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The measurement of biomarkers in the diagnosis and prognosis of neonatal sepsis: a review of the literature

A dosagem de biomarcadores no diagnóstico e prognóstico de sepse neonatal: uma revisão de literatura

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ABSTRACT: *Introduction:* Neonatal sepsis (NS) is an important cause of morbidity and mortality in newborns despite advances. Possessing a complex pathophysiology, NS presents different clinical forms and eventually scarce guiding symptoms or signs, which makes diagnosis, severity analysis and timely therapy difficult. *Objective:* To analyze the use of biomarkers for the early diagnosis and prognostic evaluation of SN. *Method:* Literature review with search in PubMed/MEDLINE, Scielo and VHL databases, conducted between February and March 2021, with analysis of 21 articles published from January 2010 to December 2020. *Results and Discussion*: The biomarkers used in SN analysis, such as C-reactive protein (CRP), procalcitonin (PCT), interleukins (IL-6, IL-8), tumor necrosis factor alpha (TNF-α) and cell surface markers are important for faster diagnosis and assistance, enabling better prognosis for neonatal cases. *Conclusion:* The study of biomarkers with the knowledge of their serum levels during disease progression can facilitate the analysis and predict the severity of SN, besides following the establishment of an early protocol, increasing the proportion of patients who receive an effective treatment and obtain better prognosis.

Keywords: Neonatal sepsis; Biomarkers; Newborn.

RESUMO: *Introdução:* A sepse neonatal (SN) é importante causa de morbimortalidade em recém-nascidos apesar dos avanços. Possuindo uma fisiopatologia complexa, a SN apresenta diferentes formas clínicas e, eventualmente, sintomas ou sinais norteadores escassos; o que dificulta o diagnóstico, a análise da gravidade e a terapêutica oportuna. *Objetivo:* Analisar o uso de biomarcadores para o diagnóstico precoce e avaliação prognóstica da SN. *Método:* Revisão bibliográfica com busca nas bases de dados PubMed/MEDLINE, Scielo e BVS, realizada entre fevereiro e março de 2021, com análise de 21 artigos publicados de janeiro de 2010 a dezembro de 2020. *Resultados e Discussão:* Os biomarcadores usados na análise da SN, como a proteína C reativa (PCR), procalcitonina (PCT), interleucinas (IL-6, IL-8), fator de necrose tumoral alfa (TNF-α) e marcadores de superfície celular, são importantes no diagnóstico e assistência mais céleres, viabilizando um melhor prognóstico para os casos neonatais. *Conclusão:* O estudo dos biomarcadores com o conhecimento acerca da modificação de seus níveis séricos durante a progressão da doença pode facilitar a análise e predizer a gravidade da SN, além de orientar a instauração de um protocolo precoce, aumentando a proporção de pacientes que recebem um tratamento eficaz e obtêm melhores prognósticos.

Palavras-chave: Sepse neonatal; Biomarcadores; Recém-nascido.

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INTRODUCTION

Neonatal sepsis (NS) is the most important cause of morbidity and mortality in newborns despite advances in the diagnostic technology selected for this function. According to data from the World Health Organization (WHO), this dysfunction represents a relevant health problem in newborns (NB) hospitalized in Neonatal or mixed Intensive Care Units (neonatal and pediatric) and is one of the main causes of neonatal death in the world, accounting for 60% to 80% of lost lives in childhood[1–3]. The incidence of SN is increasing every year, which raises an alert in the medical community, as this fact contrasts with evident advances in the area of antibiotic therapy, microbiology and life support techniques[4].

Regarding the definition of sepsis, the pioneer Roger Bone, in 1980, defined the concept of "septic syndrome", and later, in the 1991 consensus, the concept of "sepsis" (sepsis-1) was used, including the differentiation in four stages: systemic inflammatory syndrome (SIRS), sepsis, severe sepsis and septic shock[4]. Currently, the Third International Consensus for the Definition of Sepsis and Septic Shock (Sepsis-3) is in force, which took into account the heterogeneity of this syndrome as a result of the immune response, and started to characterize it as a systemic organic dysfunction , potentially fatal, due to an unregulated inflammatory response of the body to the infection[2]. This organ dysfunction is traditionally identified by increasing two or more parameters of the SOFA (Sequential Organ Failure Assessment) severity scale, which is still widely used in intensive care units (ICUs)[2.4].

