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ORIGINAL ARTICLE

Delta-Hemoglobin Equivalent and Granularity Index as Cell-Derived Biomarkers for the Detection of Bacterial Infections

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SUMMARY

Background: **Prompt and precise detection of an infection in the blood is of great clinical importance in terms of early therapy initiation and the patient's prognosis. Infection-triggered inflammatory cellular and humoral signaling cascades offer great opportunities to redefine standard tests. However, while inexpensive and easy-to-use biomarkers for the detection of infections and the concomitant inflammatory processes exist, they are rarely used in clinical practice. We aimed to investigate the correlation of Granularity Index (GI) and Delta-hemoglobin equivalent (Delta-He) as inexpensive and easy-to-use cell-derived infection markers with established acute-phase parameters in a randomly selected patient.**

Methods: **We analyzed plasma concentrations of the established C-reactive protein (CRP) and procalcitonin (PCT) and leukocyte and thrombocyte counts in blood samples of 1,787 patients undergoing routine laboratory inflammation diagnostics. We also measured the Granularity Index (GI) and Delta-hemoglobin equivalent (Delta-He) in this cohort between February 2019 and February 2020.**

Results: **Delta-He and GI Index significantly correlated with CRP concentration (AUC 0.72, 95% CI 0.71 - 0.74; $p < 0.001$ for both analytes) and thrombocyte count ($p < 0.001$ for both analytes) but not with leukocyte count (AUC 0.54, 95% CI 0.50 - 0.59, $p < 0.67$). Furthermore, Delta-He significantly correlated with PCT (AUC 0.65, 95% CI 0.63 - 0.68, $p < 0.001$) while GI Index did not. Additionally, thrombocyte count significantly correlated with CRP ($p < 0.001$) and with PCT concentrations ($p < 0.001$).**

Conclusions: **Delta-He and GI are two novel, inexpensive and easy-to-use cell-derived hematological biomarkers with the potential to be used as fully automated and highly standardized parameters. These biomarkers would be available on a 24 hours basis in the routine laboratory for the detection of bacterial infections by measuring a complete blood count (CBC) with differential and reticulocyte counts.**

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KEY WORDS

Delta-He, Granulation index, inflammation, CRP, leukocyte count, hematology analyzer

LIST OF ABBREVIATIONS

ACD - anemia of chronic disease
AUC - area under curve

CBC - complete blood count
CI - confidence interval
CRP - C-reactive protein
FSC - forward-scattered
GI - Granularity Index
Delta-He - Delta-Hemoglobin equivalent
PCT - procalcitonin
PMN - polymorphonuclear neutrophilic granulocytes
Ret-He - reticulocyte hemoglobin content
ROC - receiver operating characteristic
SFL - side-fluorescent light
SIRS - systemic inflammatory response syndrome
SSC - side-scattered
WBC - white blood cell count

INTRODUCTION

Infection, tissue damage, intoxications, advanced cancer, and autoimmune diseases can all cause severe, complex systemic acute-phase reactions that involve metabolic, neuroendocrine, and hematopoietic changes. Traditionally, along with clinical examination, C-reactive protein, procalcitonin, leukocyte and thrombocyte counts have been the parameters involved in timely diagnosis of such pathomechanisms [1]. For decades, complete blood count (CBC) has been an essential component in the diagnosis of acute phase reactions, from local and systemic bacterial infections up to septic shock [1]. The first definition of systemic inflammatory response syndrome (SIRS) included an elevated or decreased white blood cell count (WBC), or alternatively, a normal cell count with a presence of > 10% bands [1]. Today, both parameters - WBC and bandemia - tend to be less useful, since half of all patients presenting to the hospital with bacteremia have a normal white blood cell count [2]. Yet because a CBC is a ubiquitously available test that provides important clinical information about the patient's disease status, the focus is increasingly on a more careful interpretation of and nuances in the data [3]. The acute phase protein C-reactive protein (CRP) - commonly used to track bacterial infections [17] - typically displays a 24 - 48-hour delayed increase in serum concentration, which impedes its use as an infection-associated biomarker. Procalcitonin (PCT), a marker established for early diagnosis of systemic bacterial infection [21,22], has similar diagnostic disadvantages [21,22].

Modern, fully automated hematological blood analyzers make it possible not only to count and differentiate blood cells, but also to assess neutrophil granulation and activation in ethylenediaminetetraacetic acid (EDTA) anti-coagulated blood samples based on cell granularity (protein content of cytoplasm), cell shape and volume, and total nucleic acid compounds in the cytoplasm [4, 5]. Several fluorescent dyes (e.g., polymethines and oxazines) stain nucleic acids as well as different cellular protein components of blood cells. In addition, sideward fluorescence as well as sideward- and forward-scatter measurements quantify the cytoplasmic content of nucleic acids, cellular granularity and size of blood cells [4,5].

Hepcidin, the central regulatory protein of iron metabolism triggered by bacteria-associated inflammation, is responsible for endocytosis of the iron exporter protein ferroportin in duodenal enterocytes, splenic macrophages, and hepatocytes. It reduces iron availability in the blood and traps iron intracellularly [6,7]. This inflammation-triggered iron redistribution hampers hemoglobin synthesis in the bone marrow, leading to anemia of chronic disease (ACD). Serum hepcidin concentrations are increased and reticulocyte hemoglobin content (Ret-He) is decreased (e.g., in pneumonia patients) [8], reflecting iron supply and quality of erythropoiesis over the past two to three days. The difference in the hemoglobin content of newly formed reticulocytes and mature erythrocytes is defined as the Delta hemoglobin equivalent (Delta-He) [9-11]. A negative Delta-He indicates insufficient iron supply and impaired hemoglobinization of reticulocytes [9-11].

