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First trimester serum biomarkers in pregnancies complicated with placental chronic inflammation

Claire de Moreuil[a,b,*], Marie-Pierre Moineau[c], Maël Padelli[c], Françoise Lede[d], Annabelle Remoue[d], Christophe Tremouilhac[b,e], Philippe Merviel[b,e], Brigitte Pan Petesch[b,f], Emmanuelle Le Moigne[a,b], Karine Lacut[a,b], Pascale Marcorelles[d,g]

[a] Département de médecine interne et pneumologie, CHU de Brest, Hôpital La Cavale Blanche, Brest Cedex, France
[b] EA 3878, GETBO, Université Bretagne Loire, Brest Cedex, France
[c] Département de biochimie, CHU de Brest, Hôpital La Cavale Blanche, Brest Cedex, France
[d] Service d'anatomopathologie, CHU de Brest, Hôpital Morvan, Brest Cedex, France
[e] Service de gynécologie et d'obstétrique, CHU de Brest, Hôpital Morvan, Brest Cedex, France
[f] Fédération de cancérologie et d'hématologie, CHU de Brest, Hôpital Morvan, Brest Cedex, France
[g] EA 4685 LIEN, Université Bretagne Loire, Brest Cedex, France

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ABSTRACT

Objective: This study aimed at determining if first trimester serum biomarkers could predict adverse pregnancy outcomes associated with villitis (VUE) and chronic intervillositis of unknown etiology (CIUE).
Study design: Between January 2013 and June 2018, we selected from pathology department files placentas with VUE or CIUE associated with VUE and control placentas with available first trimester Down syndrome screening results. First trimester PAPP-A and βhCG levels were recorded. Placental growth factor (PlGF) levels were measured in patients with an available first trimester serum sample. Histological findings in placentas, course of pregnancies and newborns' characteristics were compared between cases and controls.
Results: 78 cases and 75 controls were included. In cases, there were 21,8% intrauterine growth restriction (IUGR), 30,8% small for gestational age (SGA). Compared to controls, placentas from cases were smaller (425 g [IQR 370–480] *vs* 460 g [IQR 390–523], p = 0,03), showed more maternal vascular malperfusion features (79,5% *vs* 22,7%, p < 0,0001) and more fetal vascular malperfusion features (33,3% *vs* 12%, p = 0,002). Cases had lower PlGF (29,74 pg/ml [IQR 19,74–36,17] *vs* 36,37 pg/ml [IQR 27,36–49,13], p = 0,007) and βhCG levels (0,74 MoM [IQR 0,53–1,12] *vs* 1,00 MoM [IQR 0,72–1,53], p = 0,002) than controls. These differences resulted from lower PlGF levels in VUE patients compared to CIUE associated with VUE patients and controls (28,35 *vs* 34,05 and 36,37 pg/ml, p = 0,01) and from lower βhCG levels in CIUE associated with VUE patients compared to VUE patients and controls (0,65 *vs* 0,86 and 1, p = 0,005).
Conclusion: Low first trimester PlGF levels in cases, especially in VUE patients, suggest that reduced angiogenesis is involved in adverse pregnancy outcomes related to VUE.

Introduction

Villitis of unknown etiology (VUE) is a common histological lesion reported in five to 15% of third trimester placentas [1], firstly described in 1975 as associated with intrauterine growth restriction (IUGR) [2]. VUE is defined by a chronic inflammation with maternal T CD8+ lymphocytes and histiocytes in the placental villi after an exhaustive investigation excluding infectious agents [1,3]. Chronic intervillositis of unknown etiology (CIUE) is a rare lesion (0,6 per 1000 placentas of second and third trimesters) defined by a chronic inflammation with histiocytes and lymphocytes in the intervillous space, after exclusion of infectious agents [4].

VUE and CIUE have been reported in association with recurrent adverse pregnancy outcomes such as early recurrent miscarriage, stillbirth, IUGR, small for gestational age (SGA) and pre-eclampsia [5–11]. Pathophysiological findings of VUE and CIUE evoke a common immune origin, *i.e.* a maternal anti-fetal rejection process [4].

* Corresponding author at: Département de médecine interne et pneumologie, CHU de Brest, Hôpital La Cavale Blanche, Boulevard Tanguy Prigent, 29609 Brest Cedex, France.
E-mail address: claire.demoreuil@chu-brest.fr (C. de Moreuil).

