Auto-Antibodies Against The Angiotensin II Type I Receptor In Women With Uteroplacental Acute Atherosclerosis And Preeclampsia At Delivery And Several Years Postpartum

Amalie Bjerke Rieber-Mohn^{a,b,1}, Meryam Sugulle^{b,1,*}, Gerd Wallukat^c, Patji Alnæs-Katjavivi^{a,b}, Gro Leite Størvold^{b,e}, Nils Bolstad^{a,f}, Christopher WG Redman^g, Ralf Dechend^{c,d}, Anne Cathrine Staff^{a,b}

^aInstitute of Clinical Medicine, Faculty of Medicine, University of Oslo, Oslo, Norway

^bDivision of Gynaecology and Obstetrics, Oslo University Hospital, Oslo, Norway

^cExperimental and Clinical Research Center, a joint cooperation between the Max-Delbrück Center for Molecular Medicine in the Helmholtz Association and the Charité Medical Faculty, Berlin, Germany

^dDepartment of Cardiology and Nephrology, HELIOS Klinikum Berlin, Germany

^eInstitute for Experimental Medical Research, Oslo University Hospital, Oslo, Norway

^fDepartment of Medical Biochemistry, Oslo University Hospital, Oslo, Norway

^gNuffield Department of Obstetrics and Gynaecology, University of Oxford, United Kingdom

¹ The authors ABRM and MS contributed equally to this work

*corresponding author at the Division of Gynaecology and Obstetrics, Oslo University Hospital and University of Oslo, Oslo, Norway.

Postbox 4956, Nydalen

0424 Oslo, Norway

Phone: +4723015589 Cell phone +47 41688655

Email: uxsume@ous-hf.no

Abstract

Background

Uteroplacental acute atherosclerosis is a pregnancy-specific lesion resembling early stages of atherosclerosis found frequently in preeclampsia. Preeclampsia is associated with an increased risk for future maternal atherosclerotic cardiovascular disease. The renin-angiotensin-system plays a role both in atherosclerosis and in preeclampsia. Circulating agonistic autoantibodies at the angiotensin-II type 1 receptor (AT₁-AA) are increased in preeclampsia. We hypothesized an association between AT₁-AA at delivery and postpartum with acute atherosclerosis in pregnancy.

Material and Methods

Maternal serum and decidua basalis tissue was collected at elective cesarean section (n=41; 24 preeclampsia, 17 normotensive controls). Circulating AT₁-AA were detected by a bioassay using spontaneously beating rat cardiomyocytes at delivery (n=41) and 5-8 years postpartum in a subgroup (n=10). Decidual acute atherosclerosis was assessed by immunohistochemistry.

Results

Significantly less normotensive controls (18%; 3/17) than women with preeclampsia (58%; 14/24) were AT₁-AA positive at delivery, $p < 0.01$. Uteroplacental acute atherosclerosis and circulating AT₁-AA at delivery were not significantly correlated. Postpartum, 2 prior preeclamptic women had circulating AT₁-AA, both without acute atherosclerosis in pregnancy.

Conclusions

Our results confirm that circulating AT₁-AA are present significantly more often in preeclampsia than in normotensive pregnancy, however without association to acute atherosclerosis. Whether circulating maternal AT₁-AA or acute atherosclerosis target young women at increased long-term cardiovascular risk warrants further investigations.

Keywords: autoantibodies; acute atherosclerosis; cardiovascular disease; preeclampsia; renin-angiotensin system

1. Introduction *

Preeclampsia is a pregnancy-specific and potentially lethal multisystem disorder defined by new-onset hypertension and proteinuria after gestational week 20, or alternatively, in the absence of proteinuria, other new onset preeclampsia-associated signs such as decreased platelet count, increased serum creatinine and liver transaminase levels, pulmonary edema or cerebral symptoms (Tranquilli et al., 2014). The clinical presentation for the mother and fetus, as well as time of onset in pregnancy, is heterogeneous, and various attempts in categorizing the syndrome have been made. Early-onset preeclampsia, typically defined as premature delivery prior to gestational week 34 or 37 (von Dadelszen et al., 2003), is associated with fetal growth restriction (Mol et al., 2016). An increased ratio of anti-angiogenic soluble fms-like tyrosine kinase receptor 1 (sFlt1) to pro-angiogenic placental growth factor (PlGF) of 85 or above in the maternal circulation has been linked to preeclamptic pregnancies with adverse outcomes (Rana et al., 2013). Pregnancy complications associated with placental dysfunction such as preeclampsia and fetal growth restriction are acknowledged as important epidemiological risk factors for future CVD in women (Nichols et al., 2014). However, the underlying molecular mechanisms are not fully understood.

