Recurrent miscarriage and the subsequent risk of cardiovascular disease

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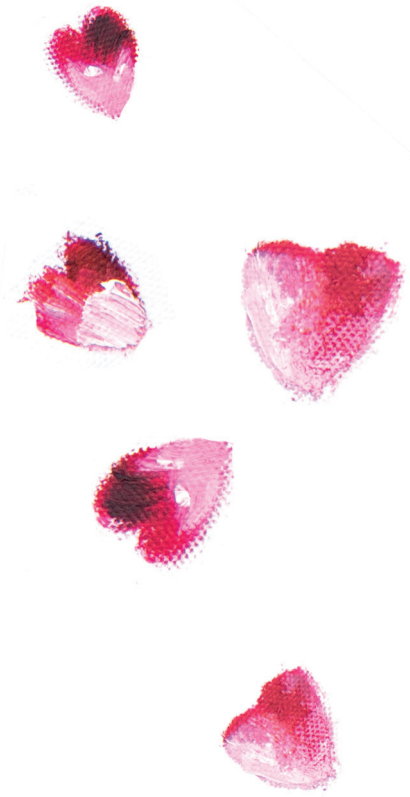

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Assessment of novel cardiovascular biomarkers in women with a history of recurrent miscarriage

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Abstract

Objectives A history of recurrent miscarriage is associated with future cardiovascular disease. The aim of this study was to determine novel cardiovascular biomarkers in women with a history of recurrent miscarriage as this might lead to a better understanding of the association.

Study design Women who visited the recurrent miscarriage clinic at Leiden University Medical Centre (between 2000 – 2010), and had three consecutive miscarriages ≤ 30 years were invited to participate in this follow-up study (between 2012-2014). The reference group consisted of women with at least one uncomplicated pregnancy and a history of no miscarriage, matched on zip code, age, and date of pregnancy.

Main outcome measures Cardiovascular biomarkers were determined, classified into; inflammation (HsCRP, lipoprotein-associated phospholipase A2), thrombosis (homocysteine, folate, anti-cardiolipin antibodies and anti-ß-2-glycoprotein antibodies), lipid metabolism (lipoprotein(a)), renal function (creatinine, microalbuminuria), myocardial damage (N-terminal pro-brain natriuretic peptide, high sensitive TroponineT) and multiple mechanisms (albumin, vitamin D).

Results In both groups, 36 women were included. Women with recurrent miscarriage had a significantly higher median HsCRP (1.49mg/L) compared to women with no miscarriage (1.01mg/L, p=0.03) and a significantly lower mean albumin (46.0 vs 47.6g/L, p=0.004) and vitamin D (55.6 vs 75.4nmol/L, p=0.007), respectively. Differences remained after adjustments for classic cardiovascular risk factors (BMI, smoking, diabetes mellitus, and hypertension).

Conclusions Our findings suggest a proinflammatory state in women with a history of recurrent miscarriage, which suggests a less optimal health, compared to women with no miscarriage. More research (observational and intervention) is warranted to investigate the association with vitamin D.

Introduction

Cardiovascular disease is the leading cause of morbidity and mortality in women in the western world[1]. There is increasing evidence that women with adverse pregnancy outcome are at increased risk of future cardiovascular disease, most well established for preeclampsia[2, 3]. Recent epidemiological research suggests that also women with a history of recurrent miscarriage have an increased risk of cardiovascular disease later in life[4-6]. Recurrent miscarriage is commonly defined as three or more consecutive pregnancy losses before 22 weeks of gestation[7]. It is a very heterogeneous condition and affects 0.5-3% of all fertile couples[8]. Several hypotheses are possible for the association between recurrent miscarriage and cardiovascular disease: shared risk factors and underlying pathology may lead to both diseases or alternatively, miscarriage could trigger a mechanism or cascade (second hit) that in turn leads to cardiovascular disease. Possibly via interactions with well-known risk factors. Two small studies are published concerning classical cardiovascular risk factors in women with a history of recurrent miscarriage with inconsistent results[9, 10]. An altered cardiovascular risk profile in women with recurrent miscarriage was found in the first study[9], although in the second study no difference in cardiovascular function and risk factors was described[10]. In addition to classic cardiovascular risk factors, there is a wide variety of novel biomarkers strongly associated with future cardiovascular disease in general [11, 12]. For example, high-sensitivity C-reactive protein (HsCRP), an inflammatory biomarker and lipoprotein(a) (Lp(a)), a lipid related biomarker. The most recent European guideline for the prevention of cardiovascular disease states that biomarkers may be useful in specific subgroups[12]. Knowledge about these markers might contribute to a better understanding of the association between miscarriage and future cardiovascular disease. Therefore, we conducted a follow-up study to determine novel cardiovascular biomarkers in women with a history of recurrent miscarriage compared to women with no miscarriage.

