Plasma citrulline during the first 48 h after onset of necrotizing enterocolitis in preterm infants

Feenstra, Froukje A.; Kuik, Sara J.; Derikx, Joep P. M.; Heiner-Fokkema, M. Rebecca; Kooi, Elisabeth M. W.; Bos, Arend F.; Hulscher, Jan B. F.

Published in:
Journal of Pediatric Surgery

DOI:
10.1016/j.jpedsurg.2020.11.020

Publication date:
2021

Journal of Pediatric Surgery 56 (2021) 476–482

Contents lists available at ScienceDirect

Journal of Pediatric Surgery

journal homepage: www.elsevier.com/locate/jpedsurg.org

Plasma citrulline during the first 48 h after onset of necrotizing enterocolitis in preterm infants

Froukje A. Feenstra[a,*], Sara J. Kuik[b], Joep P.M. Derikx[c], M. Rebecca Heiner-Fokkema[d], Elisabeth M.W. Kooi[b], Arend F. Bos[b], Jan B.F. Hulscher[a]

[a] Department of Surgery, Division of Pediatric Surgery, University of Groningen, University Medical Center Groningen, Hanzeplein 1, 9713 RB Groningen, the Netherlands
[b] Department of Neonatology, Beatrix Children's Hospital, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands
[c] Department of Surgery, Division of Pediatric Surgery, Emma Children's Hospital Academic Medical Centre and VU Medical Centre, Amsterdam, the Netherlands
[d] Laboratory of Metabolic Diseases, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands

ARTICLE INFO

Article history:
Received 11 February 2020
Revised 29 October 2020
Accepted 16 November 2020

Keywords:
Necrotizing enterocolitis
Citrulline
Full enteral feeding
Preterm infants
Survival
Treatment

ABSTRACT

Background: Levels of plasma citrulline (citrulline-P), a biomarker for enterocyte function, might be useful for the monitoring the course of necrotizing enterocolitis (NEC). Our aim was to evaluate whether citrulline-P levels during the first 48 h (h) after NEC onset were associated with need for surgery, survival, and intestinal recovery.

Methods: In preterm infants with NEC (Bell's stage ≥2) we measured citrulline-P levels during the first 48 h after NEC onset. Categorizing the measurements into 0–8 h, 8–16 h, 16–24 h, 24–36 h, and 36–48 h, we determined the course of citrulline-P using linear regression analyses. Next, we analyzed whether citrulline-P levels measured at 0–24 h and 24–48 h differed between conservative and surgical treatment, survivors and nonsurvivors, and equal/below and above total group's median time to full enteral feeding (FEFt).

Results: We included 48 infants, median gestational age 28.3 [IQR:26.0–31.4] weeks, birth weight 1200 [IQR:905–1524] grams. Citrulline-P levels decreased the first 48 h (B per time interval: -1.40 μmol, 95% CI, −2.73 to −0.07, $p = 0.04$). Citrulline-P was not associated with treatment, nor with survival. Citrulline-P at 0–24 h, but not 24–48 h, was higher in infants with FEFt ≤20 days than in infants with FEFt >20 days (20.7 [IQR:19.9–25.3] µmol/L ($n = 13$) vs. 11.1 [IQR:8.4–24.0] µmol/L ($n = 11$), $p = 0.049$), with a citrulline-P cut-off value of 12.3 μmol/L.

Conclusion: Citrulline-P levels decreased the first 48 h after NEC onset, suggesting on-going intestinal injury. In survivors, measuring citrulline-P in the first 24 h after NEC onset may provide an indication for intestinal recovery rate.

1. Introduction

One of the cornerstones of the treatment for necrotizing enterocolitis is a nil per mouth (NPO) regimen [1,2]. However, withdrawing enteral feeding results in villous atrophy and thus reduced gut function [3]. In addition, prolonged parenterally administered nutrition is associated with complications such as sepsis and cholestasis [4,5], and late re-feeding is associated with a prolonged hospital stay [6].

Conversely, premature reintroduction of enteral feeding in infants with NEC might induce disease progression [4]. Establishing a suitable biomarker to indicate which NEC infants are ready for the reintroduction of enteral feeding is therefore very relevant.

One of these potential biomarkers is plasma citrulline (citrulline-P), which is a marker for enterocyte function [7-13] . Citrulline is a non-protein amino acid and a metabolic intermediate of the urea cycle. It is synthesized from glutamine and glutamate in both liver and small intestine [14,15]. As citrulline produced by the liver is catabolized in situ, all citrulline circulating in the bloodstream can be considered as exclusively derived from the small intestine. Despite this, we should consider that other factors could possibly affect plasma citrulline levels in unstable infants, such as renal failure [12].

