Inflammation Research (2023) 72:929–932
https://doi.org/10.1007/s00011-023-01726-y

Inflammation Research

ORIGINAL RESEARCH PAPER

Reduction in the number of neutrophils in the broncho-alveolar aspirate is associated with worse prognosis in elderly patients with severe COVID-19

Raquel Duque Nascimento Arifa[1] · Marcela Helena Gonçalves-Pereira[2] · Luciana Santiago[2,4,5] · Cecilia Gómez Ravetti[5,6] · Paula Frizera Vassallo[4,5] · Fernanda de Fátima Souza Oliveira[7] · Adriano de Paula Sabino[7] · Mauro Martins Teixeira[2,3] · Vandack Nobre[5,6] · Helton da Costa Santiago[2] · Daniele G. Souza[1]

Received: 24 February 2023 / Revised: 20 March 2023 / Accepted: 24 March 2023 / Published online: 29 March 2023

Abstract

The blood levels of neutrophils are associated with the severity of COVID -19. However, their role in the pulmonary environment during COVID -19 severity is not clear. Here, we found a decrease in the neutrophil count in BAL (bronchoalveolar lavage) in non-survivors and in older patients (> 60 years). In addition, we have shown that older patients have higher serum concentration of CXCL8 and increased IL-10 expression by neutrophils.

Keywords Severe COVID-19 · Neutrophils · ARDS · Elderly patients · Mechanical ventilation

Introduction

COVID-19 is an important cause of acute respiratory distress syndrome (ARDS) [1]. ARDS is a lung injury characterized by widespread inflammation, increased permeability, pulmonary edema and loss of aerated lung tissue, leading to hypoxemia and bilateral radiographic opacities [2]. ARDS is more common and severe in elderly patients and the use of invasive mechanical ventilation (IMV) is essential for life support in these critically ill patients [3].

Neutrophils play an important role in sepsis and ARDS [4], and the impaired migration of neutrophils to the site of infection may contribute to the severity of the disease [5]. Previous studies have reported neutrophilia and increased neutrophil infiltration in the lungs of patients with severe COVID-19 [4, 6]. Elderly patients show impaired neutrophil function [7] and also poorer prognosis COVID-19. It is not clear whether and how neutrophils contribute to the higher severity and mortality in elderly patients. Here, we

Responsible Editor: John Di Battista.

Raquel Duque Nascimento Arifa and Marcela Helena Gonçalves-Pereira Contributed equally to this work.

Helton da Costa Santiago and Daniele G. Souza Contributed equally to this work.

✉ Helton da Costa Santiago
heltonsantiago@icb.ufmg.com

✉ Daniele G. Souza
dani@icb.ufmg.br

[1] Department of Microbiology, Laboratory of Microorganism-Host Interaction, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil

[2] Department of Biochemistry and Immunology, Institute of Biological Sciences, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil

[3] Center for Drug Research and Development of Pharmaceuticals, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil

[4] Clinical Hospital, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil

[5] Interdisciplinary Center for Research in Intensive Medicine, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil

[6] Faculty of Medicine, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil

[7] Department of Clinical and Toxicological Analysis, College of Pharmacy, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil

