The uteroplacental circulation in hypertensive disorders of pregnancy; Doppler ultrasound and histopathological studies
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UTERINE ARTERY DOPPLER FLOW AND UTEROPLACENTAL VASCULAR PATHOLOGY IN NORMAL PREGNANCIES AND PREGNANCIES COMPPLICATED BY PRE-ECLAMPSIA AND SMALL-FOR-GESTATIONAL-AGE FETUSES

(Provisionally accepted, Placenta)

This study was conducted to investigate the association between uterine artery Doppler flow patterns and uteroplacental vascular pathology in normal and complicated pregnancies in view of the recently described concept of heterogeneous causes of hypertensive pregnancy complications. Forty-three women whose pregnancies were complicated by pre-eclampsia, the HELLP-syndrome and/or small-for-gestational-age (SGA) fetuses and 27 women with normal pregnancies undergoing elective Caesarean section were included. We obtained uterine artery Doppler waveforms at a mean of 4 days before delivery. Placental bed biopsies were obtained at Caesarean section and analysed for physiological changes and pathological changes.

We found that abnormal uterine artery Doppler flow was strongly associated with pregnancy complications. Absence of physiological changes was seen in 58% of complicated pregnancies and 40% of normal pregnancies. Pathological changes were seen in 58% of complicated pregnancies and 53% of normal pregnancies; they occurred in spiral arteries with and without physiological changes, and there was no significant correlation to Doppler results. In conclusion, absence of physiological changes is associated with abnormal uterine artery Doppler flow and pregnancy complications. However, there is a gradient in the severity of uteroplacental vascular pathology and the correlation with pregnancy complications is not as strong as previously thought. There is also a significant degree of uteroplacental vascular pathology in normal pregnancies with normal uterine artery Doppler flow. We hypothesise that additional factors might be necessary to induce the clinical syndrome of pre-eclampsia.
INTRODUCTION

In normal pregnancy, trophoblast cells invade the spiral arteries as early as 12 weeks of gestation. This invasion induces changes in the spiral arteries, which become much larger and lose their media to fibrinoid depictions (Pijnenborg et al. 1981). Thus, these vessels lose their vasoactive properties and become capable of transporting large amounts of blood at low resistance towards the placenta. By measuring spiral artery blood flow using colour Doppler ultrasound in the second trimester, Matijevic et al. (1995) found that physiological changes are functionally complete at 17 weeks.

In pre-eclamptic pregnancies, several abnormalities in this process came to light in a number of histological studies: Firstly, the physiological adaptation of spiral arteries appears to be absent or restricted to the distal (decidual) portion of the spiral arteries (Robertson, Brosens and Dixon 1975; Khong et al. 1986). This is in accordance with the finding that spiral artery blood flow as measured by colour Doppler ultrasound is impaired in pre-eclamptic pregnancy (Matijevic and Johnston 1999). Secondly, a distinctive lesion is found in the uteroplacental vessels of pre-eclamptic women, which consists of intimal proliferation, media necrosis and lipid accumulation. The most severe form of this abnormality, with extensive tissue necrosis, is traditionally termed “acute atherosis” (Khong 1991; DeWolf, Robertson and Brosens 1975). In the presence of these abnormalities, blood flow to the placenta is compromised and the resulting placental insufficiency is thought to play a central role in pre-eclampsia and, to a lesser extent, in fetal growth restriction resulting in SGA infants (Gerretsen et al. 1986). Evidence of compromised placental blood flow in pregnancies complicated by SGA fetuses was recently given by Ferrazzi et al. (1999) who found a positive correlation between abnormal uterine artery Doppler flow and the presence of hypoxic-ischemic lesions in placentae from pregnancies complicated by SGA fetuses.
The concept of impaired physiological adaptation implies high resistance in the uteroplacental circulation throughout pregnancy. Doppler studies aimed at early detection of pre-eclampsia are based on this theory. A number of screening studies in the past decade has evaluated this possibility (Campbell et al. 1986; Jacobson et al. 1990; Steel et al. 1990; Bewley, Cooper and Campbell 1991; Bower, Schuchter and Campbell 1993; Mires et al. 1998). Unfortunately, however, they have often shown disappointing results due to a large number of false negatives. The milder forms of pre-eclampsia especially appear to be difficult to predict by second-trimester Doppler (Bower, Schuchter and Campbell 1993; Mires et al. 1998). Recently, Ness and Roberts (1996) proposed a theory that could explain these disappointing results. They proposed heterogeneous causes for the clinical entity of pre-eclampsia whereby placental as well as maternal factors could lead to placental insufficiency, with subsequent endothelial damage and the clinical syndrome of pre-eclampsia as the common end result. This theory has been reinforced by recent reports on the role of oxidative stress acting as an intermediary between maternal factors and reduced placental perfusion in pre-eclampsia. (Roberts and Hubel 1999) In this concept, defective placentation is not universally present in all cases of pre-eclampsia. This is in accordance with histopathological studies by Pijnenborg et al. (1991) and by Meekins et al. (1994). They studied the uteroplacental arteries in pre-eclamptic and normotensive women. They found that physiological adaptation is not an all or none phenomenon but varies between spiral arteries in the same patient, and that there is a spectrum of changes that do not have a strong correlation to clinical severity. This interesting finding could explain the disappointing results of the Doppler velocimetry screening studies. It is further corroborated by the study of Ghidini et al. (1997) who described a considerable overlap in the degree of placental vascular lesions between normal and pre-eclamptic pregnancies.