Methods

Study design

Follow-up study.

Exposed

Women who visited the recurrent miscarriage clinic at Leiden University Medical Centre between 2000 and 2010 and had their third consecutive miscarriage before the age of 31 years were invited to participate in this follow-up study. Recurrent miscarriage was defined as ≥ 3 consecutive miscarriages before 22 weeks of gestation. Women with

primary miscarriage (without birth ≥ 22 weeks of gestation before miscarriage) and secondary miscarriage (with a birth ≥ 22 weeks of gestation before miscarriage) were included. All women had a routine recurrent miscarriage work-up to identify possible causes for the recurrent miscarriage: a standardized history of the couple was performed, karyotyping of the couple (this was offered routinely before 2005 to all couples, after 2005 this was only offered in presence of low maternal age and/or positive family history for recurrent miscarriage[13], presence of uterus anomalies by ultrasound or hysteroscopy and presence of acquired and heritable thrombophilia was assessed. Acquired thrombophilia: Antiphospholipid syndrome was defined as the presence of elevated anticardiolipin antibodies or lupus anticoagulant in repeated samples taken 3 months apart and at least 10 weeks after a delivery [14], after revision of the classification criteria an elevated level of anti-β2 glycoprotein-I was added to the work-up [15]. Homocysteine levels were determined to exclude hyperhomocysteinemia. Heritable thrombophilia was defined by the presence of a factor V Leiden mutation, factor II (prothrombin) gene mutation, protein C or S deficiency or antithrombin deficiency. Enrolment took place between 2012 and 2014.The time interval between the diagnosis recurrent miscarriage and the time of follow-up had to be at least 2 years.

Unexposed matched group

For the reference group women with one or more uncomplicated pregnancy(ies) and no miscarriages were enrolled. In the Netherlands, it is common practice that independent primary care midwives are taking care of low-risk women (with no medical or obstetrical history) during pregnancy and child birth. The zip code of each woman with recurrent miscarriage was used to contact the nearest midwifery practice to take the impact of socio-economic status into account. Women with the same zip code, the same age (difference in birthdate maximal 1 year) and of which the time of first delivery was close to the time of the third miscarriage of the matched exposed woman (maximal 6 months before or 6 months after) were asked to participate.

In both groups pregnant and lactating women (within the last 3 months) were excluded.

Procedures and definitions

After enrolment all women were asked to fill out a web based questionnaire and were invited for risk factor screening including venous blood samples. The questionnaire included general information, medical history, family history of cardiovascular disease, use of medication, intoxications and obstetric history. Information about medical history, use of medication, intoxications and pregnancy outcome was cross checked in obstetrical records to overcome recall bias. Pregnancy outcome in at least one continuing pregnancy was recorded. Gestational diabetes was defined as a glucose intolerance resulting in hyperglycemia with onset during pregnancy[16]. Preeclampsia was defined as systolic blood pressure above 140 mmHg and/or diastolic pressure

above 90 mmHg combined with proteinuria [17], pregnancy induced hypertension as systolic blood pressure above 140 mmHg and/or diastolic pressure above 90 mmHg or higher measured on two occasions(after 20 weeks' gestation)[18], preterm birth as a delivery between 24 and 37 weeks gestation, intra-uterine growth restriction as birth weight below the 10th percentile for gestational age and sex according to the Perinatal Registry of the Netherlands birth weight percentiles [19]. Recurrent miscarriage was defined as idiopathic when the work-up for causes of recurrent miscarriage showed no abnormalities.

Cardiovascular risk factor assessment

Assessment of cardiovascular risk factors was performed by trained research nurses or physicians at the Leiden University Medical Centre or at the participants' home. Urine was collected for assessment of microalbuminuria immediately after waking up. Venous blood samples were collected after an overnight fast. A panel of novel cardiovascular biomarkers was tested in this study. Biomarkers were classified into; inflammation (HsCRP, Lipoprotein-associated phospholipase A2 (Lp-PLA2)), thrombosis (homocysteine, folate, anti-Cardiolipin antibodies (aCL) and anti-ß-2-Glycoprotein antibodies (aβ2GPI)) IgG and/or IgM, lipid metabolism (Lp(a)), renal function (creatinine, microalbuminuria), myocardial damage (N-terminal pro-brain natriuretic peptide(NT-proBNP), high sensitive Troponine T (hsTroponineT) and multiple mechanisms (albumin, 25-OH-Vitamin D). We describe these biomarkers with their possible association with cardiovascular disease in short in table 1.