We aimed to compare the concentration levels of four established and two novel cell-derived hematological biomarkers in patients with and without bacteria-associated infections who underwent a blood examination in our medical laboratory. The clinical diagnosis was used as "gold standard" in our study, consisting of anamnesis, physical examination, imaging techniques, and microbiological examination of samples suspected to be infectious.

We hypothesized that Delta-He levels, capable of distinguishing between different disease-based types of anemia, correlate with levels of classic biomarkers, and can be used to diagnose acute bacterial infections or infection-associated inflammation.

MATERIALS AND METHODS

Patients, data collection and ethics approval

We retrospectively analyzed the routine hematologic and clinical chemistry laboratory data of all 1,787 patients who underwent at least a CRP, a PCT measurement, and a complete blood count plus a reticulocyte analyzed in our medical laboratory over the course of one year. The blood withdrawals for CRP, PCT, and CBC plus reticulocyte were always performed simultaneously. All laboratory tests were directly and electronically ordered by the clinician and collected in serum and K_2-EDTA anticoagulated blood tubes (Sarstedt AG & Co. KG, Nümbrecht, Germany). No sample was stored longer than 1 hour prior to measurement. All laboratory tests were part of the hospital's routine diagnostic procedures. Clinicians ordered the blood tests for their patients as they deemed clinically appropriate, and did not follow a study protocol with a predefined set of blood tests.

In total, the data of 1,787 laboratory measurements from routine blood collections between February 4, 2019, and

February 3, 2020, were analyzed. Eight hundred and sixty-five (865) samples were collected from males (48.4%) and 922 from females (51.6%). The median age of the study population was 79.0 years, with the youngest patient being 1 day old and the oldest 100 years (age range: 0 - 100 years). The study population comprised patients from the following clinical wards: internal medicine (61%; n = 1,090), including gastroenterology, cardiology, and pulmonology); surgery (19%; n = 340), including abdominal, vascular and trauma/orthopedic surgery; gynecology (3%; n = 54); pediatrics (3%; n = 54); intermediate care unit (3%; n = 54); emergency room (2.9%; n = 52); intensive care unit (2.4%; n = 41); neurology (0.6%; n = 10); and ophthalmology and otorhinolaryngology (5.1%; n = 92). The main clinical diagnoses were sepsis 28%, bacterial infection 20%, bacterial wound and skin infections 19%, bacterial pneumonia 14%, bacterial peritonitis 8%, bacterial sinusitis 3%, bacterial tonsillitis 3%, and controls without infection/inflammation 5%. The infection-associated clinical diagnoses were microbiologically confirmed. This study was approved by the Ethics Committee of the Berliner Ärztekammer, Germany (ETH KB-2020/0011). Additionally, the entire study was carried out in accordance with the Declaration of Helsinki and all relevant local legislation.

Principle of measurement on the XN series

A Sysmex XN-1000 analyzer was used for this study. The XN-1000 utilizes impedance technology as well as fluorescence flow cytometry to count and differentiate blood cells in EDTA anticoagulated blood samples. Hematology analyzers of the XN series contain a red diode laser producing a light ray with a wavelength of 633 nm. Specific polymethine- and oxazine-based fluorescent dyes are incorporated into nucleic acids and bound to cytoplasmic proteins of white blood cells. Three different signals can be obtained: 1) forward-scattered (FSC) light, giving information on cell-size, 2) side-scattered (SSC) light, providing information about the intracellular structure and granularity of the cell, and 3) side-fluorescent light (SFL), providing information on nucleic acid content (DNA and RNA), reflecting the metabolic activitiy of the cell. The XN-1000 was calibrated at regular intervals. The manufacturer's quality control material was run on all instruments twice daily at three different levels (XN Check controls).

The granularity of neutrophils, for example induced by bacteremia, can be measured as hypogranulation or hypergranulation based on a change in the granularity of polymorphonuclear neutrophilic (PMN) granulocytes (NEUT-GI value in the Diff-Channel of the XN-1000 platform) [4-13]. A hypergranulation of neutrophils can be caused by inflammation or infection (e.g., bacteria), while hypogranulation can be observed in myelodysplastic syndromes or even in leukemias [5,13]. The measurement and calculation procedure of the GI Index is identical on the XN-1000 platform using NEUT-GI as the corresponding analyte to the former NEUT-X. Zimmermann et al. revealed in a study with 789 blood samples (543 samples from different intensive care units and 246 samples from blood-healthy control patients) a new reference interval for NEUT-GI and the corresponding GI Index on the XN-platform [14]. The new 95% reference interval was 140.91 to 160.46 (standard deviation: 4.47) on the XN-platform versus 129.20 to 142.33 (standard deviation: 3.35) on the former XE-platform. The GI Indices displayed no significant statistical difference between the XN series and the XE series in both patient cohorts [14].

Measurement of CRP and PCT

C-reactive protein and procalcitonin concentrations in serum samples were determined using commercially available turbidimetric assays (Roche Deutschland Holding GmbH, Grenzach-Wyhlen, Germany for CRP and BRAHMS GmbH, Hennigsdorf, Germany for PCT) on a Roche Cobas 6000 platform (Roche Deutschland Holding GmbH, Grenzach-Wyhlen, Germany) according to the manufacturer's protocol. The Cobas 6000 was calibrated at regular intervals. The manufacturer's quality control material was run on the instrument twice daily at two different levels (PreciControl ClinChem Multi Level 1 and 2 for CRP and Elecsys BRAHMS PCT PreciControl PCT 1 and 2 for PCT).

