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An Overview about Sepsis Biomarkers; with special Emphasis on Hepcidin and Sepsis

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Abstract: Sepsis represents a dysregulated immune response to infection that leads to organ dysfunction. Host response biomarkers play a critical role in diagnosis, early recognition of organ dysfunction, risk stratification, prognostication, and patient management, including antibiotic stewardship. Biomarkers may also be helpful for trial enrichment to identify suitable patients and/or risk categorization for an intervention. A wide range of biomarkers, measured by a host of different technologies, are being investigated to discriminate a systemic inflammatory response syndrome (SIRS) rapidly, which is an excessive defensive body's response to a harmful stressor (for example, infection, trauma, surgery, acute inflammation, ischemia or reperfusion, or cancer) or early identification of infection-triggered organ dysfunction (sepsis). The humoral innate immune response consists of multiple components, including fluid phase pattern recognition molecules (PRMs) and the complement system. PRMs include C-reactive protein (CRP), serum amyloid P component (SAP), and pentraxin 3 (PTX-3). The rise in CRP level is primarily induced by interleukin (IL)-6 and IL-1β acting on the gene responsible for CRP transcription during the acute phase of an inflammatory process. CRP is a pentameric acute-phase reactant protein whose conformation facilitates the ability to trigger complement activation and activate platelets, monocytes, and endothelial cells. Several potential biomarkers for monitoring sepsis have been investigated, including white blood cell count (WBC), C-reactive protein, (CRP), lactate, and procalcitonin (PCT). During the last decade Heparin binding protein (HBP) has attracted interest as a biomarker for severe bacterial infection including sepsis and meningitis. HBP also known as Cationic Antimicrobial Protein of 37 kDa (CAP37) was identified in 1984 and is produced by neutrophils, stored in intracellular vesicles, and rapidly released upon stimulation by pathogen associated microbial patterns, PAMPs. Elevated HBP levels have been shown to correlate with hypotension and organ dysfunction in patients with bacterial sepsis but failed to distinguish patients with septic shock from other causes of shock Hepcidin plays a role in innate immunity through its interactions with IL-6 and other pro-inflammatory cytokines. The ability to sequester iron within cells to prevent its availability for pathogenic or neoplastic growth appears to be largely dependent on hepcidin stimulation by IL-6. This innate defense may help protect against many pathogens, including streptococcal and malarial species. The severity of sepsis depends on several host factors, such as age, comorbidities, and immune status, as well as pathogen factors such as virulence, microbial species, and infectious load. In spite of the sepsis-3 criteria presented as a consensus document 2016 the diagnosis is often a challenge in critically ill patients.

Keywords: Sepsis biomarkers, Hepcidin

Introduction: Sepsis is defined as a life-threatening organ dysfunction caused by a dysregulated host response to infection. Septic shock should be considered a subset of sepsis in which underlying circulatory, cellular, and metabolic abnormalities contribute to a greater risk of mortality than that posed by sepsis alone. Both sepsis and septic shock represent a major growing global burden and a challenge for emergency physicians because of their increasing incidence and great pathophysiological, molecular, genetic, and clinical complexity ***(1).***

The incidence of sepsis and septic shock has continuously increased since the first consensus definition (Sepsis-1) in 1991, reaching around 49 million cases of sepsis and 11 million sepsis-related deaths worldwide in 2017. These data led the World Health Organization (WHO) to declare sepsis a global health priority. This alarming increase in incidence can be attributed to different factors: (i) the advanced average age among patients, especially in western countries; (ii) the increased number of invasive procedures; (iii) the wide usage of immunosuppressive drugs and chemotherapy; and (iv) antibiotic resistance. Despite significant advancements in therapeutic management, septic patients have a high risk of in-hospital mortality (IHM), accounting for approximately 20% of all-cause deaths globally, rendering this combined ailment one of the major causes of morbidity and mortality in critically ill patients ***(2)***.

The frequency of identifiable microorganisms in sepsis/septic shock has varied over time, with a current preponderance of Gram-positive bacteria and an increased clinical and epidemiological significance of fungal sepsis. Among the Gram-positive bacteria, the most frequently isolated pathogens are Staphylococcus aureus and Streptococcus pneumoniae, whereas among the Gram-negative bacteria, those most commonly identified are Escherichia coli, Klebsiella, and Pseudomonas spp. Among the fungal infections associated with the condition, the predominant role is played by Candida spp., which can often be identified in immunosuppressed or neoplastic patients undergoing long-term treatment with chemotherapeutic and immunosuppressive drugs. The main sites of infection related to sepsis are the respiratory tract/pulmonary parenchyma (43%); the urinary system (16%); the abdomen (14%); the head, which is associated with a fever of unknown origin (FUO) (14%); and other sites/causes (13%) ***(3).***

