Endothelial dysfunction and vascular stiffness in women with a previous pregnancy complicated by early or late pre-eclampsia

ENDOTHELIAL DYSFUNCTION AND VASCULAR STIFFNESS IN WOMEN WITH A PREVIOUS PREGNANCY COMPLICATED BY EARLY OR LATE PRE-ECLAMPSIA

Endothelium and PE

Rossana Orabona[1], MD; Edoardo Sciatti[2], MD; Enrico Vizzardi[2], MD; Ivano Bonadei[2], MD; Adriana Valcamonico[1], MD; Marco Metra[2], MD; Tiziana Frusca[1,3], MD

[1]Maternal Fetal Medicine Unit, Department of Obstetrics and Gynecology, University of Brescia, Italy

[2]Section of Cardiovascular Diseases, Department of Medical and Surgical Specialties, Radiological Sciences and Public Health, University of Brescia, Italy

[3]Department of Obstetrics and Gynecology, University of Parma, Italy

Corresponding Author:

Dr. Rossana Orabona, MD

Piazzale Spedali Civili 1, 25123 Brescia, Italy

Tel: +390303995679; Fax: +390303996801

Mail: oraroxy@libero.it

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ABSTRACT

Objectives: Pre-eclampsia leads to an increased cardiovascular risk later in life. The persistence of endothelial dysfunction after delivery may represent the link between pre-eclampsia and cardiovascular disease. We aimed at evaluating endothelial function and arterial stiffness after pregnancies complicated by early-onset or late-onset pre-eclampsia and their correlation with gestational age and mean uterine artery pulsatility index both considered at the diagnosis of pre-eclampsia and birth weight percentile.

Methods: 30 women who experienced early-onset pre-eclampsia, 30 with a previous late-onset pre-eclampsia and 30 controls were recalled from 6 months to 4 years after delivery. All women included were free from cardiovascular risk factors and drugs. We studied them by peripheral arterial tonometry and pulse wave analysis.

Results: All vascular parameters were ~~all~~ significantly impaired in early-onset pre-eclampsia. Late-onset pre-eclampsia showed higher vascular rigidity than controls' and normal values of reactive hyperaemia index, although it was significantly lower in respect with controls'. On the multivariate analysis gestational age and mean uterine artery pulsatility index, both considered at the diagnosis of the disease, and birth weight percentile were statistically related to the vascular indexes we studied, after correcting for confounding parameters.

Conclusions: Women with previous pregnancies complicated by pre-eclampsia, in particular cases with early-onset of the disease, showed a persistent microcirculatory dysfunction, as suggested by a significant reduction of reactive hyperaemia index value, and an increased arterial stiffness.

Keywords: arterial stiffness – cardiovascular risk – EndoPAT – endothelial dysfunction – pre-eclampsia – pulse wave velocity.

INTRODUCTION

Evidence in Literature suggests that women who experienced pre-eclampsia (PE) are at increased risk of hypertension, coronary artery disease and fatal stroke later in their life. [1] It might be due to maternal systemic endothelial dysfunction sprouting out from an oxidatevely stressed placenta. [2] Although previous investigations demonstrated the relationship between endothelial dysfunction and PE, [3,4] the majority of them have been focused on brachial artery ultrasound (BAUS), which is considered the gold standard for evaluating endothelial function measuring the flow mediated dilatation (FMD). Being highly operator-dependent it requires a specific training. Among non-invasive methods, peripheral arterial tonometry (PAT) is a more reproducible technique for endothelial function detection. It has been validated and previously used to assess peripheral arterial tone. [5-9]

In previously preeclamptics endothelial dysfunction may result in arterial stiffness [10-12] which describes the reduced arterial capability to expand and contract according to pressure changes. It is a key-point in cardiovascular (CV) risk profile evaluation because of its direct and independent association with CV risk. [13]

This study aimed to evaluate endothelial function using PAT and arterial stiffness in women with a history of early-onset (EO) or late-onset (LO) PE from 6 months to 4 years after delivery and whether vascular status was related to gestational age (GA) and mean uterine artery pulsatility index (UtA PI) both considered at the diagnosis of PE and birth weight percentile.

METHODS

Subjects:

This was a prospective single-center case-control study in compliance with the Declaration of Helsinki and approved by the local ethical committee. Results are reported following the STROBE

guidelines. We retrospectively searched our electronic database for 30 consecutive women with a previous diagnosis of EOPE and 30 with a prior LOPE treated at the Maternal Fetal Medicine Unit of the Department of Obstetrics and Gynecology, University of Brescia, Italy. EOPE and LOPE were defined using 34 weeks' gestation as cut-off. 30 healthy women, matched for age, body mass index (BMI), parity, and without CV risk factors were used as controls. All of them were recalled by phone between 6 months and 4 years after delivery to assess their eligibility and received a single appointment at the Cardiology Unit, University of Brescia, Italy, to undergo peripheral blood pressure measurement, endothelial function evaluation and vascular stiffness assessment. We paid attention to perform these exams in the same temperature-controlled room and during a morning after a fasting night. All women gave their written informed consent. According to the International Society for the Study of Hypertension in Pregnancy (ISSHP), PE was defined as a blood pressure of at least 140/90 mmHg, on two occasions 4-6 hours apart, after the 20th week of gestation in previously normotensive women, accompanied by proteinuria ≥ 300 mg/24 hours. [14]

Small for gestational age (SGA) newborn was considered as a birth weight percentile < 10th percentile for that GA. Intrauterine growth restriction (IUGR) was defined as fetal abdominal circumference < 10th percentile according to local standards [15] and abnormal umbilical artery PI > 95th percentile based on local standards, irrespective of the presence of absent or reversed end-diastolic flow.

None of the women we included had any of the following CV risk factors: smoke habit, dyslipidemia, overweight, diabetes mellitus, or chronic hypertension. We confirmed them analyzing patients' medical charts before and after pregnancy. Cited CV risk factors were defined according to accepted Italian and European guidelines. All the study population was subject to other exclusion criteria: multiple pregnancies, chromosomopathies or foetal malformations, maternal cardiopathies or immune disorders, PE superimposed on chronic hypertension and pregestational renal disease. The whole study cohort showed normal blood pressure values and absence of any pathologic

proteinuria 6 months after delivery. Data regarding demographic and clinical characteristics during pregnancy were collected from obstetrical charts of all studied women.

