Fecal S100A12: Identifying Intestinal Distress in Very-Low-Birth-Weight Infants

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ABSTRACT

Objectives: The aim of the study was to determine whether longitudinal measurements of fecal S100A12, a damage-associated molecular pattern protein, which is released from neutrophils or monocytes under stress, can detect very-low-birth-weight (VLBW) infants at risk for intestinal distress apart from necrotizing enterocolitis.

Methods: This prospective study included 46 VLBW infants with intestinal distress and 49 reference patients. Meconium and stool samples were collected prospectively on alternate days for 4 weeks, and fecal S100A12 was measured by enzyme-linked immunosorbent assay.

Results: Gestational age and weight at birth were significantly lower in patients with intestinal distress when compared to unaffected reference infants. Median levels of fecal S100A12 were significantly higher in patients with intestinal distress at onset of disease and before compared with unaffected reference infants. Median levels of fecal S100A12 declined steadily to baseline levels within 2 weeks after disease onset. The ideal cutoff value for identifying patients with intestinal distress within 7 days before disease onset was 60 μg/kg (sensitivity 0.73; specificity 0.55).

Conclusions: Fecal S100A12 levels are increased in VLBW infants with intestinal distress; however, the potential for S100A12 as an early biomarker is largely limited by overlaps between values of infants with intestinal distress and the reference population.

Key Words: disease marker, gestational age, growth, nutrition, premature neonates

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Very-low-birth-weight (VLBW) infants account for only 1.5% of live births while contributing disproportionately to neonatal morbidity (1). Whereas growth and further neurodevelopment is essentially influenced by optimal early nutrition, intestinal distress and associated feeding intolerance are the most common gastrointestinal short-term problems affecting infants with VLBW. The underlying pathophysiology remains poorly understood, but seems to involve a dysregulated immature immune response, digestive ability, and intestinal barrier function (2–5). Whereas early detection of intestinal distress and adequate intervention could improve the outcome, clinical signs are often late, unspecific, and do not allow differentiation between distinct disease entities. In addition, there are no reliable serum-based laboratory markers or imaging modalities available that allow an early diagnosis. Therefore, an early and reliable marker for intestinal distress in VLBW infants would be helpful.

We have previously shown that fecal S100A12, a damage-associated molecular pattern protein, which is released from neutrophils or monocytes under stress, is an accurate fecal marker of intestinal inflammation and disease relapse in inflammatory bowel disease (6–9). Furthermore, we have reported that longitudinal measurement of fecal S100A12 can identify VLBW infants at risk of necrotizing enterocolitis (NEC) and predict disease severity (10). In the present study, we aimed to evaluate the role of fecal S100A12 in intestinal distress in VLBW infants apart from NEC and its value as a possible early diagnostic marker.

METHODS

Study Design

Patients were recruited from April 2008 to October 2009 in 5 German tertiary neonatal intensive care units (Wuppertal, Schwerin, Erfurt, Münster, and Krefeld). Ethical approval was obtained from the ethics committee of Witten/Herdecke University for all of the participating hospitals, and fully written informed consent was obtained from all legal guardians. All preterm infants with birth weights <1500 g were included. Meconium and stool samples were prospectively collected on alternate days for at least 28 days. On admission, baseline characteristics of the infants and maternal information for enrolled infants were recorded. In addition, epidemiological parameters, for example, postnatal age, daily feeding regimen, respiratory support, laboratory and radiograph result, and clinical signs, were recorded when each stool sample was collected throughout the follow-up.

Patients

Intestinal distress was defined by clinical signs, radiologic and laboratory findings as reported by the responsible physician, or a reduction of enteral feeds by ≥30%. After clinical assessment, enteral feeding was interrupted if there were significant residues in gastric aspirates, abdominal distension, and/or blood in stool (11,12).