Sepsis Biomarkers

Sepsis represents a dysregulated immune response to infection that leads to organ dysfunction. Host response biomarkers play a critical role in diagnosis, early recognition of organ dysfunction, risk stratification, prognostication, and patient management, including antibiotic stewardship. Biomarkers may also be helpful for trial enrichment to identify suitable patients and/or risk categorization for an intervention. A wide range of biomarkers, measured by a host of different technologies, are being investigated to discriminate a systemic inflammatory response syndrome (SIRS) rapidly, which is an excessive defensive body's response to a harmful stressor (for example, infection, trauma, surgery, acute

inflammation, ischemia or reperfusion, or cancer) or early identification of infection-triggered organ dysfunction (sepsis) ***(4).***

The humoral innate immune response, cytokines, and chemokines

The humoral innate immune response consists of multiple components, including fluid phase pattern recognition molecules (PRMs) and the complement system. PRMs include C-reactive protein (CRP), serum amyloid P component (SAP), and pentraxin 3 (PTX-3). The rise in CRP level is primarily induced by interleukin (IL)-6 and IL-1β acting on the gene responsible for CRP transcription during the acute phase of an inflammatory process. CRP is a pentameric acute-phase reactant protein whose conformation facilitates the ability to trigger complement activation and activate platelets, monocytes, and endothelial cells. Furthermore, CRP is one of the most widely used and investigated biomarkers. A prospective multicenter cohort study followed 483 adult patients who survived hospitalization for sepsis for up to one year. IL-6, high-sensitivity C reactive protein (hs-CRP), soluble programmed death-ligand 1 (sPD-L1), E-selectin, and intercellular adhesion molecule 1 (ICAM-1) were evaluated at five-time points during and after hospitalization. A comparison was made between a phenotype with hyperinflammation (high levels of IL-6 and hs-CRP) and a phenotype of immunosuppression (high sPD-L1 levels). Compared with a normal phenotype, both hyperinflammation and immunosuppression phenotypes had higher 6-month hospital readmission rates and 1-year mortality rates, both all-cause and attributable to cardiovascular or cancer ***(5).***

Pentraxin (PTX-3) is secreted by macrophages, dendritic cells, macrophages, fibroblasts, mesangial cells, and glial cells under pathogen or inflammatory stimuli. Plasma PTX-3 was assessed on days 1, 2, and 7 in 958 patients with sepsis or septic shock included in the Albumin Italian Outcome Sepsis (ALBIOS) study. The researchers assessed a possible association between PTX-3 levels and clinical severity, organ dysfunction, treatment, and mortality within 90 days. PTX-3 levels were elevated at the onset of sepsis and increased with illness severity and the number of organ dysfunctions. PTX-3 levels decreased between days 1 to 7, but this was less prominent in patients with septic shock. In a prospective observational analysis, PTX-3, IL-6, procalcitonin (PCT), and lactate combined showed excellent performance in predicting 28-day all-cause mortality among patients diagnosed with sepsis or septic shock and superior to SOFA score ***(6).***

The activation of PRRs culminates in the stimulation of transcription factors resulting in the expression and secretion of proinflammatory cytokines, including tumor necrosis factor-alpha (TNF-α), IL-1-β, IL-6, and interferons (IFNs). These inflammatory mediators are required for host defense against pathogens and activation of the adaptive immune response. A retrospective study evaluated a broad panel of cytokines and found IL-1β, IL-6, IL-8, MCP-1, IL-10, and plasminogen activator inhibitor 1 (PAI-1) levels were increased in the acute phase of sepsis in both critically and non-critically ill patients. In addition, levels of IL-10 (days 1, 2, and 4), IL-6 and PAI-1 (days 2 and 4), and IL-8 (day 4) increased in critically

ill patients compared to non-critically ill. In summary, hs-CRP, IL-6, and PAI-1 circulatory levels may have utility in stratifying a hyperinflammatory patient phenotype ***(7).***

Neutrophil-to-lymphocyte ratio (NLR)

The neutrophil-to-lymphocyte ratio (NLR), calculated as a simple ratio between the neutrophil and lymphocyte counts measured in peripheral blood, is a biomarker which conjugates two faces of the immune system: the innate immune response, mainly due to neutrophils, and adaptive immunity, supported by lymphocytes. Neutrophils are responsible for the first line of host immune response against invading pathogens, through different mechanisms, including chemotaxis, phagocytosis, release of reactive oxygen species (ROS), granular proteins and the production and liberation of cytokines. Neutrophils also play an important regulatory role in adaptive immunity and are the main effector cells during the systemic inflammatory response (SIRS). As regulators of innate immunity, neutrophils recruit, activate and programme other immune cells, secreting an array of pro-inflammatory and immunomodulatory cytokines and chemokines capable of enhancing the recruitment and effector functions of other immune cells, such as dendritic cells (DCs), B cells, NK cells, CD4, CD8 and γδ T cells, as well as mesenchymal stem cells ***(6).***