Blood pressure during pregnancy, 6 months after delivery and at CV evaluation was assessed using a standard, calibrated, electronic sphygmomanometer (OMRON Healthcare, Hoofddorp, The Netherlands). It automatically inflates the cuff till over-systolic pressure and then deflates gradually, recording blood pressure by means of an oscillometric technology. During blood pressure measurement, the woman was relaxed and sitting at a 45-degree angle using an appropriate cuff size placed at the level of the heart. The mean of three blood pressure was recorded. The arm in which the highest sitting diastolic pressures was found was the arm used for all subsequent readings throughout the study. Every effort was made to have the same staff member obtain blood pressure measurements in each individual patient, at the same time of day, using the same equipment. Mean arterial pressure (MAP) was calculated as (SBP + 2*DBP)/3, being SBP and DBP systolic and diastolic blood pressures, respectively.

Endothelial function assessment:

PAT signals were obtained using the EndoPAT-2000 device (Itamar Medical Ltd., Caesarea, Israel), which has been validated and used previously to assess peripheral arterial tone in other populations. [5-9] Specially designed finger probes are placed on the middle finger of each subject's hands. These probes comprised a system of inflatable latex air cuffs connected by pneumatic tubes to an inflating device controlled through a computer algorithm. A constant counter pressure (pre-determined by baseline DBP) is applied through the air cushions. This prevents venous pooling, thus avoiding veno-arteriolar reflex vasoconstriction. There is no occlusion of arterial blood flow. Pulsatile volume changes of the distal digit induce pressure alterations in the finger cuff, which are sensed by pressure transducers and transmitted to and recorded by the EndoPAT-2000 device. A decrease in the arterial blood volume in the distal fingertip causes a decrease in pulsatile arterial column changes, reflected as a decrease in the measured PAT signal, and vice versa. Endothelial

function is measured via a reactive hyperemia (RH) protocol: it consists of a 5 min baseline measurement, after which a blood pressure cuff on the test arm is inflated to 60 mmHg above baseline SBP or at least 200 mmHg for 5 min. Occlusion of pulsatile arterial flow is confirmed by the reduction of the PAT tracing to zero. After 5 min, the cuff is deflated, and the PAT tracing is recorded for a further 5 min. The ratio of the PAT signal after cuff release compared with baseline is calculated through a computer algorithm automatically normalizing for baseline signal and indexed to the contra lateral arm. The calculated ratio is called RH-PAT index or RH index (RHI). Its normal value is > 2.00 (pure number), while a clear endothelial dysfunction is showed by a value ≤ 1.67. Values between 1.67 (excluded) and 2.00 (included) are still of unclear significance. At the same time, the software calculates peripheral augmentation index (AIx), which is a measure of arterial stiffness (see below). In particular, since this parameter is influenced by heart rate, it is automatically standardized per 75 bpm (AIx@75). According to EndoPAT software peripheral AIx@75 is normal when < 17%. Typical examples of normal and altered EndoPAT traces are shown in Figure 1.

Pulse wave analysis (PWA):

Central BP was measured by arterial tonometry, using Vascular Explorer (Enverdis GmbH, Jena, Germany); it calculates aortic stiffness parameters from oscillatory recorded pressure waves of the brachial and posterior tibial arteries and from body surface measurements (brachial-jugular, jugular-pubis symphysis, jugular-ankle). Using inflatable upper and lower arm cuffs with high fidelity sensors, pulsatile volume changes (resulting from pulsatile fluctuations of the two arteries) are transduced into pressure curves. Pulse waves are recorded when the two arteries are completely occluded at a cuff pressure of 35-40 mmHg above SBP. A dedicated computer program is used to further analyze the recorded pulse waves. Pulse transit time (PTT) is determined from the decomposition of the general aortic pressure wave using the reflection method. This measurement is based on the fact that the forward traveling pulse wave (generated by the ejection of the left

ventricle) is reflected in the periphery creating a second reflected wave. PTT is determined by the foot-foot difference in time between the forward and the beginning of the reflected pressure wave (reflection method under brachial stop/flow conditions), and aortic pulse wave velocity (PWV) is automatically calculated from PTT and travelling distance between jugulum (sternal notch) and pubis symphysis, according to manufacture recommendations. Moreover, brachial-ankle PWV is registered by means of simultaneous cuff measurements taken on the upper arm and ankle at DBP. Then, carotid-femoral PWV (cfPWV) is calculated from brachial-ankle and aortic ones. According to the European reference values and techniques, this number was multiplied for 0.8 as a correction factor for body surface distance measurements, and values > 9.6 m/s (0.8 * 12 m/s) are considered pathologic. [16]

Finally, another central haemodynamics parameter measuring arterials stiffness, the aortic AIx@75, is calculated from brachial pressure curves in combination with automated transfer algorithms. Manufacturers indications suggest that aortic AIx@75 is altered when ≥ 35%.

Statistical analysis:

All analyses were done using IBM SPSS Statistics 20 for Windows (SPSS, Inc., Chicago, IL). Continuous variables were visually tested for normality using Q-Q plots and represented by mean ± standard deviation, while categorical variables as frequency (n) and percentage of the sample. After Levene's test for homoscedasticity, Welch's unequal variances analysis of variance (ANOVA) was performed to analyze the difference between means for continuous variables (independent samples Welch's t-test if two groups), and Dunnett C test for post-hoc analysis. The χ^2 test was used for assessing differences between proportions. Bivariate Spearman's nonparametric correlations were calculated to assess the association between EndoPAT/PWA parameters and EOPE/LOPE, altered/normal mean UtA PI, SGA/non-SGA newborn (as dichotomic variables). Four multivariate linear regression analyses using "enter" method were run to test the independent ability of GA at the diagnosis of PE, mean UtA PI at diagnosis of the disease and birth weight percentile, all

considered as continuous variables (independent variables), to predict the previously cited vascular parameters, after correcting each variable for the others and for SBP/DBP at diagnosis of PE, Caesarean section and IUGR rates (the only variables which differed at Welch's t-test between EOPE and LOPE). Sample size calculation was adequate for all parameters with 85% power and a 5% Type I risk. For all statistical tests, probability values < 0.05 were considered significant.

RESULTS

In total, 288 cases of PE were found in our electronic database during the considered period, of which 41 EOPE and 57 LOPE were eligible for the study (Figure 2). Considering sample size calculation, cost effectiveness and available resources, only 60 cases (30 EOPE and 30 LOPE) were randomly chosen and definitely enrolled. Women with a previous uncomplicated pregnancy who delivered at our hospital in the same period were selected according to the exclusion criteria cited above and randomly included as controls. The whole study cohort was free from any medication at the time of assessment, including oral contraceptive.