Statistical analysis

The data set was analyzed using Prism version 8.0 for Windows (GraphPad Software, San Diego, CA, USA). All data were assessed with a Kolmogorov-Smirnov test and the Shapiro-Wilk test for normal distribution. The correlation analysis of Delta-He, GI Index, CRP, PCT, leukocyte and thrombocyte counts was performed using Spearman's correlation analysis because normal distribution was not confirmed. A p-value of less than 0.05 was considered statistically significant. Additionally, the diagnostic accuracy of Delta-He, GI Index, CRP, PCT, leucocyte and thrombocyte counts to detect bacterial infection was analyzed using receiver operating characteristic (ROC) curve analysis [15].

RESULTS

Delta-He (r_s: 0.882; 95% CI: 0.734 to 0.916; $p < 0.0001$) and GI Index (r_s: 0.725; 95% CI: 0.672 to 0.768; $p < 0.0001$) were both strongly correlated with CRP concentration (Figure 1A and 1B). Delta-He was also strongly correlated with PCT levels (r_s: 0.736; 95% CI: 0.690 to 0.773; $p < 0.001$, Figure 2A), while no correlation between GI Index and PCT concentrations was observed (Figure 2B). Interestingly, CRP and PCT values are not significantly correlated with each other in our study cohort (data not shown).

Delta-He and GI Index are significantly correlated with thrombocyte count (r_s: 0.687; 95% CI: 0.627 to 0.753; $p < 0.001$ for Delta-He and r_s: 0.674; 95% CI: 0.638 to 0.715; $p < 0.0007$ for GI Index) but not with leukocyte

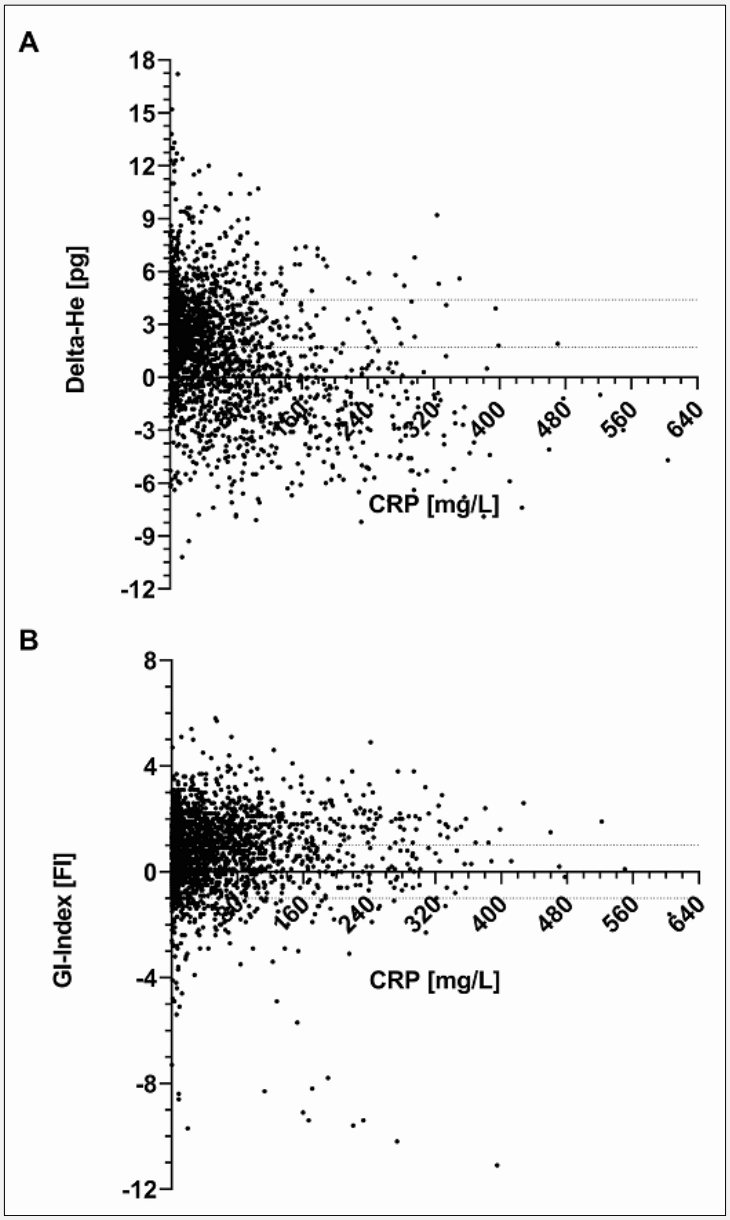

Figure 1. Scatter plots for Delta-He and GI Index versus CRP in 1,787 laboratory measurements.

A) Depicted is the correlation between Delta-He- and CRP levels (r_s: 0.882; 95% CI: 0.734 to 0.916; $p < 0.0001$). B) Illustrated is the correlation between GI Index and CRP levels (r_s: 0.725; 95% CI: 0.672 to 0.768; $p < 0.0001$).

count (Figure 3). The thrombocyte count significantly correlated with CRP (r_s: 0.740; 95% CI: 0.727 to 0.793; $p < 0.001$) and with PCT concentrations (r_s: 0.7651: 95% CI: 0.714 to 0.811; $p < 0.0001$) (Figures 4B and 4D). Furthermore, there was no obvious correlation between leukocyte count and CRP or between leukocyte count and PCT (Figures 4A and 4C).

