REVIEW

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Calprotectin levels in necrotizing enterocolitis: a systematic review of the literature

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Abstract

Background Fecal calprotectin has been extensively investigated as a screening marker for the detection of necrotizing enterocolitis (NEC). However, there is a complete lack of consensus regarding its efficacy as a diagnostic test.

Objective The purpose of the present systematic review is to evaluate the effectiveness of fecal calprotectin as a screening marker for the detection of NEC.

Materials and methods We conducted a systematic review of studies published in the Medline (1966–2016), Scopus (2004–2016), ClinicalTrials.gov (2008–2016), Cochrane Central Register of Controlled Trials CENTRAL (1999–2016), and Google Scholar (2004–2016) databases, combined with studies found in the reference lists of the included studies. All prospective and retrospective observational cohort studies were included.

Results Thirteen studies that included 601 neonates were identified in the international literature. The presence and severity of NEC was evaluated with the modified Bell's criteria. Ten studies found significantly elevated fecal calprotectin levels among infants with NEC ($p < 0.05$). One study found that this effect was observed irrespective of the stage of the disease. Five studies evaluated the efficacy of fecal calprotectin as a diagnostic test. The reported sensitivity ranged between 76 and 100 %, and the specificity varied from 39 to 96.4 %. However, the proposed cut-off values were not similar.

Conclusion Current evidence suggests that fecal calprotectin is elevated in newborns suffering from NEC. However, its significance as an early screening marker remains unknown. Future studies are needed and should focus on the identification of specific cut-off values.

Keywords Fecal calprotectin · S100A8 · S100A9 · Necrotizing enterocolitis · NEC

Introduction

Necrotizing enterocolitis (NEC) is characterized by variable damage to the intestinal tract that ranges from mild mucosal defects to full-thickness necrosis of the bowel wall. The incidence of NEC ranges from 10 to 15 % and the estimated mortality rate fluctuates between 10 and 30 % in very low birth weight (VLBW) infants [1]. Its exact pathogenetic mechanisms remain, to date, unknown. However, gastrointestinal ischemia, alimentation, and microbiota in combination with immature gastrointestinal functions and host defense mechanisms are believed to play a key role in the progression of NEC [2]. The pattern described by Bell et al. in 1978 was the first systematic description of NEC [3]. Current treatment strategies for the treatment of advanced NEC with intestinal perforation include laparotomy and primary peritoneal drainage [4].

NEC prognosis can be improved by early detection and accurate evaluation of disease severity and activity over time. To date, no specific markers of inflammation of the gastrointestinal tract exist in neonates. Recently, calprotectin has been investigated extensively as a

potential biomarker. However, there is still no consensus regarding its use in current clinical practice [5, 6]. Calprotectin is a 36-kDa calcium- and zinc-binding cytosolic protein and a member of the S100 family of proteins, which are released from neutrophils or monocytes and followed by the pro-inflammatory activation of pattern recognition receptors [7]. It is secreted into the intestinal lumen during the early phases of intestinal mucosal damage and thus could be useful for the early detection of NEC and possibly for tracking the course of the disease. Fetal calprotectin is easily found in stool and is measured by ELISA [8–10].

The purpose of our systematic review is to synthesize the current knowledge in the field and to reach firm conclusions regarding the use of calprotectin as a potential screening marker of NEC in preterm neonates.

Methods

Study design

The present study was designed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [11]. Eligibility criteria were predetermined by the authors. No language or date restrictions were applied during the literature search. All observational studies (both prospective and retrospective) were included in the systematic review. Case reports and review articles were excluded from tabulation and analysis. Two researchers tabulated the selected indices using structured forms. Any discrepancies in the methodology, article retrieval, and statistical analysis were resolved by reaching consensus among all authors.

Literature search and data collection

We used the Medline (1966–2016), Scopus (2004–2016), Popline (1974–2016), ClinicalTrials.gov (2008–2016), and Cochrane Central Register of Controlled Trials CENTRAL (1999–2016) databases in our primary search, along with the reference lists of electronically retrieved full text papers. Our search was restricted to a minimum number of keywords to assess an eligible number that could be hand searched, minimizing the potential loss of articles. All the articles that met or were presumed to meet the inclusion criteria were retrieved in full text. Search strategies and results are shown in Fig. 1.

Our search strategy included the terms calprotectin, enterocolitis, S100A8, S100A9. We specifically searched Pubmed using the MeSH terms "("leukocyte l1 antigen complex"[MeSH Terms] OR ("leukocyte"[All Fields]

AND "l1"[All Fields] AND "antigen"[All Fields] AND "complex"[All Fields]) OR "leukocyte l1 antigen complex"[All Fields] OR "calprotectin"[All Fields]) AND ("enterocolitis"[MeSH Terms] OR "enterocolitis"[All Fields])". The PRISMA flow diagram depicts the method of article retrieval (Fig. 1).