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Angiogenic biomarkers have never been studied specifically in plasma of pregnant women affected by VUE or CIUE. Yet, placental growth factor (PlGF) is a validated early biomarker of vasculoplacental disorders related to abnormal placental bed implantation and could be disturbed in adverse pregnancy outcomes associated with exclusive VUE and CIUE associated with VUE [12].

In this observational study, we described 78 placentas affected by exclusive VUE or CIUE associated with VUE and corresponding pregnancies and compared them with 75 placentas without chronic inflammation from uncomplicated pregnancies. In addition, we determined if first trimester serum biomarkers (PlGF, PAPP-A, βhCG) could predict adverse pregnancy outcomes associated with VUE and CIUE.

Materials and methods

Definition of cases and controls

Placentas with exclusive VUE or CIUE associated with VUE from pregnancies with a delivery between January 2013 and June 2018 in one of the six maternities of our county participating to "HPP-IPF" (NCT02884804) or "HEMOTHEPP" (NCT02443610) observational studies were qualified as cases. They were selected from the Pathology department files of Brest University Hospital using a computerized database (ADICAP system).

Control placentas were selected during the same period among placentas classified as "normal", "with minimal lesions" or "with acute inflammation" from uncomplicated pregnancies (*ie* without pre-eclampsia, stillbirth, IUGR or SGA).

Multiple gestation pregnancies were excluded.

Histopathological examination of the placentas

Histopathological examination of the placentas had been performed by two senior perinatal pathologists at Brest University Hospital.

Placentas were fixed in 4% buffered formalin. Standard sampling of three blocks in the central area was performed and slides were Hematoxylin, Eosin and Saffron (HES) stained.

Recorded macroscopic findings corresponded to the following items: placenta weight, fetoplacental weight ratio, placental abruption and other grossly identified lesions.

According to Amsterdam consensus [13], a placental weight < 10^{th} percentile, a thin cord < 10^{th} percentile, a placental abruption, a macroscopic infarct in a preterm placenta or a nonperipheral infarct > 5% of the chorionic plate in a term placenta were considered as features of maternal vascular malperfusion.

Recorded microscopic findings corresponded to the following items: maternal vascular malperfusion features such as microscopic infarcts, decidual arteriopathy, abnormal villi development and fetal vascular malperfusion features such as avascular villi and stem vessel obliteration.

The following chronic inflammation features were also described: VUE, CIUE, chronic chorioamniotitis and chronic deciduitis.

VUE was classified in high or low grade. High grade VUE was defined by the presence of inflammation affecting more than 10 contiguous villi in any focus, with more than one focus required for diagnosis [14]. VUE was also defined according to the location of the inflammatory infiltrate: proximal stem villi and sometimes chorionic plate (*i.e.* subchorial), distal villi (*i.e.* parenchymatous), anchoring villi embedded in the basal plate (*i.e.* basal) [13].

Criteria used for CIUE diagnosis were the same as developed by Bos *et* al [10]. CIUE could be associated with VUE and was classified as diffuse or focal according to the density of cellular infiltrate [10,13–15].

Data on pregnancies

Data on pregnancies were recorded from medical files on a computerized database. Baseline maternal characteristics included preconceptional Body Mass Index, medical history, blood and rhesus group, obstetrical history and age at delivery. Pregnancy characteristics included method of conception, medication during pregnancy, gestational diabetes, pre-eclampsia, intrahepatic cholestasis, premature rupture of membrane and term at delivery. The following fetal characteristics were recorded: presence and term at diagnosis of IUGR, SGA, stillbirth, birthweight and sex of the newborn.

Adverse pregnancy outcomes were defined as the occurrence during pregnancy of one of the following complications: stillbirth, IUGR, SGA or pre-eclampsia. Pre-eclampsia was defined according to the American College of Obstetricians and Gynecologists' definition published in 2013 [16]. IUGR was defined according to the 2013 French College of Obstetricians and Gynecologists guidelines by an estimated fetal weight below the 10^{th} percentile using locally-accepted curve (AUDIPOG) associated with clinical signs of pathology [17]. SGA was defined by a birthweight below the 10^{th} percentile.

Biological parameters

First trimester Down syndrome screening results were collected for each pregnancy.

First trimester pregnancy associated plasma protein-A (PAPP-A) and β-human chorionic gonadotrophin (βhCG) levels had been measured in international unit/liter and converted to multiples of the median (MoM) using the crown-rump length or biparietal diameter measurement when the blood sample was obtained as an estimate of gestational age.

