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Correlation of Neutrophil and Procalcitonin Levels with Multiorgan Damage Scores in an Escherichia coli-Induced Male Rat Sepsis Model

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ABSTRACT

Sepsis is a life-threatening condition caused by a dysregulated host response to infection, which may lead to multiple-organ damage. Neutrophils and procalcitonin (PCT) are inflammatory biomarkers known to increase during sepsis and are suspected to be associated with the severity of organ injury. This study aimed to analyze the relationship between neutrophil and procalcitonin levels and multiple-organ tissue damage scores in a male rat sepsis model induced by intraperitoneal *administration of Escherichia coli*. This was a true experimental laboratory study using a post-test-only control group design involving nine male *Rattus norvegicus* rats divided into a control group, an *E. coli*-induced group receiving 1×10^6 CFU/kg body weight, and an *E. coli*-induced group receiving 1.5×10^6 CFU/kg body weight. Neutrophil levels were measured using a hematology analyzer, while PCT levels were determined using an Enzyme-Linked Immunosorbent Assay (ELISA). Multiple-organ damage was assessed through histopathological examination of the liver, kidney, spleen, heart, and lungs. Data were analyzed using Spearman's correlation test. The results showed that neutrophil levels were significantly and positively correlated with damage scores of the liver ($r_s = 0.88$; $p = 0.002$), spleen ($r_s = 0.90$; $p < 0.001$), heart ($r_s = 0.92$; $p < 0.001$), and lungs ($r_s = 0.91$; $p < 0.001$). Procalcitonin levels were also significantly and positively correlated with damage scores of the liver ($r_s = 0.90$; $p < 0.001$), spleen ($r_s = 0.87$; $p = 0.002$), heart ($r_s = 0.85$; $p = 0.004$), and lungs ($r_s = 0.88$; $p = 0.002$). In conclusion, higher neutrophil and procalcitonin levels were associated with greater multiple-organ tissue damage in a male rat sepsis model induced by *Escherichia coli*.

INTRODUCTION

Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection (Singer et al., 2016). It remains a major global health problem with high morbidity and mortality. Based on Global Burden of Disease data, sepsis is estimated to cause approximately 48.9 million cases and 11 million deaths annually, representing about 19.8% of all global deaths (Rudd et al., 2020). In clinical management, the appropriateness of early antibiotic administration is crucial, as inappropriate empiric therapy is directly linked to increased mortality and prolonged hospital stays in septic patients (Nugraheni et al., 2021). An uncontrolled immune response in sepsis triggers systemic inflammation, microcirculatory disturbance, endothelial injury, and organ dysfunction, which underlies the development of multiple organ dysfunction syndrome (MODS) affecting the lung, kidney, liver, heart, and other organs (Srdić et al., 2024).

Intraperitoneal injection of live bacteria, including *Escherichia coli*, is a widely used method to induce systemic infection in experimental sepsis. *Escherichia coli*, a prominent Gram-negative bacterium, is a leading pathogen in community-acquired and nosocomial sepsis, particularly in cases involving intra-abdominal infections and bacteremia (Mahmuda et al., 2025). Its lipopolysaccharide (LPS) endotoxin activates the innate immune system, triggers the release of proinflammatory mediators, and causes cellular injury in multiple organs (van der Poll et al., 2021). This model mimics intra-abdominal infection and produces inflammatory dynamics that closely resemble live bacterial infection rather than pure endotoxemia.

Neutrophils play a central role during the early phase of bacterial infection through phagocytosis, degranulation, reactive oxygen species (ROS) production, and the formation of neutrophil extracellular traps (NETs). However, excessive neutrophil activation exacerbates tissue injury and may contribute to endothelial dysfunction, coagulation, and microthrombosis. Therefore, the neutrophil count reflects the severity of the systemic inflammatory response (van der Poll et al., 2021). Procalcitonin (PCT) is a biomarker that increases during systemic bacterial infection, stimulated by bacterial endotoxin and proinflammatory cytokines such as IL-6 and TNF-α, and a recent meta-analysis showed that PCT outperforms C-reactive protein for detecting bacterial sepsis (Chuang et al., 2025).

