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Persistent inflammation, immunosuppression, and catabolism are associated with impaired lymphocytic mitochondrial metabolism during the early phase of sepsis. A single-center, prospective cohort study

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Abstract

Background: Lymphopenia and lymphocytic metabolism have been individually associated with poor prognosis in sepsis; however, the relationship between these factors remains poorly understood. We evaluated whether lymphocyte count and lymphocytic mitochondrial metabolism are associated with the development of persistent inflammation, immunosuppression, and catabolic syndrome (PICS).

Methods: A prospective cohort study was conducted in four clinical-surgical intensive care units (ICUs). We included critically ill patients with sepsis requiring vasopressor support and assessed mitochondrial metabolism in isolated lymphocytes, including Complex I- and II-linked respiration, at sepsis diagnosis (day 1) and on day 3. Absolute lymphocyte counts and derived ratios, e.g., neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR), were evaluated at the same time points. The primary outcome was the association between mitochondrial respiratory function and PICS. Secondary outcomes included associations between lymphocyte counts, derived biomarkers, and mitochondrial respiration.

Results: We included 64 patients, 10 of whom developed PICS. At sepsis diagnosis, patients who did not develop PICS exhibited significantly higher Complex I and II-linked respiration compared with those who did (mean difference [MD] for Complex I: 115 pmol O_2 s^{-1} 10^{-6} cells; 95% CI: 13–291; $P = 0.01$; MD for Complex II: 171 pmol O_2 s^{-1} 10^{-6} cells; 95% CI: 31–397; $P = 0.01$). No significant differences were observed in the delta (day 3 minus day 1) mitochondrial respiration or lymphocyte count between the PICS and non-PICS groups. Additionally, lymphocyte counts and derived ratios showed no significant correlation with Complex I or II respiration, even when stratified by quartiles across time points.

Conclusions: Our findings suggest that reduced mitochondrial Complex I- and II-linked respiration in lymphocytes at sepsis onset may be associated with the development of PICS. Nevertheless, this association remains exploratory and requires confirmation in larger, adequately powered studies.

Key words: sepsis, septic shock, PICS, persistent inflammation, immunosuppression, and catabolism syndrome, chronic critical illness; lymphopenia, lymphocytes, mitochondrial dysfunction, oxidative phosphorylation, cell respiration, immunosuppression, catabolism.

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Sepsis should be defined as life-threatening organ dysfunction caused by a dysregulated host response to infection [1], which is shaped by both pathogen and endophenotype host factors [2]. The persistence of this dysregulated response can result in a wide range of long-term functional impairments following intensive care unit (ICU) admission [3]. Among these consequences, the deve-

lopment of persistent inflammation, immunosuppression, and catabolism syndrome (PICS; also known as PIICS) is a particularly concerning clinical event [4], in which lymphocytes play a central role in perpetuating this maladaptive response [5].

Lymphocytes represent approximately 40% of the circulating white blood cells; however, their proportion can fluctuate in response to sepsis, offering a dynamic "readout" of the patient's immune and clinical status [6]. In the acute phase of sepsis, a marked reduction in lymphocyte count, referred to as lymphopenia, is commonly observed and reflects a state of immune dysfunction. Notably, lymphopenia, typically defined as an absolute lymphocyte count of < 1000 cells mm^{-3}, has been consistently associated with poor outcomes in critically ill patients [7]. In this context, monitoring absolute lymphocyte counts and derived ratios, such as the neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (MLR), and platelet-to-lymphocyte ratio (PLR), has emerged as a simple yet valuable strategy for assessing the host immune response. Additionally, aggregated inflammatory indices, such as the systemic inflammatory index (SII), the systemic inflammatory response index (SIRI), and the aggregate inflammatory systemic index (AISI), have emerged as potential markers of systemic inflammation with a predictive role in the perioperative setting and COVID-19 [8, 9]. These blood-based parameters serve not only as potential biomarkers of immune competence but also as early indicators of progression toward PICS [7, 10, 11].

In addition to reduced cell counts, mitochondrial bioenergetic dysfunction in lymphocytes may contribute to impaired immune competence. Effective immune responses, particularly in sepsis, require lymphocytes to exhibit metabolic plasticity to meet the elevated energy demands necessary for immune activation, proliferation, and resolution of infection [12]. Lymphocyte mitochondria play a critical role in efficiently coupling electron transport through the respiratory chain with oxygen consumption and adenosine triphosphate (ATP) synthesis. ATP is essential for a wide range of immune functions, including intracellular signaling, cytokine production, and lymphocyte proliferation [6].

