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Ischemia modified albumin as a marker of hypoxia in preterm infants in the first week after birth

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ABSTRACT

Background: Tissue hypoxia remains a leading cause of morbidity and mortality in preterm infants. Current biomarkers often detect irreversible hypoxic cellular injury (i.e. lactate) and are non-specific. A new biomarker is needed which detects tissue hypoxia before irreversible damage occurs.
Aims: To investigate the relation between serum ischemia modified albumin (IMA), a marker of hypoxia; and analytic variables, patient related variables and conditions associated with hypoxia, in preterm infants.
Study design: Retrospective cohort study.
Subjects: Infants with a gestational age < 30 weeks and/or birth weight < 1000 g.
Outcome measures: We collected two remnant blood samples in the first week after birth and measured IMA. IMA/albumin ratio (IMAR) was used to adjust for albumin. We assessed correlations between IMA(R) and analytic variables (albumin, lipemia- and haemolysis index); mean-2 h SpO_2; mean-2 h variability of regional splanchnic oxygen saturation (r_sSO_2), measured using near-infrared spectroscopy; and patent ductus arteriosus (PDA).
Results: Sixty-five infants were included. Albumin, the lipemia- and haemolysis index correlated negatively with IMA (r:-0.620, $P<0.001$; r:-0.458, $P<0.001$; and r:-0.337, $P=0.002$). IMAR correlated negatively with SpO_2 (rho:-0.614, $P<0.001$). Lower r_sSO_2 variability correlated with higher IMAR values (rho:-0.785, $n=14$, $P=0.001$ and rho:-0.773, $n=11$, $P=0.005$). Infants with a hemodynamic significant PDA (hsPDA) had higher IMAR values than infants without PDA (0.13 [0.11–0.28], $n=16$ vs. 0.11 [0.08–0.20], $n=29$, $P=0.005$ and 0.11 [0.09–0.18], $n=13$ vs. 0.09 [0.06–0.17], $n=37$, $P=0.026$).
Conclusions: When adjusted for albumin, the lipemia- and haemolysis index, IMAR has potential value as a marker for systemic hypoxia in preterm infants, considering the associations with SpO_2, variability of r_sSO_2, and hsPDA.

1. Introduction

Tissue hypoxia remains a leading cause of morbidity and mortality in preterm infants [1]. When hypoxia persists, organs may experience hypoxic injury which may increase the risk of necrotizing enterocolitis (NEC) [2], poor neurodevelopmental outcome [3], and mortality [2]. To that end, higher oxygen saturation targets were advised in preterm infants [4]. Regardless, considerable variation of oxygen saturation in preterm infants persist and therefore hypoxia remains a common finding in preterm infants [5,6].

Currently, SpO_2, lactate, and pH are the most commonly used markers for hypoxia in preterm infants. However, these markers may have some disadvantages. First of all, low SpO_2 levels may only indicate hypoxemia and not represent the level of tissue hypoxia. Furthermore,

Abbreviations: IMA, Ischemia modified albumin; IMAR, Ischemia modified albumin ratio; r_sSO_2, Regional splanchnic oxygen saturation; r_cSO_2, Regional cerebral oxygen saturation; PDA, Patent ductus arteriosus; hsPDA, Hemodynamic significant patent ductus arteriosus; NEC, Necrotizing enterocolitis.

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high lactate and low pH indicate severe tissue hypoxia with irreversible cellular damage and are both non-specific markers. Besides, abnormal levels could also be present in non-hypoxic conditions [7,8]. Therefore, a different marker is needed which may detect tissue hypoxia before irreversible damage occurs.

Ischemia modified albumin (IMA), presented in absorbance units per millilitre, is a marker which is elevated during ischemia, and a biomarker for early detection of myocardial ischemia in adults [9]. IMA could also be a specific and early marker to detect a degree of tissue hypoxia in preterm infants. Although the exact cascade of the formation of IMA is under debate, it is known that within minutes after tissue hypoxia occurs, albumin is modified into IMA which results in a decreased capacity to bind metals, i.e. cobalt [9,10]. Therefore, IMA can be distinguished from normal albumin, and the degree of hypoxia can be estimated [9]. This makes IMA a rapid marker for tissue hypoxia which could potentially be used in preterm infants.

IMA has been associated with neonatal diseases such as NEC in a small observational study [11]. However, it remains unknown whether IMA can function as a reliable marker for tissue hypoxia in preterm infants. In this exploratory study we aimed to explore the reliability of IMA in very preterm infants in three steps. First, we aimed to investigate the course of IMA levels during the first week after birth. Second, we aimed to determine which analytical variables affected IMA levels. Third, we aimed to investigate the association between IMA levels and patient related variables and conditions associated with hypoxia such as patent ductus arteriosus (PDA) and NEC.

