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***Observational Study***

**Clinical significance of platelet mononuclear cell aggregates in patients with sepsis and acute respiratory distress syndrome**

Huang CM *et al*. Significance of PMAs in sepsis with ARDS

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**Author contributions:** Huang CM performed the study; Li JJ analyzed the data; Wei WK designed the research and wrote the manuscript; All authors have read and approve the final manuscript.

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**Abstract**

BACKGROUND

The diagnosis of sepsis combined with acute respiratory distress syndrome (ARDS) has increased owing to the enhanced awareness among medical professionals and the continuous development of modern medical technologies, while early diagnosis of ARDS still lacks specific biomarkers. One of the main pathogenic mechanisms of sepsis-associated ARDS involves the actions of various pathological injuries and inflammatory factors, such as platelet and white blood cells activation, leading to an increase of surface adhesion molecules. These adhesion molecules further form platelet-white blood cell aggregates, including platelet-mononuclear cell aggregates (PMAs). PMAs has been identified as one of the markers of platelet activation, here we hypothesize that PMAs might play a potential biomarker for the early diagnosis of this complication.

AIM

To investigate the expression of PMAs in the serum of patients with sepsis complicated by ARDS and its clinical significance.

METHODS

We selected 72 hospitalized patients diagnosed with sepsis as the study population between March 2019 and March 2022. Among them, 30 patients with sepsis and ARDS formed the study group, while 42 sepsis patients without ARDS comprised the control group. After diagnosis, venous blood samples were immediately collected from all patients. Flow cytometry was employed to analyze the expression of PMAs, platelet neutrophil aggregates (PNAs), and platelet aggregates (PLyAs) in the serum. Additionally, the Acute Physiology and Chronic Health Evaluation (APACHE) II score was calculated for each patient, and receiver operating characteristic curves were generated to assess diagnostic value.

RESULTS

The study found that the levels of PNAs and PLyAs in the serum of the study group were higher than those in the control group, but the difference was not statistically significant (*P* > 0.05). However, the expression of PMAs in the serum of the study group was significantly upregulated (*P* < 0.05) and positively correlated with the APACHE II score (*r* = 0.671, *P* < 0.05). When using PMAs as a diagnostic indicator, the area under the curve value was 0.957, indicating a high diagnostic value (*P* < 0.05). Furthermore, the optimal cutoff value was 8.418%, with a diagnostic sensitivity of 0.819 and specificity of 0.947.

CONCLUSION

In summary, the serum levels of PMAs significantly increase in patients with sepsis and ARDS. Therefore, serum PMAs have the potential to become a new biomarker for clinically diagnosing sepsis complicated by ARDS.

**Key Words:** Sepsis; Acute respiratory distress syndrome; Platelet leukocyte aggregates; Platelet mononuclear cell aggregates, Biomarker

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**Core Tip:** Our research aimed to investigate the expression of platelet mononuclear cell aggregates (PMAs) in the serum of patients with sepsis complicated by acute respiratory distress syndrome (ARDS) and its clinical significance. The results indicate that the serum levels of PMAs significantly increase in patients with sepsis and ARDS. Therefore, serum PMAs have the potential to become a new biomarker for clinically diagnosing sepsis complicated by ARDS.

**INTRODUCTION**

Sepsis is a common and critical clinical condition, and in recent years, the number of cases diagnosed as sepsis with concomitant acute respiratory distress syndrome (ARDS) has increased, owing to the enhanced awareness among medical professionals and the continuous development of modern medical diagnostic technologies[1]. Due to the high mortality rate associated with sepsis, it has attracted widespread attention in the clinical community. Despite various diagnostic tools available, early diagnosis of ARDS still lacks specific biomarkers. Recent studies have confirmed that one of the main pathogenic mechanisms of sepsis-associated ARDS involves the actions of various pathological injuries and inflammatory factors[2,3].

In this study, platelets and white blood cells in the patient's body are activated, leading to an increase in surface adhesion molecules. These adhesion molecules further form platelet-white blood cell aggregates, including platelet-neutrophil aggregates (PNAs), platelet aggregates (PLyAs), and platelet-mononuclear cell aggregates (PMAs)[4]. Activated platelets bind to monocytes and neutrophils, with the noteworthy observation that the binding of platelets to monocytes precedes that to neutrophils. PMAs are considered one of the markers of platelet activation[5]. The objective of this study is to investigate whether PMAs in the serum of sepsis patients with ARDS can serve as effective biomarkers for the early diagnosis of this complication.