An isolated rise in neutrophil count, and, consequently, an elevated NLR, can be observed in several conditions: bacterial or fungal infection, acute stroke, myocardial infarction, atherosclerosis, severe trauma, cancer, post-surgery complications and any condition characterized by tissue damage that activates SIRS. This is because the early hyperdynamic phase of infection is characterized by a proinflammatory state, mediated by neutrophils and other inflammatory cells. SIRS is associated with the suppression of neutrophil apoptosis, which augments neutrophil-mediated killing as part of the innate response. Thus, NLR is often characterized by an increase in neutrophils and a decline in lymphocytes ***(8).***

Moreover, NLR could predict mortality in the general population. NLR was significantly associated with higher overall mortality (HR 1.14, 95% CI 1.10–1.17, per quartile of NLR) and also with specific causes of mortality, including heart disease (HR 1.17, 95% CI 1.06–1.29, per quartile of NLR), chronic lower respiratory diseases (1.24, 1.04–1.47), influenza/pneumonia (1.26, 1.03–1.54) and kidney diseases (1.62, 1.21–2.17). On the other hand, no significant associations of NLR with mortality due to cancer, cerebrovascular disease, accidents, or diabetes mellitus were noted. In addition, in the Rotterdam study, it was shown that NLR levels were independently and significantly associated with an increased risk of all-cause mortality (HR 1.64; 95% CI 1.44-1.86) ***(6).***

NLR has been known as a reliable marker for the diagnosis of bacteremia and sepsis. A recent metanalysis showed that a higher NLR was associated with poor prognosis in patients with sepsis (mean HR 1.75) and that NLR was higher in non-survivors than in survivors from sepsis (mean HR 1.18). Moreover, in a single-center prospective observational study of septic patients admitted to an intensive care unit (ICU), NLR values correlated with sepsis severity as calculated with the SOFA score ($R = 0.65$) and also with presepsin ($R = 0.56$), with a sensitivity of 47%, a specificity of 78% and an AUC of 0.631 ($p < 0.05$). The same study also

showed that NLR was significantly higher in patients with septic shock, suggesting the potential value of NLR in assessing sepsis severity, especially when its value is above 10. It was proposed a cut-off value series for NLR according with procalcitonin (PCT) values as a tool for bacteremia or sepsis diagnosis, as well as for decision making. Compared with CRP and white blood cell count (WBC), NLR showed a moderate sensitivity (57.8%) and high specificity (84%). Several diseases associated with elevated NLR values, such as trauma, surgery, pancreatitis and rheumatic disorders, often associated with sepsis, may play confounding roles ***(9).***

In the *Gurol et al. 2015* study, patients were grouped according to the following criteria: PCT levels < 0.05 ng/mL (healthy group, HG), PCT levels 0.05–0.5 ng/mL (local infection group, LIG), PCT levels 0.5–2 ng/mL (systemic infection group, SIG), PCT levels 2–10 ng/mL (sepsis group, SG) and PCT levels > 10 ng/mL (sepsis shock group, SSG). The NLR cut-off values for the HG, LIG, SIG, SG and SSG groups were <5, ≥5–<10, ≥10–<13, ≥13–<15 and ≥15, respectively. The authors proposed a NLR cut-off value of 5 for diagnosis of infection or sepsis when exclusion criteria are used. A recent observational study demonstrated that NLR and IL-6 appeared to be independent predictors of 28-day mortality in septic patients. Moreover, Jang et al. conducted a clustering analysis showing that age, NLR and delta neutrophil index (DNI) were the most robust predictors for sepsis status in all subjects, as well as in cluster-specific groups. These findings could contribute to suggest a strategy to screen sepsis patients without leukocytosis in the emergency care units. This is because, as we said above, NLR increase precedes WBC and CRP alterations, being the first sign of the activation of the immune system during sepsis. However, there are insufficient data about the role of NLR to identify the source of sepsis ***(10).***

Platelet to lymphocyte ratio

Growing evidence indicates that immune dysregulation (especially cellular immunity), including proinflammatory or anti-inflammatory responses during different stages, is common in cases of sepsis. Recently, studies have reported that platelets play an important role in both the immunomodulatory and inflammatory process, by inducing the release of inflammatory cytokines and interacting with different kinds of bacteria and immune cells, including neutrophils, T-lymphocytes, natural killer (NK) cells and macrophages, which contribute to the initiation or exacerbation of the inflammatory process. Low lymphocyte counts, which to a certain degree represent a suppressed immune and inflammatory response, have also been reported to be associated with inflammatory diseases, such as cardiovascular disease and type 2 diabetes ***(11).***

Based on these findings, the PLR was suggested as being a novel systematic inflammatory indicator, and its use was initially reported in the prognostic prediction of neoplastic disorders, such as hepatocellular carcinoma and breast cancer. Accumulating evidence suggests that elevated PLRs are strongly associated with increased systemic inflammation, which may contribute to the progression and prognoses of many disorders, such as atherosclerosis and diabetes mellitus ***(11).***

Many studies have shown that PLR is an inflammatory indicator, independent risk factors and prognostic predictors of sepsis. Also, Platelets have also been shown to be associated with the severity and prognosis of sepsis. More than half of sepsis patients with thrombocytopenia develop during hospitalization, and septic patients with moderate to severe thrombocytopenia have higher mortality ***(12).***

