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DANIELE CRISTÓVÃO ESCOUTO

AVALIAÇÃO DO MODELO FULLPIERS E PLGF COMO PREDITORES DE DESFECHOS ADVERSOS EM MULHERES COM DOENÇA HIPERTENSIVA GESTACIONAL

EVALUATION OF THE FULLPIERS MODEL AND PLGF AS PREDICTORS OF ADVERSE OUTCOMES IN WOMEN WITH HYPERTENSIVE DISORDERS OF PREGNANCY

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Pontifícia Universidade Católica
do Rio Grande do Sul

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To my parents,
who taught me to dream big, work hard, stay focused and always search for what makes my soul happy.

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There is nothing beautiful
about the wreckage of a human being.
There is nothing pretty about damage, about pain, about heartache.
What is beautiful is their strength, their resilience, their fortitude
when they pick through the wreckage of their life to build something
beautiful brand new,
against every odd
that is stacked against them.
(Nikita Gill).

RESUMO

Introdução – A distinção adequada dos casos de alto risco para eventos graves nas doenças hipertensivas gestacionais, não apenas pré-eclâmpsia, é um desafio clínico. O modelo fullPIERS é uma ferramenta simples e de baixo custo que utiliza variáveis clínicas para estratificar a probabilidade de eventos adversos em gestantes com pré-eclâmpsia. O fator de crescimento placentário (PlGF) é um biomarcador com concentrações reduzidas no plasma de mulheres com pré-eclâmpsia e com crescente emprego na avaliação de gestantes com suspeita de pré-eclâmpsia.

Objetivos – O objetivo deste estudo é estimar a acurácia do modelo fullPIERS e do biomarcador PlGF como preditores de desfechos adversos maternos em gestantes com doença hipertensiva gestacional.

Métodos – Estudo de coorte prospectiva em um hospital terciário em Porto Alegre, Brasil, que incluiu gestantes admitidas com pressão arterial sistólica ≥ 140 e/ou pressão arterial diastólica ≥ 90 mmHg a partir da 20ª semana de gestação. Os piores valores de variáveis clínicas e laboratoriais dentro das primeiras 48 horas de admissão foram coletados. Desenvolvimento de eventos adversos foi acompanhado por um período de 14 dias. Concentrações plasmáticas maternas de PlGF do momento da admissão foram mensuradas.

Resultados – 405 gestantes foram incluídas no estudo. Entre as 351 mulheres incluídas na análise do modelo fullPIERS, 20 (5%) desenvolveram pelo menos um evento adverso materno dentro de 14 dias de internação. O modelo fullPIERS teve pouca capacidade discriminativa para prever desfechos em 48 horas [AUC 0,639 (95% CI 0,458-0,819)]. A acurácia do modelo foi ainda mais baixa dentro de sete semanas da admissão [AUC 0,612 (95% CI 0,440-0,783)]; a capacidade discriminativa manteve-se similar dentro de 14 dias da admissão [AUC 0,637 (95% CI 0,491-0,783)]. A calibração do modelo fullPIERS também foi ruim: inclinação 0,35 (95% CI 0,08-0,62) e intercepto 1,13 (95%CI -2,4-0,14). A análise do PlGF incluiu 392 gestantes. PlGF <5º percentil esteve associados a eventos adversos maternos dentro de 48 horas em gestantes incluídas antes de 35 semanas com sensibilidade de 0,80 (0,4-0,96), valor preditivo negativo (VPN) de 0,98 (0,9-0,99) e AUC ROC de 0,672 (IC 95% 0,5-0,9). PlGF <100 pg/mL apresentaram sensibilidade de 0,8 (0,4-0,96), especificidade de 0,6 (0,5-0,7) e VPN de 0,99 (0,94-0,99) em mulheres após 37 semanas de gravidez. PlGF apresentou bom desempenho para prever parto até 14 dias em gestantes incluídas antes de 35 semanas. PlGF <5º percentil esteve associado a recém-nascido pequeno para idade gestacional (PIG) com sensibilidade de 0,75 (0,6-0,9), especificidade 0,65 (0,5-0,7), NPV de

0,87 (0,79-0,94) e AUC ROC 0,698 (0,6-0,79), em gestantes com <35 semanas, a acurácia diminuiu com o aumento das idades gestacionais.
Conclusões – O modelo fullPIERS e a concentração de PLGF mostraram baixa acurácia na predição de desfechos adversos maternos em mulheres com doença hipertensiva gestacional, incluindo pré-eclâmpsia. O modelo fullPIERS teve desempenho inferior na nossa amostra quando comparado com o estudo que validou este teste. O PLGF parece ser um biomarcador para uso como ferramenta adicional na predição de parto dentro de 14 dias e recém-nascidos PIG, especialmente em gestantes antes da 35ª semana gestacional.

Palavras-chave – Hipertensão-induzida-pela-gravidez, Prognóstico, Biomarcador, Indutores-da-angiogênese.

ABSTRACT

Introduction - Singling out high-risk patients from the diverse hypertensive disorders of pregnancy, and not only preeclampsia, is a challenge for clinicians. The fullPIERS model is a simple and low-cost evaluation instrument using clinical variables to stratify the adverse outcomes probability of pregnant women with high-risk preeclampsia. Placental growth factor (PlGF) levels are reduced in preeclampsia and are increasingly being used as a biomarker in the assessment of this disease.

Objectives - The aim of the study is to evaluate the performance of the fullPIERS model and PlGF to predict adverse outcomes in women with hypertensive disorders of pregnancy.

Methods - A prospective cohort study carried out at a teaching hospital in Porto Alegre, Brazil enrolling pregnant women admitted with a systolic blood pressure ≥ 140 mmHg and/or a diastolic blood pressure ≥ 90 mmHg from the 20th week of gestation. First 48 hours of admission worst clinical and laboratory data were recorded and the development of adverse maternal and perinatal outcomes scrutinised up to 14 days. Admission maternal plasma PlGF concentrations were measured.

Results – A total of 405 women were enrolled. From the 351 women included in the fullPIERS model analysis, 20 (5%) developed at least one of the combined maternal adverse outcomes. The fullPIERS model had poor outcomes discrimination at 48h [AUC 0.639 (95% CI 0.458-0.819)]. At the seventh admission day, the model's accuracy was even lower [AUC 0.612 (95% CI 0.440-0.783)]; the model's discriminative ability remained similar [AUC 0.637 (95% CI 0.491-0.783)] at 14 days. Calibration of the fullPIERS model was poor: slope -0.35 (95% CI 0.08-0.62), intercept -1.13 (95%CI -2.4-0.14). PlGF analysis included 392 women. PlGF < 5th percentile predicted maternal adverse outcomes within 48h in women with gestation < 35 weeks with sensitivity of 0.80, NPV of 0.98 and AUC ROC of 0.672 (CI 95%0.5-0.9). The threshold of <100 pg/mL, had best accuracy in women after 37 weeks of pregnancy, sensitivity of 0.8, specificity of 0.6, negative predictive value of 0.99 and PPV of 0.04. PlGF had good performance to predict delivery within 14 days in women presenting before 35 weeks. PlGF <5th percentile predicted delivery of a SGA infant with sensitivity of 0.75, specificity 0.65, PPV of 0.45, NPV of 0.87, and AUC ROC 0.698, in women with gestation < 35 weeks, accuracy decreased at later gestational ages.

