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Circulating angiotensin II type I receptor – autoantibodies in diabetic pregnancies

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ABSTRACT

Pregnant women with either pre-existing or gestational diabetes mellitus are at increased risk of preeclampsia as well as future cardiovascular disease. The renin-angiotensin system is dysregulated in both diabetes mellitus and preeclampsia. In preeclampsia, maternal levels of circulating agonistic autoantibodies against the angiotensin II Type I receptor (AT_1-AAs) are increased. Circulating AT_1-AAs are thought to contribute to both the pathophysiology of preeclampsia and the increased risk of future cardiovascular disease. Studies exploring AT_1-AA in diabetes outside pregnancy suggest their potential for both metabolic and cardiovascular pathogenicity. No studies have investigated AT_1-AAs in diabetic pregnancies. We hypothesized elevated maternal circulating AT_1-AA levels in pregnancies complicated by any type of diabetes mellitus. Third-trimester maternal serum from 39 women (controls: n = 10; type 1 diabetes: n = 9; type 2 diabetes: n = 10; gestational diabetes=10) were analyzed for AT_1-AA using an established bioassay method. Circulating AT_1-AAs were present in 70% (7/10) of the controls and 83% (24/29) of the diabetes group ($P = 0.399$). Presence of AT_1-AA was correlated to hsCRP levels ($P = 0.036$), but neither with maternal circulating angiogenic factors (soluble fms-like tyrosine kinase-1 and placental growth factor), nor with maternal or fetal characteristics indicative of metabolic disease or placental dysfunction. Our study is the first to demonstrate presence of circulating AT_1-AAs in pregnant women with any type of diabetes. Our findings suggest AT_1-AAs presence in pregnancy independently of placental dysfunction, nuancing the current view on their pathogenicity. Whether AT_1-AAs per se contribute to increased risk of adverse pregnancy outcomes and future cardiovascular disease remains currently unanswered.

1. Introduction

Worldwide, one in six pregnancies is complicated by maternal hyperglycemia due to either pre-existing diabetes mellitus (DM) i.e. type 1 or type 2 DM, or gestational DM (IDF, 2021).

The affected women are at risk of developing hypertensive disorders of pregnancy and preeclampsia, which in turn leads to an increased risk of adverse pregnancy outcomes and future cardiovascular disease (Weissgerber and Mudd, 2015). In 2011, The American Heart Association recommended including pregnancy history to identify women at risk for developing cardiovascular disease (Mosca et al., 2011). This also includes DM in pregnancy (Shah et al., 2008). Women with gestational DM have a substantially increased risk of developing type 2 DM (Bellamy et al., 2009), but prior gestational DM has also been associated with increased cardiovascular disease risk in the absence of progression to type 2 DM (Kramer et al., 2019). This indicates that factors besides

Abbreviations: AT_1-AA, Angiotensin II Type I receptor autoantibody; AT_1-R, Angiotensin II Type I receptor; BMI, Body mass index; BP, Blood pressure; DM, Diabetes mellitus; GW, Gestation week; LDL, Low density lipoprotein; PE, Preeclampsia; PlGF, Placental growth factor; RAAS, Renin-angiotensin-aldosterone system; s-Eng, soluble endoglin; sFlt-1, soluble fms-like tyrosine kinase-1; TG, Triglyceride.

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hyperglycemia also add to the risk of cardiovascular complications following gestational DM.

Several studies have suggested that the renin-angiotensin-aldosterone system (RAAS) plays a crucial role in both cardiovascular and metabolic disease development (Hansson et al., 1999; Brenner et al., 2001). RAAS is a signaling cascade essential to blood pressure regulation. Low sodium concentrations or fluid flow in the kidneys ultimately results in angiotensin II formation (Fyhrquist and Saijonmaa, 2008). Angiotensin II mainly exerts its effect via Angiotensin II type I receptor (AT_1-R) which leads to arteriolar vasoconstriction (Fyhrquist and Saijonmaa, 2008). The net effect of Angiotensin II is an increase in arterial blood pressure. Circulating Angiotensin II Type I Receptor autoantibodies (AT_1-AAs) have been found increased in preeclampsia in several studies (Wallukat et al., 1999; Dechend et al., 2000; Dechend et al., 2003; Herse et al., 2007; Rieber-Mohn et al., 2018). By activating the AT_1-R and by generating inflammatory effects by several mechanisms, AT_1-AAs are thought to contribute to the pathophysiology of preeclampsia (Dechend et al., 2000; Dechend et al., 2003).

