

Original Article

Necrotizing funisitis is an indicator that intra-amniotic inflammatory response is more severe and amnionitis is more frequent in the context of the extension of inflammation into Wharton's jelly

Ji Su Seong ^a, Chan-Wook Park ^{a, b, *}, Kyung Chul Moon ^c, Joong Shin Park ^a,
Jong Kwan Jun ^{a, b}

^a Department of Obstetrics and Gynecology, Seoul National University College of Medicine, Seoul, South Korea

^b Institute of Reproductive Medicine and Population, Seoul National University Medical Research Center, South Korea

^c Department of Pathology, Seoul National University College of Medicine, Seoul, South Korea

ARTICLE INFO

Article history:

Accepted 27 May 2021

Keywords:

Necrotizing funisitis

Intra-amniotic inflammatory response

Amnionitis

Inflammation into Wharton's jelly

ABSTRACT

Objective: Necrotizing funisitis (NF) is defined as the presence of an arc (i.e., crescent/band/ring/halos) of infiltrated neutrophils and/or associated debris in Wharton's jelly (WJ) of umbilical-cord (UC). However, no information exists about the comparison in intra-amniotic inflammatory-response (IAIR) and inflammation in extra-placental membranes between the presence and absence of NF in the context of inflammation in WJ among spontaneous preterm births (PTBs). The objective of current study is to examine this issue.

Materials and methods: We examined IAIR and the frequency of amnionitis according to the progression of inflammation in UC (i.e. stage-1, umbilical phlebitis [inflammation in umbilical-vein(UV)] only; stage-2, involvement of at least one umbilical-artery(UA) and either the other UA or UV without extension into WJ; stage-3, the extension of inflammation into WJ without NF; stage-4, the extension of inflammation into WJ with NF) in 120 singleton spontaneous PTBs (<37weeks). IAIR was gauged by AF MMP-8 (ng/ml) within 3days before birth.

Results: 1) Stage-1, stage-2, stage-3, and stage-4 were present in 20%(24/120), 6%(7/120), 61%(73/120), and 13%(16/120) of cases respectively; 2) AF MMP-8 continuously increased (stage-1 vs. stage-2 vs. stage-3 vs. stage-4; median[ng/ml], range[ng/ml]; 207.2[16.8–1196.5] vs. 444.1[8.5–2608.0] vs. 458.8[0.4–3116.7] vs. 1859.7[912.3–5304.8]; Spearman's rank correlation-test, $\alpha = 0.454$, $P = 0.006$), and the frequency of increased AF MMP-8 (≥ 854.1 ng/ml) elevated (stage-1 vs. stage-2 vs. stage-3 vs. stage-4; 13%[1/8] vs. 33%[1/3] vs. 32%[6/19] vs. 100%[5/5]; Linear-by-linear-association, $P = 0.012$) with the progression of inflammation in UC; 3) Moreover, there was a stepwise increase in the frequency of amnionitis according to the progression of inflammation in UC (stage-1, 33%[8/24]; stage-2, 43%[3/7]; stage-3, 62%[45/73]; stage-4, 81%[13/16]; Linear-by-linear-association, $P = 0.001$).

Conclusion: NF is an indicator that IAIR is more severe and amnionitis is more frequent in the context of the extension of inflammation into WJ. Therefore, current study confirms that NF is the most advanced stage in the progression of inflammation within UC.

© 2021 Taiwan Association of Obstetrics & Gynecology. Publishing services by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

Ascending intra-uterine infection (AIUI) is one of the important pathophysiologies for preterm birth (PTB) due to either preterm

labor and intact membranes (PTL) or preterm premature rupture of membranes (preterm-PROM) [1,2]. AIUI sequentially progresses as follows: 1) firstly, vaginal-cervical canal (i.e., vaginitis and cervicitis); 2) chorio-decidua (CD) of extra-placental membranes (EPM) (i.e., choriodecidualitis); 3) amnion of EPM (i.e., amnionitis) and intra-amniotic cavity (i.e., intra-amniotic inflammatory response [IAIR]); 4) finally, fetus (i.e., inflammation in umbilical cord [UC]) [1,2]. Inflammation in UC called as funisitis is the most advanced stage of AIUI and the histologic hallmark of fetal inflammatory syndrome (FIRS) defined as an elevated UC blood IL-6 (>11 pg/ml)

* Corresponding author. Department of Obstetrics and Gynecology, Seoul National University College of Medicine, Seoul, 03080, South Korea. Fax: +82 2 762 3599.

E-mail address: hwpark0803@gmail.com (C.-W. Park).

Abbreviation list

AF	amniotic fluid
AIUI	ascending intra-uterine infection
CD	chorio-decidua
DAMPs	danger-associated molecular-patterns
EPM	extra-placental membranes
FIR	fetal inflammatory response
FIRS	fetal inflammatory response syndrome
GA	gestational age
H&E	hematoxylin and eosin
IAIR	intra-amniotic inflammatory response
MMP-8	matrix metalloproteinase-8
NF	necrotizing funisitis
Preterm-PROM	preterm premature rupture of membranes
PTB	preterm birth
PTL	preterm labor and intact membranes
ROC	receiver operating characteristics
UA	umbilical artery
UC	umbilical cord
UV	umbilical vein
WJ	Wharton's jelly

or CRP (≥ 200 ng/ml) [3,4]. Of note, among the progression of inflammation in UC, necrotizing funisitis (NF) is defined as the presence of an arc (i.e., band, ring, halos) of infiltrated neutrophils and/or associated debris in Wharton's jelly (WJ) around one or more umbilical vessels, while the extension of inflammation into WJ is diagnosed as the presence of infiltrated neutrophils in perivascular WJ and further extending deep into WJ [5,6]. Neutrophils in amniotic fluid (AF) could be considered to be a signature of fetal inflammatory response (FIR) [4,7]. Indeed, neutrophils in AF were reported to be of fetal origin in about two-thirds of cases with intra-amniotic infection or inflammation [8]. Of note, neutrophils in AF can release matrix metalloproteinase-8 (MMP-8) into AF [8,9] thereby suggesting that IAIR gauged by AF MMP-8 can be a surrogate marker for FIR. Moreover, amnionitis in the progression of inflammation in EPM may be another surrogate marker for FIR, considering that amnionitis is an independent risk factor for early onset neonatal sepsis and an indicator that FIR and IAIR are more likely and severe [10].

Up to now, there are various classifications for the progression of inflammation in UC (Table 1) [8,10–28]. However, as shown in Table 1, we cannot find any classification including both 'the extension of inflammation into WJ' and 'NF' in the progression of inflammation in UC. Indeed, '2015 Amsterdam placental workshop group consensus statement' [5,6] did not consider the presence or absence of inflammation in WJ, while 'modified Salafia's criteria' [29] did not examine NF as in the following: 1) 2015 Amsterdam placental workshop group consensus statement; stage 1, neutrophil infiltration within the umbilical vein (UV) wall with or without extension into WJ; stage 2, neutrophil infiltration within at least one umbilical artery (UA) and either the other UA or UV with or without extension into WJ; stage 3, the presence of NF (Fig. 1a and Table 2), and 2) modified Salafia's criteria; stage 0, inflammation free UC; stage 1, umbilical phlebitis; stage 2, involvement of at least one UA and either the other UA or UV without extension into WJ; stage 3, the extension of inflammation into WJ (Fig. 1b and Table 2). Moreover, although two previous studies examined surrogate markers for FIR such as IAIR [17] and amnionitis [13] according to the progression of inflammation in UC, they did not include NF (Table 1). Given that the density of fetal neutrophils extending into

WJ is generally higher in cases with NF than in those without NF, it is plausible that NF is associated with more severe IAIR and more frequent amnionitis in the context of inflammation in WJ. Our hypothesis was that IAIR and amnionitis would be more severe and frequent in cases with NF than in those without NF in the context of inflammation in WJ. The objective of this study is to examine this issue.