Abbreviations: citrulline-P, plasma citrulline; EDTA, ethylene diamine tetra-acetic acid; FEF, full enteral feeding; FEF, time to full enteral feeding; h, hours; IQR, inter quartile range; ml, millilitre; μmol/L, micromoles per litre; NEC, necrotizing enterocolitis; NPO, nil per mouth; NTR, Netherlands Trial Register; p, p-value; ROC, receiver operator characteristics; TPN, total parenteral nutrition.

* Corresponding author.
E-mail address: f.a.feenstra02@umcg.nl (F.A. Feenstra).

https://doi.org/10.1016/j.jpedsurg.2020.11.020

Previous studies have reported lower citrulline-P levels in preterm infants with NEC than in infants without NEC [8,11]. Limited data, however, are available on the course of citrulline in infants with NEC in the first days after onset of disease [16]. Serial measurements could be a useful tool to monitor disease progression and intestinal recovery from NEC. Therefore, the aim of this study was to evaluate the course of citrulline-P during the first 48 h after NEC onset. Furthermore, we determined whether citrulline-P levels in preterm infants with NEC during the first 48 h after NEC onset were associated with the type of treatment and survival, as indicator for disease progression. We also assessed whether citrulline-P levels were associated with the time to full enteral feeding (FEFt), as indirect measure for intestinal recovery. We hypothesized that infants with higher citrulline-P levels, or who displayed an increasing trend of citrulline-P levels, would reach full enteral feeding faster than infants with lower citrulline-P levels. Additionally, we hypothesized that infants who would require surgical treatment or even die because of NEC, would demonstrate lower citrulline-P levels than survivors or infants necessitating only conservative treatment.

2. Material and methods

2.1. Study design

In this explorative observational study we included preterm infants with proven NEC (Bell's stage ≥ 2). Patients were recruited from two prospective clinical observational trials previously conducted in our hospital. These studies aimed to predict the onset and course of NEC in high risk infants. First, the NoNEC trial (NTR3239) was performed between 2010 and 2012 [17]. Second, the NaNEC trial (NTR4816) was performed between 2015 and 2017 [18]. In both trials, the onset of NEC was defined as the time of the first abdominal X-ray made upon clinical suspicion of NEC including X-rays performed in referring hospitals. Proven NEC was defined as NEC Bell's stage 2 or 3, supported by plain abdominal X-ray showing pneumatosis intestinalis and/or portal venous gas or determined during surgery [2]. After NEC onset, all infants were treated according to the hospital protocol: a NPO regimen, gastric decompression, pain management, respiratory and/or cardiovascular support if necessary and broad-spectrum antibiotic therapy. While on a NPO regimen, patients received total parenteral nutrition (TPN) including glucose, electrolytes and Primène® 10% amino acid supplements (2,1 gram/100 ml TPN), which contained L-arginine (0,84 gram/100 ml Primène® 10% solution). Citrulline was not supplemented. Reintroduction of enteral feeding after the NPO regimen was based on the hospital protocol, starting with 20 mL/kg on day 6 after NEC, on condition that abdominal distension and tenderness had disappeared, bloody stools were absent, and pneumatosis intestinalis had disappeared for at least 24 h on abdominal radiographs. Exclusion criteria were large chromosomal abnormalities, major intraventricular haemorrhage (≥ grade 3), congenital bowel defects, and congenital heart deformities other than patent ductus arteriosus. Written informed consent was obtained from all parents. The Medical Ethics Committee of the University Medical center Groningen approved both studies (NTR3239 and NTR4816).

2.2. Blood sampling and citrulline measurement

When we suspected an infant of NEC, collection of blood samples occurred at regular intervals according to routine clinical practice. The time of blood collection after NEC onset was recorded. During every routine blood analysis an additional 100 microlitres (μl) of blood was obtained in an ethylene diamine tetra-acetic acid (EDTA) tube (Greiner bio-one) of 2.5 mL. The blood samples were centrifuged for 10 min at 1200 rpm at room temperature and plasma was isolated and frozen at −20 °C until the day of analysis. The measurements were performed by a laboratory technician blinded for the patient characteristics. Citrulline-P levels were analyzed by ultra-high performance liquid chromatography triple quadrupole mass spectrometry analysis (UHPLC-MS/MS), according to our local laboratory protocol. Sample preparation with AccQ•Tag reagents was conducted according to the manufacturer's protocol (Waters Corporation, Milford, MA, USA). Data were analyzed using Analyst 1.6.2 (Sciex).

2.3. Patient and NEC-specific characteristics

We collected data on gestational age, birth weight and gender from the infants' medical charts. Furthermore, we identified the postnatal day of NEC onset, Bell's stage, treatment strategy, the occurrence of NEC induced stenosis, recurrence of disease, days between NEC onset and surgical treatment, and in case of the infants' demise, the postnatal age of death, and days between NEC onset and death.