In non-pregnant individuals with DM, AT_1-AAs may be associated with hypertension, left ventricular dilatation (Zhao et al., 2014), and the pathophysiology of diabetes nephropathy (Zhao et al., 2012). Currently, no studies are published reporting AT_1-AA prevalence in women with pregnancies complicated by DM. We hypothesized that women with pregnancies complicated by either pre-existing or gestational DM have an increased prevalence of AT_1-AAs compared to healthy, euglycemic, pregnant controls and that these autoantibodies may contribute to increased risks for adverse outcomes and later maternal cardiovascular disease.

In pregnancy, including in gestational DM, there is a crosstalk between the hormonal active placenta and the maternal tissues. A dysfunctional placenta may increase the risk for maternal systemic vascular inflammation, insulin resistance and development of gestational DM (Jayabalan et al., 2017). Proangiogenic (e.g. PlGF: placental growth factor) and anti-angiogenic factors (e.g. sFlt-1: soluble fms-like tyrosine kinase-1, and sEng: soluble endoglin) are secreted by the placenta, and their concentration is dysregulated in the maternal circulation in preeclampsia, with a relative anti-angiogenic status, contributing to the clinical features with de novo hypertension and proteinuria (Levine et al., 2004). As suggested by Redman et al., syncytiotrophoblast stress, as seen in preeclampsia and other clinical forms of placental dysfunction, contributes to this anti-angiogenic state (Redman et al., 2020). In DM, hyperglycemia, hyperinsulinemia and fetal hypoxia affect the placental vasculature and stimulate the secretion of pro-angiogenetic factors (Cvitic et al., 2014). However, studies of angiogenic circulating markers in pregnant women with either pre-existing or gestational diabetes mellitus have shown conflicting results, either a more pro-angiogenic state with increased circulating PlGF (Ong et al., 2004; Eleftheriades et al., 2014) or an anti-angiogenic state with decreased PlGF (Tsiakkas et al., 2015; Yu et al., 2009; Nuzzo et al., 2021), compared to euglycemic, pregnant controls.

We aimed therefore to investigate if AT_1-AA prevalence in pregnancies complicated by DM is increased compared to euglycemic, healthy pregnant controls, and if AT_1-AAs correlate with traditional risk factors for cardiovascular disease, delivery outcome, as well as with placenta stress-related biomarkers (sFlt-1, PlGF, sFlt-1/PlGF ratio, s-Eng).

2. Material and methods

2.1. Study population and sample collection

Women included in this study were part of the ongoing Oslo Pregnancy Biobank study (recruited 2001–2015) at the Department of Obstetrics, Oslo University Hospital, Ullevål, delivering approximately 7100 women annually. A convenience sample of 39 women in the third trimester with available maternal serum was included (euglycemic: n = 10; type 1 DM: n = 9; type 2 DM: n = 10; gestational DM: n = 10). Serum was collected before delivery after fasting and frozen at – 80 °C until analyzed for circulating AT_1-AA with a bioassay (described in detail below). Inclusion criteria were gestation week > 36, age > 18 years, and fluency in Norwegian or English. Gestational age was calculated based on routine ultrasound screening at gestational week (GW) 17–20. For in vitro fertilization, gestational age was calculated from the date of embryonal transfer. DM complicating pregnancy, either pre-existing or gestational was defined according to the 1999 WHO criteria (WHO, 1999). Birth weight percentile was calculated according to Norwegian population-based sex- adjusted reference ranges (Johnsen et al., 2006).

All women provided written informed consent. The study was approved by the Regional Committee for Medical and Health Research Ethics of South-Eastern Norway C (2010/1850) and performed in accordance with the principles of the Helsinki Declaration.