Conclusion - In conclusion, in our sample the fullPIERS model and PlGF were limited predictors of maternal adverse outcomes in pregnant women with hypertensive disorders of pregnancy, including preeclampsia. The performance of the fullPIERS model in our sample

was inferior to that of the original cohort. PlGF as a biomarker appears to be an additional tool to predict delivery within 14 days and SGA newborn in women before 35 weeks gestation.

Key words: Pregnancy-induced hypertension. Prognosis. Biomarker. Angiogenic proteins.

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LIST OF ABBREVIATIONS

ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
AUC	Area under the curve
CH	Chronic hypertension
CKD	Chronic kidney disease
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
ELISA	Enzyme-linked immunoabsorbent assay
fullPIERS	Pre-eclampsia Integrated Estimate Risk Study group score
GA	Gestational age
GH	Gestational hypertension
HDP	Hypertensive disorders of pregnancy
HELLP	Haemolysis, elevated liver enzymes and low platelets
HLA	Human leukocyte antigen
HLA-C	Human leukocyte antigen locus C
HLA-E	Human leukocyte antigen locus E
HLA-G	Human leukocyte antigen locus G
HSL	São Lucas hospital
HYPITAT	Hypertension and Pre-eclampsia Intervention Trial at Term
IQR	Interquartile range
KCL	King's College London
LDH	Lactate dehydrogenase
LR	Likelihood ratio
MeSH	Medical subject heading
miniPIERS	Low-resourced centres pre-eclampsia integrated estimate risk study group score
NPV	Negative predictive value
OR	Odds ratio
PE	Preeclampsia
PIERS	Pre-eclampsia Integrated Estimate risk Study group
PlGF	Placental growth factor
PPH	Postpartum haemorrhage

PPV	Positive predictive value
PRES	Posterior reversible encephalopathy syndrome
PUCRS	Pontifical Catholic University of Rio Grande do Sul
RAAS	Renin-angiotensin-aldosterone system
ROC	Receiver-operating characteristic
RUPP	Reduced uterine perfusion pressure
SBP	Systolic blood pressure
SD	Standard deviation
sFLT-1	Soluble fms-like tyrosine kinase-1
SGA	Small for gestational age
SLE	Systemic lupus erythematosus
SPE	Superimposed preeclampsia
SpO2	Oxygen saturation by pulse oximetry
TRIPOD	Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis
UK	United Kingdom
UN	United Nations Organization
USA	United States of America
VEGF	Vascular endothelial growth factor
WHO	World Health Organization

SUMMARY

Introduction

1 INTRODUCTION

1.1 INITIAL CONSIDERATIONS

By March of 2014 I began my PhD program with a busy schedule of training and travelling. I was living in a city 140 km away from Porto Alegre, and had to travel weekly in order to complete the hours of lectures and disciplines required by the postgraduate program.

In January 2015 we started organizing, setting up and training staff to initiate recruitment for the main project of my PhD program: a cohort study that would evaluate factors associated with the development of adverse outcomes in women with hypertensive disorders of pregnancy (HDP). By March 2015 recruitment began and within less than two years we reached the target of 400 pregnant women with HDP.

Since the beginning of the postgraduate program I have been involved in the postpartum hypertension outpatient clinic at São Lucas Hospital (HSL) at Pontifical Catholic University of Rio Grande do Sul (PUCRS). My teaching tasks, a requirement of the PhD program, was conducted at this clinic as an assistant consultant and supervisor of medical students.

The clinic is part of the Nephrology Research Group from the School of Medicine/PUCRS, a group that actively studies HDP. Evaluation of L-arginine/nitic oxide pathway, endothelial function, genetic polymorphisms, immunological and other physiopathological aspects of pregnant women with and without HDP are part of the group's efforts to add knowledge to that research field.

During the main study's recruitment phase, I participated in the Foreign Sandwich PhD program supported by the Coordination for the Improvement of Higher Education Personnel (CAPES) – one of the main governmental agency that support research in Brazil. After an unexpected long wait related to visa problems, by November 2015 I became a postgraduate research student at King's College London (KCL). The British were friendlier and welcoming than I could ever imagine. The work environment was professional and generous. Soon I was included in several activities inside and outside of the unit. I started an immersion period of science and research under the supervision of Professor Lucilla Poston, Head of the Women´s Health Department of KCL. My direct mentor was Dr Kate Bramham. A nephrologist and enthusiastic of the study of

renal diseases and its relation to pregnancy, Dr Bramham shared her knowledge and included me in some of her ongoing projects.

During my time at KCL I took active part in research projects involving renal transplant and pregnancy, renin-angiotensin-aldosterone system in women with HDP, cardiovascular risk and renal disease at postpartum of HDP. The work involved data management, statistical analysis and manuscript writing. I spent countless hours studying biostatistics and taking specific classes on the subject. The most important gain from that period was the skills I developed, they will allow me to work on research anywhere. That experience also allowed me to take part in projects of scientific and technological innovative content at an institution of international excellence.

Back to Brazil, by the end of 2016, recruitment phase of the main project was nearly done, mostly due by the brilliant work of two undergraduate medical students, Miss Nathalia Paludo and Miss Rayssa Amaral, part of the investigators team. Analysis and writing periods came, and now I am presenting the results of this journey.

This thesis is part of an effort from the university to encourage internationalization, attending CAPES goals. It is one of the first thesis written and argued in English at our Postgraduate Program and, therefore, format patronization is not fully established. Thesis structure follows the formal orientation of The Central Library Irmão José Otão, PUCRS (PUCRS, 2011). It is divided in pre-textual, textual and post-textual elements. Obligatory sections of textual elements are: introduction, development and conclusion. Post-textual elements consist of appendices and annexes sections, published and unpublished data from studies developed during the PhD program, both as part of the Nephrology Research Group at PUCRS or during the period at KCL - important to mention that those studies were not included in the main objectives of this thesis.

The focus of the present study was to evaluate prognostic markers of adverse outcomes on HDP. We hope to be able to increase knowledge and contribute to the expansion of this research area.