2.4. Full enteral feeding

Full enteral feeding (FEF) was defined as an intake of 150 mL/kg/day of enteral formula or fortified breast milk tolerated for at least 24 h. Infants were categorized into two groups, i.e. one group equal/below and the other above median FEFt of all included infants.

2.5. Data and statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (IBM SPSS Statistics 23, IBM Corp., Armonk, New York, USA). Patient characteristics and citrulline-P levels were reported as median [interquartile range (IQR)]. To determine the course of citrulline-P during the first 48 h after NEC onset, the citrulline-P measurements were categorized into five time-intervals: 0–8 h, 8–16 h, 16–24 h, 24–36 h and 36–48 h after NEC onset. Next, we used linear regression analyses between citrulline-P levels and the different time-intervals. To determine whether citrulline-P levels were associated with the type of treatment (i.e. conservative vs. surgical), survival (survivors vs. nonsurvivors), and FEFt (≤ or > than total groups median), we categorized the citrulline-P measurements into two periods: 0–24 h and 24–48 h after NEC onset. Due to the relatively small sample size we were unable to perform the analyses for the different categories for all five time-intervals. In case of multiple samples of individual infants available within one of these two periods, we calculated means. Next, we used the Mann Whitney U test to determine whether citrulline-P levels differed between groups. In case of a p-value < 0.1, we determined cut-off points by generating receiver operator characteristics (ROC) curves. We chose not to correct for multiple testing because of the explorative nature of the study. A p-value <0.05 was considered statistically significant.

3. Results

3.1. Patients

We identified 120 preterm infants with suspected NEC who were eligible for inclusion, of whom 48 infants were diagnosed with proven NEC (Bell's stage ≥ 2) (Fig. 1). Our final study population consisted of these 48 preterm infants with gestational age of median 28.3 [IQR 26.0–31.4] weeks and birth weight 1200 [IQR 905–1524] grams. NEC developed at median postnatal day 9 [IQR 8–21]. Infants who survived had a higher gestational age ($p < 0.01$)

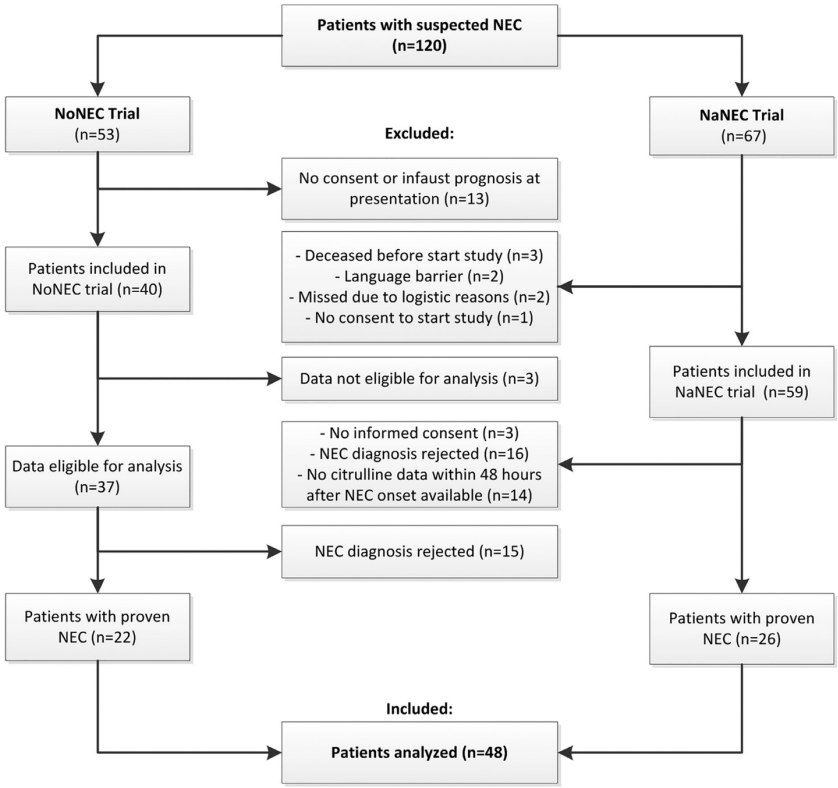

Fig. 1. Flow diagram of the study population.
Abbreviations: NEC - Necrotizing enterocolitis.

and higher birth weight ($p = 0.02$) than nonsurvivors. Fifteen infants (31%) needed surgical intervention on median day 2 [IQR 1–5]. Eleven infants (23%) did not survive. In seventeen infants we were able to collect more than one blood sample during the first 48 h after NEC onset (38%). From the remaining 28 infants (62%) we collected only one blood sample in the first 48 h after NEC onset. We provide a complete overview of the patient characteristics in Table 1.