Demographic and clinical characteristics of the study cohort are shown in Table 1 and Table 2. At 6 months-4 years after delivery, women belonging to EOPE group had significantly higher SBP ($p=0.007$), DBP ($p=0.003$) and MAP ($p=0.001$). In addition, EOPE had worse obstetrical and foetal data: higher blood pressures, more altered UtA PI at diagnosis of PE, more frequent Caesarean section and IUGR rates.

Table 3 shows EndoPAT and PWA data in the three groups. All PAT/PWA indexes were impaired significantly more in EOPE group than both LOPE one and controls. LOPE group showed higher vascular rigidity than controls but normal values of RHI, although it was significantly lower if compared to them.

At diagnosis of PE, we found an altered UtA PI in 37 patients out of 49 (75.5%). Considering PAT/PWA data in relation to UtA PI, only RHI ≤ 2.00 (p=0.04) and cfPWV*0.8 (p=0.03) were higher significantly more in those patients with an alteration of UtA Doppler velocimetry.

32 preeclamptic patients (53.3%) had a SGA newborn. AIx@75 alone was significantly higher in the SGA group (p=0.048).

The correlation between GA and mean UtA PI, both considered at the diagnosis of PE, and birth weight percentile and EndoPAT/PWA parameters was reported in Table 4. GA at the diagnosis of PE was directly correlated with all parameters except of RHI < 1.67 and RHI ≤ 2.00. An altered UtA PI correlated with RHI ≤ 2.00 (p=0.02), peripheral AIx@75 (p=0.02) and cfPWV*0.8 (p=0.03), while the presence of a SGA newborn was related to RHI ≤ 2.00 (p=0.04) and cfPVW*0.8 > 9.6 m/s (p=0.03). On the multivariate analysis (Table 5) ~~we found that~~ GA and mean UtA PI both considered at the diagnosis of PE ~~onset~~, and ~~the~~ birth weight percentile were statistically related to PAT/PWA indexes. These findings remained statistically significant even after excluding women with a time from delivery < 1 year (n=6).

Time from delivery to our visit was not statistically significant among the three groups, nor between EOPE and LOPE. Nevertheless we evaluated the changes of the values of PAT/PWA indexes according to time between delivery and cardiologic evaluation. We resumed these relationships in Figure 3.

DISCUSSION

Pregnancy itself leads to a maternal CV adaptation characterized by a high cardiac output and low systemic vascular resistance, in order to response to the increased metabolic demands. [17,18] In cases complicated by PE, arterial compliance is reduced and vascular stiffness increased. [19-21] Playing a key role in the pathogenesis of PE [2], endothelial dysfunction may represent a link between

placentation defects and CV risk later in life. [22] The acute atherosis, which characterizes the spiral arteries during PE, [23,24] is similar to the early stages of atherosclerosis (type I and II lesions, according to the American Heart Association [25]). Endothelial dysfunction seems to be related to a poor placentation with retained smooth muscle cells into the vascular walls of the placental bed, vasoconstriction, hypoxic-ischemic damage and oxidative stress. [2] Using PAT, we found a significant microcirculatory impairment in the EOPE group compared with the LOPE one and matched controls. Women who experienced LOPE showed a normal RHI value although it was significantly lower than controls'. Several studies have hypothesized the persistence of endothelial dysfunction in previously preeclamptics. In particular, they extensively showed an endothelium-dependent reduction of FMD, [4,26-30] which is actually considered the gold standard in endothelial function assessment. On the PAT technique only two studies have been reported in Literature. [31,32] Among these, Carty *et al* [31] did not observe any difference in RHI value between women who have had hypertensive and normotensive pregnancies. However they did not make a differentiation between PE and gestational hypertension (27 women at all) and their mean GA at delivery was 39.9 weeks' gestation which is consistent with LOPE. Our data extended to the postpartum those of Yinon *et al*: [32] they showed a reduced RHI during PE compared with controls. Our study is the first to clearly applying PAT technique at short-medium term after preeclamptic pregnancies. Its strength is the complete absence of CV risk factors in all the study cohort. EndoPAT is a more recent technique than BAUS. Its most important advantage is the quite absolute operator-independence. In addition, it is easy-to-use and does not need any training process. [33] On the other hand it is more expensive than BAUS because its finger probes need to be disposed after use. According to several Authors, EndoPAT and BAUS do not fully correlate each other [34] because they focus on different vascular beds: microcirculation the first, muscular arteries the second. EndoPAT is associated with Framingham CV risk factors [34,35] and it predicts future CV events [34,36-38] better than Reynolds Risk Score (which is Framingham risk score plus high-sensitivity C-reactive protein). [39]

Arterial stiffness plays a major role in hypertensive disorders, being both cause and effect of high blood pressure. In the context of pregnancy-induced hypertension there is significant evidence that arterial stiffness precedes the clinical phase of the disease both early in pregnancy and even prior to pregnancy in women at risk. [19-21] According to other Authors, [28,40] our data suggested that aortic stiffness, as quantified by cfPWV, is increased significantly more in women with previous EOPE if compared with the LOPE group and controls, except of aortic AIx@75 ≥ 35% which was altered also in LOPE. Aortic stiffness is largely predictive of future CV events. [41-43] Recently its additional value to define CV profile has been consolidated [41,42,44,45] and it has been included in the European Society of Cardiology/European Society of Hypertension guidelines. [46]

PAT/PWA indexes were related to GA at diagnosis of PE, mean UtA PI assessed at the same time and birth weight percentile suggesting that these factors predicted not only an adverse pregnancy outcome both also a subsequent development of maternal CV impairment. These findings agree and extend what already known about this topic, particularly regarding arterial stiffening. [47-49]

It is known that pregnancy transiently improves vascular compliance. This phenomenon seems to persist few years after delivery. [50-52] However, these findings are based on a slightly reduction of MAP in a subsequent pregnancy. Albeit our study was not designed for this issue, we directly evaluated microcirculation and arterial stiffness in a wide time-span from delivery and did not find any difference between short and medium term assessment. We acknowledge that the small number of patients involved may hamper our findings. Nevertheless the persistence of an inflammatory response many years after PE has been demonstrated through blood samples; [53] our data seem to go in the same direction.

