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Intestinal Intraluminal Glycerol and Plasma I-FABP Levels In Preterm Infants with Necrotizing Enterocolitis

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Abstract

Background/Purpose: Necrotizing enterocolitis (NEC) is highly associated with prematurity, and is characterized by bowel necrosis and multiple organ failure. There is a strong need for improved diagnostic methods to reduce the significant morbidity and mortality associated with NEC. The aim of this single center prospective study was to investigate the possibility of detecting early signs of NEC, by using rectal intraluminal microdialysis and plasma intestinal fatty acid binding protein (I-FABP) in preterm infants, admitted to a level III neonatal intensive care unit.

Methods: The study was performed on extremely preterm infants with a gestational age of less than 28 weeks. During a 4-week period after birth, rectal intraluminal microdialysate levels of glucose, lactate, pyruvate and glycerol were measured, and plasma was collected for I-FABP analysis. Infants not developing NEC served as controls.

Results: Microdialysis revealed signs of intestinal hypoxic or ischemic damage and cell membrane degradation, with a marked increase of both intraluminal glycerol and plasma I-FABP in infants developing NEC, as well as in infants suffering from other complications. The microdialysate levels of glucose, lactate and pyruvate were too low to be evaluated in this setting. All infants tolerated the microdialysis well without any complications.

Conclusion: Elevated levels of intraluminal glycerol and plasma I-FABP suggests mucosal cell membrane degradation and hypoxic or ischemic damage in preterm infants developing NEC, as well as in preterm infants suffering from other complications such as volvulus, sepsis or respiratory distress. However, it was not possible to predict development of NEC before clinical diagnosis using these markers.

Keywords: Necrotizing; Enterocolitis; Intraluminal; Microdialysis; Glycerol; I-FABP

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Introduction

Necrotizing enterocolitis (NEC) is the most common gastrointestinal disorder in extremely premature very low birth weight neonates (VLBW <1500 g) [1]. In this group, incidence ranges between of 10-15% with mortality rates reported as high as 50% [2-5]. The risk of developing NEC is inversely related to gestational age and birth weight, and the incidence has increased in parallel with the improved survival of extremely preterm infants [3]. The disease is characterized by inflammation of the bowel, varying degrees of intestinal necrosis, leading to sepsis and in some cases multiple organ failure.

Today, the diagnosis of NEC relies on a combination of clinical symptoms, signs, and radiologic assessment. The diagnosis is very difficult at an early stage, and no biomarker has been identified to diagnose NEC with high accuracy before clinical suspicion [1]. Commonly, NEC is evident at a late stage, when systemic levels of biomarkers are reached, and intestinal damage has been established.

Intestinal ischemia is considered to be pivotal in the pathogenesis of NEC [1,6]. NEC results in variable degrees of ischemic necrosis of the small and large intestine, ranging from mild ischemic damage of the intestinal mucosa to transmural necrosis and perforation of the gut wall. Recent studies on intestinal ischemia with the microdialysis technique [7-13], have demonstrated the typical metabolic response to anaerobic metabolism; reduced glucose levels, production of lactate leading to