3.2. The course of citrulline-P during the first 48 h after NEC onset

The individual citrulline-P values measured during the five time-intervals the first 48 h after NEC onset are presented in Fig. 2. Median citrulline-P levels were 24 [IQR: 11.1–28.5] μmol/L 0–8 h ($n = 14$), 24.0 [IQR: 16.6–27.5] μmol/L 8–16 h ($n = 17$), 17.4 [IQR: 11.0–20.3] μmol/L 16–24 h ($n = 19$), 18.0 [10.0–24.3] μmol/L hours ($n = 14$), and 14.0 [IQR: 12.0–21.5] μmol/L 36–48 h after NEC onset ($n = 18$), respectively. Citrulline-P levels decreased during the first 48 h after NEC onset (B per time-interval: −1.40 μmol, 95% CI, −2.73 to −0.07, $p = 0.04$).

3.3. The association between citrulline-P and the type of treatment

We did not find statistically significant differences in median citrulline-P levels within the first 24 h between conservatively and surgically treated infants (19.3 [IQR: 10.9–24.5] µmol/L ($n = 22$) vs. 20.5 [IQR: 15.3–23.8] µmol/L ($n = 12$), $p = 0.58$), nor within 24–48 h after NEC onset (18.2 [IQR: 12.3–27.0] µmol/L ($n = 14$) vs 12.4 [IQR: 12.0–17.3] µmol/L ($n = 9$), $p = 0.16$) (Fig. 3).

3.4. The association between citrulline-P and survival

The median time between NEC onset and death was 68 [IQR: 33–155] hours, whereof one infant died within the first 24 h and three infants between 24 and 48 h after NEC. Median citrulline-P levels did not differ between survivors and nonsurvivors 0–24 h after NEC onset (19.3 [IQR: 11.2–24.2] µmol/L ($n = 24$) vs. 20.0 [IQR: 12.1–21.8] µmol/L ($n = 10$), $p = 0.82$), and also not 24–48 h after NEC onset (17.5 [IQR: 12.2–23.8] µmol/L ($n = 20$) vs. 12.0 (range: 6.5 – 15.5) µmol/L ($n = 3$), $p = 0.12$) (Fig. 4).

Table 1
Clinical characteristics.

	Total group: $N = 48$	Survivors $n = 37$	Nonsurvivors $n = 11$
Baseline characteristics			
Gestational age (weeks)	28+3 [26.0–31.4]	29.0 [27.1–32.0] *	25.7 [25.3–27.0] *
Birth weight (grams)	1200 [905 - 1524]	1250 [1023 - 1670] *	900 [800 - 1000] *
Male	36 (75%)	27(73%)	9 (82%)
Characteristics regarding NEC			
NEC onset (postnatal day)	9 [8 - 21]	10 [8 - 25]	9 [8 - 16]
Bell's classification			
Stage 2A / 2B	20/7 (42% / 15%)	20/7 (54% / 19%)	0/0 (0% / 0%)
Stage 3A / 3B	5/16 (10% / 33%)	2/8 (5% / 22%)	3/8 (27% / 73%)
Surgical intervention	15 (31%)	9 (24%)#	6 (55%)#
Days between NEC onset and surgery	2 [1–5]	2 [1–8]	3 [1–14]
Complications			
Post NEC stricture	8 (22%)	8 (22%)	-
Recurrent NEC	1 (3%%)	1(3%)	-
Deceased	11 (23%)	–	11 (100%)
Deceased (postnatal day)	13 [11–22]	–	13 [11–22]
Time between NEC onset and death (hours)	68 [33–155]	–	68 [33–155]

Data are expressed as numbers (percentages) or median [interquartile range] unless otherwise specified.
Comparisons were made between survivors and nonsurvivors. * $p < 0.05$.
# $p < 0.10$.
Abbreviations: NEC - Necrotizing enterocolitis.

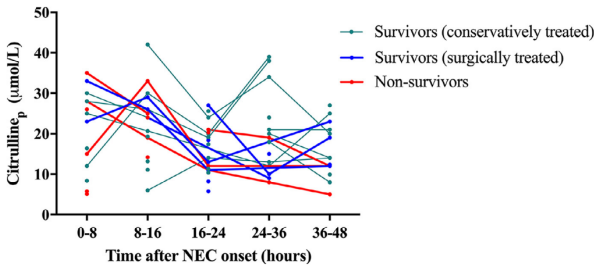

Fig. 2. The course of citrulline-P during the first 48 h after NEC onset. Each measured citrulline-P level is marked by a dot and the individual trend lines are represented by the coloured lines, reflecting the type of treatment and survival. Abbreviations: citrulline-P - plasma citrulline, μmol/L - micromoles per litre.