Definitions

The severity of NEC was assessed using the modified Bell's criteria, which stratifies cases into the following categories: suspected (Stage I), mild (Stage IIa), moderate (Stage IIb), severe without bowel perforation (Stage IIIa), and severe with bowel perforation (Stage IIIb). Evaluation is based on the presence of systemic findings (i.e., metabolic or respiratory acidosis, acidemia, neutropenia, etc.), intestinal findings (i.e., abdominal distention, blood stool, signs of peritonitis, abdominal cellulitis, etc.), radiological findings (i.e., pneumatosis intestinalis, ileus, pneumoperitoneum), and according to treatment needs (i.e., antibiotics, intropes, surgery, etc.).

Quality assessment

The methodological quality of the included studies was assessed with the Ottawa-Newcastle scale, which uses a star-based system to evaluate the strength of each paper's study group selection, the comparability of the groups, and the ascertainment of the outcome of interest [12].

Results

Heterogeneity between studies and risk of bias

Our systematic review included 13 studies that involved 601 neonates. In Table 1, we present the methodological characteristics of the included studies. The majority of the included studies used the modified Bells criteria to assess patients for NEC. However, we observed heterogeneity in the NEC population included in these studies.

Most studies used a similar methodology for conducting the calprotectin assays. The majority of the included studies used the commercially available Calprotectin ELISA Kits and conducted the assay according to the manufacturer's instructions [13–21]. The reference value given by all commercially available ELISA is 50 µg/g feces for healthy adults and children aged 4–17 years, regardless of sex [22]. Two studies used the Bühlmann Quantum Blue rapid test [23, 24]. One study did not report the exact methods used for calprotectin measurement [25].

Fig. 1 Search plot diagram
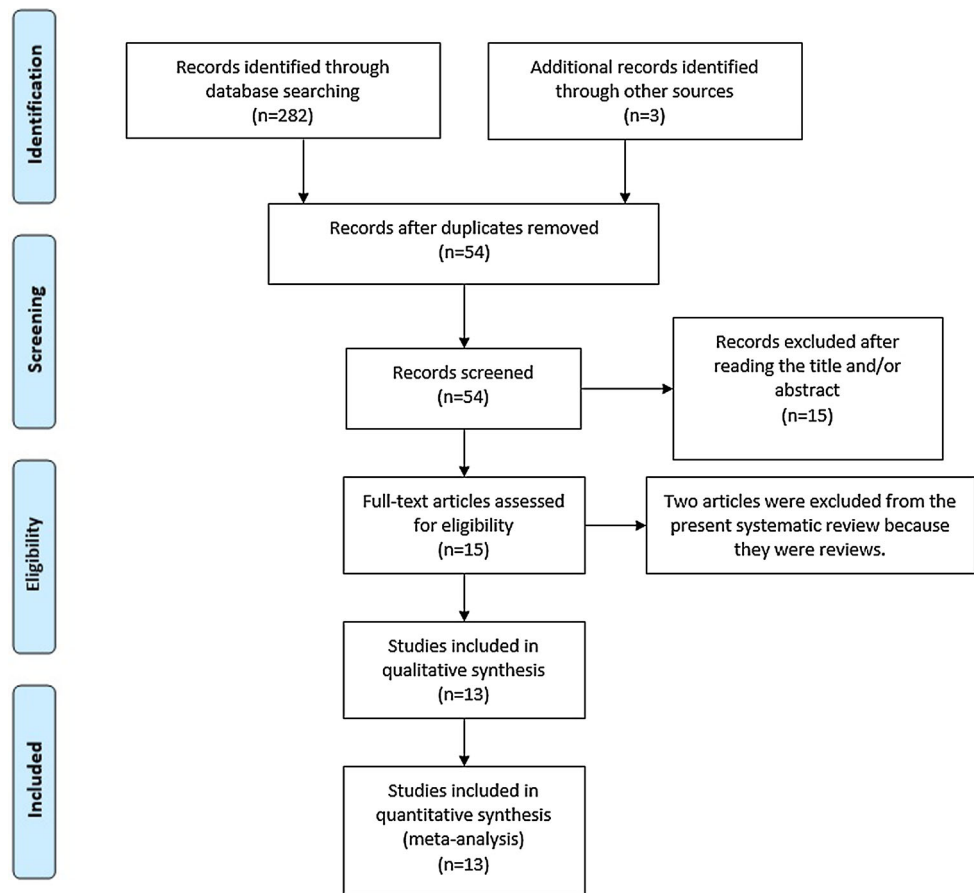
Table 1 Study characteristics (*NEC vs controls*)