NS has a complex pathophysiology, which leads to different clinical forms and may present without symptoms or guiding signs, or with nonspecific manifestations; This ends up making difficult the diagnostic conduct and the analysis of the severity of the pathology[4]. On the other hand, when analyzing the pathophysiology of SN, it is known that, after the identification of a signal originating from the pathogen or some damage caused by it, there is an increase in the generation of inflammatory and anti-inflammatory mediators of the most diverse classes, which can be acute or not, interleukins (IL), markers, among other mediating molecules, making it possible to strategically measure these substances and use them in a more accurate analysis of the development of SN[4.5].

This analysis is extremely important, because the biomarkers related to it are shown as important parameters for a better and faster diagnosis, stratification, screening, rational use of antibiotics and monitoring of prognosis, causing a significant reduction in mortality, since its variations indicate the levels of inflammatory response to infectious injury of the most diverse forms and evolutionary stages of the dysfunction[1,4,5]. These biomarkers are still relevant in this process as they can help to estimate the severity of the condition and allow the differentiation and characterization of viral, bacterial and fungal infections; in addition to identifying the spread or not of the dysfunctional infectious process[6].

Considering the relevance of this information and its prompt availability when using biomarkers, the search for these molecules in the assessment of sepsis led to the identification of a little more than 170 biomarkers; including procalcitonin (PCT), C-reactive protein (PCR), cytokines such as interleukin (IL) 1β, IL-8, IL-6, interferon (IFN-γ), tumor necrosis factor (TNF-α), chemokines and markers for cell surface[1,4]. In this sense, several studies were recently published analyzing the role of the most varied biomarkers in septic patients, and these discoveries are a great advance for science[7-9].

This fact is fundamental, since traditional diagnostic practices use blood culture as gold standard, which has many limiting factors, such as: risk of obtaining small blood volumes, variation in bacteremia, use of antibiotics by mother before sample collection in the NB, high cost and delay of the result by this method (availability in 48-72 hours). These situations can lead to some cases of true infections that have not been confirmed by false-negative blood cultures[10,11]. Thus, it is noted that blood cultures lack the necessary sensitivity and selectivity in this scenario, making the confirmation of NS a challenge.

On the other hand, the importance of diagnostic accuracy is noted, emphasizing that the brevity and accuracy in the characterization of the pathogen are of great value amid an urgent demand to start antibiotics to optimize the treatment of sepsis in RN. In addition, the excessive use of antimicrobials is a very frequent situation in neonatal units, requiring that the beginning of antibiotic therapy be overly controlled and analyzed, since empirical approaches can end up creating favorable environments for the emergence of bacterial resistance. Thus, with the modification of the NB's microbiota leading to possible short- and long-term consequences, it may lead to a potentially worse prognosis, which raises the importance of a more in-depth discussion regarding the indiscriminate use of empirical antibiotics in this age group[10,11].

Considering that the isolated use of the described biomarkers is not sufficient due to the absence of signs and symptoms compatible with sepsis in the routine of clinical practice, the objective is to analyze the combination of clinical characteristics with biomarkers for early diagnosis and prognostic assessment, since together they are essential to speed up the diagnosis and thus reduce the morbidity and mortality of SN.

MATERIAL AND METHODS

This study refers to a literature review in which the DeCS/BVS: "Biomarkers", "prognosis", "sepsis", "neonatal" were employed and associated with the AND Boolean operator. For the elaboration of the study, a search

was carried out in the PubMed/MEDLINE, Scielo and VHL databases, between the period of February and March 2021. The construction of this review was divided into stages, starting with identification of the proposed theme and selection of articles to be analyzed, followed by exclusion of those not available in full, duplicated or that did not fit the proposed objective, restricting the search for publications between the period of January 2010 to December 2020. Finally, the evaluation and interpretation of the collected data and the elaboration of the obtained were carried out.

From a total of 173 articles published between the aforementioned period, 22 articles were selected that consist of original articles, systematic reviews and editorials in English, Portuguese and Spanish, with a focus on the proposed objective.

RESULTS

All of the 22 articles delimited in the methodology were read in full and 18 of them were selected to compose this study, with 4 being excluded since, after the full reading, they weren't addressed because the content not in line with the proposed objective. Of the 18 selected articles, the oldest was published in 2012 and the most recent in 2020, confirming the delimitation proposed in the methodologies of scientific analysis on the subject in the last 10 years. All works were separated by title, authorship, year of publication, study design and analyzed biomarkers, as shown in Table 1.