The renin angiotensin system (RAS) plays an essential role for the regulation of blood pressure. We and others have previously suggested that dysregulation of the tissue-based and circulating RAS may be involved in the pathophysiology of preeclampsia, however, the underlying mechanisms are not yet fully understood (Wallukat et al., 1999, Herse et al., 2007, Walther et al., 2005, Xia et al., 2003). Preeclamptic women manifest exaggerated pressor responses to Angiotensin II (Ang II), although the circulating Ang II concentrations are lower

* Abbreviations:

AA: autoantibodies; Ang I: angiotensin I ; Ang II: angiotensin II; ACE: angiotensin converting enzyme; AT1: angiotensin II type 1; AT1-AA: angiotensin II type 1 autoantibodies ; AT1R: angiotensin II type 1 receptor; BMI: body mass index; BPM: beats per minute; CVD: cardiovascular disease; GW: gestational week PlGF: placental growth factor; RAS: renin angiotensin system; sFlt1: soluble fms-like tyrosine kinase-1; SGA: small for gestational age; VEGF: vascular endothelial growth factor

compared to control pregnancies (Hanssens et al., 1991, Gant et al., 1973). Increased angiotensin sensitivity has also been reported to persist into the postpartum period (Saxena et al., 2010). Several studies have shown maternal circulating agonist autoantibodies (AA) directed at the angiotensin II type 1 (AT₁) receptor in women suffering from preeclampsia.

We have previously described both maternal and offspring circulating AT₁-AA at delivery (Herse et al., 2007). Postpartum studies have yielded conflicting results with regard to persistence of circulating AT₁-AA (Wallukat et al., 1999), (Hubel et al., 2007).

Acute atherosclerosis is a pregnancy-specific lesion of the uteroplacental spiral artery wall, resembling early stages of atherosclerosis (Stary et al., 1994, Harsem et al., 2007, Staff and Redman, 2014, Alnaes-Katjavivi et al., 2016). Previous studies by our group showed that uteroplacental acute atherosclerosis occurs in 30-40% of preeclamptic women at cesarean delivery, and is not restricted to early-onset disease (Harsem et al., 2007, Alnaes-Katjavivi et al., 2016). We have suggested that acute atherosclerosis is the final manifestation of several inflammatory processes (Staff et al., 2014), as it occurs in pregnancies complicated by preeclampsia, fetal growth restriction, diabetes mellitus or autoimmune disease as well as pregnancies without any complications (Staff et al., 2014). We have put forth the novel concept that the subset of women who develop these lesions may have increased risk of atherosclerotic arterial disease later in life (Staff et al., 2010).

Several studies support a role of RAS and activation of angiotensin II type 1 receptor (AT₁R) in the pathogenesis of atherosclerosis, a major cause of cardiovascular disease, (da Silva et al., 2015) although studies directly linking AT₁-AA and atherosclerotic disease directly are currently lacking. Likewise, there are currently no publications exploring an association between the RAS and the atherosclerosis-resembling lesion confined to pregnancy, namely acute atherosclerosis. In the present study, we therefore investigated the presence of maternal circulating AT₁-AA at delivery both in normotensive and preeclamptic

pregnancies with regard to presence of acute atherosclerosis and maternal circulating PIGF/sFlt biomarker levels, the latter biomarkers interpreted as proxy for placental dysfunction. We also explored a possible persistence of maternal circulating AT₁-AA several years after the index pregnancy.

2. Material and Methods

2.1 Study population and sample collection.

A total of 41 women with singleton pregnancies, recruited to the ongoing Oslo Pregnancy Biobank project from 2001 onwards, were included in this study. All women underwent elective cesarean section; 24 with a diagnosis of preeclampsia and 17 normotensive, healthy women. None of the women had ruptured membranes, regular uterine contractions or signs of infection. Women recruited to the “Oslo Pregnancy Biobank” during 2001-2004 were invited to a clinical follow-up study in 2008-2009, as a part of another Oslo Pregnancy Biobank research project (the CHASE study). A subgroup of the original CHASE cohort was included in our present study: 3 prior normotensive women and 7 with prior preeclampsia. The total postpartum cohort is described previously (Kvehaugen et al., 2011).

Preeclampsia was defined as new-onset hypertension (blood pressure $\geq 140/90$ mmHg) and new-onset proteinuria (≥ 30 protein/creatinine ratio or $\geq 1+$ on dipstick) ≥ 20 weeks of pregnancy (Tranquilli et al., 2014). Euglycemic, normotensive women with pregnancies without clinical evidence of fetal growth restriction were defined as controls.

Newborn gender specific baby weight percentiles were calculated according to Norwegian fetal growth curves (Johnsen et al., 2006). Small for gestational age (SGA) was defined as birth weight below the 10th percentile. Gestational week (GW) at delivery was defined by routine ultrasound screening at GW 17-20.

Decidual tissue for acute atherosclerosis diagnosis was collected by our vacuum suction method of the the uterine wall of the placental bed, following placental delivery at elective cesarean section, as previously described (Harsem et al., 2004). Fasting blood samples from the same women were collected prior to delivery, and at 5-8 years follow-up for the longitudinal cohort, and serum was frozen at -80°C as described previously (Staff et al., 2005).

All participating women provided informed written consent.

2.2 AT₁-receptor AA bioassay

Maternal circulating AT₁-receptor AA were detected and characterized by a "bioassay" using spontaneously beating rat cardiomyocytes as previously described (Wallukat et al., 1999). Immunoglobulin (IgG) fractions from maternal serum were added to the cardiomyocytes in culture after the estimation of the basal beating rate of the cells. The AT₁-receptor AA activity was measured 60 minutes after their application at 37°C . Test results were considered to be positive if delta beats per minute exceeded 7.2. The angiotensin receptor antagonist Losartan was added to confirm specificity of the AA. The tests were performed with the examiner blinded to the diagnosis and pregnancy outcome.

