Gut barrier proteins in diagnosing necrotising enterocolitis in preterm infants: abridged secondary publication

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KEY MESSAGES

1. Our study provides the first evidence that hepatocarcinoma-intestine-pancreas (HIP) and intestinal bile acid binding protein (I-BABP) were specific, novel biomarkers for early diagnosis of necrotising enterocolitis (NEC).
2. Use of HIP followed by I-BABP significantly improved the diagnostic performance. A stepwise risk stratification scheme for preterm infants suspected of NEC is proposed.
3. This risk stratification scheme can facilitate neonatologists in identification and management of preterm infants with NEC.

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Introduction

Necrotising enterocolitis (NEC) is one of the most devastating complications of prematurity. Despite advances in neonatal management for preterm very-low-birth-weight infants, NEC-associated morbidities and mortality remain high.[1] NEC often manifests in a fulminant manner with minimal antecedent signs and symptoms; it is important to recognise the initial bowel injury early so that neonatologists can promptly initiate treatment to minimise further damages to the bowel.

Acute-phase proteins, cell surface antigens, cytokines, and chemokines have been used to identify sepsis and NEC cases, but these mediators are unable to differentiate the two conditions.[2,3] Proteins originated from the bowel such as intestinal-fatty acid binding protein, liver-fatty acid binding protein, trefoil factor-3 (TFF3), and claudin-3 can be used as biomarkers for diagnosing NEC.[4] Nonetheless, these biomarkers are only useful in differentiating severe (surgical) cases from milder (medical) cases, and they are not clinically useful for detection of early bowel injury.[4] Such biomarkers are also unable to differentiate mild NEC cases from septicaemic or control patients.

Significant and extensive changes of gene expression are associated with multiple pathways involving inflammation, hypoxia and oxidative stress, cell adhesion and chemotaxis, extracellular matrix remodelling, angiogenesis, muscle contraction, and arginine metabolism.[5] These molecular responses correspond closely with the pathophysiology of NEC. We therefore hypothesised that novel tissue-specific or NEC-specific biomarkers could be discovered through searching for (1) gut-specific genes with mRNA levels dysregulated in NEC tissues, (2) genes that were up-regulated in accordance to the pathophysiologic mechanisms (eg, microbial infection and hypoxic and oxidative stress), and (3) proteins that shared the same protein family with known NEC biomarkers. We used a systemic bioinformatic approach to discover novel biomarkers through mining of the global gene expression data in diseased small bowel tissues collected from NEC infants. The objective was to discover novel biomarkers with good diagnostic value (sensitivity and specificity >85%) for diagnosing both medical (mild) and surgical (severe) NEC at the early phase.

Methods

This study comprised three phases: a discovery phase based on analysis of transcriptomes of NEC tissues to identify genes encoding for potential biomarker candidates, an exploratory phase to confirm level changes of selected markers, and a validation phase to examine the diagnostic value of plasma levels of the candidate proteins.

In the discovery phase, we analysed gene expression microarray data[5] of surgical small bowel tissues from infants with proven NEC (n=5), spontaneous intestinal perforation (n=5), and surgical control (n=4) to identify genes in which mRNA levels were altered in the NEC tissues in the same direction as those genes-encoding NEC

biomarkers. Among the gene list, only those that were predominantly expressed in the mucosa of human small intestine were retained by examining their baseline expression patterns in normal human tissues, and formed the disease site–specific list. This step aimed to filter out genes-encoding proteins that were not specific to the disease site of NEC. Using functional annotation analysis among the site-specific gene list, we retained genes in which expressions were likely to be up-regulated in response to microbial infection, hypoxia, and/or oxidative stress and genes that encoded proteins belonging to the same protein family of previously reported NEC biomarkers. Proteins in the final gene list were considered as potential NEC biomarker candidates.

In the exploratory phase, these proteins were examined in a case-control cohort consisted of 10 NEC patients, 20 non-NEC septicaemia patients, and 20 non-NEC non-septicaemia patients.

In the validation phase, plasma levels of the potential biomarker candidates and previously reported potential NEC biomarkers (intestinal-fatty acid binding protein, liver-fatty acid binding protein, TFF3, claudin-3, and cytosolic beta-glucosidase) were measured to determine their values in differentiating NEC cases (n=20) from septicaemic cases (n=40).

The Mann-Whitney *U* test was used for between-group comparisons. The Kruskal-Wallis test was used for comparisons of more than two groups, followed by Dunn's post-hoc tests. P values were adjusted by Bonferroni correction or Benjamini-Hochberg procedure for multiple comparisons where appropriate. The receiver operating characteristic curve, drawn by plotting sensitivity against 1-specificity at various cutoff values, was used to determine the diagnostic utilities of the biomarkers. All statistical analyses were performed using SPSS (Windows version 22; IBM Corp, Armonk [NY], US).

Results

Of 16 potential NEC biomarkers identified, 11 were quantified in NEC patients, non-NEC septicaemia patients, and non-NEC non-septicaemia patients in the exploratory phase.

In the validation phase, plasma levels of galectin-4, hepatocarcinoma-intestine-pancreas (HIP), intestinal bile acid binding protein (I-BABP), liver-fatty acid binding protein, and TFF3 were significantly higher in the NEC groups than both the non-NEC septicaemia group (P<0.011) and non-NEC non-septicaemia group (P<0.001). Galectin-4, I-BABP, HIP, and TFF3 had the highest diagnostic value in distinguishing NEC cases from non-NEC septicaemia cases and non-NEC non-septicaemia cases (area under the receiver operating characteristic curve was 0.84-0.91, all P<0.001).

Combination of the best two markers (HIP and I-BABP) was evaluated for improvement of their diagnostic performance. Using a risk-stratified approach by firstly using HIP to screen patients of high NEC risk and then using I-BABP to identify NEC cases among the high-risk group, the diagnostic performance of 85% sensitivity and 91% specificity was achieved.

Discussion

This study provides evidence that plasma HIP and I-BABP are tissue-specific novel biomarkers for early diagnosis of NEC. We formulated a stepwise risk stratification scheme by combining HIP and I-BABP to achieve diagnostic performance of 85% sensitivity and 91% specificity. These novel biomarkers were discovered using a comprehensive and systemic bioinformatic approach based on tissue expression profiles, dysregulatory pathways of intestinal injury, and pathophysiologic mechanisms of NEC, as well as through mining of the global gene expression data in diseased small bowel tissues collected from infants with NEC. Our new risk stratification scheme enables neonatologists to identify NEC cases during presentation of early and non-specific symptoms, and to facilitate decisions on timely application of treatment strategies to minimise gut damage.

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