

ORIGINAL ARTICLE

Amniotic fluid rapid biomarkers are associated with intra-amniotic infection in preterm pregnancies regardless of the membrane status

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OBJECTIVE: The objective of this study was to evaluate the association of amniotic fluid lactate dehydrogenase and glucose concentrations with microbial invasion of amniotic cavity and histologic chorioamnionitis before 37 pregnancy weeks in women with or without preterm premature rupture of membranes.

STUDY DESIGN: Amniocentesis was performed on 70 women with suspected intra-amniotic infection. Standard biochemical methods, molecular microbiology and culture techniques were used. Histopathological examination of the placenta was performed.

RESULTS: Thirty (48%) women had microbial invasion of the amniotic cavity (MIAC), 53 (76%) women had histological chorioamnionitis and 28 women had both. The cutoff values for MIAC and histological chorioamnionitis were 429 IU l⁻¹ for lactate dehydrogenase and 0.7 mmol l⁻¹ for glucose. Both end points occurred equally often regardless of the membrane status.

CONCLUSION: Increased amniotic fluid lactate dehydrogenase and decreased glucose were associated with MIAC and histological chorioamnionitis. However, test performance was of limited value.

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INTRODUCTION

Chorioamnionitis is closely associated with preterm delivery and neonatal morbidity and mortality, especially at an early gestational age.^{1–4} Early diagnosis of subclinical chorioamnionitis, that is, intra-amniotic infection (IAI), and optimal timing of delivery is a major obstetrical challenge. One manifestation of IAI is microbial invasion of the amniotic cavity (MIAC). Clinical chorioamnionitis occurs in only 5 to 10% of preterm deliveries,⁵ whereas silent MIAC and histologic chorioamnionitis (HCA) are much more common.^{6–8} HCA and MIAC do not necessarily coexist.⁹ Thus, rapid biomarkers to detect IAI would be useful in clinical practice.

The usefulness of a common, non-invasive biomarker to detect chorioamnionitis—maternal blood C-reactive protein—is limited in IAI.¹⁰ Thus, more accurate biomarkers are needed.

Previous studies have linked high concentration of amniotic fluid lactate dehydrogenase (am-LD) and low concentration of glucose (am-Gluc) with IAI or HCA.^{11–13} However, studies linking these biomarkers with MIAC detected by molecular microbiology techniques (am-PCR) are lacking.

We wanted to evaluate the association of am-LD and am-Gluc with both MIAC and HCA in singleton pregnancies with suspected chorioamnionitis with subsequent preterm birth regardless of membrane status at presentation.

MATERIALS AND METHODS

This prospective study was performed at the Department of Obstetrics and Gynecology, University Hospital, Helsinki, Finland between March 2010 and March 2015. One hundred and four singleton pregnancies between 22⁺⁰ and 36⁺⁶ weeks of gestation with amniocentesis performed by our study group members for suspected chorioamnionitis were recruited, including

women with or without preterm prelabor rupture of the membranes (PPROM). IAI was suspected in the presence of contractions and at least one of the following criteria: uterine tenderness, maternal fever, fetal tachycardia, malodorous discharge from cervix, increased C-reactive protein >10 mg l⁻¹ and total white blood cell count >20 × 10⁹ l⁻¹, or sludge at ultrasound examination in those with intact membranes (Table 1). We excluded women with amniocentesis-to-delivery interval >7 days (*n* = 34). PPRM was diagnosed by clinical speculum examination or a positive bedside dipstick test (ActimProm[®], Medix Biochemica, Espoo, Finland). Gestational age was determined at the first trimester (12⁺⁰ to 13⁺⁶ weeks of gestation) ultrasound screening. After exclusions, 70 women were eligible for the final analysis.