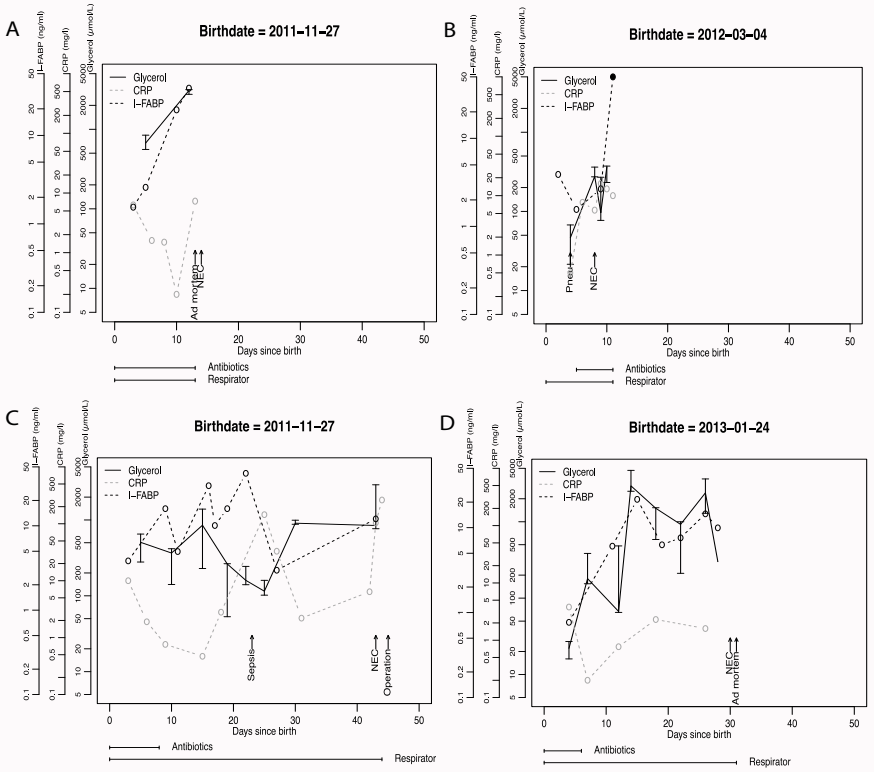


Figure 1: A-D. Glycerol, I-FABP and CRP levels in four infants developing NEC. Glycerol values are medians with bars for maximum and minimum values.

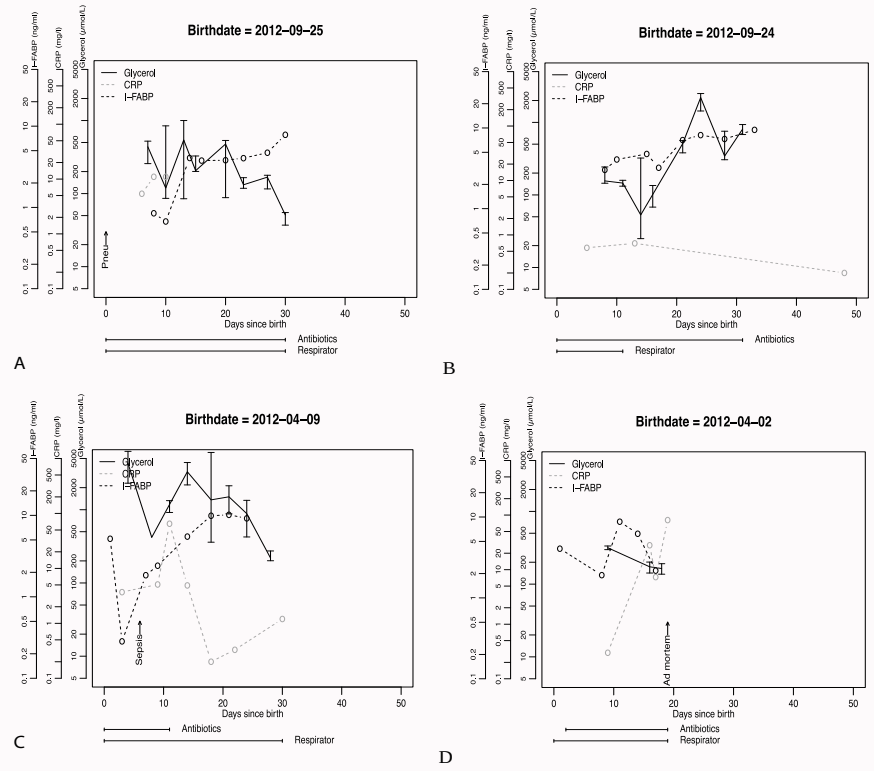


Figure 2: A-J. Glycerol, I-FABP and CRP levels in ten infants without clinical or radiological signs of NEC. Values for glycerol are medians with bars for maximum and minimum values. Glycerol and I-FABP levels varied considerably during the observation periods, with both rising and falling concentrations at different time points. Two infants (E, F) were operated for ligation of patent ductus arteriosus. K. One infant suffered from expansive intestinal necrosis due to mid-gut volvulus. All infants were intubated and ventilation was maintained on respirator during the observation periods as indicated (Figure 1A-D, 2A-K). Three of them suffered from pneumothorax due to ventilator trauma (Figure 1B, 2A, G). Antibiotic treatment was also initiated in all children, and maintained for different periods as indicated (Figure 1A-D, 2A-K). Two of the infants displayed a significant persistent ductus arteriosus, and were operated on thereafter (Figure 2E, F). Severe infection and sepsis was present in one patient (Figure 2C).

