymph nodes subsequently activating T-cells by expressing various co-receptors such as CD40, CD80 and CD86 [74,76]. While eosinophils are generally beneficial during respiratory viral infections by acting as APCs and direct viral interaction, they also have the ability to trigger a harmful inflammatory response in the respiratory tract [74,76]. Possible causes of eosinopenia include: recruitment into the lung, the initiation of eosinophil apoptosis, inhibition of eosinophil outflow, impaired eosinopoiesis in the bone marrow, and decreased production of chemokines, such as eosinophil chemotactic factor and, possibly, eotaxin [73]. Eotaxin is chemotactic for eosinophils, but not for mononuclear cells or neutrophils [73,75]. A study done in guinea pigs showed that eotaxin is the main eosinophil attractor in the lungs [73].

In addition, a weak positive correlation was found between the eosinophil count and IL-8 concentrations within surviving patients, which was absent in those individuals who died. Eosinophils can secrete IL-8, aiding in the recruitment of neutrophils to the site of infection [77]. The role of eosinopenia in COVID-19 is still poorly understood and requires further research.

Finally, TGF-β1 is a critical anti-inflammatory cytokine, which controls the magnitude and type of immune response against microbes [78,79]. In patients who develop COVID-19-related ARDS, TGF-β1 has been shown to accumulate in the lungs during the early infection period [79]. Inflammatory signals can trigger the release of activated TGF-β1 from latent stores in the lung during the initial stages of ARDS [80]. The released TGF-β1 then initiates a cascade of events, eventually leading to the stabilization of hypoxia inducible factor alpha, which, in turn, causes endothelial permeability [80]. Increased endothelial cell permeability results in leakage, ultimately causing edema, impaired cell function, and injury to ischemic tissue [81,82]. Conditions under which increased endothelial permeability has been shown to occur are ARDS due to COVID-19 infection, arthritis, asthma and other chronic inflammatory conditions [81]. Low levels of TGF-β1 could also predispose to hypercytokinemia. A study conducted on TGF-β1-null mice showed that mice died due to multiorgan inflammation, including lung inflammation, with characteristics of ARDS [80]. These results show the importance of TGF-β1 in controlling lung inflammation, specifically, as well as inflammation in general [80]. Our group, and others, have now shown lower TGF-β1 levels to be associated with mortality in COVID-19 [79].

The pattern of cytokines associated with mortality in our study differs from those reported from developed world contexts where circulating levels of IL-1β, −2 receptor, −4, −6, −8, −10 and − 17, indicative of a predominant T-helper-1 response, were the main risk factors for mortality [83]. These findings have important implications for management of COVID-19 in diverse settings. While IL-6 receptor blocking therapy has become the mainstay of immunomodulatory treatment of

COVID-19 in high-income countries, a recent clinical trial testing the efficacy of the IL-6 monoclonal antibody, tocilizumab, as a treatment option for COVID-19 in an African population found that patients treated with tocilizumab had higher rates of secondary infections compared to those not receiving the drug (17.2% versus 6%) [84]. The study also found no significant improvement in the mortality rate of COVID-19 patients receiving tocilizumab [84]. These results suggest that IL-6 therapy might not be as effective in the African context, a contention supported by the findings of our study.

The main limitation of the current study is that the immunological variables were only studied in peripheral blood and not in the lung or other tissue types. In addition, during the pandemic, due to the strain of a large influx of COVID-19 admissions and short-staffed hospital environments, some of the clinical records are incomplete, resulting in a smaller sample size available to test correlations between the clinical and immunological factors.

5. Conclusion

In conclusion, the current study of hospitalized patients with COVID-19 demonstrated a relatively low mortality and good outcomes overall, both in people with and without HIV infection. The clinical factors most associated with mortality were a lower platelet count possibly due to immunothrombosis, increased creatinine and a greater need for oxygen therapy. The immunological profile associated with COVID-19 mortality in South Africa points to the role of the innate immune system, which is different from that reported in developed countries. This could have implications for treatment. Immunological factors associated with mortality were increased concentrations of RANTES, IL-8 and eotaxin, all of which are involved in the activation and recruitment of predominantly innate immune cells, with decreased TGF-β1 indicating an impaired anti-inflammatory response.

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CRediT authorship contribution statement

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Declaration of competing interest

Authors declare that they have no conflict of interest.

Data availability

The datasets analyzed for this study can be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clim.2024.110323.

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