Previous work from our group demonstrated that lymphocytic mitochondrial respiration, both at baseline and after stimulation with Complex I and II, declined from day 1 to day 3 in patients with sepsis, and this reduction was associated with increased short- and long-term mortality [13]. These findings underscore the potential of lymphocytic mitochondrial bioenergetics, particularly respiratory parameters, as prognostic biomarkers for sepsis. However, the relationship between lymphocyte proliferation, lymphocyte mitochondrial bioenergetic function, and their combined impact on the host response to sepsis and related clinical outcomes remains unclear [14]. In this study, we investigated whether lymphocyte counts and mitochondrial bioenergetic function were associated with the development of PICS in patients with sepsis.

METHODS

We performed a *post-hoc* study derived from a cohort study that prospectively evaluated consecutive patients who had been admitted to four different ICUs in a tertiary academic hospital (Grupo Hospitalar Conceição, Porto Alegre, Brazil). The original cohort has been described in a previous study [13]. This study was approved by the local ethics committee (Comitê de Ética em Pesquisa do Grupo Hospitalar Conceição – Plataforma Brazil number 66240017.0.0000.5530). Written informed consent was obtained from the patient or their next of kin.

Adult patients (aged > 18 years) admitted to the Intensive Care Unit (ICU) for sepsis with persistent hypotension were enrolled in this study. Inclusion in the study occurred at ICU admission for sepsis management. Sepsis was defined in accordance with the current guidelines [15], and persistent hypotension was characterized by the necessity for vasopressors to maintain a mean arterial pressure (MAP) of > 65 mmHg following initial fluid administration. The patients included were admitted to the ICU within the first 24 hours of sepsis management. The exclusion criteria included known mitochondrial disease, pregnancy, refusal by the patient or next of kin to provide informed consent, imminent death, and withholding or withdrawal of treatment. The epidemiological characteristics and treatments of the patients were prospectively documented, including the Simplified Acute Physiology Score (SAPS III) and Sequential Organ Failure Assessment (SOFA) scores at ICU admission (first day, D1) and on day 3 (D3). Clinical and laboratory data were also collected for this study. Patients with PICS syndrome were identified as those with an ICU length of stay greater than 14 days; exhibiting persistent inflammation, defined as C-reactive protein (CRP) > 150 mg L^{-1}; persistent immunosuppression, defined as a total lymphocyte count < 0.8×10^9 L^{-1}; and a catabolic state, defined as a serum albumin < 3 g dL^{-1} or weight loss > 10% or body mass index < 18 during hospitalization [16]. However, patients can still manifest PICS even after discharge from the ICU and while hospitalized in a medical ward.

Blood samples and lymphocyte isolation

All blood samples for mitochondrial measurements were collected as previously described [13].

Blood samples were collected through an arterial line or venous puncture simultaneously with the blood samples for lymphocyte respirometry. Mitochondrial respiration linked to Complexes I and II was assessed on D1 and D3 and delta (D3 minus D1) in the isolated lymphocytes. Measurements were performed using high-resolution oxygraphy (Oxygraph-2k; Oroboros Instruments, Innsbruck, Austria) at 37°C. This protocol allows the assessment of Complex I (CI) and Complex II (CII)-linked respiration [13]. The researchers conducting the mitochondrial analyses were blinded to the clinical outcomes, and those responsible for clinical data collection were blinded to the mitochondrial analysis results.

Routine hemogram monitoring was performed using an automated hemocytometer analyzer (Sysmex XN 3100; Sysmex, Paraná, Brazil), which provided the total lymphocyte count (cells μL^{-1}). The neutrophil-to-lymphocyte ratio (NLR), monocyte-to-lymphocyte ratio (MLR), and platelet-to-lymphocyte ratio (PLR) were calculated as the ratios of the absolute counts of neutrophils, monocytes, and platelets to the absolute counts of lymphocytes at each time point. SII was calculated as the quotient of neutrophils and platelets divided by lymphocyte counts; SIRI was defined as the quotient of neutrophils and monocytes divided by lymphocyte counts; and AISI was defined as the quotient of neutrophils, monocytes, and platelets divided by lymphocyte count. The laboratory hospital participates in the Quality Control Program for Clinical Laboratories of the Brazilian Society of Clinical Pathology, ensuring that all analyses adhere to the international standards.