The laboratory tests performed included: am-Gluc, am-LD, am-16S rRNA sequencing (am-PCR) and amniotic fluid culture. Am-LD and am-Gluc were analyzed by using Modular P System (Roche Diagnostics, Penzberg, Germany) and according to IFCC (International Federation of Clinical Chemistry) recommendations. Am-LD was analyzed with a quantitative assay for the total enzymatic activity of lactate dehydrogenase (including the activity of all LD-isoenzymes, LD1-LD5) according to IFCC recommendations. The assay was developed for Roche automated clinical chemistry analyzers. The intra-assay coefficient of variation for am-LD was less than 2.3% and the detection limit of the assay was 5 IU l⁻¹. The detection limit of am-Gluc was 0.50 mmol l⁻¹. In the low concentration range (less than 4 mmol l⁻¹), the intra-assay coefficient of variation was 4.7%, and at concentration levels higher than 4 mmol l⁻¹ (linear range to 42 mmol l⁻¹), the intra-assay coefficient of variation was higher than 2.0%. Values of am-Gluc below 0.5 mmol l⁻¹ were recorded as 0 mmol l⁻¹ for statistical analysis. MIAC was defined as positive am-PCR or culture. Amniotic fluid bacterial culture result was available in 46 cases and am-PCR result in 59 cases. Both were available in 43 cases. An amniotic fluid sample was cultured for aerobic and anaerobic bacteria on chocolate blood agar in 5% CO₂ and on Fastidious Anaerobe Agar in anaerobic conditions at 35 ± 1 °C. A thioglycolate broth enrichment was used. The samples were in culture for 7 days and were inspected after 1, 2 and 7 days. The culture methods

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used enabled also the detection of common *Candida* species and *Mycoplasma hominis*, but not *Ureaplasma* species. A minimum of 500 μ l of amniotic fluid was subjected to ceramic bead-beating cell lysis

(Precellys24 tissue homogenizer, Bertin Technologies, Montigny le Bretonneux, France) followed by magnetic-bead-based DNA extraction method (NucliSENS kit with easyMAG automatic nucleic acid purification

Table 1. Signs and symptoms of intra-amniotic infection by membrane status

	PPROM	No PPRM	P-value
<i>n</i>	40	30	
Gestational age at amniocentesis median (range)	27.5 (22+5–34+4)	27.4 (23+1–36+5)	0.94
Gestational age at delivery median (range)	27.5 (23+2–34+4)	27.5 (23+2–36+6)	0.93
Maternal age median (range)	31 (21–44)	31,5 (18–39)	0.43
Nulliparity, <i>n</i> (%)	18 (45)	14 (47)	0.89
Uterine tenderness, <i>n</i> (%)	5 (12.5)	15 (50)	< 0.001
Maternal fever > 38 °C, <i>n</i> (%)	1 (2.5)	1 (3.3)	1.0
Maternal tachycardia > 100 b.p.m., <i>n</i> (%)	0	0	1.0
Fetal tachycardia > 160 b.p.m., <i>n</i> (%)	0	0	1.0
Malodorous discharge, <i>n</i> (%)	8 (20)	9 (30)	0.33
Maternal CRP > 10 mg l ⁻¹ , <i>n</i> (%)	34 (85)	29 (97)	0.23
Maternal WBC count > 20 × 10 ⁹ l ⁻¹ , <i>n</i> (%)	7 (17.5)	2 (6.7)	0.28
Sludge, <i>n</i> (%)	11 (27.5)	7 (23)	0.69

Abbreviations: b.p.m., beat per minute; CRP, C-reactive protein; PPRM, preterm prelabor rupture of membranes; WBC, white blood cell.

Table 2. Microbiological results and their association with amniotic fluid biomarkers, PPRM and HCA

