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Hypogelsolinemia in Patients Diagnosed with Acute Myeloid Leukemia at Initial Stage of Sepsis

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Statistical Analysis C
Data Interpretation D
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Background: Gelsolin (GSN) is an actin-binding and $\text{PIP}_2/\text{Ca}^{2+}$ -regulated protein found in the cytoplasm and blood plasma. Hypogelsolinemia occurs in a wide range of traumatic injuries and inflammatory reactions. We hypothesize that blood GSN levels will be altered in patients diagnosed with acute myeloid leukemia (AML) that develop sepsis, and assessment of GSN concentration will be a useful marker to determine their clinical outcome. To achieve this task, we evaluated the plasma gelsolin concentration in blood samples collected from patients diagnosed with acute myeloid leukemia (AML) at initial stages of sepsis.


Material/Methods: To assess if AML patients might be at risk of sepsis, a SOFA score was determined. Plasma gelsolin concentration was evaluated using an immunoblotting technique.

Results: We found that GSN concentration in the blood of the AML group with developing sepsis was significantly lower ($32 \pm 41 \mu\text{g/ml}$; $p < 0.05$) compared to the AML group ($65 \pm 35 \mu\text{g/ml}$) and control group ($176 \pm 37 \mu\text{g/ml}$; $p < 0.001$). Additionally, low gelsolin concentration in the blood of AML patients developing sepsis was associated with a high SOFA score. A decrease of GSN concentration in the blood of AML subjects with developing sepsis suggests that GSN level in blood reflects not only chronic inflammation stage associated with leukemia, but that GSN depletion also manifests the inflammation associated with sepsis development.

Conclusions: The results presented here suggest the possible utility of GSN evaluation for diagnostic purposes. Overall, these data support the that reversing plasma GSN deficiency might be a possible new strategy in sepsis treatment.

MeSH Keywords: C-Reactive Protein • Gelsolin • Leukemia, Myeloid, Acute • Sepsis

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Background

Numerous studies conducted in the last decade indicate the possibility of using blood plasma gelsolin (pGSN) concentration as a diagnostic marker of an inflammatory response occurring throughout severe tissue injuries, burns, strokes, and sepsis. pGSN belongs to the family of actin-binding proteins (ABPs) [1] that function in the blood as an actin buffer and scavenger of different bioactive lipids, including LPA, S1P, and PAF, and bacterial cell components LPS/LTA. Currently known functions of pGSN reflect the presence of amino acid sequences that preferentially bind actin and PIP₂ within the gelsolin molecule. However, a previously described correlation of a sudden decrease in pGSN content with an increased risk of patient death is still not fully understood [2–4].

The main site of gelsolin synthesis is muscle tissue [1]. Both extracellular (secreted, pGSN) and intracellular (cytoplasmic GSN, cGSN) forms of gelsolin are the product of the gene located on chromosome 9 in humans [5–8]. GSN content in the blood of a healthy person varies in the range of 150–300 µg/ml [8,9]. GSN has the ability to bind to actin, which determines its major intracellular tasks. This ability is subject to ionized calcium and PIP₂ control [10,11]. Low GSN level is a risk factor for further mortality and may indicate a life-threatening condition [2]. GSN reduction in the blood was also observed in hepatic necrosis and myocardial infarction [12,13]. A well-described example of plasma gelsolin concentration decrease is acute respiratory distress syndrome (ARDS) [14], in which the concentration is reduced to 30% of values found in healthy individuals [15]. The GSN level in plasma samples from patients after bone marrow transplantation showed that a reduction of less than 100 µg/ml in the first week after transplantation correlated with a significantly increased risk of pulmonary complications and a high probability of death in the first 3 months after transplantation [16]. Lee et al. recorded the exhaustion of plasma GSN (25–50% of normal) associated with the presence of actin circulating within 6 h of septic challenge in a murine model of sepsis. GSN substitution in sepsis induced solubilization of F-actin and significantly reduced mortality in mice. GSN induced a change in the cytotoxic profile of endotoxin mice towards the anti-inflammatory direction [17–20]. In the present study, we attempted to verify the main hypothesis that there is a decrease of plasma gelsolin concentration in patients with acute myeloid leukemia, and its further decrease in the initial stage of sepsis.