The present study suffers from some limitations. We lack a preconceptional endothelial function evaluation in order to clarify whether the alterations we found are consequences of PE or the first clinical expression of a maternal predisposition to endothelial dysfunction already present before pregnancy. EndoPAT limitations have been discussed above. Third, the small number of patients

involved, which prevented us to run more complete multivariate regressions, although the study is powered enough to its aim. Fourth, the time range of the study (6 months to 4 years after delivery) can appear too wide; we chose 6 months as inferior cut-off because we wanted to be sure that both proteinuria and hormonal alterations typical of pregnancy have normalized. We found the same alterations both 6 months and 4 years after delivery. Lastly, the method we used to evaluate arterial stiffness has not been fully validated; nevertheless a system operating on the same mechanism, namely Vicorder, has been compared with the other most used technologies and no differences has been found among them in quantifying arterial stiffness. [54]

In conclusion, in previously preeclamptics in particular in cases with early-onset of the disease, a microcirculatory dysfunction seems to persist, as suggested by a significant reduction of RHI value, and an increased arterial stiffness. These findings are associated with higher CV risk later in their life, because of an independent relationship between RHI/arterial stiffness and multiple traditional CV risk factors [35] and their predictive ability of future events. [36,37-39,41-45]

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REFERENCES

1. Irgens HU, Reisaeter L, Irgens LM, Lie RT. Long term mortality of mothers and fathers after pre-eclampsia: population based cohort study. *BMJ* 2001; **323** : 1213-1217.

2. Borzychowski AM, Sargent IL, Redman CW. Inflammation and pre-eclampsia. *Semin Fetal Neonatal Med* 2006; **11** : 309-316.

3. Cockell AP, Poston L. Flow-mediated vasodilatation is enhanced in normal pregnancy but reduced in preeclampsia. *Hypertension* 1997; **30** : 247–251.

4. Chambers JC, Fusi L, Malik IS, Haskard DO, De Swiet M, Kooner JS. Association of maternal endothelial dysfunction with preeclampsia. *JAMA* 2001; **285** : 1607–1612.

5. Goor DA, Sheffy J, Schnall RP, Arditti A, Caspi A, Bragdon EE, Sheps DS. Peripheral arterial tonometry: a diagnostic method for detection of myocardial ischemia induced during mental stress tests: a pilot study. *Clin Cardiol* 2004; **27** : 137–141.

6. Bonetti PO, Barsness GW, Keelan PC, Schnell TI, Pumper GM, Kuvin JT, Schnall RP, Holmes DR, Higano ST, Lerman A. Enhanced external counterpulsation improves endothelial function in patients with symptomatic coronary artery disease. *J Am Coll Cardiol* 2003; **41** : 1761–1768.

7. Bonetti PO, Lerman LO, Lerman A. Endothelial dysfunction: a marker of atherosclerotic risk. *Arterioscler Thromb Vasc Biol* 2003; **23** : 168–175.

8. Lavie P, Shlitner A, Sheffy J, Schnall RP. Peripheral arterial tonometry: a novel and sensitive non-invasive monitor of brief arousals during sleep. *Isr Med Assoc J* 2000; **2** : 246–247.

9. Halligan SC, Murtagh B, Lennon RJ, Pumper GM, Mathew V, Higano ST, Lerman A. Effect of long-term hormone replacement therapy on coronary endothelial function in postmenopausal women. *Mayo Clin Proc* 2004; **79** : 1514–1520.

10. Elvan-Taspinar A, Franx A, Bots ML, Bruinse HW, Koomans HA. Central hemodynamics of hypertensive disorders in pregnancy. *Am J Hypertens* 2004; **17** : 941–946.

11. Tihtonen KMH, Koobi T, Uotila JT. Arterial stiffness in preeclamptic and chronic hypertensive pregnancies. *Eur J Obstet Gynecol Reprod Biol* 2006; **128** : 180-186.

12. Kaihura C, Savvidou MD, Anderson JM, McEniery CM, Nicolaides KH. Maternal arterial stiffness in pregnancies affected by preeclampsia. *Am J Physiol Heart Circ Physiol* 2009; **297** : H759–H764.

13. Hausvater A, Giannone T, Sandoval YH, Doonan RJ, Antonopoulos CN, Matsoukis IL, Petridou ET, Daskalopoulou SS. The association between preeclampsia and arterial stiffness. *J Hypertens* 2012; **30** : 17–33.

14. Brown MA, Lindheimer MD, de Swiet M, van Assche A, Moutquin JM. The classification and diagnosis of hypertensive disorders of pregnancy: Statement from the International Society for the Study of Hypertension in Pregnancy. *Hypertens Pregnancy* 2001; **20** : IX-XIV.

15. Todros T, Ferrazzi E, Groli C, Nicolini U, Parodi L, Pavoni M, Zorzoli A, Zucca S. Fitting growth curves to head and abdomen measurements of the fetus; a multicentric study. *J Clin Ultrasound* 1987; **15** : 95–105.

16. Mattace-Raso F, Hofman A, Verwoert GC, Wittemana JC, Wilkinson I, Cockcroft J, McEniery C, Yasmin, Laurent S, Boutouyrie P, Bozec E, Hansen TW, Torp-Pedersen C, Ibsen H, Jeppesen J, Vermeersch SJ, Rietzschel E, De Buyzere M, Gillebert TC, Van Bortel L, Segers P, Vlachopoulos C, Aznaouridis C, Stefanadis C, Benetos A, Labat C, Lacolley P, Stehouwer C, Nijpels G, Dekker JM, Stehouwer C, Ferreira I, Twisk JW, Czernichow S, Galan P, Hercberg S, Pannier B, Guérin A, London G, Cruickshank JK, Anderson SG, Paini A, Agabiti Rosei E, Muiesan ML, Salvetti M, Filipovsky J, Seidlerova J, Dolejsova M. Reference Values for Arterial Stiffness' Collaboration. Determinants of pulse wave velocity in healthy people and in the presence of cardiovascular risk factors: 'establishing normal and reference values'. *Eur Heart J* 2010; **31** : 2338-2350.

17. Clapp JF 3rd, Capeless E. Cardiovascular function before, during, and after the first and subsequent pregnancies. *Am J Cardiol* 1997; **80** : 1469-1473.

18. Morris EA, Hale SA, Badger GJ, Magness RR, Bernstein IM. Pregnancy induces persistent changes in vascular compliance in primiparous women. *Am J Obstet Gynecol* 2015; **212** : 633.e1-6.

19. Savvidou MD, Kaihura C, Anderson JM, Nicolaides KH. Maternal arterial stiffness in women who subsequently develop pre-eclampsia. *PLoS One* 2011; **6** : e18703.