2.2. AT1-receptor bioassay

Maternal circulating AT_1-AAs were measured by the chronotropic responses to AT_1 receptor-mediated stimulation of cultured neonatal rat cardiomyocytes, as previously described (Wallukat et al., 1999). Immunoglobulin G was isolated from 200 μl of rat serum by protein G sepharose on bioline protein purification system and added to the spontaneously beating neonatal rat cardiomyocytes. AT_1-AAs in the tested maternal serum were measured by counting the contraction rate after 60 min. The difference between basal beating frequency and beating frequency after adding maternal sera accounts for the delta beats contraction rate. A negative delta indicates a decrease, a positive delta an increase in the contraction rate after adding the serum. Test results were considered to be positive (i.e. presence of AT_1-AA) if delta beats per minute exceeded 7.2 and if the increased beating rate was successfully blocked by losartan, an angiotensin antagonist added to confirm AT_1-AA specificity. The serum samples were examined blinded to the patient's diagnosis.

2.3. Biomarker laboratory analyses

Previously analyzed and published results for maternal Low Density Lipoprotein (LDL), triglycerides (TG) and hsCRP were available in 5/10 of the controls and in 17/29 of the diabetes group (16/29 for hsCRP). The analyses were performed at the Department of Medical Biochemistry, Oslo University Hospital, blinded for clinical information, as in previous studies (Moe et al., 2018). Likewise, previously published s-Eng results were available from a prior analyses (Human Endoglin/CD105 Immunoassay Quantikine® ELISA) study Serum levels of sFlt1 and PlGF were determined at Oslo University Hospital using Elecsys immunoassays (Roche Diagnostics, Mannheim, Germany) utilizing a fully automated electrochemoiluminiscence immunoassay platform (Cobas E 601, Roche Diagnostics) according to the manufacturer's instructions, as in previous studies (Staff et al., 2009).

2.4. Clinical cardiovascular risk markers

Blood pressure was measured at inclusion according to regular hospital protocols with validated devices approved for pregnancy at the respective inclusion time point. Body mass index (kg/m^2) was assessed both pre-pregnancy and at delivery.

2.5. Statistical analyses

Data were analyzed using IBM SPSS Statistics Data Editor, version 26. Non-parametric Mann-Whitney U test and Kruskal-Wallis test were used when comparing groups with continuous data. Chi-square test and Fisher's exact test were applied to categorical data. A probability of $\leq$ 0.05 was considered statistically significant.

3. Results

3.1. Clinical characteristics and maternal placenta-associated biomarkers

The clinical characteristics of the included pregnancy groups are shown in Table 1. In the DM group, significantly more women were overweight, delivered earlier, and delivered heavier babies, as compared to the control group (Table 1). Median newborn weight percentiles did not differ across the pre-existing and gestational DM groups ($P = 0.429$, details not shown). Further, insulin was used in 100% of women with type 1 DM, 86% of women with type 2 DM, and 40% of women with gestational DM ($P = 0.118$). Although median systolic and diastolic blood pressure values did not differ between controls and the combined DM group, all women in the control group were normotensive, whereas 11 of 29 diabetic women (type 1: n = 3; type 2; n = 5; gestational; n = 3) were hypertensive at inclusion (systolic ≥140 and/or diastolic blood pressure ≥90 mmHg), $P = 0.022$. In the control group, one had preeclampsia in a prior pregnancy, compared to four in the DM group ($P = 0.677$) (type 1 diabetes: n = 3; gestational diabetes n = 1, $P = 0.061$).

Values are given in median values (and minimum to maximum range), percentages, and rates; all P-values as compared to the control group. Ethnicity: Categorized into Scandinavian or Western immigrant (n given in Table) and Asian/African (remainder). DM: Diabetes mellitus (combined pre-existing and gestational DM), BMI: Body mass index (kg/m2), PE: Preeclampsia, BP: Blood pressure.

Other biomarkers linked to increased cardiovascular disease risk and inflammation did not differ significantly between the control and DM group: LDL: 3.7 (3.3–5.4) mmol/L vs. 4.0 (2.2.–5.6) mmol/L, $P = 0.940$; TG: 2.7 (2.1–3.8) mmol/L vs. 2.6 (1.8–12.5) mmol/L, $P = 1.000$; hsCRP: 6.4 (2.7–8.5) mg/L vs. 3.9 (0.70–32.7) mg/L, $P = 0.275$. Third-trimester maternal circulating placenta biomarkers (sFlt-1,PlGF, sFlt-1/PlGF ratio, sEng) did also not differ between the control and DM groups, as detailed in Table 2. We did likewise not identify any large or significant differences between these angiogenic markers in pregnancies with pre-existing DM as compared with gestational DM pregnancies (data not shown).