Histopathological examination can assess organ damage in sepsis and convert it into semiquantitative tissue damage scores, allowing more objective evaluation of multiorgan dysfunction (Matthay et al., 2024). However, research linking systemic inflammatory biomarkers to multiorgan damage remains limited, as most studies evaluate biomarkers separately, focus on a single organ, or describe inflammatory changes without correlating them to histopathological scores across several organs. This study simultaneously correlates neutrophil and PCT levels with multiorgan tissue damage scores in a male rat sepsis model induced by intraperitoneal *Escherichia coli*, aiming to analyze each biomarker's association with the degree of histopathological damage in the liver, kidney, spleen, heart, and lung.

METHOD

This study was a true experimental laboratory study with a post-test-only control group design and a correlational analytical approach. The relationship between neutrophil and procalcitonin (PCT) levels (independent variables) and multiorgan tissue damage scores (dependent variables) was evaluated 72 hours after induction in the liver, kidney, spleen, heart, and lung. Nine healthy male *Rattus norvegicus* rats obtained from the Faculty of Veterinary Medicine, Universitas Gadjah Mada, were used in this study. The animals were 2 months old and weighed 180–200 g. The rats were acclimatized for 7 days under standard laboratory conditions with ad libitum access to food and water. Male rats were used to minimize the potential influence of estrogen on inflammatory responses (Sun et al., 2020). The animals were randomly allocated into three groups (n = 3 per group): a control group receiving 0.5 mL of 0.9% NaCl, a treatment group induced with *Escherichia coli* at a dose of 1×10^6 CFU/kg body weight (P1), and a treatment group induced with *Escherichia coli* at a dose of 1.5×10^6 CFU/kg body weight (P2). Sepsis was induced via intraperitoneal injection of a live *E. coli* suspension. All animals were euthanized 72 hours after induction for sample collection and histopathological examination.

Rats were assigned to the three groups by simple randomization, with each animal having an equal probability of allocation to any group. The minimum number of animals per group was determined according to Federer's formula [$(t-1)(n-1) \geq 15$, where t is the number of treatment groups], which indicated a minimum total of nine animals. Three animals were therefore included in each group, consistent with the 3Rs (Replacement, Reduction, Refinement) principles for animal research and the preliminary, hypothesis-generating nature of this study. The two *Escherichia coli* doses were selected to balance a sufficient inflammatory response against adequate animal survival for biomarker and histopathological assessment at the study endpoint. Intraperitoneal bacterial induction in rats using doses of approximately $1.02 \times 10^6 - 5.6 \times 10^6$ CFU has been reported to produce approximately 20% mortality within 24 hours, with early mortality specifically attributable to *E. coli*-dominant inocula within this range, whereas doses exceeding 10^7 CFU/kg have been associated with excessive early mortality that precludes observation at

later time points (Tallósy et al., 2021). Accordingly, doses of 1×10^6 CFU/kg (P1) and 1.5×10^6 CFU/kg (P2) were selected to achieve a measurable systemic inflammatory response while maintaining adequate survival for biomarker and histopathological assessment at the 72-hour endpoint. The higher dose (P2) was included to evaluate the effect of increased bacterial exposure on the degree of organ damage, consistent with the dose-response approach commonly employed in experimental sepsis models (Matthay et al., 2024).

Blood samples were collected from the retro-orbital plexus under anesthesia. Neutrophil counts were measured using an automated hematology analyzer from EDTA-anticoagulated blood samples and expressed as $\times 10^3/mm^3$. Serum procalcitonin (PCT) levels were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions. Optical density was read at 450 nm using a microplate reader, and PCT concentrations were expressed in ng/mL.

Following euthanasia, liver, kidney, spleen, heart, and lung tissues were harvested and fixed in 10% buffered formalin. Tissue samples were processed using standard histological techniques, embedded in paraffin, sectioned at 4–5 μm thickness, and stained with hematoxylin and eosin (H&E). Histopathological evaluation was performed under light microscopy at 400× magnification in five randomly selected fields per slide. Organ damage was assessed using semiquantitative scoring systems based on histopathological alterations. Two anatomical pathologists independently evaluated all slides, and inter-rater reliability was assessed using the kappa test. Damage was graded using established semiquantitative scoring systems: the Manja Roenigk system for the liver (Ramachandran & Kakar, 2009; Restuti et al., 2026), the modified system for the kidney and heart (Saleh et al., 2017), the modified) system for the spleen (Ibrahim et al., 2018), and a modified lung injury scoring system from Mamanto & Rahmawati (2021) and Hidayah et al. (2020) assessing alveolar dilatation, alveolar septal destruction, and inflammatory cell infiltration.

