Effect of Known and Previously Undiagnosed Undifferentiated Connective Tissue Diseases on Pregnancy Outcome

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ABSTRACT

Objective: Previously undiagnosed and known UCTDs are associated with severe obstetric and perinatal outcomes compared to controls.

Methods: Previously undiagnosed UCTDs and controls were screened using a two-step approach (self-administered questionnaire and test for rheumatic autoantibodies) and evaluation by a rheumatologist. Pregnant subjects with known UCTD were recruited at the first trimester of pregnancy during their rheumatologic follow-up. Pregnancy complications such as preeclampsia, fetal growth restriction, small for gestational age, umbilical artery PI>95th percentile, prematurity<37 weeks 'gestation, were evaluated in cases and controls.

Results: Out of the 5199 women screened, 114 (2.2%) had a previously undiagnosed UCTD and 65 (1.25%) were diagnosed with major connective tissue diseases. Women with known UCTDs enrolled during first trimester were 78. Compared to controls, UCTD subjects, either previously undiagnosed and known, had increased rates of second trimester uterine artery bilateral notches, umbilical artery pulsatility index>95th percentile, and increased odds ratios of moderate to severe obstetric complications (respectively, OR 5.6 (CI 2.9-10.8) and OR 9.31 (CI 4.6-18.6)). Previously undiagnosed UCTDs, similarly to known UCTDs, were associated with the same risk of preeclampsia, respectively OR 6.3 (CI 2.4-16.7) and 6 (CI 2-18.3). UCTDs developed preeclampsia at earlier weeks (mean 32.5 vs 37.0 weeks) and delivered at earlier weeks (mean 35.5 vs 38.5 weeks) compared to control women.

Conclusions: Rheumatic autoimmune screening in pregnancy is useful because also minor connective tissue diseases, as previously undiagnosed UCTDs, could be complicated by obstetric adverse outcome, as preeclampsia.

Keywords: Connective tissue diseases; Rheumatic autoimmune screening; Preeclampsia; UCTD; Pregnancy outcomes

INTRODUCTION

The term undifferentiated connective tissue disease (UCTD) identifies a heterogeneous group of disorders characterized by signs and symptoms suggestive of a systemic rheumatic disorder but not fulfilling any of the classification criteria for a defined connective tissue disease (CTD) [1-3]. Since more than 50% of well-defined CTD are preceded by an undifferentiated or incomplete phase lasting month or years, the concept of UCTD has been often used to identify early phases of systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), or other CTD [4,5]. However, follow-up studies suggest that among subjects with an initial diagnosis of UCTD, 10-20% progress to a definite UCTD in the first few years of follow-up, 50-60% remain undifferentiated and up to 24% regress to a remission state [3,6,7]. Epidemiological data suggest that UCTDs are more common than well-defined CTD, are often paucisymptomatic and undiagnosed but responsible of autoimmune-related diseases involving lung, cardiovascular and reproductive systems

[8-10]. Although previous studies have reported increasing rates of reproductive failures and pregnancy complications among women who later develop SLE, RA or Sjögren's Syndrome (SS), data on the effect of UCTDs on pregnancy outcome are limited. A known diagnosis of UCTDs antedating pregnancy is usually associated with an increased risk of adverse pregnancy outcome [11]. Previous studies have found that is possible to identify cases of early, relatively asymptomatic, previously undiagnosed cases of UCTDs at the beginning of pregnancy. In cohort case-control prospective studies, these early rheumatic disorders impact negatively on pregnancy outcome [11,12].

The purpose of this study was to evaluate the effect of previously undiagnosed UCTDs identified during the first trimester of pregnancy and those of known UCTDs antedating gestation on pregnancy outcomes compared to healthy controls.