The reference group consisted of infants without any signs and symptoms of intestinal distress. All patients received an echocardiography between 24 and 36 hours after birth to determine the existence and significance of a persistent ductus arteriosus. Significance of the persistent ductus arteriosus was determined by

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analyzing left ventricular output (LVO), ductal diameter, and celiac artery flow (CAF). All patients with a left atrial:aortic ratio >1.4, a ductal diameter >2 mm, and a CAF:LVO ratio <0.1 received a ductal closure therapy with ibuprofen for 3 days. Echocardiographic parameters were monitored every 24 hours.

For further analysis, all patients with intestinal distress were further subdivided as follows:

1. Patients with a CAF:LVO ratio <0.1 were categorized as having impaired intestinal blood circulation (13).
2. Patients who did not pass at least 1 stool each day without enemas or stimulation for 3 consecutive days were classified as having decreased intestinal motility (14).
3. Patients at least 72 hours old with a meconium plug identified in the rectum on radiographic contrast enema were defined as having meconium plug syndrome (15–17).
4. NEC stage I was determined using the modified Bell classification (5,18). To obtain clear case definitions, patients with definite diagnosis of NEC (stage II and III) were excluded and analyzed in a separate study (10).
5. Intestinal perforation was defined as occurrence of intestinal perforation during the first 14 days of life without evidence of NEC (19).
6. Patients with a reduction of enteral feeds by ≥30% without any other clinical evidence for a specific underlying disorder were classified as having intestinal distress of unknown origin.

Stool Analysis

All of the stool samples were stored at −80°C before analysis within 24 hours after specimen collection. Concentrations of S100A12 were determined by a double sandwich enzyme-linked immunosorbent assay system, as described previously (20). Analyses were performed by investigators in Münster (Germany), who were blinded for the diagnosis and the disease. All of the analyses were performed in triplicate.

Statistical Analyses

Statistical comparisons of the data between groups (unaffected control vs intestinal distress) were tested by the Mann-Whitney *U* test. Data are presented as median and range except when otherwise stated. To determine the accuracy of S100A12 measurements, receiver-operating characteristic curves were drawn by plotting sensitivity against 1-specificity. Overall accuracy of the markers in detecting intestinal distress was represented by area under the curve with 95% confidence interval. Best cutoff point is defined as the maximum sum of sensitivity and specificity. All tests of significance were 2-tailed. $P < 0.05$ was considered significant. All calculations were performed by using the Statistical Package for the Social Sciences (version 14, SPSS Inc, Chicago, IL).

RESULTS

Intestinal Distress Among Patients

We enrolled 95 infants, of whom 46 patients (48.4%) subsequently developed intestinal symptoms (decreased intestinal motility, meconium plug syndrome, suspected NEC stage I, intestinal perforation, impaired intestinal blood circulation, intestinal distress of unknown origin) and 49 infants had no signs of intestinal distress (reference group) (Table 1). The median postnatal age at diagnosis of intestinal distress was 7.5 days after birth (1–26 days). The median weight at diagnosis was 870 g (470–2100 g). A total of 819 meconium and postmeconium stool samples were collected and analyzed. Median gestational age (GA) and birth weight (BW) were significantly lower in patients with intestinal distress (GA 27.0 weeks [23.0–32.7 weeks]; BW 825 g [436–1480 g]) compared with the reference group (GA 29.1 weeks [24.9–35.9 weeks], $P < 0.001$; BW 1185 g [570–1490 g], $P < 0.0001$). No statistically significant differences between the disease and the reference group were found in other neonatal factors (eg, sex ratio, Apgar scores, umbilical artery pH at birth, enteral feeding regime, medication use) or maternal factors (eg, preeclampsia, diabetes, clinical chorioamnionitis, premature rupture of membranes, mode of delivery).