Material and Methods

Specimen collection

Collection of human blood specimens was performed in the Holy Cross Cancer Center of Kielce. The experiments were performed

according to the principles outlined in the Declaration of Helsinki and approved by the Ethics Committee of the Jan Kochanowski University in Kielce. At the time of patient recruitment, written consent was obtained from all subjects and all patients gave their informed consent prior to inclusion in the study. We collected blood plasma from healthy subjects (control), patients diagnosed with AML, and patients with AML at the initial stage of sepsis diagnosis. The sequential sepsis-related Organ Failure Assessment score (SOFA) is proposed to indicate the diagnostic criteria for sepsis that were apply in this study for patients suffering from AML. Moreover, diagnosis of AML was confirmed by phenotypic and cytological bone marrow testing. The samples of blood were placed into EDTA tubes and were centrifuged at 3200 rpm for 10 min to separate plasma, which was then transferred to fresh plastic tubes and frozen at –80°C until use.

Immunoblotting of gelsolin in blood

Gelsolin concentration was assessed as previously described [16]. Plasma samples were denatured in the presence of 4X sample buffer at 95°C for 5 min and loaded to electrophoresis on 10% SDS-polyacrylamide (sodium dodecyl sulfate-polyacrylamide) mini-gels. Recombinant human plasma gelsolin was subjected as a positive control in each gel in a concentration range of 7.5–25 ng. Next, proteins were transferred to polyvinylidene difluoride (PVDF) membranes (Immobilon®-FL PVDF, Millipore). Membranes were incubated with Odyssey blocking buffer (Li-Cor). Transferred proteins were probed with a monoclonal anti-human gelsolin antibody (G4896, Sigma, St. Louis, MO, USA) used at a 1: 10 000 dilution in TBS-T, overnight at 4°C. Goat anti-mouse IgG (H+L) IRDye 800 CW was applied for 60 min at room temperature (1: 20 000, LI-COR). Visualization and quantification were carried out with the LI-COR Odyssey® scanner and an Image Studio™ software (LI-COR Biosciences).

Statistical analysis

Data are reported as a mean ±SD. Differences between means were evaluated using the *t* test, with *p*<0.05 as the level of significance.

Results

Clinical and laboratory characteristics of the patient groups

A group of 82 patients were examined, of which 38 were women and 44 men (Table 1). The average age was 57.3±14.3. The study group consisted of patients of the Hematology Clinic of the Holy Cross Cancer Center in Kielce diagnosed with acute myelogenous leukemia, and in some cases diagnosed with sepsis.

Table 1. Clinical characteristics of AML patients and a reference group.

FAB subtype	Patients with AML and (sepsis)	Age	Sex F/M
AML patients (n=82)			
AML M0	4 (2)	58.75±11.76	2/2
AML M1	10 (8)	57.6±12.55	6/4
AML M2	22 (13)	57.77±14.1	11/11
AML M4	14 (9)	60.21±8.22	8/6
AML M5	7 (3)	61.43±10.35	2/5
AML M5b	9 (7)	51.22±14.27	3/6
AML/MDS	16 (10)	50.38±16.04	6/10
All patients	82 (52)	56.43±13.74	38/44
Reference group (n=30)			
Control group	30	58.68±19.77	18/12

The general condition of the patients was assessed in accordance with the ECOG (Eastern Cooperative Oncology Group) performance status scale. The average ECOG result was 2.9 ± 0.9 . Mean (\bar{x}) CRP level was 183.6 ± 125.5 mg/l; PCT on average was 10.06 ± 15.7 ng/ml. Mean neutrophil count was $0.8 \pm 3.3 \times 10^9$ /L. We also analyzed the concentration of lactate dehydrogenase (LDH) in blood plasma. The mean LDH concentration in AML patients was significantly higher than in the healthy volunteers. Mean LDH concentration in the whole AML study group was 1298.6 ± 1570.9 U/l. In the plasma of patients who survived sepsis, the average LDH concentration was 965.9 ± 1086.1 U/l, whereas in the deceased it was 1989.1 ± 2382.9 U/l.

Outline of pGSN concentration

We observed a statistically significant reduction in the concentration of GSN in the blood of patients with AML ($p < 0.001$) and AML with sepsis ($p < 0.001$) compared to the control group (Figure 1A). Additionally, we assessed the correlation between SOFA score and GSN concentration in the blood plasma of the AML patients developing sepsis (Figure 1B). A high correlation according to Guilford was found ($R = 0.5647$). We showed a very low correlation between the SOFA score and the PCT level (Figure 1C) ($R = 0.0538$) and low correlation between GSN and the PCT level (Figure 1D) in patient blood ($R = 0.0472$).