METHODS

Subjects with previously undiagnosed UCTDs and controls for the study were recruited among unselected pregnant women who obtained antenatal care at our Department during the first trimester of pregnancy. The criteria of recruitment and validation methods have been reported elsewhere [12]. Briefly, all women with a singleton pregnancy attending the clinic for antenatal care each Monday during a 6-year period (May 2010 to June 2016) with fluency in Italian language, and without a previous diagnosis of rheumatic disease were tentatively enrolled in the study. The study was approved by the local ethics committee of our Department (Current Research Project No. 686 of IRCCS Foundation Policlinico San Matteo of Pavia years 2010-2016). After informed consent and before the medical evaluation, each woman was asked to complete a 10-item screening questionnaire for rheumatic diseases including any connective tissue disorder symptoms (Table 1). Women who answered positively to one or more of the questions were tested for the presence of circulating autoantibodies, including antinuclear anti-body (ANA), anti-double-stranded DNA, anti-extractable nuclear antigen (ENA), anticardiolipin antibody (aCL), anti-β_2-glycoprotein I antibodies (aβ_2GPI) and lupus anticoagulant (LA), according to standardized methods, as previously described [12]. The ANA test was considered positive at a titer $\geq$ 1:80. To ensure random sampling, the first two subjects with negative responses to all the items in the questionnaire, after each woman positive for questionnaire, were asked to participate in the study, were tested for autoantibodies and served as the control. Cases and controls were referred to the rheumatology unit of our hospital for further clinical assessment including a careful history and a physical examination. Rheumatologists were unaware of the results of the questionnaires.

Table 1: Items of the questionnaire for rheumatic disorders during pregnancy. Women were asked to answer yes or no to all the questions.

1. Have you ever had generalised or localised reddening of your skin after exposure to sunlight?
2. Have you ever had an obvious or prominent rash on your cheeks or nose?
3. Do your hands or feet become white in the cold and then blue or pink?
4. (a) Have you ever had painful and swollen joints? (b) Do you suffer from stiffness lasting one hour or more in the morning?
5. Have you ever had pericarditis or pleuritis?
6. Do you have a dry mouth?
7. Do you feel like you have sand in your eyes?
8. Have you ever had painful white mouth ulcers?
9. Have you ever had thrombophlebitis?
10. Have you had two or more miscarriages or stillbirths?

Rheumatoid factor and anti-citrullinated peptide anti-bodies were not included in the screening autoantibody profile, but they were tested only in patients with arthritis after the rheumatologic evaluation. Rheumatic diseases were classified according to widely used criteria for UCTD and other major connective tissue diseases [1,13-19].

Pregnant subjects with known UCTD were recruited during first trimester of pregnancy among women attending the outpatient clinic of the Department of Rheumatology of our hospital during the period May 2010-June 2016. After informed consent, each woman was asked to complete the same screening questionnaire for connective tissue disorder symptoms (Table 1). The diagnosis of UCTD had been made according to the criteria of Mosca et al. [1,2] and included (a) signs and symptoms suggestive of a CTD without fulfilling the criteria of any definite CTD (b) ANA positivity in two determinations; (c) absence of autoantibody markers or symptoms distinctive of any CTD; (d) signs and symptoms duration $\geq$ 3 years for stable UCTD and less than 3 years for early UCTD.

Cases (previously undiagnosed and known UCTDs pregnant subjects) were evaluated by monthly rheumatologic clinical assessment during pregnancy. After first trimester enrolment, cases and controls were followed-up with monthly obstetric clinical and ultrasonographic evaluations. The mean uterine artery pulsatility index (PI) in the first and second trimester was evaluated according to standard methods [20]. Pulsatility indices of uterine or umbilical arteries were considered abnormal when the values were higher than the 95th percentile of reference curves [20]. Fetal growth restriction (FGR) was diagnosed when the abdominal fetal circumference at ultrasonographic examination fell below the 10th percentile of our local reference curves, confirmed on at least two consecutive measurements taken two weeks apart after the standard US obtained at 18–22 weeks of pregnancy, and PI of umbilical artery was higher than the 95th percentile of reference curves signaling a reduced perfusion of fetal placental unit. Preeclampsia was diagnosed according to standard criteria [21]. Small for gestational age (SGA) infants were diagnosed when birth weight was below the 10th percentile of the Italian population [22]. Moderate-to-severe pregnancy complications included second trimester spontaneous abortion, FGR, preeclampsia, delivery before 34 weeks gestation and a low birthweight (LBW) (<2500 gr).