Fecal S100A12 Levels at Onset of Intestinal Distress

Fecal S100A12 levels were significantly higher in patients with intestinal distress at disease onset (370 μg/kg; 5–48,750 μg/kg) compared with unaffected reference infants (45 μg/kg, 5–16,000 μg/kg; $P < 0.002$). More specifically, at onset of disease, fecal S100A12 levels were highest in VLBW infants with intestinal perforation (5500 μg/kg; 2005–62,500 μg/kg; n = 3; $P < 0.004$) and impaired intestinal blood circulation (4975 μg/kg; 5–25,000 μg/kg; n = 10; $P < 0.0001$), followed by patients with intestinal distress of unknown origin (595 μg/kg; 5–30,950 μg/kg; n = 19; $P < 0.001$), meconium plug syndrome (542 μg/kg; 140–29,000 μg/kg; n = 4; $P < 0.02$), NEC stage I (490 μg/kg; 5–3115 μg/kg; n = 14; $P < 0.002$), and patients with decreased intestinal motility (260 μg/kg; 5–48,750 μg/kg; n = 26; $P < 0.005$) (Fig. 1).

Fecal S100A12 Levels in Monitoring of Intestinal Distress

Time course analysis of fecal S100A12 levels in VLBW infants showed significantly elevated S100A12 levels before clinical onset of meconium plug syndrome (388 μg/kg; 95–810 μg/kg; n = 4; $P < 0.03$), NEC stage I (188 μg/kg; 5–3050 μg/kg; n = 18; $P < 0.003$), decreased intestinal motility (125 μg/kg; 5–48,750 μg/kg; n = 25; $P < 0.05$), and intestinal distress of unknown origin (115 μg/kg; 5–72,000 μg/kg; n = 45; $P < 0.003$) compared with unaffected infants (Fig. 1A–D). Fecal S100A12 levels were also elevated in VLBW infants who subsequently showed intestinal perforation (2883 μg/kg; 5–36,350 μg/kg; n = 4) or impaired intestinal blood circulation (60 μg/kg; 5–980 μg/kg; n = 10), but these differences were not statistically significant (Fig. 1E and F). A more detailed time course analysis of fecal S100A12 at different time intervals before and after onset of intestinal distress showed that fecal S100A12 levels are steadily and statistically significant, increasing within 21 days before disease onset compared with unaffected neonates whose stool specimens were collected at a similar gestational and postnatal age (Fig. 2A).

Overall, median levels of fecal S100A12 declined steadily to baseline levels within 2 weeks after diagnosis of intestinal distress and appropriate treatment when compared with fecal S100A12 levels of unaffected reference infants (45 μg/kg; 5–16,000 μg/kg) (Fig. 2A); however, when different causes of intestinal distress in VLBW infants were considered, we observed that median fecal S100A12 levels were still significantly elevated after disease onset in patients with impaired intestinal blood circulation (570 μg/kg; 5–32,050 μg/kg; n = 24; $P < 0.02$), intestinal perforation (330 μg/kg; 5–9600 μg/kg; n = 23; $P < 0.001$), and intestinal distress of unknown origin (205 μg/kg; 5–40,700 μg/kg; n = 39; $P < 0.002$) (Fig. 1D–F). On the contrary,