Case	PPROM Yes/No	HCA Yes/No	Detected microbes		Biomarker concentrations	
			Culture	PCR	am-LD IU l ⁻¹	am-Gluc mmol l ⁻¹
<i>Culture-positive, PCR-positive</i>						
1	Yes	Yes	<i>Peptostreptococcus anaerobius</i> and <i>Streptococcus viridans</i>	<i>Peptostreptococcus anaerobius</i> and <i>Streptococcus viridans</i>	1911	0
2	No	Yes	<i>Candida albicans</i>	<i>Peptostreptococcus</i> species	2769	0.1
3	No	Yes	<i>Streptococcus pneumoniae</i>	<i>Streptococcus pneumoniae</i>	438	0
4	No	Yes	<i>Streptococcus viridans</i>	<i>Streptococcus viridans</i>	1355	0
5	No	Yes	<i>Escherichia coli</i>	<i>Fusobacterium nucleatum</i>	5276	0
6	Yes	Yes	<i>Candida albicans</i>	<i>Ureaplasma urealyticum/parvum</i>	1453	0
7	Yes	Yes	<i>Candida albicans</i>	<i>Ureaplasma urealyticum/parvum</i> , <i>Campylobacter ureolyticus</i>	4834	0
8	Yes	Yes	<i>Candida albicans</i>	<i>Candida albicans</i>	1941	0.7
<i>Culture-positive, PCR-negative</i>						
9	Yes	Yes	<i>Gardnerella vaginalis</i>	NA	4426	2.8
10	No	Yes	<i>Gardnerella vaginalis</i>	NA	1785	0
11	Yes	Yes	<i>Gardnerella vaginalis</i>	Negative	912	0
12	Yes	Yes	<i>Candida albicans</i>	Negative	1576	0
13	No	No	<i>Candida albicans</i>	Negative	1728	0.9
14	No	No	Coagulase-negative Staphylococcus	Negative	4344	0.4
<i>Culture-negative, PCR-positive</i>						
15	No	Yes	Negative	<i>Mycoplasma</i> species	1945	0
16	No	Yes	Negative	<i>Campylobacter ureolyticus</i>	5459	0.6
17	No	Yes	Negative	<i>Campylobacter ureolyticus</i> , <i>Fusobacterium nucleatum</i>	3241	0
18	Yes	Yes	Negative	<i>Streptococcus viridans</i>	330	1.8
19	Yes	Yes	Negative	<i>Hemophilus influenzae</i>	671	1.5
20	Yes	Yes	Negative	<i>Fusobacterium nucleatum</i>	292	0.7
21	Yes	Yes	Negative	<i>Ureaplasma urealyticum/parvum</i>	965	0.4
22	Yes	Yes	Negative	<i>Ureaplasma urealyticum/parvum</i>	1253	0
23	Yes	Yes	Negative	<i>Ureaplasma urealyticum/parvum</i>	1422	0
24	No	Yes	Negative	<i>Ureaplasma urealyticum/parvum</i>	482	3.4
25	No	Yes	Negative	<i>Ureaplasma urealyticum/parvum</i>	6385	0
26	Yes	Yes	Negative	<i>Ureaplasma urealyticum/parvum</i>	9698	0
27	Yes	Yes	Negative	<i>Ureaplasma urealyticum/parvum</i>	672	0
28	No	Yes	Negative	<i>Campylobacter ureolyticus</i>	860	0.9
29	Yes	Yes	NA	<i>Ureaplasma urealyticum/parvum</i>	91	1.2
30	No	Yes	NA	<i>Ureaplasma urealyticum/parvum</i>	341	0.7

Abbreviations: am-LD, amniotic fluid lactate dehydrogenase; am-Gluc, amniotic fluid glucose; HCA, histologic chorioamnionitis; NA, not available; PPRM, preterm prelabor rupture of membranes.

platform, bioMérieux, Marcy l'Etoile, France) as described by the manufacturer. The extracted DNA was amplified in duplicate by PCR using the following primers: 5'-TTG GAG AGT TTG ATC MTG GCT C-3' (forward) and 5'-GTA TTA CCG CGG CTG CTG-3' (reverse). DNA of λ -phage served as an inhibition control in the PCR reaction. Positive PCR product was verified by gel electrophoresis; 5 μ l of the PCR product was sequenced in a core facility, and the obtained sequence was compared with NCBI BLAST sequence database (www.ncbi.nlm.nih.gov/blast). Mixed sequences were analyzed by Ripseq mixed analysis tool (<https://www.ripseq.com/>) when appropriate.

Histopathological examination of the placenta ($n=70$) was performed by a placental pathologist. Placental sampling sites were the chorionic plate, umbilical cord and extraplacental membranes. Sections of tissue blocks were stained with hematoxylin and eosin. HCA was defined as a diffuse infiltration of inflammatory cells (mainly neutrophils) in any part of the tissue. Inflammatory changes in the umbilical cord (funisitis) was recorded separately. The outcome data were analyzed in relation to comparing the presence or absence of histological chorioamnionitis.