The blood samples were centrifuged after coagulation, separated and serum was frozen at -80°C within 2 hours. Routine chemistry analyses were performed on a Roche Modular P800 chemistry analyzer using reagents of Roche Diagnostics (Mannheim, Germany). Analytical variation of all analytes was well below 5%. Homocysteine was analyzed on an Immulite 2000 Xpi immunoanalyzer of Siemens Healthcare Diagnostics (Tarry town, NY, USA). Analytical variation varied between 5% and 8%. NT-proBNP, Folate, hsTroponinT,25-OH-Vitamin D and Lp(a) were analyzed on a Roche Modular E170 Immunoanalyzer. Analytic variation varied between 3% and 6%. Immunological analyses were performed on an Immunocap 250 immunoanalyzer (Thermo Fisher Scientific, Waltham, MA, USA). Analytical variation was up to 5% for aCL and 10% for aβ2GPI antibodies. Lp-PLA2 was measured using an ELISA (Diadexus, San Francisco, CA, USA). All analyses were performed by technicians blinded for obstetrical history.

Table 1. Novel cardiovascular biomarkers, possible associations and mechanisms

Biomarker	Possible association with	Mechanisms
HsCRP	Inflammation	Acute phase protein with hepatic origin [21]
Lp-PLA2	Inflammation	Vascular-specific inflammatory biomarker. (Atherosclerotic plaques in blood vessels) [36]
Homocysteine	Thrombosis	Endothelial cell damage, reduction in the flexibility of vessels, and alters the process of haemostasis. Hyperhomocysteinemia can also arise from nutritional deficiencies of folate, vitamin B6, and vitamin B12. [33]
Folate	Thrombosis	Lowering serum homocysteine.[37]
aCL	Thrombosis	Antiphospholipid antibody: changes in the coagulation cascade, inhibition of protein C, antithrombin and annexin, platelet activation and complement, increased expression of endothelial adhesion molecules. [38] aCL seem to represent the group of autoantibodies with the highest correlation and possible risk outcome with CVD. [39]
aβ2GPI	Thrombosis	Antiphospholipid antibody: changes in the coagulation cascade, inhibition of protein C, antithrombin and annexin, platelet activation and complement, increased expression of endothelial adhesion molecules, [38]. Locally, using cellular models aβ2GPI antibodies seems to have a prominent pathogenic role. [39]
Lp(a)	Lipid metabolism	The physiological function of Lp(a) is unclear, pathogenic role in atherosclerosis and thrombosis formation. [40]
Creatinine	Renal function	May be surrogate marker for generalized vascular damage as well as renal dysfunction. Renal dysfunction may enhance intermediate risk factors such as hypertension, hyperhomocysteinemia and abnormalities of thrombogenic factors. [41]
Microalbuminuria	Renal function	May be a marker of widespread vascular abnormalities, including those of the glomerular capillary wall. Renal dysfunction. [41]
NT-proBNP	Myocardial damage	NT-proBNP is synthesized in response to ventricular stretch and ischemic injury [42]
hsTroponineT	Myocardial damage	Secreted from cardiac (and skeletal) muscles. Myocardial injury and ischemia. [43].
Albumin	Multiple mechanisms	Negative acute-phase protein. Interacts with free fatty acids, and inhibits their promoting effects on platelet aggregation and thrombosis. May act as an indirect and sacrificial antioxidant and inhibits peroxidase, free radical generation. Inhibitor of endothelial apoptosis.[24]
Vitamin D	Multiple mechanisms	Modulation of inflammatory processes. Favourably influence cardiovascular health including downregulation of the renin–angiotensin system, enhancement in insulin secretion and insulin sensitivity, and protection against angiogenesis. [26]

HsCRP: high-sensitivity C-reactive protein. Lp-PLA2: Lipoprotein-associated phospholipase A2, aCL: anti-Cardiolipin, aβ2GPI: anti-ß-2-Glycoprotein, Lp(a):Lipoprotein (a), NT-proBNP : N-terminal pro-brain natriuretic peptide, hsTroponineT: high sensitive Troponine T