an elevated lactate/pyruvate ratio, accompanied by increased glycerol levels as a result of cell-membrane phospholipid degradation caused by ischemia-induced phospholipase activation.

We have previously studied experimental NEC with the microdialysis technique, using a hypoxia/re-oxygenation model of early NEC in rat pups [13]. Elevated intraluminal microdialysate levels of glycerol and lactate indicated intestinal hypoxia and enterocyte cell damage in this experimental NEC setting.

The accuracy of different plasma biomarkers in diagnosing NEC and intestinal ischemia has been studied extensively [1,14,15]. Intestinal fatty acid binding protein (I-FABP) is specifically present in mature enterocytes of small and large intestine, and is released as soon as cell membrane integrity is compromised. I-FABP is present in very small amounts in the plasma of healthy individuals, probably representing the normal turnover of enterocytes, but levels rise rapidly after episodes of acute intestinal ischemia and inflammation, including NEC [14-17]. I-FABP levels provide specific information about the number of dying intestinal epithelial cells, and can be used as an aid in early diagnosis of NEC or intestinal necrosis of other origin [16,18-20].

The aim of this single center prospective study was to investigate the possibility of detecting hypoxic or ischemic intestinal damage following NEC, by using rectal intraluminal microdialysis and measuring plasma levels of I-FABP on extremely preterm infants admitted to a level III neonatal intensive care unit.

Materials and Methods

Study population and setting

The study was approved by the regional committee on medical research ethics, and informed consent was obtained from the parents.

Preterm infants with a gestational age less than 28 weeks and weighing <1500 g were included. No abdominal symptoms or other clinical signs of illness were present in the infants, and there was no evidence of any otherwise complicating disease on inclusion. The infants were admitted to a level III neonatal intensive care unit, and followed during a 4-week period. Any routine blood testing and radiology scans were performed on a clinical basis. A total of 15 infants were included during this period. Four of these developed NEC stage 2 or 3, or NEC confirmed by histopathology. The remaining 11 infants did not develop NEC during this period, and therefore served as controls. The diagnosis of NEC was staged according to a simplification of the Bell classification (Walsh and Kliegman) [21], using 3 categories. Stage 1 was defined as suspected NEC, with lethargy, abdominal distension, bloody stools or apnea. Stage 2 was considered present when either X-rays or ultrasound revealed pneumatosis intestinalis, portal gas, intestinal perforation or ileus with dilated bowel loops. Stage 3 was defined when the occurrence of organ failure was present in addition to the stage 2 criteria.

Microdialysis

The infants were monitored during a 4-week period, with microdialysis measurements twice a week. All measurements were performed using a clinically approved CMA 70 microdialysis catheter (cut-off 20 kDa, 10 mm membrane length, Mdialysis AB, Solna, Sweden). The microdialysis catheters were connected to microinjection pumps (CMA 107, CMA Microdialysis AB, Stockholm, Sweden) and perfused with an isotonic Ringer´s solution with a flow rate of 1.0 µL/minute.

The microdialysis catheter was rectally inserted 10 mm, and secured in position with tape. Initially, in situ stabilization was allowed for 5 minutes, due to the non-traumatic placement of the catheter. Microdialysate samples were then collected every 30 minutes for a total of 90 minutes. Samples were immediately put into freezer at -20°C. Analyses of glucose, L-lactate, pyruvate and glycerol were performed using an enzymatic colorimetric technique on a CMA 600 Microdialysis Analyzer (CMA Microdialysis AB). The CMA 600 Analyzer was automatically calibrated at start-up and re-calibrated every sixth hour using standard calibration solutions from the manufacturer (CMA Microdialysis AB). Quality controls at two different concentrations for each analysate were performed every weekday. Total imprecision coefficient of variation was <10% for all analysates.