In the present study, we have investigated the association between uterine artery Doppler flow patterns and histologic findings in placental bed biopsies from
normal and complicated pregnancies. The observation from screening studies that a substantial number of women develop pre-eclampsia despite normal Doppler flow results earlier in pregnancy prompted us to the present study. We hypothesised that this discrepancy is the result of pathological changes such as acute atherosis occurring in physiologically adapted uteroplacental arteries. The possibility of atherosis in physiologically adapted vessels has been denied by some earlier studies (Robertson, Brosens and Dixon 1975; DeWolf, Robertson and Brosens 1975; Khong 1991) but would be in agreement with the more recent studies by Pijnenborg et al (1991) and Meekins et al (1994). The relationship between uteroplacental Doppler flow and physiological changes has previously been investigated by Lin et al. (1995) and Voigt and Becker (1992). Both these studies found a significant correlation between impaired Doppler flow and absence of physiological changes in normal and complicated pregnancies. However, both studies also report a considerable overlap in the degree of physiological changes between normal and complicated pregnancies. In contrast to these studies, we have evaluated pathological changes as a separate entity.
Materials and Methods

Patients
Over a period of four years, all women admitted to our antenatal ward with pregnancies complicated by PIH, pre-eclampsia, the HELLP-syndrome and/or SGA fetuses who were planned to undergo caesarean section during daytime hours were asked to participate in this study. Forty-three women were included. All women had singleton pregnancies and gestational age at delivery ranged from 26 to 40 weeks. In addition, all women with normal pregnancies undergoing elective Caesarean section at term over a period of fourteen months were asked to participate. Twenty-seven women were included in this group. Clinical data for both groups are given in table 1. In both groups, Doppler measurements were performed at a mean of 4 days before delivery.

In accordance to the definition proposed by Davey and MacGillivray (1988) and adopted by the International Society for the Study of Hypertension in Pregnancy, pregnancy-induced hypertension was defined as a diastolic blood pressure > 90 mmHg occurring after 20 weeks of gestational age. Pre-eclampsia was defined as pregnancy-induced hypertension and significant proteinuria (300mg/24h or 2 plus on urinalysis). Small-for-gestational-age (SGA) was defined as a birthweight below the 10th percentile for foetal sex and gestational age (Kloosterman 1970). The study was approved of by the hospital medical ethics committee. All women gave informed consent.
<table>
<thead>
<tr>
<th></th>
<th>Normal Pregnancy (n=15)</th>
<th>Complicated Pregnancy (n=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-eclampsia</td>
<td>0</td>
<td>6 (25%)</td>
</tr>
<tr>
<td>Pre-eclampsia + SGA fetus</td>
<td>0</td>
<td>8 (33%)</td>
</tr>
<tr>
<td>HELLP-syndrome</td>
<td>0</td>
<td>2 (8%)</td>
</tr>
<tr>
<td>HELLP-syndrome + SGA fetus</td>
<td>0</td>
<td>4 (17%)</td>
</tr>
<tr>
<td>SGA fetus, non-hypertensive</td>
<td>0</td>
<td>4 (17%)</td>
</tr>
<tr>
<td>Gestational age at delivery (wks)</td>
<td>37.4 ± 2.0</td>
<td>32.0 ± 3.2 *</td>
</tr>
<tr>
<td>Birthweight (grams)</td>
<td>2825 ± 587</td>
<td>1230 ± 492 *</td>
</tr>
<tr>
<td>Maximum diastolic blood pressure (mmHg)</td>
<td>75 ± 9</td>
<td>112 ± 13 *</td>
</tr>
<tr>
<td>Interval Doppler-delivery (days, range)</td>
<td>5 (0-21)</td>
<td>3 (0-21)</td>
</tr>
</tbody>
</table>