20. Hale S, Choate M, Schonberg A, Shapiro R, Badger G, Bernstein IM. Pulse pressure and arterial compliance prior to pregnancy and the development of complicated hypertension during pregnancy. Reprod Sci 2010 ; **17** : 871-877.

21. Hale SA, Badger GJ, McBride C, Magness R, Bernstein IM. Prepregnancy Vascular Dysfunction in Women who Subsequently Develop Hypertension During Pregnancy. *Pregnancy Hypertens* 2013; **3** : 140-145.

22. Roberts JM, Redman CW. Pre-eclampsia: more than pregnancy-induced hypertension. *Lancet* 1993; **341** : 1447–1451.

23. Staff AC, Dechend R, Pijnenborg R. Learning from the placenta: acute atherosis and vascular remodeling in preeclampsia-novel aspects for atherosclerosis and future cardiovascular health. *Hypertension* 2010; **56** : 1026-1034.

24. Staff AC, Dechend R, Redman CW. Review: Preeclampsia, acute atherosis of the spiral arteries and future cardiovascular disease: two new hypotheses. *Placenta* 2013; **34 Suppl** : S73-78.

25. Stary HC, Chandler AB, Glagov S, Guyton JR, Insull Jr W, Rosenfeld ME, Schaffer SA, Schwartz CJ, Wagner WD, Wissler RW. 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* 1994; **89** : 2462-78.

26. Agatisa PK, Ness RB, Roberts JM, Costantino JP, Kuller LH, McLaughlin MK. Impairment of endothelial function in women with a history of preeclampsia: an indicator of cardiovascular risk. *Am J Physiol Heart Circ Physiol* 2004; **286** : H1389–H1393.

27. Hamad RR, Eriksson MJ, Silveira A, Hamsten A, Bremme K. Decreased flow-mediated dilation is present 1 year after a preeclamptic pregnancy. *J Hypertens* 2007; **25** : 2301–2307.

28. Germain AM, Romanik MC, Guerra I, Solari S, Reyes MS, Johnson RJ, Price K, Karumanchi SA, Valdés G. Endothelial dysfunction: a link among preeclampsia, recurrent pregnancy loss, and future cardiovascular events? *Hypertension* 2007; **49** : 90-95.

29. Lampinen KH, Rönnback M, Kaaja RJ, Groop PH. Impaired vascular dilatation in women with a history of pre-eclampsia. *J Hypertens* 2006; **24** : 751-756.

30. Yinon Y, Kingdom JC, Odutayo A, Moineddin R, Drewlo S, Lai V, Cherney DZ, Hladunewich MA. Vascular dysfunction in women with a history of preeclampsia and intrauterine growth restriction: insights into future vascular risk. *Circulation* 2010; **122** : 1846-1853.

31. Carty DM, Anderson LA, Duncan CN, Baird DP, Rooney LK, Dominiczak AF, Delles C. Peripheral arterial tone: assessment of microcirculatory function in pregnancy. *J Hypertens* 2012; **30** : 117-123.

32. Yinon D, Lowenstein L, Suraya S, Beloosesky R, Zmora O, Malhotra A, Pillar G. Preeclampsia is associated with sleep-disordered breathing and endothelial dysfunction. *Eur Respir J* 2006; **27** : 328–333.

33. Vizzardi E, Gavazzoni M, Della Pina P, Bonadei I, Regazzoni V, Sciatti E, Trichaki E, Raddino R, Metra M. Noninvasive assessment of endothelial function: the classic methods and the new peripheral arterial tonometry. *J Investig Med* 2014; **62** : 856-864.

34. Hamburg NM, Palmisano J, Larson MG, Sullivan LM, Lehman BT, Vasan RS, Levy D, Mitchell GF, Vita JA, Benjamin EJ. Relation of brachial and digital measures of vascular function in the community: the Framingham heart study. *Hypertension* 2011; **57** : 390-396.

35. Hamburg NM, Keyes MJ, Larson MG, Vasan RS, Schnabel R, Pryde MM, Mitchell GF, Sheffy J, Vita JA, Benjamin EJ. Cross-sectional relations of digital vascular function to cardiovascular risk factors in the Framingham Heart Study. *Circulation* 2008; **117** : 2467–2474.

36. Rubinshtein R, Kuvin JT, Soffler M, Lennon RJ, Lavi S, Nelson RE, Pumper GM, Lerman LO, Lerman A. Assessment of endothelial function by non-invasive peripheral arterial tonometry predicts late cardiovascular adverse events. *Eur Heart J* 2010; **31** : 1142-1148.

37. Matsuzawa Y, Sugiyama S, Sumida H, Sugamura K, Nozaki T, Ohba K, Matsubara J, Kurokawa H, Fujisue K, Konishi M, Akiyama E, Suzuki H, Nagayoshi Y, Yamamuro M, Sakamoto K, Iwashita S, Jinnouchi H, Taguri M, Morita S, Matsui K, Kimura K, Umemura S, Ogawa H. Peripheral endothelial function and cardiovascular events in high-risk patients. *J Am Heart Assoc* 2013; **2** : e000426.

38. Xu Y, Arora RC, Hiebert BM, Lerner B, Szwajcer A, McDonald K, Rigatto C, Komenda P, Sood MM, Tangri N. Non-invasive endothelial function testing and the risk of adverse outcomes: A systematic review and meta-analysis. *Eur Heart J Cardiovasc Imaging* 2014; **15** : 736-746.

39. Matsuzawa Y, Sugiyama S, Sugamura K, Nozaki T, Ohba K, Konishi M, Matsubara J, Sumida H, Kaikita K, Kojima S, Nagayoshi Y, Yamamuro M, Izumiya Y, Iwashita S, Matsui K, Jinnouchi H, Kimura K, Umemura S, Ogawa H. Digital assessment of endothelial function and ischemic heart disease in women. *J Am Coll Cardiol* 2010; **55** : 1688-1696.

40. Khalil A, Jauniaux E, Harrington K. Antihypertensive therapy and central hemodynamics in women with hypertensive disorders in pregnancy. *Obstet Gynecol* 2009; **113** : 646-654.

41. Sehestedt T, Jeppesen J, Hansen TW, Wachtell K, Ibsen H, Torp-Pedersen C, Hildebrandt P, Olsen MH. Risk prediction is improved by adding markers of subclinical organ damage to SCORE. *Eur Heart J* 2010; **31** : 883–891.