Materials and methods*Study design and patient population*

The study population included one hundred and twenty pregnant women who delivered at Seoul National University Hospital (SNUH) from December 1993 to March 2013 and met the following criteria: 1) singleton pregnancy; 2) gestational age (GA) at delivery: 20.0–36.6 weeks; 3) PTB due to either PTL (45 cases) or preterm-PROM (75 cases); 4) placental pathologic slides available; and 5) the presence of inflammation in UC. At our institution, trans-abdominal amniocentesis for the identification of intra-amniotic infection/inflammation and placental pathologic examination after delivery were routinely recommended and performed on all pregnant women hospitalized with either PTL or preterm-PROM. The relationship between AF MMP-8 concentrations and the progression of inflammation in UC was examined in 41 patients delivered within 3 days of amniocentesis. The criterion of amniocentesis-to-delivery interval was applied to preserve a meaningful temporal relationship between the results of AF studies and the pathologic findings of placenta obtained at delivery. PTL and preterm-PROM were diagnosed with previously published criteria [29]. Written informed consent was gained from the study population. The Institutional Review Board of our institute (SNUH) specifically approved current study.

Clinical characteristics and pregnancy outcomes

Clinical characteristics and pregnancy outcomes were investigated from the medical records. Data included maternal age, parity, GA at amniocentesis, cause of preterm delivery, gender of newborn, delivery mode, GA at delivery, birth weight, 1 min and 5 min Apgar scores, antenatal use of corticosteroids, antenatal use of antibiotics, and antenatal use of tocolytics.

Diagnosis of inflammation in umbilical cord (UC) and amnionitis

Placental tissue samples for pathologic examination included EPM (i.e., CD and amnion), chorionic plate, and UC. These samples were fixed in 10% neutral buffered formalin and embedded in paraffin. Sections of prepared tissue blocks were stained with hematoxylin and eosin (H&E). Clinical information regarding the placental tissues was not disclosed to pathologists. Inflammation in UC was diagnosed in the presence of neutrophil infiltration into the umbilical vessel walls or WJ according to previously published criteria [26], and NF was defined neutrophils with or without associated debris in concentric bands-rings-halos around one or more umbilical vessels according to previously reported criteria [5]. The progression of inflammation in UC was classified into four groups as follows; 1) stage-1: neutrophil infiltration within the UV wall; 2) stage-2: neutrophil infiltration within at least one UA and either the other UA or UV without extension into WJ; 3) stage-3: the extension of neutrophil infiltration into WJ without NF; 4) stage-4: the extension of neutrophil infiltration into WJ with NF. Amnionitis was diagnosed in the presence of one or more focus of at least five neutrophils in amnion according to the previously published criteria [26].

Table 1

Previous studies reporting the relationship among intra-amniotic inflammatory response (IAIR), advanced stage of inflammation in extra-placental membranes (EPM) (i.e., amnionitis) and the progression of inflammation in umbilical cord (UC).

Primary Author (year) [Reference number]	n	GA at delivery	Cause of PTB	Other inclusion criteria	Diagnostic criteria of inflammation in UC	Inclusion of either NF or inflammation of WJ	The relationship of IAIR with either funisitis or NF		The relationship of IAIR with the progression of inflammation in UC		The relationship of advanced stage of inflammation in EPM (i.e., amnionitis) with either funisitis or NF		The relationship of advanced stage of inflammation in EPM (i.e., amnionitis) with the progression of inflammation in UC	
							Funisitis	NF	Regardless of the inclusion of NF	Including NF	Funisitis	NF	Regardless of the inclusion of NF	Including NF
Jacques et al. (1992) [11]	45	18–40 wks	NA	NF (+)	1) mild; narrow, but still distinct, bands of neutrophils that usually unrounded only one or two of the blood vessels; 2) moderate; no information; 3) severe; typically marked calcification or wide bands of necrosis filled with neutrophils and cellular debris surrounding at least two blood vessels	NF (+) No comment about inflammation in WJ	NA	Not done	NA	NA	NA	Acute HCA was present in 100% of NF cases.	NA	NA (The severity of acute HCA did not correlate with the degree of NF.)
Romero R et al., (1992) [12]	92	22–36 wks	PTL	Delivery within 48h after amniocentesis	ref. [26] ^a	NF (–) WJ (+)	Not done	NA	Not done (AF cul [+]; [grade of inflammation in UC] : grade 0, 17.6% grade 1, 75% grade 2, 83.3% grade 3, 66.7% grade 4, 90% : P < 0.001 in chi-square for trend)	NA	The values indicate excellent agreement for readings from UC and amnion.	NA	Not done	NA
K. H. Van Hoesen et al. (1996) [13]	2899	NA	NA	–	Acute funisitis: stage 1; limited to the vein alone; stage 2; involving one or more arteries	NF (–) WJ (–)	Not done	NA	Not done	NA	^d Acute funisitis was more frequent with increasing stages of membrane inflammation.	N	^e Acute funisitis stage 2 was more frequent with increasing stages of membrane inflammation.	NA
Yoon BH et al., (2000) [14]	315	20–35 wks	PTL, preterm-PROM, MFI	Delivery within 3 days of amniocentesis (n = 106)	ref. [27] ^b	NF (–) WJ (+)	Not done (AF cul [+]; funisitis [–] < [+])	NA	NA	NA	Not done ^h	NA	NA	NA
Ohya et al., (2002) [15]	383	23–32 wks	NA	Clinically ill infant or grossly abnormal placenta	ref. [28] ^c	NF (+) WJ (–)	NA	Not done	NA	NA	NA	Not done	NA	NA
SE Lee et al. (2006) [16]	832	>37 wks	NA	Delivery within 72h of amniocentesis	ref. [27] ^b	NF (–) WJ (+)	AF WBC; funisitis(–) < (+)	NA	NA	NA	Not done ^h	NA	NA	NA
CW Park et al., (2008) [8]	139	<35 wks	PTL, preterm-PROM, MFI	Delivery within 72h of amniocentesis	ref. [27] ^b	NF (–) WJ (+)	AF MMP-8 kit(+), AF IL-6 & AF WBC; funisitis(–) < (+)	NA	NA	NA	Not done ^h	NA	NA	NA
Buhimschi et al. (2009) [17]	132	≥23.1 wks	PTL, preterm-PROM	–	ref. [26] ^a	NF (–) WJ (+)	^f funisitis; MR 0 < MR 1-2 < MR 3-4	NA	Increase in funisitis grade; ^f MR 0 < MR 1-2 < MR 3-4	NA	Not done	NA	NA	NA

CW Park et al., (2009) [10]	290	<36 wks	PTL, preterm-PROM, MFI	HCA	ref. [27] ^b	NF (–) WJ (+)	AF MMP-8 & WBC; funisitis(–) < (+)	NA	NA	NA	Amnionitis: funisitis(–) < (+)	NA	NA	NA
J. Lee et al. (2011) [18]	301	24–32 wks	iPTB, sPTB	–	ref. [27] ^b	NF (–) WJ (+)	Not done	NA	NA	NA	Not done ^h	NA	NA	NA
T. Cobo et al. (2012) [19]	107	23–36 wks	preterm-PROM	–	ref. [26] ^a	NF (–) WJ (+)	AF IL-8, IL-6, IL-10 sIL-6r, sTNF-RI, etc; funisitis(–) < (+)	NA	NA	NA	NA	NA	NA	NA
Lee SY et al. (2012) [20]	306	23–35 wks	PTL, preterm-PROM	Delivery within 72h of maternal CRP measurement	ref. [27] ^b	NF (–) WJ (+)	Not done	NA	NA	NA	Not done ^h	NA	NA	NA
M. Kacerovsky et al., (2012) [21]	115	24–37 wks	preterm-PROM	–	NA	NA	1) 24–32 wks funisitis; ^g MIAC(–) < (+) 2) 32–37 wks funisitis; ^g MIAC(–) = (+)	NA	NA	NA	NA	NA	NA	NA
S. M. Kim et al., (2015) [22]	412	24–35 wks	NA	Delivery within 120h of amniocentesis	ref. [27] ^b	NF (–) WJ (+)	Not done	NA	NA	NA	Not done	NA	NA	NA
JW Park et al., (2017) [23]	153	20–34 wks	PTL, preterm-PROM	Delivery within 48h of Amniocentesis	ref. [27] ^b	NF (–) WJ (+)	Not done	NA	NA	NA	Not done	NA	NA	NA
Musilova et al., (2018) [24]	159	34–37 wks	preterm-PROM	–	ref. [26] ^a	NF (–) WJ (+)	funisitis; [MIAC-IAI] : All (+) = IAI alone = MIAC alone = All (–)	NA	NA	NA	NA	NA	NA	NA
RJ Martinez-Portilla et al. (2019) [25]	47	26–37 wks	preterm-PROM	–	NA	NA	Not done	NA	NA	NA	Not done ⁱ	NA	NA	NA