Statistical analyses were carried out with non-parametric one-way analysis of variance and the Bonferroni post-hoc test to compare continuous variables between the groups studied. Categorical variables were compared by Pearson's x^2. Partitioning of x^2 statistics with the Bonferroni correction for multiple comparisons was used to evaluate the statistical significance of pairwise comparisons in two × K tables. Associations between previously undiagnosed and known UCTDs and pregnancy outcomes were evaluated using penalized logistic regression analysis with odds ratios (ORs) and 95% confidence intervals (95% CIs), adjusted for potential confounders (Stata 13.0 for Windows). Logistic equations included complications overall of pregnancy (preeclampsia, FGR, SGA, umbilical artery PI>95th percentile, prematurity<37 weeks gestation) and moderate-severe pregnancy complications as outcome variables and first trimester smoking (yes/no), multiparity (yes, no), first trimester maternal body mass index (continuous), and the type of UCTD as explanatory variables. Odds ratios in logistic regression were compared according to Scott and Freese [23].

RESULTS

During the period of the study 5348 pregnant subjects were eligible for the screening of UCTDs by a dual approach using a questionnaire plus antibody detection and 5199 (97.2%) gave their consent. The rate of positivity to one or more questions as detailed in questionnaire was 9.5% (494/5199). Of the 494 subjects with a positive questionnaire, 331 (67%) tested positive for autoantibodies and were sent to rheumatology unit for evaluation. The prevalence of previously undiagnosed UCTD in the entire cohort was 2.2% (114/5199). The prevalence of major rheumatic diseases was 1.25% (65/5199) with major connective tissue diseases.

During the period of enrolment of subjects with undiagnosed UCTDs, 87 subjects with a known diagnosis of UCTDs, already followed at the outpatient clinic of the Rheumatology Division of our hospital, reported a confirmed initial pregnancy. Nine (10.3%) of these women had a spontaneous abortion before enrolment and were excluded from the study, leaving 78 subjects for analysis. The median gestational age at enrolment of the entire population study was 12.1 weeks (IQR=11.8-12.6).

Symptoms, obtained from the questionnaire, and autoantibody characteristics of subjects with previously undiagnosed and known UCTDs at the beginning of pregnancy are reported in Table 2. Rheumatologic symptoms, ANA titre and other autoantibodies were similarly represented in UCTD subjects, either previously undiagnosed and known. Compared to women with previously undiagnosed disease, women with known UCTDs had increased rates of joint pain and dry mouth, ENA antibodies detection and ANA at high (>1:160) titre.

Table 2: Autoimmune symptoms and laboratory findings in known and previously undiagnosed undifferentiated connective tissue diseases (UCTDs).

	known UCTDs (n= 78)	Previously undiagnosed UCTDs (n= 114)	p-value
	N (%)	N (%)	
Photosensitivity	29 (37.18)	50 (43.86)	0.37
Erythema and/or malar rash	19 (24.36)	34 (29.82)	0.51
Raynaud's phenomenon	38 (48.72)	50 (43.86)	0.55
joint pain	49 (62.82)	50 (43.86)	0.012
serositis	0 (0.0)	3 (2.63)	0.27
Dry mouth	17 (21.79)	11 (9.65)	0.023
Dry eyes	13 (16.67)	13 (11.40)	0.39

Painful mouth ulcers	16 (20.51)	21 (18.42)	0.71
Thrombophlebitis	4 (5.13)	2 (1.75)	0.23
Pregnancy loss	20 (25.64)	33 (28.95)	0.74
ANA 1:80	21 (26.92)	43 (37.72)	
ANA 1:160	22 (28.2)	38 (33.33)	
ANA>1:160	35 (44.8)	33 (28.9)	0.02
anti ds-DNA	4 (5.13)	2 (1.75)	0.23
ENA	27 (34.62)	15 (13.16)	<.001
aCL IgG	6 (7.69)	6 (5.26)	0.55
aCL IgM	8 (10.25)	7 (6.14)	0.4
a β 2GPI IgG	4 (5.13)	4 (3.51)	0.72
a β 2GPI IgM	3 (3.85)	3 (2.63)	0.69
LA	1 (1.28)	2 (1.75)	1

ANA: antinuclear antibodies; ENA: anti-extractable nuclear antigens antibodies; aCL: Anticardiolipin antibodies; a β 2GPI: anti- β 2glycoprotein I; LA: Lupus anticoagulant.