2. Methods

2.1. Patient population

In this exploratory pilot study we included all infants with a gestational age below 30 weeks and/or birth weight below 1000 g and admitted to the neonatal intensive care unit of the University Medical Center Groningen within the first three days after birth between September 2018 and October 2019, with at least one clinical blood sample taken within the first week after birth. Infants with congenital heart disease other than PDA, were excluded. The medical ethical committee of the University Medical Center Groningen approved this study (No. 2017/563). Because this study concerns pseudononymised remnant material from patients whose parents did not opt-out for using these samples, as was offered routinely, our ethical board waived the need for parental consent.

2.2. Blood sample collection

We used the remnant of two regular blood samples collected in lithium-heparin tubes which were obtained in the first week after birth. The first sample was collected between day one and day four and the second between day five and day eight after birth. Samples were centrifuged for 5 min at 1885 G (relative centrifugal force). Plasma samples were initially stored at room temperature and moved within hours to the refrigerator (2–8 °C). Within 60 h after blood collection, samples were stored at −80 °C.

2.3. IMA and albumin assay

For IMA detection, the rapid colorimetric method described by Bar-Or et al. was used [10], but modified and optimized using the principles of the IMA-assay of the Szybio assay (Szybio Biotech, Wuhan, China) and the findings by Lee et al. [12] Using 96-well clear flat bottom polystyrene ELISA-plates (Corning, NY, USA) 20 μL of plasma was added per well, each well containing 133.3 μL of a buffer made of 6.9 μL 0.5 M $CoCl_2$ in 10 mL 20 mM TRIS buffer (pH 7.4). Plates were covered by foil to prevent cross contamination. After shaking the ELISA-plate for 10 s at 600 rpm and an incubation for five minutes at 37 °C, absorbance (A1) was measured at 505 nm (reference 690 nm) on a multimode plate reader (Victor Nivo, Perkin Elmer, USA). Next 66.7 μL of a buffer of 2.3 mg DTT in 10 mL of 50 mM MOPS buffer (pH 7.4) was added to the plate. After shaking the ELISA-plate for 10 s at 600 rpm and an incubation for five minutes at 37 °C, absorbance (A2) was measured at 505 nm (reference 690 nm). Finally we subtracted A2 from A1 to correct for background noise. IMA was measured in duplicate and expressed in absorbance units per millilitre (U/mL).

Plasma albumin (g/L) was measured on the Cobas c501 analyzer using the bromocresol green colorimetric assay (Roche diagnostics GmbH, Mannheim). The IMA-assay is based on reduced metal binding capacity due to significant molecular changes of albumin during ischemia. Because the IMA-assay measures normal albumin and indirectly IMA, this automatically result in a negative correlation between albumin and IMA. Therefore, we calculated the IMA/albumin ratio (IMAR = IMA/albumin) to adjust for total albumin, expressed in absorbance units per millilitre / grams of albumin per litre [13].

2.4. The effect of analytical variables on IMA

To investigate the precision and reproducibility of IMA, we measured intra- and inter-assay variability ten times with pooled samples of low (0.06), middle (0.11) and high (0.21) IMAR levels [14]. Next, we investigated whether other colorimetric substances in the plasma interfere with the IMA-assay by measuring the haemolytic, icteric and lipemic indices measured on the Cobas c501 analyzer (Roche diagnostics GmbH, Mannheim). Finally, we investigated the effect of total albumin on IMA.

2.5. Patient related variables and conditions associated with hypoxia and IMAR

From the hospital records we collected demographic variables and variables associated with hypoxia such as: sex, gestational age, birth weight, Apgar score at 1 and 5 min, acidosis based on an arterial pH of the umbilical cord <7.3, a newborn illness severity and mortality risk score: SNAPPE-II score (missing variables were scored as normal) [15], death within 14 days after birth, acidosis based on a pH <7.3 (measured within two hours of IMA blood sample collection); and several variables of systemic and tissue oxygenation, collected within two hours before blood sample collection: mean two-hour SpO_2, mean two-hour regional cerebral oxygen saturation (r_cSO_2), and mean two-hour regional splanchnic oxygen saturation (r_sSO_2) measured by near-infrared spectroscopy according to our local protocol [16]. We calculated variability of r_sSO_2 -a potential marker for intestinal hypoxic injury- with coefficients of variation (CoVAR = SD/mean) in epochs of 30 min [17].