Interestingly, the initial level of gelsolin might be of great value to assess the outcome of sepsis treatment in AML patients, as the initial concentration of gelsolin in survivors was significantly higher compared to patients who died in the course of sepsis (Figure 2A). We also compared leukocytosis in both groups, showing higher (high, above 5 G/L) leukocyte counts in the deceased patients (Figure 2B). We observed a higher level of PCT in both surviving AML patients developing sepsis and AML

patients who died in the course of sepsis (Figure 2C). A significantly higher level of CRP protein ($p < 0.05$) was observed in patients who died (Figure 2D). Interestingly, in AML patients who died from sepsis, the low GSN level was positively correlated with a higher level of PCT ($R = 0.44$), and we found the opposite observation in the survival group, in which higher PCT level was recorded for patients with higher GSN level (Figure 3A, 3B, respectively). This might indicate that low GSN level is a better marker to predict clinical outcome compared to PCT.

Discussion

In patients with cancer, a diagnosis of infection is usually problematic. The clinical challenge is to differentiate infection and fever of unknown importance [21]. Early diagnosis and stratification of sepsis risk increases the chances of initiating well-timed treatment, which improves the prognosis of patients [22]. The diagnosis of sepsis is most often based on clinical symptoms, including general condition, hemodynamic disturbances, and organ dysfunctions. For the sepsis diagnosis, a quick, sensitive, and specific biomarker is needed [23]. For many years, biomarkers such as C-reactive protein and PCT have been used to evaluate sepsis, its severity, and treatment, including response to antibiotics. The exact role of biomarkers in the treatment of septic patients remains undefined. The specificity of CRP has been widely questioned. PCT may be a better prognostic indicator than CRP, but its value has also been questioned, especially in patients with certain cancers [22]. An increase in PCT levels was noted in conditions associated with an inflammatory response, such as trauma, heart surgery, and other major surgery. Although CRP is considered an inferior sepsis index compared to PCT, it is often used in clinical practice due to its low cost and wide availability. Increased serum CRP