Furthermore, Delta-He, GI Index, CRP, PCT, as well as leucocyte and thrombocyte counts were analyzed as a means to detect a bacterial infection using ROC curve analysis. ROC curve analysis revealed an area under the curve (AUC) for the capability to detect a bacterial infection of 0.748 for Delta-He (Figure 5A; 95% CI: 0.7243 to 0.7674; $p < 0.001$), 0.639 for GI Index (Fig-

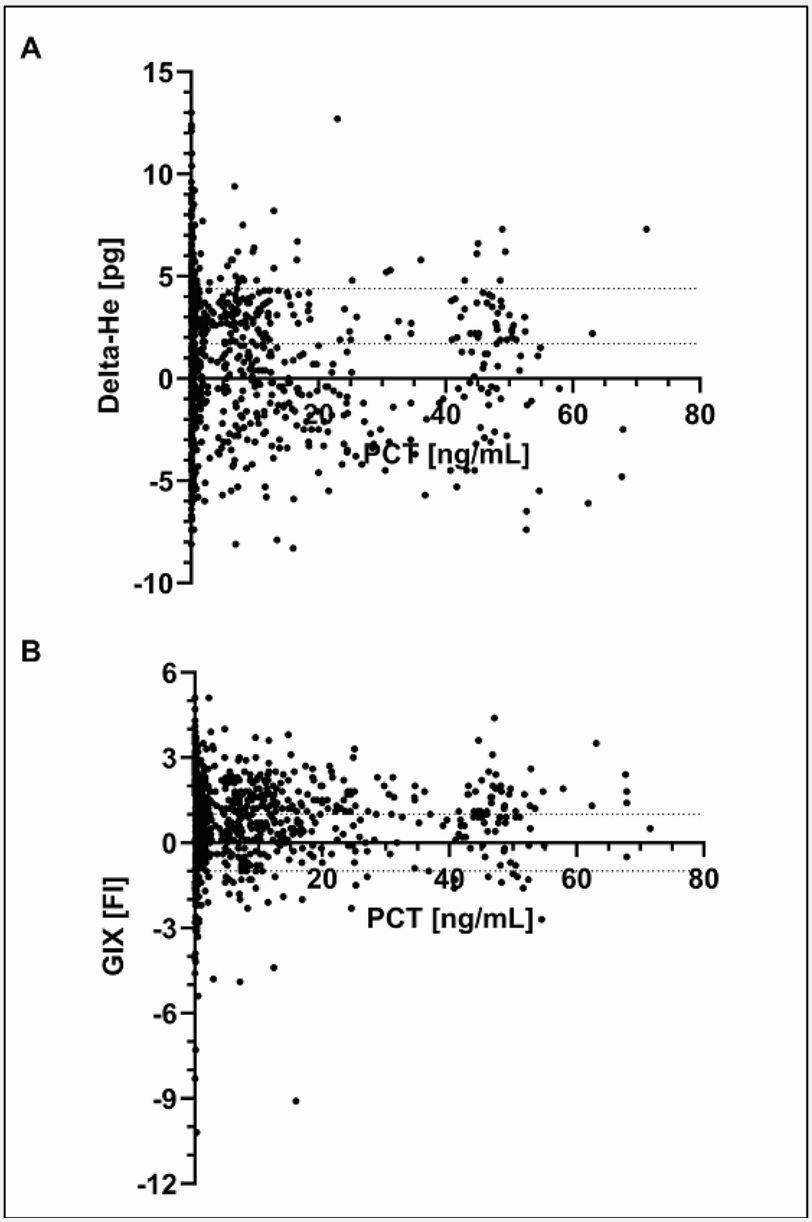

Figure 2. Scatter plots for Delta-He and GI Index versus PCT in 1,787 laboratory measurements.

A) Exhibited is the correlation between Delta-He and PCT levels (r_s: 0.736; 95% CI: 0.690 to 0.773; p < 0.001). B) GI Index does not correlate with PCT levels.

ure 5B; 95% CI: 0.5914 to 0.6783; p < 0.004), 0.721 for CRP (Figure 5C; 95% CI: 0.7093 to 0.7413; p < 0.001), 0.653 for PCT (Figure 5D; 95% CI: 0.6268 to 0.6842; p < 0.001), 0.542 for leucocyte count (Figure 5E; 95% CI: 0.4985 to 0.5931; p = 0.67), and 0.683 for thrombocyte count (Figure 5F; 95% CI: 0.6575 to 0.7186; p < 0.001), respectively.

DISCUSSION

In comparisons with conventional acute-phase proteins CRP and PCT and leukocyte and thrombocyte counts, we measured Delta-He and GI Index to determine the cell-derived hematological biomarkers' diagnostic ability to detect bacteria-induced inflammation in blood