DAMPs

DAMPs are endogenous danger molecules released from damaged or stressed cells. These molecules activate the innate immune system through interaction with PRRs. DAMPs contribute to the host defense but can also promote pathological inflammatory responses. Calprotectin, a protein found in the cytosol of neutrophils and macrophages, is released under cell stress or damage. In a mixed population study, plasma calprotectin levels were higher in sepsis than in trauma patients and other medical conditions. Calprotectin levels were higher in patients who did not survive for 30 days. Plasma PCT did not differ between the groups or as a prognosticator of the outcome. Receiver operating characteristic (ROC) analysis, used as a sepsis biomarker, had a higher area under the curve (AUC) value for calprotectin (AUC: 0.79) compared to PCT (AUC: 0.49) ***(13).***

A prospective study evaluated IL-6, HMGB-1, and neutrophil gelatinase-associated lipocalin (NGAL) in 14 septic patients and 16 patients without sepsis admitted to the ICU. In patients with sepsis, IL-6 decreased levels were associated with ICU survival; NGAL levels rose in non-survivors, while HMGB-1 levels were unchanged in both survivors and non-survivors regardless of complications ***(14).***

Endothelial cells and BBB markers

The first step in endothelial and BBB injury is the breakdown and destruction of proteins followed by release into the bloodstream. These proteins or peptides can be evaluated as a marker of endothelial cells and BBB integrity. Plasma levels of occludin (OCLN), claudin-5 (CLDN-5), zonula-occludens 1 (ZO-1), PCT, and lactate were assessed in 51 septic patients. OCLN and ZO-1 were elevated with disease severity and positively correlated with the Acute Physiology and Chronic Health Evaluation II (APACHE-II) and SOFA scores and lactate levels. The predictive value for in-hospital mortality of ZO-1 was comparable to that of lactate levels, APACHE-II, and SOFA scores but superior to OCLN and PCT ***(15).***

In a case series of brain autopsies from adults who died from sepsis, 38% had no OCLN expression in the endothelium of cerebral microvessels. BBB damage was associated with higher maximum SOFA scores and PCT levels > 10 µg/L. BBB damage in the cerebellum was more common with CRP values > 100 mg/L. Soluble fms-like tyrosine kinase 1 (sFlt-1), soluble E-selectin (sE-selectin), soluble intercellular adhesion molecule 1 (sICAM-1), soluble vascular cell adhesion molecule 1 (sVCAM-1), and PAI-1 were evaluated in another studies. All these evaluated endothelial biomarkers were associated with sepsis severity. sFlt-1 had the strongest association with the SOFA score, while sFlt-1 and PAI-1 had the highest area under the operating receiver characteristic curve for mortality ***(15).***

Syndecan-1 is a structural component of the endothelium. Antithrombin, PAI-1, syndecan-1, VCAM-1, E-selectin, IL-1β, IL-6, IL-8, HMGB-1, and histone-H3 were increased in septic patients compared with healthy controls. Non-survivors had a higher syndecan-1 level compared with survivors. On day one, an association was seen between syndecan-1 levels and APACHE-II, SOFA, DIC scores, hemostatic markers, IL-1β, IL-8, and PAI-1. Day 1 syndecan-1 levels were also significantly higher in patients with DIC and had reliable discriminative power to predict both DIC development and subsequent mortality ***(16).***

The serum biomarker, calcium-binding protein B (S100B), reflects BBB disruption, glial cell injury, and activation. S100B is used to evaluate brain injury severity and predict outcomes from stroke, traumatic brain injury, encephalopathy, and delirium. A prospective cohort study demonstrated that day three values for predicting 180-day mortality were superior to day one (0.731 vs. 0.611). Patients with sepsis-associated encephalopathy also had elevated levels. In another observational study of 22 patients with septic shock, delirium was present in ten. The odds ratio for the risk of developing delirium with an S100B > 0.15 μg/L was 18.0. Patients with delirium had higher plasma levels of IL-6. S100B and IL-6 levels were positively correlated. S100B, PAI-1, angiopoietin (Ang)-2, ZO-1, and OCLN are the main biomarkers currently used to evaluate vascular injury and BBB permeability ***(17).***

Gut permeability markers

Critically ill patients show an increase in gut permeability, which may trigger systemic inflammatory response syndrome and multiple organ dysfunction syndromes (MODS). Plasma zonulin levels were higher in sepsis patients compared to a post-surgical control group or healthy volunteers. In another study, serum levels of intestinal fatty acid-binding protein (I-FABP) were higher in patients with sepsis and higher still in those with septic shock. Serum D-lactic acid levels were also elevated with sepsis but did not differentiate severity. Neither I-FABP nor D-lactic acid could prognosticate ***(18).***