TABLE 1. Patient characteristics

	Control	Decreased intestinal motility	Meconium plug syndrome	Suspected NEC (Bell stage I)	Intestinal perforation	Impaired blood circulation	Distress of unknown origin
Patients, n (%)	49 (52)	14 (15)	6 (6)	7 (7)	5 (5)	4 (4)	10 (11)
Gestational age, wk (range)	29.1 (25–36)	26.9 (23–33)	29.2 (25–32)	27.7 (23–30)	25.3 (24–27)	27.2 (24–29)	27.0 (25–32)
Birth weight, g (range)	1185 (570–1490)	825 (510–1480)	717 (625–1470)	680 (466–1108)	696 (436–900)	905 (650–1130)	960 (496–1446)
Sex ratio (male/female)	1.4	2.5	0.2	0.4	1.5	0.0	0.7
Maternal age, y (range)	29 (17–42)	30 (21–45)	31 (28–44)	36 (22–39)	29 (21–36)	29 (26–41)	29 (17–36)
Apgar score 1 min, sum (range)	6 (0–9)	6 (0–9)	7 (1–7)	5 (2–7)	5 (2–7)	2 (1–4)	5 (4–8)
Apgar score 5 min, sum (range)	7 (2–10)	6 (0–9)	7 (4–9)	7 (4–9)	7 (4–8)	4 (3–6)	7 (5–9)
Apgar score 10 min, sum (range)	9 (5–10)	7 (0–10)	9 (7–10)	8 (6–10)	8 (7–9)	7 (6–9)	9 (7–9)
Umbilical artery pH value (range)	7.34 (7.04–7.50)	7.33 (7.24–7.41)	7.36 (7.33–7.45)	7.28 (7.15–7.31)	7.38 (7.18–7.42)	7.35 (7.29–7.43)	7.37 (7.14–7.43)
Stool samples, n (%)	381 (47)	134 (16)	63 (8)	64 (8)	30 (4)	44 (5)	103 (13)
Weight at diagnosis, g (range)	—	960 (593–1570)	730 (550–1260)	800 (680–1115)	730 (470–800)	1040 (620–1270)	1105 (700–2100)
Age at diagnosis, days (range)	—	8 (1–25)	4 (2–6)	10 (1–26)	6 (2–10)	9 (4–13)	13 (2–26)
Feeding at diagnosis, % parenteral (range)	—	43 (0–92)	95 (25–100)	56 (20–85)	88 (81–91)	68 (16–88)	0 (0–51)

NEC = necrotizing enterocolitis.

median fecal S100A12 levels of patients with meconium plug syndrome (35 μg/kg; 5–32,450 μg/kg; n = 55), NEC stage I (18 μg/kg; 5–2795 μg/kg; n = 31), or impaired intestinal blood circulation (5 μg/kg; 5–8000 μg/kg; n = 83) did not differ or were even lower during the time after diagnosis when compared with unaffected reference infants (Fig. 1A–C).

Predictive Value of Fecal S100A12 and Influence of Gestational Age

Next, we analyzed the value of fecal S100A12 levels in detecting intestinal distress in different gestational age groups. Median fecal S100A12 levels did not differ in patients with a GA between 24 and 27 weeks between infants without intestinal distress (95 μg/kg; 5–16,000 μg/kg; n = 63) and those who subsequently developed intestinal distress (158 μg/kg; 3–72,000 μg/kg; n = 162; $P = 0.385$) (Fig. 2B). In contrast, median fecal S100A12 levels were significantly higher in patients with intestinal distress at a GA of 28 to 31 weeks (68 μg/kg; 5–42,500 μg/kg; n = 184) and 32 to 37 weeks (103 μg/kg; 5–25,950 μg/kg; n = 92) compared with reference infants at a GA of 28 to 31 weeks (42 μg/kg; 5–6500 μg/kg; n = 182; $P < 0.01$), and 32 to 37 weeks (39 μg/kg; 5–2645 μg/kg; n = 136; $P < 0.02$), respectively (Fig. 2B).

The cutoff value for differentiating all patients with intestinal distress from those without intestinal symptoms at the time of disease onset was 250 μg/kg (Fig. 3A), whereas the ideal cutoff value for identifying VLBW infants with intestinal distress within 7 days before disease onset was 60 μg/kg (Fig. 3B). This resulted in an overall sensitivity of 73%, a specificity of 55%, a positive predictive value of 29%, and a negative predictive value of 91%. More important, serum levels of standard inflammatory markers (C-reactive protein, interleukin-6) were not related to disease activity (data not shown).