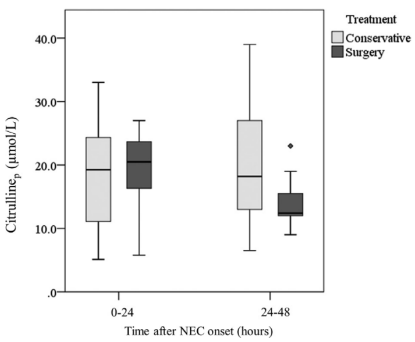

Fig. 3. Citrulline-P 0–48 h in conservatively and surgically treated infants.
The boxes represent the individual citrulline-P values of conservatively and surgically treated infants between the 25th and 75th percentiles (interquartile range); the whiskers represent the range of the values with the exception of outliers (◆) if present. Abbreviations: NEC - Necrotizing enterocolitis, citrulline-P - plasma citrulline, μmol/L - micromoles per litre.

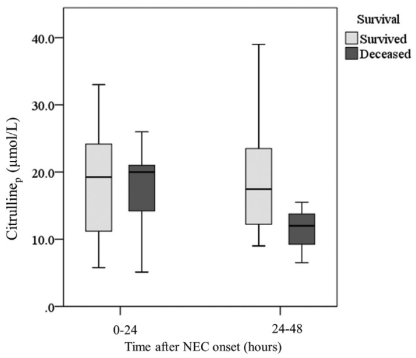

Fig. 4. Citrulline-P 0–48 h in survivors and nonsurvivors.
The boxes represent the individual citrulline-P values of survivors and nonsurvivors between the 25th and 75th percentiles (interquartile range); the whiskers represent the range of the values with the exception of outliers (◆) if present.Abbreviations: NEC - Necrotizing enterocolitis, citrulline-P - plasma citrulline, μmol/L - micromoles per litre.

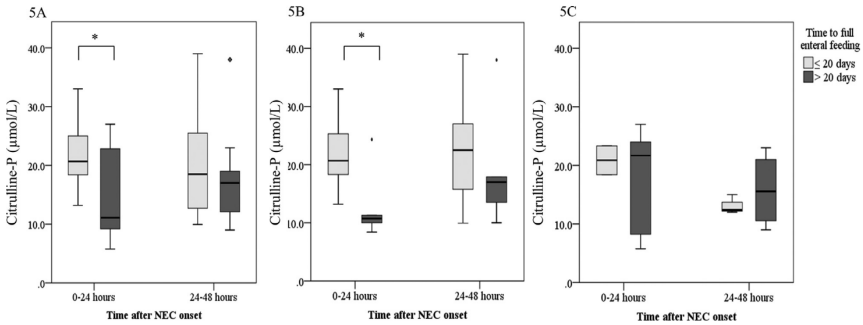

Fig. 5. Citrulline-P 0–48 h after NEC onset and time to full enteral feeding.
The boxes represent the individual citrulline-P values of the entire study population (5A), conservatively treated infants (5B), and surgically treated infants (5C) between the 25th and 75th percentiles (interquartile range) between infants who reached full enteral feeding ≤ 20 days and > 20 days; the whiskers represent the range of the values with the exception of outliers (◆) if present. Significant differences are marked with an asterisks: * $p < 0.05$. Abbreviations: citrulline-P - plasma citrulline, µmol/L – micromoles per litre.

3.5. The association between citrulline-P and time to full enteral feeding

In survivors, median FEFt was 20 [IQR: 17–27] days. We found higher median citrulline-P levels during the first 24 h after NEC onset in infants with FEFt of 20 days or less than in those with FEFt more than 20 days (20.7 [IQR: 17.9–25.3] µmol/L ($n = 13$) vs. 11.1 [IQR: 8.4–24.0) µmol/L ($n = 11$), $p = 0.049$). Particularly in the conservatively treated group, citrulline-P levels during the first 24 h after NEC onset were higher in infants with FEFt of 20 days or less than in those with FEFt of more than 20 days (20.7 [IQR: 17.4–25.6] µmol/L ($n = 11$) vs. 10.8 [IQR: 9.6–14.6] µmol/L ($n = 6$), $p < 0.01$) (Fig. 5). We did not find an association between citrulline-P levels and FEFt in the subgroup of surgically treated infants. Median citrulline-P levels measured between 24 and 48 h after NEC onset did not differ between in infants with FEFt of 20 days or less than in those with FEFt more than 20 days.