Available blood samples collected at the end of first trimester of pregnancy (between 11 weeks of gestation (WG) and 13+6 WG) for Down syndrome screening stored at −20 °C in Biochemistry department of Brest University Hospital were used for PlGF quantification. Measurements were done with the automated B·R·A·H·M·S KRYPTOR compact PLUS system (B·R·A·H·M·S PlGF plus KRYPTOR: Thermo Fisher Scientific, Hennigsdorf, Berlin) according to the manufacturer's instructions described elsewhere [18]. PlGF levels were expressed in pg/ml. We compared our results to norm values of PlGF determined by Verlohren et al. [19] between 10 and 14 W G and we determined the number of values <$2,5^{th}$ percentile (corresponding to 26,80 pg/ml) in each group.

The institutional review board of Brest University Hospital approved "HPP-IPF" and "HEMOTHEPP" studies. Informed oral consents were obtained from all participants. Informed written consents were obtained from patients whose blood samples had been collected at the end of first trimester and who had not been enrolled in one of these two studies.

Statistical analysis

Quantitative variables were summarized using median and interquartile range (IQR). Categorical variables were summarized as frequency (%) of categories.

Difference between groups for categorical variables were analyzed by the Chi square test (or Fisher's exact test if the number of individuals was <5). For continuous variables, differences between groups were analyzed by nonparametric tests: the Mann and Whitney U test for comparison between two groups and the Kruskall Wallis test for comparison between more than two groups. The significance level used was $p < 0,05$.

All statistical analyses were performed with GraphPad Prism V5.

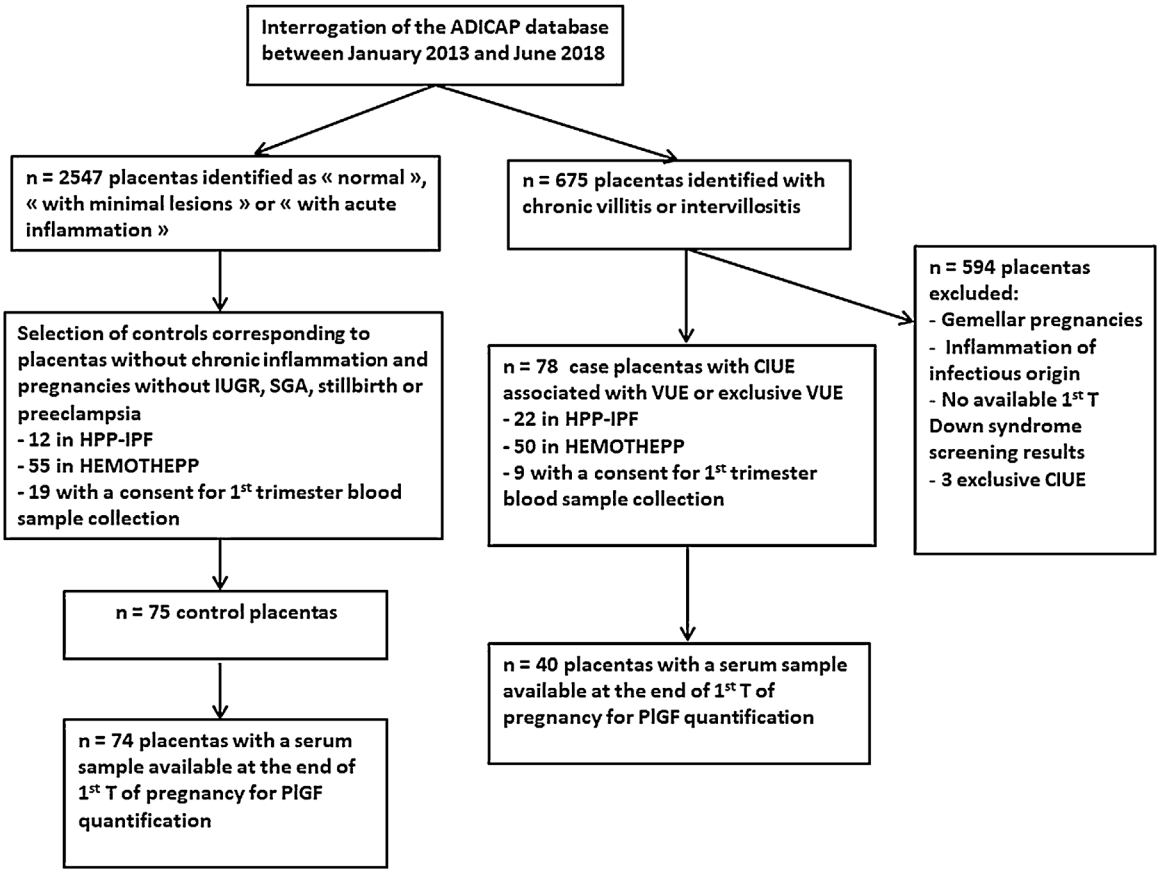

Fig. 1. Flow chart of the study.