In addition to the three individual semiquantitative histopathological subscores (each analyzed separately for its correlation with neutrophil and PCT levels; Table 3), a composite lung score ("lung mean of three parameters") was additionally calculated as the unweighted mean of these subscores to provide an overall summary of pulmonary injury. The authors developed this composite score, which was not included in the original histopathological scoring methods described by Mamanto and Rahmawati (2021), and Hidayah et al. (2020), from which the three individual subscores were adapted. Although such summary indices may facilitate interpretation of overall tissue injury, averaging ordinal histopathological scores assumes comparable intervals between score categories and equal contribution of each parameter to the composite score, assumptions that cannot be formally verified for semiquantitative scoring systems (Klopfleisch, 2013).

Data were analyzed using Statistics software. Continuous data were presented as mean ± standard deviation (SD). The Shapiro–Wilk test was performed to assess data normality. Because the pooled dataset (N = 9) comprised one control group and two treatment groups expected a priori to differ biologically, we interpreted the pooled distribution cautiously, as any departure from normality could reflect the experimental design rather than the underlying distribution within each group. Given the ordinal nature of the histopathological scores and the small sample size within each group (n = 3), Spearman's rank correlation test was selected to assess associations between neutrophil count, PCT level, and histopathological findings. Correlation coefficients (rs) were reported to two decimal places, and p-values < 0.05 were considered statistically significant. Ethical approval was obtained from the Health Research Ethics Committee, Faculty of Medicine, Universitas Muhammadiyah Surakarta, Indonesia (Ethical Clearance No. 6419/A.2/KEPK-FKUMS/VI/2026). All procedures followed institutional guidelines for laboratory animal welfare.

RESULTS

Descriptive statistics for all study variables are presented in Table 1. The mean neutrophil level was $5.48 \pm 3.07 \times 10^3/mm^3$ (range 1.40–7.78), and the mean PCT level was 3.97 ± 2.69 ng/mL (range 0.39–5.82). The highest mean damage was found in alveolar septal destruction (1.51 ± 0.99) and the liver (1.50 ± 0.78), and the lowest in the heart (0.52 ± 0.45). The inter-rater

agreement between the two pathologists ranged from fair to almost perfect across the scored parameters (Cohen's Kappa: liver κ = 0.63, kidney κ = 0.65, heart κ = 0.67, spleen κ = 0.43, alveolar dilatation κ = 0.48, alveolar septal destruction κ=0.29, and inflammatory cell infiltration κ = 0.86; all $p < 0.05$). Among the evaluated parameters, alveolar septal destruction showed the lowest inter-rater agreement (κ = 0.29), whereas inflammatory cell infiltration demonstrated almost perfect agreement (κ = 0.86).

Table 1. Descriptive statistics of neutrophil, PCT, and multiorgan histopathology scores

Variable	N	Min	Max	Mean	SD
Neutrophil ($\times 10^3/mm^3$)	9	1.40	7.78	5.48	3.07
Procalcitonin (ng/mL)	9	0.39	5.82	3.97	2.69
Liver score	9	0.70	2.60	1.50	0.78
Spleen score	9	0.30	1.90	1.19	0.70
Heart score	9	0.10	1.20	0.52	0.45
Renal score	9	0.30	1.50	0.87	0.49
Lung – alveolar dilatation	9	0.20	1.50	0.90	0.42
Lung – alveolar septal destruction	9	0.20	2.30	1.51	0.99
Lung – inflammatory cell infiltration	9	0.40	1.80	1.01	0.55
Lung – mean of 3 parameters	9	0.40	1.78	1.14	0.58

Note: N = number of subjects; Min = minimum; Max = maximum; SD = standard deviation; PCT = procalcitonin.

Table 2. Shapiro–Wilk normality test for neutrophil and PCT levels

Variable	W	df	p	Conclusion
Neutrophil ($\times 10^3/mm^3$)	0.665	9	<0.001	Not normal
Procalcitonin (ng/mL)	0.626	9	<0.001	Not normal

Note: W = Shapiro–Wilk statistic; df = degrees of freedom; data are considered normally distributed if p>0.05; PCT = procalcitonin.