42. Mattace-Raso FU, van der Cammen TJ, Hofman A, van Popele NM, Bos ML, Schalekamp MA, Asmar R, Reneman RS, Hoeks AP, Breteler MM, Witteman JC. Arterial stiffness and risk of coronary heart disease and stroke: the Rotterdam Study. *Circulation* 2006; **113** : 657–663.

43. Mitchell GF, Hwang SJ, Vasan RS, Larson MG, Pencina MJ, Hamburg NM, Vita JA, Levy D, Benjamin EJ. Arterial stiffness and cardiovascular events: the Framingham Heart Study. *Circulation* 2010; **121** : 505–511.

44. Sehestedt T, Jeppesen J, Hansen TW, Rasmussen S, Wachtell K, Ibsen H, Torp-Pedersen C, Olsen MH. Thresholds for pulse wave velocity, urine albumin creatinine ratio and left ventricular mass index using SCORE, Framingham and ESH/ESC risk charts. *J Hypertens* 2012; **30** : 1928–1936.

45. Boutouyrie P, Tropeano AI, Asmar R, Gautier I, Benetos A, Lacolley P, Laurent S. Aortic stiffness is an independent predictor of primary coronary events in hypertensive patients: a longitudinal study. *Hypertension* 2002; **39** : 10–15.

46. Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Böhm M, Christiaens T, Cifkova R, De Backer G, Dominiczak A, Galderisi M, Grobbee DE, Jaarsma T, Kirchhof P, Kjeldsen SE, Laurent S, Manolis AJ, Nilsson PM, Ruilope LM, Schmieder RE, Sirnes PA, Sleight P, Viigimaa M, Waeber B, Zannad F, Redon J, Dominiczak A, Narkiewicz K, Nilsson PM, Burnier M, Viigimaa M, Ambrosioni E, Caufield M, Coca A, Olsen MH, Schmieder RE, Tsioufis C, van de Borne P, Zamorano JL, Achenbach S, Baumgartner H, Bax JJ, Bueno H, Dean V, Deaton C, Erol C, Fagard R, Ferrari R, Hasdai D, Hoes AW, Kirchhof P, Knuuti J, Kolh P, Lancellotti P, Linhart A, Nihoyannopoulos P, Piepoli MF, Ponikowski P, Sirnes PA, Tamargo JL, Tendera M, Torbicki A, Wijns W, Windecker S, Clement DL, Coca A, Gillebert TC, Tendera M, Rosei EA, Ambrosioni E, Anker SD, Bauersachs J, Hitij JB, Caulfield M, De Buyzere M, De Geest S, Derumeaux GA, Erdine S, Farsang C, Funck-Brentano C, Gerc V, Germano G, Gielen S, Haller H, Hoes AW, Jordan J, Kahan T, Komajda M, Lovic D, Mahrholdt H, Olsen MH, Ostergren J, Parati G, Perk J, Polonia J, Popescu BA, Reiner Z, Rydén L, Sirenko Y, Stanton A, Struijker-Boudier H, Tsioufis C, van de Borne P, Vlachopoulos C, Volpe M, Wood DA. 2013 ESH/ESC guidelines for the management of arterial hypertension: the Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *Eur Heart J* 2013; **34** : 2159-2219.

47. Prefumo F, Muiesan ML, Perini R, Paini A, Bonzi B, Lojacono A, Agabiti-Rosei E, Frusca T. Maternal cardiovascular function in pregnancies complicated by intrauterine growth restriction. *Ultrasound Obstet Gynecol* 2008; **31** : 65-71.

48. Spaanderman ME, Willekes C, Hoeks AP, Ekhart TH, Aardenburg R, Courtar DA, Van Eijndhoven HW, Peeters LL. Maternal nonpregnant vascular function correlates with subsequent fetal growth. *Am J Obstet Gynecol* 2005; **192** : 504-512.

49. Mongraw-Chaffin ML, Cirillo PM, Cohn BA. Preeclampsia and cardiovascular disease death: prospective evidence from the child health and development studies cohort. *Hypertension* 2010; **56** : 166-171.

50. Mikolajczyk RT, Zhang J, Ford J, Grewal J. Effects of interpregnancy interval on blood pressure in consecutive pregnancies. *Am J Epidemiol* 2008; **168** : 422–426.

51. Bernstein IM, Thibault A, Mongeon JA, Badger GJ. The influence of pregnancy on arterial compliance *Obstet Gynecol* 2005; **105** : 621–625.

52. Basso O, Christensen K, Olsen J. Higher risk of pre-eclampsia after change of partner. An effect of longer interpregnancy intervals? *Epidemiology* 2001; **12** : 624–629.

53. Östlund E, Al-Nashi M, Hamad RR, Larsson A, Eriksson M, Bremme K, Kahan T. Normalized endothelial function but sustained cardiovascular risk profile 11 years following a pregnancy complicated by preeclampsia. *Hypertens Res* 2013; **36** : 1081–1087.

54. Davies JM, Bailey MA, Griffin KJ, Scott DJ. Pulse wave velocity and the non-invasive methods used to assess it: Complior, SphygmoCor, Arteriograph and Vicorder. *Vascular* 2012; **20** : 342-349.

TABLES

Table 1. Clinical data of the study cohort at cardiovascular evaluation.

Variable	EOPE group (n=30)	LOPE group (n=30)	Controls (n=30)	p
Time from delivery (years)	2.3±0.7	2.5±0.8	2.2±0.6	0.1
Maternal age at assessment (years)	38±4	36±6	37±4	0.08
BMI (kg/m^2)	23.2±2.3	22.3±2.4	23.1±2.5	0.3
BSA (m^2)	1.68±0.14	1.67±0.12	1.62±0.08	0.1
SBP (mmHg)	125±13$^{\$}$	116±11	119±8	0.007
DBP (mmHg)	80±9*$^{\$}$	73±9	74±6	0.003
MAP (mmHg)	95±10*$^{\$}$	87±9	89±4	0.001
HR (bpm)	78±9	77±10	79±7	0.6

EOPE = early-onset preeclampsia; LOPE = late-onset preeclampsia; BMI = body mass index; BSA = body surface area; SBP = systolic blood pressure; DBP = diastolic blood pressure; MAP = mean artery pressure; HR = heart rate. * $p<0.05$ vs controls; $^{\#}$ $p<0.05$ vs controls; $^{\$}$ $p<0.05$ vs LOPE.