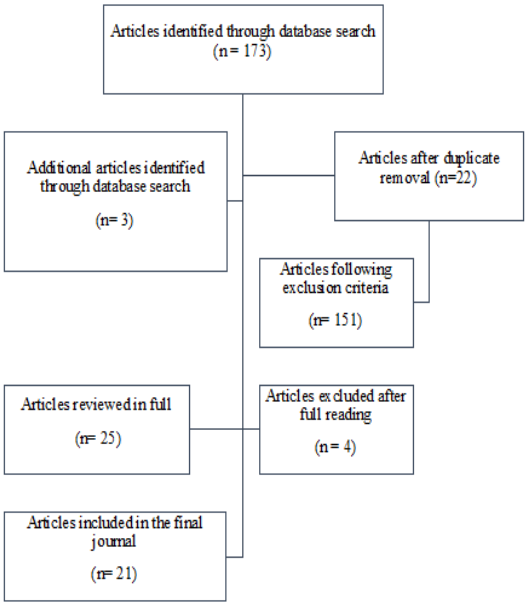

Figure 1: Study selection flowchart.

Table 1: Studies on the relationship of biomarker dosage in the prognosis and diagnosis of neonatal sepsis.

Title	Authors	Year	Study design	Biomarkers analyzed
Presepsis (soluble CD14 subtype): Reference ranges of a new sepsis marker in term and preterm neonates	Pugni L, et al.	2015	observational study	PCR; PCT; Presepsin;
The systemic inflammation mortality risk assessment contingency table for severe sepsis	Carcillo JA, et al.	2018	Prospective cohort study	PCR; PCT; IL; TNF-α;
Serum calprotectin: a potential biomarker for neonatal sepsis	Decembrino L, et al.	2015	Prospective cross-sectional study	serum calprotectin; PCR; nCD64;
Comparative assessment of cytokines and other inflammatory markers for the early diagnosis of neonatal sepsis–a case control study	Prashant A, et al.	2013	case-control study	IL-6; IL-8; TNF-α; sCD163; PCR;
Evaluation of new diagnostic biomarkers in pediatric sepsis: matrix metalloproteinase-9, tissue inhibitor of metalloproteinase-1, mid-Regional pro-atrial natriuretic peptide, and adipocyte fatty-acid binding protein	Alqahtani MF, et al.	2016	Prospective observational study	TIMP-1; mrProANP; A-FaBPs; MMP9; PCR;
Diagnostic value of mean platelet volume for neonatal sepsis: a systematic review and meta-analysis	Wang J, et al.	2020	Meta analysis	PCR; PCT; MPV;
Presepsis as a diagnostic marker of sepsis in children and adolescents: a systemic review and meta-analysis	Yoon S, et al.	2019	Meta analysis	PCT; PCT; Presepsin;
Neonatal sepsis and Inflammatory Mediators	Machado RJ, et al.	2014	Review article	TNF-α; IL-1; IL-1 β IL-4; IL-6; IL-7; IL-8; IL-10; IL-12TGF-β; IFN-γ;

Table 1: Studies on the relationship of biomarker dosage in the prognosis and diagnosis of neonatal sepsis.

Title	Authors	Year	Study design	Biomarkers analyzed
Use of biomarkers in pediatric sepsis: literature review	Lanziotti VS, et al.	2016	Review article	PCR; PCT; IL-6; IL-8; IL-18; NGAL; proADM;
Biomarkers of endothelial dysfunction predict sepsis mortality in young infants: a matched case-control study.	Wright JK, et al.	2018	Paired case-control study	Ang-1; Ang-2; ICAM-1; sICAM;
Neonatal sepsis: pathophysiological aspects and biomarkers	Cortes JS, et al.	2019	Literature review	PCR; PCT; WING; PUL; Hepcidin; IL-6; IL-8; TNF-α; IL-10;CD64; Resistin;
Clinical value of plasma soluble urokinase-type plasminogen activator receptor levels in term neonates with infection or sepsis: a prospective study.	Siahanidou T, et al.	2014	case-control study	PCR; SuPAR;
Procalcitonin as a Biomarker for sepsis in children	González-Rangel D, et al.	2016	Retrospective, observational and descriptive study	PCR; PCT;
Identification of low risk factors in small infants with fiber without clear focus	Cisneros BG, et. al	2018	Review article	PCR; PCT; Total neutrophils;
Predictive value of procalcitonin in children with suspected sepsis.	Bustos R, et al.	2015	prospective study	PCR; PCT; Lactate;
Serious bacterial infections in febrile young children: Lack of value of biomarkers.	Karsas M, et al.	2016	Prospective cross-sectional study	PCR; PCT;
Procalcitonin for the diagnosis of late sepsis in newborns of low birth weight	Bustos R, et al.	2012	Prospective, observational study	PCR; PCT; IL-6; IL-10; IL-8;
The paradigm of sepsis: in search of the perfect biomarker	Ledesma CA, et al.	2018	Editorial	PCR; PCT; IL-1;