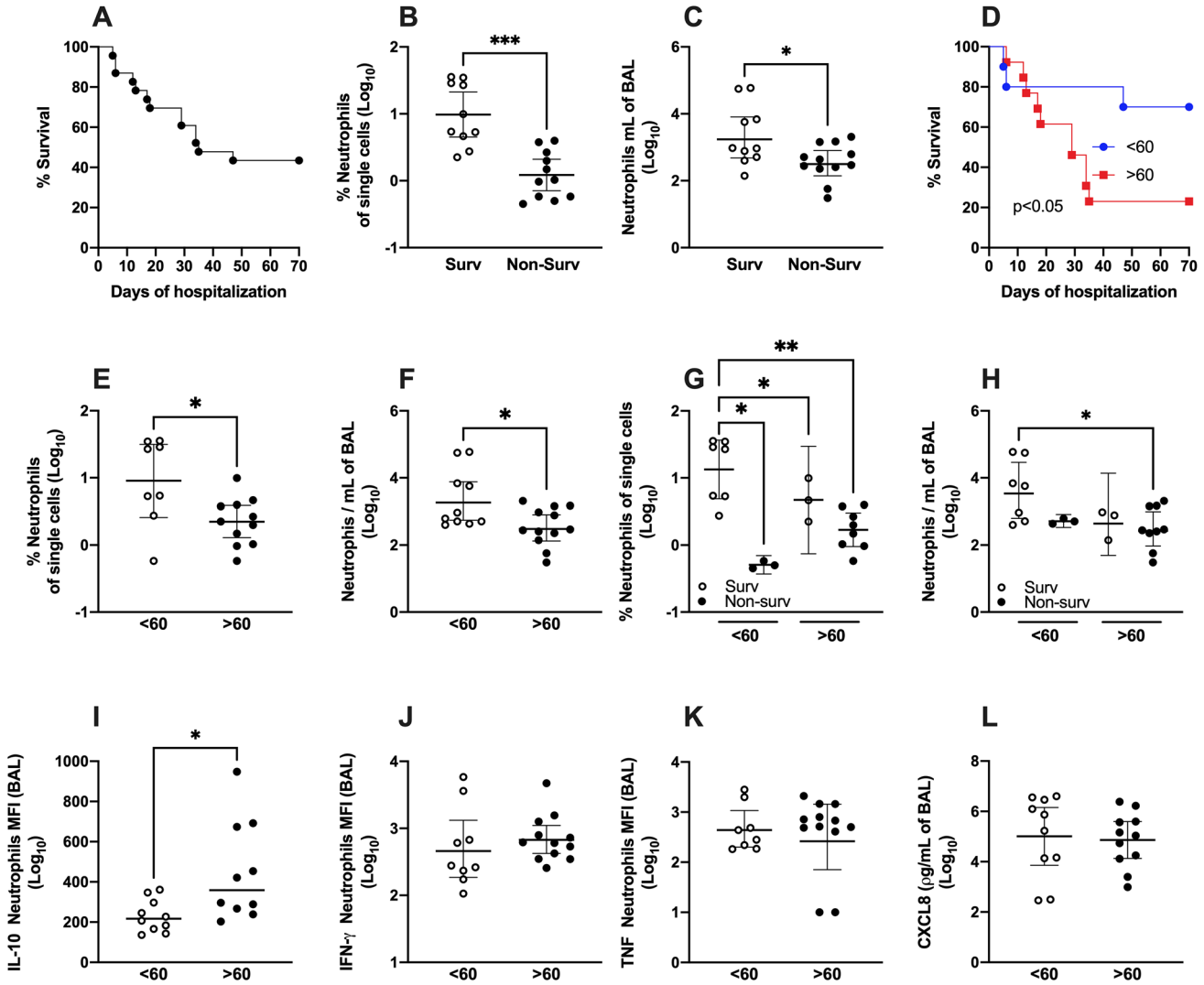

Fig. 1 Neutrophils are reduced in the BAL of non-survivors and patients over than 60 years with severe COVID-19. Curve of survival from COVID-19 patients under mechanical ventilation (**A**). Flow cytometry analysis of neutrophils frequencies (**B**) and numbers (**C**) in survivor group (n = 10) and non-survivor group (n = 13). Survival curve of patients younger (< 60 years of age) and older (> 60 years of age) (**D**). Flow cytometry analysis of the neutrophil frequencies (**E**) and numbers (**F**). Frequencies (**G**) and numbers (**H**) of neutrophils in younger survivors (n = 7), younger non-survivors (n = 3), older survivors (n = 3), and older non-survivors (n = 10). Flow cytometry analysis of MFI of IL-10 (**I**), IFN-γ (**J**) and TNF (**K**) expressed by neutrophils. CXCL8 concentration (**L**) in the BAL of younger and older patients with COVID-19. Horizontal lines represent geometric mean ± 95% CI. *P < 0.05, **P < 0.01, ***P < 0.001 by one-way ANOVA followed by Newman–Keuls multiple comparisons post-test or by a t-test, to compare medians of the two groups. Survival analysis was made by Log Rank test. Outliers were identified using the ROUT (Q = 2.0%) method of GraphPad Prism program version 9.0 and removed from the analysis

investigated the differences in the number of neutrophils in BAL and blood between young and elderly patients with severe COVID-19 and assessed factors related to their migration and activation.