Table 1
Clinical characteristics of the included pregnancy groups.

	Controls (n = 10)	DM (n = 29)	*P*-value
Maternal age	35 (32–39)	36 (19–37)	1.0
BMI pre pregnancy (kg/m^2)	22.7 (21.1–30.9)	25.9 (19.8–37.2)	0.064
BMI at delivery (kg/m^2)	29.4 (25.5–36.0)	31.3 (26.6–44.0)	0.365
BMI ≥ 25 (kg/m^2) (n)	3	7	0.031
Para	1 (0–2)	1 (0–4)	0.092
Previous PE (n)	1 Missing 2	4 Missing 8	0.677
Systolic BP at inclusion (mmHg)	125.5 (100–137)	130 (98–189)	0.418
Diastolic BP at inclusion (mmHg)	81 (60.89)	75 (60–98)	0.382
Systolic BP < 20 weeks (mmHg)	113 (93–132)	115 (98–149)	0.692
Diastolic BP < 20 weeks (mmHg)	67.5 (50–84)	70 (60–84)	0.788
Gestational age at inclusion (weeks)	39.3 (38.7–40.4)	38.1 (36.1–40.1)	0.031
Gestational age at delivery (weeks)	39.3 (38.7–40.4)	38.6 (36.4–40.4)	0.015
Cesarean section (%)	100	76.9 Missing 3	0.096
Newborn sex (% girls)	50	48.3	0.925
Newborn weight (grams)	3575 (3470–3732)	4035 (2530–5857)	0.024
Placenta weight (grams)	736 (396–1000)	700 (190–1895) Missing 6	0.923
Ethnicity (n: Scandinavian or Western immigrant)	9	23	0.644

Table 2
Third-trimester maternal circulating placenta-associated biomarkers in the included pregnancy groups.

	Control n = 10	DM n = 29	*P*-value
sFlt-1 (pg/mL)	3859 (2795–9073)	5050 (1976–9756)	0.456
PlGF (pg/mL)	166.4 (71–474)	143.8 (73.3–990)	1.000
sFlt-1 /PlGF ratio	24 (7–104)	33 (3–125)	0.740
s-Eng (ng/mL)	7.6 (5.5–17.8) Missing 3	10.4 (4.9–36.8) Missing 14	0.359

Values are given in median values (and minimum to maximum range). DM: Diabetes mellitus (combined pre-existing and gestational DM).

3.2. Maternal circulating AT_1-AA

In total, 79.5% (31/39) of the included women were AT_1-AA positive. In the DM group, 83% (24/29) were AT_1-AA positive, which was not significantly different compared to 70% (7/10) AT_1-AA positivity in the control group, $P = 0.399$. Positivity for AT_1-AA was similarly distributed between the DM groups (gestational DM: n = 7/10; type 1 DM: n = 7/9; type 2 DM, n = 10/10, $P = 0.296$). Circulating maternal levels of AT_1-AAs, expressed as median delta beats per minute, did not differ significantly between the control group and the total DM group (11.7 vs. 15.3 beats per minute, $P = 0.623$) (Fig. 1), nor across all groups ($P = 0.697$) or between the DM groups ($P = 0.839$).

Irrespective of diagnosis, the AT_1-AA positive group did not differ from the AT_1-AA negative in delivery outcomes (in terms of newborn weight ($P = 0.798$), newborn weight percentile ($P = 0.746$) or delivery week ($P = 0.132$)), or most of the tested cardiovascular disease risk markers. However, AT_1-AA positive women had significantly higher levels of hsCRP (Table 3).

For the DM group (n = 29), AT_1-AA positive and negative women did not differ significantly in delivery outcomes or cardiovascular disease risk markers. Among the 11 diabetic patients with blood pressures ≥ 140/90 at inclusion, 72% (8/11) were AT_1-AA positive, which did not differ significantly from the normotensive diabetic group, with 88% (16/18) AT_1-AA positive pregnant women ($P = 0.615$).

3.3. Maternal circulating AT_1-AA and circulating levels of placenta-associated biomarkers

For the total pregnancy group, median maternal circulating levels of placenta-associated biomarkers did not differ between AT_1-AA positive and negative women (Fig. 2). In the DM group, AT_1-AA positive and negative women did not have different median concentration levels of s-Eng (12.4 ng/m vs. l 8.8 ng/mL, $P = 0.082$), sFlt-1 (5066 pg/mL vs.