We furthermore collected data regarding conditions associated with hypoxia such as small for gestational age defined as <p10 according to Dutch reference values [18], NEC development (Bell's stage 2 or higher) later on during admission to the NICU [19], and sepsis developed in the first week after birth (defined as sepsis symptoms with a positive blood culture and/or C-reactive protein >20 mg/L). We collected data regarding PDA, categorized as: no clinical signs of PDA or no PDA on echocardiograph (no PDA); non-hemodynamically significant PDA (non hsPDA); and hemodynamically significant PDA (hsPDA) that required treatment. The categorization of PDA was defined in both the first half of the week and second half of the week. If the occurrence of a PDA was not determined in the first half of the week, we categorized the occurrence of the PDA similar as that of the second half. Finally, we collected data regarding neurodevelopment with the Bayley-III-NL score for cognition and motor function during standard follow-up at the age of 23–33 months corrected age. We used composite scores for cognitions and individual scaled scores for fine- and gross motor function [20].

2.6. Statistical analysis

Data are presented as mean ± SD for parametric data, median (range) for nonparametric data and number (percentage) for nominal data. In order to answer the first research question, to investigate the course of IMA in the first week after birth, paired *t*-test and Wilcoxon signed rank test were used to assess differences between sample 1 and 2 for paired parametric and non-parametric data, respectively. Spearman's rank correlation coefficients was used to assess the correlation between IMAR and postnatal age. For the second research question, to assess whether analytical variables affect our IMA results, we calculated Pearson's and Spearman's rank correlation coefficients for parametric and non-parametric data in infants with two samples available in the first week after birth. For the third research question, to investigate patient related variables and conditions associated with hypoxia and IMAR, the IMA/albumin ratio (IMAR) was used to adjust for albumin. We calculated Spearman's rank correlation coefficients for continuous data and used Mann Whitney *U* tests for nominal data. To adjust for possible confounders of the relation between hypoxia and IMAR, all variables with a significant correlation ($P < 0.05$) with IMAR in the first and/or second sample were used as potential confounder in the multivariable regression analysis model, which we adjusted for gestational age and postnatal age [21]. When a variable was correlated with IMAR in only one of the two samples, we performed multivariable linear regression analyses for both samples separately. We considered two-tailed *P*-values of <0.05 statistically significant. As this was a pilot study, in which we conducted to evaluate feasibility, and generate hypotheses for further investigations, we chose not to correct for multiple testing. IBM SPSS Statistics 23 (IBM Corp., Armonk, NY, USA) was used for statistical analyses and GraphPad Prism 7.02 (GraphPad Software Inc., La Jolla, California, USA) for graphical displays.

3. Results

Out of 82 eligible infants, 65 were included. We excluded 14 infants without available remnant blood samples and three infants with congenital heart disease, other than PDA (Fig. 1). One sample was collected in 24 infants while in 41 infants two samples were collected during the first week after birth. This resulted in 51 samples during the first half of the week (sample 1) and 55 samples during the second half of the week (sample 2). Reasons for missing samples were: not enough plasma for analysis (*n*=14), no blood sample collected (*n*=3), transferred to other hospital (n=1), or death (n=1). Patient characteristics are shown in Table 1.

Table 1
Patient characteristics.

	Total (*n* = 65)	
Gestational age (weeks)	27.4 (24.1–30.0)	
Birth weight (grams)	1000 (600–1650)	
Head circumference (cm)[a]	25.3 (21.5–29.0)	
Small for gestational age	13 (20)	
Male, n (%)	40 (61.5)	
Apgar score at 1 min[a]	5 (0–9)	
Apgar score at 5 min[a]	7 (3–9)	
Multiple births, n (%)	19 (29)	
Patent ductus arteriosus, n (%)	First sample	Second sample
No PDA	36 (55)	43 (66)
Non hsPDA	10 (15)	6 (9)
hsPDA	19 (30)	16 (25)

Data are presented as median (range) or as number (percentage). HsPDA (hemodynamic significant patent ductus arteriosus.
[a] 63 infants used for analysis.

3.1. The time course of ischemia-modified albumin in the first week after birth

IMA absorbance was 0.35 ± 0.03 U/ml in the first sample while it was 0.33 ± 0.03 U/ml in the second sample (*n*=41, *P*<0.001). The distribution of the samples over the days is shown in Supplemental Table S1. Albumin was 28.6 ± 5.2 g/L in the first sample and 31.8 ± 6.0 g/L in the second sample (*n*=41, *P*<0.001). The IMA/albumin ratio (IMAR) was 0.12 (0.08–0.28) in the first sample and 0.10 (0.07–0.18) in the second sample (n=41, *P*<0.001). After clustering all samples at various time points, we found that IMAR decreased with a higher postnatal age (rho: −0.392, *n*=41, *P*<0.001) with a median IMAR of 0.13 (0.11–0.28) on day one and 0.09 (0.07–0.14) on day seven after birth (Fig. 2).