Table 3. Spearman correlation between neutrophil and PCT levels and multiorgan damage scores

Organ damage score	Neutrophil rs	Neutrophil p	PCT rs	PCT p
Liver	0.88**	0.002	0.90**	<0.001
Spleen	0.90**	<0.001	0.87**	0.002
Heart	0.92**	<0.001	0.85**	0.004
Renal	0.65	0.06	0.54	0.14
Lung – alveolar dilatation	0.55	0.12	0.75*	0.021
Lung – alveolar septal destruction	0.98**	<0.001	0.93**	<0.001
Lung – inflammatory cell infiltration	0.75*	0.019	0.60	0.09
Lung – mean of 3 parameters	0.91**	<0.001	0.88**	0.002

Note: rs = Spearman correlation coefficient; p = 2-tailed significance. *significant at p<0.05; **significant at p<0.01; N = 9 for all tests; PCT = procalcitonin.

Both neutrophil (W = 0.665; p < 0.001) and PCT (W= 0.626; p < 0.001) levels were not normally distributed. As described in the statistical analysis, this finding was interpreted cautiously because the pooled dataset comprised one control group and two treatment groups with biologically distinct biomarker levels. Therefore, the non-parametric Spearman rank correlation test was used for the bivariate analysis, consistent with the ordinal nature of the histopathological scores and the small sample size.

Neutrophil level correlated positively and significantly with the damage scores of the liver (rs = 0.88; p = 0.002), spleen (rs = 0.90; p < 0.001), heart (rs = 0.92; p < 0.001), alveolar septal destruction (rs = 0.98; p < 0.001), inflammatory cell infiltration (rs = 0.75; p = 0.019), and the lung mean of three parameters (rs = 0.91; p < 0.001). The correlation with the renal score was moderate but not significant (rs = 0.65; p = 0.06), and the correlation with alveolar dilatation was not significant (rs = 0.55; p = 0.12).

PCT level correlated positively and significantly with the damage scores of the liver (rs = 0.90; p < 0.001), spleen (rs = 0.87; p = 0.002), heart (rs = 0.85; p = 0.004), alveolar dilatation (rs=0.75; p = 0.021), alveolar septal destruction (rs = 0.93; p < 0.001), and the lung mean of three parameters (rs = 0.88; p = 0.002). The correlations with inflammatory cell infiltration (rs = 0.60; p = 0.09) and the renal score (rs = 0.54; p = 0.14) were not significant. A strong and significant positive correlation was also found between neutrophil and PCT levels (rs=0.87; p=0.002).

DISCUSSION

The treatment groups showed substantially higher neutrophil and PCT levels than the control group, producing the bimodal distribution seen in the normality analysis and confirming successful induction of a systemic inflammatory response by the Gram-negative bacterium. Mechanistically, lipopolysaccharide (LPS) from the *E. coli* cell wall is recognized by Toll-like receptor 4 (TLR4) on macrophages and monocytes, activating nuclear factor kappa B (NF-κB) and increasing transcription of proinflammatory cytokines such as TNF-α, IL-1β, and IL-6, which mobilize neutrophils from the bone marrow into the circulation and stimulate PCT synthesis in parenchymal tissues (van der Poll et al., 2021). Measurement at 72 hours post-induction reflects an inflammatory response that has entered a relatively stable phase, consistent with the dynamic evolution of sepsis from an early proinflammatory phase (Liu & Liang, 2026).

Neutrophil level correlated positively with the damage scores of almost all target organs, most strongly with alveolar septal destruction. This is consistent with the pathophysiology of acute respiratory distress syndrome (ARDS) in sepsis, which positions the lung as a primary target organ owing to massive neutrophil sequestration in the pulmonary capillaries (Matthay et al., 2024). Activated neutrophils marginate and adhere to the pulmonary microvascular endothelium and release ROS, elastase, and myeloperoxidase that degrade the basement membrane and interalveolar septa (Xie et al., 2025). This process is aggravated by NET formation. (Fang et al., 2025) showed in an LPS sepsis model that blocking the Mac-1 adhesion molecule reduces adhesion-dependent NET formation and attenuates lung injury, providing strong mechanistic support for the role of neutrophils in alveolar septal disruption. A significant correlation was also found with the lung three-parameter mean and with inflammatory cell infiltration, whereas the correlation with alveolar dilatation did not reach significance, suggesting that neutrophils are more closely linked to severe structural damage and inflammatory infiltration than to the largely reversible vascular-tone changes underlying alveolar dilatation.

