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The severity of chronic histiocytic intervillositis is associated with gestational age and fetal weight

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ABSTRACT

Introduction: Chronic histiocytic intervillositis (CHI) is a rare histopathological lesion in the placenta that is associated with poor reproductive outcomes. The intervillous infiltrate consists mostly of maternal mononuclear cells and fibrin depositions, which are both indicators for the severity of the intervillous infiltrate. The severity of the intervillous infiltrate as well as the clinical outcomes of pregnancy differ between cases. Our objective is to determine the relation between the severity of the intervillous infiltrate and the clinical outcomes of CHI.
Methods: Cases of CHI were semi-quantitatively graded based on histopathological severity scores. Hereto, CD68 positive mononuclear cells were quantified, fibrin depositions visualized by both a PTAH stain and an immuohistochemical staining, and placental dysfunction was assessed via thrombomodulin staining.
Results: This study included 36 women with CHI. A higher CD68 score was significantly associated with a lower birthweight. Loss of placental thrombomodulin was associated with lower gestational age, lower birthweight, and a lower placenta weight. The combined severity score based on CD68 and PTAH was significantly associated with fetal growth restriction, and the joint score of CD68 and fibrin was associated with birthweight and placental weight.
Discussion: More severe intervillous infiltrates in CHI placentas is associated with a lower birth weight and placental weight. Furthermore, this study proposes thrombomodulin as a possible new severity marker of placental damage. More research is needed to better understand the pathophysiology of CHI.

1. Introduction

Chronic histiocytic intervillositis (CHI) is a relatively uncommon histopathological lesion of the placenta, first described by Labarrere and Mullen in 1987 [1]. The lesion predominantly consists of maternal mononuclear cells which infiltrate the intervillous space of the placenta. Furthermore, CHI is often accompanied by perivillous fibrin depositions, villitis and trophoblast damage [2,3]. CHI is a serious condition and is associated with poor perinatal outcomes, like fetal growth restriction (FGR), intrauterine fetal death (IUFD) and spontaneous miscarriages [2–5]. CHI can arise at any gestational age. Prevalence rates of CHI are varying between 0.80 and 0.96% for first trimester losses and in the second and third trimester the prevalence is estimated at 0.0006%. An overall prevalence rate of CHI of 0.17% was found by a large retrospective cohort study, which included nearly 30.000 specimens [6,7]. Thereby, CHI has a high recurrence rate which is estimated between 18% and 100% in subsequent pregnancies [2,6]. The combination of the high recurrence rate and the unfavorable clinical outcomes of CHI accentuate its clinical pertinence [8].

Histopathological studies revealed that the severity of the intervillous infiltrate differs in various cases of CHI [9]. Some cases present an extensive amount of mononuclear cells in the intervillous space, with or without pronounced damage to the placenta, while other CHI cases only show mild histopathological changes. Furthermore, there is inconsistency in the literature about the relation between the severity of

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Abbreviations

CHI	Chronic histiocytic intervillositis
FGR	Fetal growth restriction
IUFD	Intra uterine fetal demise
TOP	Termination of pregnancy

the intervillous infiltrate and the severity of clinical outcome. In some studies a more severe lesion was found to be associated with a higher incidence of fetal loss, more FGR and a lower gestational age [2,3,10]. A positive association between CHI lesion severity and odds of pregnancy loss was also shown in a meta-analysis [11]. However, four other publications did not find proof of such a relation [6,12–14]. In addition, there is no generally agreed severity score for CHI. Numerous articles defined the severity of the intervillous infiltrate in CHI placentas by the volume of mononuclear cells of maternal origin, frequently combined with the amount of fibrin depositions [2,3,7]. Besides these markers, thrombomodulin could be eligible as a marker for severity of placental damage [15] in CHI. Thrombomodulin is essential for the development and maintenance of endothelial cells and it covers three distinct pathways: inflammation, coagulation and cell survival [16]. Furthermore, thrombomodulin is essential for the development and maintenance of endothelial cells; thrombomodulin knockout models are lethal in the embryonic phase and result in massive thrombosis [17,18]. Under inflammatory circumstances, thrombomodulin is cleaved by metalloproteases, this cleaved thrombomodulin breakdown product is believed to be non-functional [19]. Thrombomodulin is also expressed by the syncytiotrophoblast of the placenta. Previous studies highlight the preventive capacity of placental thrombomodulin in the development of pre-eclampsia [20,21].

In this study, a possible relation between the severity of CHI based on the volume of mononuclear cells, the extent of fibrin depositions and the severity of placental damage will be determined. Our aim is to gain a better understanding of the clinical outcomes in CHI in order to better understand the pathophysiology of CHI.

2. Methods

2.1. Study population

This retrospective study analyzed all patients diagnosed with CHI in the University Medical Center Utrecht (UMCU) between 2000 and 2015. These cases have been described previously [5,22]. The placental samples from the UMCU were appraised in agreement with a standardized protocol. Samples of the fetal membranes, umbilical cord and three full-thickness samples of placenta parenchyma, which macroscopically showed no deviations, were obtained for all placentas. When macroscopically visible lesions were present, these were also sampled. The diagnosis for CHI was based on the standardized diagnostic criteria written by Bos et al. [8] The presence or absence of villitis, perivillous fibrin depositions or other histopathological changes were not used as a selection criterium. Cases with available formalin fixed paraffin embedded tissue blocks were included in this study. Clinical data was collected from medical records. This study was authorized by the UMCU biobank committee (TC-BIO number: 16–434).

2.2. Immunohistochemistry

An immunohistochemical staining was performed for CD68, thrombomodulin, and fibrin. First, the placental sections were deparaffinized and rehydrated. Antigen retrieval was performed using citrate buffer (pH 6.0, fibrin and thrombomodulin) or TRIS/EDTA buffer (pH 9.0, for CD68). Endogenous peroxidase was blocked for 20 min with H_2O_2/distilled water (dilution 1:250) for fibrin and CD68. The sections were incubated in 1% BSA/PBS with mouse anti-fibrine (dilution 1:200, Immunotech), mouse anti-CD68 (dilution 1:2000, DAKO) or mouse anti-thrombomodulin (dilution 1:200, Novocastra) for 60 min at room temperature. As a negative control, non-specific isotype matched antibodies were used (DAKO). Thereafter, the sections were washed 3 × 5 min in 1× PBS and were incubated for 30 min with anti-mouse envision (DAKO). DAB substrate was used to visualize the primary antibody (10 min, chromogen:substrate = 1:50, DAKO) and the slides were counterstained with hematoxylin for 10 s. After dehydration a coverslip with mounting medium was applied.