2.3 Evaluation of decidua basalis tissue for the presence of acute atherosclerosis

The decidual tissue acute atherosclerosis diagnosis of the included patients has been performed and published previously for a larger cohort (Alnaes-Katjavivi et al., 2016). In brief, vacuum suction collected and rinsed decidual tissue (endometrium of pregnancy) was formalin fixed, routinely processed and paraffin embedded and used for immunohistochemistry. According to our published method, a woman is diagnosed with decidua basalis acute atherosclerosis in the presence of at least one foam cell lesion (defined as ≥ 2 adjacent intramural vacuolated, CD68 + cells) in one section of the spiral arterial wall (Alnaes-Katjavivi et al., 2016).

2.4 Biomarker analyses

Serum levels of sFlt1 and PlGF were determined at Oslo University Hospital using Elecsys immunoassays (Roche Diagnostics, Mannheim, Germany) utilizing a fully automated electrochemoluminescence immunoassay platform (Cobas E 601, Roche Diagnostics) according to the manufacturer's instructions.

2.5 Statistical analyses

Data were analysed using SPSS version 23. Non-parametric Mann-Whitney test was used when comparing groups with continuous data. Chi Square test and Fisher were applied on categorical data. A probability of ≤ 0.05 was considered statistically significant.

2.6 Ethical approval

The study complied with the Helsinki declaration and was approved by the Regional Committee for Medical and Health Research Ethics of South-Eastern Norway (reference number 529-02162).

3. Results

3.1 Clinical characteristics and maternal placental derived biomarkers at index pregnancy

Clinical characteristics of the preeclampsia and normotensive pregnant control patient groups are shown in Table 1. Most of the women with preeclampsia were delivered prematurely (75% prior to GW 37, 50% prior to GW 34). Almost half of the preeclampsia group delivered a SGA baby (46%), all of which were delivered $<GW$ 34. Most of the preeclamptic women (75%) fulfilled the "severe preeclampsia" criteria according to ACOG, with blood pressures over 160/110 (Tranquilli et al., 2014).

One third of the women in the preeclampsia group (38%; 9/24) had received antihypertensive drugs prior to delivery. Predelivery maternal circulating sFlt1 and PlGF concentrations were available in most preeclamptic (23/24) and normotensive controls (13/17). As expected, median maternal sFlt1 as well as the sFlt1/PlGF ratio were significantly increased in the preeclampsia group compared to the normotensive controls (sFlt1: 12 504 pg/mL vs 4 152 pg/mL, $p<0.001$; sFlt1/PlGF ratio: 200 vs 41, $p<0.001$). In most women with preeclampsia (91%; 21/23) the sFlt1-1/PlGF ratio was >85 . Further, as expected, median maternal PlGF was significantly lower in the preeclampsia group than in the control group at delivery (68 pg/mL vs 133 pg/mL, $p=0.002$).

3. 2 Maternal circulating AT₁-AA in normotensive and preeclamptic pregnancies

In the preeclampsia group, significantly more women had maternal AT₁-AA at delivery (58%; 14/24) compared to normotensive controls (18%; 3/17; $p<0.01$). Median beats per minute (bpm) of spontaneously beating neonatal rat cardiomyocytes exposed to immunoglobulins from maternal serum was 16.0 bpm for the preeclampsia group and 0.6 bpm for the control group (Figure 1). Three women in the normotensive pregnant group yielded a remarkably high increase in the cardiomyocyte contraction assay ($\Delta 17.32$ -17.52 bpm). Additionally one of the women in the normotensive group yielded a high increase in the rat cardiac beating rate ($\Delta 13.2$ bpm), but was not blocked by the angiotensin antagonist Losartan, meaning AT₁-AA specificity could not be confirmed. As a consequence, the woman is therefore considered to be AT₁-AA negative.

No association was found between gestational age at delivery and the presence of AT₁-AA, neither in the preeclampsia group nor the normotensive control group. We found no difference in the rate of AT₁-AA positivity between the early- and late-onset preeclampsia

groups (< 34 GW: 58% AT1-AA positive (7/12), versus ≥ 34 GW: 58% AT1-AA positive (7/12), $p=1.0$). There were no significant differences in median bpm between the early and late onset preeclampsia groups (14.1 vs 17.92 bpm, $p=1.0$; cut-off 34 GW). Further, we found no significant correlations between maternal age or BMI and increase in bpm within each study group (controls and preeclampsia; data not shown).

3.3 Maternal circulating AT₁-AA and circulating levels of PlGF and sFlt1

For the total pregnancy group, median maternal sFlt1 concentration at delivery was higher in AT₁-AA positive women (n=16) compared to AT₁-AA negative women (n=20) (12 009 pg/mL vs 7 080 pg/mL, $p=0.048$). For the total pregnancy group, there was no significant difference between PlGF concentrations in women with or without AT₁-AA (73 pg/mL vs 82 pg/mL, $p=0.4$) or as a sFlt1/PlGF-ratio (175 vs 78, $p=0.1$). In the preeclampsia group, there was, however, no significant difference in median maternal sFlt1 concentration between AT₁-AA positive (n=13) and negative women (n=10) (11 390 pg/mL vs 12 668 pg/mL, $p=0.6$). Likewise, there was no significant difference between median maternal PlGF (68 pg/mL vs 66 pg/mL, $p=0.6$), nor the sflt1/PlGF ratio (200 vs 190, $p=0.9$) (Figures 2A and B).