A very strong correlation was found with the heart, reflecting sepsis-induced cardiomyopathy, in which activated neutrophils recruited to the myocardium through chemokine axes release ROS and proteases that injure cardiomyocytes, alongside the depressant effect of proinflammatory cytokines on myocardial contractility (Li, 2024; Nong et al., 2023). The relatively low cardiac histopathology score (mean 0.52) suggests that structural myocardial injury was less severe than pulmonary, hepatic, and splenic injury at the time of evaluation. The strong correlation with the spleen (rs = 0.90; p < 0.001) reflects its role as the principal reticuloendothelial organ in the systemic immune response, with neutrophil infiltration of the red pulp during the hyperinflammatory phase (van der Poll et al., 2021). The correlation with the liver was also strong and significant (rs = 0.88; p = 0.002); as the gateway for detoxifying endotoxin

delivered through the portal circulation, the liver responds to LPS through Kupffer cell activation, sinusoidal congestion, and hepatocyte apoptosis (Guo et al., 2025), and Murao et al. (2025) showed that neutrophil-derived NETs activate Kupffer cells and aggravate hepatocyte injury.

In contrast, neutrophil levels did not show a statistically significant correlation with the renal damage score, although the coefficient indicates a moderate trend just at the threshold of significance. The most likely explanation is the limited sample size, which left the analysis underpowered to confirm a moderate correlation rather than indicating an absence of association; this is compatible with the complex mechanism of sepsis-associated acute kidney injury (SA-AKI), which involves factors beyond systemic inflammation, hypoperfusion, microcirculatory disturbance, and tubular oxidative stress, that evolve more slowly than injury in the lung or liver (Manrique-Caballero et al., 2021).

Procalcitonin reflects the intensity of systemic bacterial infection more specifically than the direct cellular inflammatory response represented by neutrophils, and a systematic review and meta-analysis by Chuang et al. (2025) showed that PCT outperforms C-reactive protein for detecting bacterial sepsis. PCT correlated most strongly with the liver ($rs = 0.90$; $p < 0.001$), slightly stronger than neutrophils with the same organ ($rs = 0.88$; $p = 0.002$). This has a strong biological basis given the dual role of the liver: it is a major site of PCT production after LPS and cytokine stimulation and, simultaneously, a vulnerable target of Kupffer cell activation, sinusoidal congestion, and inflammation-mediated hepatocyte apoptosis (Guo et al., 2025; Murao et al., 2025). PCT also correlated strongly with alveolar septal destruction and the lung three-parameter mean, reaffirming the lung as a primary target organ; the correlation with alveolar dilatation was significant, whereas that with inflammatory cell infiltration did not reach significance.

PCT correlations with the spleen and heart were also strong and significant, though slightly weaker than the neutrophil correlations with these organs, consistent with splenic damage being more closely tied to the cellular immune response represented by neutrophils and cardiac injury proceeding through multifactorial pathways including coronary microcirculatory dysfunction and cytokine toxicity (Hao et al., 2024; Li, 2024; Nong et al., 2023). As with neutrophils, the PCT–kidney correlation was not significant; although moderate, the non-significant p indicates that rising PCT was not consistently accompanied by greater renal damage within the 72-hour observation window, consistent with the distinct dynamics of SA-AKI (Manrique-Caballero et al., 2021).

The consistent positive correlations between systemic inflammatory biomarkers and damage scores in most organs are compatible with multiple organ dysfunction syndrome (MODS) as a central pathophysiological process in sepsis, in which mitochondrial dysfunction and oxidative stress form the final pathway bridging systemic inflammation and multiorgan tissue damage (Liu & Liang, 2026; Srdić et al., 2024). Within this limited cohort, damage was not distributed homogeneously: the lung, particularly alveolar septal destruction, was the most affected organ with the strongest correlations to both biomarkers, whereas the kidney showed the weakest. This pattern is consistent with organs that have dense vascularization and vulnerable endothelium, such as the lung and liver, being injured earlier than organs with multifactorial injury mechanisms such as the kidney, with endothelial dysfunction serving as the principal bridge between systemic inflammation and organ damage (Matthay et al., 2024; Srdić et al., 2024). The strong, significant correlation between neutrophil and PCT levels indicates that the two biomarkers moved in parallel during systemic bacterial infection and may provide complementary information regarding inflammatory activity and organ injury severity. These findings support their potential value as indicators of disease severity, given that progression toward MODS (Evans et al., 2021). As this was a preliminary study with a small sample, these exploratory observations require validation in larger studies before broad clinical application.