3.2. The effect of analytical variables on IMA

Intra- and inter-assay variability of IMA was 1.6 % and 6.6 %, respectively. The lipemia index and haemolysis index correlated negatively with IMA (r:-0.458, *n*=41, *P*<0.001 and r:-0.337, n=41, *P*=0.002) while the icteric index did not correlate with IMA (r:-0.100, n=41, *P*=0.371). We adjusted for these indices in a regression analysis which we will present separately. There was a negative correlation between IMA and albumin (*P*<0.001).

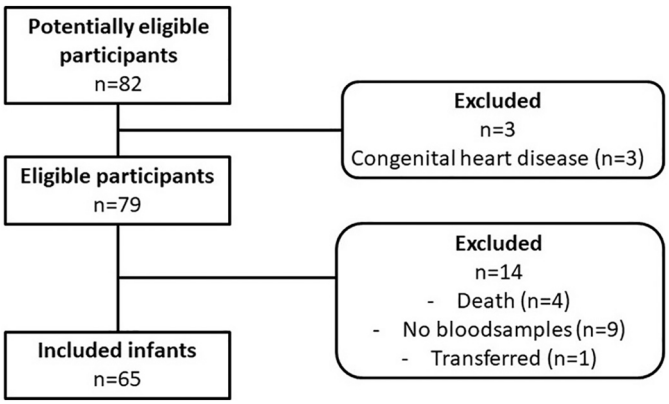

Fig. 1. Flowchart of included patients.

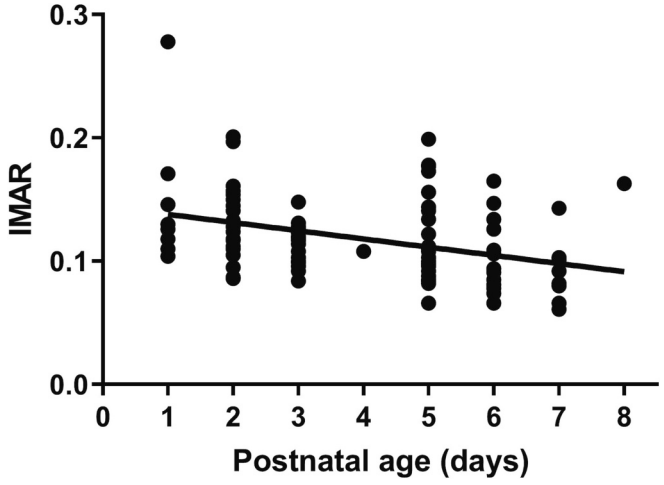

Fig. 2. IMAR in the first week after birth of all samples.
IMAR: ischemia modified albumin / albumin ratio.

3.3. Patient related variables and conditions associated with hypoxia and IMAR

IMAR was not significantly different between males and females in either sample. Gestational age correlated negatively with IMAR in the first sample but not in the second (Table 2). When Apgar score at 1 and 5 min was lower, IMAR-levels were higher in both samples (Table 2). SNAPPE-II score correlated positively with IMAR in the first and second sample (Table 2). The median two-hour mean SpO_2 was 95 % (79–100 %) in the first sample and 95 % (87–100 %) in the second sample. Lower median SpO_2-levels correlated with higher IMAR-levels in both the first and second sample (Table 2). IMAR was not significantly different in infants with acidosis of the umbilical cord in the first sample (0.12 [0.09–0.15], n=6 vs. 0.12 [0.08–0.28], n=46, P=0.599) and second sample (0.10 [0.07–0.018], n=5 vs. 0.10 [0.06–0.20], n=50, P=0.703), nor in infants with acidosis in the first (0.13 [0.08–0.28], n=16 vs. 0.12 [0.09–0.20], n=35, P=0.383) and second sample (0.11 [0.06–0.20], n=16 vs. 0.10 [0.07–0.17], n=39, P=0.251). IMAR was also not significantly different between infants who died and survived during NICU admission (Table 2).

Regarding organ oxygenation, r_cSO_2 did not correlate with IMAR in the first sample, but was negatively correlated with IMAR in the second sample (Table 2). r_sSO_2 did not correlate with IMAR in both samples (Table 2). However, when variability of r_sSO_2 was lower, IMAR was significantly higher in the first (rho: −0.785, n=14, P=0.001) and second sample (rho: −0.773, n=11, P=0.005) (Table 2).