3.4 Maternal circulating AT₁-AA and presence of acute atherosclerosis

Of the original cohort of 41 patients, decidual basalis tissue for acute atherosclerosis evaluation was available for 36 patients: 14 normotensive controls and 22 women with preeclampsia.

Diagnosing decidual acute atherosclerosis immunohistochemically, 5 of 14 normotensive controls had acute atherosclerosis, and 10 of 22 women with preeclampsia had acute atherosclerosis.

In women with acute atherosclerosis (preeclampsia and controls together), maternal circulating AT₁-AA at delivery were present in 53% (8/15), compared to 38% (8/21) in women without acute atherosclerosis, however the difference was not statistically significant ($p=0.364$). In

preeclamptic women with AT₁-AA at delivery, 46% (6/13) also had acute atherosclerosis, which was similar to the rate in PE women without AT₁-AA at delivery (44%; 4/9) (Figure 3).

3.5 Clinical characteristics and presence of AT₁-AA 5-8 years postpartum

Postpartum blood samples from 10 women were available for analysis. Mean interval to the postpartum follow-up was 6.0 years (range 4.8- 7.25 years) after the index pregnancy delivery. None of the 10 included postpartum women had hypertension or diabetes postpartum, none were using antihypertensive drugs or were pregnant at the time of blood sampling. Five of the women had AT₁-AA present at the index pregnancy, all from the prior preeclampsia group. Within the postpartum group of prior preeclampsia, acute atherosclerosis was present in 3 of 7 women at index delivery. None of the prior normotensive pregnancy group had acute atherosclerosis in the index pregnancy.

Of these ten women with postpartum blood samples available for analyses, only two were found to have circulating AT₁-AA 5-8 years postpartum, both with late-onset preeclampsia in their index pregnancy, but without presence of decidual acute atherosclerosis. Only one of these two women also had detectable circulating AT₁-AA at delivery in the index pregnancy. The second woman, without AT₁-AA in index pregnancy, had however undergone another normotensive pregnancy since the index delivery. The woman with circulating AT₁-AA both present at delivery and postpartum had a decrease from 18.52 bpm at delivery to 12 bpm postpartum. Both women were non-smokers and apart from abdominal obesity in one of the women, no remarkable clinical phenotype was recorded (data not shown).

Characteristic	Controls n=17	Preeclampsia n=24	P-values
Age at delivery (years)	31 (25-33)	30.5 (28-33)	0.832 ^c
Primigravidae (%)	59% (10/17)	58% (14/24)	0.975 ^a
Nulliparous (%)	76% (13/17)	79% (19/24)	1.0 ^a
BMI pre-pregnancy (kg/m ²)	22.3 (19.5-24.2)	24 (21.2-29)	0.024 ^c
BMI at delivery (kg/m ²)	27.9 (24.3-31.3)	30.8† (28-32.6)	0.036 ^c
Systolic BP <20 gestational weeks (mmHg)	112 (105-120)	115 (110-125)	0.207 ^c
Diastolic BP <20 gestational weeks (mmHg)	65 (60-70)	73 (63-80)	0.031 ^c
Systolic BP at delivery (mmHg)	120 (115-120)	160 (150-170)	<0.001 ^c
Diastolic BP at delivery (mmHg)	75 (65-80)	103 (100-105)	<0.001 ^c

PE in prior pregnancy	12%	13%	1.0 ^b
(%)	(2/17)	(3/24)	
Gestational age at delivery	38.9	33.9	<0.001 ^c
(weeks)	(38.6-39)	(32.3-36.6)	
Preterm delivery	0%	75%	<0.001 ^c
<37 ⁺⁰ gestational weeks		(18/24)	
Preterm delivery	0%	50%	<0.001 ^c
<34 ⁺⁰ gestational weeks		(12/24)	
Fetal gender	53%	71%	0.241 ^a
(% female)	(9/17)	(17/24)	
Newborn weight percentile(Johnsen et al., 2006)	67	16	0.003 ^c
	(40-77)	(2-50)	
Newborn weight	3460	2224	<0.001 ^c
(grams)	(3248-3642)	(1471-2764)	
Small for gestational age	0%	46%	0.001 ^b
(cut-off at birthweight ≤10 th centile)		(11/24)	
Small for gestational age	0%	33%	0.013 ^b
(cut-off at birthweight ≤3 rd centile)		(8/24)	
Placenta gross weight, membranes and umbilical cord	518†	385‡	0.002 ^c
(grams)	(460-555)	(275-480)	

Table 1.

Clinical characteristics of the included pregnancy groups.