Outcomes

The primary outcome was the relationship between mitochondrial variables (Complex I and Complex II respiration) and PICS. The secondary outcomes included the association between the total lymphocyte count and lymphocyte-derived biomarkers (NLR, MLR, PLR, SII, SIRI, and AISI) and mitochondrial variables. We also examined the association between Complex I and Complex II-linked respiration and the use of tracheostomy (TQT), and the incidence of reinfection in these patients. These factors serve as additional indicators of chronic critical illness and immunosuppression, such as in PICS syndrome. As an exploratory secondary outcome, we assessed the association between PICS diagnosis and in-hospital mortality, along with hospital-free days at day 60, and the association between mitochondrial and lymphocyte-derived outcomes and 28-day mortality. Patients who died during the observation period were assigned zero hospital-free days on day 60.

Statistical analysis

Descriptive statistics included frequencies and percentages for categorical variables and means (or medians) and standard deviation (SD) (or interquartile range, IQR), and confidence intervals (CI) for continuous variables. To compare continuous variables, we used the Student's *t*-test, Mann-Whitney *U* test, Kruskal-Wallis test, and Pearson or Spearman correlation test, as appropriate, according to normal distribution. Categorical variables were analyzed using the χ^2 test. We evaluated the association between lymphocyte counts and Complex I and Complex II respiration quartiles on day 1 using one-way ANOVA with Tukey's post-hoc test. We also evaluated the association between PICS and in-hospital mortality using a Kaplan-Meier curve with a Cox proportional hazards model adjusted for SOFA improvement on day 3. PICS may be a competing event with mortality because patients with early mortality do not develop the syndrome. Therefore, we also analyzed the data using days free of hospitalization at 60 days, counting zero days for patients who died within this period. All P-values were two-tailed, and a 2-sided unadjusted *P*-value of ≤ 0.05 was considered statistically significant. All analyses were performed using the software packages BlueSky Statistics (BlueSky Statistics LLC, Chicago, IL, USA) and Jamovi (The Jamovi project) version 1.6.23.

RESULTS

A total of 64 patients were included in this study (Figure 1). Ten patients developed PICS, whereas 54 did not. The 28-day mortality rate was 35% ($n = 23$). The baseline clinical variables and their associations with PICS are presented in Table 1. The criteria used to define PICS for each patient are described in Supplementary material.

Patients who did not develop PICS exhibited higher lymphocyte Complex I and Complex II mitochondrial respiration at sepsis diagnosis compared to those who developed PICS (Complex I mean difference [MD] 115 pmol O_2 s^{-1} 10^{-6} cells, 95% CI: 13–291, $P = 0.01$; Complex II MD 171 pmol O_2 s^{-1} 10^{-6} cells, 95% CI: 31–397, $P = 0.01$). Delta Complex I and delta Complex II respiration did not differ between patients who developed PICS and those who did not develop PICS: MD –88 pmol O_2 s^{-1} 10^{-6} cells, 95% CI: –244 to 41, $P = 0.18$; MD –186 pmol O_2 s^{-1} 10^{-6} cells, 95% CI: –516 to 107, $P = 0.14$. Complex I and II respiration on day 3 did not differ between patients who developed PICS and those who did not (Table 1). PLR and NLR on day 3, but not on day 1, were associated with PICS status. Interleukin (IL)-1, IL-6, IL-10, CRP, MLR, SII, AISI, and SIRI were not associated with PICS development on either day 1 or day 3 (Table 1). Patients who developed reinfection during hospitali-

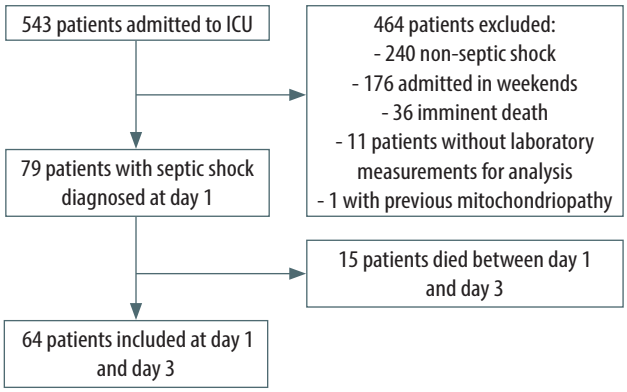

FIGURE 1. Flowchart of patient inclusion in the study

zation had significantly lower Complex I respiration levels on day 3 than those who did not develop reinfection. These patients also had lower levels of Complex II respiration on day 3; however, the difference was not statistically significant. In addition, patients who required TQT had lower levels of Complex I and Complex II respiration on day 1, but the difference was not statistically significant (Figure 2).