1.2 LITERATURE REVIEW

1.2.1 Hypertensive disorders of pregnancy: definitions, diagnosis and classification

Hypertensive disorders of pregnancy (HDP) refer to important medical complications occurring during pregnancy and can present as different syndromes. The initial clinical presentation and laboratory features define the diagnosis and classification of HDP (Malachias *et al.*, 2016). Classification of HDP varies on literature. (Bulletins--Obstetrics, 2002; Visintin *et al.*, 2010; Magee *et al.*, 2014; Tranquilli *et al.*, 2014; Lowe *et al.*, 2015). The present study have employed the definitions proposed by the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy (Chart 1) (NHBPEPWG, 2000), which has the same categories as the American College of Obstetricians and Gynaecologists and has been adopted by the Brazilian Hypertension Guidelines (Malachias *et al.*, 2016). It is also in line with the research recommendations of the Society of Obstetric Medicine of Australia and New Zealand guidelines (Lowe *et al.*, 2015). Chart 1 presents the categories mainly based on NHBPEPWGHBPP, 2000.

Hypertension in pregnancy is defined as blood pressure above 139/89 mmHg during the gestational period, and may be classified in different categories. Gestational hypertension, (GH) presence of isolated hypertension from the 20th gestational week in the absence of proteinuria. Chronic hypertension (CH) is the presence of hypertension or use of anti-hypertensive drug before the 20th gestational week. Preeclampsia (PE) requires presence of either: hypertension from the 20th gestational week and proteinuria; or haemolysis, elevated liver enzymes and low platelets known as HELLP syndrome (NHBPEPWG, 2000). Superimposed preeclampsia (SPE) is defined as the new onset of proteinuria; if both hypertension and proteinuria present, sudden increase in proteinuria or blood pressure, or the development of one additional biochemical alteration: thrombocytopenia (platelet count <100 $10^9/mm^3$ or abnormally increased levels of alanine aminotransferase (ALT) or aspartate aminotransferase (AST) (NHBPEPWG, 2000).

Chart 1 – Classification of hypertensive disorders of pregnancy				
Hypertensive disorder of pregnancy	Hypertension*		Proteinuria **	
Gestational age (GA)	GA < 20	GA ≥ 20	GA < 20	GA ≥ 20
Gestational hypertension		+		
Chronic hypertension	+	+		
Preeclampsia		+		+
Superimposed Preeclampsia	+	+	-/+	+/++

Source: Escouto (2018). Modified from Hentschke (2014) (Nhbpepwg, 2000; Tranquilli *et al.*, 2014; Malachias *et al.*, 2016) *Hypertension: SBP >140 mmHg and/or DBP > 90 mmHg. ** Proteinuria: > 300mg/ 24h; ≥ 30 mg/ mmol by random urinary sample protein: creatinine ratio, or ≥ 2+ of protein by dipstick.

Hypertension is defined as the presence of systolic blood pressure (SBP) ≥ 140 mmHg and/or a diastolic blood pressure (DBP) ≥ 90 mmHg; or the use of anti-hypertensive medication. Diagnosis is confirmed after two measurements by trained professional, at different times and ideal conditions (Malachias *et al.*, 2016). Those measurements should respect a minimum of 4-6 hours to a maximum of seven days interval. Some cautions must be taken during assessment of arterial blood pressure (Brown et al., 2001):

- The individual should be seated, rested for at least 2-3 minutes;
- Upper arms are preferable for measurement;
- Adequate cuff size;
- DBP is identified by cessation of Korotkoff sounds (Korotkoff 5).

Proteinuria is defined as the presence of ≥ 300 mg of protein 24-h urine collection, random urinary sample protein to creatinine ratio ≥ 30, or ≥ 2+ of protein by dipstick. Dipstick proteinuria, however, must be confirmed due to elevated cases of false results. (Brown et al., 2001)

Preeclampsia presents as severe when associated with worsen features. The definition varies, but it can be considered when SBP is >160mmHg or DBP is > 110mmHg. Early onset PE, is associated with severity of disease, and is defined as disease onset < 34 weeks of gestational age (GA) (Tranquilli *et al.*, 2014).

Eclampsia is the occurrence of seizures without an explainable cause in the presence of a hypertensive disorder. Eclampsia may occur during pregnancy or early

puerperium (Douglas e Redman, 1994).HELLP syndrome, is characterized by the presence of schistocytes in peripheral blood smear and lactate dehydrogenase (LDH) > 600 U/L, plasma levels of AST > 70 U/L and thrombocytopenia <100,000/ mm3. The use of partial HELLP syndrome is not recommended by some authors, since the absence of complete syndrome criteria is not associated with the same amount of complications. (Audibert et al., 1996)

It is important to keep in mind that diagnostic criteria of HDP are restrictive and may not identify atypical cases of PE. Up to 40% of eclampsia cases and 15% of women with HELLP syndrome do not present with hypertension or proteinuria. (Douglas e Redman, 1994; Sibai, 2004) Although more strict definition is usually used in research projects, most guidelines suggest that even in the absence of hypertension and proteinuria, the presence of signs or symptoms highly suggestive of PE should suggest special attention. Headache, flashlights, abdominal pain, thrombocytopenia and abnormal liver enzymes are some features that should be followed closely (Nhbpepwg, 2000; Tranquilli *et al.*, 2014; Lowe *et al.*, 2015).

1.2.2 Maternal mortality and epidemiology of hypertensive disorders of pregnancy

Data from the World Health Organization (WHO) showed a global estimative of 287.000 maternal deaths during the year 2010. (WHO, 2012) Reduction of maternal mortality was one of the main goal of the Millennium Declaration of the United Nations Organization (UN) (UN, 2000). The target to reduce 75% of maternal mortality by the year 2015, however, was not achieved. The UN admitted that, despite of the preventable nature of most maternal deaths, progress in the area are slow and only interventions with strong political support would have a true impact on mortality (UN, 2013). Considerable advances have been made in several countries, including in Brazil.

Hypertension disorders are the first cause of maternal mortality in Latin America (Khan et al., 2006), responsible for about a quarter of the maternal deaths and 18% worldwide (Abalos et al., 2014). Also, for each maternal death, 20 women develop severe adverse outcomes or permanent disabilities (Canada, 2004). The maternal death rate in 2014 in Brazil and Porto Alegre were, respectively, 58 and 21 for 100.000 livebirths. Maternal mortality due to severe arterial hypertension in Brazil was reported by the National Vigilance on Severe Maternal Morbidity as 51 deaths for 100.000 livebirth at 27 obstetric centres (Zanette et al., 2014).