**MATERIALS AND METHODS**

***Study subjects and diagnostic criteria***

This study included 72 adult sepsis patients admitted to our hospital between March 2019 and March 2022. The diagnosis was in accordance with the " International Guidelines for Management of Sepsis and Septic Shock: 2016[6]." Following the 2012 Berlin definition[7], patients were categorized into the study group (sepsis with ARDS, *n* = 30) and the control group (sepsis alone, *n* = 42). Exclusion criteria comprised pregnancy with blood system diseases, pure blood system diseases, HIV infection, ongoing chemotherapy, use of immunosuppressive agents or antiplatelet drugs, pulmonary interstitial fibrosis, and acute exacerbation of chronic obstructive pulmonary disease. The study was approved by our hospital's medical ethics committee, and written informed consent was obtained from all patients or their authorized representatives.

***Sample collection***

Three milliliters of peripheral venous blood were collected from all confirmed sepsis patients immediately upon admission, placed in anticoagulant tubes, and preserved and transported to the Shanghai Lanwei Medical Laboratory for further testing *via* ice pack refrigeration. To ensure accuracy, the entire blood collection process strictly adhered to standardized procedures to prevent platelet activation-induced errors.

***Sample testing***

Patients underwent blood gas analysis, and the oxygenation index PaO2/FiO2 was calculated. Flow cytometry was employed to classify platelet-mononuclear cell aggregates in the peripheral blood of both sepsis patient groups, including PLyAs, PMAs, and PNAs. The site of infection was recorded, and the nature of the pathogenic bacteria was determined through blood culture.

***Acute physiology and chronic health evaluation II Score***

Within 24 h of admission, all confirmed sepsis patients had their physiological indicators meticulously recorded by the attending physician, who then calculated the Acute Physiology and Chronic Health Evaluation (APACHE) II score, noting the worst values.

***Statistical analysis***

Statistical analysis was conducted using SPSS 20.0 software. Descriptive data are presented as mean ± SD, and *t*-tests were used for comparisons. Receiver operating characteristic (ROC) curves were generated to determine the optimal cutoff value, sensitivity, and specificity of serum PMAs in diagnosing sepsis with ARDS. The significance level was set at *P* < 0.05.

**RESULTS**

***General data comparison between the two groups***

According to the data in Table 1, there were no significant differences (*P* > 0.05) observed in general information, such as age, gender, infection site, pathogen, and oxygenation index PaO2/FiO2, between sepsis patients with ARDS and those with sepsis alone.

***Comparison of PNAs, PLyAs, and PMAs between the two groups***

As shown in Table 2, the serum levels of PNAs and PLyAs in sepsis patients with ARDS were slightly higher than those in the sepsis-alone group; however, these differences were not statistically significant (*P* > 0.05). Nevertheless, the serum levels of PMAs in sepsis patients with ARDS were significantly higher than those in the sepsis-alone group, and this difference was statistically significant (*P* < 0.05).

***Comparison of APACHE II scores between the two groups***

According to the data in Table 3, the APACHE II scores of sepsis patients with ARDS were significantly higher than those of sepsis patients without ARDS, and this difference was statistically significant (*P* < 0.05).

***Correlation analysis between PMAs and APACHE II scores***

As illustrated in Figure 1A, the PMAs levels in sepsis patients with ARDS were significantly higher than those in sepsis patients without ARDS. Further linear correlation analysis revealed a positive correlation between PMAs and APACHE II scores in patients (*r* = 0.671, *P* < 0.05).

***Diagnostic value of various indicators for ARDS***

Using PMAs and APACHE II scores as test variables and ARDS as the state variable, ROC curves were fitted. When using PMAs as the test variable, the area under the curve (AUC) was 0.957, indicating a significant diagnostic value (*P* < 0.05). The optimal cutoff value for PMAs was 8.418%, with a diagnostic sensitivity of 0.819 and specificity of 0.947. When using APACHE II scores as the test variable, the AUC was 0.940, indicating a significant diagnostic value (*P* < 0.05). The optimal cutoff value for APACHE II scores was 17.115, with a diagnostic sensitivity of 0.837 and specificity of 0.844. Refer to Tables 4 and 5, and Figure 1B for detailed results.