DISCUSSION

Previous studies investigating the role of fecal calprotectin in the management of intestinal distress in neonates have focused almost exclusively on the diagnosis of NEC (11,21–28). Nonetheless, the real clinical challenge is to separate patients with gastrointestinal symptoms (eg, feeding intolerance, spontaneous intestinal perforation, meconium ileus/plug) from true NEC patients. We have recently reported that longitudinal measurement of fecal S100A12 is superior to fecal calprotectin in identifying VLBW infants at risk for NEC at an early stage and predicting disease severity (10). In the present study, we therefore aimed to provide data evaluating whether fecal S100A12 is a helpful marker for early risk assessment of intestinal distress in VLBW infants apart from NEC and whether it may allow the differentiation of NEC from other causes of intestinal distress in VLBW infants. Our results clearly show that fecal S100A12 is an early biomarker for the diagnosis of intestinal distress in VLBW infants apart from NEC; however, the utility of fecal S100A12 in differentiating true NEC from other causes of intestinal symptoms may be limited.

Median levels were highest in VLBW infants with intestinal perforation (5500 μg/kg) and impaired intestinal blood circulation (4975 μg/kg), followed by S100A12 levels in patients with intestinal distress of unknown origin (595 μg/kg), meconium plug syndrome (542 μg/kg), NEC stage I (490 μg/kg), and patients with decreased intestinal motility (260 μg/kg) (Fig. 1). These values were within or even higher than the median fecal S100A12 levels we found at onset of NEC (510 μg/kg) (10). Thus, measurement of fecal S100A12 in VLBW infants may provide important clinical information to pediatricians and pediatric surgeons but does not allow differentiating patients with NEC from those