A recent international study conducted by the WHO included over 300.000 pregnancies with 2% prevalence of PE. The disease distribution varied between regions. Latin American countries and low-income countries had more cases of PE. In this study, 70.000 Brazilian pregnancies were included and 4.6% had PE diagnosis, while 1% had CH and 0.2% eclampsia (Abalos 2014).

Chronic hypertension affects over 30% of the Brazilian (Malachias *et al.*, 2016). Women are a majority and prevalence increases proportionally with age (Saúde, 2008). The association with cardiovascular diseases (CVD), such as ischemic cardiac disease, cerebrovascular diseases and chronic kidney disease (CKD) augment diseases impact. Cardiovascular diseases were the cause of death in 17% of women below 50 years-old during 2014 (Saúde, 2008).

The increase in maternal age worldwide suggests that CH in pregnancy may become a more prevalent clinical problem. An American survey showed an increase in CH, especially with development of SPE, from years 1995 to 2004. Risk was higher among black women and women over 35 years old (Savitz *et al.*, 2014).

Chronic hypertension increases risk for SPE. A 2014 meta-analysis included almost 800,000 pregnancies from 25 countries showed that the risk of developing PE was almost eight times higher in chronic hypertensive women than in general population. Also, other maternal and neonatal adverse outcomes such as severe hypertension, preterm delivery and small for GA were 2 to 3-fold more frequent among North-American women with CH (Bramham *et al.*, 2014). Superimposed preeclampsia further increases the risk of complications. Diagnostic difficulties to differentiate SPE in women with previous hypertension or proteinuria only adds to the challenge of dealing with HDP.

1.2.3 Causes and mechanisms of preeclampsia

Preeclampsia is a disease associated with the presence of the syncytiotrophoblast and the maternal response to placentation. Inadequate placentation and the consequent local hypoxia were long considered as the main causes of disease. We now know that those were not the cause, but strong predisposing factors involved in the development and pathophysiology of PE (Sibai et al., 2005).

In 2005, Redman suggested a model of two stages, pre-clinical and clinical to explain the pathogenesis of PE (Redman e Sargent, 2005). In order to understand this

model, previous knowledge of normal placental development is necessary. Uterine spiral arteries are partially occluded by cytotrophoblast plugging in the beginning of pregnancy. Placental flow is kept as limited as possible during embryogenesis to avoid potential pathogenesis associated with oxidative stress (Burton *et al.*, 2003). As pregnancy evolves, the increase in blood flow is assured by the interaction between placenta and uterine surface by elimination of cytotrophoblast plugging and remodelling of spiral arterioles (Schneider, 2011). Placental trophoblastic cells invade the muscle layer of spiral arterioles causing dilatation of its lumen, altering the original endothelium and forming a pseudo-endothelium structure. The process begins at the 9th gestational week and lasts around 12 weeks. The final result is a wide complex of dilated ducts composed by maternal and foetal faces (Burton *et al.*, 2009).

In PE part of the remodelling process may be inhibited. Failure of conversion of the maternal spiral arteries promotes a turbulent blood flow to the intervillous space, possibly causing damage to the villous architecture and, also, vasoconstriction and ischemia-reperfusion injury (Burton *et al.*, 2009). The limited increase of utero-placental blood flow is called inadequate placentation. A dysfunctional placenta, with inadequate perfusion, would release pro-inflammatory substances on maternal circulation. Hypoxia, endothelial activation, oxidative stress, placental necrosis with release of cell debris would all serve as stimulus to inflammatory cytokines production. At the two-stage model a misbalance of homeostasis and alterations of the adaptive hemodynamic changes of pregnancy occur. This process gives place to a syndrome of elevated vascular peripheral resistance and inflammatory microangiopathy manifested at the clinical phase of disease (Redman e Sargent, 2005).

This course seems to depend on the interaction between allele genes of human leukocyte antigen (HLA) expressed by trophoblast, HLA-C, HLA-E, HLA-G, with maternal natural killer cells and dendritic cells. These cells are modified at pregnancy to allow trophoblastic invasion. An inadequate or exaggerated maternal immune response can be involved at pre-clinical stage of PE (Steegers et al., 2010).

Based on the reproductive immunology studies and considering the hypothesis that the base alteration in PE could be immunologic, Redman & Sargent proposed a new model for PE involving three stages. During a normal pregnancy, exposition to paternal biological material would induce tolerance to paternal alo-antigens. A dysfunction of this immunoregulatory process causing an incomplete tolerance to the allogenic foetus (stage I), could initiate the abnormal placentation (stage II). Since immune regulation

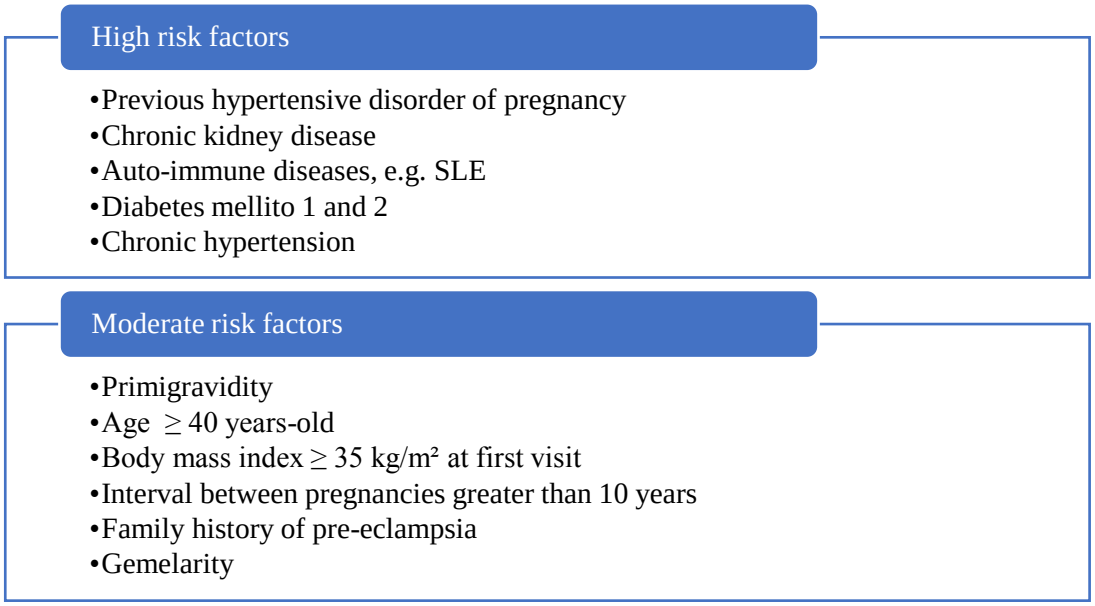
tends to diminish thorough pregnancy, stage III, instead of being caused by maternal "rejection" of a genetically different organ, would result of the maternal inflammatory reaction to a dysfunctional placenta (Redman e Sargent, 2010).