Subtitle: A-FaBPs = adipocyte fatty acid binding proteins; Ang-1 = Angiopoietin-1; Ang-2 = Angiopoietin-2; ASA = Serum Amyloid A; CAM-1 = Intercellular adhesion molecule-1; IFN-γ = interferon-γ; IL = interleukin; MMP-9 = matrix metalloproteinase-9; mrProANP = regional mean pro-atrial natriuretic peptide; NGAL = human neutrophil gelatinase; CRP = C-reactive protein; PCT = procalcitonin; proADM = Adrenomedullin; PUL = lipopolysaccharide binding protein; sCD163 = soluble CD163; sICAM = Soluble intercellular adhesion molecule-1; SuPAR = Soluble urokinase-type plasminogen activating receptor; TGF-β = transforming growth factor-beta; TIMP-1=tissue inhibitor of metalloproteinase-1; TNF-α = Tumor Necrosis Factor Alpha; MPV = mean platelet volume;

DISCUSSION

Sepsis is defined by the Third International Consensus for the Definition of Sepsis and Septic Shock (Sepsis-3) as an organic dysfunction that carries the risk of death caused by a dysregulation of the host's response to infection[2,12]. In NB, it continues to be a disease with increasing prevalence, high morbidity and mortality, being characterized as a major public health problem. According to data from the World Health Organization (WHO), SN is responsible for about five million deaths annually. In view of the possible absence of symptoms or signs that guide the conduct and accurate analysis of the condition, NS ends up having a difficult diagnosis and the severity of the disease underestimated[1].

In SN, for diagnostic confirmation, the presence bacterial infection (suspected or confirmed by blood culture or other complementary diagnostic methods, such as the use of biomarkers) is necessary. Finally, the analysis of clinical findings such as petechiae and purpura, fever, cough also help in the diagnosis[13]. These factors are nonspecific and often present at a more advanced stage of the infection, making early diagnosis of the condition difficult. Thus, the development of biomarkers capable of anticipating the diagnosis of sepsis could eventually increase in the proportion of patients who receive empirical treatment in a timely manner, thus leading to an improvement in the prognosis[7].

C-Reactive Protein (CRP)

C-reactive protein (CRP) was the first pattern recognition receptor identified in the scientific literature, being one of the longest used biomarkers in the analysis of

SN[14.15]. It is defined as a nonspecific, acute-phase reacting protein that has high plasma levels in acute or chronic inflammatory diseases. Its production is carried out by the liver after an inflammatory response or tissue injury, with the presence of IL-6 as a stimulus[7].

In a more accurate analysis of its production levels, studies have observed that it increases between 4 to 6 hours after interaction with an inflammatory trigger, which may or may not be infectious, being able to reach a peak in 48–72 hours after the onset of the event[14.16]. This rise in the CRP level within 72 hours was shown to be associated with an inadequate response to antibiotic therapy for sepsis in adults, which could reflect a lack of control in the fight against the disease, also related to cases in NB. In contrast, normal CRP levels for a period longer than 72 hours could indicate discontinuation of antibiotic use in newborn patients with sepsis[15]. Finally, in a case-control study, composed of 150 NB, it was concluded that CRP levels are high not only in infected newborns but also in the uninfected group at the time of diagnosis, which would be an indication that the increase in levels could be seen in both infectious and inflammatory conditions, endorsing that CRP is an unspecific biomarker in cases of unconfirmed infections, but it has high sensitivity in predicting mortality[17].