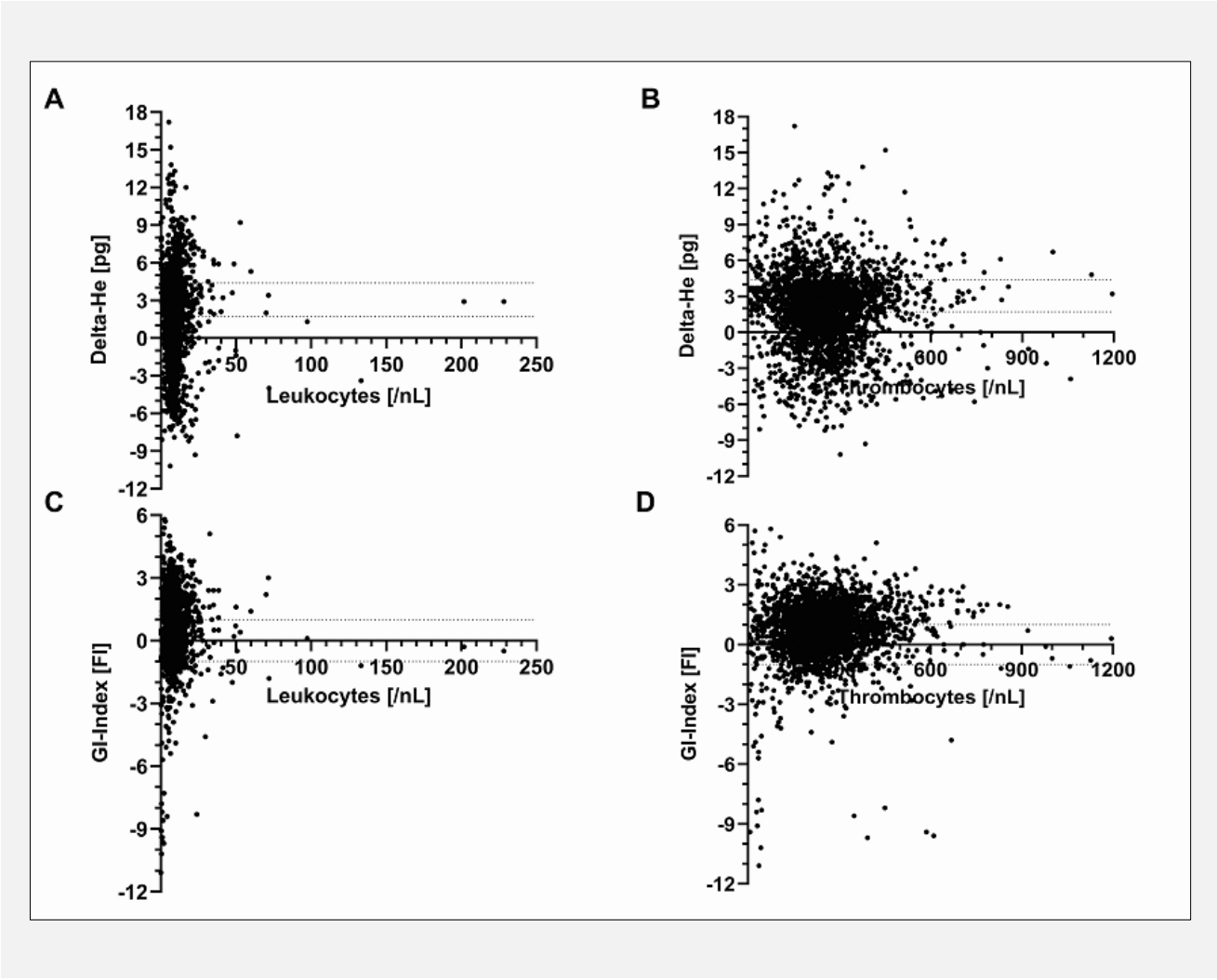

Figure 3. Scatter plots for Delta-He and GI Index versus leucocyte and thrombocyte counts in 1,787 laboratory measurements.

A) Delta-He concentrations and leucocyte count are not correlated with each other. B) Illustrated is the correlation between Delta-He concentrations and thrombocyte count (r_s: 0.687; 95% CI: 0.627 to 0.753; p < 0.001). C) GI Index values and leucocyte count are not correlated with each other. D) Exhibited is the correlation of GI Index concentrations and thrombocyte count (r_s: 0.674; 95% CI: 0.638 to 0.715; p < 0.0007).

samples from 1,787 patients. We found that Delta-He and GI Index are two novel, inexpensive and easy-to-use cell-derived hematological biomarkers which can be used as fully automated and highly standardized parameters available in the routine laboratory for the detection of bacteria-induced inflammation.

Timely detection of severe infections and subsequent administration of an appropriate antibiotic therapy improves outcome, especially in critically ill patients suffering from systemic bacterial infections [22,23,27-29, 33-35]. In combination with a reticulocyte count, a complete blood count usually contains significant information and can often suggest a diagnosis of systemic bacterial infection or septic shock. This blood count-derived data may regularly be overlooked, however, due to the focus on white blood cell count [2,3]. Cell-derived hematological parameters like Delta-He or GI Index provide deeper insights into the erythron and its red precursor cells (e.g., reticulocytes) as well as into the infection-associated granularity of neutrophils during acute-phase reactions [4-10,12,13]. As a result of hepcidin-25-mediated intracellular 'iron trapping' there is reduced iron availability in the blood and consequently iron overload in the reticulo-endothelial system [9,24, 25]. An increase in hepcidin-25 levels caused by infection directly influences the hemoglobinization rate of reticulocytes, and subsequent comparison of complete blood count with reticulocyte count is sufficient for the analysis of the erythron [26]. Within a few hours, the infection-triggered hepcidin-25 levels increase, leading to