Chronic hypertensive women have an increased risk of developing superimposed PE. Considering abnormal placentation and uteroplacental circulation involved in the pathophysiology of PE, it is possible that hypertension itself is not the only factor involved in predisposition to the disease. It is known that systemic renin-angiotensin-aldosterone system (RAAS) activity is augmented in non-pregnant women with CKD and CH. A United Kingdom (UK) prospective cohort with 195 CKD and CH women showed reduced concentrations of plasma renin during pregnancy in comparison to healthy controls. Renin plasma concentrations were further supressed in women that developed SPE. A comparison of renin plasma concentrations between women with SPE and those previously normotensive that developed PE, at time-of-disease, showed no difference (Escouto *et al.*, Jul 2016). This finding suggests a common pathophysiological pathway to renin suppression in both presentations of the disease. The RAAS and other possible mechanisms involved in development PE superimposed to chronic hypertensive still need elucidation.

1.2.4 Preeclampsia: risk factors

Despite of elevated morbimortality associated with PE, to date, the only concrete option for prevention of PE is contraception. (Dekker e Sibai, 2001) During pre-conception counselling of women of childbearing age should address main risk factors of PE (Figure 1).

Figure 1 - Risk factors for Preeclampsia



Source: Escouto (2018).

Preeclampsia is known as a disease of primigravity (Dekker e Sibai, 2001). The risk of having the disease during the first pregnancy is almost threefold higher (Duckitt e Harrington, 2005). This association is possibly associated to a shorter exposition to paternal sperm and poor immune adaptation. That would also explain the elevated risk in teenagers (Dekker e Sibai, 2001). However, there is also augmented risk of PE in pregnancies whose fathers were involved in a previous pregnancy complicated by PE, suggesting a role of paternal factors to the pathophysiology of PE (Lie et al., 1998).

A previous history of PE is associated with 20 to 25% increase risk of PE on index pregnancy (Dukler et al., 2001; Bramham et al., 2011). Risk also increases with maternal age and longer interval between pregnancies (Lie et al., 1998). Women with CH have 8 times more chance to develop PE (Bramham *et al.*, 2014).

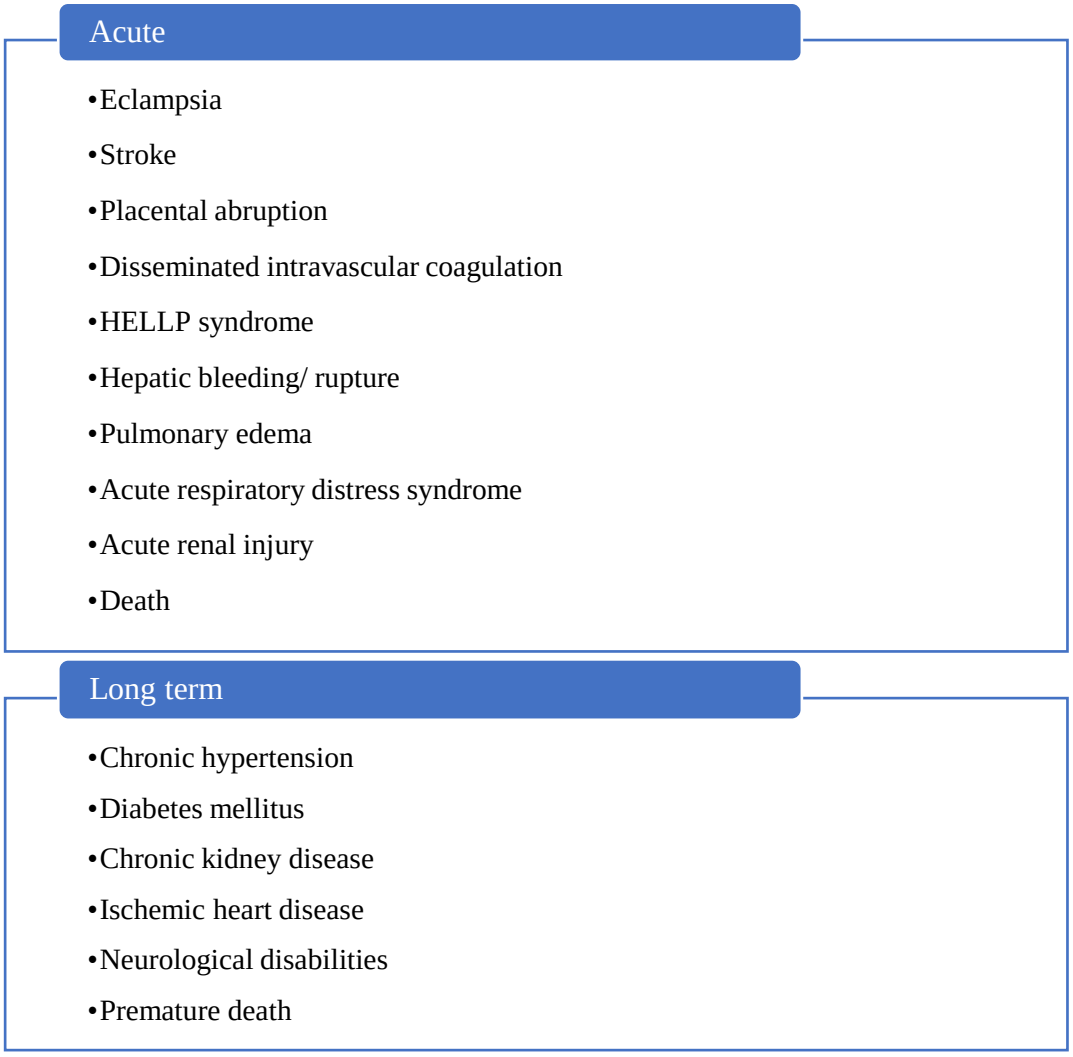
Body mass index, a modifiable risk factor, is positively associated with HDP and PE (Duckitt e Harrington, 2005). An excessive weight gain during pregnancy, especially during first weeks, also increases PE risk, independently of weight previous to pregnancy (Macdonald-Wallis et al., 2013).