The total lymphocyte count at the time of sepsis diagnosis was not associated with Complex I respiration (Spearman's $r = 0.03$, 95% CI: –0.23 to 0.29, $P = 0.8$). This lack of association did not differ between survivors (Spearman's $r = 0.03$, 95% CI: –0.29 to 0.34, $P = 0.85$) and non-survivors (Spearman's $r = –0.01$, 95% CI: –0.39 to 0.43, $P = 0.96$). Moreover, lymphocyte counts at this time point were not associated with Complex II respiration (Spearman's $r = 0.003$, 95% CI: –0.25 to 0.26, $P = 0.97$). This lack of association was also not different between survivors (Spearman's $r = 0.06$, 95% CI: –0.26, 0.37, $P = 0.73$) and non-survivors (Spearman's $r = –0.15$, 95% CI: –0.56 to 0.31, $P = 0.51$). When we stratified Complex I respiration on day 1 by quartiles, there was no difference in the total lymphocyte count between the different quartiles ($P = 0.42$), and the same was observed for the stratification of Complex II respiration in quartiles ($P = 0.94$). The same results were obtained when we analyzed Complex I and II respiration on day 3, stratified by quartiles, with lymphocyte counts on day 3 ($P = 0.55$ and $P = 0.61$, respectively).

The mitochondrial respiratory variables at baseline were not correlated with the lymphocyte-derived biomarkers (NLR, PLR, and MLR) (Table 2). In addition, the delta values of CI and CII did not correlate with the lymphocyte-derived biomarkers (Table 2). Complex I respiration was not associated with the SII on day 1 (Spearman's $r = –0.04$, 95% CI: –0.29, 0.22, $P = 0.78$). Complex I respiration on day 1, however, was associated with SIRI on day 1 (Spearman's $r = –0.27$, 95% CI: –0.5 to –0.01, $P = 0.03$) but not with AISI on day 1 (Spearman's $r = –0.11$, 95% CI: –0.36, 0.16, $P = 0.41$). Complex II respiration on day 1 was not associated with SII on day 1 (Spearman's $r = –0.06$, 95% CI: –0.31 to 0.2, $P = 0.64$). Complex II respiration on day 1, however, was associated with SIRI on day 1 (Spearman's $r = –0.27$, 95% CI: –0.49 to –0.01, $P = 0.04$) but not with AISI on day 1 (Spearman's $r = –0.16$, 95% CI: –0.4, 0.1, $P = 0.22$). Complex I respiration was not associated with SII at day 3 (Spearman's $r = –0.21$, 95% CI: –0.48 to 0.09, $P = 0.16$), nor with SIRI at day 3 (Spearman's $r = 0.24$, 95% CI: –0.07 to 0.51, $P = 0.11$) or AISI at day 3 (Spearman's $r = –0.07$, 95% CI: –0.36 to 0.23, $P = 0.65$). The results of the correlation coefficients between the mitochondrial variables and the derived biomarkers are presented in Table 2.

Patients who developed PICS had a lower hazard of in-hospital mortality than those who did not develop PICS: HR 0.20 (95% CI: 0.05–0.86, $P = 0.03$) in univariate analysis (Figure 3). When we evaluated the association between PICS and in-hospital mortality adjusted by SOFA score improvement at day 3, patients who developed PICS still had a lower hazard of in-hospital mortality: HR = 0.20 (95% CI: 0.05–0.87, $P = 0.03$), HR for SOFA improvement 1.14 (95% CI: 0.39–3.34, $P = 0.81$). When censoring at 60 days of hospitalization and evaluating hospitalization-free days at day 60, patients who developed PICS did not have increased mortality (log-rank $P = 0.06$).