Statistics

Matched data were analyzed using SPSS software version 22.0 (Statistical Package for Social Science; SPSS, Chicago, Illinois, USA). Comparisons of normal distributed data were performed using paired T- test. Comparisons of categorical data were performed using the McNemar's test. Skewed variables were log transformed, differences on the logarithmic scale were calculated and then back transformed to the original scale. This results in estimates of the ratio of the (geometric) mean in the recurrent miscarriage group over the mean in the no miscarriage group. For all tests, a p-value < 0.05 is indicated statistical significant. Multivariate analyses were performed using unianova test, to adjust for the following potential confounders (classical cardiovascular risk factors): Model 1: BMI; Model 2: BMI and smoking; and Model 3 BMI, smoking, diabetes mellitus and hypertension. The calculations were repeated in a subgroup analysis including women with idiopathic recurrent miscarriage.

Ethics

Approval from the medical ethics committee of Leiden University Medical Centre (P04-020; October 3, 2012) was obtained and all participants gave informed consent. The study was registered with the Dutch trial registry NTR3408. This study is part of the REMI (REcurrent MIscarriage) studies, studies which investigate consequences and causes of recurrent miscarriage.

Results

A flowchart of the inclusion of the participants is shown in Figure 1. Of the 76 included women (38 women with recurrent miscarriage and 38 women with at least one uncomplicated pregnancy and no miscarriage) 4 women were excluded from analysis, because they did not meet the inclusion criteria for this study as described in Figure 1, leaving 36 matched pairs.

Women with recurrent miscarriage were slightly younger at follow-up and had a significantly higher gravidity and lower parity than those in the no miscarriage group (Table 2). Women with recurrent miscarriage were more often smokers during pregnancy (p=0.05). On all other variables groups were comparable.