1.2.5 Adverse outcomes of hypertensive disorders of pregnancy

Complications associated with HDP can be divided into acute and long term (Ghulmiyyah e Sibai, 2012) (Figure 2). PE is the cause of 20% of antenatal hospital

admissions in the United Kingdom (Rosenberg e Twaddle, 1990). Severe adverse outcomes such as pulmonary oedema, stroke and acute renal failure are associated with HDP. Need for intensive care unit admissions, and specialized treatments such as mechanical ventilation and haemodialysis might be necessary. The lack of resources in low and middle-income countries aggravate risk for those women (Duley, 2009). Near miss cases are 8 times more frequent among women with PE than other HDP. Coagulopathies and other haematological disorders are the most frequent acute complications, followed by respiratory distress, cardiovascular and hepatic dysfunctions (Abalos et al., 2014).

Figure 2 – Maternal complications associated to Hypertensive Disorders of Pregnancy



Source: Escouto (2018). Modified from Ghulmiyyah e Sibai, 2012. HELLP, haemolysis, elevated liver enzymes and low platelets.

Ninety percent of maternal mortality associates to HDP occur in low and middle-income countries (Lerberghe, 2005). The main cause of death is intracranial haemorrhage (Moodley, 2004). A survey conducted in 2014, showed 4% of mortality among women with eclampsia (Abalos et al., 2014). It is associated to mortality of any cause, especially in women with PE before 37th gestational week, when mortality risk is almost four times higher than in normal pregnancies (Bellamy et al., 2007).

Women with previous history of HDP have a life-long risk of CVD (Brown et al., 2013). Meta-analyses of observational studies have demonstrated a two-fold increase in the risk for CVD later in life in women with previous PE compared to those normotensive pregnancies (Mcdonald *et al.,* 2008; Brown *et al.,* 2013).Bellamy et al demonstrated threefold increase risk of CH, twofold increase risk of stroke and fatal and non-fatal acute myocardial ischemia. (Bellamy et al., 2007) It is undetermined whether the association between PE and CVD is secondary to underlying common risk factors, such as dyslipidemia and obesity, or if PE is an independent factor causing vascular damage (Garovic e Hayman, 2007). Increased cardiovascular risk in women with PE is strongly associated with severity of disease, including need for preterm delivery, and has been estimated to be two-fold higher in those with severe compared with mild disease (Mcdonald *et al.*, 2008).

Chronic kidney disease is also observed long term after a preeclamptic pregnancy. A meta-analysis with a weighted mean of 7 years postpartum follow-up of 273 women with previous PE, showed a 4-fold increase in microalbuminuria when compared to healthy controls, whereas women with severe PE had an 8-fold increase (Eder e Mcdonald, 1987). An American survey from a cohort of 34,000 women who gave birth from years 1976 to 2010, identified that the odds of having end stage renal disease were 4 times higher in women with history of previous PE comparing with those without, after adjustment for age and parity (Kattah *et al.,* 2017).

Perinatal adverse outcomes associated with HDP occur due to placental dysfunction, complications associated to preterm delivery and maternal complications (Bokslag *et al.,* 2016). Neonates of pre-eclamptic pregnancies have an average of 5% lower birthweight than those born of healthy pregnancies. A further reduction (23%) may occur in cases of early-onset of disease (Odegård *et al.*, 2000). Preeclampsia is a significant contributor to preterm birth, with odds ratio (OR) four times higher than healthy pregnancies (Davies *et al.,* 2016). Preterm birth, in turn, is associated with higher rates of infant respiratory distress syndrome, intraventricular haemorrhage,

sepsis, bronchopulmonary dysplasia and neurodevelopmental disability in childhood (Saigal e Doyle, 2008).

According to the WHO perinatal mortality is 3 to 7 times higher in pregnancies complicated by PE and eclampsia (Abalos et al., 2014). A population-based study evaluated 57 million live and stillbirths in periods 1990-91 and 2003-04 in the United States of America (USA). Women with HDP had increased OR for stillbirth and neonatal deaths from 1.3 to 2.3, risk increased with the number of previous pregnancies and was higher in the 2003-04 periods. The study included chronic hypertensive and previous normotensive women with and without development of PE. However, differentiation between groups was not possible (Ananth e Basso, 2010).

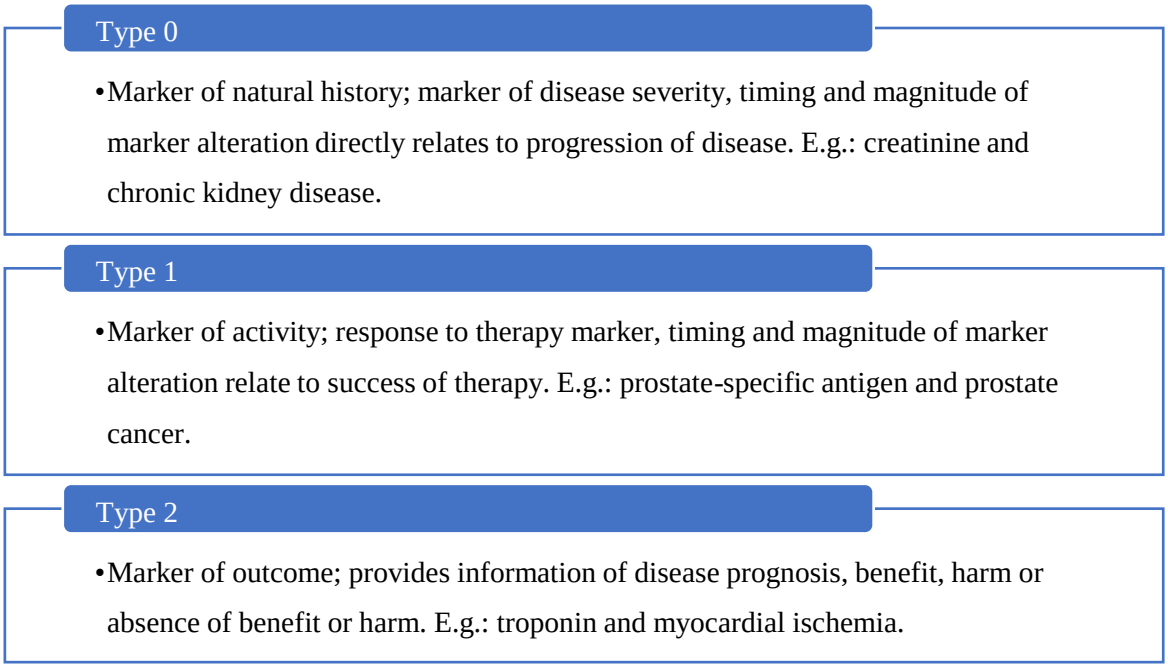
Long term outcomes of children of HDP pregnancies are unclear. A recent systematic review demonstrated association of HDP with cognitive function impairment, hypertension and increased cardiovascular risk later in life. However, differences in outcome measurements and covariate adjustment among the included studies limited the finding's strength (Pinheiro *et al.*, 2016).