Values are given in median values (and 95% confidence interval for the median), percentages and rates; all *P*-values as compared to the control group. Applied statistical tests as indicated: ^a= Pearson chi-square; ^b=Fisher's exact test; ^c=Mann-Whitney test. Number of cases in which information is missing as indicated by: †=1; ‡= 4.

C: healthy, normotensive pregnancies, named controls

PE: Preeclampsia

BMI: Body mass index (kg/m²)

BP: Blood pressure

4. Discussion

Our study is the first to explore an association of circulating AT₁-AA at delivery and several years postpartum in women with the presence of acute atherosclerosis diagnosis in decidua basalis at delivery. We confirm in the present study previous findings that women with preeclampsia more frequently have circulating AT₁-AA as compared to controls (Herse et al., 2007, Herse et al., 2008). However, in our study we found that only 58% of the women with preeclampsia had circulating AT₁-AA compared to previous studies presenting higher rates, up to 70-100% (Walther et al., 2005, Wallukat et al., 1999, Velloso et al., 2016, Herse et al., 2009, Herse et al., 2007, Xia et al., 2003). Clinical differences in the studied populations may account for some of the differences in the rate of “AT₁-AA positivity” in our cohort as opposed to others. Herse et al included only women with preeclampsia delivered up to 36 GW (Herse et al., 2007), and although the majority (76%) of the women with preeclampsia in our study also delivered before GW 37, we could not find that AT₁-AA were more prevalent in early-onset preeclampsia in our present cohort. Interestingly, when applying the presence of SGA (birthweight <10th centile) as a proxy for severity of placental dysfunction and thereby also severity of preeclampsia, our study cohort included 11 of 24 newborns with a birthweight <10th centile. In line with a high rate of placental dysfunction and SGA newborns in our cohort, we demonstrated a high rate of an antiangiogenic profile (sFlt1/PlGF ratio >85) in the preeclampsia group. In contrast, Herse et al reported only one SGA newborn in their cohort (Herse et al., 2007), which hypothetically may have resulted in a lower rate of AT₁-AA in their study, if placental dysfunction would contribute to AT₁-AA presence in maternal circulation. However, this was not the case, since AT₁-AA were detected in 80% of women with PE in that study (Herse et al., 2007). The finding of a higher median sFlt1 concentration, as well as sFlt1/PlGF ratio in AT₁-AA positive women compared to AT₁-AA negative women is presumably due to the fact that women with preeclampsia are more likely to develop AT₁-

AA, and account for 81% of the AT₁-AA positive group with available sFlt1 and PlGF values in our cohort. Our results suggest that AT₁-AA presence during pregnancy may not depend on placental dysfunction per se, but rather on the presence of other preeclamptic features. In line with this suggestion, we found no difference in angiogenic biomarkers at delivery between preeclamptic pregnancies with or without AT₁-AA presence.

Interestingly, only one other study reported a rate of maternal circulating AT₁-AA as high in normotensive controls as the one detected in our study (17%, 3/17), in contrast to other reports with rates as low as 0% (0/12) (Wallukat et al., 1999), 11 % (2/18) (Xia et al., 2003), 0% (0/10) (Herse et al., 2007) and 0% (0/29) (Velloso et al., 2016). Notably, the increase in the rat cardiac beating rate in the cardiac contraction assay was quite high for these three normotensive women, above the median increase for preeclamptic women in our study. A dose-response relationship between activating IgG or Ang II concentrations and cardiomyocyte contraction rate has been described (Wallukat et al., 1999), implying that these controls do not represent “mild cases”. Prior studies have concluded that the cardiomyocyte contraction assay can distinguish IgG from normotensive and preeclamptic patients (Wallukat et al., 1999). Our study may contribute to a more differentiated view, since this conclusion cannot be applied in the same “clear-cut” manner to our study population. None of these AT₁-AA positive normotensive women had any specific present clinical characteristics, although one of these had a history of preeclampsia prior to the index pregnancy. AT₁-AA is not a pregnancy-specific marker, it is also described in non-pregnant women with dilative cardiomyopathy (Wallukat et al., 2002), essential hypertension (Fu et al., 2000) and renal allograft rejections (Dragun et al., 2005). However, there was no clinical suspicion of disease in any of the clinically very well described normotensive women included in our study.

The only other study reporting higher rates of AT₁-AA in maternal circulation than both ours and others is by Walther et al, reporting AT₁-AA in maternal circulation in 50% of

healthy pregnant women at delivery without adverse pregnancy outcome (5/10) (Walther et al., 2005). However, the authors related their findings to abnormal uterine perfusion measured in mid-pregnancy (Walther et al., 2005). As it is neither medically indicated to perform Doppler measurements of the uterine arteries in healthy pregnant women nor a routine according to our national pregnancy surveillance guidelines, such data are not available for the normotensive AT₁-AA positive women in our study.

When focusing on cardiovascular risk-related factors, we found no significant associations between maternal AT₁-AA positivity and maternal age, BMI, blood pressure below gestational week 20 or birthweight percentiles/SGA. Also, our present study indicates that maternal circulating AT₁-AA positivity in pregnancy is not associated with the presence of decidual acute atherosclerosis in pregnancy. We do not know the exact mechanisms of its development, but we have suggested that acute atherosclerosis is a consequence of several inflammatory processes.