2.3. Histological staining

A histological PTAH stain was performed to analyze fibrin depositions in CHI placentas. First, the placental sections were deparaffinized and rehydrated. Then, the sections were incubated in potassium permanganate 0.25% for 15 min. Afterward, the sections were washed in distilled water before they were incubated in oxalic acid 5% for 5 min. Again, the sections were washed in distilled water and subsequently incubated in a PTAH solution for 24 h. After incubation in the PTAH solution, the sections were washed in distilled water. To dehydrate the sections, they were treated with 70% ethanol, twice with 100% ethanol and twice with xylene, and after that they were air-dried. Finally, a coverslip with mounting medium was applied.

2.4. Semi-quantitative scoring

From each placenta one representative formalin fixed paraffin embedded block was selected based on the histopathological analysis by a pathologist for histological and immunohistochemical staining. Slides were scored on a 20× magnification on 10 different locations. Scoring was performed by two independent observers blinded for clinical outcome. Severity of the intervillous infiltrate was semi-quantitatively scored according to the classifications described by Parant et al. [3] Thrombomodulin semi-quantitative score was based on a previous publication of Turner et al. [15,21] Semi-quantitative scores are displayed in Table 1. When observers scored differently, consensus was

Table 1
Semi-quantitative classification system for severity.

CD68	**Score 0; Mild (<10% infiltration of the intervillous space)**	**Score 1; Moderate (10–50% infiltration of the intervillous space)**	**Score 2; Severe (>50% infiltration)**
PTAH	Score 0; Mild (<10% of the intervillous space)	Score 1; Moderate (10–50% of the intervillous space)	Score 2; Severe (>50% of the intervillous space)
Fibrin	Score 0; Mild (<10% of the intervillous space)	Score 1; Moderate (10–50% of the intervillous space)	Score 2; Severe (>50% of the intervillous space)
Thrombomodulin	Score 1; Diffuse (>50% of syncytiotrophoblast positive for thrombomodulin)	Score 0; Focal loss (0–50% of syncytiotrophoblast positive for thrombomodulin)	
Severity score based on CD68 + PTAH	Mild (the sum of CD68 and PTAH scores between 0 and 2)	Severe (the sum of CD68 and PTAH scores between 3 and 4)	
Severity score based on CD68 + Fibrin	Mild (the sum of CD68 and Fibrin scores between 0 and 2)	Severe (the sum of CD68 and Fibrin scores between 3 and 4)	

obtained. Interobserver agreement was minimally sufficient in all semi-quantitative scores ($\kappa \geq 0.5$).

2.5. Clinical definitions

Fetal loss before 24 weeks of gestational age was subdivided between miscarriage, termination of pregnancy (TOP) and abortion. Miscarriage was defined as a spontaneous fetal loss before 24 weeks of gestation [23]. In addition, early miscarriage was defined as a spontaneous pregnancy loss before 10 weeks of pregnancy and late miscarriage as a spontaneous pregnancy loss between 10 and 24 weeks of pregnancy [23]. TOP was defined as an induced pregnancy loss before 24 weeks of pregnancy for medical reasons, such as severe fetal growth restriction and congenital defects [24]. Induced pregnancy loss for psychosocial reasons before 24 weeks of pregnancy was defined as abortion [25]. IUFD refers to spontaneous fetal loss after 24 weeks of pregnancy. An early neonatal death was defined as death during birth and within the first 7 days after birth. A pregnancy was considered term from 37 weeks of gestation onwards and preterm if birth occurred between 24 and 37 weeks of pregnancy. FGR was referred to as a birthweight below the 3rd percentile [26].

2.6. Statistical analysis

Data was processed and analyzed with SPSS statistics software (version 25). Baseline characteristics are represented using percentages for binary data and a combination of means and standard deviations or medians, minima and maxima for continuous data, depending on the normality of data. To compare categorical data between groups, a Fisher exact test was used to test significance, because there was a low number of cases. Spearman's rank correlation coefficient was determined to research any possible associations between different outcomes. Only associations were tested between chosen variables for severity of CHI (semi-quantitative CD68 score, semi-quantitative PTAH score, semi-quantitative fibrin score and semi-quantitative thrombomodulin staining) and gestational age, birthweight, birthweight percentile, FGR and placenta weight. The significance level was set to $P \leq 0.05$.

3. Results

3.1. Study population

In the period from 2000 to 2015, 45 placentas with an intervillous infiltrate were diagnosed at the department of pathology at the UMCU [5,22]. Four twin pregnancies, three cases with an underlying infection and two cases without availability of remaining placental sections were excluded. Therefore, 36 cases were eligible for this study. Patient characteristics are summarized in Table 2. The mean maternal age during the index pregnancy was 34.1 years. Ten women (27.8%) were primigravida and fifteen women (41.7%) had no living children. Only 36% of the pregnancies term. At least one hypertensive disorder during pregnancy, either pregnancy induced hypertension, pre-eclampsia or HELLP-syndrome, was diagnosed in eleven women (30.6%). Eighty-one percent of babies was born alive of whom fifty-two percent had FGR.

3.2. Placental characteristics

Placental characteristics are displayed in Table 3. CHI was present in all 36 placenta samples, the histopathological classification of the intervillositis was mild in 21 samples (58%), moderate in 9 samples (25%) and severe in 6 samples (17%). Signs for maternal vascular malperfusion were present in 29 placentas (81%); the placenta weight was small in relation to gestational age in 24 placentas (71%), ischemic changes were present in 5 placentas, infarcts were present in 6 placentas (17%) and an accelerated villous maturation occurred in 12 placentas (33%). Decidual arteriopathy was not present in any of the placentas.

Table 2
Patient characteristics.

