Review Article

Hematological and Biological Markers of Neonatal Sepsis

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ABSTRACT

Septicemia in neonates is the commonest cause of mortality. Early recognition and diagnosis of neonatal sepsis remains a challenge because of the variable and nonspecific clinical presentation. The laboratory criteria are often non specific and not fully reliable. The objective of this review is to highlight the various hematological and biological markers of neonatal sepsis. We searched PubMed and Elsevier's web of science from studies evaluating the hematological and biological markers of neonatal sepsis. The key words used were "neonatal sepsis", "hematological marker" and "biomarker". Since a battery of markers of neonatal sepsis are available, it is always better to rely on a combination of markers along with the clinical correlation.

Key words: **Neonate, Septicemia, Biological Marker**

Introduction

The incidence of neonatal sepsis still remains high in spite of the advancement in the perinatal and neonatal care. The reasons are mainly related to the combination of the neonatal reduced immune defense and the complex interactions between the infecting microorganisms and the host response (1-3).

The blood stream infection rates in developed countries range from 10-25% for all neonates to around 50% in preterm very low birth weight (VLBW) infants (4-5). The figures in the developing countries are considerably higher even though the exact figure is not known. World Health Organization (WHO) estimates that out of the four million neonatal deaths all over the world every year, over 35% are due to infection in the neonatal period (6). In India, the incidence of neonatal sepsis varies from 11-24.5/1000 live births (7).

Rapid diagnosis and therapeutic intervention of the sepsis is of great importance for a better outcome of patients. However it still remains a challenge to diagnose neonatal sepsis early as the clinical symptoms are not reliable (8). Even

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though blood culture has been the gold standard method for diagnosis (9), the result is available late, usually within 24- 72 hours.

An intensive search for a variety of hematological and biological markers has been carried out by many investigators for the evaluation of clinical sepsis. There is an increasing battery of laboratory tests available for diagnosis of sepsis but most have failed to reach the level of accuracy and consistency (10).

The purpose of this article is to review the currently available hematological and biological markers for neonatal sepsis and to speculate on the possible utility of novel biomarkers under investigation.

Methods

We used an electronic search of the PubMed and Elsevier's web of science database from studies evaluating the hematological and biological markers of neonatal sepsis. First PubMed was queried for "neonatal sepsis" and "biomarker". The search was restricted to reviews in English. The entire Medline databases were searched in august 2012 and all the studies both clinical and experimental which evaluated a biomarker were included. For each identified biomarker, the Medline databases were screened again using the biomarker name and the keyword "biomarker". We also searched using the key words "neonatal sepsis", "hematological marker" and also "blood culture". After the extensive search, the following informations were analyzed.

Culture

The isolation of microorganism from blood, cerebrospinal fluid (CSF), urine or other body fluids remains the gold standard for a definitive diagnosis (11). Blood culture being the gold standard for diagnosis of septicemia, should be ideally done in all suspected cases before starting antibiotics. Conventionally it takes around 72 hours before labeling it sterile (12). The reasons for low sensitivity of the culture method are because of the concomitant antibiotic therapy or a combination of small blood sample volume and low colony counts (11). Ideally two sets of blood culture bottles are recommended for optimum isolation of the organism. Although the recommended volume of blood is 1-5 ml in pediatrics patients, smaller volume of blood in neonates less than 1ml have a sensitivity comparable to a larger volume. The reason is because of the higher bacterial count in neonates and infants and lower immune status (13). However, a count of at least four CFU/ml and one ml blood volume are necessary to reach a probability of isolation of 98% (14). It is now possible to detect growth in 12-24 hours by BACTEC or BACT/ALERT culture system which can detect bacteria at a much lower concentration of 1-2 CFU/ml. Blood samples collected from indwelling catheters or lines should be avoided as they are likely to be contaminated.(12)

Search for an ideal diagnostic marker:

Researchers have been trying hard for years to find a test or panel of tests able to identify septic neonates accurately and rapidly while awaiting culture results. Diagnostic test characteristics significantly change depending on sensitivity, specificity and predictive value. Considering the fact that neonatal sepsis has high mortality and serious morbidity, an ideal diagnostic marker should have a very high sensitivity (infected infants have a positive test) and negative predictive value (a negative tests confidently rules out infection) preferably reaching 100% (15, 16). However, the lack of reliable clinical signs often results in injudicious use of antimicrobial agents. So an ideal diagnostic marker should have a high specificity and a good positive predictive value preferably better than 85% in order to minimize unnecessary use of antibiotics in false positive cases (16).