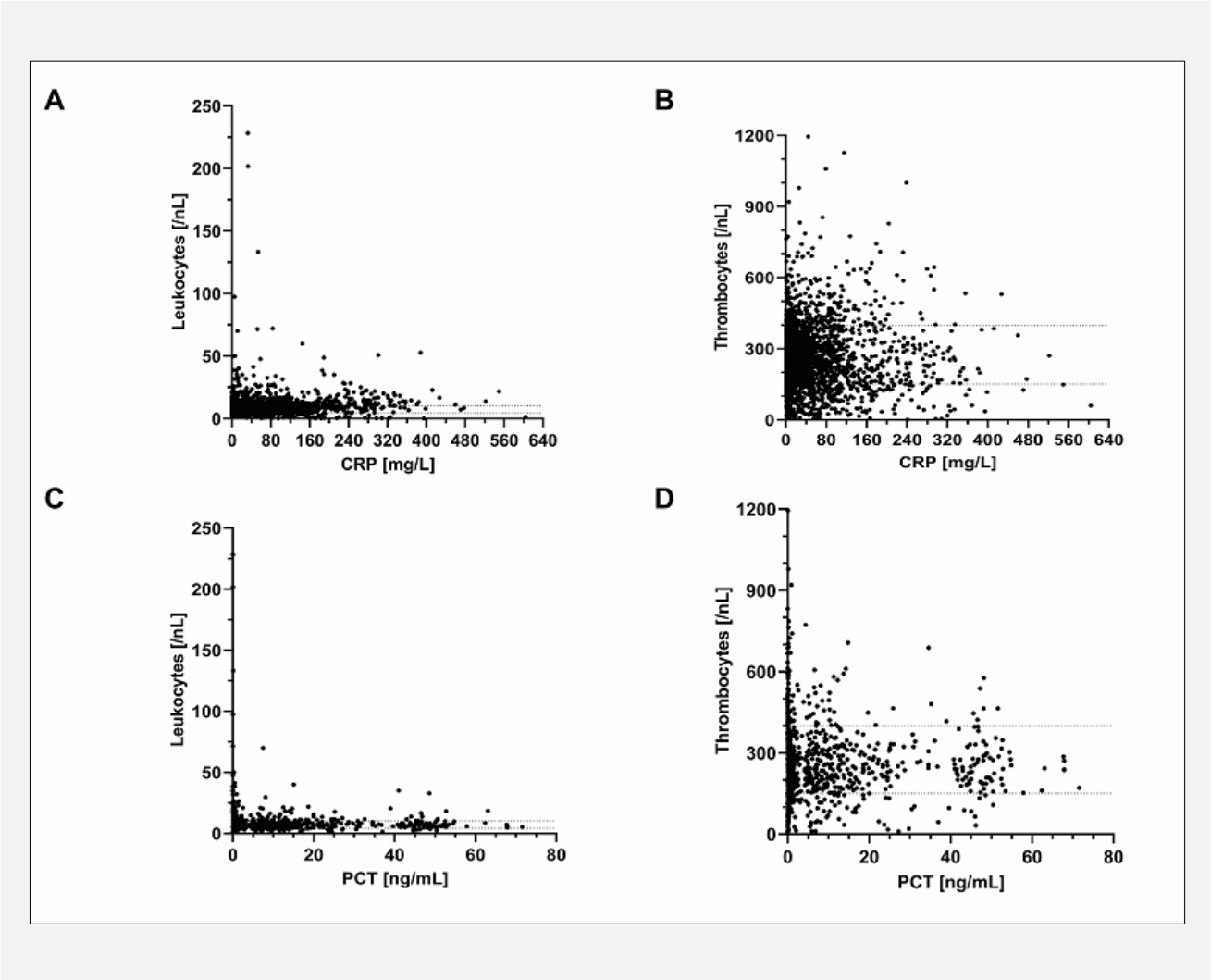

Figure 4. Scatter plots for leucocyte and thrombocyte counts versus CRP and PCT concentrations in 1,787 laboratory measurements.

A) Leucocyte count and CRP levels are not correlated with each other. B) Thrombocyte count and CRP concentrations display a statistically significant correlation with each other (r_s: 0.740; 95% CI: 0.727 to 0.793; $p < 0.001$). C) Leucocyte count and PCT levels are not correlated with each other. D) Thrombocyte count and PCT concentrations exhibit a statistically significant correlation with each other (r_s: 0.7651: 95% CI: 0.714 to 0.811; $p < 0.0001$).

less iron in the blood stream. This results in a significantly reduced hemoglobinization rate in reticulocytes, leading to a negative value for Delta-He [9,27].

Delta-He has gained attention in three recent studies, first as a cell-derived biomarker for the onset and resolution of inflammation (e.g., induced by infections or sepsis), second as an independent predictor of all-cause mortality and the response to erythropoiesis-stimulating agents (ESAs), and third as a new biomarker clearly distinguishing between different types of inflammation-based anemias before and after medical therapy [6,9, 28]. Whereas those studies focused on chronic inflammatory diseases and iron-deficiency anemia (IDA), we are the first specifically studying Delta-He as a biomarker for bacterial infection.

We observed significant changes in Delta-He levels within a few hours during our daily work in the hematology laboratory. In untreated patients suffering from bacterial sepsis or severe inflammatory diseases (e.g., inflammatory bowel disease), Delta-He levels were negative before the administration of any therapy. Promptly after adequate medical intervention and recovery from the severe acute-phase reaction (e.g., administration of antibiotics in patients suffering from bacterial infections up to sepsis), the Delta-He levels became positive again [26]. In such cases, the hepcidin-25 levels declined due to the decreased acute-phase reaction and iron was again available in the blood stream, leading to an in-