DISCUSSION

In this study, we found that patients who progressed to PICS exhibited reduced mitochondrial Complex I and II-linked respiration in lymphocytes at the time of sepsis diagnosis. This finding supports emerging evidence that mitochondrial bioenergetic dysfunction is not only an early marker but is strongly associated with the development of immunosuppression in sepsis [1–3]. The absence of significant differences in respiratory changes between days 1 and 3 suggests that early mitochondrial impairment, rather than short-term respiratory recovery, may serve as a potential prognostic biomarker for PICS. PICS is increasingly recognized as a state of prolonged immunosuppression characterized by T cell exhaustion, expansion of regulatory T cells (Tregs), monocyte/macrophage paralysis, and neutrophil dysfunction (4). Lymphocytes are downstream effectors of the immune response and respond to cytokines released by innate immune cells such as monocytes, macrophages, dendritic cells, and neutrophils. When activated, these phagocytes release pro-inflammatory cytokines, such as IL-1 and IL-6 [18, 19], a process that is energetically demanding and can lead to cellular energy depletion, theoretically linking the PICS state to mitochondrial dysfunction in lymphocytes [20, 21].

Previous studies have shown that the recovery of Complex I and II activity within three days of sep-

TABLE 1. Clinical and laboratory characteristics and their associations with outcomes

Variable	Frequency (%) or mean (SD)/median (IQR)					
	No-PICS ($n = 54$)	PICS ($n = 10$)	*P*-value	Survivors ($n = 41$)	Non-survivors ($n = 23$)	*P*-value
Age (years)	71 (58–79)	70 (51–78)	0.424	71 (55–78)	71 (62–79)	0.31
SAPS 3	76 (64– 85)	77 (70–82)	0.531	75 (65–83)	77 (71–87)	0.25
SOFA score day 1	7 (6–9)	9 (7–10)	0.503	7 (6–9)	9 (6–11)	0.04
SOFA score day 3	3 (2–7)	6 (4–9)	0.56	3 (1–5)	7 (3–15)	< 0.01
IL-6 day 1 (pg mL^{-1})	72.5 (31.6–213)	57.7 (30.5–134)	0.875	63.1 (30.3–195)	123 (32.8–217)	0.58
IL-6 day 3 (pg mL^{-1})	67.3 (34.8–159)	77 (17.3–117)	0.55	58.6 (34.8–110)	89.9 (37–179)	0.32
IL-1β day 1 (pg mL^{-1})	28 (12.3–514)	78.7 (27.3–374)	0.409	23.6 (13.9–458)	56.3 (17.8-739)	0.14
IL-1β day 3 (pg mL^{-1})	38.1 (11.6 –480)	50.1 (9.5–572)	0.143	22.3 (9.7–287)	91.7 (19.2–621)	0.06
IL-10 day 1 (pg mL^{-1})	184 (133–251)	221 (184–233)	0.619	175 (126–225)	200 (168–247)	0.10
IL-10 day 3 (pg mL^{-1})	179 (148–351)	331 (167–725)	0.296	170 (132–241)	308 (168–741)	0.01
Platelet count day 1 ($\times$ 1000 cells μL^{-1})	217 (139–278)	191 (117–247)	0.649	219 (150–313)	141 (89–243)	0.02
Platelet count day 3 ($\times$ 1000 cells μL^{-1})	210 (140–259)	149 (119–225)	0.82	214 (142–321)	144 (73–226)	0.04