The various hematological and other biological markers available for the diagnosis of neonatal sepsis are discussed separately.

Hematological markers:

Hematological indices were probably used as the initial markers for early diagnosis of neonatal sepsis. The following hematological parameters have been studied either singly or in combination (17-20).

1. Total Leukocyte count
2. Total neutrophil count
3. Immature neutrophil count
4. Immature to total neutrophil (IT ratio)
5. Immature to mature neutrophil ratio
6. Morphological or degenerative changes in neutrophil such as vacuolization, Dohle bodies, intracellular bacteria, toxic granules.
7. Platelet count.

The WBC count and differential count are useful in assessing a neonate with sepsis. The marrow reserves of leukocytes in a new born are relatively smaller compared with older children and adults. Thus leucopenia is reported more frequently as a frequent sign of overwhelming infection (21). The normal peripheral WBC count of newborns ranges from 5000 to 20,000/cumm (22), but even values outside this range has a poor specificity for predicting sepsis. There is some evidence that absolute neutrophil count plus bands in the differential is more predictive of sepsis than the total WBC count (21). Results of while cell counts and ratios varied widely from studies with sensitivity and specificity ranging from 17-90% to 31-100%, respectively (18). In general, the abnormal leukocyte ratios including the IT ratio ≥ 0.2, tend to have high sensitivity, whereas abnormal leukocyte counts, such as leukopenia and neutropenia tend to have high specificity (17-19, 23). The IT ratio of ≥ 0.2 may reach a sensitivity of 90% and negative predictive value of 98% (18,24). The reliability of IT ratio is that it is less influenced by non infectious factors (25). Using the hematological scoring system (HSS) involving seven above mentioned variables (one point allocated to each abnormal variable) suggested that the higher the score, the greater is the sensitivity. A cut off score of ≥ 3 had a high sensitivity of 96%, but a disappointingly low positive predictive value of 31% (19). However this scoring system was not widely accepted because of its unfavorable diagnostic values and complexity of the scoring method. Conversely, many authors have emphasized the utility of HSS as a reliable screening technique, especially when used in conjunction with other biological markers (26-28).

Thrombocytopenia and morphological changes in neutrophils were often more and usually denote late signs of infection (16, 17, 23).

Micro erythrocyte sedimentation rate (Micro ESR):

The technique of estimating micro ESR by using a microhematocrit tube has been developed over 50 years ago. Normal values changes significantly during the first two weeks of life, and can be calculated by adding 2 or 3 to the age of the infant in days. Even though micro ESR has high specificity, it has a low sensitivity because of the delay in rise and a long time requirement for normalization following recovery. Thus it is considered of little value in diagnosis of neonatal sepsis (29).

Granulocyte colony stimulating factor, a mediator produced by the bone marrow for promoting proliferation and differentiation of neutrophils, has been recently highlighted to be a reliable infection markers for early diagnosis of neonatal sepsis (30, 31). With a cut off value of 200pg/ml, it is thought to have high sensitivity (95%) and negative predictive value (99%) for predicting early neonatal bacterial and fungal infections (30).

As septic neonates are prone to develop hemorrhagic and thrombotic complications, activation of the clotting and fibrinolytic system has been seen both in adult and preterm neonates with severe infection (32-34).

Circulating thrombin-antithrombin III complex, plasminogen activator inhibitor-I, plasminogen tissue activator, fibrinogen and D-dimer

concentrations are significantly raised in infected infants compared with non infected patients (32-34).

The utility of granulocyte colony stimulating factor (G-CSF) and coagulation products as a clinical indicator of sepsis will require further investigation and direct comparison with other infection markers.

Acute phase reactants:

These are the immediate inflammatory proteins produced by liver in response to infection or tissue injury. The most extensively used and investigated acute phase reactant is C-reactive protein (CRP).