1.2.6 Biomarkers and hypertensive disorders of pregnancy

Biomarker, or biological marker, was introduced as a Medical Subject Heading (MeSH) term in 1989. "Measurable and quantifiable biological parameters (e.g., specific enzyme level, specific hormone level, specific gene phenotype distribution in a population, presence of biological substances) which serve as indices for health- and physiology-related assessments, such as disease risk, psychiatric disorders, environmental exposure and its effects, disease diagnosis; metabolic processes; substance abuse; pregnancy; cell line development; epidemiologic studies; etc" (Ncbi)

Figure 3 shows the classification is used to validate biomarkers that consist of a hierarchical classification of three categories (Mildvan et al., 1997).

Figure 3 - Classification of biomarkers



Source: Escouto, 2018.

The search for biomarkers for PE is an active field of research. Uric acid (Paula *et al.*, 2008; Bramham *et al.*, 2013); urinary podocytes (Konieczny et al., 2013); MicroRNA-376c (Baker e Delles, 2013); gene expression of placenta proteins such as β-HCG and beta subunit of luteinizing hormone, (Lapaire et al., 2012) are some of recent investigation targets.

The permeability of the glomerular basement membrane proteins is key to diagnosis of PE. Studies have proved the efficiency of random urine sample of protein to creatinine ratio for the diagnosis of PE and SPE, a possible type 1 marker (Waugh *et al.*, 2005; Morris *et al.*, 2012). The ability of proteinuria for predicting adverse outcomes have also been previously addressed, type 2 marker (Payne *et al.*, 2011; Bramham *et al.*, 2013).

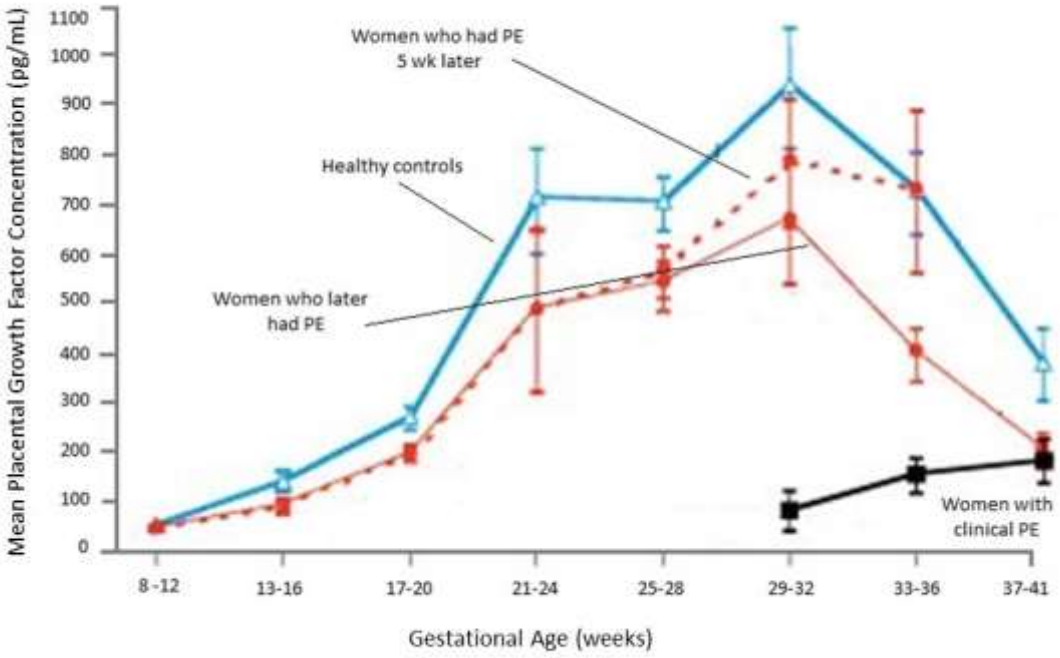
Hyperuricemia is described as being common in pregnancies affected by PE and often precedes the diagnosis of this disease. Historically uric acid was used as a marker of this condition, but lost space as a sign of maternal hypertensive renal damage to proteinuria. Uric acid is a marker of oxidative stress, tissue injury and renal dysfunction, and thus may be useful in predicting PE complications, a type 2 marker (Martin e Brown, 2010). Angiogenic factors are presented as promising candidates for PE biomarkers. Levine et al showed that plasma concentrations of soluble fms-like tyrosine kinase-1 (sFLT-1) and placental growth factor (PlGF) are altered in pregnant women

that developed PE weeks before its clinical presentation (Figure 4). sFLT-1 is an anti-angiogenic protein, a soluble split of the vascular endothelial growth factor (VEGF) receptor, that compete with vascular receptors of PlGF and VEGF inhibiting its pro-angiogenic actions, and promoting endothelial dysfunction. Due to lack of stimuli, reduced concentrations of PlGF and VEGF also lead to endothelial dysfunction (Levine et al., 2004).

Previous studies have demonstrated the ability of the ratio of sFlt-1 and PlGF to distinguish women with and without PE with the use of automated assays with sensitivities and specificities >95% for preterm PE (Ohkuchi *et al.*, 2010; Sunderji *et al.*, 2010; Verlohren *et al.*, 2010). Also, the measurement of antiangiogenic proteins seems to distinguish presence of PE in women that present with hypertension or proteinuria for other reasons, including Diabetes mellitus and Systemic Lupus Erythematosus (Cohen *et al.*, 2007; Qazi *et al.*, 2008). Moreover, superimposed PE requiring delivery within 14 days in women with CH and CKD who may have lower maternal PlGF concentrations. also showed good accuracy (Bramham *et al.*, 2016).

The implication of angiogenic factors in the pathogenesis of PE also encourage the development of new targeted therapies (Karumanchi, 2016). Administration of PlGF to reduced uterine perfusion pressure (RUPP) rats stopped hypertension and restored reductions in glomerular filtration rates of pregnant rats (Spradley *et al.*, 2016).

Figure 4 - Concentrations of PlGF during pregnancy



Source: Escouto (2018). Modified from Levine et al (2004). Shows the mean free PlGF concentrations before and after the onset of PE according to the gestational age of the foetus. I bars represent standard errors. PE, preeclampsia. PlGF, placental growth factor

1.2.7 Estimating risk and prognosis of Hypertensive Disorders of Pregnancy: state of art

To date we have not been able to predict which women will develop PE. Most women are only identified once disease is fully established. The broad spectrum of presentations of HDP severity adds complexity to the management of those women. The lack of adequate biomarkers to predict disease progression and adverse outcomes in patients with HDP results in substantial financial burden to the health care system (e.g. frequent medical visits, hospitalizations, intensive laboratory surveillance (Chaiworapongsa *et al.*, 2014). Finding possible tools of prognostic value is an area of active research.