CRP day 1 (mg L^{-1})	188 (97–273)	156 (83–216)	0.41	155 (82–208)	186 (115–316)	0.12
CRP day 3 (mg L^{-1})	71 (34–131)	135 (58–169)	0.151	87 (45–163)	61 (36–171)	0.74
Leukocytes day 1 (cells μL^{-1})	12830 (9663–20370)	13800 (10788–19088)	0.483	12650 (10330–20320)	13590 (9710–20160)	0.77
Leukocytes day 3 (cells μL^{-1})	11098 (8710–15590)	12115 (9853–19610)	0.811	10200 (8633–13295)	14010 (10420–20886)	0.06
Lymphocytes day 1 (cells μL^{-1})	1001 (626–1599)	1048 (670–1503)	0.448	1000 (644–1595)	1017 (629–1496)	0.70
Lymphocytes day 3 (cells μL^{-1})	1252 (928–1587)	1049 (700–1186)	0.354	1208 (981–1440)	1040 (684–1911)	0.61
Neutrophils day 1 (cells μL^{-1})	10095 (7462–18356)	12043 (7727–16776)	0.566	10310 (7467–18389)	12326 (7445–17973)	0.79
Neutrophils day 3 (cells μL^{-1})	8201 (5618–11227)	10157 (7177–17423)	0.333	6395 (1807–8782)	8030 (0–14085)	0.61
NLR day 1	10.1 (5.97–20.3)	7.82 (5.8–18.1)	0.491	8.3 (5.64–18.2)	11.6 (7–21)	0.53
NLR day 3	6.27 (4.27–10.5)	13.1 (6.99–17)	0.041	5.95 (4.27–8.76)	11.6 (6.9–21.1)	0.01
Monocytes day 1 (cells μL^{-1})	812 (577–1162)	796 (533–1001)	0.585	796 (634–1091)	951 (558–1191)	0.88
Monocytes day 3 (cells μL^{-1})	738 (489–1017)	767 (354–925)	0.479	718 (516–926)	858 (415–1064)	0.48
MLR day 1	0.253 (0.181–0.376)	0.26 (0.174–0.327)	0.815	0.25 (0.19–0.35)	0.21 (0.16–0.39)	0.79
MLR day 3	0.414 (0–0.747)	0.654 (0.302–0.788)	0.51	0.512 (0.044–0.738)	0.299 (0–0.809)	0.80
PLR day 1	181 (122–389)	203 (139–289)	0.364	233 (138–383)	171 (81–299)	0.22
PLR day 3	160 (101–244)	182 (102–479)	0.041	161 (122–245)	203 (60–290)	0.53
SIRI day 1	7.71 (4.77–17.9)	7.23 (4.27–13.7)	0.69	6.98 (4.69–14.31)	7.89 (4.68–21.88)	0.62
SIRI day 3	4.58 (2.24–9.59)	7.35 (2.93–10.6)	0.65	5.17 (2.23–9.95)	3.58 (2.49–9.3)	0.61
AISI day 1	1629 (650–2993)	1365 (715–4462)	0.704	1930 (715–3050)	1330 (425–2000)	0.34
AISI day 3	865 (482–1624)	1240 (399–1566)	0.912	831 (359–2060)	1330 (425–2000)	0.57
SII day 1	2046 (1011–3466)	2373 (671–4957)	0.767	2111 (1030–3980)	1710 (696–3390)	0.37
SII day 3	1247 (761–2180)	1670 (757–2551)	0.423	1160 (793–2200)	1690 (725–2530)	0.76
Albumin day 1	2.5 (2–2.8)	2.35 (2.2–2.8)	0.984	2.5 (2.05–2.9)	2.25 (2.02–2.58)	0.15
Complex I respiration day 1 (pmol O_2 s^{-1} 10^{-6} cells)	326 (178–578)	207 (160–244)	0.022	397 (245–612)	176 (167–227)	< 0.01
Complex I respiration day 3 (pmol O_2 s^{-1} 10^{-6} cells)	476 (317–670)	351 (261–473)	0.213	526 (403–738)	338 (266–363)	< 0.01
Complex II respiration day 1 (pmol O_2 s^{-1} 10^{-6} cells)	554 (400–843)	384 (356–433)	0.021	575 (438–999)	419 (374–578)	0.03
Complex II respiration day 3 (pmol O_2 s^{-1} 10^{-6} cells)	818 (634–1094)	747 (540–1053)	0.507	931 (703–1241)	596 (435–775)	< 0.01