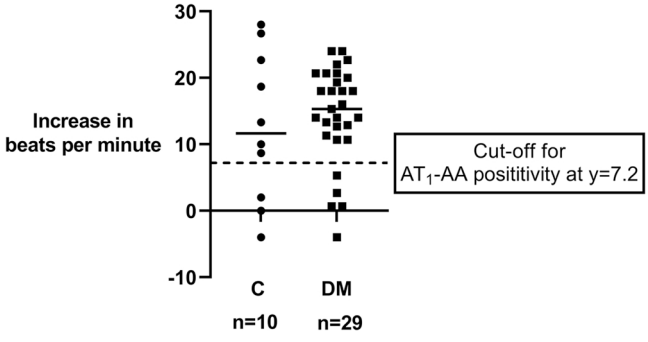

Fig. 1. Maternal AT_1-AA serum levels in the pregnancy groups. The Y-axis of Fig. 1 shows the increase in beat number per minute of spontaneously beating neonatal rat cardiomyocytes when exposed to immunoglobulin from maternal serum for the study groups (C = healthy, euglycemic pregnancies, named controls; DM = Diabetes mellitus). Values above the line y = 7.2 represent the AT_1-AA positive group, whereas values below are defined as AT_1-AA negative.

Table 3
Cardiovascular disease risk markers in AT_1-AA negative and AT_1-AA positive pregnant women in the total study group (n = 39).

	AT_1-AA negative n = 8	AT_1-AA positive n = 31	*P*-value
BMI pre pregnancy (kg/m^2)	26.6 (19.8–32.7)	25.9 (21.1–34.8)	0.959
BMI at delivery (kg/m^2)	31.2 (26.3–43.9)	31.3 (25.3–41.8)	0.798
Systolic BP at inclusion (mmHg)	135.5 (120–148)	121 (98–189)	0.085
Diastolic BP at inclusion (mmHg)	75.5 (70–90)	78 (60–98)	0.621
Systolic BP < week 20 (mmHg)	111.5 (98–132)	115 (93–149)	0.670
Diastolic BP < week 20 (mmHg)	70 (60–84)	68 (50–81)	0.401
LDL (mmol/L)	3.7 (3.3–5.2) Missing 3	4.3 (2.3–5.6) Missing 15	1.000
TG (mmol/L)	3.4 (2.1–4.6) Missing 3	2.5 (1.8–6.1) Missing 15	0.367
hsCRP (mg/L)	2.0 (0.70–6.90) Missing 3	4.3 (1.2–32.7) Missing 16	0.036

Levels are given in median values (and minimum to maximum range). BMI: Body mass index (kg/m2), BP: Blood pressure, TG: Triglycerides.

4128 pg/mL, $P = 0.594$), PlGF (145.9 pg/mL vs. 141.2 pg/mL, $P = 0.716$), or sFlt-1/PlGF ratio (32.5 vs. 39, $P = 0.634$).

4. Discussion

Our study is to our knowledge the first to describe the presence of circulating AT_1-AAs in diabetic pregnancies and their association to maternal and neonatal characteristics, as well as their lacking associations to placenta-associated biomarkers. Contrary to our hypothesis, we found no differences in circulating AT_1-AAs between women with pregnancies complicated by any type of diabetes and normotensive, euglycemic pregnant controls. The high rates of AT_1-AA positive diabetic women correspond to the high rates previously described in pregnancies complicated by preeclampsia (Wallukat et al., 1999; Walther et al., 2005; Herse et al., 2009; Velloso et al., 2016), however, rates of elevated levels of AT_1-AAs in euglycemic and normotensive controls were only slightly lower. Although women in the DM group were included earlier than the control group, all samples analyzed were from the late third trimester. "Our study adds information on the range in AT1-AA rates in healthy controls, with earlier reports of rates ranging from as low as 0% (Wallukat et al., 1999; Velloso et al., 2016), 11% (Xia et al., 2003), 17% (Rieber-Mohn et al., 2018), and 50% (Walther et al., 2005) in healthy controls and our present study with 70%."