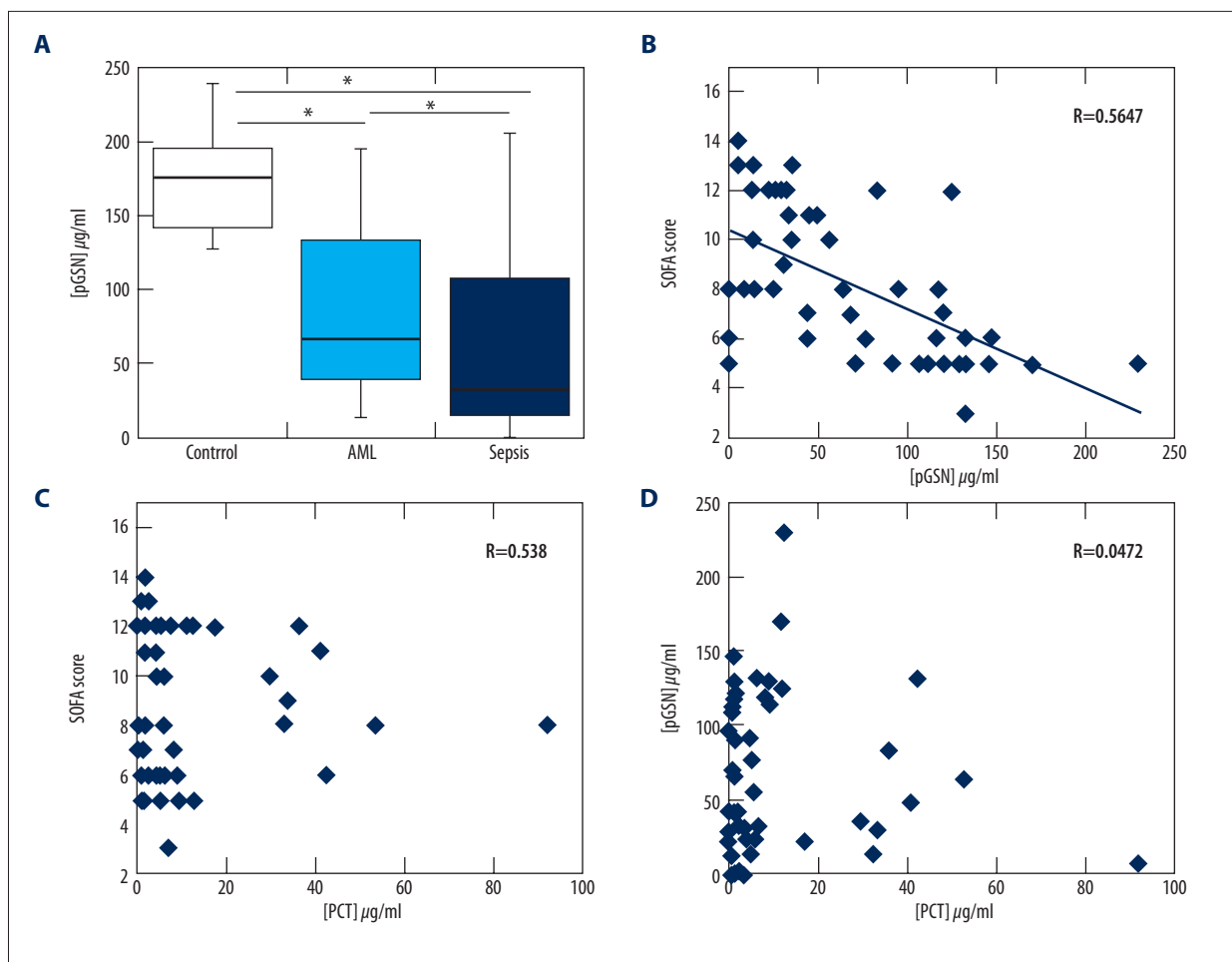


Figure 1. (A) Box plots representing plasma GSN, concentration in control (n=30 healthy volunteers), patients diagnosed with AML (n=30), and patients diagnosed with AML that developed sepsis (n=52). (B) Scatter plots of plasma GSN concentration versus SOFA score of patients diagnosed with AML that developed sepsis. (C) Scatter plots of PCT concentration versus SOFA score of patients diagnosed with AML that developed sepsis. (D) Scatter plots of PCT concentration versus GSN concentration of patients diagnosed with AML that developed sepsis. * Statistically significant.

concentration correlates with an increased risk of organ failure or death. Testing the dynamics of CRP during the process can be helpful in assessing the response to treatment [24–26]. Indeed, in all our patients, higher CRP levels was correlated with unfavorable prognosis.

Compared to a previous report indicating a strong correlation between GSN and PCT, in AML patients developing sepsis, such a correlation was not strongly pronounced [23]. The authors of the publication (Horvath-Szalai, et al.) conducted an analysis of patients with a diagnosis of sepsis staying in the Intensive Care Unit. Sepsis was diagnosed as a complication of surgical intervention (acute abdomen treatment or Whipple's procedure) and other medical events such as pneumonia. The group of patients with sepsis and SIRS was compared with the control group. In our analyzes, we confirmed the results: a higher level of pGSN and CRP and a lower SOFA score in survivors.

This finding might indicate that the anti-inflammatory effects of gelsolin are based on signaling pathways that are not connected with signaling pathways that govern the CRP synthesis. PCT levels in both our groups were not statistically different and were high in both groups. It is possible that the difference in the obtained PCT levels also results from differences at the time of material collection. The material to be evaluated in the patient group with sepsis in agranulocytosis patients with AML was collected at the time of the first symptoms of sepsis, and the PCT level increased in subsequent determinations [23].