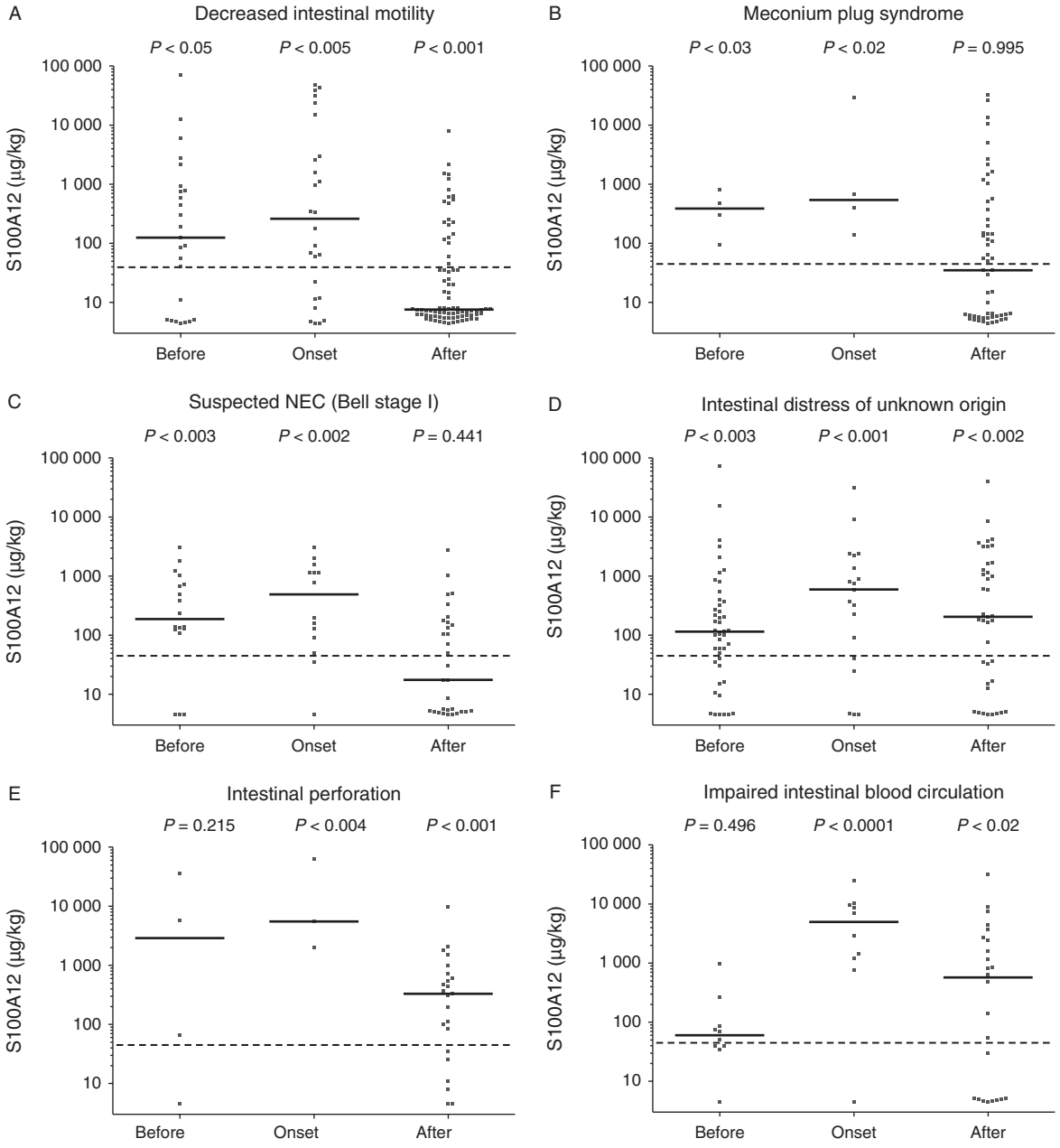

FIGURE 1. Fecal S100A12 levels in very-low-birth-weight (VLBW) infants with intestinal distress. A total of 438 stool samples of 46 VLBW infants with intestinal distress were analyzed before, after, and at the time of disease onset. The scatter plots show the median levels (central horizontal line) of fecal S100A12. The median S100A12 level of 381 stool samples of 49 matched controls (45 µg/kg) is represented by the dotted line. *P* values are shown. NEC = necrotizing enterocolitis.

with other gastrointestinal disorders. This missing accuracy can be attributed most likely to the large overlap in fecal S100A12 levels of infants with and without intestinal distress, which in turn is related to the wide range of fecal S100A12 levels in VLBW infants without gastrointestinal symptoms (Fig. 2B). This seems to be a general characteristic of fecal biomarkers of gastrointestinal inflammation in premature infants because fecal calprotectin levels in VLBW infants also show wide variations in healthy individuals and decreasing levels during the first month of life (28).

Interestingly, fecal S100A12 levels were also elevated 21 days before and 7 days after onset of intestinal distress (apart from NEC) when compared with reference infants without gastrointestinal symptoms (Fig. 2A). This may indicate that the pathophysiology of intestinal distress may include a prolonged period of inflammation before clinical diagnosis (eg, spontaneous intestinal perforation). The cutoff value for identifying patients with intestinal distress (apart from NEC) within 7 days before disease onset was 60 µg/kg (Fig. 3B). Sensitivity, specificity, and positive and negative predictive values for fecal S100A12 for the detection