Blood samples

Routine testing of blood samples included C-reactive protein (CRP) using the standard clinical laboratory method. Blood was drawn for analysis of I-FABP. A volume of 100 µL in EDTA was centrifuged, and plasma was stored at -70°C until analysis. I-FABP was analyzed by a commercial sandwich-ELISA (DY3078, R&D Systems, Minneapolis, MN, USA), in which a monoclonal antibody specific for I-FABP was coated onto microtitre plates. Standards and samples were pipetted into the wells and the peptide was bound to the immobilized antibodies. After washing, a biotinylated anti-I-FABP antibody was added. Following incubation and washing, a streptavidine-HRP conjugate was added to the wells. After incubation and washing, a substrate solution was added. The development was stopped and the absorbance was measured in a SpectraMax 250 (Molecular Devices, Sunnyvale, CA, USA). The concentrations in the samples were determined by comparing the optical density of the sample with the standard curve. The assays were calibrated against highly purified recombinant human I-FABP. Measurements of I-FABP were performed without knowledge of the clinical diagnoses.

Results

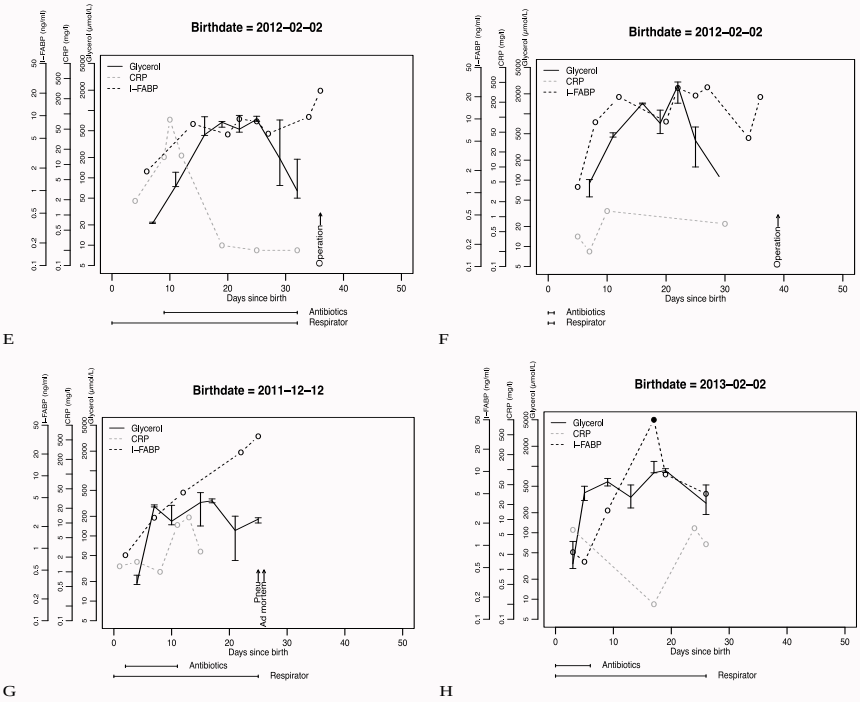
Microdialysis

Intraluminal microdialysate levels of glycerol were detectable. However, the concentrations of lactate, glucose and pyruvate were too low for analysis in all infants. During the observation periods, the concentrations of glycerol, I-FABP and CRP varied considerably in infants with NEC (Figure 1A-D). The mean levels of glycerol or I-FABP at NEC diagnosis were not higher than before diagnosis, or compared to controls.

The infants who did not develop NEC served as controls. In these infants, both glycerol and I-FABP levels also revealed a high degree of variation, with rising and falling concentrations during the observation periods (Figure 2A-K). One infant developed symptoms of intestinal ischemia but no signs of NEC on x-ray (Figure 2K). Laparotomy revealed expansive intestinal necrosis, as a result of mid-gut volvulus. In this patient, I-FABP clearly displayed an early elevation, accompanied by a later rise of glycerol and CRP.