AF, amniotic fluid; EPM, extra-placental membrane; GA, gestational age; HCA, histologic chorioamnionitis; IAI, intra-amniotic inflammatory; IAI, intra-amniotic inflammatory response; IL-8, interleukin-8; iPTB, indicated PTB; MFI, maternal–fetal indication; MIAC, Microbial invasion of the amniotic cavity; MR score, mass-restricted score; NA, not applicable; NF, necrotizing funisitis; PMN, polymorphonuclear leukocyte; preterm-PROM, preterm premature rupture of membranes; PTB, preterm birth; PTL, preterm labor; SNF, subacute necrotic funisitis; sIL-6r, soluble IL-6 receptor; sPTB, spontaneous PTB; sTNF-R1, soluble TNF receptor-1; UC, umbilical cord; WJ, Wharton's jelly.

^a Grade of inflammation in UC, grade 1: PMN within inner third of umbilical vein wall; grade 2: PMN within inner third of at least two umbilical vessel walls; grade 3: PMN in perivascular WJ; and grade 4: panvasculitis and funisitis extending deep into WJ.

^b Grade of inflammation in UC, grade 1: PMN infiltration confined to umbilical vessel walls; and grade 2: extension of PMN infiltration into WJ.

^c SNF: degenerating neutrophils, lymphocytes, monocytes, occasional plasma cells; and cord stripes (rings or crescents on cross section).

^d There is no exact data about the frequency of acute funisitis according to the stage of membrane inflammation.

^e There is no exact data about the frequency of acute funisitis stage 2 according to the stage of membrane inflammation.

^f MR score: a proteomic profile that is highly characteristic of intra-amniotic inflammation. (MR 0: no inflammation, MR 1–2: minimal inflammation, and MR 3–4: severe inflammation).

^g MIAC was defined as a positive polymerase chain reaction analyses for genital mycoplasmas and/or *C. trachomatis* and/or the growth of any bacteria in the amniotic fluid.

^h Inflammation in at least one placental compartment was more frequent in cases with funisitis than in those without funisitis.

ⁱ Funisitis was more frequent in cases with inflammation in membranes than in those without inflammation in membranes.

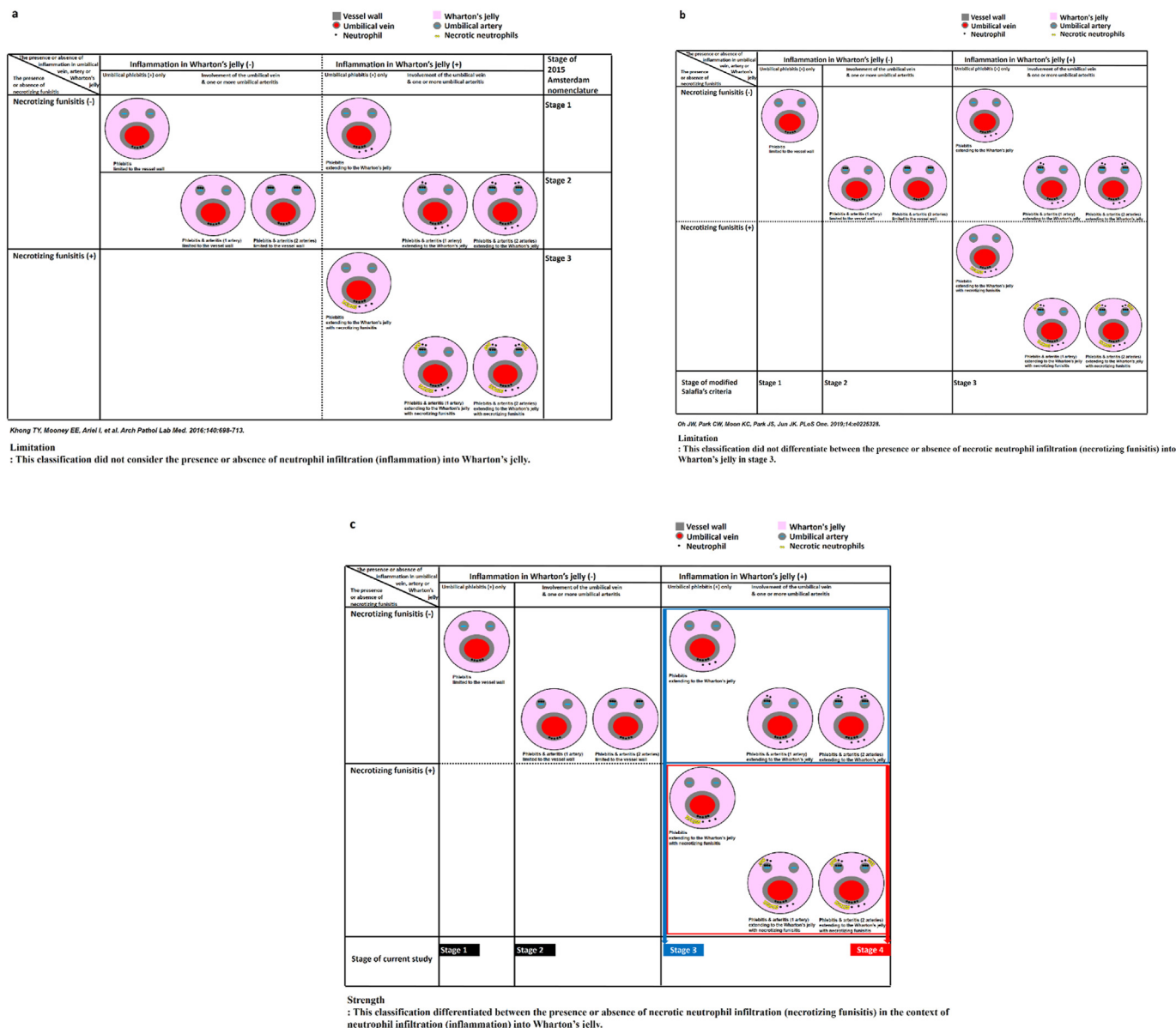


Fig. 1. Schema of classifications for the progression of inflammation in umbilical cord (UC) in previous studies and current study. 1) 2015 Amsterdam placental workshop group consensus statement (7, 8); stage 1, neutrophil infiltration within the umbilical vein (UV) wall with or without extension into Wharton's jelly (WJ); stage 2, neutrophil infiltration within at least one umbilical artery (UA) and either the other UA or UV with or without extension into WJ; stage 3, the presence of necrotizing funisitis (NF) (a); 2) modified Salafia's criteria (32); stage 0, inflammation free UC; stage 1, umbilical phlebitis [inflammation in UV] only; stage 2, involvement of at least one UA and either the other UA or UV without extension into WJ; stage 3, the extension of inflammation into WJ (b); and 3) the classification of current study; stage-1, umbilical phlebitis only; stage-2, involvement of at least one UA and either the other UA or UV without extension into WJ; stage-3, the extension of inflammation into WJ without NF; stage-4, the extension of inflammation into WJ with NF (c).

Amniotic fluid (AF) studies

AF was centrifuged and stored in polypropylene tubes at -70°C [9]. MMP-8 concentrations in stored AF were measured with a commercially available enzyme-linked immunosorbent assay (Amersham Pharmacia Biotech, Inc., Little Chalfont, Bucks). Details about this assay and its performance have been previously reported [9]. The intensity of IAIR was gauged by AF MMP-8 level (ng/ml) as previously described [9]. AF MMP-8 results were available in 35 patients because IAIR was not examined in 6 patients due to the limited amount of remained AF amount.