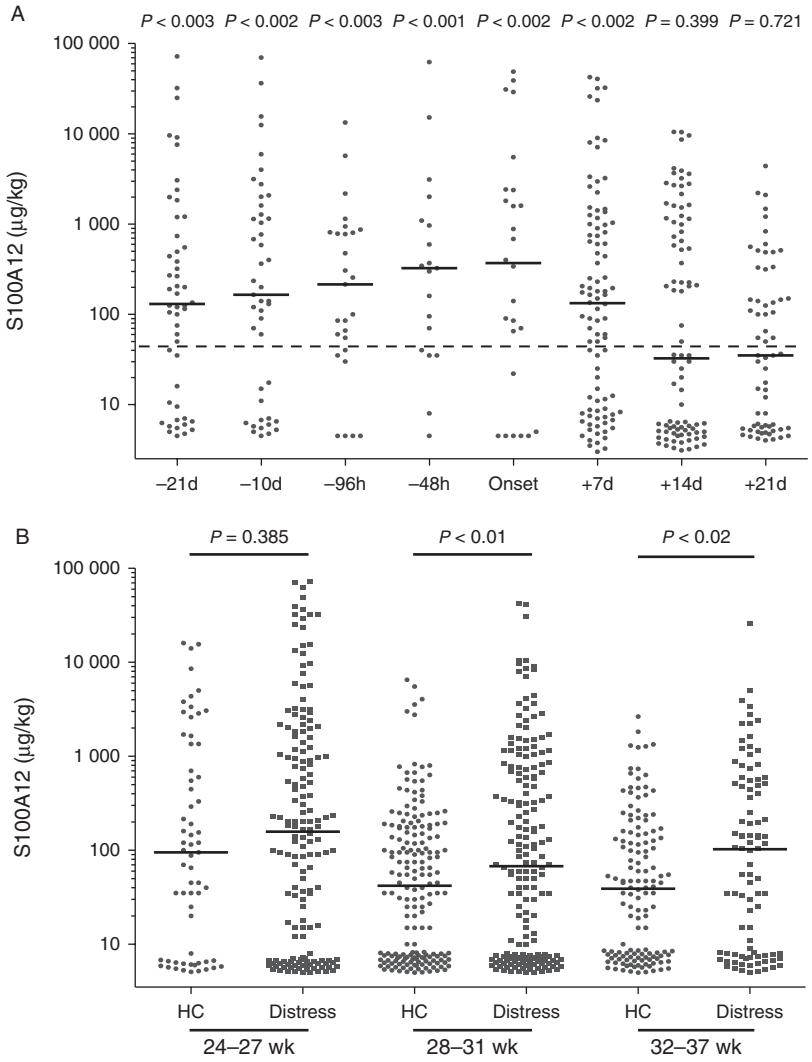

FIGURE 2. Time course analysis of fecal S100A12 levels in very-low-birth-weight (VLBW) infants. The scatterplots show the median (central horizontal line) of fecal S100A12 levels. *P* values are shown. A, Stool samples (n = 438) of 46 VLBW infants with intestinal distress were analyzed at different time points before disease onset (12–48 hours, 48–96 hours, 5–10 days, and 11–21 days), after disease onset (1–7 days, 8–14 days, and 15–21 days), and at the time of disease onset. The median S100A12 level of 381 stool samples of 49 matched healthy controls (HC, 45 µg/kg) is represented by the dotted line. B, S100A12 concentrations in stool samples of infants without intestinal disease (HC) were compared with fecal S100A12 levels of infants experiencing intestinal distress. The graph compares S100A12 levels on the background of the gestational age of 24 to 27 weeks, 28 to 31 weeks, and 32 to 37 weeks.

of intestinal distress apart from NEC were 73%, 55%, 29%, and 91%, respectively. This limited sensitivity and specificity is most likely because of the physiological high levels of fecal S100A12 during the first week of life (10). This is similar to the test performance of fecal S100A12 we have previously reported for the prediction of NEC within 7 days before disease onset (cutoff value 65 µg/kg: sensitivity 70%, specificity 68%, positive predictive value 37%, negative predictive value 89%) (10). Whereas S100A12, therefore, seems to be not suitable for early diagnostic confirmation of intestinal distress or NEC in premature infants, it may be helpful in the exclusion of such pathologies of the digestive tract.

In line with previous studies on VLBW infants with NEC, we found that gestational and birth weight were significantly lower in VLBW infants with intestinal distress compared with the reference group (Table 1) (10,28). This points to the immaturity of the gastrointestinal tract of preterms, including the intestinal barrier function and innate immune defense (2–5). Accordingly, intestinal distress was diagnosed predominantly during the first and second week after birth (Table 1) in contrast to NEC, which was usually diagnosed during the second and third week of life. Otherwise, we found no significant correlations between fecal S100A12 level and various neonatal and maternal factors apart from the diagnosis of intestinal distress. We also found no correlation between C-reactive protein and interleukin-6 levels and fecal S100A12 in reference infants, confirming our previous assumption that systemic infection does not affect fecal S100A12 levels in the absence of severe gastrointestinal disease (10). Furthermore, standard inflammatory markers (C-reactive protein, interleukin-6) were suitable neither