Among previously undiagnosed UCTD 48/114 subjects (42.10%) required treatment (Corticosteroids, hydroxychloroquine and/or aspirin and/or low-molecular weight heparin) during pregnancy, while among known UCTD 56/78 subjects (71.79%) were already treated at the beginning of pregnancy. During pregnancy and puerperium, 3 subjects developed SLE (one among previously undiagnosed and 2 among known UCTD), 2 antiphospholipid syndrome (APS) (one among previously undiagnosed and one among known UCTD), one spondiloarthritis and one undifferentiated arthritis (one among previously undiagnosed and one among known UCTD). Overall the progression to a definite CTD was 5.1%

(4/78) among known and 2.6% (3/114) among previously undiagnosed UCTDs (p=0.49).

Compared to healthy controls, UCTD subjects, either previously undiagnosed and known, had increased first and second trimester uterine artery and third trimester umbilical artery pulsatility indices suggesting defective placentation and increased resistance in placental bed (Table 3). In addition, gestational age at delivery, birthweight and percentile of birthweight were all lower in UCTD subjects compared to controls (Table 3).

Table 3: Pregnancy variables, weeks of delivery and neonatal birthweight among known and previously undiagnosed undifferentiated connective tissue diseases (UCTDs) *: p<0.05 compared to controls.

	Controls	Known UCTDs	Previously Undiagnosed UCTDs	Overall p-value
	(n=612)	(n=78)	(n=114)	
	median (IQR 25-75)	median (IQR 25-75)	median (IQR 25-75)	
maternal age (yrs)	33.5 (30.0-36.0)	33.0 (31.0-37.0)	34.0 (30.0-37.0)	0.5
Maternal BMI (kg/m^2)	22.6 (20.4-24.5)	22.2 (20.2-24.8)	21.6 (19.8-25.5)	0.43
Gestational age at enrolment	12. 3(11.9-13.1)	12 (11.6-12.4)	12.4 (11.7-12.3)	0.7

1st trimester mean uterine artery PI	1.27 (0.77-1.41)	1.29 (1.03-1.59)*	1.40 (1.16-1.79)*	<0.001
2nd trimester mean uterine artery PI	0.76 (0.60-1.00)	0.91 (0.80-1.13)*	1.03 (0.84-1.26)*	<0.001
3rd trimester umbilical artery PI	0.92 (0.82-1.01)	0.90 (0.86-1.00)	0.92 (0.84-1.02)	0.7
Weeks of delivery (wks)	39.3 (38.5-40.2)	38.5 (37.3-40.1)*	39.1 (38.1-40.0)*	<0.001
Umbilical cord artery pH	7.31 (7.27-7.33)	7.28 (7.24-7.32)	7.31 (7.26-7.33)	0.07
Neonatal weight (g)	3290.0 (3023.7-3551.2)	3025 (2580.0-3380.0)*	3210 (2825.0-3550.0)*	<0.001
Percentile of neonatal weight	51 (28-75)	37 (16-54)*	47 (20-69)*	<0.001

Table 4 reports main pregnancy complications among cases and controls. The rates of second trimester uterine artery bilateral notches, umbilical artery pulsatility index>95th percentile, preeclampsia, FGR and SGA were all higher among UCTD subjects, either already established or undiagnosed during the first trimester of pregnancy, compared to controls.