Our study is the first to present data on circulating AT₁-AA as long as 5-8 years postpartum in women with a previous normotensive pregnancy or preeclampsia. Hubel et al reported that maternal AT₁-AA persisted up to 27 months after pregnancy in 17.2% of women with previous preeclampsia and 2.9% in women with a previous normotensive pregnancy (Hubel et al., 2007). In our smaller cohort with a postpartum observational period exceeding the observational period in Hubel's study by minimum 33 months, this finding could not be confirmed. Wallukat et al previously reported that spontaneous beating rate decreased by 50% after delivery in samples obtained longitudinally up to 29 days postpartum (Wallukat et al., 1999). This is in contrast to our finding, where the woman with AT₁-AA present at delivery and postpartum had a remaining high activation of the AT₁ receptor.

None of the women with post-partum AT₁-AA in our study had a diagnosis of acute atherosclerosis at index delivery, but the small numbers restrict our ability to conclude with regard to persistence of circulating AT₁-AA postpartum and concomitant acute atherosclerosis diagnosis.

Important strengths of our study are the clinically well-defined study population, the constant method of biological sample collection and storage, as well as targeted decidua basalis tissue sampling and thoroughly defined criteria for decidua basalis acute atherosclerosis (Alnaes-Katjavivi et al., 2016). Another advantage is the sophisticated bioassay approach to identify AT₁-AA, based on spontaneously beating neonatal rat cardiomyocytes exposed to serum from the pregnant women, where the exact binding site of the AT₁-AA to the AT₁R is identified. This method has previously been evaluated by confirmation studies (Herse et al., 2008). However the complexity of this time-consuming manual technique restricts the number of samples processed in our study.

An obvious weakness of our study is the limited number of included women both during pregnancy and the extended postpartum period as well as the lack of follow-up over decades, thus not allowing for more generalizable conclusions.

5. Conclusion

In summary, we found a higher rate of AT₁-AA in maternal circulation in preeclamptic pregnancies than in healthy controls. Presence of AT₁-AA did not associate with the presence of acute atherosclerosis. Whether AT₁-AA positivity or decidual acute atherosclerosis at delivery target young women at increased long-term cardiovascular risk remains to be investigated.

6. Figure legends

Figure 1. AT₁-AA in sera of normotensive controls and preeclamptic women.

The Y-axis shows the increase in beat number per minute of spontaneously beating neonatal rat cardiomyocytes when exposed to immunoglobulin from maternal serum for the study groups (C= healthy, normotensive pregnancies, named controls; PE=preeclampsia). Filled circles represent late-onset PE, empty circles represent early-onset PE, cut off 34 GW. Values above the line $y=7.2$ represent the AT₁-AA positive group, whereas values below are defined as AT₁-AA negative. # Denotes normotensive patient with significant increase in bpm in the AT₁-AA bioassay, but without blocking effect by Losartan, i.e. AT₁-AA specificity could not be verified. As a consequence, the woman is therefore considered to be AT₁-AA negative.

* $p<0.01$.

Figures 2A-B. Angiogenic factor concentrations [A: sFlt1 concentration (pg/mL); B: PlGF concentration (pg/mL), respectively] presented for each pregnancy group in relation to positivity for AT₁-AA in maternal serum (C= healthy, normotensive pregnancies, named controls; PE=preeclampsia). * $p<0.01$.

Figure 3. AT₁-AA in sera of normotensive controls and preeclamptic women with and without acute atherosclerosis. Values above the line $y=7.2$ increase in beats per minute represent the AT₁-AA positive group, whereas values below are defined as AT₁-AA negative. The control marked with # is considered to be AT₁-AA negative (as in Figure 1).

Figure 4. Change in beats per minute of spontaneously beating neonatal rat cardiomyocytes when exposed to immunoglobulin from serum 5-8 years postpartum in women with prior normotensive index pregnancy (C, n=3) and prior preeclampsia in index pregnancy (PE, n=7) women compared to pregnancy data in the same women. Values above the line $y=7.2$ represent the AT₁-AA positive group, whereas values below are considered to be AT₁-AA negative.

Acknowledgements

We thank Lise Øhra Levy and previous PhD students for patient recruitment and for essential biobanking help, at Oslo University Hospital.

Funding sources

This work was partly supported by the South-Eastern Norway Regional Health Authority [grant number 2014026], the research Council of Norway [grant number 230652], and Oslo University Hospital.

Amalie Bjerke Rieber-Mohn received a medical student research grant from the University of Oslo and The Research Council of Norway.

Ralf Dechend was supported by the German Research Foundation [DE 631/9-1].