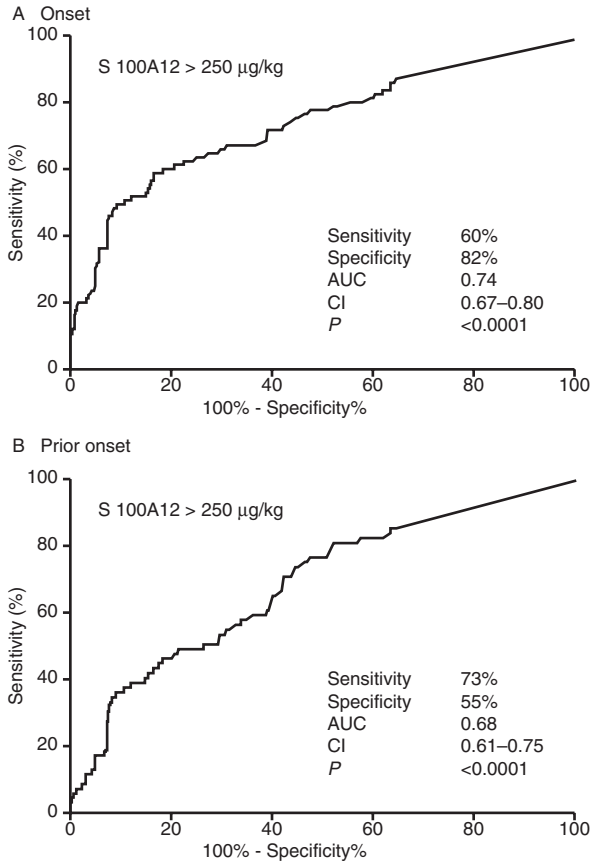

FIGURE 3. Receiver-operating characteristic (ROC) curve analyses. ROC curve analyses were performed to analyze the sensitivity and specificity of fecal S100A12 in differentiating very-low-birth-weight infants with gastrointestinal distress from those without gastrointestinal disease (control) at disease onset (A) and within 1 week before onset (B). Area under the ROC curve, 95% confidence interval (CI), and *P* value are shown for each curve. The fecal S100A12 value that gives the best accuracy is shown.

for the prediction nor for the diagnosis of intestinal distress in VLBW infants.

Appropriate and early nutrition plays a key role in the health care of preterm infants, but increasing feeding volumes is often limited by individual feeding tolerance (29). Interpreting the clinical and prognostic significance of common and aspecific signs of feeding intolerance is challenging, and decisions regarding the initiation, increase, or reduction of feeding depend in part on the balance between the risks of complications such as NEC. Our clinical criteria to reduce enteral feeds (gastric residuals, abdominal distension and/or blood in stool) were in accordance with previous studies, which used similar definitions as we did in our study (11,28). Nevertheless, standardized and optimized nutrition protocols in preterm infants would be helpful in the management of enteral feeding and food intolerance (12,30–32).

Overall, the early increase of fecal S100A12 levels in our patients with intestinal distress suggests that monitoring fecal S100A12 may provide useful early warning signals; however, even though more reliable than fecal calprotectin levels (10), S100 proteins still have a high inter- and intraindividual variability as well as an age dependency during the first weeks of life. Nevertheless, we have previously reported stable S100A12 levels in stool samples of reference infants obtained on days 8 to 28 after birth (10), and the majority of patients with intestinal distress experienced onset of disease during that time period; however, the age dependence and the large overlaps between values for infants who developed intestinal disease and healthy infants limit the potential for S100 proteins as biomarkers in VLBW infants. Nevertheless, the present data suggest that sequential measurement of fecal S100A12 seems to be a promising noninvasive clinical screening test for intestinal distress in VLBW infants, at least to rule out severe intestinal disorders.

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