The results of am-LD, am-Gluc, am-PCR and microbial culture were available to the clinicians. The cutoff values of am-Gluc < 0.8 mmol I^{-1} and am-LD > 419 were considered consistent with IAI during the study period.¹¹

Women with PPROM were managed according to the local clinical guidelines. All patients with PPROM received intravenous antibiotics (Azithromycin 500 mg \times 1 and cefuroxime 1.5 g \times 3 for three consecutive days or until delivery in cases with PPROM before 33+0 gestational weeks. Cases with PPROM after 33+0 gestational weeks received cefuroxime 1.5 g \times 3 for three consecutive days or until delivery). Women with PPROM had expectant management until 34 gestational weeks, after which the labor was induced, and earlier if symptoms and signs of clinical chorioamnionitis were present.

All calculations were carried out using Microsoft Statistical Package for Social Sciences (SPSS, Chicago, IL, USA) for Windows v22.0. Comparisons between proportions were performed with the Chi-square test or Fisher's exact test when the number of cases was under five. Because the data did not follow a normal distribution, data with continuous variables were analyzed with Mann-Whitney U test. The receiver operating characteristics curves were derived and area under the curve was determined. The sensitivity, specificity, positive predictive value and negative predictive value were derived. All statistical tests were two-sided. A P -value < 0.05 was considered statistically significant.

All patients provided a written informed consent for the study. The local Ethics Committee and the hospital district of Helsinki and Uusimaa approved the study protocol (75/03/03/2013).

RESULTS

Of the 70 women, 32 (45.7%) were primiparous. The mean (s.d.) age was 30.9 (± 5.9) years. The median (range) gestational age was 27.5 (range 22⁺⁵ to 36⁺⁵) weeks at the time of amniocentesis, and 27.5 (range 23⁺² to 36⁺⁶) weeks at the time of delivery. Forty (57%) women had PPROM.

Microbiologic results were available in 62 (87%) women. Of those, 30 (48%) had MIAC. Table 2 shows the specific amniotic fluid microbiologic findings. Both am-PCR and culture were positive in eight women. Six women had only culture positive, and 16 women had only am-PCR result positive. Of all women, 53 had HCA (76%). A total of 28 women (45%) had both MIAC and HCA. Funisitis occurred in 19 (36%) cases, and all had HCA.

Am-LD and am-Gluc concentrations in relation to MIAC are shown in Figures 1 and 2. The median concentrations of am-LD were higher in those with MIAC than in those without MIAC (1514.5 IU I^{-1} (range from 91 to 9698 IU I^{-1}) vs 694 IU I^{-1} (range from 62 to 3882 IU I^{-1}), $P=0.012$). The median concentrations of am-Gluc were lower among those with MIAC than among those without MIAC (0.05 mmol I^{-1} (range from 0 to 3.4 mmol I^{-1}) vs 1.1 mmol I^{-1} (range from 0 to 6 mmol I^{-1}), $P=0.002$). In the prediction of MIAC and HCA, the cutoff value of am-LD based on receiver operating characteristic curve was 429 IU I^{-1} for both. The corresponding value for am-Gluc was 0.7 mmol I^{-1} (Figures 3

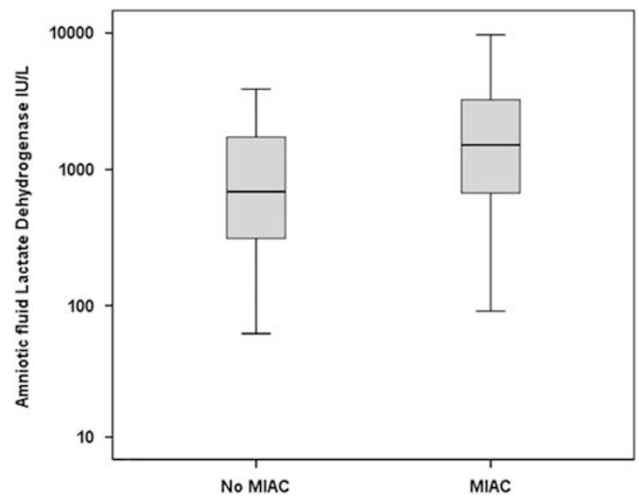


Figure 1. The median concentrations of am-LD were higher in those with MIAC than in those without MIAC (1514.5 IU I^{-1} (range from 91 to 9698 IU I^{-1}) vs 694 IU I^{-1} (range from 62 to 3882 IU I^{-1}), $P=0.012$).