Considering that the measurement of CRP levels has consolidated its use in clinical practice, is readily available and has a low cost, it reaffirms itself as a good biomarker in the analysis of the response to antibiotic therapy. However, this statement is only correct when performed dynamically, considering that the predictive value of CRP levels increases with serial dosing. This is due to the fact that CRP is not a specific biomarker to distinguish an infection from an inflammation, nor does it allow a precise analysis of the infectious agent. Therefore, it must be associated with other biomarkers and with the clinical assessment of the patient[1.14].

Procalcitonin (PCT)

Procalcitonin is a pro-peptide precursor of the hormone calcitonin produced in the parafollicular neuroendocrine cells of the lungs and intestine[18]. It has no hormonal activity, but it shows up at high levels when in the presence of systemic infectious conditions and is considered a reliable biomarker to differentiate septic conditions from non-infectious systemic inflammatory response syndrome (SIRS)[7,9,14]. In response to endotoxins or inflammatory mediators released by bacteria, such as cytokines, the increase in its levels is related to its responsiveness to the severity of the infection[7.9].

In analyzing its specificity, PCT is seen as useful for identifying bacterial infections since, in the course of a viral infection, its production is restricted by the action of interferon gamma (IFN-γ)[14.19]. However, its values may depend on factors such as gestational age and postnatal age, especially in the first days of life, and may increase in non-infectious causes, such as SIRS, trauma and major cardiac surgery, in a physiological way, during the first days of life[19].

Therefore, PCT has better sensitivity, specificity and predictive values than CRP, IL-6 and INF-alpha, since, as previously reported, it remains at reduced levels in viral infections and inflammatory processes[18]. In a meta-analysis, PCT had good sensitivity (combined, 0.78) and low specificity (combined, 0.57), at a cutoff value of < 2.0 ng/mL in pediatric patients outside the neonatal period[16]. The analyzed results corroborate the relevance of the combined use of biomarkers in the approach to the diagnosis of NS[7,14].

Considered as an acute phase reactant, in response to inflammatory stimuli, mainly bacterial, PCT starts its synthesis within 2-4 hours, reaching maximum values between 24-36 hours from the development of the infection[16]. In an analysis of a prospective study, the daily maintenance of high concentrations of PCT was associated with high mortality in the neonatal ICU, and the reduction of these levels within 48 hours was related to a favorable prognosis[7].

Cytokines and Chemokines

Cytokines designate a group of small-sized molecules that present short serum half-life. They can be found in dosages produced in minutes to a few hours. They play a central role in the immune response in NB sepsis. In the early 1990s, studies on sepsis showed a strong association of this pathology with an exacerbated release of pro-inflammatory cytokines, including: tumor necrosis factor (TNF-α), interleukin (IL-6, IL-8) and interferon-γ (IFN-γ)[13]. Together with chemokines, cytokines are important biomarkers of NS, as they have greater potential than PCT in the early diagnosis of sepsis, in addition to contributing to the assessment of the severity of sepsis. However, despite their favorable characteristics, the use of these mediators faces barriers in the NICU routines, as they present high costs and little practicality in performing its analysis[11].

Interleukin – 6

Interleukin-6 (IL-6) is a cytokine with great evidence in the literature regarding NS. Stimulated by the contact of the immune system with microbial products, its importance is due to its property of acting by signaling the activation of the immune system and, in this way, influencing the release of other cytokines, such as TNF-α

and IL-1β. This cytokine indicates an early response to the inflammatory condition present in sepsis, which may precede the elevation of CRP levels by interfering with its production during bacterial infection[13]. The increase in the serum concentration of IL-6 may precede the appearance of signs and symptoms of the infection, in addition to being associated with more severe cases of NS. Furthermore, its accuracy can be increased if used in combination with other diagnostic biomarkers. Thus, it is understood that its clinical use is of great relevance as a predictor of severe sepsis in these patients[1.14].

A case-control study, with analysis of morbidity and mortality in cases of NS compared to cases of morbid outcome, reverberates the central idea that this cytokine is a good predictor for the evolution of sepsis, as it showed a significant increase in serum levels of IL- 6 due to severe sepsis[17].