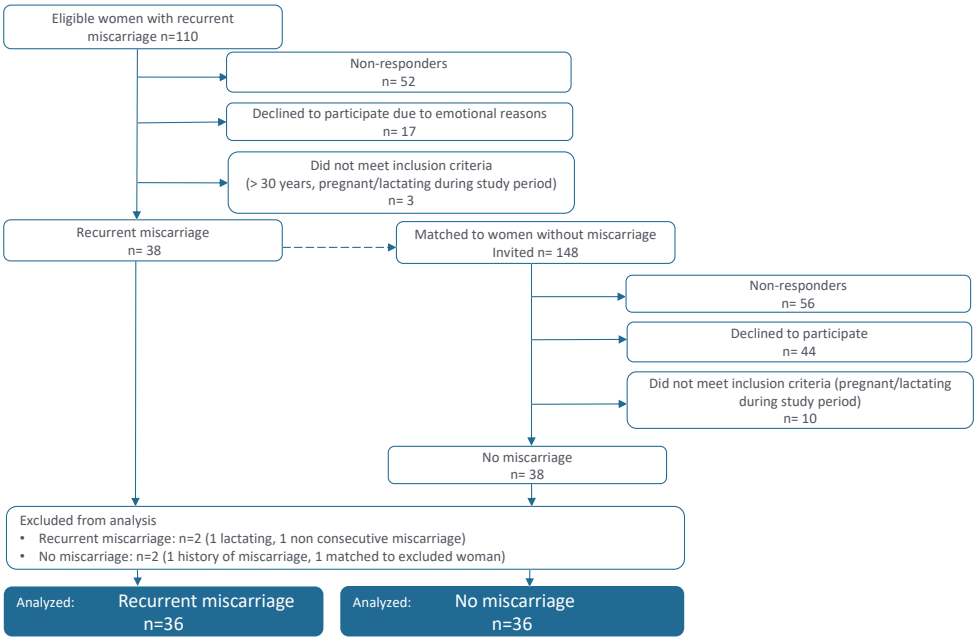

Figure 1. Flow chart Selection of participants

Table 2. Characteristics of participants

	No miscarriage n=36	Recurrent miscarriage n=36	p-value
Maternal age at index pregnancy[a]	26.36 (2.65)	26.47 (2.69)	0.70
Maternal age[b]	34.50 (3.59)	33.28 (3.51)	<0.001
Caucasian (%)	32 (88.9)	30 (83.3)	0.63
University level education (%)	16 (44.4)	8 (22.2)	0.08
BMI[b]	23.78 (3.49)	25.89 (7.08)	0.09
Smoking during pregnancy[d] (%)	5 (13.9)	14 (38.9)	0.05
Smoking[b,c] (%)	5 (13.9)	10 (27.8)	0.23
Diabetes mellitus[b,c] (%)	0 (0)	4 (11.1)	0.04
Antihypertensive medication use[b,c] (%)	0 (0)	3 (8.3)	0.08
Gravidity	2.28 (0.62)	7.11 (2.07)	<0.001
Parity	2.25 (0.60)	1.64 (0.83)	0.001
Primary miscarriages (%)	--	27 (75.0)	--
At least one continuing pregnancy[c] (%)	37 (100)	35 (97.2)	0.31
Gestational diabetes[d] (%)	0 (0)	0 (0)	--
Preeclampsia/Pregnancy induced hypertension[c,d] (%)	0 (0)	3 (8.3)	0.08
Preterm birth[d] (%)	1 (2.8)	4 (11.1)	0.38
Intra uterine growth restriction[d] (%)	4 (11.1)	4 (11.1)	1.00

BMI body mass index
Data are presented as mean (SD)
[a] Age at first pregnancy for unexposed women, age at third consecutive miscarriage for exposed women
[b] At time of follow-up
[c] Chi-squared test. Mc Nemars test not possible (at least one variable in each 2-way table is a constant)
[d] In at least one pregnancy

Table 3. Novel cardiovascular biomarkers in women with recurrent miscarriage

	No miscarriage n=36	Recurrent miscarriage n=36	Mean difference (95% CI)	p-value	Mean difference (95% CI) Model 1	Mean difference (95% CI) Model 2	Mean difference (95% CI) Model 3
Inflammation							
HsCRP mg/L	1.01 (0.32; 1.97)[a]	1.49 (0.65; 5.56)[a]	1.95 (1.17; 3.31)	0.03	1.66 (1.12; 2.40)	1.66 (1.10; 2.51)	1.58 (1.05; 2.45)
Lp-PLA2 ng/ml	360 (75.8)	355 (79.9)	-5.68 (-42.26; 30.90)	0.76	-1.77 (-41.61; 38.07)	-6.12 (-48.14; 35.93)	-12.81 (-56.35; 30.73)
Thrombosis							
Homocysteine µmol/L	9.61 (2.93)	8.17 (2.27)	-1.44 (-2.62; -0.26)	0.02	-1.72 (-2.97; -0.46)	-1.97 (-3.27; -0.67)	-1.96 (-3.34; -0.58)
Folate nmol/L	19.2 (7.05)	20.0 (7.48)	0.79 (-2.41; 4.00)	0.62	1.35 (-2.00; 4.68)	1.54 (-2.01; 5.08)	1.02 (-2.65; 4.69)
aCL[b]				0.51			
weak positive (%)	2 (5.6)	5 (13.9)					
positive (%)	1 (2.8)	1 (2.8)					
aβ2GPI[bc]				0.31			
weak positive (%)	0 (0)	0 (0)					
positive (%)	0 (0)	1 (2.8)					
Lipid metabolism							
Lp(a) nmol/L	20.0 (6.05; 110)[a]	10.6 (6.50; 34.3)[a] *Missing 1*	-1.45 (-2.82; 1.32)	0.25	-1.45 (-2.95; 1.45)	-1.48 (-3.16; 1.48)	-1.23 (-2.63; 1.74)
Renal function							
Creatinine µmol/L	67.4 (7.55)	62.9 (9.58)	-4.50 (-9.20; 0.19)	0.06	-4.81 (-9.96; 0.35)	-5.54 (-10.95; -0.13)	-5.91 (-11.63; -0.19)
Microalbuminuria mg/L	3.00 (3.00; 5.38)[a]	3.00 (3.00; 5.30)[a] *Missing 1*	1.04 (-1.38; 1.55)	0.66	1.05 (-1.38; 1.48)	1.02 (-1.48; 1.41)	1.05 (-1.51; 1.38)
Myocardial damage							
NT-proBNP ng/L	55.9 (53.6)	56.9 (35.0)	1.06 (-21.48; 23.59)	0.93	10.35 (-13.00; 33.70)	7.90 (-16.76; 32.56)	5.67 (-19.35; 30.69)
hsTroponineT ng/L	3.67 (3.66)	3.19 (1.17)	-0.47 (-1.31; 0.37)	0.27	-0.17 (-1.07; 0.72)	0.12 (-0.76; 1.01)	0.10 (-0.77; 0.97)
Multiple mechanisms							
Albumin g/L	47.6 (1.95)	46.0 (2.37)	-1.61 (-2.66; -0.56)	0.004	-1.33 (-2.44; -0.21)	-1.28 (-2.47; -0.09)	-1.19 (-2.43; 0.06)
Vitamin D nmol/L	75.4 (26.8)	55.6 (29.4)	-19.75 (-33.71; -5.79)	0.007	-20.61 (-35.76; -5.45)	-22.09 (-38.11; -6.06)	-24.43 (-40.32; -8.54)
Vitamin D ≤ 60nmol/L N (%)	10 (27.8)	21 (58.3)		0.02			