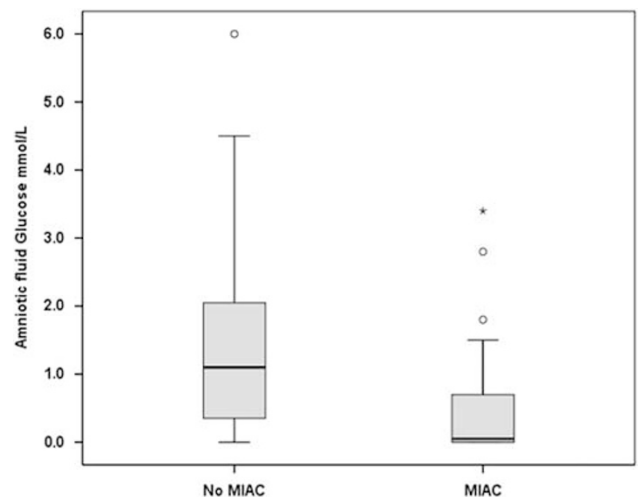


Figure 2. The median concentrations of am-Gluc were lower in those with MIAC than in those without MIAC (0.05 mmol I^{-1} (range from 0 to 3.4 mmol I^{-1}) vs 1.1 mmol I^{-1} (range from 0 to 6 mmol I^{-1}), $P=0.002$).

and 4). The overall performance of these cutoff values for MIAC and HCA is shown in Table 3. The concomitant use of both thresholds for am-LD (> 429 IU I^{-1}) and am-Gluc (< 0.7 mmol I^{-1}) were associated with MIAC (odds ratio 3.8, 95% confidence interval 1.3 to 10.9) and HCA (odds ratio 5.9, 95% confidence interval 1.5 to 23.0). If both biomarkers were normal (am-LD < 429 IU I^{-1} and am-Gluc ≥ 0.7 mmol I^{-1}), MIAC occurred in 6/19 (32%) cases and HCA in 13/23 (56%) cases.

The overall rates of MIAC or HCA did not differ between those with or without PPROM (for MIAC 43 vs 56%, $P=0.32$; for HCA 78 vs 73%, $P=0.69$). The median concentrations of am-LD (1092 IU I^{-1} vs 1297 IU I^{-1} , $P=0.64$) and am-Gluc (0.7 mmol I^{-1} vs 0.6 mmol I^{-1} , $P=0.5$) did not differ either. Both in cases with HCA and in cases without HCA, the membrane status did not have any effects on the rate of MIAC or the am-LD and am-Gluc concentrations (Table 4).