Discussion

Today, NEC is diagnosed by a combination of clinical, laboratory, and radiological findings. These diagnostic methods lack high specificity and sensitivity for NEC, especially in the early phase. Certain indications of NEC are evident at a late stage during the



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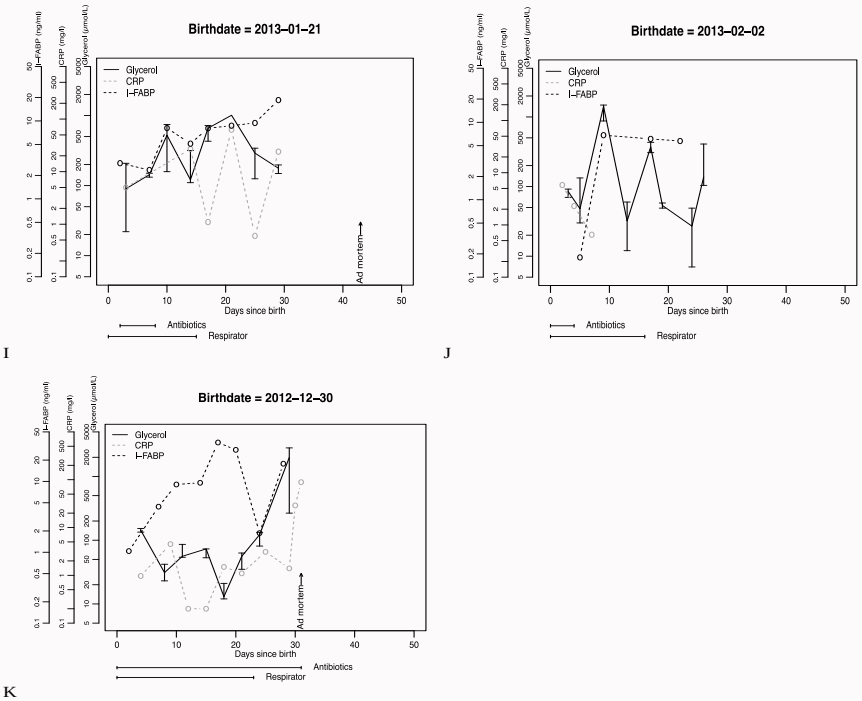

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course of the disease, when intestinal necrosis is manifest. Early diagnosis and treatment is important to reduce the morbidity and mortality associated with NEC. Thus, there is a strong need for improved diagnostic methods.

Microdialysis has previously been used to study intestinal ischemia, both on humans and on rats. The advantage of the microdialysis approach is that it measures metabolites of ischemia, locally, in the organ of interest. In early stage experimental NEC, the mucosa is primarily affected, as it is the most vulnerable part of the gut wall [13]. This raises the opportunity to use intestinal intraluminal microdialysis to identify anaerobic stress at an early stage, before systemic levels of the metabolites are reached, and before the organ of interest is severely damaged.

In our previous study on experimental intestinal ischemia [12], signs of intestinal damage in the lumen were measured by microdialysis, before systemic levels of the anaerobic metabolites were reached. The intraluminal levels of glycerol also had a positive correlation with aggravated histological mucosal damage. We have previously studied intestinal intraluminal microdialysis in experimentally induced early NEC in rat pups [13]. In the study, elevated levels of glycerol and lactate were measured by placement of a rectally inserted microdialysis catheter.

In humans, patients with recent abdominal surgery have been monitored with microdialysis [22,23], and these studies have demonstrated that microdialysis is a valuable tool for detecting visceral ischemia using the intraperitoneal approach. However, no clinical studies have previously been performed using the intraluminal approach, partly because of the difficulty of placing the microdialysis catheters in the gut lumen. In one study, subcutaneous microdialysis was used in neonates that had recently undergone surgery to monitor metabolic changes [24]. The rationale for using the intraluminal approach instead of the intraperitoneal, is that any early mucosal damage would be first detected in the gut lumen, whereas any intraperitoneal detection of metabolites would reflect a later stage in the disease.

