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Biomarkers of necrotising enterocolitis

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SUMMARY

Different categories of biomarkers of necrotising enterocolitis (NEC), including (i) non-specific mediators of the inflammatory cascade, e.g. acute phase reactants, chemokines, cytokines, and cell surface antigens, (ii) enhanced non-specific biomarkers, and (iii) specific gut-associated proteins, have distinctive biochemical characteristics and properties. The appropriateness of using these mediators in specific clinical situations, and the pros and cons of their applications as indicators or predictors of intestinal injury and NEC are highlighted. Many potentially new biomarkers such as micro-RNA, volatile organic compounds and gut microbiomes are currently under investigation. A stringent protocol for biomarker discovery is revealed so that investigators can consider this methodology as a reference for future discovery of organ-specific and/or disease-specific biomarkers for preterm infants.

1. Introduction

Necrotising enterocolitis (NEC) is the most frequently occurring acute abdominal emergency affecting premature infants [1,2]. The incidence is estimated to range from 7% to 10% in very low birth weight (VLBW) infants in the USA [3,4]. NEC causes intense inflammation and necrosis of tissues in the neonatal gastrointestinal (GI) tract. It has been demonstrated that the exaggerated immunological response associated with bowel inflammation may cause devastating damage to distant organs, especially the central nervous system, resulting in neurocognitive impairment [5,6]. Affected infants requiring surgery and those associated with pan-necrosis of the bowel are particularly vulnerable to severe long-term morbidities such as short bowel syndrome, parenteral nutrition-associated cholestasis and sepsis, and increased mortality. Yet, it is difficult to diagnose NEC in the early phase of presentation when inflammation is mild, as both clinical and radiological features are subtle and non-specific [7]. Abdominal distension, feeding intolerance and increased gastric residuals are signs common to both NEC and other neonatal GI conditions, such as GI dysmotility of prematurity [8] and intestinal ileus from sepsis or electrolyte disturbances [9]. Early radiological signs of thickened bowel wall, dilated bowel loops, and paucity of intestinal gas are not pathognomonic of NEC. Features of localised GI perforation (50–60%) and pneumatosis intestinalis are frequently absent in early plain abdominal radiographs [10,11]. It is also important to accurately identify infants with NEC from those with sepsis without NEC because the clinical management is substantially different with regard to antibiotic coverage, duration of fasting and subsequent parenteral nutrition requirements [9]. Thus, early diagnosis and differentiation of NEC from neonatal sepsis remain a major challenge for neonatologists. A biomarker or a panel of biomarkers that can facilitate early diagnosis or predict the severity or prognosis of NEC at or before the clinical presentation would be of tremendous value to neonatologists and paediatric surgeons for providing prompt treatment and targeting the sickest patients.

This article reviews different classes of biomarkers currently available for clinical assessment of NEC. They include: (i) non-specific biomarkers, (ii) 'enhanced' non-specific biomarkers, and (iii) gut-specific biomarkers, for early diagnosis, differentiation of NEC from other inflammatory/sepsis conditions, and prediction of the severity and the need for surgical intervention at the initial presentation. Potentially new GI biomarkers for diagnosing and assessing bowel inflammation are also highlighted. In addition, this review examines new and emerging molecular techniques for discovering novel biomarkers.

2. Biomarkers of NEC

2.1. Non-specific biomarkers of NEC

Non-specific biomarkers are mediators of the inflammatory cascade and can be broadly subdivided into three main categories: (i) acute phase reactants and other proteins, (ii) cytokines and chemokines, and (iii) cell surface antigens. Each category has its own biochemical characteristics, and therefore different types of biomarkers may be more applicable to specific clinical situations.

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2.1.1. Acute phase reactants and other proteins

The most widely used acute phase reactant for identifying neonatal sepsis and NEC is C-reactive protein (CRP). It is a 'late-warning' biomarker and, hence, not effective for early diagnosis of infection or inflammatory conditions [12]. However, CRP is useful for monitoring the disease progress and guiding treatment. In infants with suspected NEC, normal serial CRP levels favour the early discontinuation of antimicrobial therapy and prompt reintroduction of enteral feeding, whereas persistently elevated levels in NEC infants receiving medical treatment suggest the development of complications, e.g. peritonitis, intra-abdominal abscess, or formation of an inflammatory mass with concealed perforation and/or matted necrotic bowels [13]. Another useful acute phase protein is serum amyloid A (SAA) [14–16]. Its biochemical characteristics are similar to those of CRP but with earlier upregulation [16]. We have demonstrated that the combined use of SAA and apolipoprotein CII (ApoSAA score) can identify all cases of neonatal sepsis and NEC at the time of initial sepsis screening [16]. By stratifying suspected infected/NEC infants into different risk categories, the ApoSAA score allows withholding and early cessation of antibiotic treatment within 24 h in 45% and 16% of cases, respectively [16]. Although some investigators further suggest that serum SAA concentration correlates with the severity or stage of NEC [14,15], it is impractical to define different cut-off levels to quantify the severity of infection and/or NEC [7].

Other proteins, including procalcitonin [14,17], anaphylatoxin (C5a) [18], arginine, [19] endotoxins [20], platelet-activating factor and related lipids [21], e-selectin [12,22], inter-α inhibitor proteins [23], S100A8/A9 [24], and various GI hormones (e.g. gastrin and neurotensin) [25], have also been suggested as useful indicators for either early diagnosis [21], monitoring progress [21], or predicting surgical and lethal NEC [18,19]. As most are isolated reports and have not been validated by larger studies, more research is required to delineate their usefulness in screening and diagnosing the condition.