Table 2. Demographic and clinical data of the study population

Variable	EOPE group (n=30)	LOPE group (n=30)	Controls	p
Maternal age at delivery (years)	36±4	34±6	35±4	0.06
Parity (%): - nulliparity - primiparity - multiparity	 18 (60.0%) 9 (30.0%) 3 (10.0%)	 24 (80.0%) 3 (10.0%) 3 (10.0%)	 21 (70.0%) 6 (20.0%) 3 (10.0%)	0.1
GA at diagnosis of PE (weeks)	$27^{+5}\pm2^{+4}$	$36^{+4}\pm1^{+2}$	-	<0.001
Mean UtA PI at diagnosis of PE	1.56±0.39	1.13±0.43	-	0.001
SBP at diagnosis of PE (mmHg)	163±13	161±27	-	0.007
DBP at diagnosis of PE (mmHg)	117±33	104±8.0	-	0.003
Proteinuria (mg/24 h)	3258±926	3012±2618	-	0.8
GA at delivery (weeks)	$30^{+6+}\pm3^{+6}$ *	$37^{+1}\pm1^{+2\,\#}$	$39^{+1}\pm1^{+0}$	0.03
Caesarean section	30 (100.0%)*$	17 (56.7%)#	5 (16.7%)	<0.001
IUGR	23 (76.7%)	13 (43.3%)	-	<0.001
Male sex	15 (50.0%)	10 (33.3%)	17 (56.7%)	0.4
Birth weight (g)	928±539$	2483±561#	3315±485	<0.001
Birth weight percentile	14.1±20.7*	20.7±22.3#	48.0±21.9	0.02
Maternal complications (%) - HELLP syndrome - Eclampsia - Abruptio placentae - DIC	 - - 1 (3.3%) 1 (3.3%)	 - - - -	 - - - -	-

EOPE = early-onset preeclampsia; LOPE = late-onset preeclampsia; GA = gestational age; SBP = systolic blood pressure; DBP = diastolic blood pressure; UtA = uterine arteries; PI = pulsatility index; IUGR = intrauterine growth restriction; HELLP = haemolysis, elevated liver enzymes, low platelets; DIC = disseminated intravascular coagulation.

Table 3. EndoPAT and pulse wave analysis parameters divided according to gestational age at diagnosis of preeclampsia.

Variable	EOPE group (n=30)	LOPE group (n=30)	Controls (n=30)	p
RHI	1.70±0.42*$	2.51±0.49#	2.89±0.35	<0.001
RHI < 1.67	11 (36.7%)*$	0 (0.0%)	0 (0.0%)	<0.001
RHI ≤ 2.00	23 (76.7%)*$	2 (6.7%)	0 (0.0%)	<0.001
Peripheral AIx@75 (%)	17±19*$	6±13#	-2±6	<0.001
Peripheral AIx@75 ≥ 17%	12 (40.0%)*$	6 (20.0%)#	0 (0.0%)	<0.001
Aortic AIx@75 (%)	44.0±12.5*$	19.1±10.1	14.3±6.0	<0.001
Aortic AIx@75 ≥ 35%	7 (23.3%)*$	5 (16.7%)#	0 (0.0%)	<0.001
cfPWV*0.8 (m/s)	8.42±1.92*$	6.16±1.11	5.86±0.50	<0.001
cfPWV*0.8 > 9.6 m/s	3 (10.0%)*$	0 (0.0%)	0 (0.0%)	<0.001

EOPE = early-onset preeclampsia; LOPE = late-onset preeclampsia; RHI = reactive hyperemia index; AIx@75 = augmentation index corrected for 75 bpm; cfPWV = carotid-femoral pulse wave velocity. * $p<0.05$ vs controls; # $p<0.05$ vs controls; $ $p<0.05$ vs LOPE.

Table 4. Spearman's correlation between EndoPAT/pulse wave analysis parameters and early/late-onset preeclampsia, altered/normal mean uterine artery pulsatility index, small/appropriate for gestational age newborn. The analysis focused on preeclamptics alone.

Variable	EOPE/LOPE (n=60)		Altered/Normal UtA PI (n=49)		SGA/Non-SGA (n=60)	
	rho	p	rho	p	rho	p
RHI	-0.272	0.009	-0.118	0.4	-0.159	0.2
RHI < 1.67	0.061	0.6	0.252	0.08	0.145	0.3
RHI ≤ 2.00	<0.001	1	0.323	0.02	0.265	0.04
Peripehral AIx@75 (%)	0.243	0.02	0.353	0.02	0.238	0.08
Peripheral AIx@75 ≥ 17%	0.279	0.04	0.000	1	0.228	0.09
Aortic AIx@75 (%)	0.677	<0.001	0.221	0.2	0.044	0.8
Aortic AIx@75 ≥ 35%	0.504	0.001	0.141	0.4	0.022	0.9
cfPWV*0.8 (m/s)	0.542	<0.001	0.368	0.03	-0.142	0.4
cfPWV*0.8 > 9.6 m/s	0.493	0.001	0.221	0.2	0.349	0.03

EOPE = early-onset preeclampsia; LOPE = late-onset preeclampsia; SGA = small for gestational age; UtA = mean uterine arteries; PI = pulsatility index; RHI = reactive hyperemia index; AIx@75 = augmentation index corrected for 75 bpm; cfPWV = carotid-femoral pulse wave velocity

Table 5. Multivariate linear regressions between EndoPAT/pulse wave analysis parameters and gestational age at preeclampsia onset, mean uterine artery pulsatility index at diagnosis of the disease, birth weight percentile (considered as independent variables), after correcting for possible confounders (variables which differed between EOPE and LOPE in Table 2). The analysis focuses on preeclamptics alone.

Variable	**RHI**		**Peripheral AIx@75**		**Aortic AIx@75**		**cfPWV*0.8**	
	p	**β**	**p**	**β**	**p**	**β**	**p**	**β**
Gestational age	<0.001	0.966	<0.001	-0.198	<0.001	-0.996	<0.001	-1.281
Mean UtA PI	0.041	-0.282	<0.001	0.276	<0.001	0.737	<0.001	0.894
Birth weight percentile	0.036	0.162	0.001	-0.262	<0.001	-0.406	<0.001	-0.818
SBP	0.001	-1.110	<0.001	0.867	<0.001	0.121	<0.001	0.219
DBP	0.014	-0.618	<0.001	1.339	<0.001	0.307	<0.001	0.517
Caesarean section rate	0.998	0.000	0.005	0.107	<0.001	0.072	<0.001	0.384
IUGR	0.638	0.057	0.422	-0.030	<0.001	0.812	<0.001	0.656
Adjusted R^2	*0.724*		*0.862*		*0.505*		*0.497*	

RHI = reactive hyperemia index; AIx@75 = augmentation index corrected for 75 bpm; cfPWV = carotid-femoral pulse wave velocity; UtA = mean uterine artery; PI = pulsatility index.