Results

Cases

675 placentas with chronic inflammation were identified by interrogation of ADICAP system between January 2013 and June 2018 (Fig. 1). At the same period, 6685 placentas had been examined. Among these 675 placentas, after excluding placentas from multiple gestation pregnancies, placentas with inflammation of infectious etiology and placentas without first trimester Down syndrome screening results, 81 placentas with VUE and/or CIUE were included. 3 placentas with exclusive CIUE were finally excluded because this sub-group was too small to be representative. Of the 78 remaining placentas, 40 corresponding patients had a stored serum sample available at first trimester of pregnancy for PlGF quantification.

Among these 78 cases, 16 (20,5%) were CIUE associated with VUE, accepting their co-occurrence in a placenta, and 62 (79,5%) were exclusive VUE.

Controls

2547 placentas labelled “normal” or “with minimal lesions” or “with acute inflammation” were identified at the same period (Fig. 1). After excluding multiple gestation pregnancies, pregnancies complicated with pre-eclampsia, stillbirth, IUGR or SGA, placentas with chronic inflammation and placentas without first trimester Down syndrome screening results, 75 control placentas were included. Of these, 74 corresponding patients had a stored serum sample available at first trimester of pregnancy for PlGF quantification.

Comparison between cases and controls

Baseline maternal characteristics were similar in cases and controls (Table 1).

In the case group, adverse pregnancy outcomes were frequent: 21,8% IUGR, 30,8% SGA and 10,3% pre-eclampsia (Table 2).

Cases had less gestational diabetes (12,8% *vs* 26,7%, p = 0,04) than controls. Median term at delivery was similar in cases and controls (39 and 38 W G).

Table 1
Comparison of maternal characteristics between cases (CIUE associated with VUE and exclusive VUE) and controls.

	n = 78 cases	n = 75 controls	p
Median age at delivery (year)	31 (27–34)	30 (25–35)	0.9
Medical history			
Asthma	16 (20.5%)	9 (12%)	0.2
Chronic hypertension	0	2 (2.7%)	0.2
Autoimmune disease	5 (6.4%)	2 (2.7%)	0.4
Systemic lupus erythematosus	1 (1.3%)	0	
Autoimmune thyroiditis	4 (5.1%)	0	
Median BMI before pregnancy (kg/m^2)	23 (22–28)	24 (21–27)	0.6
Obese women	10 (12.8%)	10 (13.3%)	1
Obstetrical history			
Nulliparous	37 (47.4%)	29 (38.7%)	0.3
Vasculoplacental disorders	8 (10.3%)	5 (6.7%)	0.6
Blood group			
Group 0	24 (30.8%)	29 (38.7%)	0.3
Any group with negative rhesus	12 (15.4%)	17 (22.7%)	0.3

Continuous variables are expressed as median and interquartile range and categorical variables are expressed as frequency (percent). Chi square test or Fisher's exact test were used for categorical variables and Mann and Whitney U test was used for continuous variables. CIUE = chronic intervillositis of unknown etiology; VUE = villitis of unknown etiology; BMI = body mass index.

Table 2
Comparison of pregnancies between cases (CIUE associated with VUE and exclusive VUE) and controls.

	n = 78 cases	n = 75 controls	p
Method of conception			
Natural	74 (94.9%)	70 (93.3%)	0.7
Assisted	4 (5.1%)	5 (6.7%)	
Medication use during pregnancy			
Aspirin	6 (7.7%)	2 (2.7%)	0.3
Long term corticosteroid therapy	1 (1.3%)	1 (1.3%)	
Gestational diabetes	10 (12.8%)	20 (26.7%)	0.04
Pre-eclampsia	8 (10.3%)	0	
Intrahepatic cholestasis	2 (2.6%)	2 (2.7%)	
Premature rupture of membrane	13 (16.7%)	20 (26.7%)	0.2
Median term at delivery (WG)	39 (37–40)	38 (35–40)	0.08
IUGR	17 (21.8%)	0	
SGA	24 (30.8%)	0	
Birth weight (grams)	2645 (2270–2883)	2970 (2340–3385)	
Birth weight (percentiles)	8.9 (2.3–30.6)	53.5 (30.4–70)	
Sex			
Male	31 (39.7%)	38 (50.7%)	0.2
Female	47 (60.3%)	37 (49.3%)	