References

- ALNAES-KATJAVIVI, P., LYALL, F., ROALD, B., REDMAN, C. W. & STAFF, A. C. 2016. Acute atherosclerosis in vacuum suction biopsies of decidua basalis: An evidence based research definition. *Placenta*, 37, 26-33.
- BREWER, J., LIU, R., LU, Y., SCOTT, J., WALLACE, K., WALLUKAT, G., MOSELEY, J., HERSE, F., DECHEND, R., MARTIN, J. N., JR. & LAMARCA, B. 2013. Endothelin-1, oxidative stress, and endogenous angiotensin II: mechanisms of angiotensin II type I receptor autoantibody-enhanced renal and blood pressure response during pregnancy. *Hypertension*, 62, 886-92.
- DA SILVA, A. R., FRAGA-SILVA, R. A., STERGIOPOULOS, N., MONTECUCCO, F. & MACH, F. 2015. Update on the role of angiotensin in the pathophysiology of coronary atherothrombosis. *Eur J Clin Invest*, 45, 274-87.
- DRAGUN, D., MULLER, D. N., BRASEN, J. H., FRITSCHKE, L., NIEMINEN-KELHA, M., DECHEND, R., KINTSCHER, U., RUDOLPH, B., HOEBEKE, J., ECKERT, D., MAZAK, I., PLEHM, R., SCHONEMANN, C., UNGER, T., BUDDE, K., NEUMAYER, H. H., LUFT, F. C. & WALLUKAT, G. 2005. Angiotensin II type 1-receptor activating antibodies in renal-allograft rejection. *N Engl J Med*, 352, 558-69.
- FU, M. L., HERLITZ, H., SCHULZE, W., WALLUKAT, G., MICKE, P., EFTEKHARI, P., SJOGREN, K. G., HJALMARSON, A., MULLER-ESTERL, W. & HOEBEKE, J. 2000. Autoantibodies against the angiotensin receptor (AT1) in patients with hypertension. *J Hypertens*, 18, 945-53.
- GANT, N. F., DALEY, G. L., CHAND, S., WHALLEY, P. J. & MACDONALD, P. C. 1973. A study of angiotensin II pressor response throughout primigravid pregnancy. *J Clin Invest*, 52, 2682-9.
- HANSENS, M., KEIRSE, M. J., SPITZ, B. & VAN ASSCHE, F. A. 1991. Measurement of individual plasma angiotensins in normal pregnancy and pregnancy-induced hypertension. *J Clin Endocrinol Metab*, 73, 489-94.
- HARSEM, N. K., ROALD, B., BRAEKE, K. & STAFF, A. C. 2007. Acute atherosclerosis in decidual tissue: not associated with systemic oxidative stress in preeclampsia. *Placenta*, 28, 958-64.
- HARSEM, N. K., STAFF, A. C., HE, L. & ROALD, B. 2004. The decidual suction method: a new way of collecting decidual tissue for functional and morphological studies. *Acta Obstet Gynecol Scand*, 83, 724-30.
- HERSE, F., DECHEND, R., HARSEM, N. K., WALLUKAT, G., JANKE, J., QADRI, F., HERING, L., MULLER, D. N., LUFT, F. C. & STAFF, A. C. 2007. Dysregulation of the circulating and tissue-based renin-angiotensin system in preeclampsia. *Hypertension*, 49, 604-11.