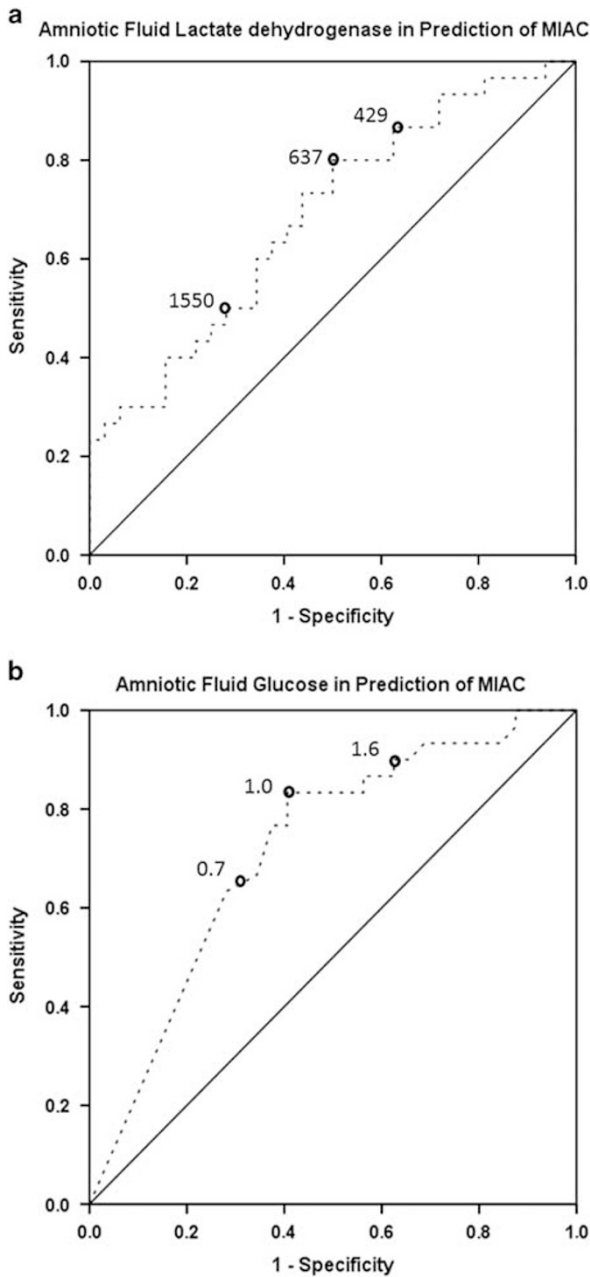


Figure 3. (a and b) Receiver operating characteristic (ROC) curves of amniotic fluid lactate dehydrogenase (am-LD) (a) and amniotic fluid glucose (am-Gluc) (b) for predicting microbial invasion of the amniotic cavity (MIAC). The most optimal cutoff value for am-LD was 429 IU l⁻¹ (AUC 0.69 (95% confidence interval (CI) 0.55 to 0.82)) and for am-Gluc 0.7 mmol l⁻¹ (AUC 0.72 (95% CI 0.59 to 0.85)). The sensitivity and 100-specificity points for am-LD values of 429, 637 and 1550 IU l⁻¹ and for am-Gluc values of 0.7, 1.0 and 1.6 mmol l⁻¹ are marked.