Statistical analysis

We used the Kruskal–Wallis test and Pearson's chi-square test for the comparison of continuous and categorical variables respectively. Multiple comparisons of continuous and categorical variables between the groups according to the progression of inflammation in UC were performed with 1-way ANOVA with posthoc Tukey test and Fisher's exact test with Bonferroni's correction, respectively. Spearman's rank correlation test was used to examine the correlation between AF MMP-8 concentrations and the progression of inflammation in UC. The receiver operating

Table 2

Previous classifications of the progression of inflammation in umbilical cord (UC).

Article [Reference]	Stage	Description	Remarks
Khong TY et al. (2016) [6]	1	Umbilical phlebitis with or without inflammation in WJ	* This classification did not consider the presence of absence of inflammation in WJ.
	2	At least one umbilical arteritis and/or either the other umbilical arteritis or umbilical phlebitis with or without inflammation in WJ	
	3	The presence of NF	
Oh JW et al. (2019) [29]	1	Umbilical phlebitis	* This classification did not consider the presence of absence of NF in inflammation in WJ.
	2	At least one umbilical arteritis and/or either the other umbilical arteritis or umbilical phlebitis	
	3	Inflammation in WJ	
Current study	1	Umbilical phlebitis	* This classification differentiated the presence of absence of NF in the context of inflammation in WJ.
	2	At least one umbilical arteritis and/or either the other umbilical arteritis or umbilical phlebitis	
	3	Inflammation in WJ without NF	
	4	Inflammation in WJ with NF	

WJ, Wharton's jelly; NF, necrotizing funisitis.

characteristics (ROC) curve was used to estimate the best cut-off values (maximum sum of sensitivity and specificity) at which to identify AF MMP-8 as being raised or not raised for the detection of NF. Using these cut-off values, we compared the frequency of increased AF MMP-8 and amnionitis according to the progression of inflammation in UC with Pearson's chi-square test. Moreover, linear by linear association was used to investigate the trend about the frequency of increased AF MMP-8 and amnionitis according to the progression of inflammation in UC. Diagnostics indices (sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, negative likelihood ratio) were determined for AF MMP-8 and amnionitis to identify NF. Multiple logistic regression analysis was performed for the exploration of the relationship between various variables and NF. Statistical significance was defined as $P < 0.05$.

Results

Clinical characteristics and pregnancy outcomes according to the progression of inflammation in umbilical cord (UC)

Table 3 demonstrated clinical characteristics and pregnancy outcomes according to the progression of inflammation in UC. Stage-1 (umbilical phlebitis only), stage-2 (involvement of at least one UA and either the other UA or UV without extension into WJ), stage-3 (the extension of inflammation into WJ without NF), and stage-4 (the extension of inflammation into WJ with NF) were present in 20.0% (24/120), 5.8% (7/120), 60.8% (73/120), and 13.3% (16/120) of cases respectively. GA at delivery was different without reaching statistical significance ($P = 0.133$), resulting in almost but not quite statistically significant difference in birth weight ($P = 0.054$) and Apgar score < 7 at 1 min ($P = 0.079$) closely related to GA at delivery among the groups according to the progression of inflammation in UC.

Amniotic fluid (AF) MMP-8 concentrations according to the progression of inflammation in umbilical cord (UC)

Fig. 2 shows AF MMP-8 concentrations (ng/ml) according to the progression of inflammation in UC. AF MMP-8 concentrations significantly and continuously increased according to the progression of inflammation in UC (Kruskal–Wallis test, $P = 0.0031$ and Spearman's rank correlation, $P = 0.006$).

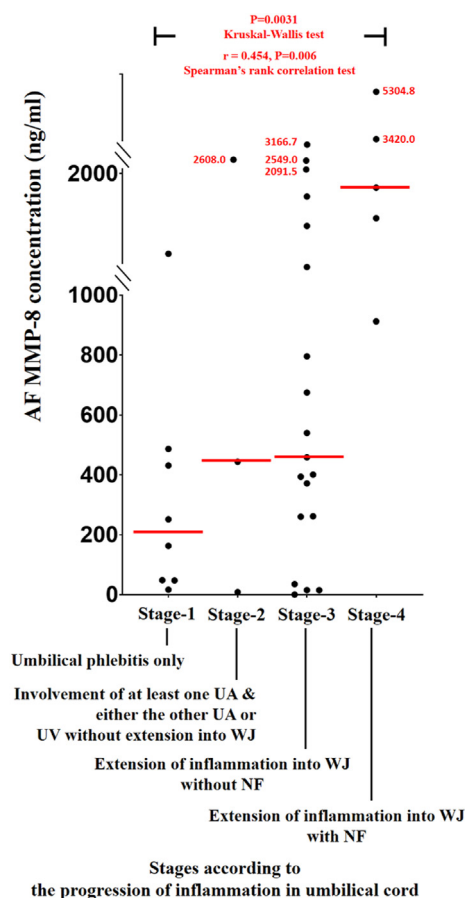


Fig. 2. Amniotic fluid (AF) MMP-8 concentrations (ng/ml) according to the progression of inflammation in umbilical cord (UC). (i.e., stage-1, umbilical phlebitis [inflammation in umbilical vein (UV)] only; stage-2, involvement of at least one umbilical artery [UA] and either the other UA or umbilical vein [UV] without extension into Wharton's jelly [WJ]; stage-3, the extension of inflammation into WJ without necrotizing funisitis [NF]; and stage-4, the extension of inflammation into WJ with NF). AF MMP-8 concentrations continuously increased according to the progression of inflammation in UC (stage-1 vs. stage-2 vs. stage-3 vs. stage-4; median [ng/ml], range [ng/ml]; 207.2 [16.8–1196.5] vs. 444.1 [8.5–2608.0] vs. 458.8 [0.4–3116.7] vs. 1859.7 [912.3–5304.8]). Each P value is shown in the graph. AF MMP-8 concentration results were available in 35 patients, and those were not examined in 6 patients due to the limited amount of remained AF amount.

Table 3

Clinical characteristics and pregnancy outcomes according to the progression of inflammation in umbilical cord (UC).

	Stage-1 ^g (n = 24)	Stage-2 ^g (n = 7)	Stage-3 ^g (n = 73)	Stage-4 ^g (n = 16)	P value ^a
	20.0% (24/120)	5.8% (7/120)	60.8% (73/120)	13.3% (16/120)	
Maternal age, year (mean ± SD)	32.8 ± 3.8	29.0 ± 3.2	32.1 ± 4.9	31.4 ± 4.3	NS (0.218)
Parity (≥1)	75.0% (18/24)	57.1% (4/7)	53.4% (39/73)	62.5% (10/16)	NS (0.312)
Median GA at amniocentesis, wks [range] ^f	32.7 [27.9–35.7]	29.0 [25.3–32.3]	29.1 [23.7–36.9]	27.5 [25.6–36.0]	NS (0.067)
Cause of preterm birth					NS (0.443)
Preterm-PROM	37.5% (9/24)	14.3% (1/7)	37.0% (27/73)	50.0% (8/16)	
PTL	62.5% (25/24)	85.7% (6/7)	63.0% (46/73)	50.0% (8/16)	
Male Newborn	50.0% (12/24)	85.7% (6/7)	45.2% (33/73)	43.8% (7/16)	NS (0.225)
Cesarean delivery	54.2% (13/24)	28.6% (2/7)	38.4% (28/73)	25.0% (4/16)	NS (0.265)
Median GA at delivery, wks [range]	33.5 [21.0–36.7]	29.1 [24.3–35.0]	30.1 [23.3–36.9]	29.4 [24.3–36.3]	NS (0.133)
Birth weight, g (mean ± SD)	1945 ± 705	1369 ± 606	1618 ± 683	1463 ± 611	NS (0.054)
1 min Apgar score of <7	33.3% (8/24)	71.4% (5/7)	61.6% (45/73)	50.0% (8/16)	NS (0.079)
5 min Apgar score of <7	20.8% (5/24)	28.6% (2/7)	31.5% (23/73)	31.2% (5/16)	NS (0.793)
Antenatal corticosteroids use ^b	52.2% (12/23)	57.1% (4/7)	70.8% (51/72)	75.0% (12/16)	NS (0.317)
Antenatal antibiotics use ^c	56.5% (13/23)	100% (7/7)	84.3% (59/70)	87.5% (14/16)	0.011 ^e
Antenatal tocolytics use ^d	39.1% (9/23)	57.1% (4/7)	60.0% (42/70)	68.8% (11/16)	NS (0.246)

