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NEONATAL AND PAEDIATRIC SEPSIS

Biochemical markers of neonatal sepsis

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Summary

The use of biochemical markers in neonatal infection has remained an important area of research in the past decades. Many infection markers are components of the inflammatory cascade and reflect the host's immunological status and response to infection. Cytokines and chemokines such as interleukin (IL)-6 and IL-8 have been demonstrated to have good diagnostic utilities as early phase markers, while acute phase reactants such as C-reactive protein and procalcitonin have superior diagnostic properties during the later phases. Other markers, including inter-α-inhibitor proteins, IL-10 and regulated upon activation normal T cells expressed and secreted (RANTES) have been demonstrated to yield important prognostic information and may help the clinician identify infants who will develop fulminant infection from the outset of presentation. The advent of flow cytometry and molecular techniques have made crucial contributions to the field and promise to further improve the diagnostic accuracy and clinical management of infected infants.

Key words: Chemokines, cytokines, infection markers, leukocyte surface antigens, neonatal infection.

Abbreviations: CRP, C-reactive protein; GRO, growth-related oncogene; IFN, interferon; IL, interleukin; LBP, lipopolysaccharide-binding protein; PCT, procalcitonin; RANTES, regulated upon activation normal T cells expressed and secreted; SAA, serum amyloid A; TGF, transforming growth factor; TNF, tumour necrosis factor; VLBW, very low birth weight.

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INTRODUCTION

Investigation of biochemical markers for neonatal infection is an important area of research, because morbidity and mortality in neonatal sepsis remains substantial[1,2] despite continued advances in neonatology[3] and choices of novel antibiotics. Most current biochemical markers are derived from components of the intricate and complex inflammatory response to invading pathogens. This review focuses on recent studies of chemokines, cytokines, cell surface antigens and acute phase proteins as potential infection markers. The role of other modalities of investigation of neonatal infection will also be addressed. Table 1 contains a glossary of infection markers discussed in this review.

ROLE OF INFLAMMATORY MEDIATORS

Inflammatory mediators play important roles in the host response to pathogens. These molecules can have pro-inflammatory or anti-inflammatory properties. Pro-inflammatory mediators, such as interleukin (IL)-1β, IL-6 and tumour necrosis factor-α (TNF-α) activate host defences against infective agents, while anti-inflammatory mediators, such as IL-4, IL-10 and transforming growth factor β (TGF-β), are important in regulating and limiting the inflammatory response, preventing an excessive reaction which may itself cause host organ damage and tissue cell death.[4–6] Over the past decades, research into various actions and interactions of these mediators has further increased our understanding of the inflammatory cascade and provided us with new avenues of investigation into their clinical application. One such important area is the field of neonatal infection. Despite advances in neonatal intensive care,[3] stringent infection control measures, and an increasingly wide range of newer generations of antimicrobial agents, neonatal infection remains an important cause of morbidity and mortality amongst neonates, especially very low birth weight (VLBW) and extremely premature infants.[7,8] Early identification of infected neonates is fraught with difficulties as the clinical features during the early phases of infection may be non-specific and inconspicuous. Routine microbiological and haematological investigations that are currently in common use have substantial limitations, e.g., the wide normal variation of total and differential white cell counts, subjective nature of white cell morphology, difficulty in obtaining culture specimens from various tissues, low sensitivity of blood culture in neonates and often long periods of time required for clinically useful results by conventional culture techniques. The development of newer biochemical markers will further increase the ability of neonatal clinicians to differentiate between infected and non-infected infants. A comprehensive account of all the biochemical markers that have been studied in the past decades is beyond the scope of this review and, thus, only a select collection of the more important and promising recent infection markers will be described.

APPLICATIONS OF BIOCHEMICAL MARKERS

Each of the plethora of infection markers, e.g., acute phase reactants, chemokines, cytokines and leukocyte cell surface antigens, have distinct characteristics. The distinct properties of various biochemical markers suggest that each mediator has a different set of indications and restrictions when applied to different types of infection (e.g., viral versus bacterial), or even different phases of the infective process.[9] Infection markers have traditionally been used to assist neonatologists decide on which patients to

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TABLE 1 Glossary of potential diagnostic markers in neonatal sepsis

