Novel diagnostic markers for necrotising enterocolitis in the premature infant

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Ethical review	Approved WMO
Status	Recruitment stopped
Health condition type	Gastrointestinal inflammatory conditions
Study type	Observational non invasive

Summary

ID

NL-OMON34937

Source
ToetsingOnline

Brief title
NoNEC

Condition

- Gastrointestinal inflammatory conditions

Synonym
necrotising enterocolitis

Research involving
Human

Sponsors and support

Primary sponsor: Universitair Medisch Centrum Groningen
Source(s) of monetary or material Support: De Cock subsidie

Intervention

Keyword: diagnosis, gut wall integrity, near infrared spectroscopy, necrotising enterocolitis complications

Outcome measures

Primary outcome

urine I-FABP/Claudin to Creatinine ratio, tissue oxygenation as measured via

NIRS

Secondary outcome

inflammatory/metabolic markers such as WBC, CRP, TNFa, IL6 and IL10 and

lactate/base excess as measured in blood and urine, location/quantity of FABP*s

in the resected bowel and faecal cultures.

Study description

Background summary

Necrotising enterocolitis is the most prevalent acute gastro-enterological disease in the Neonatal Intensive Care Unit (NICU). The disease often progresses rapidly, with potentially life-threatening complications, e.g. bowel perforation necessitating laparotomy. Morbidity and mortality are therefore high, in some series up to 40%. However, diagnosis is often difficult as signs are often non-specific, and to date specific markers for the disease or its complications are not available. Early diagnosis allows for early treatment, which may improve outcome in patients with NEC.
Promising markers for the diagnosis of NEC are Fatty Acid Binding Proteins (FABPs). FABPs are proteins which are important in the fat-metabolism of the enterocyte. When enterocytes are damaged, FABPs are released in the circulation and subsequently secreted by the kidney. This also holds true for parts of the tight junctions, the bridges between enterocytes. When these are damaged, Claudin-3 is released. Claudin-3 can also be assessed in urine. Preliminary data suggest that both urine FABP and Claudin levels (expressed as ratios to urine Creatinine) are early indicators for loss of gut integrity and therefore possible early indicators for NEC and its complications. Although I-FABP and Claudin have been suggested as early markers for NEC, little is known about the location and quantification of FABP*s in the bowel wall during the development

of (complications of) NEC.

As ischemia and reperfusion are supposedly involved in the pathophysiological mechanisms underlying NEC, early identification of ischemia of the gut can be of vital importance.This is possible using near-infrared spectroscopy (NIRS). NIRS is a continuous, non-invasive portable technique allowing for the determination of regional tissue oxygenation and thus the presence of ischemia. NIRS is increasingly being used to monitor cerebral oxygenation, but can also be used to monitor oxygenation of abdominal tissues. As the cerebral oxygenation of the brain remains relatively constant, the ratio between local oxygenation and cerebral oxygenation has been shown to provides an adequate estimate of local tissue oxygenation.

It is known that neonates with NEC have a disturbed intestinal flora. As yet, it is unknown whether this is cause or effect of the disease. Although regular culture is able to identify most pathogens, there are pathogens which can not be cultured using the routine techniques. Recently a new technique has been developed to investigate the presence of bacteria on the DNA/RNA level using the so-called denaturing gradient gel electrophoresis (DGGE) GGE-16SrRNA-technique. This offers the possibility to investigate pathogens which can not be identified using routine culturing techniques.

Finally, cytokines may allow for early diagnosis of NEC. Increased serum levels of cytokines are found in early phases of inflammatory conditions. As yet, many assays for cytokines can only be performed on serum samples. Recently, new techniques have become available which makes it possible to analyse urine cytokine profiles. When this method would also be possible in neonates, obtaining bloodsamples for these assays will not be necessary anymore.

Study objective

The overall objective of this study is an improvement in (early) diagnosis of NEC and its life-threatening complications, thus to be able to detect children who are *at risk* for NEC and its complications as early as possible.

To this end we will as a first objective assess urine FABP/Claudin to Creatinine ratio as early markers for NEC and its possible complications and compare them with regional tissue oxygenation as measured by NIRS. This should give insight in the relation between tissue oxygenation and enterocyte damage. It also should provide insight in the use of NIRS and FABP/Claudin levels as diagnostic markers for (complicated) NEC and might offer new modalities to guide therapy based on these results.