Maternal characteristics	N = 36
Maternal age, mean (SD)	34 (4,4)
Gravidity, median [range]	3 [1–8]
Primi-gravida, n (%)	10 (28)
Parity, median [range]	1 [0–5]
Nulli-parous, n (%)	15 (42)
Pregnancy characteristics	
Term, n (%)	13 (36)
Gestational age [days], median [range [days]]	267 [259–289]
Sectio	3 (23)
IUFD, n (%)	2 (15)
Pre-term, n (%)	19 (53)
Gestational age [days], median [range [days]]	230 [178–258]
Sectio	13 (68)
IUFD, n (%)	1 (5)
Gestational age <24 weeks, n (%)	4 (11)
Miscarriage, n (%)	1 (25)
Late miscarriage, n (%)	1 (100)
Abortion, n (%)	0
TOP, n (%)	3 (75)
Hypertensive complications of pregnancy, n (%)	11 (31)
PIH, n (%)	4 (11)
Pre-eclampsia, n (%)	7 (20)
Diabetes gravidarum, n (%)	2 (6)
Fetal characteristics	**N = 36**
Number of live born fetuses	29 (81)
Male, n (%)	15 (52)
Birthweight, mean (SD)	1765 g (1057 g)
FGR, n (%)	15 (52)
Early neonatal death, n (%)	2 (6)

Table 3
Placental characteristics.

Placental weight, median [range]	329.5 [96–626]
Nucleated erythrocytes, n (%)	21 (58)
Severe, n (%)	9 (43)
Maternal vascular malperfusion, n (%)	**29 (81)**
Small placenta (weight for gestational age < p10), n (%)	24 (71)
Accelerated villous maturation, n (%)	12 (33)
Infarct, n (%)	6 (17)
Ischemic changes, n (%)	5 (14)
Increased syncytial knotting, n (%)	3 (8)
Distal villous hypoplasia, n (%)	0
Decidual arteriopathy, n (%)	0
Fetal vascular malperfusion, n (%)	**9 (25)**
A-vascular villi, n (%)	6 (17)
Fetal thrombosis, n (%)	5 (14)
Villous stromal vascular karyorrhexis n (%)	5 (14)
Intramural fibrin deposition, n (%)	3 (8)
Delayed villous maturation, n (%)	2 (6)
Stem vessel obliteration, n (%)	0
Intervillositis, n (%)	**36 (100)**
Mild	21 (58)
Moderate	9 (25)
Severe	6 (17)
Villitis, n (%)	**29 (81)**
Mild	16 (44)
Moderate	7 (19)
Severe	6 (17)
Perivillous fibrin, n (%)	**24 (67)**
Mild	13 (36)
Moderate	4 (11)
Massive	7 (20)

Signs for fetal vascular malperfusion were present in 9 placentas (9%); fetal thrombosis was present in 5 placentas (14%), a vascular villi were present in 6 placentas (17%), intramural fibrin depositions were present in 3 placentas (8%) and villous stromal vascular karyorrhexis was present in 5 placentas (14%). Stem vessel obliteration was not present. Villitis was present in 29 placentas (81%) and perivillous fibrin in 24 placentas (67%). Severity of CHI was not associated with the severity of villitis ($r = -0.129$, $p = 0.452$, data not shown) nor with the severity of perivillous fibrin depositions ($r = -0.050$, $p = 0.773$, data not shown). The 9 cases with fetal vascular malperfusion were all present in a case with chronic villitis.

3.3. CD68 is associated with birthweight

Representative examples for the semi-quantitative scores of CD68, PTAH, fibrin and thrombomodulin are shown in Fig. 1. Semi-quantitative grading resulted in different scores for CD68, PTAH, fibrin and thrombomodulin. According to CD68 stain, eleven cases were classified as mild (<10% infiltration of the intervillous space, Fig. 1 A), thirteen as moderate (10–50% infiltration of the intervillous space, Fig. 1 B) and twelve as severe (>50% infiltration of the intervillous space, Fig. 1C). A higher CD68 score was significantly associated with a lower birthweight ($r = -0.45$, $p < 0.01$, Fig. 2 A) and a lower birthweight percentile ($r = -0.58$, $p < 0.05$, Fig. 2 B). The semi-quantitative CD68 score was positively associated with the histopathological graded severity of CHI ($r = 0.343$, $p = 0.041$, data not shown).

3.4. Fibrin depositions are not related to clinical outcomes

Following the PTAH classification, ten cases were scored as mild (<10% of the intervillous space, Fig. 1 D), eighteen cases as moderate (10–50% of the intervillous space, Fig. 1 E) and eight cases as severe (>50% infiltration of the intervillous space, Fig. 1 F). Clinical characteristics were not significantly different between PTAH scores and we did not find any significant associations. According to the semi-quantitative score for the fibrin stain, seven cases were classified as mild (<10% of the intervillous space, Fig. 1 G), fifteen cases were classified as moderate (10–50% of the intervillous space, Fig. 1H) and twelve cases were classified as severe (>50% of the intervillous space, Fig. 1 I). For two cases slides were not available for the fibrin stain. Clinical outcome was not significantly different between semi-quantitative fibrin scores and no significant associations were observed. A higher score was allocated for the severity of fibrin depositions if the immunohistochemically staining was used compared to the PTAH stain. Semi-quantitative PTAH scores and semi-quantitative fibrin scores were positively associated ($r = 0.458$, $p = 0.006$, data not shown).

Focal loss of thrombomodulin is associated with a lower gestational age, lower birthweight and lower placental weight.

Following the thrombomodulin stain, 24 cases were classified as diffuse (Score 1, >50% of syncytiotrophoblast positive for thrombomodulin, Fig. 1 J) and twelve cases were classified as focal loss (Score 0, 0–50% of syncytiotrophoblast positive for thrombomodulin, Fig. 1 K). Clinical characteristics were not significantly different between thrombomodulin scores. Focal loss of thrombomodulin expression was significantly associated with a lower gestational age ($r = 0.39$, $p < 0.05$, Fig. 2C), a lower birthweight ($r = 0.41$, $p < 0.05$, Fig. 2 D) and lower placenta weight ($r = 0.34$, $p < 0.05$, Fig. 2 E).

3.5. Combined severity score

According to the combined severity score of CD68 and PTAH, 26 cases were classified as mild (the sum of CD68 and PTAH score between 0 and 2) and ten cases as severe (the sum of CD68 and PTAH score between 3 and 4). Analysis showed that SGA occurred significantly more often in the group which was classified as severe (80% $p = 0.047$, Fig. 3 A). Furthermore, a higher severity score was associated with a lower birthweight percentile ($r = -0.344$, $p < 0.05$, Fig. 3 B). Gestational age and birth weight were not associated with the combined severity score based on CD68 and PTAH. Besides the sum of CD68 and PTAH scores, also the severity based on the accumulation of CD68 and fibrin scores was analyzed. The severity was classified as mild in 23 (cases the sum of CD68 and fibrin score between 0 and 2) and severe in eleven cases (the sum of CD68 and fibrin between 3 and 4). Clinical outcomes did not differ between both groups. A significant association was present with birthweight ($r = -0.427$, $p < 0.05$, Fig. 3C) and placenta weight ($r = -0.394$, $p < 0.05$, Fig. 3 D).