**DISCUSSION**

Sepsis, as a common complication of severe infections, trauma, acute abdomen, and major surgeries in clinical practice, spans multiple disciplines such as internal medicine, surgery, and gynecology. It leads to multi-organ dysfunction, poor prognosis, and a high mortality rate[8]. The lungs are particularly susceptible to the effects of sepsis, causing pathological damage that is closely related to patient prognosis. Sepsis-induced multi-organ pathology includes the aggregation of white blood cells and platelets at the site of infection, disseminated intravascular coagulation, endothelial damage, resulting in the loss of surfactant on the alveolar surface, and activation of oxidative stress responses. These mechanisms collectively contribute to the development of severe lung injury[9]. Due to the activation of inflammatory reactions and coagulation mechanisms in sepsis patients, they are prone to developing ARDS, with pathological manifestations in the lungs characterized by increased permeability of the alveolar-capillary barrier, pulmonary tissue edema, and severe hypoxemia. After the onset of typical injury symptoms, some sepsis patients may rapidly deteriorate within a short period, progressing to ARDS, thus affecting their prognosis[10]. Venous blood samples are the most readily available and suitable for laboratory testing. Among various specimens, patient serum is primarily used as a biological specimen for accurately and rapidly assessing the severity of sepsis.

In sepsis, damage to endothelial cells leads to activating inflammatory cells and platelets. Activated inflammatory cells release a large number of inflammatory and cellular factors through a cascade reaction, promoting endothelial cell apoptosis and monocyte release of chemokines. Platelets and white blood cells interact in the microcirculation of damaged tissues, forming platelet-white blood cell aggregates. This process further accelerates the release of inflammatory factors such as interleukin and tumor necrosis factor-alpha[11]. The worsening of the inflammatory response leads to endothelial cell swelling, necrosis and shedding, further worsening the patient's condition. Therefore, platelet-white blood cell aggregates in the serum play a crucial intermediary role between platelet activation and inflammatory response.

The results of this study indicate that the serum levels of PMAs in sepsis patients with ARDS were significantly higher than those in sepsis patients without ARDS (*P* < 0.05), confirming the utility of PMAs as a beneficial indicator for diagnosing sepsis with ARDS. The APACHE II scoring system is commonly used to assess the severity and prognosis of critically ill patients[12], have confirmed the application of the APACHE II score in predicting mortality in sepsis patients. The current study demonstrates that the APACHE II scores of sepsis patients with ARDS were significantly higher than those of sepsis patients without ARDS and were positively correlated with serum PMAs levels (*P* < 0.05). This further confirms the clinical importance of serum PMAs in the early diagnosis of sepsis with ARDS.

***Study limitation***

One limitation of this study is the relatively small sample size, with a total of 72 hospitalized patients included in the analysis. The limited sample size may affect the generalizability of the findings to a broader population. Additionally, the study focused on patients from a single hospital, which may introduce institutional biases and limit the external validity of the results. Including a more diverse and larger sample from multiple medical centers could enhance the robustness and applicability of the study findings. Furthermore, the retrospective nature of the study poses inherent limitations. The reliance on historical data collected from medical records may lead to incomplete or missing information. The retrospective design also prevents the researchers from controlling the data collection process, potentially introducing biases in the selection of patients or in the measurement of variables. A prospective study with a carefully designed protocol and standardized data collection procedures would provide stronger evidence and allow for better control of confounding variables. The study primarily focused on the expression of PMAs in the serum as a potential biomarker for sepsis complicated by ARDS. While the findings suggest a significant association, the study does not explore the underlying mechanisms or causality between elevated PMAs levels and the development of ARDS in sepsis patients. Further mechanistic studies are needed to elucidate the pathways through which PMAs may contribute to the pathogenesis of ARDS in sepsis. Finally, the study does not address the specificity of PMAs as a biomarker, and its utility in distinguishing sepsis with ARDS from other conditions that may present with similar clinical manifestations. Future research should explore the specificity and sensitivity of PMAs in differentiating various respiratory and systemic disorders to better understand its diagnostic value in a broader clinical context.

**CONCLUSION**

In conclusion, the elevation of serum PMAs levels is closely associated with the release of inflammatory factors. Although the exact mechanism of PMAs still requires further research, current studies suggest that its changes have important clinical significance in early diagnosing sepsis with ARDS. Therefore, PMAs may serve as one of the biomarkers for early diagnosing sepsis with ARDS.