Table 1. Clinical data from the different study groups with representative biopsies. Data are n (%) or mean (SD). * p<0.05 compared to controls.
**Methods**

Doppler measurements were performed using an Acuson 128 XP duplex scanner (Acuson, Mountainview, Calif.) or a Hitachi EUB 515A (Tokyo, Japan). Waveforms were obtained from both uterine arteries at the crossing with the external iliac artery as previously described (Oosterhof and Aarnoudse 1992). The Pulsatility Index (PI) was calculated according to Gosling and King (1975). The mean PI of the left and right uterine artery was used. Reference values for the Pulsatility Index at various gestational ages were determined by fourweekly assessment of the uterine arteries in 63 normal pregnancies (unpublished observations). An abnormal result was defined as a PI above the mean + 2SD for gestational age in the reference population. Thus, the cut-off PI value at 26 weeks was 1.38, decreasing with gestational age to a cut-off value of 0.94 at 40 weeks.

Placental bed biopsies were taken at delivery by means of a punch biopsy clamp. Biopsies were fixed in formaldehyde and embedded in paraffin. We used haematoxylin-eosin and Periodic Acid Schiff staining to assess the physiological changes and pathological alterations. Keratin 7 staining (monoclonal mouse antihuman cytokeratin 7, OV-TL 12/30 from Dako) was used to identify trophoblastic cells in the vessel wall.

Placental bed biopsies were considered representative if spiral arteries were seen in decidual segments as well as in myometrial segments. Furthermore, to ascertain that the biopsy was taken from the placental site, biopsies were only considered representative if trophoblastic cells were present in the decidual stroma (Robertson et al. 1986).

Placental bed biopsies were assessed independently by two observers, without knowledge of clinical details or the Doppler flow results. Biopsies were scored on the presence of physiological changes and pathological alterations. They were analysed as follows:
Physiological changes:
Physiological vascular changes were defined as a loss of the muscular components of the vessel wall, replacement of the vessel wall by fibrinoid depositions, and the presence of trophoblastic cells in the vessel wall. Biopsies were classified into three groups:
- Complete physiological changes: complete physiological changes are observed in the myometrium as well as the decidua (Figure 1).
- Partial physiological changes: physiological changes are observed in spiral arteries in the myometrium, but the original vessel wall is not completely replaced by fibrinoid deposits and the arterial character was still partially recognisable.
- Absent physiological changes: physiological changes are not seen anywhere in the myometrial vessels (Figure 2).

Pathological changes:
Pathological changes were defined based on morphological characteristics as intimal lipid accumulation or hyperplasia, medial necrosis, and acute atherosis with (sub)total occlusion of the lumen wall by intimal proliferation or mononuclear infiltrate. The presence of arterial thrombosis was also recorded. Biopsies were classified into three groups:
- No pathological changes.
- Mild pathological changes: pathological alterations such as mucoid changes, foam cells or hyalinisation are present in either decidual or myometrial vessels, but the lumen is not significantly narrowed. Figure 3 shows an example of mild pathological changes in a biopsy from a normal pregnancy.
- Severe pathological changes/acute atherosis: the lumen is (sub)totally occluded by acute atherosis, infiltrate or thrombosis (Figure 4).
Figure 1. Normal pregnancy at 38 weeks; normal Doppler results. Complete physiological changes are seen in this spiral artery segment at the decidual-myometrial junction. The media has been replaced by PAS-positive fibrinoid. (PAS x 100).

Figure 2. Pre-eclampsia complicated by the HELLP-syndrome, delivery at 30 weeks. The Doppler results were abnormal. Absence of physiological changes in myometrial segment of spiral artery. (Haematoxylin-eosin x 100).
Figure 3. Pre-eclampsia without IUGR at 34 weeks. The Doppler results were normal. Mild pathological changes consisting of foam cells and lipid depositions in the intima and media. (Haematoxylin-eosin x 150).

Statistical methods:
As PI does not show a normal distribution in a larger population, non-parametric tests (Mann-Whitney) were used to compare PI values. A two-tailed p < 0.05 was considered significant.
Kappa-statistics were calculated to assess inter-observer agreement for both physiological changes and pathological alterations. For physiological changes, inter-observer agreement was 86%, with a kappa-value of 0.72; for pathological changes inter-observer agreement was 76% with a kappa-value of 0.53. To compare physiological and pathological changes between Doppler outcome groups, we used the Chi-square test (Fisher' Exact test) and Spearman's test for nonparametric correlation.
RESULTS

A total of 70 biopsies from 43 complicated and 27 normal pregnancies were taken at caesarean section. Fourteen biopsies were classified as not representative because of the absence of trophoblastic cells; in addition, 17 biopsies were classified as not representative because of the absence of spiral arteries in the myometrium. Of the 39 biopsies (59%) which were considered representative, 24 were from complicated pregnancies and 15 from normal pregnancies.