In the first and second sample IMAR was higher in hsPDA infants than infants with no PDA (P=0.005, P=0.026) (Fig. 3). IMAR was not significantly different in infants with and without sepsis in both the first (P=0.423) and second sample (P=0.589). IMAR was higher in infants who were small for gestational age than in those who were not, but only in the second sample (P=0.003), and not in the first (P=0.092). NEC was diagnosed in 12 (19 %) infants at a median postnatal age of 13 days after birth (range 5–39). IMAR was not different between infants who later on developed NEC and who did not develop NEC for both the first (P=0.174) and second sample (P=0.725). Neurodevelopmental outcome at 23–33 months corrected age, was not associated with IMAR in both the first and second sample (Table 2). Remarkably, the infant with the highest IMAR level was unable to complete the Bayley-III-NL test due to major neurodevelopmental problems.

3.4. Regression analyses: factors associated with IMAR

Of all variables which were significantly correlated with IMAR; SNAPPE-II score, SpO_2, variability of r_sSO_2, small for gestational age, and hsPDA remained significantly associated with IMAR after correction for gestational age, postnatal age on the day of blood collection, the lipemia index, and the haemolytic index (Table 3). This means that with every 1 point increase in SNAPPE-II score, IMAR increases approximately 0.001 point in both samples, while with every 1 % increase in SpO_2, IMAR decreases with approximately 0.005 in the first sample. Due to the limited sample size, variability of r_sSO_2 was only adjusted for gestational age. R_cSO_2 did not significantly correlate with IMAR in univariate linear regression analyses and was therefore not included in the multiple regression model (Table 3).

4. Discussion

In this exploratory pilot study we demonstrated that IMA is a stable marker and when adjusted for serum albumin level (IMAR), it is related to several markers and conditions associated with hypoxia in preterm infants. Moreover, we found that several analytical variables affect IMA. The total amount of albumin, the lipemia index and to a lesser extent the haemolytic index of the sample should therefore be taken into account when measuring IMA.

In this study we investigated if IMA could be a reliable marker in preterm infants. First of all we found that IMAR levels showed a relatively stable course in the first week after birth. In the first four days

Table 2
Correlations between IMAR and patient related variables.

Variable		Sample 1			Sample 2		
		rho	n	P-value	rho	n	*P*-value
Gestational age		−0.538	51	<0.001‡	−0.190	55	0.164
Apgar score 1 min		−0.466	49	0.001**	−0.348	54	0.010*
Apgar score 5 min		−0.412	49	0.003**	−0.295	55	0.029*
pH umbilical cord		−0.072	46	0.635	−0.207	49	0.153
SNAPPE-II score		0.596	51	<0.001‡	0.561	55	<0.001‡
SpO_2		−0.497	44	0.001**	−0.280	55	0.038*
pH		−0.212	28	0.279	−0.068	36	0.692
R_cSO_2		−0.286	28	0.141	−0.314	43	0.040*
R_sSO_2		0.374	13	0.209	0.500	11	0.117
Variability of r_sSO_2		−0.785	14	0.001**	−0.773	11	0.005**
Bayley-III cognition		−0.052	32	0.779	0.017	33	0.923
Bayley-III fine motor function		0.000	31	0.999	0.044	33	0.951
Bayley-III gross motor function		0.178	17	0.495	−0.307	18	0.215
Variable		Sample 1			Sample 2		
		Median (range)	n	P-value	Median (range)	n	P-value
SGA	Yes	0.14 (0.10–0.20)	10	0.092	0.15 (0.08–0.20)	10	0.003**
	No	0.12 (0.08–0.28)	41		0.09 (0.06–0.18)	45	
Sepsis	Yes	0.13 (0.12–0.15)	3	0.423	0.12 (0.06–0.20)	6	0.589
	No	0.12 (0.08–0.28)	48		0.10 (0.07–0.18)	49	
NEC	Yes	0.11 (0.09–0.16)	9	0.174	0.10 (0.07–0.15)	10	0.725
	No	0.13 (0.08–0.28)	42		0.10 (0.06–0.20)	43	
Death	Yes	0.15 (0.16–0.17)	2	0.056	0.09 (0.09–0.13)	3	0.929
	No	0.12 (0.11–0.14)	49		0.10 (0.09–0.13)	52	

R_cSO_2: regional cerebral oxygen saturation. R_sSO_2: regional splanchnic oxygen saturation. SGA: small for gestational age. NEC: necrotizing enterocolitis. Death: death within 14 days after birth.

* Indicates P-value < 0.05.

** Indicates P-values < 0.01.

‡ Indicates P-values < 0.001.