Table 4: Pregnancy outcome in known and previously undiagnosed undifferentiated connective tissue diseases (UCTDs) *: p<0.05 compared to controls.

	controls (n=612)	known UCTDs (n=78)	Previoulsy undiagnosed UCTDs (n=114)	p-value
	n (%)	n (%)	n (%)	
Parity ≥ 1	186 (30.4)	34 (43.6)*	44 (38.6)	<0.001
Smoke	110 (18.0)	11 (14.1)	13 (11.4)	0.2
2nd trimester abortion	1 (0.2)	2 (2.6)*	2 (1.7)	0.03
1st trimester uterine artery bilateral notch	12 (2.0)	5 (6.4)*	24 (21.0)*	<0.001
2nd trimester uterine artery bilateral notch	8 (1.3)	3 (3.8)	11 (9.6)*	<0.001
Preeclampsia	11 (1.8)	6 (7.7)*	9 (11.5)*	<0.001
Fetal growth restriction	9 (1.5)	11 (14.1)*	6 (5.3)*	<0.001
Umbilical artery PI>95th pct	56 (9.15)	19 (24.4)*	19 (16.7)*	<0.001
Small for gestational age	51 (8.3)	13 (16.7)*	11 (14.1)*	0.05
Low birthweight (<2500 gm)	18 (2.9)	15 (19.2)*	11 (9.6)*	<0.001
Prematurity<37 weeks	7 (1.1)	11 (14.1)*	7 (6.1)*	<0.001
Prematurity ≤ 31 weeks	0	4 (5.1)*	2 (1.7)*	<0.001

Gestational diabetes	11 (1.8)	4 (5.1)*	12 (10.5)*	<0.001
Cesarean Section	176 (28.6)	32 (41.0)*	41 (36.0)	0.04
Moderate-severe complications	25 (4.1)	19 (24.3)*	19 (16.7)*	<0.001

After correction in penalized logistic regression for the confounding effect of smoking, parity, presence of antiphospholipid antibodies and maternal BMI, UCTDs, either previously undiagnosed or known, were significantly associated with increased odds ratios of preeclampsia, SGA, FGR and moderate to severe complications when compared to healthy controls (Table 5). Previously undiagnosed UCTDs, similarly to known UCTDs, were associated with the same risk of preeclampsia, respectively OR 6.3 (CI 2.4-16.7) and 6 (CI 2-18.3).

Table 5: Odds Ratios (OR) of complications of pregnancy among known and previously undiagnosed UCTDs compared to controls.

	Controls	Known UCTDs	Previously undiagnosed UCTDs
Pregnancy outcomes		OR (95% CI)	OR (95% CI)
Preeclampsia	Reference	6 (2-18.3)	6.3 (2.4-16.7)
Fetal growth restriction	Reference	11.4 (4.5-29.1)*#	3.84 (1.3-11.1)
Small for gestational age	Reference	2.4 (1.2-4.8)	1.4 (0.7-2.8)
Umbilical artery Doppler PI>10th percentile	Reference	3.2 (1.7-5.7)	1.9 (1.1-3.3)
Prematurity (<37 wks)	Reference	15.4 (5.4-43.6)*#	5.1 (1.7-15.6)
Moderate/severe pregnancy complications	Reference	9.31 (4.6-18.6)	5.6 (2.9-10.8

Moreover, on average, women with UCTDs developed preeclampsia earlier in pregnancy (mean 33.1 vs 37.0 weeks) compared to control women developing preeclampsia, and delivered at earlier weeks (mean 35.5 vs 38.5 weeks) compared to control women developing preeclampsia. In addition, previously undiagnosed UCTDs had higher, not statistically significant, rates (5/9, 55.5%) of early preeclampsia (mean 32.2 weeks) compared to known UCTDs (2/6, 33.3%, mean 33.4 weeks).

Considering the presence of antiphospholipid antibodies, as risk factor of developing preeclampsia, 4/6 (66.6%) of known UCTDs and 7/9 (77.7%) of previously undiagnosed UCTDs developed preeclampsia without antiphospholipid antibodies.

DISCUSSION AND CONCLUSION

Our study showed that UCTDs are associated with increasing risk of adverse outcomes such as defective placentation, preeclampsia and FRG, compared to healthy controls, confirming previous results. Moreover, the study highlighted as new result that previously undiagnosed UCTDs had higher risk of developing preeclampia, compared to control group, similarly to known UCTDs, but, differently than known UCTDs, previously undiagnosed UCTDs developed preeclampsia at earlier weeks, without confounder factors such as antiphospholipid antibodies, BMI and smoking. These data are in accord with Simard et al. [24] that found that women with SLE are at increased risk of early-preeclampsia, independently by traditional risk factors such as pregestational hypertension, antiphospholipid antibodies, BMI or smoking.