Methods

Patients with severe COVID-19, confirmed by qRT-PCR, were recruited in the Intensive Care Unit of Hospital das Clínicas of Belo Horizonte (MG). All patients or their representatives signed an informed consent (IRB approval #30437020.9.3001.5124). Patients (n = 23) were divided into four groups: Survivors (n = 13) or non-survivors (n = 10) and younger (n = 10) or older than 60 years (n = 13).

Table 1 Neutrophil profile in blood and CXCL8 concentration in serum in patients with severe COVID-19

Blood Variables	Younger (< 60 years) (N = 10)	Older (> 60 years) (N = 13)
% of neutrophils (95% CI)	49.13 (41.17–58.62)	61.89 (52.61–72,80)*
# of neutrophils/mL of blood (95% CI)	4.527 (4.085–5.016)	4.769 (4.582–4.964)
MFI of IL-10 expression by neutrophils (95% CI)	0.816 (0.167–3.994)	0.258 (0.035–1.914)
MFI of IFN-γ expression by neutrophils (95% CI)	2.781 (2.496–3.099)	2.789 (2.591–3.002)
MFI of TNF expression by neutrophils (95% CI)	2.692 (2.517–2.879)	2.731 (2.642–2.823)
CXCL8 (ρg/mL of serum)	2.263 (1.974–2.593)	2.827 (2.506–3.189)*

Data are expressed as geometric mean ± 95% confidence intervals (lower, upper). Values are expressed as frequency of neutrophils and as logarithm number of neutrophils, mean of intensity fluorescence (MFI) of IL-10, IFN and TNF in neutrophils, and serum concentration of CXCL8 in patients with severe COVID-19. *P < 0.05

Mini broncho alveolar lavage (BAL) samples were collected in the first week after intubation at the same days of blood collection. Cells were labeled with anti-CD16, anti-CD14, anti-IL10, anti-TNF, and anti-IFN-γ and analyzed by flow cytometry. The populations of granulocytes and monocytes (SSC-A x FSC-A) and singlet (FSC-H x FSC-A) were selected. Then, the dot plot of CD16 x CD14 was used to identify the neutrophil subpopulation ($CD16^{hi}CD14^{int}$).

CXCL8 concentrations in plasma and BAL was determined using the Bio-Plex Pro™ Human Cytokine 17-plex Assay, Cat #M5000031YV (Bio-Rad).

Results

The mortality rate of patients enrolled in this study was 57% (Fig. 1A). In the survivor group, 80% were male and the median age was 56 ± 13 years old. In contrast, non-survivors were 62% male, and the median age was 70 ± 14 years (P = 0.066 compared to survivors). The frequency and number of neutrophils were reduced in the BAL of non-survivors (Fig. 1B, C). These results suggest an association between neutrophils in BAL and severity of COVID-19.

Since older age is a risk factor for COVID -19 mortality and neutrophil dysfunction [7], we investigated whether this factor could be related to neutrophil count in BAL and blood. Patients were divided into younger (< 60-year-old) and older (> 60-year-old) groups. In the older group, 62% were male and the mean age was 74 ± 8 years, significantly higher than in the younger group, 49 ± 8 years (P < 0.001) and 80% of male. The older group had higher mortality than the younger group (80% versus 25%, P < 0.05) (Fig. 1D). The number and frequency of neutrophils were reduced in the BAL of older patients (Fig. 1E, F). However, in blood, the frequency of neutrophils, but not their absolute number, was higher in the older group than in the younger group (Table 1). Interestingly, the younger patients who died of COVID-19 also had a lower frequency of neutrophils (Fig. 1G), but not in absolute numbers (Fig. 1H), in BAL compared to survivor younger patients.

To determine the activation status of neutrophils, we examined the expression of cytokines. We found that IL-10 expression by BAL neutrophils was higher in the older group than in younger group (Fig. 1I), but no difference was observed in IFN- γ or TNF expression (Fig. 1J, K). No difference was observed in cytokine expression by blood neutrophils between the groups (Table 1).