Year; author	NOS	No. of patients	NEC definition
2003; Carroll	5	14 (7 vs. 7)	Modified Bell's criteria (IB, IIB, IIIA, IIIB)
2011; Altamimi	7	55	N/A
2012; Aydemir O	7	50 (25 vs. 25)	Walsh and Kliegman criteria (Stages 2 and 3)
2012; Selimoglu	7	77 (14 vs. 63)	Modified Bell's classification
2012; Reisinger	7	62 (33 vs. 29)	Modified Bell's criteria (Stage II or above)
2012; Aydemir G	4	19 (10 vs. 9)	Modified Bell's criteria (IA, IB, IIA, IIB, IIIA, IIIB)
2012; Terrin	7	72 (34 vs. 41)	Modified Bell's criteria (Stage II or above)
2013; Wei-Hong	6	60 (30 vs. 30)	Modified Bell's criteria (I, II, III)
2014; Bin-Nun	4	15	Modified Bell's criteria (Stage II or above)
2014; Min Yoon	4	16 (4 vs. 12)	Modified Bell's criteria (Stage IA, IB, IIA, IIB)
2014; Albanna	5	35 (15 vs. 20)	Modified Bell's criteria (I, IIA, IIB, IIIA, IIIB)
2014; Shenoy	7	102 (48 vs. 54)	Modified Bell's criteria (IA, IB, IIA, IIB, IIIA, IIIB)
2015; Avula	4	24 (8 vs. 16)	Modified Bell's criteria Stage (IIB or greater)

Calprotectin values among necrotizing enterocolitis and healthy neonates

Table 2 presents the neonatal characteristics (i.e., gestational age, birth weight, age at diagnosis, and gender) and calprotectin values for the NEC and healthy neonates. We observed that ten studies found significantly elevated fecal calprotectin (FC) levels among infants with NEC ($p < 0.05$) [13, 15–17, 19, 21, 23, 26, 27]. One study noted that this effect was observed at all stages of the stage of the disease (Wei-Hong et al.) ($p = 0.01$) [27]. The remaining three studies did not find significant differences between NEC and healthy neonates [20, 28, 29].

Table 2 Neonatal characteristics and calprotectin values (NEC vs. controls)

Year; author	Gestational age (days)	Birth weight (g)	Age at diagnosis (days)	Gender (males/total)	Calprotectin levels
2003; Carroll	210 vs. 210	N/A	12 vs. 12	4/7 vs. 4/7	288.4 (210.0–340.0) vs. 98.0 (22.0–171.0) mg/L*
2011; Altamimi	N/A	<1500 g	3–60	N/A	475 vs. 190 μg/g
2012; Aydemir O	113.2 ± 10 vs. 115.6 ± 8	1048 ± 323 vs. 1044 ± 232	11 (6–29) vs. 11 (6–27)	10/25	1282 (241–3337) vs. 365 (58–1006) μg/g*
2012; Selimoglu	N/A	N/A	N/A	N/A	167.56 ± 143.43 vs. 166.44 ± 158.06 μg/mL
2012; Reisinger	215 (184–268) vs. 213 (175–289)	1400 (860–1960) vs. 1270 (585–3570)	N/A	11/29 vs. 21/33	402.2 (107.6–847.6) vs. 79.6 (1.0–625.1) μg/g*
2012; Aydemir G	203 vs. 203	950 (825–970) vs. 935 (840–990)	14 (11–16) vs. 14 (12–15)	N/A	185 (165–198) vs. 104 (92–123) mg/dL*
2012; Terrin	202.3 (198.8–205.8) 95 % CI	1094 (1047–1141) 95 % CI	13.3 (12.4–14.2) 95 % CI	42/72	3.2–4.5 mg/mL*
2013; Wei-Hong	N/A	N/A	N/A	N/A	NEC I (294.63 ± 67.15)* NEC II (322.00 ± 25.23)* NEC III (397.50 ± 17.68)* vs. (187.93 ± 55.2) mg/g
2014; Bin-Nun	111.6 ± 9.6 vs. 115.2 ± 7.2	1051 ± 425 vs. 822 ± 484	17.6 ± 8.9	N/A	3000 (2075–7875) vs. 195 (110–440) μg/g*
2014; Min Yoon	206.83 ± 24.57 vs. 203.30 ± 21.57	1235.00 ± 353.67 vs. 1240.28 ± 554.29	18.17 ± 17.22 vs. 26.10 ± 17.19	N/A	5.75 ± 1.98 vs. 4.34 ± 1.94 mg/kg*
2014; Albanna	225.4 ± 16.1 vs. 214.9 ± 14.7	1520 ± 500 vs. 1250 ± 300	8.7 ± 2.2	9/15 vs. 10/20	241.1 ± 57.5 vs. 72.6 ± 10.6 mg/dL*
2014; Shenoy T	196–238	700–2300	N/A	N/A	NA*
2015; Avula	N/A	N/A	N/A	N/A	131.6 ± 34.7 vs. 119.0 ± 12.9 (ng/mg)