AISI – aggregate inflammatory systemic index, CRP – C-reactive protein, MLR – monocyte-to-lymphocyte ratio, NLR – neutrophil-to-lymphocyte ratio, PICS – persistent inflammation, immunosuppression, and catabolic syndrome, PLR – platelet-to-lymphocyte ratio, SAPS – Simplified Acute Physiology Score, SII – systemic inflammatory index, SIRI – systemic inflammatory response index, SOFA – Sequential Organ Failure Assessment

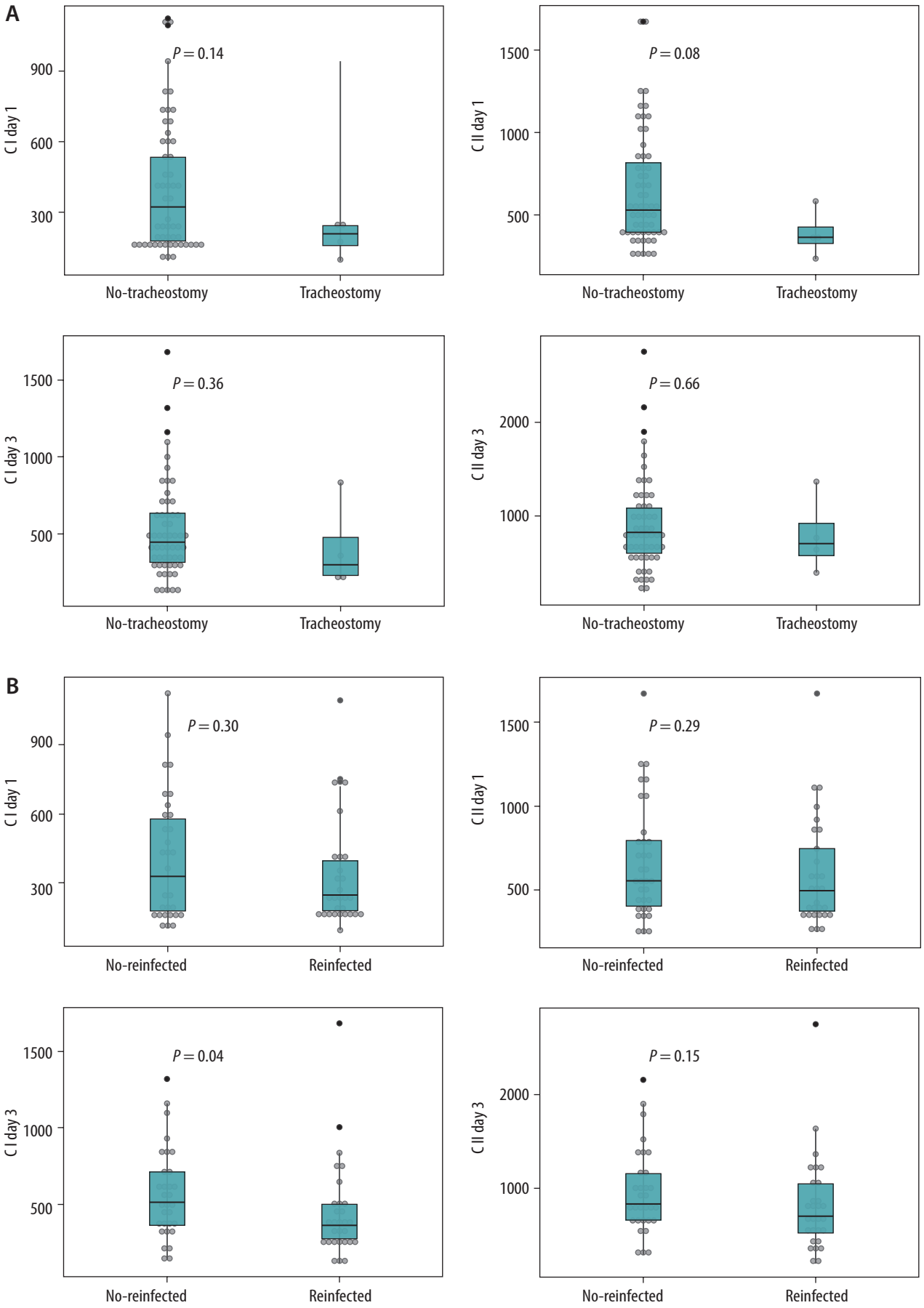

FIGURE 2. Association between mitochondrial respiration variables and the need for tracheostomy (**A**) and reinfection during hospitalization (**B**)
C I – complex I respiration, C II – complex II respiration.

TABLE 2. Correlation between variables at day 1

Variable	Complex I respiration		Complex II respiration	
	Spearman coefficient	*P*	Spearman coefficient	*P*
NLR (day 1)	–0.15	1	0.14	1
PLR (day 1)	0.07	1	0.08	1
MLR (day 1)	–0.19	0.80	0.25	0.30
	Delta Complex I respiration		Delta Complex II respiration	
	Spearman coefficient	*P*	Spearman coefficient	*P*
Delta NLR	–0.17	0.18	–0.13	0.32
Delta PLR	–0.02	0.86	–0.04	0.72
Delta MLR	0.03	0.77	–0.01	0.88

MLR – monocyte-to-lymphocyte ratio, NLR – neutrophil-to-lymphocyte ratio, PLR – platelet-to-lymphocyte ratio