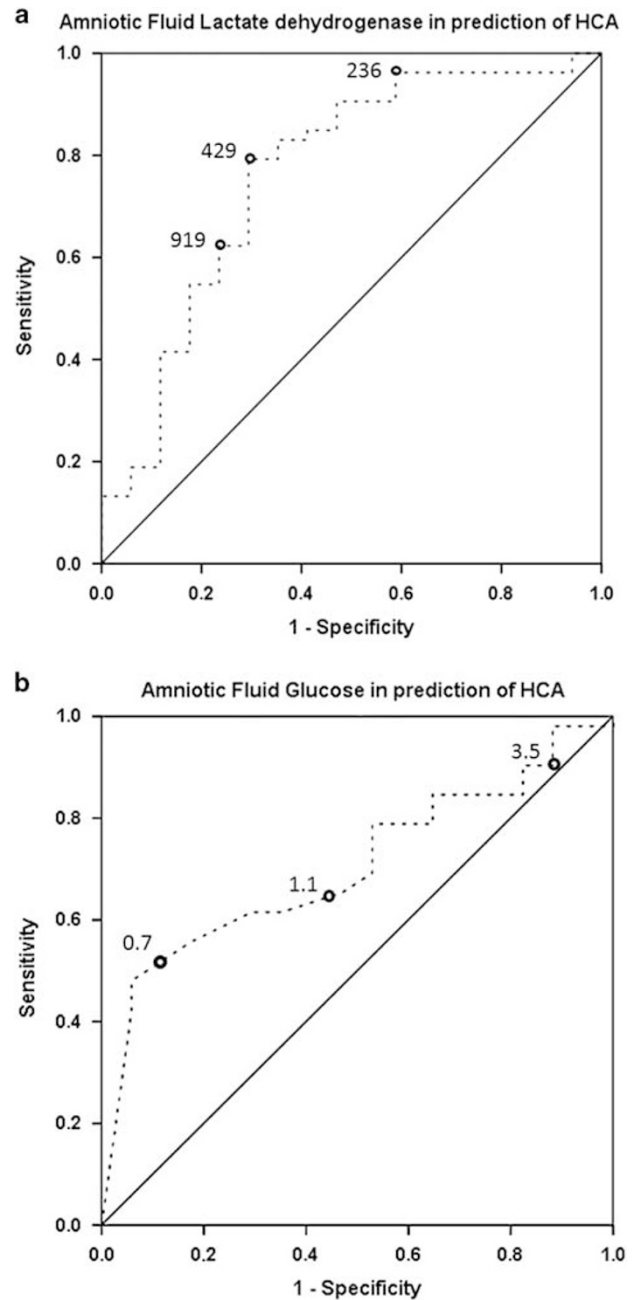


Figure 4. (a and b) Receiver operating characteristic (ROC) curves of amniotic fluid lactate dehydrogenase (am-LD) (a) and amniotic fluid glucose (am-Gluc) (b) for predicting histologic chorioamnionitis (HCA). The most optimal cutoff value for am-LD was 429 IU l⁻¹ (AUC 0.76 (95% confidence interval (CI) 0.62 to 0.9)) and for am-Gluc 0.7 mmol l⁻¹ (AUC 0.70 (95% CI 0.58 to 0.83)). The sensitivity and 100-specificity points for am-LD values of 236, 429 and 919 IU l⁻¹ and for am-Gluc values of 0.7, 1.1 and 3.5 mmol l⁻¹ are marked.

DISCUSSION

Our principal finding was that increased am-LD and decreased am-Gluc concentrations were associated with MIAC and HCA regardless of the membrane status. The use of both am-PCR and bacterial culture almost doubled the number of MIAC cases we found. The rate of MIAC was high regardless of the membrane status, higher than in one previous study,¹⁴ although in the present study, most women were not asymptomatic. Therefore,

amniocentesis is indicated also in women with intact membranes with nonspecific symptoms and signs. Despite the fact that am-LD levels were significantly higher and am-Gluc levels lower in cases with MIAC and HCA, these markers were not accurate enough to augment clinical decision-making. Nevertheless, the high positive predictive value of the am-LD and am-Gluc combination for HCA might justify the use of the combination as a provisional diagnostic tool for IAI.

Table 3. The accuracies of am-LD >429 IU l⁻¹, am-Gluc <0.7 mmol l⁻¹ and their combination for the MIAC and HCA

	MIAC (n = 30)			HCA (n = 52)		
	am-LD >429 IU l ⁻¹	am-Gluc <0.7 mmol l ⁻¹	Combined	am-LD >429 IU l ⁻¹	am-Gluc <0.7 mmol l ⁻¹	Combined
n	24	20	20	39	29	29
Sensitivity	87	67	67	83	56	56
Specificity	38	66	66	65	82	82
PPV	57	65	65	88	91	91
NPV	75	66	66	55	38	38

Abbreviations: am-LD, amniotic fluid lactate dehydrogenase; am-Gluc, amniotic fluid glucose; HCA, histologic chorioamnionitis; MIAC, microbial invasion of the amniotic cavity; NPV, negative predictive value; PPV, positive predictive value.

Table 4. Amniotic fluid lactate dehydrogenase (am-LD) and amniotic fluid glucose (am-Gluc) association with preterm prelabor rupture of membranes (PPROM) in patients with or without histologic chorioamnionitis (HCA)

	PPROM+	PPROM-	P-value
HCA			
n	31	22	
am-LD IU l ⁻¹ , median (range)	1422 (83–16246)	1585.5 (247–6385)	0.470
am-Gluc mmol l ⁻¹ , median (range) ^a	0.55 (0–6.0)	0.05 (0–3.4)	0.386
MIAC, n (%) ^b	16 (55.2)	12 (63.2)	0.301
No HCA			
n	10	7	
am-LD IU l ⁻¹ , median (range)	349 (62–3170)	259 (125–4344)	0.669
am-Gluc mmol l ⁻¹ , median (range) ^a	1.1 (0–4.5)	1.25 (0.4–1.9)	0.887
MIAC, n (%) ^b	0	2 (33.3)	