Because our data indicated a disturbance of neutrophil migration in the older group (high neutrophil levels in blood and low levels in BAL), we examined CXCL8, a chemokine that regulates neutrophil traffic. BAL CXCL8 levels were similar in the older and the younger patients (Fig. 1L), but older patients had higher blood CXCL8 concentrations when compared to younger patients (Table 1).

Discussion

Severe COVID-19 is characterized by changes in the profile of circulating neutrophils, with higher neutrophil/lymphocyte rate [4]. Several studies have reported an increase in neutrophil infiltrate in the lungs of severe COVID-19 patients, but it is not clear whether neutrophils are related to disease severity [8, 9]. We found that mortality due to COVID-19 was associated with decreased neutrophil count in the lungs, especially in older patients (> 60-year-old). Interestingly, neutrophils from older patients produced increased levels of IL-10, which may regulate the inflammatory response [10]. Coherently, studies have also shown increased PD-L1 surface expression [11] and downregulation of CD62L in neutrophils from severe COVID-19 patients, suggesting a suppressive phenotype [12]. In addition, we found higher concentrations of CXCL8 in the blood of older patients, but not in BAL, which may lead to changes in the profile and impaired migratory

capacity to the lungs of neutrophils due to desensitization of CXCR2 [5].

In conclusion, our data show that a reduction in the number of neutrophils in BAL is associated with worse prognosis in severe COVID-19 elderly patients.

Acknowledgements We are grateful to study participants, and the Institutional Laboratory of Biomarkers Research (LINBIO) for support with the flow cytometry.

Author contributions MHG-P, LS, CGR, PF V, VN and HCS conceived the study design. LS, CGR and PFV recruited study participants and collected biological samples. MHG-P performed cell culture and flow cytometry staining. FFSO and APS provided support with the flow cytometry platform. RDNA, MHG-P, MMT, HCS and DGS analyzed and interpreted data. RDNA, MHG-P, MMT, HCS and DGS prepared the manuscript.

Funding This work was supported by SESU-MEC (public notice "Fighting COVID-19"), INCT-Vacinas, CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nivel Superior) and INCT-dengue e Interação Microrganismo hospedeiro.

Data availability statement The data that support the findings of this study are available from the corresponding author DGS request.

Declarations

Conflicts of interest The authors declare they have no conflict of interest.

References

1. World Heath Organization . WHO Coronavirus (COVID-19) Dashboard. 2023. Available Online:https://covid19.who.int. Accessed 18 Mar 2023.
2. Lamers MM, Haagmans BL. SARS-CoV-2 pathogenesis. Nat Rev Microbiol. 2022;20(5):270–84.
3. Grasselli G, Cattaneo E. Mechanical ventilation parameters in critically ill COVID-19 patients: a scoping review. Crit Care. 2021;25(1):115.
4. Fu J, Kong J. The clinical implication of dynamic neutrophil to lymphocyte ratio and D-dimer in COVID-19: a retrospective study in Suzhou China. Thromb Res. 2020;192:3–8.
5. Sonego F, Castanheira FV. Paradoxical roles of the neutrophil in sepsis: protective and deleterious. Front Immunol. 2016;7:155.
6. Goncalves-Pereira MH, Santiago L. Dysfunctional phenotype of systemic and pulmonary regulatory T cells associate with lethal COVID-19 cases. Immunology. 2022;2:1.
7. Bartleson JM, Radenkovic D. SARS-CoV-2, COVID-19 and the ageing immune system. Nat Aging. 2021;1(9):769–82.
8. Liew PX, Kubes P. The Neutrophil's role during health and disease. Physiol Rev. 2019;99(2):1223–48.
9. Reusch N, De Domenico E. Neutrophils in COVID-19. Front Immunol. 2021;12: 652470.
10. Ocuin LM, Bamboat ZM. Neutrophil IL-10 suppresses peritoneal inflammatory monocytes during polymicrobial sepsis. J Leukoc Biol. 2011;89(3):423–32.
11. MacDonald L, Alivernini S. COVID-19 and RA share an SPP1 myeloid pathway that drives PD-L1+ neutrophils and CD14+ monocytes. JCI Insight. 2021;6:13.
12. Schulte-Schrepping J, Reusch N. Severe COVID-19 is marked by a dysregulated myeloid cell compartment. Cell. 2020;182(6):1419–40.