As for the variation in IL-6 levels, it shows its initial increase between 2-6 hours, with a peak of 36 hours after the onset of infection. And in patients with effective antibiotic therapy and good therapeutic response, it declines within 24 hours. Thus, this cytokine is useful as an early marker contributing to an assertive evolutionary analysis of the disease and its treatment[1,13].

Interleukin - 8

Belonging to the class of pro-inflammatory chemokines, IL-8 is a molecule produced in placental and immunological cells (monocytes and macrophages), being generated from an infectious stimulus originated in the uterus. In the 1990s, this interleukin has been widely discussed and investigated in the scientific literature as a predictive biomarker of early NS. Analogously to IL-6, IL-8 shows a pattern of development whose characteristics limit the role of this molecule as a biomarker of routine use in clinical practice for analyzing the evolution of the septic condition, since it has limited serum levels for detection, although it can be useful in initial diagnostic investigation and treatment conduct[13].

Since its function is to guide the process of chemotaxis and neutrophil activation, it can be important for use in risk stratification. This statement is supported by studies analyzing serum levels of IL-8, in which patients with a septic history with higher levels of this interleukin had lower survival rates compared to those with lower levels[14.17]. When combined with other biomarkers, such as IL-6 and CRP, IL-8 obtained higher levels in infected neonates when compared to uninfected infants in a case-control study, suggesting its influence on infectious and non-inflammatory cases[17]. With regard to the variation in serum IL-8 levels, it has high specificity and sensitivity in confirming infection within 24-48 hours, in addition to predicting the risk of mortality in this period. Furthermore, it is noteworthy that it presents an initial increase in 90 minutes, reaching its peak in 120 minutes after the onset of the infectious condition, finally having its decline around 48 hours after the birth of these newborns[13.17].

Tumor necrosis factor alpha (TNF-α)

TNF-α is considered one of the main septic biomarkers in neonates, in addition to being an indicator of tissue damage and acting in the regulation of IL-1 β secretion. Through its systemic release, it causes vasodilation and increased vascular permeability, which ultimately generates systemic edema and reduced blood volume and other changes that contribute to the evolution of the septic condition. In view of its mechanism of action, this IL appears as a "danger factor" since its high levels lead to the formation of intravascular clots that in a disseminated way can lead to multiple organ failure due to leukocyte and platelet adhesion. Thus, its high levels are a good evaluative predictor of the severity of the condition, showing greater sensitivity (95%) when combined with IL-6[13.17].

When evaluating the serum levels of TNF-α, an initial release is noted in about 30 minutesafter immunological stimulation in the face of inflammatory condition, reaching a peak in 1 hour and a half, with a short half-life estimated at about 70 minutes. Finally, its decline starts reaching concentrations close to zero after 3 hours of the initial stimulus[13].

Cell surface marker

When analyzing the pathophysiology of SN, several factors are shown to be active within the infectious process, which could be used as important biomarkers in the study of SN development, including cell surface markers. In the midst of the inflammatory response to the infection, it is possible to notice that there is an increase in the expression of antigens and other proteins in the membrane of immune cells, such as leukocytes. Among these, there is a more pronounced presence of the Fcγ I receptor expressed on neutrophil membranes, where it acts by promoting the phagocytic process, its action and expression being remarkable through the installation of the bacterial infection[1].

Among other markers, it is worth mentioning the soluble urokinase-type plasminogen activator receptor (uPAR). This membrane receptor presents protease activity by promoting the transduction of intracellular signaling pathways, and its expression is found on the surface of immune cells that participate in the development of the inflammatory process, such as monocytes and lymphocytes. In its soluble form, suPAR is used as a biomarker of

infections in adults and its properties are still little explored in neonatal infectious conditions. By analyzing their serum levels in biological fluids such as blood, urine and cerebrospinal fluid, elevations in response were detected not only in adults, but also in newborns[8].

In a case-control study, in which plasma levels of suPAR were analyzed in terms of neonates with infection (viral and bacterial), the importance of this marker was demonstrated. Mean basal levels of suPAR were higher in the group containing infected neonates (cases) compared to levels of control group, which may suggest that this may be a biomarker used in diagnosis. However, it does not distinguish between bacterial and viral infections; and the suPAR values did not show a decline after complete remission of the infection, with significant values being observed even 7 to 10 days after recovery, which suggests that this marker has continued release or production even after improvement in the infectious condition[8]. Such results raised questions in the literature about its use in the analysis of the response to the instituted treatment.