GA, gestational age; NS, not significant; *preterm-PROM*: preterm premature rupture of membranes; *PTL*, preterm labor and intact membranes; *SD*, standard deviation.^a Intergroup difference by Chi-square test (categorical variables) and Kruskal–Wallis test (continuous variables).^b Of 120 cases, 118 patients were included in this analysis, because the information about antenatal corticosteroids use in the medical records was omitted in 2 patients.^c Of 120 cases, 116 patients were included in this analysis, because the information about antenatal antibiotics use in the medical records was omitted in 4 patients.^d Of 120 cases, 116 patients were included in this analysis, because the information about antenatal tocolytics use in the medical records was omitted in 4 patients.^e There was no significant difference in the frequency of antenatal antibiotics use between each group with the use of Fisher's exact test with Bonferroni's correction.^f Forty-one patients who underwent amniocentesis within 3 days of birth were included in this analysis to preserve a meaningful temporal relationship among the results of amniotic fluid studies and those of placental histologic examination and perinatal outcome.^g Stage-1, umbilical phlebitis [inflammation in umbilical vein (UV)] only. Stage-2, involvement of at least one umbilical artery (UA) and either the other UA or UV without extension into Wharton's jelly (WJ). Stage-3, the extension of inflammation into WJ without necrotizing funisitis (NF). Stage-4, the extension of inflammation into WJ with NF.

Diagnostic indices, predictive values, and likelihood ratios of increased AF MMP-8 concentration and amnionitis for the identification of necrotizing funisitis (NF)

A ROC curve was constructed to select the cut-off values at which to identify AF MMP-8 (area under curve, 0.887; standard error, 0.062; $P = 0.006$) as being raised or not raised for the identification

of NF, and a cut-off values of 854.1 for AF MMP-8 (ng/ml) was chosen respectively. Table 4 describes diagnostic indices, predictive values and likelihood ratios of AF MMP-8 concentration (≥ 854.1 ng/ml) and amnionitis to identify pregnant women with NF.

The frequency of increased AF MMP-8 concentration (≥ 854.1 ng/ml) and amnionitis according to the progression of inflammation in umbilical cord (UC)

Fig. 3a and b demonstrated a significant and gradual increase in the frequency of increased AF MMP-8 concentration (≥ 854.1 ng/ml) (Fig. 3a) and amnionitis (Fig. 3b) according to the progression of inflammation in UC (increased AF MMP-8 concentration [≥ 854.1 ng/ml], each for $P < 0.05$ in Pearson's chi-square test and linear-by-linear association; and amnionitis, each for $P < 0.05$ in Pearson's chi-square test and linear-by-linear association). Moreover, Table 5 demonstrated increased AF MMP-8 concentration (≥ 854.1 ng/ml) was a significantly independent risk factor for NF even after the correction for potential confounding variables including GA at delivery.

The progression of inflammation in umbilical cord (UC)

Fig. 4 shows representative images for umbilical phlebitis only (Fig. 4a, stage-1), involvement of at least one UA and either the other UA or UV without extension into WJ (Fig. 4b, stage-2), the

extension of inflammation into WJ without NF (Fig. 4c, stage-3), and the extension of inflammation into WJ with NF (Fig. 4d, stage-4) in H&E stained histologic sections of UC.

Discussion

Principal findings of the study

The principal finding of this study is that the IAIR and amnionitis, the surrogate markers for FIR, are more severe and frequent in cases with NF than in those without NF in the context of inflammation in WJ among spontaneous PTBs.

The limitations of previous studies reporting the relationship between surrogate markers for fetal inflammatory response (FIR) and the progression of inflammation in umbilical cord (UC) with or without necrotizing funisitis (NF)

Almost all studies except Buhimschi's report [17] did not examine the relationship between IAIR and the progression of the inflammation in UC with or without NF although substantial numbers of studies examined the association between IAIR and the presence of either funisitis or NF [8,10,16,17,19,21,24] (Table 1). Moreover, several studies only reported the relationship between amnionitis and the presence of either funisitis or NF [10–13], but not the progression of inflammation in UC regardless of the inclusion of NF. Although only one study reported that umbilical arteritis was more frequent with increasing stages of membrane inflammation, it did not include NF in the progression of inflammation in UC [13]. However, current study firstly demonstrated that surrogate markers for FIR (i.e., IAIR and amnionitis as an advanced stage of inflammation in EPM) continuously increased according to the progression of inflammation in UC including both 'the extension of inflammation into WJ' and 'NF'.

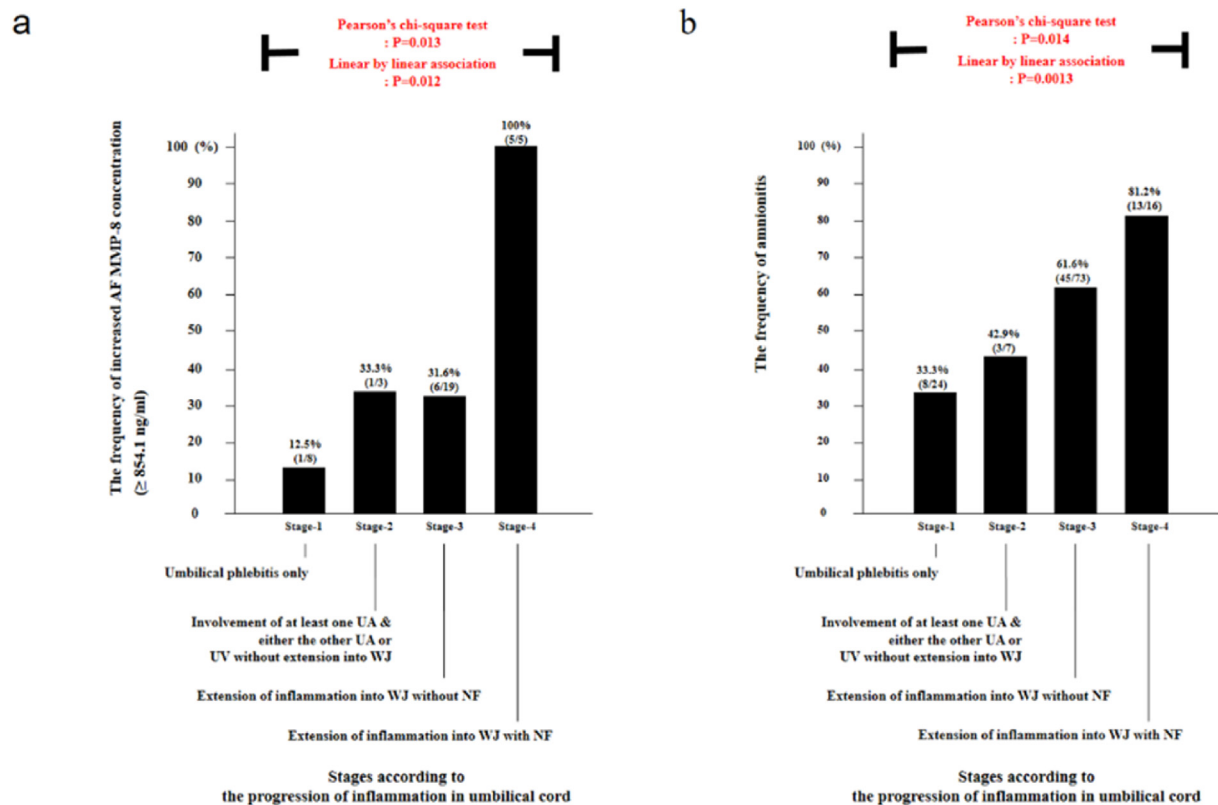


Fig. 3. The frequency of increased amniotic fluid (AF) MMP-8 concentration (≥ 854.1 ng/ml) (a) and amnionitis (b) according to the progression of inflammation in umbilical cord (UC). (i.e., stage-1, umbilical phlebitis [inflammation in umbilical vein (UV)] only; stage-2, involvement of at least one umbilical artery (UA) and either the other UA or UV without extension into Wharton's jelly [WJ]; stage-3, the extension of inflammation into WJ without necrotizing funisitis [NF]; and stage-4, the extension of inflammation into WJ with NF). Each frequency and P value is shown in the graphs. AF MMP-8 concentration results were available in 35 patients, and those were not examined in 6 patients due to the limited amount of remained AF amount.