FIGURE CAPTIONS

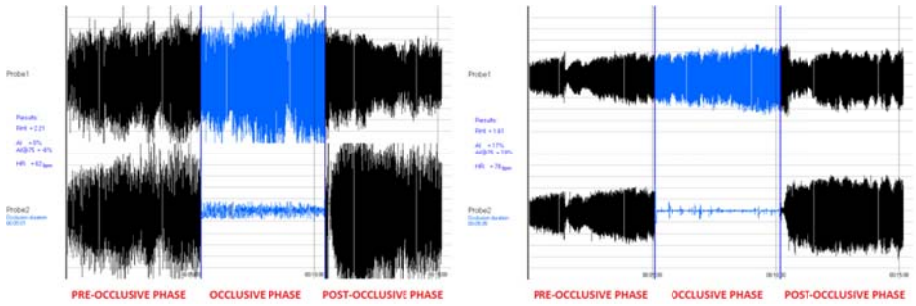

Figure 1. EndoPAT report: endothelial good function (left) and dysfunction (right). In this case (right), note the lower signal amplitude in the post-occlusive phase, that is similar to pre-occlusive phase, respect of good endothelial function (left) case.

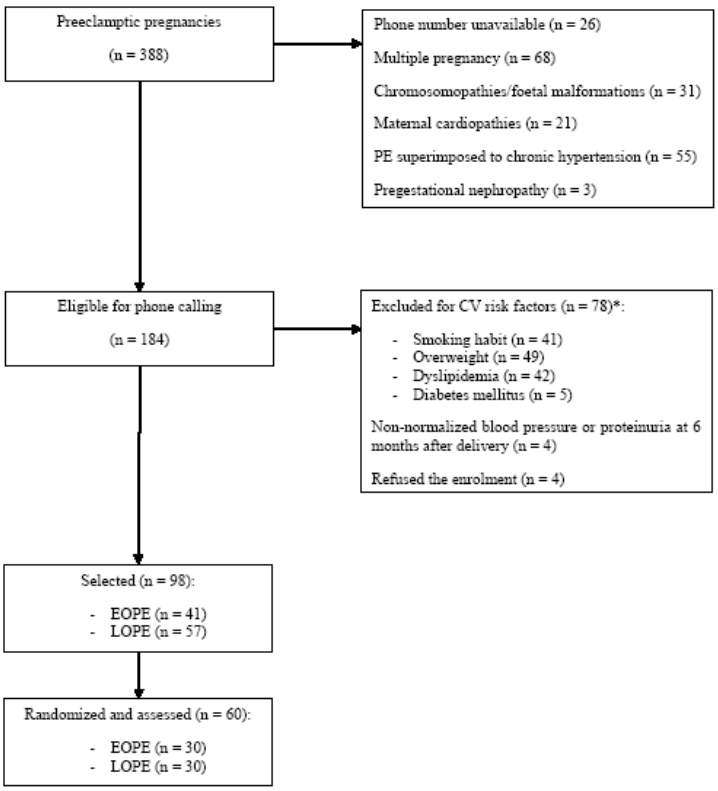

Figure 2. Flow chart of the preeclamptic pregnancies considered for the study (*58 subjects had more than one CV risk factor). CV = cardiovascular; EOPE = early-onset preeclampsia; LOPE = late-onset preeclampsia.

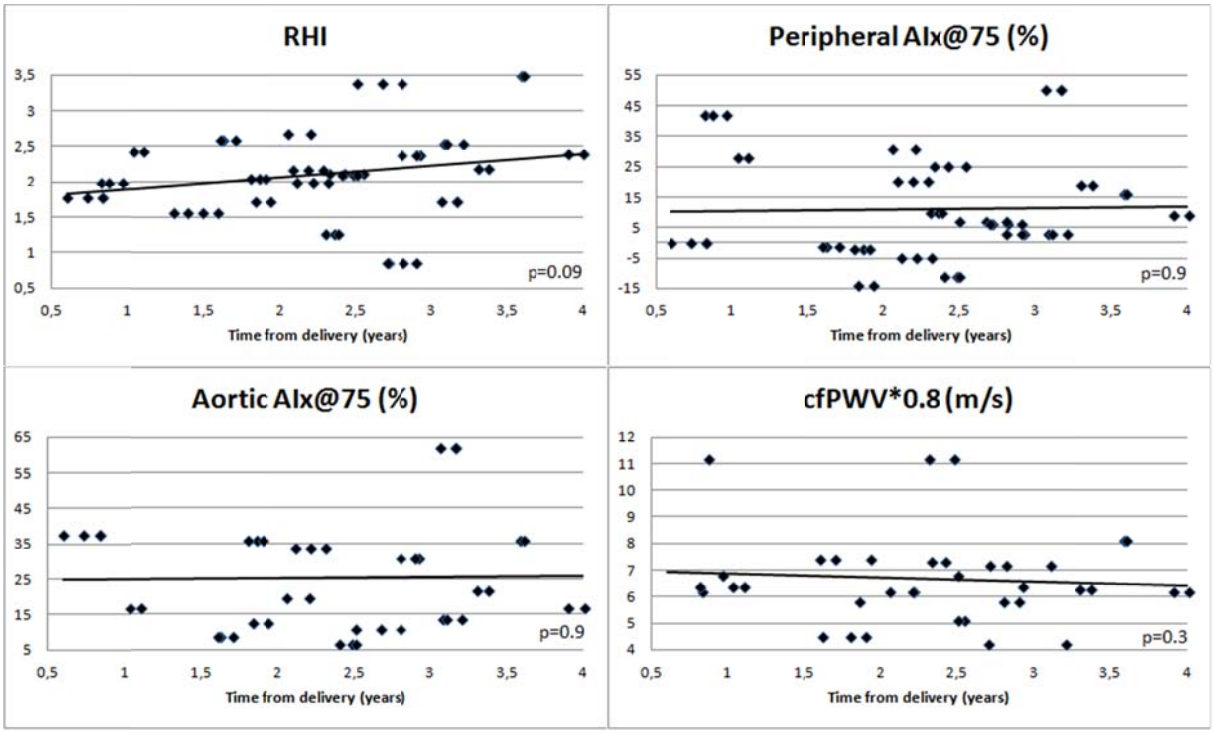

Figure 3. Temporal changes in the four vascular parameters according to time assessment from delivery.