4. Discussion

The purpose of this study was to investigate the relationship between clinical outcome and the severity of the intervillous infiltrate in placentas of women with CHI. We found a significant association between a higher CD68 score and a lower birthweight and birthweight percentile. The combined severity score based on CD68 and PTAH was significantly associated with lower birthweight percentiles, whereas the joint score of CD68 and fibrin was associated with lower birthweight and lower placenta weight. Finally, a focal loss in thrombomodulin expression was significantly associated with a lower gestational age, lower birthweight, and lower placenta weight.

In previous studies the severity of CHI was associated with a higher incidence of fetal loss, a higher incidence of FGR and a lower gestational age [2,3,7,10,27–29]. On the other hand, other publications did not find proof of such a relation [6,12–14]. In earlier studies the relation between the severity of CHI and clinical outcomes was based on $CD68^+$ staining [10,28] or combination of $CD68^+$ staining with fibrin depositions [2,3,6,9,27,29]. Our results are in line with findings by Reus et al. [10] who found that a higher grade of CD68 is associated with a lower birth weight. Additionally, the combined severity score of CD68 with PTAH showed that FGR appeared significantly more often in cases with a severe intervillous infiltrate, comparable to findings by others [2,3,29].

4.1. Markers of severity

Currently, the severity of the intervillous infiltrate is most often characterized by the volume of mononuclear cells and the amount of fibrin depositions [8]. Since these severity markers lack consistent results, other markers can be included. Thrombomodulin could be a new marker for the severity of the intervillous infiltrate. Thrombomodulin is a protein which plays a vital role in the maintenance of the endothelium by inhibiting coagulation and inflammatory pathways [21]. Besides its presence on endothelial cells, thrombomodulin is also found on the surface of the syncytiotrophoblast in the placenta [15,20]. Inflammatory processes lead to a loss of thrombomodulin or decreased expression of thrombomodulin on the cell surface and enhance the release of a soluble form into the circulation [15]. Decreased expression of thrombomodulin suggests placental damage, which may contribute to the adverse clinical outcomes of CHI [30]. The results in the current study showed that focal loss of thrombomodulin is significantly associated with a lower gestational age, lower birthweight and lower placenta weight. This is in line with our expectations and support the other results of this study; a more prominent intervillous infiltrate and placental damage are associated with less favorable clinical outcome. Thrombomodulin could also be a prognostic factor for the risk and outcomes of subsequent pregnancies. Furthermore, soluble thrombomodulin can be detected in the maternal circulation [31] so it might be possible to use soluble thrombomodulin levels in the maternal serum as a biomarker in subsequent pregnancies. More research is needed. Another possible marker of severity is the ectonucleotidase CD39 [32]. CD39 plays a role in the prevention of cell injury and inflammatory stress. The authors showed that placentas with CHI express significantly less CD39 than placentas with no