Continuous variables are expressed as median and interquartile range and categorical variables are expressed as frequency (percent). Chi square test or Fisher's exact test were used for categorical variables and Mann and Whitney U test was used for continuous variables. CIUE = chronic intervillositis of unknown etiology; VUE = villitis of unknown etiology; WG = weeks of gestation; IUGR = intrauterine growth restriction; SGA = small for gestational age.

When compared to control placentas, case placentas were smaller (425 g [IQR 370–480] *vs* 460 g [IQR 390–523], p = 0,03), in correlation with smaller birthweight (2645 g [IQR 2270–2883] *vs* 2970 g [IQR 2340–3385]) (Table 3). Case placentas showed more excessive fibrin deposition (87,2% *vs* 61,3%, p = 0,0004), more maternal vascular malperfusion features (79,5% *vs* 22,7%, p < 0,0001) and more fetal vascular malperfusion features (33,3% *vs* 12%, p = 0,002).

Median PAPP-A levels were similar in cases and in controls (0,83 MoM [IQR 0,55–1,26] *vs* 1,02 MoM [IQR 0,70–1,29], p = 0,09). Cases had lower βhCG levels than controls (0,74 MoM [IQR 0,53–1,12] *vs* 1,00 MoM [IQR 0,72–1,53], p = 0,002) (Table 4 and Fig. 2C).

First trimester PlGF levels were measured in 40 cases and in 74 controls. Median PlGF levels were lower in cases than in controls (29,74 pg/ml [IQR 19,74–36,17] *vs* 36,37 pg/ml [IQR 27,36–49,13], p = 0,007) (Table 4 and Fig. 2A).

Table 3
Comparison of histological findings between cases (CIUE associated with VUE and exclusive VUE) and controls.

	n = 78 cases	n = 75 controls	p
Placenta weight (grams)	425 (370–480)	460 (390–523)	0.03
Placenta weight (percentiles)	30 (9–54)	52 (40–76)	<0.0001
Fetoplacental weight ratio	6.3 (5.6–6.8)	6 (5.5–6.9)	0.8
Maternal vascular malperfusion	62 (79.5 %)	17 (22.7 %)	<0.0001
Fetal vascular malperfusion	26 (33.3 %)	9 (12 %)	0.002
Excessive fibrin deposition	68 (87.2 %)	46 (61.3 %)	0.0004
Chronic intervillositis (CIUE)	16 (20.5 %)	0	
Diffuse	1	0	
Focal	5	0	
Unknown	10	0	
Chronic villitis (VUE)	78 (100 %)	0	
High grade	28 (35.9 %)	0	
Low grade	50 (64.1 %)	0	
Basal	4 (5.1 %)	0	
Parenchymatous (P)	73 (93.6 %)	0	
Sub Chorial (SC)	24 (30.8 %)	0	
P + SC	22 (28.2 %)	0	
Chronic deciduitis	5 (6.4 %)	0	
Chronic chorioamniotitis	35 (44.9 %)	0	

Continuous variables are expressed as median and interquartile range and categorical variables are expressed as frequency (percent). Chi square test or Fisher's exact test were used for categorical variables and Mann and Whitney U test was used for continuous variables. CIUE = chronic intervillositis of unknown etiology; VUE = villitis of unknown etiology; P = parenchymatous; SC = subchorial.

In the case group, 24 pregnancies complicated with adverse pregnancy outcomes (*i.e.* 9 IUGR, 13 SGA and 4 pre-eclampsia) had a PlGF measurement at first trimester. 14 among 24 of these pregnancies complicated with adverse pregnancy outcomes (*i.e.* 58,3%) had a PlGF level < 2,5th percentile.

Comparison between exclusive VUE and CIUE associated with VUE

CIUE associated with VUE and 62 exclusive VUE were included in this study (Table 5).

Baseline maternal characteristics were similar between the CIUE associated with VUE group and the exclusive VUE group.

Adverse pregnancy outcomes rate was similar in the two groups, as well as median term at delivery.

Median newborn birthweight was lower in the exclusive VUE group in comparison with the CIUE associated with VUE group (2580 *vs* 2805 g, p = 0,04).