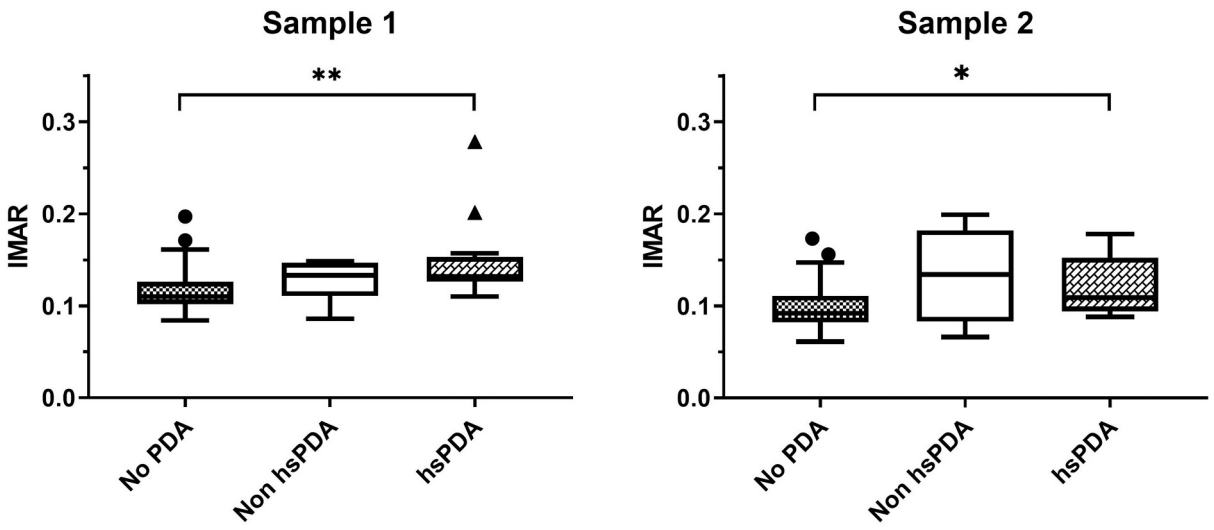

Fig. 3. Association of patent ductus arteriosus and ischemia modified albumin.
In the first sample, 29 infants were included in the no PDA group, 6 in the non hsPDA group and 16 in the hsPDA group. In the second sample, 37 infants were included in the no PDA group, 5 infants in the non hsPDA group, and 13 infants in the hsPDA group. IMAR: ischemia modified albumin / albumin ratio. PDA: patent ductus arteriosus. hsPDA: hemodynamic significant patent ductus arteriosus. *: $P < 0.05$, **: $P < 0.01$.

after birth, IMAR was approximately 20 % higher compared to the period between day five and eight after birth. This decrease in IMAR could be caused by the transition of a relative hypoxic fetal to a more hyperoxic neonatal circulation. Another explanation of the decrease of IMAR could be the increase of albumin with a constant IMA level during the first week after birth. The linear decrease suggest that IMAR could be a useful marker in preterm infants.

Second, we found that IMA is a relatively stable marker with a low intra- and inter assay variability. Nevertheless, we demonstrated that several analytical variables may affect the IMA-assay. The lipemia and haemolysis index were negatively correlated with IMA which may be caused by interference in the absorbance measurements of IMA. A high lipid concentration increases the absorbance between 500 and 600 nm and may therefore decrease the relative increase of the absorbance of the IMA-assay [22]. We speculate that a high degree of haemolysis may increase the degree of metals in the plasma which may interfere with the binding of cobalt and DTT and therefore underestimates the level of IMA [23]. Given the method to detect IMA through albumin, not surprisingly albumin was strongly correlated with IMA, warranting the use of the ratio with albumin. Although these indices may affect IMA-levels, these indices are easily measured, easily corrected for and are already commonly determined for several other assays.

Finally, we found several associations between IMAR and markers associated with hypoxia. We found associations between IMAR and SNAPPE-II score, SpO_2, and variability of r_sSO_2. We found that in the first sample IMAR was higher if the two-hour mean SpO2-levels before sampling were lower. This reversed association indicates that IMAR may indeed be a good marker for hypoxemia in preterm infants, because these infants generally have a less stable hemodynamic condition during transition the first days after birth. Regardless of the attempts to keep SpO2 within strict ranges, we still found a negative association between SpO2 and IMAR. A lower mean 2-h SpO2 may result from brief desaturations, i.e. due to apneas or insufficient oxygen supplementation, which may have resulted in a higher IMAR. Interestingly, although there was no relation between r_sSO_2 and IMAR, there was a strong inverse relation between variability of splanchnic oxygenation and IMAR. A lower variability in splanchnic oxygenation has been associated with a poorer, potentially hypoxic intestinal condition, and may be present as an early sign of a poor hemodynamic condition, when blood preferentially flows to the vital organs [17,24]. This may also explain why we did not find a relation with cerebral oxygenation. We also found a positive association between IMAR and SNAPPE-II score, which is a validated predictor of mortality and morbidity and includes several (indirect) markers of ischemia. Besides associations between IMAR and markers of hypoxia, we found an association between IMAR and a condition associated with hypoxia, a hsPDA. Infants with hsPDA had higher IMAR levels than infants without. This is in line with a study by Kahveci et al. in which they reported a higher level of IMA in infants with hsPDA than infants without PDA in the 24 h before treatment [25]. We speculate that IMAR can be used to detect hsPDA, which needs to be studied prospectively.