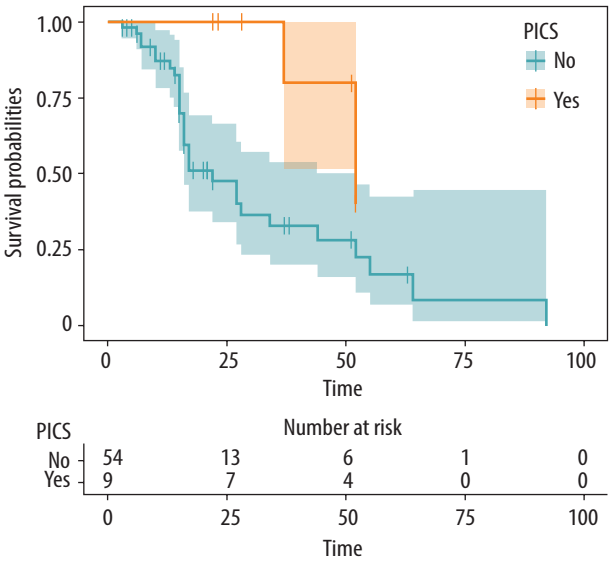

FIGURE 3. Kaplan-Meier survival curves for patients with and without persistent inflammation, immunosuppression, and catabolic syndrome (PICS)

sis onset correlates with reductions in circulating IL-6 levels, potentially reflecting a shift toward increased OXPHOS and resolution of hyperinflammatory signaling [13]. In contrast, our study found no significant differences in cytokine levels (IL-6, IL-1β, and IL-10) between patients who developed PICS and those who did not. While this may suggest that bioenergetic dysfunction contributes to PICS via mechanisms independent of these cytokines, we cannot exclude the possibility that unmeasured inflammatory mediators or signaling pathways are involved in this process. Alternatively, the narrow temporal windows of metabolic, inflammatory, and clinical assessments may have limited our ability to detect the relevant associations. These variables may also exert independent effects on sepsis prognosis, irrespective of lymphocyte count.

Changes in mitochondrial function, particularly OXPHOS and mitochondrial dynamics (fusion and fission), have been shown to impair immune competence, especially in effector and memory T cells [6]. Despite the central role of mitochondria in immune cell function, our data did not show an association between lymphocyte counts and mitochondrial respiration or between mitochondrial parameters and derived immunological ratios, such as NLR, PLR, MLR, SII, and AISI. This suggests that lymphopenia and mitochondrial dysfunction may represent parallel, yet distinct, pathogenic processes in the immunobiology of sepsis. Both persistent lymphopenia [11] and sustained metabolic dysfunction in lymphocytes have been independently associated with increased mortality in sepsis [7, 17], potentially reflecting either an inadequate host inflammatory response or vulnerability to secondary infections and prolonged organ dysfunction [2]. The association between Complex I and Complex II respiration at sepsis diagnosis and a more integrative index, such as the SIRI, should be explored in future studies.

This study had several limitations. First, we did not perform immunophenotyping of lymphocyte subsets (e.g., CD4+ T cells, CD8+ T cells, and Tregs), which may differ in their metabolic profiles and roles in sepsis and PICS. Thus, we cannot rule out subtype-specific contributions to the observed mitochondrial dysfunction. Although such analyses would enhance mechanistic insights, they remain challenging to implement in real-time clinical settings. Second, we lacked follow-up assessments of mitochondrial function beyond day 3, which may have provided a more comprehensive view of bioenergetic dynamics during critical illness. Although our short-term findings were associated with clinical outcomes, they may have underestimated long-term trajectories. We performed multiple analyses of several variables and different outcomes addressed in the study. We did not correct the *P*-value for multiple interactions, which is a potential limitation of this study. However, our study was intended to generate hypotheses,

pointing out potential interactions to be better explored in future studies, thus justifying the adopted methodology. Finally, the study was conducted at a single center with a relatively small and severely ill cohort, which limits the generalizability of the results to other sepsis phenotypes and care settings. In addition, the limited sample size reduced statistical power and increased the risk of both type I and type II errors. Consequently, these findings should be interpreted with caution and considered hypothesis-generating. Validation in larger, multicenter studies is warranted.

CONCLUSIONS

Patients who did not develop PICS exhibited higher lymphocytic mitochondrial respiration at the time of sepsis diagnosis, particularly in Complexes I and II. Lymphocyte counts and mitochondrial variables showed no significant association, regardless of survival status. Furthermore, stratification by mitochondrial respiration quartiles had no impact on lymphocyte counts. These findings suggest that mitochondrial dysfunction may be associated with immune dysregulation in patients with sepsis. However, owing to the nature of our study, these findings should be considered hypothesis-generating and require validation in larger cohorts, together with a broader analysis of the different immune mechanisms involved in the clinical response to sepsis.

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