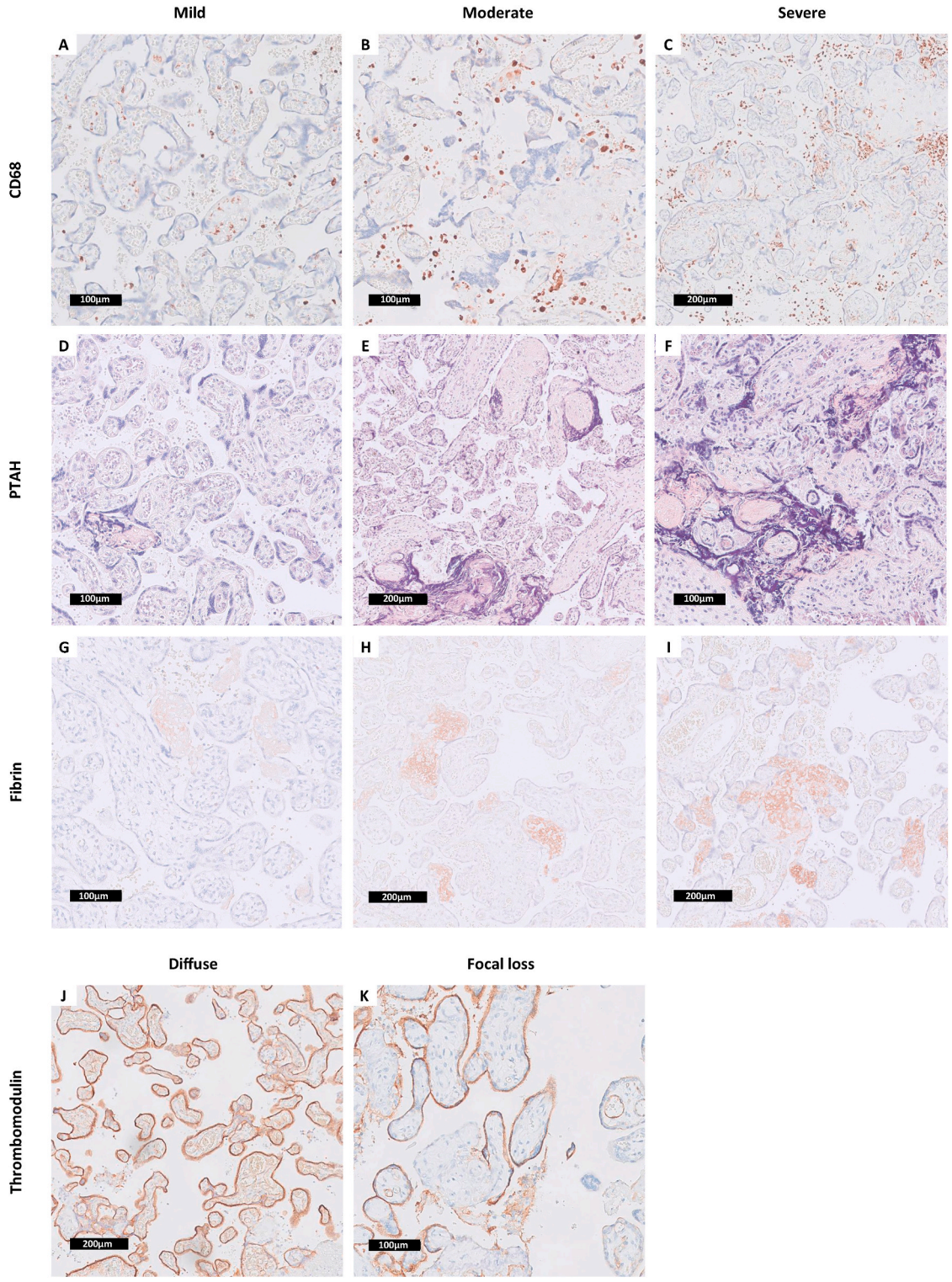

Fig. 1. Semi-quantitative scoring of CD68, PTAH, Fibrin and Thrombomodulin.
(A), (B), (C) Representative examples of the semi-quantitative scores of CD68 immunohistochemical staining to visualize the intervillous infiltrate. **(A)** Score 0; Mild (<10% infiltration of the intervillous space), **(B)** Score 1; Moderate (10–50% infiltration of the intervillous space) and **(C)** Score 2; Severe (>50% infiltration).**(D), (E), (F)** Representative examples of the semi-quantitative scores of PTAH staining to visualize fibrin depositions. **(D)** Score 0; Mild (<10% infiltration of the intervillous space), **(E)** Score 1; Moderate (10–50% infiltration of the intervillous space) and **(F)** Score 2; Severe (>50% infiltration).**(G), (H), (I)** Representative examples of the semi-quantitative scores of immunohistochemical staining to visualize fibrin depositions. **(G)** Score 0; Mild (<10% infiltration of the intervillous space), **(H)** Score 1; Moderate (10–50% infiltration of the intervillous space) and **(I)** Score 2; Severe (>50% infiltration).**(J), (K)** Representative examples of the semi-quantitative immunohistochemical staining to visualize thrombomodulin. **(J)** Score 1; Diffuse (>50% of syncytiotrophoblast positive for thrombomodulin), **(K)** Score 0; Focal loss (0–50% of syncytiotrophoblast positive for thrombomodulin).

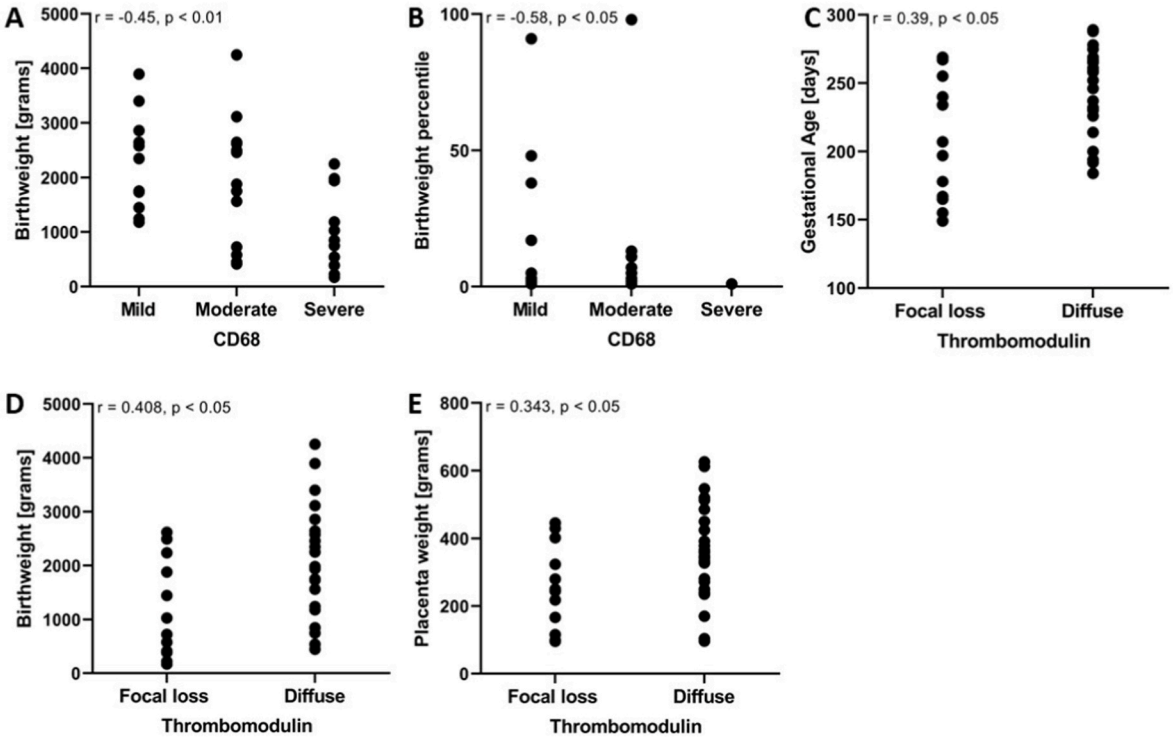

Fig. 2. Severity of CD68-positive cell infiltrate and placental thrombomodulin expression are associated with gestational age, birthweight and placenta weight. **(A)** Severity of the intervillous infiltrate is negatively associated with birthweight and **(B)** birthweight percentile. **(C)** Loss of placental thrombomodulin is associated with a lower gestational age, **(D)** decreased birthweight and **(E)** a lower placental weight.