Table 4

Diagnostic indices, predictive values, and likelihood ratios of amniotic fluid (AF) MMP-8 concentration ≥ 854.1 ng/ml and amnionitis for the identification of necrotizing funisitis (NF) among cases with either preterm labor and intact membranes (PTL) or preterm premature rupture of membranes (preterm-PPROM) (Prevalence of NF is 13.3% [16/120]).

	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Positive LR (95% CI)	Negative LR (95% CI)
AF MMP-8 ≥ 854.1 ng/ml	100.0% (5/5)	73.3% (22/30)	38.5% (5/13)	100% (22/22)	NC	0.2677 [0.1473–0.4827]
Amnionitis	81.2% (13/16)	46.2% (48/104)	18.8% (13/69)	94.1% (48/51)	2.4615 [0.8693–6.9705]	0.6627 [0.4934–0.8902]

AF, amniotic fluid; CI, confidence interval; LR, likelihood ratio; NC, not calculable; MMP-8, matrix metalloproteinase-8.

Intra-amniotic inflammatory response (IAIR), amnionitis and a more dense neutrophil infiltration into Wharton's jelly (WJ) in cases with necrotizing funisitis (NF)

Neutrophils recruitment and infiltration develop in the CD of EPM, and subsequently activate and operate the pro-survival program (i.e., anti-apoptosis) leading to the more accumulation of neutrophils in the context of intrauterine infection and/or inflammation [30]. In general, the recruitment, infiltration, or activation of neutrophils is known to be increased according to the severity of the infection and/or inflammation [31]. Based on the representative images of stage-3 (the extension of neutrophil infiltration into WJ without NF) and stage-4 (the extension of neutrophil infiltration into WJ with NF) in Fig. 4, the density of neutrophil infiltration in WJ is much more in stage-4 with NF than in stage-3 without NF. Moreover, it should be noted that IAIR elevates according to the density of neutrophil infiltration in CD in cases with inflammation restricted to CD among spontaneous PTBs [32]. Given that the density of neutrophil infiltration in WJ is much more in stage-4

with NF than in stage-3 without NF and IAIR increases according to the density of neutrophil infiltration in the CD of EPM, one can expect that IAIR is more severe in stage-4 with NF than in stage-3 without NF in the context of inflammation into the WJ of UC. Indeed, cases with stage-4 (inflammation in WJ [+]) and NF [+]) had a more severe IAIR than stage-3 (inflammation in WJ [+]) and NF [-]) in cases with inflammation of UC. Moreover, we also found that amnionitis, as another surrogate marker for FIR, is more frequent in cases with stage-4 with NF than in those with stage-3 without NF among cases with inflammation of UC.

The effect of necrosis on inflammatory response

We should explain the reason why NF is associated with more intense surrogate markers for FIR (i.e., IAIR and amnionitis). Our explanation is as follows. Firstly, some neutrophils are reported to undergo necrosis in the tissue if the infection is serious enough, while neutrophils generally die in apoptosis [33]. In the setting of in vitro study, neutrophils are destined to be apoptotic or necrotic

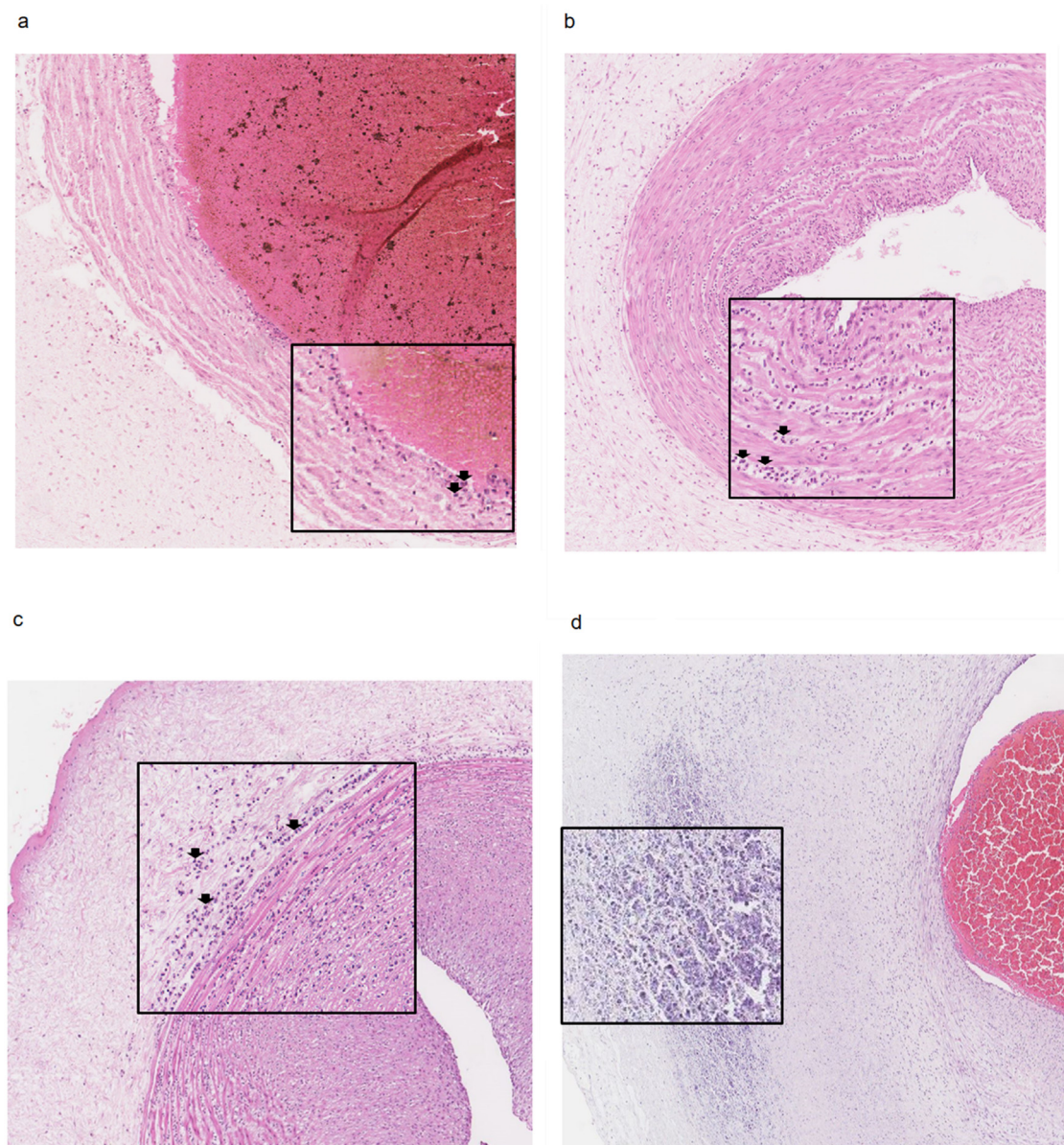


Fig. 4. Histopathology of the progression of inflammation in umbilical cord (UC). Hematoxylin and eosin stained histologic sections of UC are shown as follows: neutrophil infiltration within the umbilical vein (UV) wall (**a**), neutrophil infiltration within at one umbilical artery (UA) without extension into Wharton's jelly (WJ) (**b**), neutrophil infiltration into WJ without necrotizing funisitis (NF) (**c**), neutrophil infiltration into WJ with NF (**d**). These images are based on the magnification setting x200, and the insets of panels are based on the magnification setting x400. Arrows in the insets of panels in (a, b, c) indicate neutrophil infiltration within the UV wall (a), within at the UA wall (b) and into WJ (c) respectively. The inset of panel in d shows neutrophils with associated debris in concentric bands-rings-halos around umbilical vessel.