3.6. Citrulline-P cut-off points for reaching full enteral feeding in 20 days or less

After generating a ROC-curve with the outcome ≤ or > *a* FEFt of 20 days, we found an area under the curve of 0.74 (95% CI 0.51–0.96, $p = 0.049$) in the entire group of survivors ($n = 37$), regarding citrulline-P levels measured within the first 24 h after NEC onset (Supplemental Fig. 6). In the conservatively treated subgroup we found an area under the curve of 0.89 (95% CI 0.69–1.00, $p < 0.01$). Selecting a cut-off value for citrulline-P of 12.3 μmol/L, we found a specificity of 100%, a sensitivity of 64%, with a positive predictive value of 100% and a negative predictive value of 76% to reach FEF ≤ 20 days in the entire group, and a specificity of 100%, a sensitivity of 83%, with a positive predictive value of 100%, and a negative predictive value of 92% in the conservatively treated group.

4. Discussion

In this study we demonstrated, in preterm infants with NEC, that citrulline-P levels decrease during the first 48 h after NEC onset. We also demonstrated that citrulline-P levels were higher during the first 24 h, but not 24–48 h, after NEC onset in infants with a faster intestinal recovery than in infants with a longer intestinal recovery time, particularly in the subgroup of conservatively treated infants. Selecting a cut-off value for citrulline-P in this subgroup of 12.3 μmol/L, we found a specificity of 100%, a sensitivity of 83%, a positive predictive value of 100%, and a negative predictive value of 92% to reach FEF ≤ 20 days. Our findings suggest that citrulline-P measurements during the first 24 h after NEC onset may provide an indication for intestinal recovery rate. Finally, we found that citrulline-P levels were not different between conservatively and surgically treated infants, nor between survivors and nonsurvivors.

Our first aim was to determine the course of citrulline-P levels during the first 48 h after NEC onset, measured during multiple time-intervals. As citrulline-P is a marker for functional intestinal enterocyte mass, a decrease of citrulline-P levels suggests that the functional enterocyte mass further reduces during the first 48 h after NEC onset and that the initiation of intestinal recovery seem to occur after this period. Recently it has been reported that citrulline levels were lower in infants who developed NEC than in infants without NEC, but that these levels were similar to baseline on days 4 and 7 after NEC onset [8,19]. Whether the initiation of intestinal recovery occurs between days 2 to 4 after NEC onset, however, should be further investigated.

We also aimed to determine whether citrulline-P levels during the first 48 h after NEC onset were different between infants who were treated conservatively and surgically, and between survivors and non-survivors. As citrulline-P is a marker for functional intestinal enterocyte mass, our finding suggests that the degree of enterocyte dysfunction is relatively similar in conservatively and surgically treated infants, as well as in survivors and nonsurvivors, although we hypothesized to find lower citrulline-P levels in surgically treated infants and nonsurvivors. We do have to point out that we were able to collect blood samples of only three out of the eleven nonsurviving infants 24–48 h after onset of NEC, hampering interpretation of our results.

We observed that 76% of all infants with a citrulline-P of 12.3 μmol/L or higher during the first 24 h after NEC onset recovered relatively fast, as they were able to be fully enterally fed within 20 days. Additionally, we showed that 92% of the conservatively treated infants with a citrulline-P of 12.3 μmol/L or higher during the first 24 h after NEC onset recovered relatively fast. In line with our hypothesis, this suggests that within the group of

surviving infants, a larger maintained enterocyte function might result in a shorter FEFt, hence possibly a faster intestinal recovery. It might be that infants who recover relatively fast have less intestinal inflammation and/or necrosis than infants who need a longer time to recover, resulting in a faster recovery of the enterocyte function. Our finding is supported by a recently reported association between lower citrulline levels measured at NEC onset and a more prolonged course of disease, also requiring parenteral nutrition for a longer period of time [8]. Additionally, it has been reported that citrulline levels can reliably distinguish infants with short bowel syndrome who could be successfully weaned off parenteral nutrition [7], suggesting that it could be used to identify infants with a recovering intestinal function. Even though these two studies do not directly mention the association between citrulline levels and the intestinal recovery rate in infants with NEC, the rationale behind their findings is comparable to our results. In contrast to our findings, a previous study focusing on citrulline-P levels in infants with NEC has reported that citrulline-P on the first day after NEC onset did not correlate with FEFt [11]. Compared with our study design, this study assessed time to FEF in days [11], while we categorized the infants into $\leq$ vs. $>$ total group's median FEFt, which may be a more robust assessment.

We did not find an association between citrulline-P levels and FEFt in surgically treated infants. This suggests that intestinal recovery, including recovery of enterocyte function, is different after surgical intervention than during conservative treatment, possibly due to the removal of affected tissue. Similarly, in the NaNEC-trial we previously found that intestinal oxygen saturation measurements after the first enteral re-feed after NEC was associated with FEFt, particularly in the conservatively treated group, while not in the surgically treated group [18].