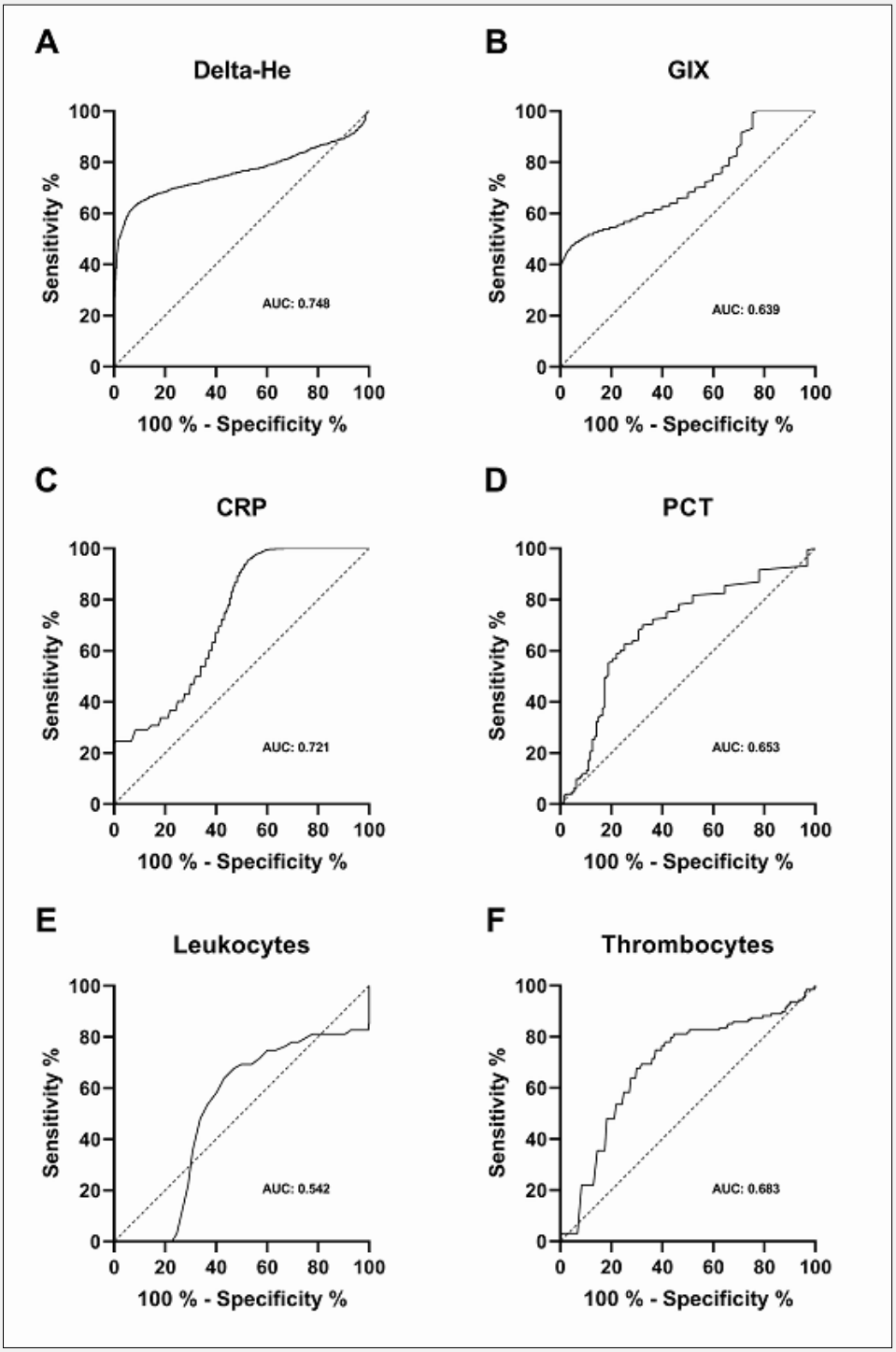

Figure 5. Receiver operating characteristic curve analysis for the six biomarkers comparing the power to predict a bacterial infection in 1,787 laboratory measurements.

AUC for Delta-He: 0.748 (Figure 5A; 95% CI: 0.7243 to 0.7674; p < 0.001), AUC for GI Index: 0.639 (Figure 5B; 95% CI: 0.5914 to 0.6783; p < 0.004), AUC for CRP: 0.721 (Figure 5C; 95% CI: 0.7093 to 0.7413; p < 0.001), AUC for PCT: 0.653 (Figure 5D; 95% CI: 0.6268 to 0.6842; p < 0.001), AUC for leucocyte count: 0.542 (Figure 5E; 95% CI: 0.4985 to 0.5931; p < 0.67 n.s.), AUC for thrombocyte count: 0.683 (Figure 5F; 95% CI: 0.6575 to 0.7186; p < 0.001).

crease in hemoglobinization of erythrocytes and reticulocytes in the bone marrow, followed by elevated levels of Delta-he within a few hours [27].

These findings are clearly supported by the data of patients in our present study: Delta-He is significantly correlated with CRP (Figure 1A) and PCT (Figure 2A) concentrations in our cohort. A ROC-curve analysis underlined this finding and revealed an AUC of 0.748 for Delta-He as a means of detecting an infection (Figure 5A), while CRP and PCT exhibited similar AUCs of 0.721 (Figure 5C) and 0.653 (Figure 5D), respectively. These results emphasize the diagnostic accuracy of Delta-He with regard to the rapid detection of a bacterial infection in a patient's blood, which is comparable to the diagnostic value of CRP or PCT when used for the same purpose.

The turn-around time for the detection of Delta-He using fluorescence flow-cytometry is below two minutes, compared to approximately 40 minutes for the measurement of CRP or PCT concentrations using an immunoassay, which requires a 10-minute centrifugation step for the corresponding serum sample. Especially in septic patients, every minute counts between diagnosis and the administration of antibiotic drugs. The quantification of Delta-He by comparing a complete blood count with a differential and reticulocyte count significantly speeds up the diagnostic procedure and the initiation of a first antibacterial treatment, especially in the emergency room (ER) setting.

In addition to Delta-He, hemoglobin content in reticulocytes (Ret-He) is a sensitive indicator for monitoring short-term changes in iron availability for erythropoiesis. Reticulocyte maturation coincides with a progressive decrease in RBC volume and hemoglobin content; consequently, Ret-He is always greater than RBC-He in healthy individuals. When demand for iron exceeds supply, Ret-He rapidly drops below RBC-He, which is easily identified by a negative Delta-He. And while inflammation-associated iron-blockade is typically associated with negative Delta-He values, as shown in this study, so is early-onset iron deficiency.

A limitation of this study is that the presence of iron deficiency as a possible confounder was not definitely excluded in our patient group. In consideration of the mean levels of ferritin and Ret-He, the prevalence of iron deficiency is up to 11.5% (on average, 10% of ferritin levels < 30 µg/L and 13% of Ret-He levels < 28 pg).

An additional approach used to obtain detailed information about the infection status of a patient is to detect and quantify another cell-derived hematological parameter reflecting the granularity of neutrophils. Those large, dark and irregular granules represent intracytoplasmic proteins with 'anti-bacterial function'. Within 30 minutes after stimulation of neutrophils with bacterial lipopolysaccharides (LPS) *in vitro*, the neutrophils' granularity demonstrated *hypogranulation* leading to a negative GI Index, followed by a subsequent and significant *hypergranulation* of neutrophils with a positive GI Index within a period of 3 hours following LPS stimulation [5]. The initial phase of hypogranulation seems to result from the release of cytotoxic/anti-bacterial granules from the cytoplasm of neutrophils due to the LPS effect. This finding exhibits a more dynamic kinetic activity of the GI Index compared to classical acute-phase proteins like CRP and PCT, which are known to increase between 12 and 48 hours after the onset of an inflammatory stimulus [5,22,29,35].