**ARTICLE HIGHLIGHTS**

***Research background***

The diagnosis of sepsis combined with acute respiratory distress syndrome (ARDS) has increased owing to the enhanced awareness among medical professionals and the continuous development of modern medical technologies, while early diagnosis of ARDS still lacks specific biomarkers. One of the main pathogenic mechanisms of sepsis-associated ARDS involves the actions of various pathological injuries and inflammatory factors, such as platelet and white blood cells activation, leading to an increase of surface adhesion molecules. These adhesion molecules further form platelet-white blood cell aggregates, including platelet-mononuclear cell aggregates (PMAs). PMAs has been identified as one of the markers of platelet activation, here we hypothesize that PMAs might play a potential biomarker for the early diagnosis of this complication.

***Research motivation***

To investigate whether PMAs could be a potential biomarker for the early diagnosis of sepsis combined with ARDS.

***Research objectives***

To investigate the clinical significance of PMAs in patients with sepsis complicated by ARDS.

***Research methods***

72 patients diagnosed with sepsis were enrolled in the study between March 2019 and March 2022. Among them, 30 patients with sepsis and ARDS formed the study group, while 42 sepsis patients without ARDS comprised the control group. After diagnosis, venous blood samples were immediately collected from all patients. Flow cytometry was employed to analyze the expression of PMAs, platelet neutrophil aggregates (PNAs), and Platelet Aggregates (PLyAs) in the serum. Additionally, the Acute Physiology and Chronic Health Evaluation (APACHE) II score was calculated for each patient, and Receiver operating characteristic curves were generated to assess diagnostic value.

***Research results***

The levels of PNAs and PLyAs in the serum of the study group were higher than those in the control group, but the difference was not statistically significant (*P* > 0.05). However, the expression of PMAs in the serum of the study group was significantly upregulated (*P* < 0.05) and positively correlated with the APACHE II score (*r*=0.671, *P* < 0.05). When using PMAs as a diagnostic indicator, the area under the curve value was 0.957, indicating a high diagnostic value (*P* < 0.05). Furthermore, the optimal cutoff value was 8.418%, with a diagnostic sensitivity of 0.819 and specificity of 0.947.

***Research conclusions***

The serum levels of PMAs significantly increase in patients with sepsis and ARDS, which might have the potential to become a new biomarker for clinically diagnosing sepsis complicated by ARDS.

***Research perspectives***

Our study provides a new method for the early diagnosis of sepsis combined with ARDS, which is the detection of serum PMAs. More samples should be enrolled to confirm this method in the future study.

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**Footnotes**

**Institutional review board statement:** The study was reviewed and approved by the Institutional Review Board of the Second People's Hospital of Haining City.

**Informed consent statement:** All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

**Conflict-of-interest statement:** All the authors report no relevant conflicts of interest for this article.

**Data sharing statement:** No additional data are available.

**STROBE statement:** The authors have read the STROBE Statement—checklist of items, and the manuscript was prepared and revised according to the STROBE Statement—checklist of items.

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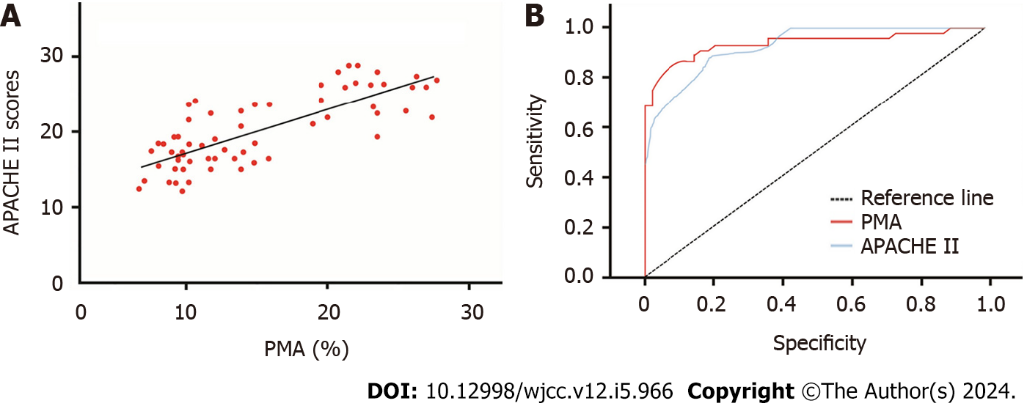
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**Figure Legends**

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**Figure 1 Platelet-mononuclear cell aggregates and Acute Physiology and Chronic Health Evaluation II scores.** A: Linear correlation between platelet-mononuclear cell aggregates and Acute Physiology and Chronic Health Evaluation (APACHE) II scores; B: Diagnostic Value of platelet-mononuclear cell aggregates and APACHE II Scores for acute respiratory distress syndrome. APACHE II: Acute Physiology and Chronic Health Evaluation II Scores; PMA: Platelet-mononuclear cell aggregate.