Non-coding RNAs and miRNA

A non-coding RNA (ncRNA) is an RNA molecule transcribed from DNA but not translated into proteins. A microRNA is a small non-coding RNA molecule that functions in RNA silencing and post-transcriptional gene expression regulation. ncRNAs and mRNAs are being studied as sepsis biomarkers. For example, long non-coding metastasis-associated lung adenocarcinoma transcript 1 (lnc-MALAT1) and micro RNA (miR)-125a were increased in sepsis patients compared with healthy controls and positively correlated with APACHE-II score, SOFA score, serum creatinine, CRP, TNF-α, IL-1β, IL-6, and IL-8. The lnc-MALAT1/miR-125a axis was also a predictor of increased 28-day mortality risk. In another study lnc-MALAT1 expression was increased in acute respiratory distress syndrome (ARDS) patients compared to non-ARDS patients (AUROC: 0.674). Non-survivors compared to survivors (AUROC: 0.651) and positively correlated with APACHE-II and SOFA scores, CRP, PCT, TNF-α, IL-1β, IL-6, and IL-17 ***(19).***

Long non-coding RNA maternally expressed gene 3 (lnc-MEG3), and the lnc-MEG3/miR-21 axis were increased, while miR-21 expression was decreased in sepsis patients compared

with healthy controls. lnc-MEG3 (AUROC: 0.887) and the lnc-MEG3/miR-21 ratio (AUROC: 0.934) had high values for predicting elevated sepsis risk, while miR-21 (AUROC: 0.801) gave excellent predictive value for a reduced sepsis risk. A further study showed miR-125a and miR-125b expressions were elevated in sepsis patients compared with healthy controls and were predictive of sepsis risk—miR-125a (AUROC: 0.749) and miR-125b (AUROC: 0.839). No correlation was seen between miR-125a and CRP, TNF-α, IL-6, IL-17, and IL-23 in however, miR-125b was positively associated with these cytokines. miR-125a failed to predict 28-day mortality risk (AUROC: 0.588) in sepsis patients, whereas miR-125b was superior (AUROC: 0.699) ***(15).***

Membrane receptors, cell proteins, and metabolites

Cell surface receptors are receptors incorporated into the plasma membrane of cells and act on cell signaling by receiving or binding to extracellular molecules. After detecting such molecules, the production of metabolites occurs. In one study, the cluster of differentiation (CD)-13, CD14, CD25, CD64, and human leukocyte antigen (HLA-DR) showed acceptable sensitivity and specificity for mortality prediction (CD13 AUROC:0.824; CD64 0.843; and HLA-DR 0.804) while CD14 and CD25 did not predict mortality. nCD64 expression, in a further study, nCD64, PCT, CRP, and SOFA scores were higher in septic patients, with nCD64 having the highest AUC (0.879) for differentiating a positive microbial culture. This was superior to PCT (0.868), SOFA score (0.701), CRP (0.609), and white blood cell (WBC) count. In predicting 28-day mortality, the combination of nCD64 and SOFA score had an AUROC of 0.91 versus 0.882 for the combination of PCT and SOFA ***(20).***

A meta-analysis of 19 studies enrolling 3012 patients evaluated the value of PCT (AUROC 0.84) and presepsin (0.87 AUROC) for diagnosing sepsis. The pooled sensitivities and specificities were 0.80 and 0.75 for PCT and 0.84 and 0.73 for presepsin. In one study, levels of presepsin, PCT, CRP, and WBC were higher in sepsis patients than in a SIRS group with AUROC values of 0.954 (presepsin), 0.874 (PCT), 0.859 (CRP), and 0.723 (WBC). The cut-off of presepsin for discriminating between sepsis and SIRS was 407 pg/ml, with sensitivity and specificity values of 98.6% and 82.6%, respectively. In a study of septic children, TREM-1 levels were higher in septic shock patients ***(20).***

Hormones and peptide precursors

Adrenomedullin (ADM) is synthesized in different tissues, including the adrenal cortex, kidney, lung, blood vessels, and heart. It has biological properties, including vasodilating, inotropic, diuretic, natriuretic, and bronchodilating. In one study, mid-regional pro adrenomedullin (MR-proADM) was an independent predictor of five different organ failures (respiratory, coagulation, cardiovascular, neurological, and renal), compared to lactate which predicted three (coagulation, cardiovascular and neurological), PCT two (cardiovascular and renal) and CRP (none). MR-proADM most accurately identified patients

with a high likelihood of further disease progression compared to other biomarkers and clinical scores. A total of 1089 individuals with either sepsis (142) or septic shock (977) were included in a randomized controlled study. The MR-proADM level within the first 24 h after sepsis diagnosis was associated with 7-day mortality (AUROC 0.72 and $p < 0.001$) and 90-day mortality (AUROC 0.71 and $p < 0.001$). Patients with declining PCT levels but persistently high MR-proADM levels on day-1 or day-4 had a substantially higher mortality risk of 19.1 (8.0–45.9) and 43.1 (10.1–184.0), respectively. Adult patients hospitalized to ICU had their bioactive-ADM levels measured in this retrospective observational study. This study comprised a total of 1867 patients, 632 septic patients, and 267 septic shock patients. The median bioactive-ADM was 74 pg/mL in sepsis patients, 107 pg/mL in septic shock, and 29 pg/mL in non-septic patients. The association of elevated bioactive-ADM and mortality in sepsis patients and the ICU population resulted in O.R.s of 1.23 and 1.22, respectively. In addition, the MR-proADM is potentially removal by continuous renal replacement therapy (CRRT) ***(15).***