Median first trimester PAPP-A and βhCG levels were similar in the two groups (0,85 MoM *vs* 0,82 MoM, p = 0,8 for PAPP-A and 0,65 MoM *vs* 0,86 MoM, p = 0,3 for βhCG) (Table 6 and Fig. 2D).

PlGF levels were lower in CIUE associated with VUE and in exclusive VUE cases compared to controls (respectively 34,05 pg/ml *vs* 28,35 pg/ml and 36,37 pg/ml, p = 0,01; Table 6 and Fig. 2B). In the CIUE associated with VUE group, one among five adverse pregnancy outcomes had a PlGF level < 2,5th percentile, whereas in the exclusive VUE group, 13 among 19 adverse pregnancy outcomes (*i.e.* 68,4%) had a PlGF level < 2,5th percentile.

Comment

Our results confirm that IUGR and SGA are frequent in VUE and CIUE associated with VUE pregnancies. Additionally, we found lower first trimester PlGF levels in exclusive VUE cases than in controls, suggesting that reduced angiogenesis is involved in adverse pregnancy outcomes related to VUE.

We identified 14 studies on VUE or CIUE in PubMed database with a healthy control group, among which only two studies had a design similar to ours [20–33]. A high prevalence of autoimmune diseases (systemic lupus erythematous, antiphospholipid syndrome, Sjögren's syndrome . . .) was described among patients with a history of CIUE [34], what we did not confirm.

We found similar adverse pregnancy outcomes rates in exclusive VUE cases and in CIUE associated with VUE cases. Only

Table 4
Comparison of first trimester biological parameters between cases (CIUE associated with VUE and exclusive VUE) and controls.

	n = 78 cases	n = 75 controls	p
Median PAPP-A level at 1st T (MoM)	0.83 (0.55–1.26)	1.02 (0.70–1.29)	0.09
Median βHCG level at 1st T (MoM)	0.74 (0.53–1.12)	1.00 (0.72–1.53)	0.002
	n = 40 cases	n = 74 controls	p
Median PlGF level at 1st T (pg/ml)	29.74 (19.74–36.17)	36.37 (27.36–49.13)	0.007

Continuous variables are expressed as median and interquartile range. CIUE = chronic intervillositis of unknown etiology; VUE = villitis of unknown etiology; PAPP-A = Pregnancy associated plasma protein-A; βhCG = β-human chorionic gonadotrophin; PlGF = placental growth factor; MoM = multiples of the median; 1st T = first trimester of pregnancy.