AML is a disease characterized by uncontrolled growth of myeloid cells. This leads to a number of clinical problems such as infection and organ damage. Epidemiological data available in the literature indicate septic shock as one of the main causes of death in patients with AML [27,28]. In a population study from Texas Hospitals, in 5501 adult patients with a

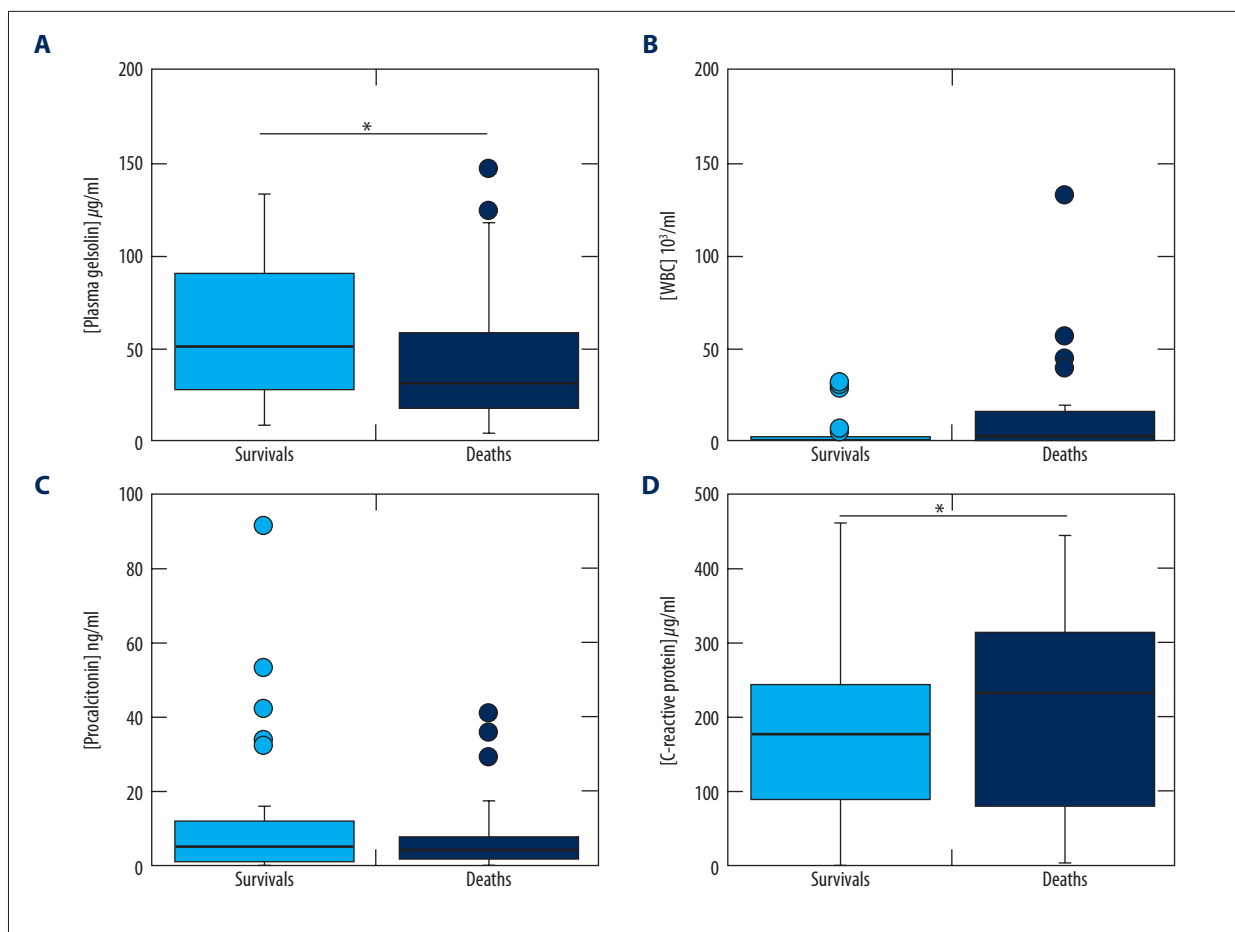


Figure 2. (A) Box plots representing plasma GSN, (B) total count of WBC, (C) procalcitonin, and (D) C-reactive protein concentration in survival of septic AML patients (n=29) and septic AML patients with negative outcomes (n=23). * Statistically significant.

diagnosis of AML, the rate of sepsis was 16% compared to 4% for non-AML patients. Sepsis in AML patients was associated with pneumonia (34%), acute renal failure (32%), and hematologic dysfunctions (29%). Among in-hospital deaths due to sepsis, mortality in AML patients was 30% [29]. Researchers constantly are looking for better sepsis biomarkers. A modern septic marker should not only help identify sepsis, but should also be useful in monitoring its progression. We have shown that in patients with AML, hypogelsolinemia progresses when sepsis develops. It is very likely that pGSN is correlated with the severity of inflammation during sepsis in AML patients, as it does in some other diseases [30,31]. GSN together with Gc-globulin (a protein binding vitamin D), acts as an actin buffer in the blood, and probably also in other body fluids [32,33], and its decrease potentializes actin toxicity [33]. Based on new molecular biology techniques, the lactate dehydrogenase (LDH) enzyme characteristic has been shown to be a very sensitive indicator of cellular metabolic status, activation status, and neoplastic transformation [34]. The increased level of LDH in AML plasma was extensively studied and results from the destruction of cells and the growth of tumor cells.