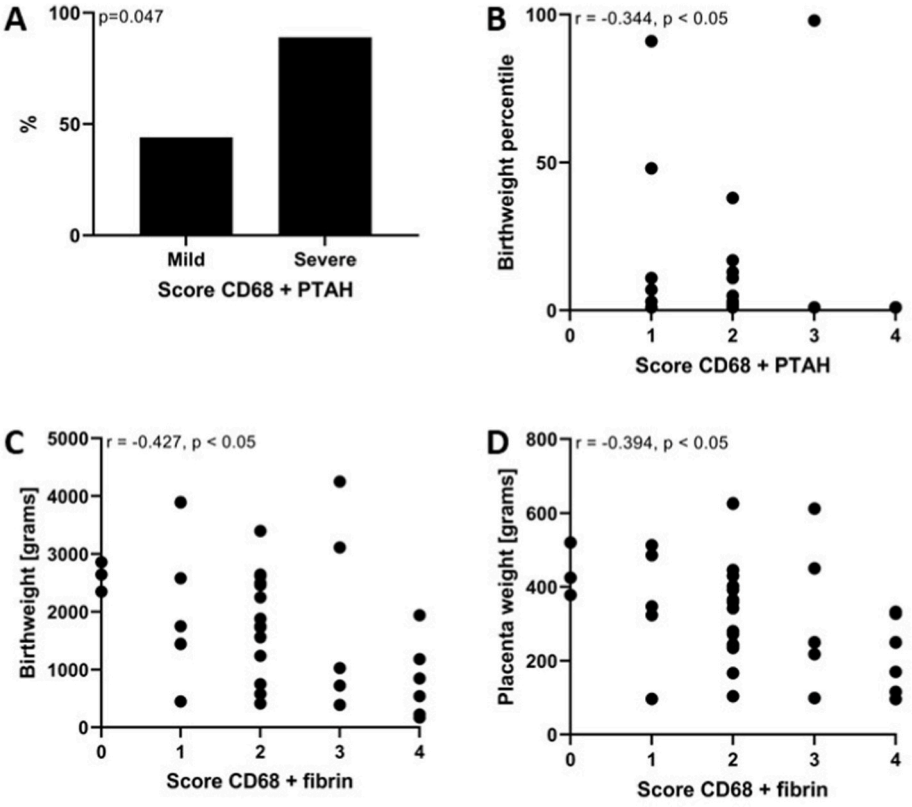

Fig. 3. The combined severity score is associated with birthweight and placenta weight.
(A) A birthweight below the 3rd percentile is more often seen in cases categorized as severe. **(B)** The combined severity score based in CD68 and PTAH is negatively associated with birthweight percentile. **(C)** The combined severity score based on CD68 and fibrin was negatively associated with birthweight and **(D)** placenta weight.

complications. In addition, cases with poorer clinical outcomes expressed significant lower amount of CD39. Lastly, various articles described markers which are involved in inflammatory pathways and might even play a role in the pathophysiology of CHI [33–36]. The contribution of these markers to the classification of the severity of CHI is not demonstrated yet, more studies on this topic are warranted. Future research could focus on the relation between the severity score of the index pregnancy and the outcomes of subsequent pregnancies. This will support medical doctors to inform women about their future perspective, to determine more suitable treatment opportunities and establish a way of personalized medicine for CHI [11].

4.2. Intervillositis due to infectious causes

Chronic intervillositis is also described in the placenta of women with a malaria infection or cytomegalovirus infection [37,38]. Furthermore, an intervillous infiltrate is also seen in women with a SARS-Cov-2 infection [39–42], and intervillositis was identified as a marker for placental SARS-CoV-2 infection [43] and a placental risk factor for intrauterine viral transmission [44]. Our samples were collected in the pre-Covid era and other causes of an infection were ruled out by excluding cases with clinical or histopathological signs for an infection. Furthermore, in cases with SARS-Cov-2 placentitis [45] the most profound clinical outcome was an intra uterine fetal demise and the majority of these cases were not growth restricted [46]. Insight into the severity of intervillositis in relation to clinical outcomes in cases with an infectious cause for the intervillositis are probably not transferable to CHI.

4.3. Strengths and limitations of this study

The strength of the current study is the use of a well-defined cohort of cases with CHI. Cases were included by experienced perinatal pathologists only if they fulfilled the standardized diagnostic criteria, which were set up by Bos et al., in 2018 [8]. In contrast, the retrospective character of this study may have led to selection bias. Furthermore, our study was too small to correct for possible confounders such as gestational age and associated complications such as pre-eclampsia. Furthermore, the significant correlations between the severity scores of CHI and clinical outcomes should be interpreted with caution since these are merely moderate correlations. Therewith, the lack of correction for possible confounders creates an uncertainty to what extent the worse outcome in cases classified as severe CHI is attributed to solely CHI or might be caused by the possible confounders stated above. A prospective multicenter study can overcome these limitations as it overcomes selection bias and will contain more cases.

5. Conclusion

In conclusion, a more severe intervillous infiltrate and more fibrin depositions in CHI placentas is associated with a higher incidence of FGR and a lower birthweight. Furthermore, this study proposes thrombomodulin loss as a severity marker of placental damage. More research is needed to better understand the pathophysiology of CHI.

Author contributions

MB, MK, ME, and MLvdH developed the idea for this study. MB, MK, KD and JB executed the immunohistochemical staining and MB and MK performed the semi-quantitative analysis of immunohistochemical staining. LvdM and PN revised the histopathology of the selected cases. MB and KB enabled collecting the clinical data. MB, MK, ME and MLvdH drafted the manuscript. All authors contributed to the writing and reviewing of the manuscript, and gave final approval of the version to be published.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