C-reactive protein:

C-reactive protein, a globulin is thought to form a precipitate when combined with the C-polysaccharide of streptococcus pneumonia (11). It is synthesized within six to eight hours of exposure to an infective process or tissue damage. It has a half life of 19 hours and may increase more than 1000 fold during an acute phase response (35). CRP as a diagnostic marker in neonates has higher sensitivity and specificity than total neutrophil count and IT ratio (18). Serial measurements at 24 and 48 hours after the onset of illness considerably improve the sensitivity (82% and 84% respectively). The specificity and the positive predictive value of CRP range from 93% to 100% (15). Thus CRP can be considered as a specific but a late marker of neonatal sepsis. On the other hand, increased CRP concentrations have been seen in non infected clinical conditions such as meconium aspiration, tissue necrosis, recent vaccination and surgery (15, 36).

Procalcitonin (PCT):

PCT is a propeptide of calcitonin with 116 amino acids secreted by the C- cells of the thyroid gland in normal situations but its level may increase in severe infections like septicaemia, meningitis, pneumonia and urinary tract infections (37, 38). Although the exact sites of production of PCT in sepsis have not been recognized, monocytes and hepatocytes are believed to be potential sources. Serum concentration of PCT begin to rise four hours after exposure to bacterial endotoxin, peak at 6-8 hrs and remains raised for at least 24 h (39). The half life is longer than CRP and is estimated to be about 25-30 h (34). PCT has been claimed to be superior to other acute phase proteins including CRP with sensitivity and specificity ranging from 87% to 100% (39, 40). Infants with viral infection, non infected inflammatory stress like birth trauma, aspiration syndrome and hypoxaemia, have normal or slightly elevated concentrations (41). Many recent studies also considered PCT to be a superior marker of neonatal sepsis compared to CRP with the added advantage of differentiating bacterial and viral infection (42-44). Thus we can avoid unnecessary use of antibiotics.

Neopterin:

It is a pyrazino-pyrimidine derivative formed from guanosione triphosphate within the biosynthetic pathway of biopterin. It is thought to be synthesised by the human macrophages when stimulated by interferon gamma released from activated T lymphocytes (45). Thus, neopterin production appears to be closely associated with activation of cellular immune system. The sensitivity and specificity of neopterin are 78.9% and 95% respectively. The positive predictive value and the negative predictive value are 93.8% and 82.6% respectively (46).

Many other acute phase reactant proteins like α -1 antitrypsin (47), fibronectin (48), haptoglobin (47), lactoferrin (49), and lipopolysaccharide binding protein (50), have been studied as a marker of neonatal sepsis. Although these markers show a significant rise in concentrations in neonatal sepsis, none of them have been routinely used.

Chemokines, cytokines, adhesion molecules and component of the immune pathway:

Neonates initially depend on natural (innate) immunity because the antigen specific immunity develops at around two years of age. The innate immunity is assumed to be provided by phagocytes (monocytes, tissue macrophages and neutrophils, natural killer cells) and humoral mediators (CRP, complements and transplacentally acquired maternal antibodies) (12). In response to antigens like bacterial endotoxins, activated tissue macrophages produce tumor necrosis factor-α(TNF- α) and interleukin (IL) which initiates the cytokine cascade increasing the production of IL-6, IL-8 and chemokines (12).

Recent studies have shown that neonates have a higher percentage of IL-6 and IL-8 positive cells than do adults (12, 51). Since the leukocyte indices and CRP are late markers and not sensitive enough for early diagnosis of neonatal sepsis, study of this diverse group of intercellular messengers was initiated (15). Among the mediators investigated, much importance has been focused on IL-6, IL-8 and TNF- α.

Interleukin-6:

The level of IL-6 rises abruptly on exposure to bacterial products after the release of TNF- α but preceding the rise of CRP. It is synthesized by mononuclear phagocytes, endothelial cells, fibroblasts, deciduas, chorion, amnion and trophoblastic cells (52). Umbilical cord blood IL-6 has been shown to be a sensitive marker for diagnosing neonatal infection within 72 hrs of birth, the sensitivities and the negative predictive values being 87% to 100% and 93% to 100% respectively (53-55). IL-6 has the highest sensitivity (89%) and a negative predictive value of 91% in neonatal sepsis compared with other biochemical markers including CRP, TNF-α, E selectin etc., at the onset of infection. But IL-6 has a very short half life and its concentration falls rapidly with treatment within 24 hours (15). Thus IL-6 can be considered as an important and significant sensitive early marker of neonatal infection.