This study has several limitations that should be stated transparently. First, the small sample size provided limited statistical power to detect moderate correlations, which explains the results that only approached or fell just below significance (neutrophil–kidney, $p=0.06$; PCT–inflammatory cell infiltration, $p=0.09$; neutrophil–alveolar dilatation, $p=0.12$; PCT–kidney, $p=0.14$); larger studies are needed to confirm these relationships and to enable reliable multivariate analysis. Second, the strong and significant correlation between the two predictors ($rs=0.87$; $p=0.002$) indicates multicollinearity, making the independent contribution of each biomarker difficult to separate analytically, future work could use staged analyses or add biomarkers reflecting different inflammatory dimensions (e.g., IL-6, HMGB1, or suPAR) for a more

comprehensive profile. Third, histopathological evaluation was performed only at a single terminal endpoint, preventing baseline-to-injury mapping over time. Therefore, the analysis captures a static snapshot of multi-organ damage at the late acute phase rather than the full cross-temporal evolution of tissue injury. Fourth, sixteen bivariate correlation tests (neutrophil and PCT against eight organ-damage parameters) were reported without correction for multiple comparisons (e.g., Bonferroni or false discovery rate), may increase the risk of Type I error given the small sample size; consequently, these correlations should be interpreted as exploratory and hypothesis-generating rather than confirmatory, and larger, appropriately powered studies with correction for multiplicity are needed before the associations can be considered conclusive. Fifth, although inter-rater agreement for alveolar septal destruction was fair (κ=0.29), this parameter consistently showed the strongest correlations with both neutrophils (rs=0.98) and PCT (rs=0.93). This intriguing divergence, where high correlation strength coexists with lower inter-observer consensus, highlights the inherent challenge of semi-quantitative visual scoring in subtle acute lung injuries. Rather than undermining the biological trend, this variability underlines the exploratory nature of the findings and strongly emphasizes the need to incorporate automated digital image analysis or standardized morphometric software in future replication studies to minimize subjective variance. Sixth, the composite "lung mean of three parameters" was an author-introduced modification, not part of the original lung scoring parameters, constructed as an unweighted average across three ordinal categories; this assumes comparable inter-category intervals and equal contribution of each parameter, assumptions that cannot be formally verified for semiquantitative histopathology scores, so readers requiring parameter-specific resolution should refer to the individual subscore correlations in Table 3.

Although circulating neutrophil counts and PCT levels do not exclusively reflect organ-specific injury, because neutrophil counts partly represent systemic marrow release rather than tissue-infiltrating neutrophil activity alone (Zhang et al., 2025), and PCT primarily reflects the systemic host response to bacterial infection, being hypersecreted diffusely from multiple non-endocrine tissues throughout the body rather than from the injured organ itself (Becker et al., 2010), the consistently strong correlations observed across multiple organs in this study support their potential utility as candidate biomarkers associated with multiorgan injury. However, further longitudinal experimental and clinical studies are required to determine their diagnostic thresholds and predictive performance for early sepsis recognition. Finally, animal sepsis models do not fully replicate the complexity of human sepsis, particularly regarding variability in age, comorbidities, baseline immune status, and pharmacogenetic responses. Nevertheless, the male Rattus norvegicus model with intraperitoneal E. coli induction remains valuable for investigating inflammatory mechanisms, biomarker activation, and multiorgan injury in Gram-negative sepsis, providing a rationale for future translational studies.

CONCLUSION

This preliminary study demonstrates an association between neutrophil level and multiorgan tissue damage scores, with higher levels related to greater damage, particularly in the lung, heart, and spleen. PCT levels also showed a significant association with multiorgan damage scores, particularly in the liver and lung. The kidney showed a distinct pattern and did not correlate significantly with either biomarker. The two biomarkers provide complementary information, but the limited sample size prevented us from conclusively establishing a simultaneous (multivariate) relationship. Larger studies are recommended to confirm these findings and to enable reliable multivariate analysis.

AUTHOR'S DECLARATION

Authors' contributions and responsibilities

NH: Conceptualization, investigation, formal analysis, writing original draft; **MA**: Investigation, data curation, writing, review, and editing; **IN**: Supervision, validation, conceptualization, writing, review, and editing. All agreed to take responsibility for every aspect of the work presented

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