Overall, the association of IMAR and several markers and conditions associated with hypoxia suggest that IMA could be a useful marker for detection of hypoxia in preterm infants. However, other markers of hypoxia/ischemia were not associated with IMAR. For example, IMAR was not associated with acidosis measured in the two hours before or after the IMA sample was collected. We speculate that the lack of this association may be caused by a high variability of carbon dioxide in preterm infants which can lead to respiratory compensation of the metabolic acidosis [26]. Small for gestational age, which is often the result of chronic fetal hypoxia [27], was only related with IMAR in the second sample and not the first. As we also saw a trend towards a higher IMAR in infants with SGA in the second sample, we believe that the lack of statistical significance in the first sample may be caused by a lack of power. Furthermore, not all SGA infants are SGA as a result of fetal hypoxia [28]. Finally, IMAR was not higher in infants who later on developed NEC. Whether impaired intestinal oxygenation already exist in first week after birth or more towards NEC onset is still under debate [29,30]. Furthermore, because IMAR is not organ specific, it may not be sensitive enough to predict NEC.

In our study, neurodevelopmental outcome measured by the Bayley-III-NL, was not associated with IMAR measured in the first week after birth. Apparently, IMAR during the first week after birth is not a good marker for neurodevelopmental outcome at 23–33 months corrected age. We suggest that this is caused by the fact that other conditions that occurred after the first week after birth, such as intracerebral hemorrhage, sepsis or NEC, will also have had a large impact on neurodevelopmental outcome. Additionally, in our study there was a high frequency of loss of follow-up due to mortality, follow-up in other hospitals and refusal of parents to attend to the follow-up. Moreover, an unavoidable disadvantage of the Bayley-III-NL test is that children with major neurodevelopmental problems were unable to complete the test.

Table 3
Relation between ischemia modified albumin and other related variables using univariate and multiple linear regression analyses.

Variable		Unadjusted			Adjusted for GA			Adjusted for GA and PNA			Adjusted for GA, PNA,lipemia index, and hemolysis index		
	Sample	B	95 % CI	P-value	B	95 % CI	P-value	B	95 % CI	P-value	B	95 % CI	P-value
Apgar score at 1 min	1	−0.508	−0.861 to −0.156	0.006**	−0.425	−0.783 to −0.068	0.021*	−0.341	−0.687 to 0.004	0.053	−0.228	−0.588 to 0.132	0.208
	2	−0.383	−0.731 to −0.036	0.031*	−0.361	−0.717 to −0.005	0.047*	−0.327	−0.687 to 0.033	0.074	−0.155	−0.476 to 0.166	0.335
Apgar score at 5 min	1	−0.746	−1.263 to −0.228	0.006**	−0.669	−1.180 to −0.158	0.011*	−0.576	−1.068to −0.083	0.023*	−0.394	−0.911 to 0.123	0.131
	2	−0.402	−0.902 to 0.098	0.113	−0.399	−0.899 to 0.100	0.115	−0.356	−0.863 to 0.152	0.165	−0.204	−0.645 to 0.237	0.357
Gestational age (weeks)[a]	1	−0.693	−1.286 to −0.100	0.023*				−0.636	−1.193 to −0.078	0.026*	−0.464	−1.011 to 0.083	0.095
	2	−0.308	−0.886 to 0.270	0.290				−0.387	−0.976 to 0.202	0.193	−0.325	−0.850 to 0.201	0.220
SNAPPE-II score	1	0.085	0.052 to 0.117	<0.001‡	0.078	0.045 to 0.112	<0.001‡	0.072	0.040 to 0.104	<0.001‡	0.064	0.030 to 0.099	<0.001‡
	2	0.088	0.052 to 0.123	<0.001‡	0.087	0.050 to 0.123	<0.001‡	0.085	0.047 to 0.123	<0.001‡	0.060	0.020 to 0.100	0.004**
SpO2 (%)	1	−0.514	−0.706 to −0.323	<0.001‡	−0.480	−0.673 to −0.286	<0.001‡	−0.406	−0.611 to −0.202	<0.001‡	−0.361	−0.564 to −0.157	0.001‡
	2	−0.266	−0.541 to 0.009	0.058	−0.244	−0.543 to 0.055	0.108	−0.207	−0.521 to 0.107	0.192	−0.043	−0.323 to 0.237	0.761
R_cSO_2 (10 %) [b]	1	−0.385	−1.577 to 0.506	0.512									
	2	−1.024	−2.196 to 0.148	0.085									
Variability of r_sSO_2 (×100) [c]	1	−0.121	−0.183 to −0.059	0.001**	−0.121	−0.188 to −0.053	0.002**						
	2	−0.113	−0.179 to −0.046	0.004**	−0.117	−0.194 to −0.040	0.008**						
Small for gestational age	1	1.339	−1.024 to 3.702	0.260	1.811	−0.454 to 4.075	0.114	1.666	−0.459 to 3.792	0.122	1.346	−0.868 to 3.561	0.227
	2	3.971	1.891 to 5.943	<0.001‡	4.118	2.112 to 6.124	<0.001‡	4.314	2.345 to 6.283	<0.001‡	3.402	1.639 to 5.164	<0.001‡
hsPDA	1	2.797	0.947 to 4.646	0.004**	2.247	0.186 to 4.309	0.033*	2.292	0.380 to 4.203	0.020*	2.292	0.427 to 4.158	0.017*
	2	1.067	−0.826 to 2.960	0.263	0.786	−1.301 to 2.872	0.453	0.845	−1.232 to 2.922	0.418	0.787	−0.986 to 2.561	0.377