The strengths of our study are the prospective design and the multiple measurements within an 48 hour timeframe, while, to the best of our knowledge, previously published studies on citrulline levels in infants with NEC were solely based on single or twice weekly measurements [8,9,11,16,20]. Recently, Fragkos et al. stated that serial citrulline measurements seem to reflect patterns of mucosal barrier injury, for example in patients with small bowel syndrome. Serial citrulline measures may assist towards the decision of the absorptive capacity of the bowel and hence the need for parenteral nutrition [19]. The insight on the possible predictive value of citrulline levels to help monitor intestinal integrity and predict FEFt in infants with NEC, will be an incentive to perform more research on this topic. We acknowledge several limitations. A limitation of our study is the relatively small sample size as well as missing data, because it was not possible to collect blood samples in all time-intervals for each patient. Blood samples were only collected when the clinical condition of the patient required blood sampling, and for some only after transfer to our NICU. As a result of the small sample size, we were unable to determine whether the course of citrulline-P during the first 48 h after NEC onset, categorized in five time-intervals, differed between type of treatment, survival, and FEFt. The small sample size may have introduced type-II error, leading to insufficient power to detect differences between groups. Furthermore, infants who were more severely ill potentially had more blood samples, inducing bias. The multiple comparisons, given the explorative nature of our study, may have led to change finding. In addition, we were unable to correct for differences in GA and BW due to the small sample size. Our two trials, however, are among the larger cohorts specifically focusing on NEC [17,18]. We used FEFt as a surrogate for intestinal recovery. Possibly, FEFt is influenced by subjective interpretation by caretakers in charge of the feeding regimen. FEFt, however, is generally used for this purpose in absence of a gold standard [11,18]. Also, we have to keep in mind that other factors in unstable infants with NEC might affect plasma citrulline levels. As plasma citrulline is catabolized in situ by the liver, an impaired liver function could hypothetically lead to changes in circulating citrulline. Fluid balance and blood transfusion might also affect plasma citrulline levels, due to haemoconcentration or dilution. And finally, renal failure could possibly affect plasma citrulline levels [12]. However, the literature is not conclusive on this topic. Adding a control group to the studies would have given more insight on plasma citrulline changes in neonates without NEC, but who for example did follow a NPO regimen and/or received TPN.

Future prospective studies performed in larger patient cohorts and over a longer period of time after NEC onset will be necessary to confirm the validity of our findings and correct for other potentially influencing factors. As arginine, proline and glutamine are amino acids related to citrulline, it would be interesting to also gather data on these amino acids in future studies. Furthermore, it would be interesting to evaluate whether the course of citrulline-P during the first 48 h after NEC onset differs significantly between conservatively treated infants and surgically treated infants or between survivors and nonsurvivors when analyzed in a larger cohort. Future studies will be necessary to provide more insight in the clinical relevance of citrulline-P levels in infants with NEC to predict disease progression, but also its use for individualizing enteral feeding regimens after NEC. Serial citrulline measures might prove to be an additional tool, guiding the clinician in whether patients are recovering and possibly support treatment strategies on for example timing of reintroduction of enteral feeding.

5. Conclusions

This study demonstrated that citrulline-P levels decrease during the first 48 h after NEC onset, suggesting on-going intestinal injury during these first 48 h. In addition, citrulline-P levels were higher during the first 24 h after NEC onset in infants who showed a faster intestinal recovery than in infants whose intestine needed a longer time to recover, with a citrulline-P cut-off value of 12.3 μmol/L to reach FEF in 20 days or less. Citrulline-P was not associated with survival or need for surgery. Whether monitoring citrulline-P during the first 48 h after NEC onset has potential to determine when intestinal recovery is initiated to aid clinicians in individualizing feeding regimens after NEC warrants further prospective studies in larger cohorts.

Funding sources

These two studies were part of the research program of the postgraduate school for Behavioral and Cognitive Neurosciences, University of Groningen. S.J. Kuik was financially supported by a grant from the Junior Scientific Master Class of the University of Groningen, Groningen, the Netherlands. Additionally, we received grants from the NutsOhra Foundation, University Medical center Groningen 'Doelmatigheidsfonds' (intramural funding) and the Jan Kornelis de Cock Foundation.

Financial disclosure statement

The authors have no financial relationships relevant to this article to disclose.

Clinical trial registry name and registration number

The NoNEC trial was registered in the Netherlands Trial Register with number NTR3239.

The NaNEC trial was registered in the Netherlands Trial Register with number NTR4816.