Second objective is to correlate the FABP/Claudin levels and NIRS data with the other serum markers of the inflammatory and metabolic response such as serum IL1, IL6, IL10, TNFa and lactate and base excess.

Third objective is - when surgery has become necessary - to analyse resectional specimens for the exact location and quantification of FABPs.

Fourth objective is to validate the Biosource* Multiplex Assay (Invitrogen), a novel assay to test multiple cytokines and chemokines in small volumes of urine, by comparing urine results with results obtained in serum.

Fifth objective is to gain insight in the inflammatory and metabolic process, including gut wall integrity and cytokine profiles during the development of NEC and its complications.

Sixth objective is is to assess faecal bacterial flora using both routine cultures and the DGGE-16SrRNA-technique.

Study design

Prospective cohort study

When there is a suspicion of NEC, urine samples and blood will be collected every six to eight hours and abdominal X-rays will be performed upon the first suspicion of NEC until confirmation of the diagnosis by either clinical or surgical means. This is, except for the urine samples, according to routine clinical practice. The diagnosis NEC will be established using the Bells criteria, with the presence of NEC defined as signs of pneumatosis intestinalis on the abdominal X-ray as assessed by an independent pediatric radiologist.(see appendix 1)
For the serum analysis 100µl of blood will suffice. This can be obtained during the daily routine bloodtests without any further burden for the child. Urine (at least 3 ml) is collected via a small cotton wool placed into the diaper for at least one hour. Urine is subsequently removed from the cotton wool by compressing the cotton wool in a syringe.
At the same time as the interventions mentioned above, regional tissue oxygenation of the gut and the brain is evaluated using the NIRS system.
Daily 30 grams of faeces will be collected via the diaper for culture.
Specimens will be stored at -80°C and subsequently analysed in the MUMC.

After the confirmation of NEC, children will be examined every eight hours using the parameters mentioned above until the time of first resumption of enteral feeding or surgery, whichever comes first. When surgery will become necessary, patients will also be assessed every eight hours postoperatively, until the first enteral feeding is resumed.
After enteral feeding has been started, blood/urine and NIRS data will be collected daily until full enteral feeding is resumed. This holds true for both the surgical and non-surgical NEC patients. In patients in whom the diagnosis NEC can be discarded, urine/blood/NIRS data will be obtained daily until resumption of full enteral feeding.

Surgery

When surgery becomes necessary and bowel is resected, small parts of the resected bowel and adjacent normal tissue will be collected. One part is snap frozen, the other part is stored in Formalin. We will use routine Hematoxin-Eosin staining for histologic evaluation and use immunohistochemistry for the determination of the localisation of the FABPs. These proteins will also be assessed on the mRNA and/or protein level. We will also perform immunohistochemistry for carbohydrases to examine the gut maturity. All resectional specimens will be analysed in the Maastricht University Medical Center.

Study burden and risks

Urine (at least 3 ml) will be collected via tissues placed in the diaper. Patient will therefore not undergo extra procedures. Regarding the bloodtests: 100µl will be enough, which can be obtained during the daily routine bloodtests without any further burden for the child. NIRS measurements are non-invasive and do not interfere with routine care.
Data from this study can not be obtained in another population, as (premature) neonates are the only children who develop NEC. The results from this study might offer new and non-invasive tools for the early diagnosis of NEC, or complications of NEC, thereby improving allowing for early treatment and improving outcome.

Contacts

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Trial sites

Listed location countries

Netherlands

Eligibility criteria

Age
Children (2-11 years)

Inclusion criteria

Neonates with suspected necrotising enterocolitis

Exclusion criteria

Abdominal wall defects, hyperbilirubinaemia necessitating intensive phototherapy

Study design

Design

Study type:	Observational non invasive
Intervention model:	Other
Allocation:	Non-randomized controlled trial
Masking:	Open (masking not used)
Control:	Active
Primary purpose:	Diagnostic

Recruitment

NL

Recruitment status:	Recruitment stopped
Start date (anticipated):	01-10-2010
Enrollment:	40
Type:	Actual

Ethics review

Approved WMO Date:	09-04-2010
Application type:	First submission
Review commission:	METC Universitair Medisch Centrum Groningen (Groningen)
Approved WMO Date:	12-07-2012
Application type:	Amendment
Review commission:	METC Universitair Medisch Centrum Groningen (Groningen)

Study registrations

Followed up by the following (possibly more current) registration

No registrations found.

Other (possibly less up-to-date) registrations in this register

No registrations found.

In other registers

Register	ID
CCMO	NL30132.042.10