according to the intensity of infection as in the following: 1) High doses *E. coli* induced necrosis of neutrophils although stimulation with low dose *E. coli* resulted in apoptosis [34]; 2) Exposure of neutrophils to viable pneumococci caused necrosis of the cells while heat-killed pneumococci accelerated the process of apoptosis observed in cultivated non-stimulated neutrophils in vitro [35]. Indeed, our study demonstrated that although not being statistically significant, intra-amniotic infection was more frequent in stage-4 with NF (inflammation in WJ [+] and NF [+]) than in stage-3 without NF (inflammation in WJ [+] and NF [-]) (stage-4 vs. stage-3; 60% vs. 42.9%). Secondly, viable neutrophils enhance the control of infection [36] and apoptosis typically minimizes inflammation through sequestration and inactivation of intracellular danger-

associated molecular-patterns (DAMPs) as a consequence of early recognition and removal of apoptotic cells via local phagocytes [37]. However, necrotic cell death promotes the liberation of intracellular DAMPs (i.e., HMGB1, heat-shock proteins, DNA-chromatin complexes and members of the extended IL-1 cytokine family) to induce the generation of cytokines and chemokines on neighboring cells or tissues ultimately leading to neutrophils recruitment [34,37–40]. Therefore, the presence of necrosis is likely to be associated with more intense inflammatory responses in the neighboring compartments (i.e., IAIR in intra-amniotic cavity, and amnionitis as an advanced stage of inflammatory response in EPM) as compared with the absence of necrosis in the context of the extension of inflammation into WJ.

Table 5

The relationship of various variables with necrotizing funisitis (NF) by multiple logistic regression analysis.

	OR	95% CI	P value
AF MMP-8 ≥ 854.1 ng/ml	28.597	[1.887, 433.484]	0.016
GA at delivery	1.066	[0.265, 4.284]	NS (0.928)
Antenatal corticosteroids use	0.609	[0.046, 8.004]	NS (0.706)
Antenatal antibiotics use	140010602.8	[0.000,]	NS (0.999)
Preterm-PROM as a cause of preterm delivery	3.720	[0.227, 61.067]	NS (0.357)
Birth weight	1.001	[0.992, 1.009]	NS (0.878)
Apgar score < 7 at 1 min	1.000	[0.074, 13.559]	NS (1.000)
Apgar score < 7 at 5 min	8.789	[0.427, 180.773]	NS (0.159)
Male gender of newborn	0.888	[0.075, 9.432]	NS (0.841)
Vaginal delivery	3.264	[0.206, 51.697]	NS (0.401)

AF, amniotic fluid; CI, confidence interval; GA, gestational age; MMP-8, matrix metalloproteinase-8; NS, not significant; OR, odd ratio; Preterm-PROM, preterm premature rupture of membranes.

Strength of this study

Unfortunately, although previous several classifications [5,6,11,15] included NF, these studies had limitations in the classification for the progression of inflammation in UC as follows: 1) the presence or absence of inflammation in WJ was not considered [5,6]; and 2) only NF, but not inflammation in umbilical vessels and WJ, was focused [11,15]. In addition to these limitations, they did not examine IAIR [5,6,11,15]. Notably, our current study demonstrated that NF is the most advanced stage in the inflammation of UC based on the finding that the surrogate markers for FIR (i.e., IAIR and amnionitis) are continuously increased with the progression of inflammation in the whole subdivisions of UC (i.e., stage-1, umbilical phlebitis only; stage-2, involvement of at least one UA and either the other UA or UV without extension into WJ; stage-3, the extension of inflammation into WJ without NF; and stage-4, the extension of inflammation into WJ with NF). This finding was reaffirmed by multiple logistic regression analysis showing that a very highly increased AF MMP-8 concentration (≥ 854.1 ng/ml) was a significantly independent risk factor for NF even after the correction for variables (i.e., GA at delivery, antenatal corticosteroids use, antenatal antibiotics use, cause of preterm birth, Apgar score < 7 at 5 min, gender of newborn, and delivery mode) that could be considered as potential confounding factors for NF and clinical parameters such as birth weight and Apgar score < 7 at 1 min that were different according to the progression of inflammation in UC.

Significance of this study

To the best of our knowledge, this is the first study reporting that the presence of NF is associated with more severe and frequent surrogate markers for FIR (i.e., IAIR and amnionitis) than the absence of NF in the context of inflammation in WJ. One can expect that necrosis in UC easily provokes an intense inflammatory response in both AF and amnion as adjacent biologic fluid and placental compartment. Indeed, our current study demonstrated surrogate markers for FIR (i.e., IAIR and amnionitis) are continuously increased with the progression of inflammation in UC. Therefore, the progression of inflammation in UC should be revisited as classified by current study. Indeed, meticulous classification of inflammation in umbilical cord is needed for the detailed subdivision of FIR ultimately leading to precise postnatal prediction for neonatal morbidities which is closed related to FIR.

Unanswered questions and proposals for future research

To confirm that NF is the most advanced stage in the progression of inflammation in UC, we should show the molecular signatures

about the positive relationship among NF, DAMPs (i.e., HMGB1) and neutrophil infiltration in UC. Moreover, the comparison in the inflammatory responses between narrow and wide bands of NF should be examined to ascertain the impact of NF on inflammatory responses.

Conclusions

NF is the most advanced stage in the progression of inflammation within UC based on IAIR and outside in neutrophil migration in EPM. This finding suggests that inflammation in WJ should be divided into the presence and absence of NF in the progression of inflammation within UC.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

Acknowledgements

This research was supported by Research Program (No. 800-20160447) funded by Seoul National University College of Medicine Research Foundation.

References

- [1] Park CW, Park JS, Moon KC, Jun JK, Yoon BH. Preterm labor and preterm premature rupture of membranes have a different pattern in the involved compartments of acute histologic chorioamnionitis and/or funisitis: pathophysiological implication related to different clinical manifestations. *Pathol Int* 2016;66:325–32.
- [2] Kim CJ, Romero R, Chaemsaitong P, Chaiyasit N, Yoon BH, Kim YM. Acute chorioamnionitis and funisitis: definition, pathologic features, and clinical significance. *Am J Obstet Gynecol* 2015;213:S29–52.
- [3] Yoon B, Romero R, Shim JY, Shim SS, Kim CJ, Jun JK. C-reactive protein in umbilical cord blood: a simple and widely available clinical method to assess the risk of amniotic fluid infection and funisitis. *J Matern Fetal Neonatal Med* 2003;142:85–90.
- [4] Gomez R, Romero R, Ghezzi F, Yoon BH, Mazor M, Berry SM. The fetal inflammatory response syndrome. *Am J Obstet Gynecol* 1998;179:194–202.
- [5] Redline RW, Faye-Petersen O, Heller D, Qureshi F, Savell V, Vogler C, Society for Pediatric Pathology. Amniotic infection syndrome: nosology and reproducibility of placental reaction patterns. *Pediatr Dev Pathol* 2003;6:435–48.
- [6] Khong TY, Mooney EE, Ariel I, Balmus NC, Boyd TK, Brundler MA, et al. Sampling and definitions of placental lesions: Amsterdam placental workshop group consensus statement. *Arch Pathol Lab Med* 2016;140:698–713.
- [7] Pacora P, Chaiworapongsa T, Maymon E, Kim YM, Gomez R, Yoon BH, et al. Funisitis and chorionic vasculitis: the histological counterpart of the fetal inflammatory response syndrome. *J Matern Fetal Neonatal Med* 2002;11:18–25.
- [8] Park CW, Lee SM, Park JS, Ju JK, Romero R, Yoon BH. The antenatal identification of funisitis with a rapid MMP-8 bedside test. *J Perinat Med* 2008;36:497–502.
- [9] Park JS, Romero R, Yoon BH, Moon JB, Oh SY, Han SY, et al. The relationship between amniotic fluid matrix metalloproteinase-8 and funisitis. *Am J Obstet Gynecol* 2001;185:1156–61.
- [10] Park CW, Moon KC, Park JS, Jun JK, Romero R, Yoon BH. The involvement of human amnion in histologic chorioamnionitis is an indicator that a fetal and an intra-amniotic inflammatory response is more likely and severe: clinical implications. *Placenta* 2009;30:56–61.
- [11] Jacques SM, Qureshi F. Necrotizing funisitis: a study of 45 cases. *Hum Pathol* 1992;23:1278–83.
- [12] Romero R, Salafia CM, Athanassiadi AP, Hanaoka S, Mazor M, Sepulveda W, et al. The relationship between acute inflammatory lesions of the preterm placenta and amniotic fluid microbiology. *Am J Obstet Gynecol* 1992;166:1382–8.
- [13] van Hoesen KH, Anyaegbunam A, Hochster H, Whitty JE, Distant J, Crawford C, et al. Clinical significance of increasing histologic severity of acute inflammation in the fetal membranes and umbilical cord. *Pediatr Pathol Lab Med* 1996;16:731–44.
- [14] Yoon BH, Romero R, Park JS, Kim M, Oh SY, Kim CJ, et al. The relationship among inflammatory lesions of the umbilical cord (funisitis), umbilical cord plasma interleukin 6 concentration, amniotic fluid infection, and neonatal sepsis. *Am J Obstet Gynecol* 2000;183:1124–9.
- [15] Ohyama M, Itani Y, Yamanaka M, Goto A, Kato K, Ijiri R, et al. Re-evaluation of chorioamnionitis and funisitis with a special reference to subacute chorioamnionitis. *Hum Pathol* 2002;33:183–90.