**Table 1 Comparison of general data between the two groups of patients (mean ± SD), *n* (%)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Study group (*n* = 30)** | **Control group (*n* = 42)** | ***χ*2/*t*** | ***P* value** |
| Male/female | 16/14 | 25/17 | 0.475 | 0.523 |
| Age | 51.6 ± 11.4 | 48.6 ± 14.7 | 1.234 | 0.224 |
| Infection | |  | 1.587 | 0.904 |
| Urinary tract infection | 4 (13.3) | 5 (11.9) |  |  |
| Hematogenous infection | 3 (10) | 4 (9.5) |  |  |
| Abdominal infection | 6 (20) | 8 (19.1) |  |  |
| Pulmonary infection | 15 (50) | 22 (52.4) |  |  |
| Others | 2 (6.7) | 3 (7.1) |  |  |
| Microbiology |  |  | 2.88 | 0.518 |
| Fungus | 3 (10) | 5 (11.9) |  |  |
| G- | 12 (40) | 17 (40.5) |  |  |
| G+ | 5 (16.7) | 8 (19) |  |  |
| Mixed infection | 6 (20) | 7 (16.7) |  |  |
| Unknown cause | 4 (13.3) | 5 (11.9) |  |  |
| PaO2/FiO2 | 141.85 ± 29.44 | 145.35 ± 30.28 | 11.27 | 0.912 |

**Table 2 Comparison of platelet neutrophil aggregates, platelet aggregates, and platelet-mononuclear cell aggregates between the two groups (mean ± SD)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Groups** | ***n*** | **PNAs (%)** | **PLyAs (%)** | **PMAs (%)** |
| Study group | 30 | 14.15 ± 8.93 | 15.42 ± 6.97 | 27.18 ± 6.141 |
| Control group | 42 | 13.87 ± 9.24 | 14.78 ± 3.24 | 17.29 ± 2.05 |

1Compared to the Control groups, the serum levels of platelet-mononuclear cell aggregates increased in sepsis patients with acute respiratory distress syndrome (*P* < 0.05).

APACHE II: Acute Physiology and Chronic Health Evaluation II Scores; PMAs: Platelet-mononuclear cell aggregates; PNAs: platelet neutrophil aggregates; PLyAs: Platelet aggregates.

**Table 3 Comparison of Acute Physiology and Chronic Health Evaluation II Scores between the two groups (mean ± SD)**

|  |  |  |
| --- | --- | --- |
| **Groups** | ***n*** | **APACHE II** |
| Study group | 30 | 35.17 ± 5.441 |
| Control group | 42 | 23.39 ± 4.24 |

1Compared to the Control groups, the Acute Physiology and Chronic Health Evaluation II scores increased in sepsis patients with acute respiratory distress syndrome (*P* < 0.05).

APACHE II: Acute Physiology and Chronic Health Evaluation II Scores.

**Table 4 Area under the curve for various parameters**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Parameter** | **AUC** | **SE** | ***P* value** | **95%CI** | |
| **Upper limit** | **Lower limit** |
| PMAs | 0.957 | 0.022 | < 0.05 | 0.914 | 0.974 |
| APACHE Ⅱ | 0.93 | 0.021 | < 0.05 | 0.872 | 0.981 |

APACHE II: Acute Physiology and Chronic Health Evaluation II Scores; PMAs: Platelet-mononuclear cell aggregates; AUC: Area under the curve.

**Table 5 Diagnostic values for various parameters**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Parameter** | **Cutoff value** | **Sensitivity** | **Specificity** | **Positive predictive value** | **Negative predictive value** |
| PMAs | 8.418 | 0.819 | 0.947 | 0.956 | 0.819 |
| APACHE Ⅱ | 17.115 | 0.837 | 0.844 | 0.829 | 0.877 |

APACHE II: Acute Physiology and Chronic Health Evaluation II Scores; PMAs: Platelet-mononuclear cell aggregates.



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