2.1.2. Cytokines and chemokines

Proinflammatory cytokines, e.g. interleukin (IL)-6 and tumour necrosis factor (TNF)-α, and chemokines, e.g. IL-8, interferon-γ inducible protein-10 (IP-10), regulated upon activation of normal T-cell expression and secretion (RANTES), are 'early warning' biomarkers for diagnosing neonatal sepsis and NEC [12,26,27]. Typically, these mediators are promptly and substantially upregulated in infective or inflammatory processes, but their levels tend to fall precipitously within 24 h with appropriate treatment [12]. Thus, the combined use of early and late-warning biomarkers can provide comprehensive coverage for detection of these conditions within the first 48 h of disease onset. More importantly, the inclusion of anti-inflammatory cytokines, e.g. IL-10, in a proinflammatory:anti-inflammatory cytokine ratio, may further reflect the severity of illness and predict mortality [27,28]. The sequential use of IL-10, IL-6 and RANTES can accurately prognosticate the development of disseminated intravascular coagulation (DIC) in neonatal sepsis and NEC at the onset of clinical presentation with 100% sensitivity and 97% specificity [27]. Thus, cytokines and chemokines can complement acute phase reactants in extending the duration of biomarker coverage and predicting the severity of illness and prognosis.

2.1.3. Cell surface antigens

Cell surface antigens, e.g. natural killer cell CD69, neutrophil CD64, and CD11b are important inflammatory biomarkers in preterm infants [29–32], as they are promptly expressed within minutes of cell contact with bacteria or their products. Also, flow cytometry analysis of cell surface antigens requires only a very small volume of blood sample (0.05 mL whole blood) and each test can be performed individually on an ad-hoc basis rather than in batches [33]. As the antigen expression persists >24 h, blood samples can always be obtained within normal working hours [33]. These biochemical properties make cell surface antigens ideal for surveillance and early diagnosis of neonatal sepsis and NEC in high-risk infants [29–32]. A recent study on early diagnosis of intra-abdominal catastrophes, including NEC, intestinal necrosis, perforation, and peritonitis, indicates that neutrophil CD64 is a more sensitive biomarker than CRP for identification of these conditions [29]. Both biomarkers are, however, unsuccessful in accurately identifying cases of spontaneous intestinal perforation (SIP) with minimal peritoneal contamination [29]. Such findings are not unexpected, as we have recently demonstrated in a plasma cytokine array study that many inflammatory and hypoxia/oxidative mediators are severely dysregulated in NEC but not in SIP [34]. Although cell surface antigens possess favourable properties for early diagnosis and screening of systemic infection and inflammatory conditions, they are not organ-specific nor disease-specific biomarkers, and thus are unable to differentiate neonatal sepsis from NEC or other intra-abdominal catastrophes [7,29].

2.2. Enhanced non-specific biomarkers of NEC

This category of mediators comprises non-specific biomarkers of inflammation, but the nature of the specimen, e.g. stool, renders these mediators more indicative of the site of tissue injury. Enhanced non-specific biomarkers are especially relevant to NEC because the presence of a large quantity of inflammatory proteins in faecal matter signifies increased intestinal mucosal permeability and/or disruption of mucosal integrity. This setting in a preterm infant is most likely associated with NEC or hypoxic–ischaemic injury of the bowel. In the past decade, many investigators have focused on finding suitable biomarkers of NEC in a faecal specimen.

Calprotectin, a heterodimeric peptide secreted by neutrophils and macrophages, is highly resilient to bacterial degradation. Calprotectin has been proposed as a useful laboratory biomarker for diagnosing intestinal inflammation and NEC [35–38]. However, the very wide variation of calprotectin concentrations in meconium and post-meconium faecal matter (12–9386 μg/g and 9–1867 μg/g, respectively) [36], substantial intra- and inter-individual variability [36,37,39], and dependence on gestational and postnatal age [40] render the normal reference range for preterm infants very difficult to define and interpret. The recommended optimum cut-off level for identification of NEC ranges from 200 to 2000 μg/g among studies [35,37,38]. Although most reports demonstrate increases in the faecal calprotectin concentration in proven NEC [35–38], a recent study indicates that the level can be paradoxically decreased (<24 μg/g) in fulminant cases [40]. The very wide reference range and marked discrepancy between studies severely undermine the usefulness of faecal calprotectin as an early diagnostic or surveillance biomarker of NEC [36,40,41].

S100A12 (calgranulin C), a proinflammatory protein, is also released in large quantity into the bowel lumen by neutrophils and monocytes following GI mucosal inflammation. Similar to calprotectin, S100A12 is remarkably resistant to degradation by faecal bacteria, and has been suggested to be a potentially useful indicator of neonatal intestinal distress. Faecal S100A12 has been reported to be significantly elevated 4–10 days before and up to 2 weeks after disease onset in preterm NEC patients compared with disease-free control infants [42]. However, there is much overlap in faecal S100A12 concentrations between the two groups, rendering its usefulness questionable in clinical situations. In fact, the biochemical properties of S100A12 bear close resemblance to calprotectin in that there is wide variability in intra- and inter-individual faecal concentrations. In addition, the