N-terminal (N.T.)-prohormone BNP (NT-proBNP) is a non-active prohormone produced by the heart and released in response to changes in intracardiac pressure. Higher levels of NT-proBNP at 24 h after sepsis onset were associated with lower short physical performance battery (SPPB) scores at 12 months and lower handgrip strength at 6-month and 12-month follow-up. NT-proBNP levels during the acute phase of sepsis may thus be a valuable indicator of a greater risk of long-term impairment in physical function and muscle strength in sepsis survivors. In another study, NT-proBNP levels on admission were higher in-hospital non-survivors (7908 pg/mL) compared with survivors (3479 pg/mL). AUROC curves of admission and 72-h NT-proBNP levels for hospital mortality were 0.631 and 0.648, respectively ***(21).***

PCT is produced in the thyroid's C cells and converted to calcitonin, with no PCT released into the bloodstream. During inflammatory processes, PCT is produced directly by stimulating bacterial components or induced by various inflammatory mediators such as IL-6 and TNF-α. PCT and presepsin had similar performance in predicting positive sepsis results with AUROC values of 0.75 and 0.73, respectively. Another investigation gave AUROC values of 0.87 for PCT and 0.78 for presepsin in predicting bacteremia. Plasma levels of presepsin and PCT were progressively higher in sepsis and septic shock than in non-septic patients. Presepsin was superior to PCT in diagnosing severe community-acquired pneumonia, while PCT was marginally superior in another study of septic patients admitted to intensive care. On the other hand, MR-proADM had a better predictive value for septic shock. This study concluded that PCT, MR-proADM, and presepsin are complementary biomarkers that could have utility in the management of septic patients. In an intention-to-treat study comparing PCT versus no PCT guidance, there was no significant difference in 28-day mortality (25.6% PCT versus 28.2% no PCT). The use of procalcitonin did not impact investment decisions as measured by the frequency of therapeutic and diagnostic interventions. ***(15).***

Neutrophil-related biomarkers

High levels of resistin collected on day 1 of ICU admission were associated with an increased likelihood of developing new organ failure, whereas high myeloperoxidase (MPO) levels on day one were associated with an increased risk of developing incident organ failure for clotting and kidney systems ***(22).***

Soluble receptors

Soluble trigger receptor expressed in the myeloid cell-1 (sTREM-1) is a TREM family member. This receptor offers excellent potential as a biomarker for infectious diseases as it can be measured in different biological fluids, including serum, pleural fluid, sputum, and urine. However, a meta-analysis of 2418 patients enrolled in 19 studies showed serum sTREM-1 had only moderate accuracy in diagnosing patients with suspected sepsis. Combining sTREM-1 with clinical variables offered more significant mortality discrimination compared to clinical variables alone. In a multicenter prospective cohort study, soluble tumor necrosis factor receptor type 1 (sTNFR1) levels > 8861 pg/ml predicted 30-day mortality ***(23).***

Patients with sepsis or septic shock displayed higher levels of the soluble form of the urokinase plasminogen activator receptor (suPAR), PCT, and lactate on days 1, 2, 4, and 7 of admission, with lactate and suPAR being the best risk stratifies for suspected infection. Levels of suPAR and PCT levels were higher in sepsis patients than in a SIRS group with AUROC values of 0.89 and 0.82, respectively. Serum sPD-L1 levels were increased in non-survivors compared with survivors with similar prognostic accuracy for 28-day mortality as APACHE-II and SOFA scores ***(24).***

Hepcidin

Hepcidin is a cysteine-rich disulphide bonded 25 amino acid peptide identified in year 2000 as primarily a key regulator of iron homeostasis but also exhibiting antibacterial and antifungal activity. Hepcidin is synthesized by hepatocytes and is upregulated by high serum iron levels. Hepcidin acts as an early responding acute phase reactant regulated by IL-6 and in response to lipopolysaccharide. Infusion of IL-6 in humans increases urine hepcidin levels and decreases serum iron and transferrin saturation within a few hours ***(25).***

Hepcidin is a peptide hormone produced in the liver that plays a crucial role in iron homeostasis. Iron is an essential part of oxygen transport within the body and is present in hemoglobin, myoglobin, and the electron transport chain. Serum iron levels must be tightly regulated to ensure an adequate supply is available for hemoglobin synthesis during erythropoiesis, without allowing iron overload to occur in the body. Hepcidin decreases the level of iron by reducing dietary absorption and inhibiting iron release from cellular storage. Hepcidin production increases when iron levels rise above the normal range of 65 to 175 mcg/dL in males and 50 to 170 mcg/dL in females ***(26).***