Declaration of Competing Interest

The authors have no conflict of interest to disclose.

Acknowledgments

We would like to acknowledge the nurses, medical staff of the NICU of Beatrix Children's Hospital in Groningen and the medical students, for helping with data collection and for creating the opportunity to carry out this study. We also greatly acknowledge E.Z. Jonkers, laboratory technician, for performing the citrulline-P analyses.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jpedsurg.2020.11.020.

References

[1] Neu J, Walker WA. Necrotizing enterocolitis. N Engl J Med 2011;364(3):255–64.
[2] Walsh MC, Kliegman RM. Necrotizing enterocolitis: treatment based on staging criteria. Pediatr Clin North Am 1986;33(1):179–201.
[3] Bohnhorst B, Muller S, Dordelmann M, Peter CS, Petersen C, Poets CF. Early feeding after necrotizing enterocolitis in preterm infants. J Pediatr 2003;143(4):484–7.
[4] Puntis JW. Nutritional support in the premature newborn. Postgrad Med J 2006;82(965):192–8.
[5] Veenstra M, Danielson L, Brownie E, Saba M, Natarajan G, Klein M. Enteral nutrition and total parenteral nutrition components in the course of total parenteral nutrition-associated cholestasis in neonatal necrotizing enterocolitis. Surgery 2014;156(3):578–83.
[6] Arbra CA, Oprisan A, Wilson DA, Ryan RM, Lesher AP. Time to reintroduction of feeding in infants with nonsurgical necrotizing enterocolitis. J Pediatr Surg 2018;53(6):1187–91.
[7] Rhoads JM, Plunkett E, Galanko J, Lichtman S, Taylor L, Maynor A, et al. Serum citrulline levels correlate with enteral tolerance and bowel length in infants with short bowel syndrome. J Pediatr 2005;146(4):542–7.
[8] Ioannou HP, Diamanti E, Piretzi K, Drossou-Agakidou V, Augoustides-Savvopoulou P. Plasma citrulline levels in preterm neonates with necrotizing enterocolitis. Early Hum Dev 2012;88(7):563–6.
[9] Becker RM, Wu G, Galanko JA, Chen W, Maynor AR, Bose CL, et al. Reduced serum amino acid concentrations in infants with necrotizing enterocolitis. J Pediatr 2000;137(6):785–93.
[10] Gondolesi G, Ghirardo S, Raymond K, Hoppenhauer L, Surillo D, Rumbo C, et al. The value of plasma citrulline to predict mucosal injury in intestinal allografts. Am J Transplant 2006;6(11):2786–90.
[11] Celik IH, Demirel G, Canpolat FE, Dilmen U. Reduced plasma citrulline levels in low birth weight infants with necrotizing enterocolitis. J Clin Lab Anal 2013;27(4):328–32.
[12] Crenn P, Messing B, Cynober L. Citrulline as a biomarker of intestinal failure due to enterocyte mass reduction. Clin Nutr 2008;27(3):328–39.
[13] Lutgens L, Lambin P. Biomarkers for radiation-induced small bowel epithelial damage: an emerging role for plasma Citrulline. World J Gastroenterol 2007;13(22):3033–42.
[14] Curis E, Nicolis I, Moinard C, Osowska S, Zerrouk N, Benazeth S, et al. Almost all about citrulline in mammals. Amino Acids 2005;29(3):177–205.
[15] Windmueller HG, Spaeth AE. Source and fate of circulating citrulline. Am J Physiol 1981;241(6):E473–80.
[16] Babu S, Prasad M, Miller M, Morrissey M, Bhutada A, Rojas M, et al. Use of serum citrulline concentrations from routine newborn screen as a biomarker for necrotizing enterocolitis. Pediatr Surg Int 2019;35(6):715–22.
[17] Schat TE, Heida FH, Schurink M, van der Laan ME, Hulzebos CV, Bos AF, et al. The relation between splanchnic ischaemia and intestinal damage in necrotising enterocolitis. Arch Dis Child Fetal Neonatal Ed 2016;101(6):F533–9.
[18] Kuik SJ, Kalteren WS, Mebius MJ, Bos AF, Hulscher JBF, Kooi EMW. Predicting intestinal recovery after necrotizing enterocolitis in preterm infants. Pediatr Res 2019.
[19] Fragkos KC, Forbes A. Citrulline as a marker of intestinal function and absorption in clinical settings: a systematic review and meta-analysis. United European Gastroenterol J 2018;6(2):181–91.
[20] Englund A, Rogvi RA, Melgaard L, Greisen G. Citrulline concentration in routinely collected neonatal dried blood spots cannot be used to predict necrotising enterocolitis. Acta Paediatr 2014;103(11):1143–7.