The prospective design, the large number of women screened for UCTDs, the distinction between known UCTDs and previously undiagnosed UCTDs and follow-up in pregnancy and puerperium are the main strengths of the present study. However, since we enrolled women at 11-13 weeks gestation, the design of this investigation does not capture the potential adverse effect of undifferentiated autoimmune disorders on early pregnancy outcome. Several case-control studies suggest that UCTDs, as CTDs, could be associated with an increasing risk of first-trimester abortion [25-27].

In our study we found a progression to major rheumatic diseases in 3.6% among subjects with UCTDs. Previous post-partum follow-up of subjects diagnosed with UCTDs in pregnancy has demonstrated a 6.7% progression to a definite CTD [28]. These data are comparable to those reported in old and new follow-up studies of non-pregnant subjects with UCTDs confirming the prognostic validity of this approach [3-6].

A dual screening approach with a questionnaire for rheumatic symptoms and subsequent autoantibody evaluation of subjects

with positive response was a reliable and effective method to diagnose UCTD during the first trimester of pregnancy in previous studies [12].

Recent studies supported the beneficial role of hydroxychloroquine in controlling disease activity during pregnancy [29]. In our study, subjects with known UCTDs already taking hydroxichloroquine at the beginning of pregnancy were counseled to continue treatment. Uncontrolled studies suggest an acceptable benefit/risk ratio of oral glucocorticoids, in controlling SLE activity during pregnancy [30]. According with these data, in our study known and previously undiagnosed UCTDs continued and/or started hydroxychloroquine and corticosteroids treatment. Aspirin and low molecular weight heparin were administered in presence of antiphospholipid antibodies and/or increased uterine artery resistance.

The effect of UCTDs on pregnancy outcome, rather than the effect of pregnancy on UCTDs, was mainly investigated in uncontrolled studies [26,27]. However, overall data on the effect of known UCTDs on pregnancy outcome suggest a 25-35% rate of pregnancy complications, according to our study.

The mechanisms underlying the deleterious effect of UCTDs in pregnancy outcome are mediated by the negative interference of autoantibodies on the immune-mediated process of early placentation [29,30]. The proinflammatory state associated with systemic autoimmune disorders could influence the balance between immune inflammatory and tolerance response and between angiogenic and anti-angiogenic soluble factors, leading to defective placentation and increasing risks of preeclampsia, FGR and preterm delivery[25-29].

Consisting with previous investigations [31], UCTDs were associated with increasing resistance in both uterine and umbilical arteries compared to healthy controls, suggesting a defective placentation and thus increasing the risk of adverse obstetric outcomes. Moreover, previously undiagnosed UCTDs were associated with the same risk of preeclampsia compared to known UCTDs, but in these patients preeclampsia seems to arise earlier in pregnancy, leading to earlier delivery, without difference in BMI or presence of antiphospholipid antibodies, traditionally associated with preeclampsia. Probably, we could explain this result by suggesting that previously undiagnosed UCTDs were diagnosed during the first trimester and so, they were treated for preeclampsia risk only in case of uterine artery abnormality, when the earlier phase of placentation had been already started and set up. These results supported that the pro-inflammatory state of UCTD, independently by the presence of antiphospholipid antibodies, could impaired early placentation and give rise to obstetric complications, as preeclampsia, FGR and preterm delivery.

In conclusion, the results of this study have shown that UCTDs, either antedating or previously undiagnosed at the beginning of pregnancy, are associated with increasing risks of moderate-severe adverse pregnancy outcomes compared to healthy controls. Moreover, previously undiagnosed UCTDs were associated with the same risk of preeclampsia compared to known UCTDs, at earlier weeks of development.

At present, although screening measures in the field of rheumatology are strongly encouraged, there are no data on the efficacy of these measures during pregnancy [32]. However, subjects with previously undiagnosed and known UCTDs, similarly to subjects with major CTDs, should be screened at the beginning of pregnancy and followed by a definite surveillance protocol taking into account potential obstetric complications, such as preeclampia and possible evolution to major connective tissue diseases during puerperium.

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