Hepcidin is an acute-phase reactant, one of many molecules whose plasma concentration changes in response to inflammation. During states of acute or chronic inflammation, levels of hepcidin and other acute-phase reactants increase, leading to a decrease in serum iron levels as hepcidin levels rise. Increased hepcidin correlates with the pathophysiology of anemia of chronic disease; the increase in inflammation causes a reduction in serum iron levels because the increase in hepcidin reduces iron transport out of cells. Conversely, a deficiency in hepcidin production can result in iron overload, as seen in hereditary hemochromatosis ***(26).***

Cellular Level

Hepatocytes are primarily responsible for the synthesis of hepcidin. Hepcidin is produced initially as a preprohormone with eighty-four amino acids. It is then cleaved into a prohormone, which gets cleaved again, forming hepcidin. The final hepcidin protein has 25 amino acids. Many factors influence hepcidin gene expression. Up-regulation occurs during inflammatory states and is primarily mediated by IL-6, a pro-inflammatory cytokine released from a variety of cell types. Transferrin, an iron-binding transport molecule in the blood, can also up-regulate hepcidin production, signaling that iron storage in the serum is adequate and that the release of iron from intracellular storage is not currently needed. Erythroferrone is a hormone produced by erythroblasts during erythropoiesis. It down-regulates the hepcidin gene expression. Hepcidin gene is also downregulated during hypoxic conditions. Both erythroferrone and hypoxia signal a demand for iron to construct new hemoglobin molecules ***(27).***

Organ Systems Involved

Iron plays a central role in the maturation and proper functioning of erythrocytes. It is essential to hematologic function. Hepcidin acts as a critical regulator for serum iron levels by modulating the release of iron from intracellular storage sites. When hepcidin levels become elevated, iron remains in its intracellular storage form, bound to the molecule ferritin. Hepcidin forms a connection between the immune system and the hematologic system. The theory is that during inflammatory states, hepcidin levels increase, so that serum iron levels decrease. The decreased serum iron levels prevent the invading pathogen from using the host's iron stores for its growth. Therefore, hepcidin is an essential mediator for immune defenses as well as hematologic functioning ***(26).***

Function

Once released into circulation from hepatocytes, hepcidin regulates plasma iron levels through interactions with ferroportin-1. Ferroportin is an iron export transmembrane protein present in the macrophages and the enterocytes. When hepcidin binds to ferroportin, it causes the cell to target the hepcidin-ferroportin complex for lysosomal degradation. The cell types most affected by this interaction are duodenal enterocytes and reticuloendothelial macrophages. Duodenal enterocytes absorb dietary iron, and reticuloendothelial macrophages store iron recovered from degraded erythrocytes in the

bone marrow, liver, and spleen. The degradation of ferroportin blocks iron absorption from enterocytes and iron mobilization from the macrophages ***(28).***

Pathophysiology

- ***Hereditary Hemochromatosis***

An autosomal recessive defect in the HFE gene, resulting in decreased hepcidin production. HFE mutations are more prevalent in individuals of European descent. Decreased hepcidin results in increased iron uptake from diet and increased iron mobilization from macrophages. Continued iron absorption despite adequate serum levels can lead to iron overload (total body iron exceeds 20g). Symptoms of hemochromatosis are secondary to iron deposition in bodily tissue and typically present in the 4th and 5th decade of life for men and women, respectively. The classic triad includes skin hyperpigmentation, liver cirrhosis, and diabetes mellitus. Additional findings include dilated cardiomyopathy, hypogonadism, arthropathy, and hypothyroidism. Hemochromatosis patients also have increased infection risk now that serum iron levels cannot decrease during inflammatory states. The diagnostic basis is iron panel results showing an increased serum iron level with increased ferritin (> 200 mcg/L) and transferrin saturation levels (> 45%). Treatment involves lifestyle modifications, therapeutic phlebotomy, and medications. Dietary changes include a diet low in iron, restriction of vitamin C supplements and alcohol, and consuming tea because tannates reduce iron absorption by binding to it. About 1 to 2 therapeutic phlebotomy sessions per week initially to bring ferritin and hemoglobin to the target level and then every 2 to 4 months. Iron-chelating agents like deferoxamine to remove iron from the circulation ***(29).***

- ***Anemia of Chronic Disease***

Anemia of chronic disease is the second most common cause of anemia after iron deficiency anemia. It is associated with a variety of disease states, including infection, neoplasm, chronic kidney disease, and autoimmune conditions like systemic lupus erythematosus. Hepcidin is an acute-phase protein, and upregulation is by interleukin-6 (IL-6) and other proinflammatory cytokines. As a result, hepcidin causes enterocytes and macrophages to degrade ferroportin, reducing absorption and promoting storage, respectively. Serum iron levels decline in an attempt to deprive rapidly dividing cells and invading microbes from nutrients. Anemia of chronic disease typically begins as a mild to moderate normocytic normochromic anemia denoted by a hemoglobin concentration of 8 to 9.5 g/dL. The anemia can progress to microcytic and hypochromic if the inflammatory conditions remain. Presenting symptoms are often nonspecific signs of anemia, including fever, pallor, and fatigue. An iron panel would show a decrease in serum iron level despite an increase in ferritin because of intracellular iron sequestration. Treatment with iron supplementation is often not beneficial as the issue lies with iron availability rather than deficiency. It is crucial to treat the underlying condition to prevent further inflammation ***(30).***