Normal pregnancy group

Doppler results
Uterine artery Doppler flow was normal in the majority of control pregnancies. An abnormally high PI was found in only three controls (11%).

Placental bed biopsies
Results from the 15 controls with representative biopsies are given in table 2. Only one representative biopsy was obtained from the three controls with abnormal flow. This biopsy showed absence of physiological changes and severe pathological changes.
Complete physiological changes were seen in 9 biopsies (60%) (Figure 1). The remaining biopsies showed absence of physiological changes; partial changes were not seen in this group.
Surprisingly, pathological changes were seen in the majority of biopsies from normal pregnancies. In the control group as well as the patient group, there was no correlation between the absence of physiological changes and the presence of pathological changes.
Table 2. Normal and complicated pregnancy groups: distribution of physiological and pathological changes in normal and abnormal PI groups.

**Complicated pregnancy group**

**Doppler results**

Pregnancy complications were strongly associated with an abnormal uterine artery PI; 24 patients (74%) had abnormal Doppler results. Eleven patients had normal Doppler results; these were four patients with the HELLP-syndrome, four patients with pre-eclampsia, one patient with a SGA fetus and two patients with both pre-eclampsia and SGA fetuses.
Placental bed biopsies

Results from the 24 patients with complicated pregnancies who had representative biopsies are given in table 2. Complete absence of physiological changes was seen in 14 biopsies (58%) (Figure 2). The remaining biopsies showed (at least partial) physiological changes. Pathological changes, such as acute atherosis or thrombosis, were seen in 14 biopsies (58%). They were not related to Doppler results. In eight cases, pathological changes were seen in biopsies which also showed physiological changes. Figure 4A is an example of extensive pathological changes in a spiral artery which also shows evidence of physiological changes as indicated by the presence of trophoblast by keratin 7 staining (Figure 4B). In addition, there is a mononuclear infiltrate which consists of granulocytes and macrophages, as earlier described by Reister et al. (1999). Macrophages were identified in this infiltrate by CD68 staining (monoclonal mouse antihuman macrophage CD68, KP-1 from Dako; figure not shown). Figure 4C shows a spiral artery in the myometrium in the same biopsy. This biopsy was obtained from a pre-eclamptic pregnancy at 33 weeks; the Doppler results were normal.

There was no significant correlation between the absence of physiological changes and the presence of pathological changes.
Figure 4A. Pre-eclamptic pregnancy without IUGR at 33 weeks. The Doppler results were normal. Partial physiological changes were seen in this spiral artery and elsewhere in the biopsy. There are pathological changes consisting of a mononuclear infiltrate (granulocytes and macrophages) and obliteration of the vessel lumen in this segment from the decidual-myomtrial junction. (PAS x 100).

Figure 4B. The same biopsy as shown in figure 4A; Keratin 7 staining x 100 indicating trophoblastic cells in the vessel wall.
DISCUSSION

In the present study, the relationship between Doppler velocimetry of the uteroplacental circulation and the presence of physiological and pathological changes in the placental bed was investigated; we analysed biopsies from a group of pregnancies complicated by PIH, pre-eclampsia, the HELLP-syndrome and SGA fetuses as well as from a control group of uncomplicated pregnant women undergoing Caesarean section.

Uterine artery PI was easy to obtain in all patients at a mean of 4 days before delivery. Fifty-nine percent of biopsies were representative. Kappa scores of inter-observer agreement were adequate for both physiological as well as pathological changes.
The PI was strongly correlated to pregnancy outcome; 11% of controls had abnormal Doppler flow, compared to 74% of patients with complicated pregnancies. The absence of physiological changes was more common in complicated pregnancies. Because of the small numbers in the subgroups, it was not valid statistically to perform Chi-contingency tests in the separate groups. In both outcome groups combined, there was a significant correlation between abnormal Doppler results and negative or partial physiological changes (Chi-square Fisher's Exact test, p=0.014; Spearman's nonparametric correlation 0.34, p=0.037). However, it is doubtful whether combining the two outcome groups is statistically valid due to possible confounding by the group classification.