The presence and amount of neutrophil granularity have been measured in past decades using manual microscopy of blood smears. Recently, Zimmermann et al. demonstrated a significant correlation between manual microscopy of toxic granulation neutrophils (TGNs), GI Index, and CRP concentrations [5]. They suggested that the GI Index be used as a replacement for manual microscopy of TGNs and as a new and cell-derived biomarker to detect inflammatory processes in blood samples [5,6]. Our findings are in accordance with that study: GI Index was significantly correlated with CRP values ($p < 0.0001$) (Figure 1B) but not with PCT levels ($p = 0.081$) (Figure 2B). A ROC curve analysis showed an AUC of 0.609 for the GI Index to detect bacteria-induced inflammation in the blood (Figure 5B) and confirmed the statistically significant correlation with the acute-phase protein CRP.

It is not known why there is no statistically significant correlation between GI Index and PCT, or between CRP and PCT, in our study. Probably, this phenomenon depends on the biochemical performance of PCT leading to a different 'diagnostic profile' when compared with other well-known acute-phase proteins like CRP or interleukin-6 (IL-6) [36]. PCT has been called a "hormokine" by Mueller et al. due to its cytokine-like behavior during infection or inflammation [37]. The cause for the diagnostic differences between PCT and other acute-phase proteins like CRP or GI Index might be this "chemokine-like" biochemical profile of PCT and the fact that PCT is a well-known substrate of (e.g.,) dipeptidyl-peptidase IV, leading to impaired diagnostic accuracy of PCT due to the following clinical parameters [36,38]: 1) local bacterial infection/colonization (e.g., tonsillitis, minor soft tissue infection, abscess, appendicitis, etc.); 2) patient status following liver transplantation, cardiogenic shock, severe pancreatitis or rhabdomyolysis; 3) certain types of autoimmune disorders; 4) no significant PCT response; 5) recently performed surgery, or recent trauma such as smoke inhalation or burns; 6) end-stage tumor disease; 7) severe renal or liver dysfunction; 8) low immunogenic responses; 9) severe immunosuppression [36-38]. Twenty-one percent of our study patients suffered from chronic inflammatory diseases or Tonsillitis, sinusitis or local skin infections. This fact may have negatively affected the discriminative diagnostic power of PCT in our study as well.

A blood count is inexpensive and can be easily measured within a few seconds. Especially in case of infection and inflammation diagnostics, clinicians usually order a complete blood count in combination with acute-

phase biomarkers like CRP and/or PCT. It is a known phenomenon that during inflammatory processes (such as infection or sepsis), thrombocytopenia can be observed in the blood and activation of coagulation can be part of the pro-inflammatory response as well [16]. Furthermore, specific bacterial enzymes can be released during infections, leading to an increased clearance of thrombocytes via the Ashwell receptor on hepatocytes [30]. Our results are in accordance with the findings of Grewal et al. [30] and other groups demonstrating a significant statistical correlation between cell-derived bacteria-induced inflammation markers, Delta-He (Figure 3B) and the GI Index (Figure 3D), based on the thrombocyte count. In addition, there was also a statistically significant correlation between the thrombocyte count and the acute-phase protein CRP (Figure 4B), but not with PCT (Figure 4D).

Leukocytosis and leukopenia have been part of diagnostic pathways for the detection of inflammation and infectious diseases in blood samples, but with an existing risk of non-specific diagnostic findings [31]. We were able to demonstrate the diagnostic limitations of the leukocyte count in our study: many patients suffering from bacterial infections with concomitant low-grade, high-grade or severe inflammation had a leukocyte count within the reference range. No significant statistical correlation was observed between Delta-He (Figure 3A), the GI Index (Figure 3C) and the leukocyte count. In addition, the leukocyte count exhibited no statistically significant correlation with CRP (Figure 4A) or PCT (Figure 4C).

Our findings are underlined by the results of other groups. In a stepwise logistic regression model, Bates et al. showed that neither leukocytosis nor leukopenia was an independent parameter reflecting a true-positive blood culture [32]. Jekarl et al. demonstrated an area under the curve (AUC) in a receiver operating characteristic (ROC) analysis regarding a leukocyte count of 0.62 for the diagnosis of bacterial infection and 0.54 for the diagnosis of severe sepsis and septic shock [33]. Ljungstrom et al. showed that biomarker combination (e.g., CRP, PCT and neutrophil-lymphocyte-count ratio) can be used to improve the timely diagnosis of sepsis and septic shock in critically ill patients, compared to the detection of CRP, PCT or neutrophil-lymphocyte-count-ratio (NLCR) alone [34]. Hoffmann et al. presented laboratory data in a retrospective study of 53,968 patients suffering from different grades of inflammation according to the serum CRP levels. But even in the cohort with high-grade inflammation, the leukocyte counts of most patients did not exceed the reference range [6].

In conclusion, the biomarkers Delta-He and the GI Index are standardized and automated parameters that can be timely (detection time: < 2 minutes) and cost-effective (obtained on a 24/7 basis from a single EDTA blood tube via a CBC with differential and reticulocyte count analysis. Delta-He and GI Index support the differential diagnosis of infection-associated inflammation in the patient's blood, and should be further evaluated, preferably in a prospective study. Focusing on the high frequency of blood counts ordered for infection and inflammation diagnostics, Delta-He and GI Index could easily be implemented in automated hematological analysis.

Souce of Funds:
None.

Declaration of Interest:
None.

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