[1] C. Labarrere, E. Mullen, Fibrinoid and trophoblastic necrosis with massive chronic intervillositis: an extreme variant of villitis of unknown etiology, Am. J. Reprod. Immunol. Microbiol. 15 (3) (1987) 85–91.
[2] F.M.D. Sauvestre, A.M.D. Mattuizzi, L.M.D.P. Sentilhes, M.M.D. Poingt, P.M.D. P. Blanco, C.M.D. Houssin, D.M.D. Carles, F.M.D. Pelluard, G.M.D. Andre, E.M.D. P. Lazaro, Chronic intervillositis of unknown etiology: development of a grading and scoring system that is strongly associated with poor perinatal outcomes, Am. J. Surg. Pathol. 44 (10) (2020) 1367–1373.
[3] O. Parant, J. Capdet, S. Kessler, J. Aziza, A. Berrebi, Chronic intervillositis of unknown etiology (CIUE): relation between placental lesions and perinatal outcome, Eur. J. Obstet. Gynecol. Reprod. Biol. 143 (1) (2009) 9–13.
[4] A. Chen, D.J. Roberts, Placental pathologic lesions with a significant recurrence risk - what not to miss, Apmis 126 (7) (2018) 589–601.
[5] M. Bos, E.T.M.S. Harris-Mostert, L.E. van der Meeren, J.J. Baelde, D.J. Williams, P. G.J. Nikkels, K.W.M. Bloemenkamp, M.L.P. van der Hoorn, Clinical outcomes in chronic intervillositis of unknown etiology, Placenta 91 (2020) 19–23.
[6] N.K. Simula, J. Terry, N.E. Kent, J. Robertson, S. Purkiss, D. Bloomenthal, C. Williams, M.A. Bedaiwy, Chronic Intervillositis of Unknown Etiology (CIUE): prevalence, patterns and reproductive outcomes at a tertiary referral institution, Placenta 100 (2020) 60–65.
[7] B.J. Doss, M.F. Greene, J. Hill, L.J. Heffner, F.R. Bieber, D.R. Genest, Massive chronic intervillositis associated with recurrent abortions, Hum. Pathol. 26 (11) (1995) 1245–1251.
[8] M. Bos, P.G.J. Nikkels, D. Cohen, J.W. Schoones, K.W.M. Bloemenkamp, J. A. Bruijn, H.J. Baelde, M.L.P. van der Hoorn, R.J. Turner, Towards standardized criteria for diagnosing chronic intervillositis of unknown etiology: a systematic review, Placenta 61 (2018) 80–88.
[9] V. Marchaudon, L. Devisme, S. Petit, H. Ansart-Franquet, P. Vaast, D. Subtil, Chronic histiocytic intervillositis of unknown etiology: clinical features in a consecutive series of 69 cases, Placenta 32 (2) (2011) 140–145.
[10] A.D. Reus, N.M. van Besouw, N.M. Molenaar, E.A.P. Steegers, W. Visser, R.P. de Kuiper, R.R. de Krijger, D.L. Roelen, N. Exalto, An immunological basis for chronic histiocytic intervillositis in recurrent fetal loss, Am. J. Reprod. Immunol. 70 (3) (2013) 230–237.
[11] L. Moar, C. Simela, S. Nanda, A. Marnerides, M. Al-Adnani, C. Nelson-Piercy, K. H. Nicolaides, P. Shangaris, Chronic histiocytic intervillositis (CHI): current treatments and perinatal outcomes, a systematic review and a meta-analysis, Front. Endocrinol. 13 (2022), 945543.
[12] P. Downey, P. Kelehan, E.E. Mooney, Chronic placental intervillositis and villitis: relationship to pregnancy outcome, Virchows Arch. 455 (2009) 163–164.
[13] A. Norgan, E. Cheek, R. Quinton, Chronic histiocytic intervillositis: clinical and pathologic characteristics in a series of cases, Lab. Invest. 100 (SUPPL 1) (2020) 1742–1743.
[14] J. Traeder, D. Jonigk, H. Feist, V. Bröcker, F. Länger, H. Kreipe, K. Hussein, Pathological characteristics of a series of rare chronic histiocytic intervillositis of the placenta, Placenta 31 (12) (2010) 1116–1119.
[15] R.J. Turner, K.W. Bloemenkamp, J.A. Bruijn, H.J. Baelde, Loss of thrombomodulin in placental dysfunction in preeclampsia, Arterioscler. Thromb. Vasc. Biol. 36 (4) (2016) 728–735.
[16] I. Maruyama, C.E. Bell, P.W. Majerus, Thrombomodulin is found on endothelium of arteries, veins, capillaries, and lymphatics, and on syncytiotrophoblast of human placenta, J. Cell Biol. 101 (2) (1985) 363–371.
[17] B. Isermann, S.B. Hendrickson, M. Zogg, M. Wing, M. Cummiskey, Y.Y. Kisanuki, M. Yanagisawa, H. Weiler, Endothelium-specific loss of murine thrombomodulin disrupts the protein C anticoagulant pathway and causes juvenile-onset thrombosis, J. Clin. Invest. 108 (4) (2001) 537–546.
[18] B. Isermann, R. Sood, R. Pawlinski, M. Zogg, S. Kalloway, J.L. Degen, N. Mackman, H. Weiler, The thrombomodulin-protein C system is essential for the maintenance of pregnancy, Nat. Med. 9 (3) (2003) 331–337.
[19] M.W. Boehme, U. Raeth, P.R. Galle, W. Stremmel, W.A. Scherbaum, Serum thrombomodulin-a reliable marker of disease activity in systemic lupus erythematosus (SLE): advantage over established serological parameters to indicate disease activity, Clin. Exp. Immunol. 119 (1) (2000) 189–195.
[20] M. Uszynski, S. Sztenc, E. Zekanowska, W. Uszynski, Thrombomodulin in human gestational tissues: placenta, fetal membranes and myometrium, Adv. Med. Sci. 51 (2006) 312–315.
[21] M. Bos, H.J. Baelde, J.A. Bruijn, K.W. Bloemenkamp, M.P. van der Hoorn, R. J. Turner, Loss of placental thrombomodulin in oocyte donation pregnancies, Fertil. Steril. 107 (1) (2017) 119–129 e5.
[22] L.E. van der Meeren, J. Krop, K.L. Dijkstra, K.W.M. Bloemenkamp, E.F. Cornish, P. G.J. Nikkels, M.P. van der Hoorn, M. Bos, One-sided chronic intervillositis of unknown etiology in dizygotic twins: a description of 3 cases, Int. J. Mol. Sci. 22 (9) (2021).
[23] A.M. Kolte, L.A. Bernardi, O.B. Christiansen, S. Quenby, R.G. Farquharson, M. Goddijn, M.D. Stephenson, E.P. Eshre Special Interest Group, Terminology for