A positive correlation between absence of physiological changes and abnormal Doppler results has already been described in earlier studies (Lin et al. 1995, Voigt and Becker 1992). However, we found absence of physiological changes in a substantial number of normal pregnancies as well. In addition, complete or partial physiological changes were observed in 10 out of 24 biopsies from pregnancies complicated by pre-eclampsia, SGA fetuses, and the HELLP-syndrome.

Pathological changes were seen in the majority of biopsies in both normal and complicated pregnancies. This high prevalence is in contradiction with earlier studies (Meekins 1994). Our inclusion of "mild" pathological changes (based on morphological characteristics such as foam cells and mucoid changes) could explain this difference. This specific category of pathological changes has not been described before and its significance could be doubted. We feel, however, that these changes represent the earlier stages of acute atherosis and are therefore relevant. We were surprised by the presence of pathological changes in normal pregnancy, and particularly by the fact that pathological changes in normal pregnancies did not always fall into the "mild" category. Ten biopsies showed severe pathological changes, four of which came from normal pregnancies.
Pathological changes, mild as well as severe, were observed in biopsies with complete physiological changes, although they were more common in biopsies with absent or partial physiological changes. This finding is in agreement with the results from the studies by Pijnenborg et al. (1991) and by Meekins et al. (1994) and contradicts the earlier belief that acute atherosis only occurs in vessels without physiological changes. In contrast to physiological changes, pathological changes do not appear to be associated with abnormal Doppler flow. To our knowledge, this study is the first to investigate the relationship between Doppler flow and pathological changes as a separate histopathological entity.

How do these findings fit within the prevailing theory concerning the pathophysiology of pre-eclampsia? Evidently, physiological adaptation is not an all-or-none phenomenon, a finding in agreement with observations previously reported by Meekins et al (1994). Rather, it varies between one spiral artery and another in the same patient as well as even within one spiral artery. In most biopsies, only one or two spiral arteries are present; it is therefore probable that some degree of sampling error exists. There may also have been a sample bias due to the small number of subjects (alpha error).

Another explanation, in concordance with the theory presented by Ness and Roberts (1996) and Roberts and Hubel (1999), is the presence of other (maternal) factors responsible for increases of uteroplacental vascular resistance independently of impaired spiral artery adaptation. Possible mechanisms include vasospasm due to increased endothelial reactivity (Roberts et al. 1989) and hyperhomocysteinemia and thrombofilias (Dekker et al. 1995; Kupferminc et al. 1999).

The relevance of (mild) pathological changes in spiral arteries remains to be elucidated. Pathological changes appear to be common in complicated as well as normal pregnancies. It should be remembered, however, that in the present study the biopsies from the complicated pregnancies were obtained at an average gestational age of 32 weeks, whereas the biopsies from controls were obtained at
term. “Pathological” changes such as shown in figure 3 may be more or less normal at term (Meekins 1994), just as a certain degree of placental infarction is considered normal in term placentas. It is conceivable that pathological changes occur earlier in complicated pregnancies compared to normal pregnancies.

In the present study, uterine artery PI’s were recorded in complicated third trimester pregnancies after the onset of clinical symptoms, and comparisons cannot be readily made with second trimester screening studies. However, the findings of the present study provide an explanation for the disappointing results of Doppler screening studies in predicting pre-eclampsia. Absence of physiological changes alone may not be sufficient to impair uteroplacental blood flow. We found normal Doppler flow with absent physiological changes in five normal pregnancies. We believe that other factors may be important in increasing vascular resistance. These factors may occur later in pregnancy, past the stage where screening studies take place. The early occurrence of pathological changes, which are more or less normal in term pregnancies, could play a role. The early occurrence of atherosis could be related to maternal thrombophilia or hyperhomocysteinemia (Dekker et al. 1995; Kupferminc et al. 1999). However, we did not find an association between pathological changes and Doppler results. Another possibility is vascular spasm due to endothelial damage and/or vasoactive factors such as endothelin which would result in increased vascular tone but could not be traceable in biopsies.

In conclusion, the association of pregnancy complications with increased uteroplacental resistance as indicated by abnormal Doppler flow can not be solely explained by abnormal uteroplacental vessel histopathology. Our findings indicate that impaired physiological adaptation of the spiral arteries is not the single causal factor in pre-eclampsia, and thereby support the concept of heterogeneous caused of pre-eclampsia as recently proposed by Ness and Roberts (1996). The frequent finding of pathological changes in normal as well as in complicated pregnancies needs to be further elucidated, in particular to find out whether these changes appear earlier in the latter group. Finally, the general concept that these
pathological changes solely occur in spiral arteries with lacking or impaired physiological adaptation has to be revised in view of the present observations.

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