- HERSE, F., STAFF, A. C., HERING, L., MULLER, D. N., LUFT, F. C. & DECHEND, R. 2008. AT1-receptor autoantibodies and uteroplacental RAS in pregnancy and pre-eclampsia. *J Mol Med (Berl)*, 86, 697-703.
- HERSE, F., VERLOHREN, S., WENZEL, K., PAPE, J., MULLER, D. N., MODROW, S., WALLUKAT, G., LUFT, F. C., REDMAN, C. W. & DECHEND, R. 2009. Prevalence of agonistic autoantibodies against the angiotensin II type 1 receptor and soluble fms-like tyrosine kinase 1 in a gestational age-matched case study. *Hypertension*, 53, 393-8.
- HUBEL, C. A., WALLUKAT, G., WOLF, M., HERSE, F., RAJAKUMAR, A., ROBERTS, J. M., MARKOVIC, N., THADHANI, R., LUFT, F. C. & DECHEND, R. 2007. Agonistic angiotensin II type 1 receptor autoantibodies in postpartum women with a history of preeclampsia. *Hypertension*, 49, 612-7.
- JOHNSEN, S. L., RASMUSSEN, S., WILSGAARD, T., SOLLIEN, R. & KISERUD, T. 2006. Longitudinal reference ranges for estimated fetal weight. *Acta Obstet Gynecol Scand*, 85, 286-97.
- KVEHAUGEN, A. S., DECHEND, R., RAMSTAD, H. B., TROISI, R., FUGELSETH, D. & STAFF, A. C. 2011. Endothelial function and circulating biomarkers are disturbed in women and children after preeclampsia. *Hypertension*, 58, 63-9.
- MOL, B. W. J., ROBERTS, C. T., THANGARATINAM, S., MAGEE, L. A., DE GROOT, C. J. M. & HOFMEYR, G. J. 2016. Pre-eclampsia. *Lancet*, 387, 999-1011.
- NICHOLS, M., TOWNSEND, N., SCARBOROUGH, P. & RAYNER, M. 2014. Cardiovascular disease in Europe 2014: epidemiological update. *Eur Heart J*, 35, 2929.
- RANA, S., SCHNETTLER, W. T., POWE, C., WENGER, J., SALAHUDDIN, S., CERDEIRA, A. S., VERLOHREN, S., PERSCHEL, F. H., ARANY, Z., LIM, K. H., THADHANI, R. & KARUMANCHI, S. A. 2013. Clinical characterization and outcomes of preeclampsia with normal angiogenic profile. *Hypertens Pregnancy*, 32, 189-201.
- SAXENA, A. R., KARUMANCHI, S. A., BROWN, N. J., ROYLE, C. M., MCEL RATH, T. F. & SEELY, E. W. 2010. Increased sensitivity to angiotensin II is present postpartum in women with a history of hypertensive pregnancy. *Hypertension*, 55, 1239-45.
- STAFF, A. C., BENTON, S. J., VON DADELSZEN, P., ROBERTS, J. M., TAYLOR, R. N., POWERS, R. W., CHARNOCK-JONES, D. S. & REDMAN, C. W. 2013a. Redefining preeclampsia using placenta-derived biomarkers. *Hypertension*, 61, 932-42.
- STAFF, A. C., BRAEKKE, K., HARSEM, N. K., LYBERG, T. & HOLTHE, M. R. 2005. Circulating concentrations of sFlt1 (soluble fms-like tyrosine kinase 1) in fetal and maternal serum during pre-eclampsia. *Eur J Obstet Gynecol Reprod Biol*, 122, 33-9.
- STAFF, A. C., DECHEND, R. & PIJNENBORG, R. 2010. Learning from the placenta: acute atherosclerosis and vascular remodeling in preeclampsia—novel aspects for atherosclerosis and future cardiovascular health. *Hypertension*, 56, 1026-34.
- STAFF, A. C., DECHEND, R. & REDMAN, C. W. 2013b. Review: Preeclampsia, acute atherosclerosis of the spiral arteries and future cardiovascular disease: two new hypotheses. *Placenta*, 34 Suppl, S73-8.
- STAFF, A. C., JOHNSEN, G. M., DECHEND, R. & REDMAN, C. W. 2014. Preeclampsia and uteroplacental acute atherosclerosis: immune and inflammatory factors. *J Reprod Immunol*, 101-102, 120-6.
- STAFF, A. C. & REDMAN, C. W. 2014. IFPA Award in Placentology Lecture: preeclampsia, the decidual battleground and future maternal cardiovascular disease. *Placenta*, 35 Suppl, S26-31.
- STAFF, A. C., REDMAN, C. W., WILLIAMS, D., LEESON, P., MOE, K., THILAGANATHAN, B., MAGNUS, P., STEEGERS, E. A., TSIGAS, E. Z., NESS, R. B., MYATT, L., POSTON, L. & ROBERTS, J. M. 2016. Pregnancy and Long-Term Maternal Cardiovascular Health: Progress Through Harmonization of Research Cohorts and Biobanks. *Hypertension*, 67, 251-60.
- STARY, H. C., CHANDLER, A. B., GLAGOV, S., GUYTON, J. R., INSULL, W., JR., ROSENFELD, M. E., SCHAFFER, S. A., SCHWARTZ, C. J., WAGNER, W. D. & WISSELER, R. W. 1994. A definition of initial, fatty streak, and intermediate lesions of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. *Circulation*, 89, 2462-78.

- TRANQUILLI, A. L., DEKKER, G., MAGEE, L., ROBERTS, J., SIBAI, B. M., STEYN, W., ZEEMAN, G. G. & BROWN, M. A. 2014. The classification, diagnosis and management of the hypertensive disorders of pregnancy: A revised statement from the ISSHP. *Pregnancy Hypertens*, 4, 97-104.
- VELLOSO, E. P., PIMENTEL, R. L., BRAGA, J. F., CABRAL, A. C., REIS, Z. S., BADER, M., SANTOS, R. A. & WALLUKAT, G. 2016. Identification of a Novel Agonist-Like Autoantibody in Preeclamptic Patients. *Am J Hypertens*, 29, 405-12.
- VON DADELSZEN, P., MAGEE, L. A. & ROBERTS, J. M. 2003. Subclassification of preeclampsia. *Hypertens Pregnancy*, 22, 143-8.
- WALLUKAT, G., HOMUTH, V., FISCHER, T., LINDSCHAU, C., HORSTKAMP, B., JUPNER, A., BAUR, E., NISSEN, E., VETTER, K., NEICHEL, D., DUDENHAUSEN, J. W., HALLER, H. & LUFT, F. C. 1999. Patients with preeclampsia develop agonistic autoantibodies against the angiotensin AT1 receptor. *J Clin Invest*, 103, 945-52.
- WALLUKAT, G., MULLER, J. & HETZER, R. 2002. Specific removal of beta1-adrenergic autoantibodies from patients with idiopathic dilated cardiomyopathy. *N Engl J Med*, 347, 1806.
- WALTHER, T., WALLUKAT, G., JANK, A., BARTEL, S., SCHULTHEISS, H. P., FABER, R. & STEPAN, H. 2005. Angiotensin II type 1 receptor agonistic antibodies reflect fundamental alterations in the uteroplacental vasculature. *Hypertension*, 46, 1275-9.
- XIA, Y., WEN, H., BOBST, S., DAY, M. C. & KELLEMS, R. E. 2003. Maternal autoantibodies from preeclamptic patients activate angiotensin receptors on human trophoblast cells. *J Soc Gynecol Investig*, 10, 82-93.