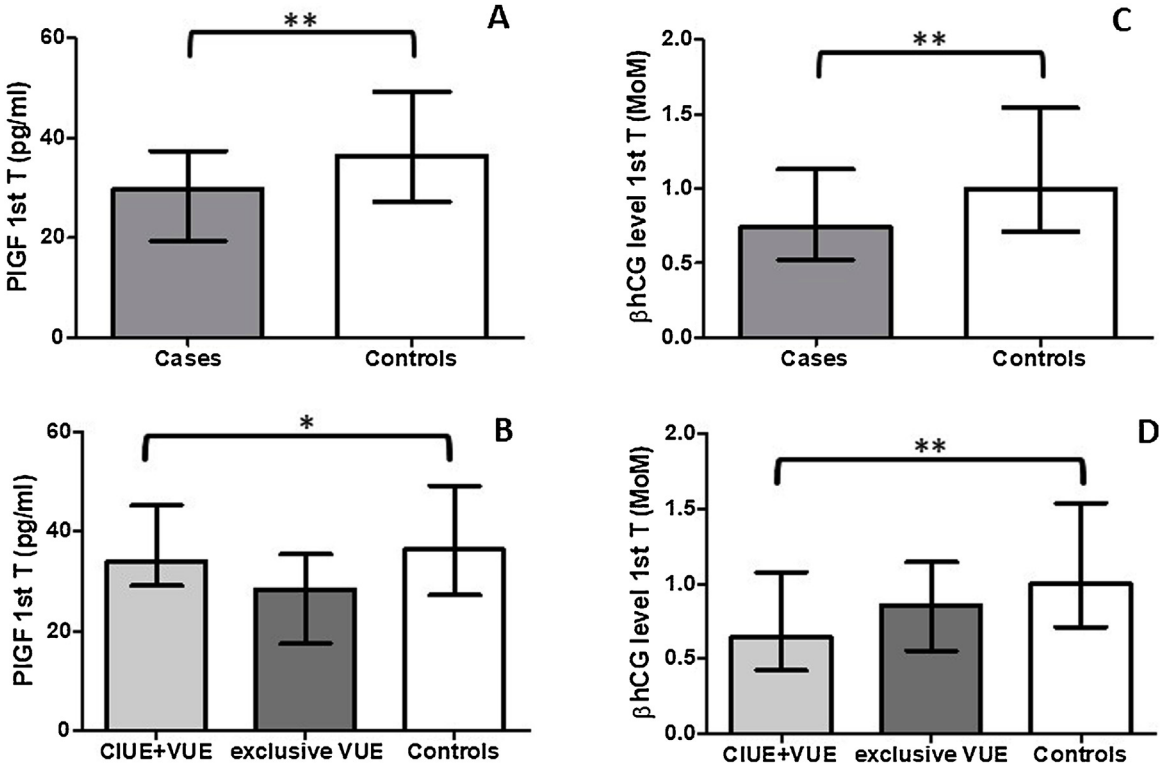

Fig. 2. Comparison of PlGF levels (A,B) and βHCG levels (C,D) at first trimester of pregnancy between cases and controls and between CIUE associated with VUE, exclusive VUE and controls (B,D).

two other studies compared obstetrical prognosis between VUE and CIUE. Nowak et al found more IUGR in the CIUE group in comparison with VUE and combined VUE-CIUE [6] whereas Labarrere et al found no difference in terms of SGA and pre-eclampsia between VUE and CIUE [27].

VUE and CIUE share a similar immune composition and a common pathophysiology. Lee et al considered VUE, chronic chorioamniotitis and chronic deciduitis as one global and unique placental manifestation of the maternal anti-fetal rejection process, characterized by a fetal systemic inflammation with increased fetal plasma angiostatic CXC motif ligand chemokine 10 (CXCL10) called "FIRS type 2" [35]. This chemokine could be the Ariane thread connecting chronic inflammation, growth restriction and reduced angiogenesis suspected in VUE [36].

A high rate of maternal vascular malperfusion features in placentas of IUGR and SGA suggests VUE and CIUE associated with VUE generate placental underperfusion. This significant patho-physiological association had already been reported by Salafia et al in a study investigating preterm pre-eclampsia [37].

To our knowledge, this is the first study evaluating the relevance of angiogenic biomarkers in prediction of adverse pregnancy outcomes in the specific population of VUE and CIUE associated with VUE pregnancies. Our study design aimed at finding a first trimester predictive biomarker of adverse pregnancy outcomes associated with exclusive VUE and CIUE associated with VUE. We documented lower βhCG levels at the end of first trimester in cases compared to controls. Low first trimester βhCG levels have already been described in association with IUGR [38] and pregnancy loss before 24 WG [39]. Nevertheless, elevated first trimester βhCG levels have been associated with vasculoplacental disorders such as pre-eclampsia, IUGR and gestational diabetes [40], which makes our results difficult to interpret.

We found lower first trimester PlGF levels in cases compared to controls. Levine et al identified that PlGF levels were lowered from 13 WG in women affected by pre-eclampsia and even lower when pre-eclampsia was associated with IUGR [12]. Poon LC et al added that PlGF was also lowered in SGA cases and that PlGF could be used in combination with PAPP-A, uterine artery pulsatility index and maternal factors to predict SGA at first trimester [41,42].

Our findings are consistent with an implication of early angiogenesis dysregulation in adverse pregnancy outcomes

Table 5
Overall comparison between CIUE associated with VUE, exclusive VUE and controls.