* Statistically significant results

Some studies investigated the correlation of FC with gestational age, birth weight, age at diagnosis, and gender. They did not find significant differences. Yoon et al. were the sole exception, who showed a possible relation between FC and gestational age in healthy neonates that were born prior to 26 weeks ($p = 0.001$). Interestingly, however, the same researchers reported a clear negative linear relationship for neonates born between 26 and 30 weeks of gestational age ($p = 0.03$) [21]. Furthermore, Carroll et al. mentioned that there was a wide variation in the concentration of calprotectin in controls and many values were outside the normal reference range for adults [13].

Accuracy of the test

Five studies evaluated the efficacy of FC as a diagnostic test. Aydemir et al. observed that a cut-off value of 792 μg/g had a 76 % sensitivity, 92 % specificity, and that the area under the curve (AUC) was 0.89 ($p < 0.001$, 95 % CI 0.81–0.98) [16]. Reisenger et al. used a lower cutoff value (286.3 μg/g) and reported a sensitivity of 81 %, a specificity of 79 %, and an AUC of 0.82 (95 % CI 0.68–0.96) [17]. However, these findings were not reproduced by Shenoy et al., who found that a cut-off value of 280 μg/g had a sensitivity of 93.3 %, specificity of 39 %, and that the AUC was 0.652 (95 % CI 0.516–0.789) [26]. Bin-Nun et al. used a cut-off value of 480 μg/g, which resulted in 100 % sensitivity and 84.6 % specificity [23]. Finally, Terrin et al. used a cutoff value of 3.0 mg/ml and reported that the sensitivity was 100 % (79.4–100 %) and the specificity was 96.4 % (87.6–99.6 %) [18].

Discussion

Necrotizing enterocolitis is a life-threatening disease that presents in preterm neonates. Early identification reduces morbidity and mortality. However, to date no specific

markers to screen for the disease have been identified. The association of FC with intestinal inflammatory diseases has been thoroughly investigated during the last decade [30]. It is believed that the presence of calprotectin in stool indicates increased neutrophil migration towards the intestinal mucosa, which is triggered by bowel inflammation [31]. Although the actual pathophysiologic pathways remain vague, it seems that the activated neutrophils gradually degranulate and secrete calprotectin, which inhibits matrix metalloproteinases and triggers apoptosis [32].

In 2010 Thuigls et al. suggested that FC might be a useful diagnostic marker for NEC [8]. Since then, several studies have investigated its efficacy. In our systematic review we sought to gather all available evidence from the international literature to evaluate the effectiveness of using FC levels in the diagnosis of NEC. According to our findings, FC is significantly elevated in neonates that suffer from NEC. To date, we do not know whether this effect takes place early during this process. However, at least one study points towards early elevation of FC levels. Although several studies have studied the differences in FC levels between NEC and healthy neonates, there seems to be a complete lack of consensus regarding the utility of specific cut-off values. This might be partially influenced by the fact that FC values show high inter-individual variation during the neonatal period. This observation is encountered both in term and preterm infants [33–36]. Therefore, the efficacy of this protein in detecting NEC remains unknown.

In this context, we strongly believe that future prospective studies that use similar techniques of protein detection are needed and that the results should be reported in specific units of measurement. Furthermore, future studies should first evaluate the cut-off values that have previously been described before proposing new ones, so as to assess the reproducibility of the test and to specifically direct future research.

Strengths and weaknesses of our study

The findings of our study are based on a systematic review of the literature. No language or date restriction was applied; therefore, there is only a small possibility that articles were omitted.

However, the risk of bias in our findings remains high due to the presence of several factors. First, all included studies were retrospective; therefore, selection bias might be an issue. Furthermore, their methodological heterogeneity in terms of patient recruitment (stage of the disease), units of measurement, and implemented assays precludes safe interpretation of results.

Last, despite the fact that the majority of included studies reported high sensitivity and the specificity of the test, the suggested cut-off points vary widely. Therefore,

we could not perform a meta-analysis of these results to provide a summary receiver operating characteristic curve.

Conclusion

The current evidence suggests that FC is elevated in newborns suffering from NEC. Its significance as an early screening marker remains unknown; however, at least one study found elevated calprotectin levels during the early stages of the disease. The reported sensitivity and specificity of the test remain unknown, as there is a lack of consensus regarding the implementation of specific cut-off values. Given the absence of specific early markers for the detection of NEC, further research is needed to reach firm conclusions.

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