In this multiple linear regression, IMAR and variability of r_sSO_2 were multiplied by 100 for a better interpretation of the effect of the different variables.
[a] Not corrected for gestational age.
[b] Multivariate linear regression was not performed due to a P-value higher than 0.05 in the univariate linear regression.
[c] Corrected for one variable due to limited number of 14 infants with available data. CI: Confidence interval. GA: gestational age. PNA: postnatal age at the time of blood collection. R_cSO_2: regional cerebral oxygenation. R_sSO_2: regional splanchnic oxygenation. hsPDA: hemodynamic significant patent ductus arteriosus. PDA: patent ductus arteriosus.
* Indicates *P*-value < 0.05.
** Indicates P-values < 0.01.
‡ Indicates P-values < 0.001.

This resulted in a missing value of a neonate with the highest IMAR level. These missing values may all have biased the neurodevelopment outcome in relation to IMAR.

Several other studies also found associations between IMAR and conditions associated with hypoxia such as anaemia of prematurity, respiratory distress syndrome and late onset sepsis [31–33]. However, these studies did not correct IMA for individual albumin levels which may be essential due to the nature of the IMA-assay in which IMA is measured indirectly. Moreover, this study adds that also other analytical variables have to be accounted for. In our study, IMA and IMAR-levels were rather low compared to other studies in neonates [25,31,33]. This may be caused by differences in the IMA-assay such as a low sample volume or it may indicate that in our study preterm infants had a lower degree of hypoxia.

Strong aspects of our study are the facts that we presented both analytical and patient related associations with IMA(R), and that we presented associations with known hypoxic conditions and biochemical and clinical markers in a relatively large cohort of preterm infants. We recognize several limitations of this pilot study. This was a retrospective explorative study using remnant clinical blood samples, implying missing values and a potential selection bias. Second, in our hospital, pO_2 and lactate are not routinely measured in the first week after birth, which disabled us to compare IMA with those variables.

5. Conclusions

This study demonstrates that, taken several analytical variables into account such as albumin, the lipemia- and haemolysis index, IMAR has potential value as a marker for systemic hypoxia in preterm infants, considering the associations with SpO_2, variability of r_sSO_2, SNAPPE-II score and hsPDA. We propose to prospectively analyse the potential of IMAR as marker for hypoxia and hypoxic conditions in a larger population of preterm infants.

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CRediT authorship contribution statement

Martin van der Heide: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. **Anneke C. Muller Kobold:** Conceptualization, Methodology, Writing – review & editing, Investigation. **Karin K.R. Koerts-Steijn:** Conceptualization, Writing – review & editing, Investigation, Methodology. **Christian V. Hulzebos:** Conceptualization, Methodology, Writing – review & editing. **Jan B.F. Hulscher:** Writing – review & editing, Supervision. **Simon Eaton:** Investigation, Methodology, Writing – review & editing. **Michael Orford:** Investigation, Methodology, Writing – review & editing. **Arend F. Bos:** Supervision, Writing – review & editing. **Jan Koerts:** Conceptualization, Investigation, Methodology, Writing – review & editing. **Elisabeth M.W. Kooi:** Conceptualization, Formal analysis, Investigation, Methodology, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no competing interests.

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