HsCRP: high-sensitivity C-reactive protein. Lp-PLA2: Lipoprotein-associated phospholipase A2, aCL: anti-Cardiolipin, aβ2GPI: anti-ß-2-Glycoprotein, Lp(a):Lipoprotein (a), NT-proBNP : N-terminal pro-brain natriuretic peptide, hsTroponineT: high sensitive Troponine T.
Model 1: BMI, Model 2: BMI, smoking, Model 3: BMI, smoking, diabetes mellitus, hypertension
Data are presented as mean (SD)
[a] median (25%; 75%)
[b] comparison 'weak positive + positive' vs negative
[c] Chi-squared test. Mc Nemars test not possible (at least one variable in each 2-way table is a constant)

Table 4. Novel cardiovascular biomarkers in women with idiopathic recurrent miscarriage

	No miscarriage n=28	Idiopathic Recurrent miscarriage n=28	Mean difference (95% CI)	p-value	Mean difference (95% CI) Model 1	Mean difference (95% CI) Model 2	Mean difference (95% CI) Model 3
Inflammation							
HsCRP mg/L	0.89 (0.25; 1.56)[a]	1.37 (0.46; 1.38)[a]	1.95 (1.12; 3.47)	0.02	1.51 (-1.01; 2.34)	1.55 (-1.02; 2.40)	1.48 (-1.10; 2.40)
Lp-PLA2 ng/ml	365 (7.14)	354 (80.1)	-11.34 (-51.36; 28.69)	0.57	-10.59 (-55.33; 34.15)	-11.31 (-58.12; 35.50)	-17.77 (-67.35; 31.81)
Thrombosis							
Homocysteine µmol/L	9.55 (2.42)	8.33 (2.32)	-1.22 (-2.54; 0.10)	0.07	-1.45 (-2.93; 0.04)	-1.62 (-3.13; -0.12)	-1.60 (-3.24; 0.04)
Folate nmol/L	19.4 (7.60)	20.7 (6.43)	1.31 (-2.12; 4.74)	0.44	1.78 (-1.91; 5.46)	1.77 (-2.09; 5.63)	1.27 (-2.76; 5.30)
aCL[b]							
weak positive (%)	1 (3.6)	4 (14.3)		0.69			
positive (%)	1 (3.6)	0					
aβ2GPI[bc]							
weak positive (%)	0 (0)	0 (0)					
positive (%)	0 (0)	1 (3.6)					
Lipid metabolism							
Lp(a) nmol/L	20.5 (5.53; 126)[a] *Missing 1*	10.1 *(6.50; 28.5)*[a]	-1.51 (-3.24; 1.38)	0.25	-1.41 (-3.16; 1.62)	-1.41 (-3.31; 1.62)	-1.15 (-2.69; 2.04)
Renal function							
Creatinine µmol/L	66.8 (6.86)	62.9 (10.0)	-3.93 (-9.37; 1.51)	0.15	-4.24 (-10.07; 1.58)	-4.80 (-10.79; 1.19)	-5.13 (-11.45; 1.19)
Microalbuminuria mg/L	3.00 (3.00; 5.23)[a] *Missing 1*	3.00 (3.00; 5.30)[a]	1.07 (-1.45; 1.66)	0.74	1.07 (-1.48; 1.66)	1.01 (-1.55; 1.58)	1.01 (-1.55; 1.51)
Myocardial damage							
NT-proBNP ng/L	53.6 (56.8)	59.4 (32.7)	5.79 (-18.94; 30.51)	0.64	13.80 (-12.79; 40.40)	10.84 (-16.32; 38.00)	8.78 (-19.35; 36.91)
hsTroponineT ng/L	3.86 (4.15)	3.25 (1.32)	-0.61 (-1.71; 0.49)	0.27	-0.30 (-1.46; 0.85)	-0.05 (-1.14; 1.04)	-0.12 (-1.18; 0.94)
Multiple mechanisms							
Albumin g/L	47.5 (2.10)	46.4 (2.27)	-1.11 (-2.27; 0.06)	0.06	-0.75 (-1.89; 0.39)	-0.79 (-1.98; 0.40)	-0.67 (-1.95; 0.61)
Vitamin D nmol/L	77.3 (25.3)	58.1 (30.6)	-19.18 (-35.12; -3.24)	0.02	-21.39 (-38.75; -4.02)	-22.17 (-40.28; -4.07)	-24.76 (-43.76; -5.76)
Vitamin D ≤ 60nmol/L N (%)	8 (28.6)	14 (50.0)		0.18			