Increased circulating AT_1-AAs are associated with severe forms of hypertensive renal disease and autoimmune disease (Fu et al., 2000; Dragun, 2007; Riemekasten et al., 2011), none of which was present in any of the clinically thoroughly described women included to our study. We thus relate new information on the spectrum of circulating AT_1-AA at term in healthy, normotensive and euglycemic pregnant women without other known clinical complications. In preeclampsia, AT_1-AAs have been linked to the generation of anti-angiogenetic placental factors, inducing oxidative stress, endothelial dysfunction, hypertension and fetal growth restriction (Herse and Lamarca, 2013). In our included pregnant women, there were no clinical signs of placental dysfunction in terms of low birth weight, nor other adverse delivery outcomes. In line with (Stepan et al., 2006) and (Herse et al., 2009), as well as our previous findings (Rieber-Mohn et al., 2018), AT_1-AAs did not correlate with levels of placenta- associated angiogenetic factors per se, further implying that the presence and pathogenicity of AT_1-AAs could depend on other immunological, placental or maternal features. Thus, it remains unclear whether the AT_1-AA contributes to increased risk of adverse pregnancy outcomes in diabetic pregnancies.

In a healthy pregnancy, circulating RAAS components are increased, but Angiotensin II sensitivity is decreased, partly due to AT_1-R

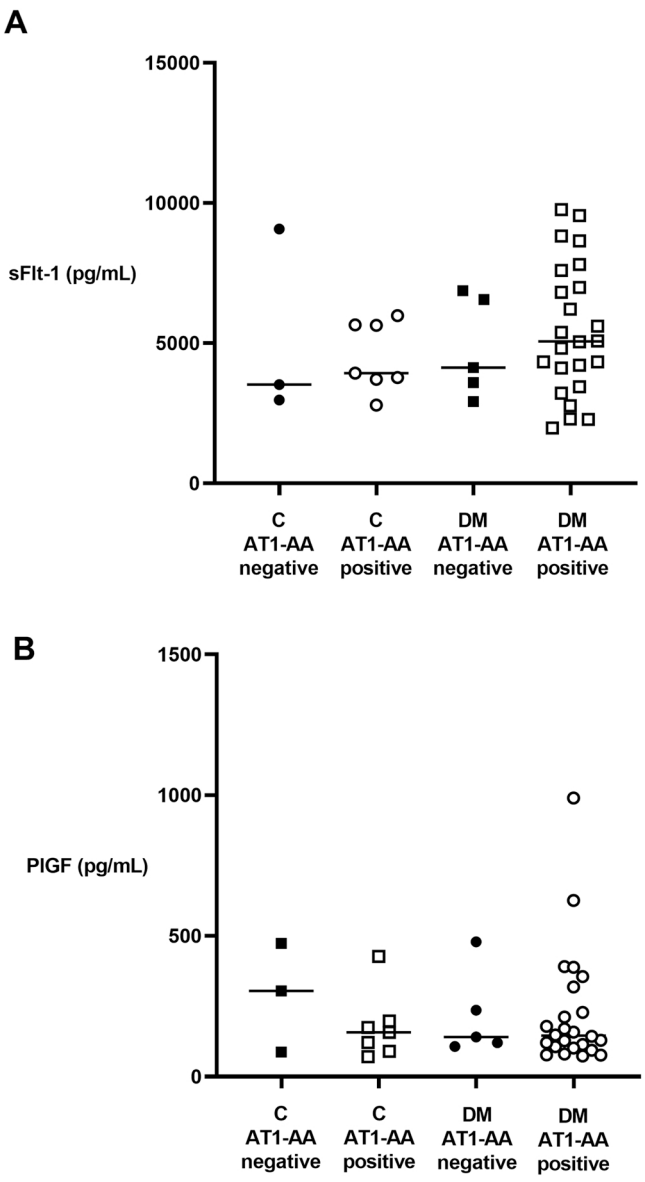

Fig. 2. Maternal circulating AT_1-AA and circulating levels of sFlt-1 and PlGF. Fig. 2. A–B Angiogenic factor concentrations [A: sFlt-1 concentration (pg/mL); B: PlGF concentration (pg/mL), respectively] presented for each pregnancy group according to positivity or negativity for AT_1-AA presence in maternal serum (C = healthy, euglycemic pregnancies, named controls; DM = Diabetes mellitus).