Interleukin-8:

It is a proinflammatory cytokine predominantly produced by monocytes, macrophages and endothelial cells with similar kinetic properties with IL-6 (12). IL-8 is considered to be one of the accurate marker having sensitivities ranging from 80-91% and specificities from 76-100% (12, 55-57) . The diagnostic accuracy is enhanced by concomitant measurement of CRP (56).

Tumor necrosis factor (TNF-α):

It is a proinflammatory cytokine that simulates IL-6 production and having broad spectrum of biological actions. Even though TNF-α has been shown to be an important mediator in the pathophysiology of septic shock but its usefulness as a diagnostic marker has not been found to be as good as either IL-6 or IL-8 (12,15).

Other markers studied recently include adhesion molecules (intercellular adhesion molecule-1, vascular cell adhesion molecule-1, E selectin, L-selectin) and complements activation products (C3a-des Arg, C3bBbP, s C5b-9 (12). But these markers require further evaluation for their use in the diagnosis of neonatal sepsis.

Cell surface markers:

With the advent of flow cytometric analysis of cell surface markers, it has enabled the study of these antigens in sepsis (58). Among the various cell surface markers, the important ones include CD11b, CD64 and CD45 RO.

CD11b is an α subunit of the β 2 integrin adhesion molecule which is normally expressed at a very low concentration on the cell surface of non activated neutrophils (59). Its expression increases within a few minutes after the inflammatory cells come in contact with the bacterial endotoxins (60).

CD64 expression was elevated significantly when compared to the non infected neonates (61). It has been reported that the CD64 index with a cutoff point value of 2.38 had sensitivity,

specificity and a negative predictive value of 100%, 68% and 100% respectively (62).

There is an increase in the lymphocyte (CD3, CD19, CD25<CD71 and CD69) and neutrophil (CD116, CD11c, CD13, CD15, CD33, and CD66b) antigens in preterm newborns in response to infection, however the diagnostic utilities of these markers are yet to be evaluated (63, 64).

PCR arrays:

Polymerase chain reaction (PCR) based tests usually take longer time to be useful at bedside. However PCR has been proven to be useful in the identification of enteroviral (65), adenoviral (66), S agalactiae (group B streptococcus) (67), and fungal infections.

Future biomarkers:

With the advancement in the technology of quantitative flow cytometry which measures a wide variety of inflammatory mediators or cell surface markers, the search for an "ideal diagnostic marker" will be a revolutionary movement. The advantage of flow cytometry especially in neonatal patients is that it requires only a minimal volume of blood. Many new chemokines, antimicrobial peptides, acute phase reactants and cell surface antigens are being investigated. Some of them include interferonY inducible protein (IP-10), monokine induced by interferon Y (MIG), monocyte chemoattractant protein-1 (MCP-1), growth related oncogenes α (GRO-α). The new markers especially IP-10 with a plasma cut off concentration of ≥ 1250pg/ml has a sensitivity and specificity of 93% and 89% respectively in detecting neonatal sepsis (68).

Calprotectin another new marker identified is a complex of two calcium binding proteins belonging to the S100 protein family is abundant in the cytosolic fraction of neutrophils (69). Its level correlated well with other laboratory markers of sepsis and proved itself as both a sensitive and specific marker of neonatal sepsis (70, 71).

Soluble CD14 is a glycoprotein expressed on the membrane surface of monocytes/ macrophages and serves as a receptor for complexes of lipopolysacchrides(LPS) and LPS binding protein. It has been projected as a new diagnostic biomarker of sepsis (72).

Other PCR based molecular diagnostic techniques have also been used to detect the genetic materials of pathogens in blood, body fluids and tissue samples. This technique is particularly useful in identifying the exact pathogen causing the infection.

Another technique is proteomics based study. Proteomics is the study of expressed proteins and as such, is much more clinically relevant than genomics which studies DNA. The most recent advancement in proteomics is the discovery of Calgranulis (S-100) protein which is thought to play a critical role in protecting fetus. Certain intracellular "alarmins" known as damage associated molecular proteins (DAMPs) which includes high mobility group box -1 protein (HMGB1), heat shock proteins, altered matrix proteins and S100 proteins, represent important danger signals that mediate inflammatory response (73).

Conclusion

It is not practicable to subject a patient to all the markers available. Hence, it is always better to consider the clinical situations while interpreting the battery of tests. Combinations of markers are always superior to the consideration of a single test. The quest for better markers is on the rise.

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