Abbreviations: am-LD, amniotic fluid lactate dehydrogenase; am-Gluc, amniotic fluid glucose; HCA, histologic chorioamnionitis; MIAC, microbial invasion of the amniotic cavity; PPROM, preterm prelabor rupture of membrane. ^amissing n = 1 HCA, PPROM+. ^bmissing n = 8: (HCA: PPROM+ n = 2, PPROM- n = 3 and no HCA: PPROM+ n = 1, PPROM- n = 2).

We acknowledge the relatively small sample size. However, the clinical setting strengthens the data and the results. Antibiotics, tocolysis and glucocorticoids were universally used according to the uniform clinical guidelines. Therefore, we did not specifically analyze the role of these potential confounding factors on the biomarker levels.

The strength of the study was the use of both am-PCR and bacterial culture instead of culture only. Most earlier studies have used only bacterial culture or Gram stain to detect MIAC.¹⁵ By am-PCR, we were able to detect cases not detected by culture.¹⁶ The overall number of MIAC cases found nearly doubled. In the future, larger studies with am-PCR, culture and placental histopathological examination are certainly needed.

Some women had HCA in the absence of MIAC. However, we did not study the role of viral infections. This or other inflammatory conditions, such as so called sterile inflammation, could explain some of the cases.^{17–19}

Clinical chorioamnionitis is only the tip of the iceberg of all IAI cases. Most cases are subclinical.¹ Amniocentesis remains the most accurate test to detect such cases. In our study, three quarters of clinically suspected IAI patients had HCA and almost half had intact membranes. On the other hand, 1 in 10 symptomatic patients had neither HCA nor PPROM. In such situations, the result of amniocentesis provides additional information and augments clinical decision-

making. Previous observational studies have already suggested that implementation of amniocentesis in the management of the preterm labor and PPROM is important.²⁰ The biomarker results may improve neonatal outcome,^{21–24} which is the ultimate goal of our approach. Amniocentesis is an invasive but safe procedure.^{25,26} If PPROM occurs following amniocentesis, it may in fact reflect infection or inflammation in the amniotic membranes at the time of amniocentesis and not necessarily be a complication of the procedure.²⁷

One major problem in current clinical practice is the lack of highly sensitive and specific biomarkers for MIAC and HCA. Am-LD reflects the inflammation and neutrophil activation in the amniotic cavity. Garry *et al.*²⁸ were the first to describe that am-LD exceeding 419 IU l⁻¹ predicts MIAC. This cutoff value has also been used in other studies.^{13,29,30} Our cutoff was almost similar. In a previous study by Kidokoro *et al.*,³¹ the optimal cutoff value for HCA was 250 IU l⁻¹. They performed amniocentesis between 16 and 35 gestational weeks which may have affected the results.³¹ In our study, the sensitivity and positive predictive value of am-LD for HCA were relatively high, but the negative predictive value was still low which is an obvious limitation.

Am-Gluc has been previously linked to MIAC with values less than 0.8 mmol l⁻¹ (refs. 11, 32) and to HCA with values less than 1.1 to 1.6 mmol l⁻¹.³³ In our study, low am-Gluc concentration predicted HCA with lower cutoff value than previously used. We found that am-Gluc was not a sensitive marker for HCA. This is in line with one previous study.³⁴

HCA can occur with MIAC (infectious) or without MIAC (sterile).^{19,35} In sterile inflammation, am-Gluc concentration may stay within the normal range.³⁴ We found that am-Gluc was associated with MIAC only in the presence of HCA.

Over half of those with normal am-LD and normal am-Gluc had HCA and almost one-third had MIAC. This is the subgroup for which more accurate biomarkers of inflammation are desperately needed.³⁶

CONCLUSION

MIAC and HCA occurred equally often regardless of the membrane status. Increased am-LD and decreased am-Gluc levels were associated with MIAC and HCA, but the diagnostic accuracy was limited. Better biomarkers for identifying inflammatory changes potentially harmful to the fetus are needed.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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