	CIUE associated with VUE n = 16	Exclusive VUE n = 62	Controls n = 75	p
Baseline maternal characteristics				
Nulliparous	7 (43.8%)	30 (48.4%)	29 (38.7%)	0.5
Assisted conception	2 (12.5%)	2 (3.2%)	5 (6.7%)	
Autoimmune disease in mother	3 (18.8%)	2 (3.2%)	2 (2.7%)	
Asthma in mother	2 (12.5%)	14 (22.6%)	9 (12%)	0.2
Chronic hypertension in mother	0	0	2 (2.7%)	
Median mother's prepregnancy BMI (kg/m^2)	26	23	24	0.5
Obesity in mother	2 (12.5%)	8 (12.9%)	10 (13.3%)	1
Median age at delivery (years)	31	30	30	1
Pregnancy characteristics				
IUGR	1 (6.3%)	16 (25.8%)	0	
Median term at diagnosis of IUGR (WG)	37	32		
SGA	4 (25%)	20 (32.3%)	0	
Pre-eclampsia	3 (18.8%)	5 (8.1%)	0	
Median term at diagnosis of pre-eclampsia (WG)	38	35		
Intrahepatic cholestasis	1 (6.3%)	1 (1.6%)	2 (2.7%)	
Gestational diabetes	2 (12.5%)	8 (12.9%)	20 (26.7%)	0.1
Premature rupture of membrane	5 (31.3%)	8 (12.9%)	23 (30.7%)	0.04
Median term at delivery (WG)	39	39	38	0.2
Median newborn weight (grams)	2805	2580	2970	
Median newborn weight (percentiles)	19.5	5.8	53.5	
Placenta characteristics				
Median placenta weight (grams)	428	418	460	0.07
Median placenta weight (percentiles)	38	29	52	<0.0001
Median fetoplacental weight ratio	6.4	6.2	6	0.8
Maternal vascular malperfusion	15 (93.8%)	47 (75.8%)	17 (22.7%)	<0.0001
Fetal vascular malperfusion	4 (25%)	22 (35.5%)	9 (12%)	0.005
Excessive fibrin deposition	12 (75%)	56 (90.3%)	46 (61.3%)	0.0005
Chronic chorioamniotitis	5 (31.3%)	30 (48.4%)	0	<0.0001
Chronic deciduitis	2 (12.5%)	3 (4.8%)	0	0.03

Continuous variables are expressed as median and categorical variables are expressed as frequency (percent). Chi square test or Fisher's exact test were used for categorical variables and Kruskall Wallis test was used for continuous variables. CIUE = chronic intervillositis of unknown etiology; VUE = villitis of unknown etiology; BMI = body mass index; WG = weeks of gestation; IUGR = intrauterine growth restriction; SGA = small for gestational age.

Table 6
Comparison of biological parameters between CIUE associated with VUE, exclusive VUE and controls.

Biological parameters	CIUE associated with VUE N = 16	Exclusive VUE n = 62	Controls n = 75	p
Median PAPP-A at 1st T (MoM)	0.85	0.82	1.02	0.2
Median βHCG at 1st T (MoM)	0.65	0.86	1.00	0.005
Biological parameters	**CIUE associated with VUE N = 16**	**Exclusive VUE n = 33**	**Controls n = 74**	**p**
Median PlGF at 1st T (pg/ml)	34.05	28.35	36.37	0.01

Continuous variables are expressed as median and interquartile range. Kruskall Wallis test was used for statistical analysis. CIUE = chronic intervillositis of unknown etiology; VUE = villitis of unknown etiology; PAPP-A = Pregnancy associated plasma protein-A; βhCG = β-human chorionic gonadotrophin; PlGF = placental growth factor; MoM = multiples of the median; 1st T = first trimester of pregnancy.

associated with exclusive VUE. On the other hand, CIUE associated with VUE cases seem to be unrelated to reduced angiogenesis, as suggested by the low frequency of very low PlGF values in this group. In exclusive VUE cases, the immunological conflict between mother and fetus might trigger reduced angiogenesis through early and abnormal placental hypoxia, as suggested by the high frequency of maternal vascular malperfusion features observed in placentas from IUGR and SGA. First trimester PlGF quantification in pregnant women with a past history of adverse pregnancy outcome related to exclusive VUE could help to predict recurrence of obstetrical complications. Therefore, a low PlGF level could justify aspirin prophylaxis throughout the ongoing pregnancy.

Our study has some limitations. Due to small-sized groups, we were not able to observe a significant association between maternal clinical characteristics and occurrence of VUE or CIUE associated with VUE. Then, PlGF was measured in only half of the cases. Finally, we tried to have a control group with normal placentas from uncomplicated pregnancies. Nevertheless, as this study was conducted retrospectively, our controls were not completely healthy, as attested by the high proportion of gestational diabetes.

Our study has some strengths: we described VUE and CIUE associated with VUE but also compared them with controls, trying by this approach to find potential predictors of VUE and CIUE associated with VUE. This last point was rarely reported in previous literature.

In summary, exclusive VUE and CIUE associated with VUE seem similarly associated with maternal vascular malperfusion features in placenta and with IUGR and SGA pregnancies. First trimester low PlGF levels in cases suggest that reduced angiogenesis is involved in adverse pregnancy outcomes related to VUE.

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