- [16] Lee SE, Romero R, Kim CJ, Shim SS, Yoon BH. Funisitis in term pregnancy is associated with microbial invasion of the amniotic cavity and intra-amniotic inflammation. *J Matern Fetal Neonatal Med* 2006;19:693–7.
- [17] Buhimschi CS, Dulay AT, Abdel-Razeq S, Zhao G, Lee S, Hodgson EJ, et al. A Fetal inflammatory response in women with proteomic biomarkers characteristic of intra-amniotic inflammation and preterm birth. *BJOG* 2009;116:257–67.
- [18] Lee J, Oh KJ, Park CW, Park JS, Jun JK, Yoon BH. The presence of funisitis is associated with a decreased risk for the development of neonatal respiratory distress syndrome. *Placenta* 2011;32:235–40.
- [19] Cobo T, Kacerovsky M, Palacio M, Hornychova H, Hougaard DM, Skogstrand K, et al. A prediction model of histological chorioamnionitis and funisitis in preterm prelabor rupture of membranes: analyses of multiple proteins in the amniotic fluid. *J Matern Fetal Neonatal Med* 2012;25:1995–2001.
- [20] Lee SY, Park KH, Jeong EH, Oh KJ, Ryu A, Park KU. Relationship between maternal serum C-reactive protein, funisitis and early-onset neonatal sepsis. *J Kor Med Sci* 2012;27:674–80.
- [21] Kacerovsky M, Musilova I, Khatibi A, Skogstrand K, Hougaard DM, Tambor V, et al. Intraamniotic inflammatory response to bacteria: analysis of multiple amniotic fluid proteins in women with preterm prelabor rupture of membranes. *J Matern Fetal Neonatal Med* 2012;25:2014–9.
- [22] Kim SM, Romero R, Park JW, Oh KJ, Jun JK, Yoon BH. The relationship between the intensity of intra-amniotic inflammation and the presence and severity of acute histologic chorioamnionitis in preterm gestation. *J Matern Fetal Neonatal Med* 2015;28:1500–9.
- [23] Park JW, Park KH, Jung EY. Clinical significance of histologic chorioamnionitis with a negative amniotic fluid culture in patients with preterm labor and premature membrane rupture. *PloS One* 2017;12:e0173312.
- [24] Musilova I, Andrys C, Drahosova M, Zednikova B, Hornychova H, Pliskova L, et al. Late preterm prelabor rupture of fetal membranes: fetal inflammatory response and neonatal outcome. *Pediatr Res* 2018;83:630–7.
- [25] Martinez-Portilla RJ, Hawkins-Villarreal A, Alvarez-Ponce P, Chinolla-Arellano ZL, Moreno-Espinosa AL, Sandoval-Mejia AL, et al. Maternal serum interleukin-6: a non-invasive predictor of histological chorioamnionitis in women with preterm-prelabor rupture of membranes. *Fetal Diagn Ther* 2019;45:168–75.
- [26] Salafia CM, Weigl C, Silberman L. The prevalence and distribution of acute placental inflammation in uncomplicated term pregnancies. *Obstet Gynecol* 1989;73:383–9.
- [27] Yoon BH, Romero R, Kim CJ, Jun JK, Gomez R, Choi JH, et al. Amniotic fluid interleukin-6: a sensitive test for antenatal diagnosis of acute inflammatory lesions of preterm placenta and prediction of perinatal morbidity. *Am J Obstet Gynecol* 1995;172:960–70.
- [28] Navarro C, Blanc WA. Subacute necrotizing funisitis: a variant of cord inflammation with a high rate of perinatal infection. *J Pediatr* 1974;85:689–97.
- [29] Oh JW, Park CW, Moon KC, Park JS, Jun JK. The relationship among the progression of inflammation in umbilical cord, fetal inflammatory response, early-onset neonatal sepsis, and chorioamnionitis. *PloS One* 2019;14:e0225328.
- [30] Presicce P, Park CW, Sentharamaikkannan P, Bhattacharyya S, Jackson C, Kong F, et al. IL-1 signaling mediates intrauterine inflammation and chorio-decidua neutrophil recruitment and activation. *JCI insight* 2018;3.
- [31] Witko-Sarsat V, Rieu P, Descamps-Latscha B, Lesavre P, Halbwachs-Mecarelli L. Neutrophils: molecules, functions and pathophysiological aspects. *Lab Invest* 2000;80:617–53.
- [32] Park CW, Lee SM, Park JS, Jun JK. 667: intra-amniotic inflammatory response elevates according to the increase in the grade of chorio-decidualitis in the context of inflammation restricted to chorio-decidua, but not inflammation in the compartments beyond chorio-decidua: 'relationship between the grade of chorio-decidualitis and intra-amniotic inflammatory response in spontaneous preterm birth' revisited. *Am J Obstet Gynecol* 2018;218:S401.
- [33] Iba T, Hashiguchi N, Nagaoka I, Tabe Y, Murai M. Neutrophil cell death in response to infection and its relation to coagulation. *J Intensive Care* 2013;1:13.
- [34] Martin SJ. Cell death and inflammation: the case for IL-1 family cytokines as the canonical DAMPs of the immune system. *FEBS J* 2016;283:2599–615.
- [35] Zysk G, Bejo L, Schneider-Wald BK, Nau R, Heinz HP. Induction of necrosis and apoptosis of neutrophil granulocytes by *Streptococcus pneumoniae*. *Clin Exp Immunol* 2000;122:61–6.
- [36] Lowe DM, Demaret J, Bangani N, Nakiwala JK, Goliath R, Wilkinson K, et al. Differential effect of viable versus necrotic neutrophils on *Mycobacterium tuberculosis* growth and cytokine induction in whole blood. *Front Immunol* 2018;9:903.
- [37] Kearney CJ, Martin SJ. An inflammatory perspective on necroptosis. *Mol Cell* 2017;65:965–73.
- [38] Wallach D, Kang TB, Dillon CP, Green DR. Programmed necrosis in inflammation: toward identification of the effector molecules. *Science* 2016;352:6281.
- [39] Wang J. Neutrophils in tissue injury and repair. *Cell Tissue Res* 2018;371:531–9.
- [40] de Oliveira S, Rosowski EE, Huttenlocher A. Neutrophil migration in infection and wound repair: going forward in reverse. *Nat Rev Immunol* 2016;16:378–91.