Analysis of the future use of biomarkers in neonatal sepsis

Calprotectin is an antimicrobial protein that can be used as a marker for activation of granulocytes and mononuclear phagocytes. It has been proposed for the diagnosis of inflammatory conditions and has been studied recently as a biomarker of SN. Data from a cross-sectional study demonstrated the use of serum calprotectin as a marker in the early diagnosis of SN, considering it a promising, specific and sensitive test in NB with sepsis, due to the important action exerted by this protein on the immune system[19].

Other biomarkers that have been investigated in detail in sepsis in adults are: adipocyte fatty acids (A-FaBPs), matrix metalloproteinase-9 (MMP-9), tissue inhibitor of metalloproteinase-1 (TIMP-1) and the regional medium pro-atrial natriuretic peptide (mrProANP). Considering the absence of reports in the literature on children and NB, a recent prospective and observational study analyzed the concentrations of these elements in a group of septic patients under 18 years of age, identifying the presence of these biomarkers in the child population during the infectious condition[20].

A finding present in this study was the relationship between lower MMP-9/TIMP-1 and greater sepsis severity and mortality risk. Furthermore, A-FaBP and mrProANP levels were higher in septic children compared to healthy children; these molecules were considered to be biomarkers directly associated with greater morbidity and length of hospital stay. The research concluded that further studies of these biomarkers should be promoted not only in the adult population, but also in the pediatric population and, more specifically, in neonates[20].

In addition to the biomarkers presented, several studies addressed the use of mean platelet volume (MPV) as a predictive marker for NS, since platelets, after being activated, release high amounts of cytokines and inflammatory mediators that act as important signaling agents of immune response. It has been shown that with the increase in MPV, there is greater platelet activation, which will trigger the early development of thrombi, promoting adhesion and aggregation of these molecules. Thus, measuring MPV can be an indication of serious risk for complications and death. Although several studies have recognized the association between neonatal sepsis and MPV, the exact relationship is not well established yet[21].

In a meta-analysis study, it was concluded that MPV measurement can be used as a marker of early SN diagnosis during clinical analysis, since MPV values were found to be higher in patients with SN compared to controls thus, it is noteworthy that new studies on the dynamic alteration of the MPV will promote greater validity of this finding[21].

FINAL CONSIDERATIONS

Considering the pathophysiology of SN, after a signal promoted by a pathogen or endogenous damage, there is an increase in the production levels of several classes of inflammatory and anti-inflammatory mediators, such as CRP, PCT, IL, TNF-alpha, among others; some of which have already been described in detail in the literature, while others are under development. Thus, a more in-depth study of these biomarkers, with knowledge about the modification of their serum levels over the time of disease progression, could improve care, facilitate diagnosis and predict the severity of NS. The practical use of these biomarkers can guide the establishment of an early protocol, increasing the proportion of patients who receive effective treatment at an opportune time, thus achieving better prognosis.

From the detailed and isolated analysis of the concentrations of biomarkers by time, observing their variations and peaks, it is possible to obtain important complementary data that signal the progression of SN. In summary, CRP increases between 4-6 hours with a peak between 48-72 hours after the onset of infection, being currently classified as an important biomarker, while PCT increases between 2-4 hours with its peak between 24 - 36 hours, also demonstrating its effectiveness. In turn, interleukins 6 and 8 and TNF-α play an important role in predicting the development of infection, since the increase

in serum levels occurs prematurely, in the earliest stages of the disease. Thus, IL-6 has a period between 2-6 hours for the beginning of its increase, while its peak is established after 36 hours of infection. IL-8 rises 90 minutes after the onset of the infection, with its peak after 120 minutes. TNF-α, on the other hand, demonstrates its increase in about 30 minutes, with a peak after 1 hour and a half, resulting from the immunological stimulus against the inflammatory condition caused by NS.

Therefore, it is extremely important that there is a commitment by the scientific community to the development of techniques that allow the practical, widespread and early use of combined biomarkers, since the isolated use of biomarkers is not sufficient for the establishment of a reliable diagnosis in the case of NS. Thus, in view of the excellent results presented with the implementation and use of biomarkers to aid in the diagnosis, treatment and prognosis of SN, it is understood that the dosage of these molecules can be of great value when implemented for this purpose.

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