Pro-inflammatory cytokines	
IFN-γ	Interferon-γ: predominantly produced by Th1 cells and facilitates activation of pro-inflammatory cells, e.g., macrophages
IL-1β	Interleukin-1β: mainly produced by macrophages to help regulate the pro-inflammatory process
IL-6	Interleukin-6: predominantly produced by leukocytes and hepatocytes in response to infection and trauma
TNF-α	Tumour necrosis factor-α: mainly produced by macrophages in response to bacterial and other inflammatory products
Anti-inflammatory cytokines	
IL-4	Interleukin-4: predominantly produced by Th2 cells
IL-10	Interleukin-10: inhibits production of pro-inflammatory cytokines by Th1 cells
TGF-β	Transforming growth factor β: regulates cellular proliferation and differentiation, and suppresses T helper cell function
CC chemokines	
MCP-1	Monocyte chemoattractant protein-1: attracts natural killer cells and activates mast cells
RANTES	Regulated upon activation normal T cells expressed and secreted: attracts eosinophils, lymphocytes and monocytes
CXC chemokines	
GRO-α	Growth-related oncogene-α: attracts neutrophils and suppresses myeloid colony formation
IL-8	Interleukin-8: attracts neutrophils and stimulates phagocytic activity
IP-10	Interferon-γ-inducible protein-10: attracts activated T lymphocytes, and may be induced by interferon-γ. It has anti-tumour activity and a regulatory role in angiogenesis
MIG	Monokine induced by interferon-γ: biological activity similar to IP-10
Acute phase reactants	
CRP	C-reactive protein: an acute phase protein produced by the liver that increases in response to IL-6
IαIp	Inter-α-inhibitor proteins: a group of plasma proteins that inhibit serine proteases and possess anti-inflammatory properties
LBP	Lipopolysaccharide-binding protein: an acute phase protein that is mainly produced by the liver
PCT	Procalcitonin: the precursor of the peptide hormone calcitonin mainly produced by the liver and by monocytes
SAA	Serum amyloid A: a group of structurally related proteins that are released in response to injury and infection by a broad range of cell types, e.g., hepatocytes, smooth muscle cells, endothelial cells, monocytes
Leukocyte surface antigens	
CD11b	Cluster of differentiation 11b: a β_2-integrin adhesion molecule present on leukocyte cell surfaces and binds molecules such as complement components and lipopolysaccharide
CD64	Cluster of differentiation 64: a receptor found on leukocyte cell surfaces that binds the Fc portion of IgG antibodies with high affinity

discontinue antibiotics early.[9,10] In recent years, however, there has been new insight into how these markers should be best utilised, especially in infants who appear to be clinically stable,[11,12] or whether to start antibiotic treatment when daily monitoring of markers indicate an abnormal level for seemingly healthy infants.[13] Other applications of infection markers that have been studied recently include not only their utilities as diagnostic tools but also their provision of vital prognostic information.[4,14,15]

Cytokines and chemokines

Inflammatory cytokines and chemokines, such as IL-1β, IL-6, IL-10, interferon γ (IFN-γ), TNF-α, IL-8, regulated upon activation normal T cells expressed and secreted (RANTES), monokine induced by interferon-γ (MIG), monocyte chemoattractant protein-1 (MCP-1), growth-related oncogene-α (GRO-α) and interferon-γ-inducible protein-10 (IP-10), mediate the host response to infection in complex inflammatory pathways. Varying levels and ratios of these mediators, therefore, provide the clinician with valuable diagnostic and prognostic information regarding the status of the inflammatory process.[4,6,15,16]

Cytokines Of the pro-inflammatory cytokines, IL-6 has been one of the most widely studied for its potential as an infection marker in neonatal infection.[4,17–24] It is produced by both T and B cells.[25] It serves many functions, including regulation of the host response to infection.[26] Exposure of the host to bacterial products results in a rapid and substantial increase in blood IL-6 concentrations.[16,20,27] IL-6 in turn stimulates hepatocytes to produce acute phase reactants such as C-reactive protein (CRP).[25] Therefore, IL-6 is potentially a more useful marker than CRP during the early phase of infection with a sensitivity of 89 versus 60% at the onset of clinical suspicion of nosocomial neonatal infection, respectively.[19] More importantly, in the same study, the negative predictive value of IL-6 (91%) was much higher than CRP (75%). This early rise in blood concentrations in response to infection has also been demonstrated in early-onset neonatal infection. In a study where cord blood IL-6 levels were measured, it was found that sensitivities and negative predictive values remained high (87–100% and 93–100%, respectively).[28,29]

In view of the short half-life of IL-6, successful treatment of infection leads to rapid reduction in its circulating concentrations to undetectable levels by 24 hours.[6,16,20] Although this characteristic suggests that IL-6 can be used to monitor the host response to treatment and progress of infection, it also implies that the window of opportunity for specimen sampling is narrow. Whilst the sensitivity of CRP increases to 82% by 24 hours and 84% by 48 hours after the onset of infection, the corresponding parameters for IL-6 decrease to 67% and 58%, respectively.[19] In view of these limitations, combining IL-6 with markers that are more sensitive during the later phases of infection has been studied to improve the diagnostic utilities.[19,24] For example, the combination of IL-6 and TNF-α is more sensitive than IL-6 or TNF-α alone in early-onset neonatal infection (98.5, 90 and 87.9%, respectively),[24] while various combinations of IL-6, CRP and TNF-α can maintain sensitivities and negative predictive values above 90% for late-onset infection from presentation until 48 hours afterwards.[19]

The inflammatory process is regulated not only by pro-inflammatory chemokines and cytokines, but also anti-inflammatory mediators. Anti-inflammatory cytokines, such as IL-10 and transforming growth factor β (TGF-β)