HsCRP: high-sensitivity C-reactive protein. Lp-PLA2: Lipoprotein-associated phospholipase A2, aCL: anti-Cardiolipin, aβ2GPI: anti-ß-2-Glycoprotein, Lp(a):Lipoprotein (a), NT-proBNP : N-terminal pro-brain natriuretic peptide, hsTroponineT: high sensitive Troponine T.
Model 1: BMI, Model 2: BMI, smoking, Model 3: BMI, smoking, diabetes mellitus, hypertension
Data are presented as mean (SD)
[a] median (25%; 75%)
[b] comparison 'weak positive + positive' vs negative
[c] Chi-squared test. Mc Nemars test not possible (at least one variable in each 2-way table is a constant)

In 78% of the women with recurrent miscarriage (n=28) no abnormalities were found. Parental chromosomal abnormality was found in one case, antiphospholipid syndrome in one case, hyperhomocysteinemia in three cases and heritable thrombophilia was found in three cases.

Novel cardiovascular biomarkers were presented in Table 3. Women with recurrent miscarriage had a significantly higher HsCRP compared to women with no miscarriage. Significantly lower values of homocysteine, albumin and vitamin D were found in women with recurrent miscarriage compared to women with no miscarriage. These significantly differences remained in the multivariate analyses adjusting for BMI, smoking, diabetes mellitus and hypertension (using three different models), except for albumin adjusted for all four classic cardiovascular risk factors (model 3). A significant lower value of creatinine was found in multivariate analysis, model 2 and 3, in women with recurrent miscarriage compared to women with no miscarriage.

The results for the subgroup analysis including women with idiopathic recurrent miscarriage (table 4) are consistent with the results of the total group. Not all differences were statistically significant.

Discussion

Main findings

Increased levels of HsCRP and decreased levels of albumin and vitamin D were found in women with a history of recurrent miscarriage compared to women with no miscarriage, also after adjustments for classic cardiovascular risk factors (BMI, smoking, diabetes mellitus and hypertension). These cardiovascular biomarkers are involved in mechanisms regarding inflammation.

Interpretation

Our findings indicate a proinflammatory state in women with a history of recurrent miscarriage, which cannot be explained by confounders as BMI, smoking, diabetes mellitus and hypertension. Inflammation plays an important pathogenic role in all stages of atherosclerosis [20]. HsCRP can detect low grade inflammation and adds prognostic information on cardiovascular risk comparable to blood pressure or cholesterol [21]. Values <1, 1 to 3, and >3 mg/l indicate lower, average, or higher relative cardiovascular risk, respectively [21]. Therefore, we can conclude that we found a clinically relevant elevation in the recurrent miscarriage group (unadjusted mean difference HsCRP mg/L 1.95, 95%CI 1.17; 3.31). Lower values of albumin were found in women with recurrent miscarriage. Albumin is inversely associated with cardiovascular disease [22, 23] and several potential biological mechanisms might explain this (Table 1). The most obvious mechanism is inflammation; it is a negative acute-phase protein and concentration falls