Clinical Significance

Hepcidin plays a role in innate immunity through its interactions with IL-6 and other pro-inflammatory cytokines. The ability to sequester iron within cells to prevent its availability for pathogenic or neoplastic growth appears to be largely dependent on hepcidin stimulation by IL-6. This innate defense may help protect against many pathogens, including streptococcal and malarial species ***(31).***

Several hepcidin agonists are currently in development and may become a viable treatment for hereditary hemochromatosis. Currently, phlebotomy is the mainstay of treatment for iron overload states, but a hepcidin agonist could help alleviate the symptoms from the deficient natural hepcidin ***(31).***

Hepcidin plays a central role in iron transport and utilization and is, therefore, an important marker of iron bioavailability ***(28).***

Hepcidin and sepsis

The severity of sepsis depends on several host factors, such as age, comorbidities, and immune status, as well as pathogen factors such as virulence, microbial species, and infectious load. In spite of the sepsis-3 criteria presented as a consensus document 2016 the diagnosis is often a challenge in critically ill patients ***(32).***

Several potential biomarkers for monitoring sepsis have been investigated, including white blood cell count (WBC), C-reactive protein, (CRP), lactate, and procalcitonin (PCT). During the last decade Heparin binding protein (HBP) has attracted interest as a biomarker for severe bacterial infection including sepsis and meningitis. HBP also known as Cationic Antimicrobial Protein of 37 kDa (CAP37) was identified in 1984 and is produced by neutrophils, stored in intracellular vesicles, and rapidly released upon stimulation by pathogen associated microbial patterns, PAMPs. Elevated HBP levels have been shown to correlate with hypotension and organ dysfunction in patients with bacterial sepsis but failed to distinguish patients with septic shock from other causes of shock ***(25).***

More recently hepcidin, identified as a major regulator of iron metabolism, has emerged as a potential marker for bacterial sepsis. Serum levels of hepcidin are increased both in children with severe infections and in adults with sepsis. It has also been reported to be a marker of acute kidney injury in patients at the ICU. In a pilot study, it reported that hepcidin levels increased in septic shock patients, similar to PCT, and prior to increases in CRP levels during disease progression. Hepcidin levels decreased within the first 24 h of admittance to the ICU ***(25).***

Since the discovery of hepcidin in 2000 most published reports investigated the role of hepcidin in anemia, but also in critically ill adult patients including infectious diseases such as sepsis. Previous studies investigated patients admitted to a General Medicine Ward who fulfilled two criteria for systemic inflammatory response syndrome (SIRS), though they were not diagnosed in accordance with the currently used international criteria for septic shock. These studies reported that hepcidin levels in serum were raised in sepsis patients ***(33).***

A recent study reported that HBP and CRP concentrations increased during the first 24 h after admittance making the evaluation of these biomarkers less valuable at the early stage of sepsis. Hepcidin as well as CRP are known to be upregulated by IL-6. In a report on surgical critical care patients, levels of CRP and IL-6 correlated with hepcidin and high CRP concentrations correlated with high same day hepcidin. Highest levels of hepcidin were noted on admission, whereas the highest CRP levels were noted after 24 h in accordance with our findings ***(25).***

Hepcidin analysis could add value at an early phase of disease to the commonly used lab analyses in support of a septic cause of disease. Hepcidin could indicate which patients are at risk for a more severe course of illness if several inflammatory markers with different origins of synthesis are affected e.g. CRP and hepcidin ***(34).***

Increased HBP levels have been reported not only in septic shock but also associated with both circulatory and respiratory failure in critically ill patients ***(34).***

A direct antimicrobial effect of hepcidin has been shown that could potentiate the antimicrobial effect of hepcidin via reduced iron concentration thereby limiting nutrition for microbes. This is in line with experimental studies in murine models where the data show that hepcidin-induced hypoferremia can act as a defense mechanism against bacterial infection. Administration of exogenous hepcidin has been suggested to be of therapeutic value in selected cases of hyperferremia. High iron concentrations have been reported to increase the lethal effects of siderophile bacteria in vivo and also in fungal infections and the reduction of available plasma iron is most likely beneficial for the host unless prolonged and leading to anemia ***(25).***

In the short-term perspective, it seems to be favourable for the host to reduce available iron in sepsis. A prolonged persistent iron deficiency though, may lead to harmful adverse effects for patients with a long ICU stay. This can inflict a higher risk of cognitive, and cardiovascular dysfunction, as well as higher risk of nosocomial co-infections and poor recovery after ICU treatment ***(35).***

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