properties and desensitization by reactive oxygen species (Irani and Xia, 2008). Few studies have explored the composition of RAAS in diabetic pregnancies. Noeguria et al. found that similarly to preeclampsia (Irani and Xia, 2008), women with gestational DM had decreased levels of the protective vasodilator Angiotensin (1−7) compared to healthy pregnant women, suggesting a link to endothelial dysfunction and insulin resistance (Nogueira et al., 2007). In non-pregnant individuals, it has been demonstrated that the beta cells of the pancreas express the AT_1-R (Rein and Bader, 2017), entailing increased expression of the receptor following beta cell hypertrophy (Graus-Nunes and Souza-Mello, 2019). AT_1-R stimulation would compromise the blood supply to the beta cells and contribute to the destruction of the cells (Graus-Nunes and Souza-Mello, 2019). AT_1-AAs could hence contribute to a pathogenic role in diabetic pregnancies, accelerating glucose intolerance and endothelial dysfunction.

Although 11 women with DM had hypertensive blood pressures,

these women were not more often positive for the AT_1-AA than diabetic women without hypertension. Similarly, there were no differences in median lipid levels between AT_1-AA positive and AT_1-AA negative women. Endothelial function and insulin sensitivity have also been associated with levels of CRP in pregnant women (Pendeloski et al., 2017). We did find an association between hsCRP and AT_1-AA, indicating increased inflammation in AT_1-AA positive women, which could support AT_1-AA as a possible marker for future cardiovascular disease (Pendeloski et al., 2017). We do not believe BMI to have impacted significantly this associations, as there were no significant differences in BMI between the AT1-AA positive and negative groups (Table 3). Also, when dichotomizing our DM and control group into normal or overweight/obese women (below compared to above 25 kg/m2), we did not find any significant differences in the rate of AT1-AA positivity (data not shown). Important with regard to this observation is the fact that there were no significant differences in BMI between the AT1-AA positive and negative group. However, levels of hsCRP were only available for five in the control group and 16 in the DM group. Whether the autoantibodies imposes a long-term cardiovascular and metabolic disease risk remains to be answered. However, Zhao et al. found that levels of AT_1-AAs were elevated in non-pregnant patients with type 2 DM with hypertension and that AT_1-AAs further correlated to the degree of left ventricular dilatation (Zhao et al., 2014), indicating that the autoantibodies could have potential for cardiovascular pathogenicity in patients with diabetes.

A strength of our study is the stringent method of biological sample collection and storage, a well-defined population concerning maternal characteristics as well as a thorough check of pregnancy complications and delivery outcomes. However, gestational DM diagnosing criteria were recently modified, leading to an increased incidence in Norway (Friis et al., 2020). Our controls were considered healthy at the time of inclusion. The bioassay method using neonatal rat cardiomyocytes is well evaluated and sophisticated, but its complexity and time consumption limits the number of samples that can be analyzed, thus representing both a strength and a weakness. The small sample size limits a generalized conclusion. However, we believe our findings provide a more nuanced view on the AT_1-AA presence as compared to previous studies.

5. Conclusion

Our study is the first to show that a large rate of pregnant women with DM have circulating AT_1-AAs present. Our current findings suggest that circulating AT_1-AAs can present at term independently of a dysfunctional placenta. Their pathogenicity may rely on other immunological, cardiovascular maternal or placental features. Whether AT_1-AAs per se contribute to increased risk of adverse pregnancy outcomes and future cardiovascular disease, remains currently unanswered. Studies exploring RAAS and AT_1-AA in diabetes outside of pregnancy suggest their potential for both metabolic and cardiovascular pathogenicity. Thus, future studies should investigate circulating AT_1-AAs postpartum in groups of women with prior pregnancy complications linked to placental dysfunction, that epidemiologically are at increased risk for cardiovascular disease (Staff et al., 2016).

Conflict of interest statement

Meryam Sugulle and Annetine Staff declare that they received in-kind reagents for the sFlt-1 and PlGF biomarker analyses referred to in the present study from Roche Diagnostics, but the company had no impact on planning, performance or any other aspects of the study. The remaining authors declare no conflict of interest. The authors declare no other conflict of interest.

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Conflict(s) of interest/Disclosure(s)

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