during the inflammatory process [24, 25]. Vitamin D (concentrations of 20 to 60 nmol/L) is also inversely associated with cardiovascular disease risk [26]. Vitamin D is involved in many processes of potential relevance to cardiovascular disease (Table 1). A deficiency could lead to increased inflammation, endothelial dysfunction, elevated blood pressure, decreased insulin sensitivity and secretion, arterial stiffness and degradation of atherosclerotic plaque[27]. Vitamin D is produced by the action of UVB light on the skin. Skin pigmentation may reduce capacity to synthesize vitamin D and due to a lower exposure in winter months there are seasonal variations. To a lesser extent, it is also provided in the diet from foods, mostly of animal origin[27]. We found a significantly lower vitamin D concentration in women with recurrent miscarriage, suggesting a decreased intake of vitamin D precursors, whether a decreased exposure to sunlight plays a role is uncertain. As the percentage of Caucasian women was comparable in both groups, we don't expect an impact of ethnicity on our findings. We assessed vitamin D levels only at follow-up. In addition, it is possible that women with recurrent miscarriage already have decreased values of vitamin D during pregnancy. Only one study assessed vitamin D levels and immunological implications, such as presence of autoantibodies and cytokine production, in women with recurrent miscarriage (N=133) and found that 47.4% of these had a vitamin D deficiency (<30 ng/ml) [28]. Over the last decade, the role of vitamin D in human reproduction has been increasingly considered as important. Adverse outcomes linked to vitamin D insufficiency in pregnancy includes pre-eclampsia, gestational diabetes, small-for-gestational age and preterm birth [29-31]. Many potential underlying mechanisms of vitamin D in regulating each of the outcomes are hypothesized, including that vitamin D could act as an immune regulator during implantation [32]. Our findings call for more (observational and intervention) studies to investigate the association between vitamin D and recurrent miscarriage.

Surprisingly, in contrast with the previous described findings, we found that women with recurrent miscarriage had lower levels of homocysteine compared to women with no miscarriage. Hyperhomocysteinemia is associated with recurrent miscarriage and with cardiovascular disease [33, 34]. Our findings could be a confounding effect of the folate and vitamin B supplementation in the recurrent miscarriage group. Homocysteine evaluation is part of the routine work-up in women with recurrent miscarriage. In case of elevated levels, women are advised to use folate and vitamin B supplementation. Women might continue this after their pregnancies and/or at least in between pregnancies. All women are advised to use periconceptional folate supplementation. As women with recurrent miscarriage have a higher gravidity this could also have influenced our results. We found comparable results in the sub analysis including women with idiopathic recurrent miscarriage (although not all significant, probably due to a loss of power). Indicating that our results are independent of a supposed cause for the recurrent miscarriage, such as thrombophilia.

Strengths and limitations

To our knowledge, this is the first study which examined novel cardiovascular biomarkers after recurrent miscarriage. Strengths of our study include a unique well-defined cohort. As recurrent miscarriage is a highly heterogenic condition we strived to more homogeneity and therefore included only women who had their third consecutive miscarriage before the age of 31. A younger age at diagnosis reduces the change of miscarriages due to fetal abnormalities and makes a maternal cause of recurrent miscarriage more plausible [35]. All women had a routine work-up at baseline to identify possible causes for the recurrent miscarriage and therefore we could perform a subgroup analysis including women with idiopathic recurrent miscarriage (Table 3). Because of the matching on zip code, age and time of pregnancy we took several confounding factors into account. Our study has also some limitations. Because women with recurrent miscarriage were included in the study before the matched controls were invited to participate, there was a small difference between the age at time of the follow-up measurements. We don't expect that this will influence the results. Almost 50% of the eligible women did not respond on the invitation and 17 women declined to participate (due to emotional reasons) in this follow-up study (Figure 1), which could possible introduce selection bias. Given the high percentage of women who had at least one continuing pregnancy in the recurrent miscarriage group (97.3%), it is likely that women without live births (women with possibly the most unfavorable profile) more often declined or did not respond. Therefore, our findings in women with recurrent miscarriage could even be an underestimate. Selection bias is also possible in the unexposed group; probably women with a higher education were more likely to participate. Another limitation is the relatively small group of patients, especially in our subgroup analysis. Therefore, this study may partly be underpowered and we should be cautious drawing conclusions. There is a wide range of novel cardiovascular biomarkers which makes it impossible to study all of them. Based on the most recent literature we have selected biomarkers which have the potential to link recurrent miscarriage and cardiovascular disease [11, 12]. Unfortunately, we did not take the accurate blood sample (sodium citrate tube) for the examination of potential interesting biomarkers fibrinogen and lupus anticoagulant.

Perspectives

Our findings suggest a proinflammatory state in women with a history of recurrent miscarriage compared to women with no miscarriage. This suggests an overall poorer health in women with a history of recurrent miscarriage, which could partly explain their increased risk for cardiovascular disease later in life. No differences were found in more specific biomarkers, for example regarding lipid metabolism and myocardial damage. Routine screening of novel cardiovascular biomarkers on patient level seems with the current insight not warranted, although screening for vitamin D status seems plausible. Women with a history of recurrent miscarriage should be given healthy lifestyle advises, such as abstention of smoking, improving dietary habits and sufficient exposure to sunlight.

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