A favorable correlation was found between the tumor and elevated LDH [35]. In studies conducted in AML patients, performed at the time of diagnosis before any therapeutic intervention, LDH in the serum was significantly higher, regardless of the type of leukemia, in comparison to the group of healthy volunteers. It was also shown that serum LDH concentration was not correlated with the number of peripheral blood leukocytes [36]. This probably indicates that regardless of the number of leukocytes in the blood, they are damaged in other places and their contents are released. We have shown that in the entire group (leukemia/leukemia with sepsis), LDH was significantly higher than in healthy subjects. We also found a significant difference in LDH concentration, with higher levels in those who died. Therefore, it may be assumed that the low level of pGSN is correlated in the studied patients with the degree of cell damage and release of actin, which is confirmed by LDH levels.

The experimental results suggest that disturbances in GSN concentration capable of scavenging actin may interfere with the inflammatory response in the host during sepsis. Examination

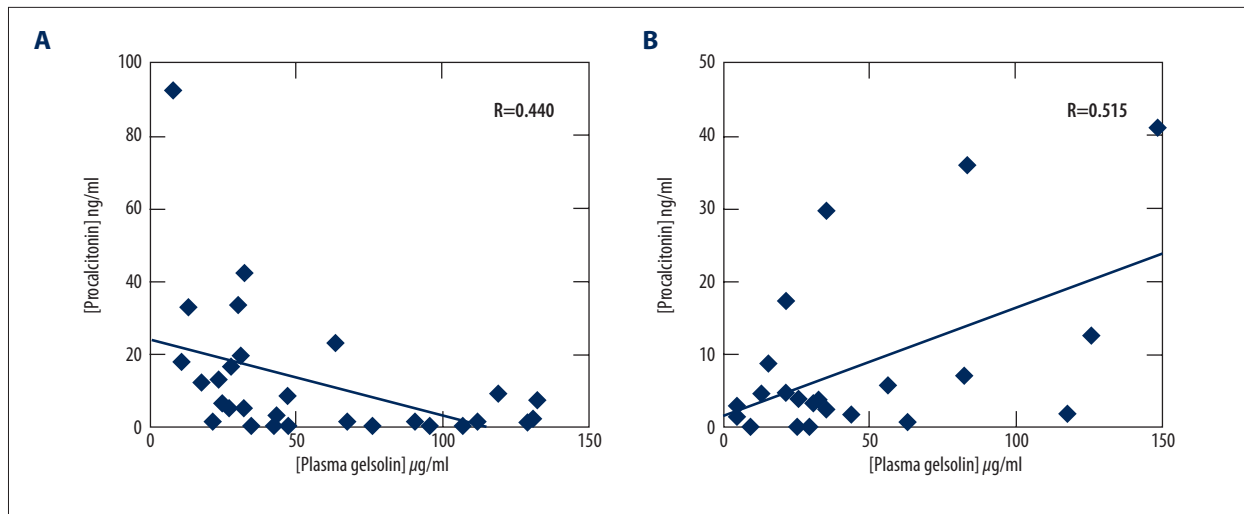


Figure 3. Scatter plots of plasma GSN concentration versus procalcitonin concentration in septic AML patients in (A) survivors and (B) negative outcomes group.

of the GSN levels may indicate the severity of the patient's condition and allow faster therapeutic intervention, while restoring levels of GSN might improve treatment outcomes.

Conclusions

None of the biomarkers previously used for inflammation so far allowed for detailed characterization of patients with sepsis. Based on the results we obtained, it seems that the simultaneous evaluation of several inflammatory biomarkers (CRP, PCT, pGSN, and LDH) in patients with AML in sepsis may be a new diagnostic approach to stratification of patient risk.

Conflicts of interest

None.

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