pregnancy loss prior to viability: a consensus statement from the ESHRE early pregnancy special interest group, Hum. Reprod. 30 (3) (2015) 495–498.
[24] J.D.G. Kleiverda, Zwangerschapsafbreking Tot 24 Weken [Termination of Pregnancy until 24 Weeks of Gestational Age], Guidelines of the Netherlands Association for Obstetrics and Gynaecology (NVOG), 2015.
[25] R.M. Silver, D.W. Branch, R. Goldenberg, J.D. Iams, M.A. Klebanoff, Nomenclature for pregnancy outcomes: time for a change, Obstet. Gynecol. 118 (6) (2011) 1402–1408.
[26] F.C. Battaglia, L.O. Lubchenco, A practical classification of newborn infants by weight and gestational age, J. Pediatr. 71 (2) (1967) 159–163.
[27] C. Capuani, F. Meggetto, I. Duga, M. Danjoux, M. March, O. Parant, P. Brousset, J. Aziza, Specific infiltration pattern of FOXP3+ regulatory T cells in chronic histiocytic intervillositis of unknown etiology, Placenta 34 (2) (2013) 149–154.
[28] N. Molenaar, N.H. van Besouw, E.A.P. Steegers, W. Visser, P. de Kuiper, R. de Krijger, N. Exalto, Chronic histiocytic intervillositis and recurrent pregnancy loss, Hum. Reprod. 26 (2011). I152-I152.
[29] C. Rota, D. Carles, V. Schaeffer, F. Guyon, R. Saura, J. Horovitz, Pronostic périnatal des grossesses compliquées d'intervillites chroniques placentaires, J. Gynécol. Obstétrique Biol. Reproduction 35 (7) (2006) 711–719.
[30] M. Bos, D. Cohen, K. Bloemenkamp, J. Bruijn, M.-L.v.d. Hoorn, P. Nikkels, H. Baelde, R. Turner, Placental complement activation and loss of placental thrombomodulin in patients with massive chronic intervillositis, Placenta 57 (2017) 327.
[31] H. Minakami, T. Takahashi, A. Izumi, T. Tamada, Increased levels of plasma thrombomodulin in preeclampsia, Gynecol. Obstet. Invest. 36 (4) (1993) 208–210.
[32] Y. Sato, K. Maekawa, M. Aman, A. Yamashita, Y. Kodama, Y. Maki, H. Sameshima, Y. Asada, CD39 downregulation in chronic intervillositis of unknown etiology, Virchows Arch. 475 (3) (2019) 357–364.
[33] R.W. Bendon, S. Coventry, M. Thompson, E.R. Rudzinski, E.M. Williams, A.P. Oron, Significance of C4d immunostaining in placental chronic intervillositis, Pediatr. Dev. Pathol. 18 (5) (2015) 362–368.
[34] D.A. Clark, J.M. Dmetrichuk, E. McCready, S. Dhesy-Thind, J.L. Arredondo, Changes in expression of the CD200 tolerance-signaling molecule and its receptor (CD200R) by villus trophoblasts during first trimester missed abortion and in chronic histiocytic intervillositis, Am. J. Reprod. Immunol. 78 (1) (2017).
[35] C.A. Labarrere, E. Bammerlin, J.W. Hardin, H.L. Dicarlo, Intercellular adhesion molecule-1 expression in massive chronic intervillositis: implications for the invasion of maternal cells into fetal tissues, Placenta 35 (5) (2014) 311–317.
[36] K. Hussein, A. Stucki-Koch, H. Kreipe, H. Feist, Expression of toll-like receptors in chronic histiocytic intervillositis of the placenta, Fetal Pediatr. Pathol. 34 (6) (2015) 407–412.
[37] J. Ordi, M.R. Ismail, P.J. Ventura, E. Kahigwa, R. Hirt, A. Cardesa, P.L. Alonso, C. Menendez, Massive chronic intervillositis of the placenta associated with malaria infection, Am. J. Surg. Pathol. 22 (8) (1998) 1006–1011.
[38] M. Taweevisit, K. Sukpan, S. Siriaunkgul, P.S. Thorner, Chronic histiocytic intervillositis with cytomegalovirus placentitis in a case of hydrops fetalis, Fetal Pediatr. Pathol. 31 (6) (2012) 394–400.
[39] R. Raschetti, A.J. Vivanti, C. Vauloup-Fellous, B. Loi, A. Benachi, D. De Luca, Synthesis and systematic review of reported neonatal SARS-CoV-2 infections, Nat. Commun. 11 (1) (2020) 5164.
[40] L. Patanè, D. Morotti, M.R. Giunta, C. Sigismondi, M.G. Piccoli, L. Frigerio, G. Mangili, M. Arosio, G. Cornolti, Vertical transmission of coronavirus disease 2019: severe acute respiratory syndrome coronavirus 2 RNA on the fetal side of the placenta in pregnancies with coronavirus disease 2019-positive mothers and neonates at birth, Am J Obstet Gynecol MFM 2 (3) (2020), 100145.
[41] F. Facchetti, M. Bugatti, E. Drera, C. Tripodo, E. Sartori, V. Cancila, M. Papaccio, R. Castellani, S. Casola, M.B. Boniotti, P. Cavadini, A. Lavazza, SARS-CoV2 vertical transmission with adverse effects on the newborn revealed through integrated immunohistochemical, electron microscopy and molecular analyses of Placenta, EBioMedicine 59 (2020), 102951.
[42] A.J. Vivanti, C. Vauloup-Fellous, S. Prevot, V. Zupan, C. Suffee, J. Do Cao, A. Benachi, D. De Luca, Transplacental transmission of SARS-CoV-2 infection, Nat. Commun. 11 (1) (2020) 3572.
[43] D.A. Schwartz, D. Morotti, Placental pathology of COVID-19 with and without fetal and neonatal infection: trophoblast necrosis and chronic histiocytic intervillositis as risk factors for transplacental transmission of SARS-CoV-2, Viruses 12 (11) (2020).
[44] D.A. Schwartz, M. Baldewijns, A. Benachi, M. Bugatti, R.R.J. Collins, D. De Luca, F. Facchetti, R.L. Linn, L. Marcelis, D. Morotti, R. Morotti, W.T. Parks, L. Patanè, S. Prevot, B. Pulinx, V. Rajaram, D. Strybol, K. Thomas, A.J. Vivanti, Chronic histiocytic intervillositis with trophoblast necrosis is a risk factor associated with placental infection from coronavirus disease 2019 (COVID-19) and intrauterine maternal-fetal severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) transmission in live-born and stillborn infants, Arch. Pathol. Lab Med. 145 (5) (2021) 517–528.
[45] J.C. Watkins, V.F. Torous, D.J. Roberts, Defining severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) placentitis, Arch. Pathol. Lab Med. 145 (11) (2021) 1341–1349.
[46] S. Stenton, J. McPartland, R. Shukla, K. Turner, T. Marton, B. Hargitai, A. Bamber, J. Pryce, C.L. Peres, N. Burguess, B. Wagner, B. Ciolka, W. Simmons, D. Hurrell, T. Sekar, C. Moldovan, C. Trayers, V. Bryant, L. Palm, M.C. Cohen, SARS-COV2 placentitis and pregnancy outcome: a multicentre experience during the Alpha and early Delta waves of coronavirus pandemic in England, EClinicalMedicine 47 (2022), 101389.