To our knowledge this study is the first to apply intestinal intraluminal microdialysis to detect hypoxic stress in extremely preterm infants. Despite the low number of patients in this study, we have found that intraluminal microdialysis of the bowel is easily accessible by rectal placement of the microdialysis catheter, which has the advantage of being a minimally invasive method. Although it was noted that an elevation of glycerol was seen in infants with NEC, it was not possible to detect any significant increase in glycerol concentrations prior to clinical diagnosis. In the infants with other complications than NEC, intraluminal glycerol values also varied considerably, both rising and falling during the observation period. Therefore it was not possible to differentiate between NEC and the controls, by observing any increase in glycerol concentrations.

We have also been able to analyze plasma levels of I-FABP in extremely preterm infants born before 28 weeks of gestation. Plasma levels of I-FABP displayed a similar pattern as glycerol, with high concentrations before the development of NEC, as well as in the control infants. In a previous study of I-FABP as a diagnostic marker of intestinal ischemia, suggested cut-off point for non-reversible intestinal ischemia was 1.3 ng/ml [25]. A study on healthy preterm infants with gestational age between 28 and 33 weeks, plasma concentrations ranged between 0.46-4.5ng/ml [17]. An interesting finding in our present study is that the concentrations of I-FABP, in controls as well as infants with NEC, exceeded these levels even at an early stage. These findings may suggest that the previous suggested cut-off point or normality range of I-FABP is not relevant in this patient category of extremely preterm infants. Infants with high enterocyte turnover should theoretically display higher levels of I-FABP. Another explanation could be that the high concentrations of I-FABP in the present study reflect intestinal enterocyte damage. This fact is supported by the high intraluminal concentrations of glycerol at an early stage in controls as well as in infants later developing NEC. It is highly valuable to establish knowledge regarding the normality range of I-FABP levels in this patient category.

In the present pilot study, elevated intraluminal glycerol levels, as well as plasma I-FABP, were detected in infants developing NEC as well as in those who had no abdominal symptoms. Therefore, we were not able to differentiate patients with NEC from control patients with other diseases. Many of these controls, however, were severely ill, with complications following extreme prematurity. Primarily, respiratory- and ventilation-associated problems dominated, resulting in long periods with low blood oxygen saturation levels. This relative hypoxic state may result in a compromised oxygenation of the intestines. In particular, the sensitive mucosal cell-layer might be affected, which could result in a hypoxia-induced mucosal cell membrane decay and release of glycerol and I-FABP into the intestinal lumen. Other complications and diseases as sepsis, infections, persistent ductus arteriosus, and anemia were also present in the control group, which aggravates the intestinal distress.

A potential methodological problem of microdialysis is that it only measures a relative concentration of the metabolites in the compartment of interest. This fact makes it difficult to compare the absolute values of two different measurements performed at different time intervals. To overcome the problem of relative concentrations, ratios such as the lactate/pyruvate ratio are often used. The lactate/pyruvate ratio is considered to be independent of changes in relative recovery, making it a useful quantitative measure [18]. In this study however, intraluminal levels of lactate and pyruvate were too low to be measured, regardless of NEC or not. The lactate/pyruvate ratio, therefore, could not serve as an indicator of hypoxic damage in the intestines in this setup. The concentrations of glycerol, on the other hand, were much higher but varied considerably during the course of the 4-week observation period. A higher relative recovery could be achieved by using microdialysis catheters with longer membranes or by using a lower perfusion flow rate. This theoretically results in higher concentrations of lactate and pyruvate, enabling calculation of the lactate/pyruvate ratio. Initially, we tried to use a 30 mm membrane, but it was not possible due to the anatomical limitations of the extremely preterm infants. A lower perfusate flow rate would also increase the recovery, but this was not practically possible in the set up.

In conclusion, this preliminary study has shown that rectal intraluminal microdialysis is safe and could provide a valuable non-invasive aid to detect hypoxia-induced intestinal damage or ischemic stress in extremely preterm infants. However, it was not possible to predict or differentiate NEC from other diagnoses, by detecting elevated levels of glycerol or I-FABP.

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