The HYPITAT (Hypertension and Pre-eclampsia Intervention Trial at Term) group published in 2011 a prediction model of progression to high risk situation in women with pregnancy hypertension and mild PE. Using clinical variables the model

obtained an area under the curve of the receiver-operating characteristic (AUC ROC) of 0.71 (95% CI, 0.67-0.74). (Van Der Tuuk et al., 2011) Aiming to identify HDP women at increased risk of developing postpartum haemorrhage (PPH), Koopmans et al analysed clinical and laboratorial prognostic variables concluding that the prediction of occurrence of PPH is possible when antepartum and intrapartum variables are combined at the time of delivery, as noted by the AUC ROC of 0.64 (95% CI 0.59-0.70) (KOOPMANS, 2014).

Thangaratinam et al published a series of systematic reviews on predictors of maternal complications. They found no relationship between elevated liver transaminases, uric acid or proteinuria values and maternal adverse outcomes. (Thangaratinam et al., 2006; Thangaratinam et al., 2009; Thangaratinam et al., 2011) Within a cohort of women admitted to hospital with PE, Payne et al also found that proteinuria should not be used in isolation for decision-making in women with PE (Payne *et al.*, 2011). Bramham et al, conducted a nested case-control study of women who participated in the Vitamins In PE trial, suggesting that women who developed PE and proteinuria of 300-499mg/24h have more severe hypertension, early deliveries and small for gestational age (SGA) infants than women GH and CH. Proteinuria >500mg/24h was associated with greater risk of pregnancy complications than a proteinuria of 300 mg/24h. Suggesting that proteinuria levels maybe a useful tool to be used in clinical practice (Bramham *et al.*, 2013).

Lactic dehydrogenase, an indicative of cellular damage and dysfunction, demonstrated to elevate the risk of poor maternal and foetal/ infant outcome when plasma levels were >800IU/l in women with PE (Qublan *et al.*, 2005). Uric acid, a marker of oxidative stress, tissue injury and renal dysfunction, have also been evaluated as predictor of adverse outcomes. Hawkins et al found that uric acid plasma levels are associated with a higher likelihood for maternal and fetal complications in high-risk women with HDP. However, uric acid had lower predictive ability for adverse pregnancy outcomes than blood pressure, maternal symptoms or proteinuria (Hawkins *et al.*, 2012). Urato *et al* found negative correlation between admission uric acid levels and length of expectant management in preterm patients with PE (Urato *et al.*, 2012). A meta-analysis of 2009, included eight studies and concluded that the serum uric acid has good accuracy to predict maternal complications in the management of women with PE. And speculate that, in patients with increased serum uric acid values, labour should be

induced due to their increased risk of complications (Koopmans, Van Pampus, *et al.*, 2009).

The search for a unique marker, of easy assessment, low cost and accurate distinction of women with HDP at higher risk for adverse outcomes continues. The focus of the next items will be on two prognostic tools that, if proven externally validated, could be potential assets for clinical practice.

1.2.7.1 Pre-eclampsia Integrated Estimate Risk Study Group score

Pre-eclampsia Integrated Estimate RiSk Study group (PIERS) started from a comparative cohort study before and after the use of the Guidelines for Management of HDP at the Women's Hospital of British Columbia in Canada (Menzies et al., 2007). The study showed that the systematic implementation of surveillance measures reduced the number of adverse maternal outcomes. Subsequently, the group collected data in several Canadian regional centres (Von Dadelszen et al., 2009).

They developed a clinical score, called fullPIERS. This logistic model was developed and validated in an international prospective study. Well-resourced centres in Canada, Australia, England and New Zealand included 2023 women with PE. Clinical variables of easy collection and low-cost were used to predict maternal adverse outcomes. Adverse outcomes were defined previously using the interactive Delphi consensus, a widely used method to obtain controlled feedback from a group of experts (Diamond *et al.*, 2014). Independent variables included in the final model were: gestational age at admission, chest pain and/or dyspnoea, oxygen saturation by pulse oximetry, platelets count, and plasma levels of AST and creatinine (Von Dadelszen et al., 2011).

Two-hundred and sixteen (13%) adverse outcomes were observed. The most frequent outcome was pulmonary oedema, followed by transfusion of blood components. The fullPIERS model demonstrated good stratification ability. With a predictive probability cut-off of 0.05 it identified over 75% of women at risk who had an adverse outcome. The model accurately discriminated pregnant women with PE at high risk of adverse outcomes from 48h (AUC ROC 0.88 [95% CI 0.84-0.92]) up to 7 days after hospital admission (AUC ROC >0.7). (Von Dadelszen et al., 2011)

Targeting to include low-resourced centres, the miniPIERS score was developed. Conducted and validated at five centres in low to middle-income countries, including

Brazil, miniPIERS evaluated 2081 women with HDP. Variables used in the final model were parity, gestational age at admission, chest pain and/or dyspnoea, headache and/ or visual disturbance, abdominal pain and vaginal bleeding, SBP and dipstick proteinuria. With an outcome rate of 19%, miniPIERS predicted adverse outcomes in women with HDP with a AUC ROC of 0,77 (95% CI 0,74-0,80) and a positive predictive value (PPV) of 85%.(Payne et al., 2014)

The same group developed an external validation of the fullPIERS model using the miniPIERS development cohort as the validation sample (Ukah, Payne, *et al.*, 2017). The analysis included 757 women, and the rate of adverse outcomes within 48 hours of admission was 14%. Despite the larger sample size and high adverse outcomes rate, the model lost discriminatory performance (AUC ROC: 0.77; 95% CI, 0.72–0.82 versus AUC ROC: 0.88; 95% CI, 0.84–0.92) and had poor calibration performance.

A large randomized controlled trial is ongoing in Africa and Asia countries. With a goal to include 80.000 pregnant women and evaluate interventions in HDP at community level and in first-level clinics (Payne *et al.*, 2013). As part of the group initiative, the utilization of a low cost, easy-to-use, mobile health (mHealth) platform, the PIERS on the Move mobile app. This tool provides a simple and fast guide to diagnosis, risk estimative and initial management of pregnant women with PE (Lim *et al.*, 2015). An on-line calculator is also available at the study group web site, where there is an interface for data input and simultaneous result of risk of outcome estimative (PRE-EMPT. Pre-eclampsia and eclampsia monitoring, prevention and treatment., 2010)

There is still need for external validation of those tools, as stressed by the authors themselves. The implementation of the fullPIERS model on clinical practice, despite of apparently promising, needs validation on our population.

1.2.7.2 Placental Growth Factor

Placental growth factor is produced by the syncytiotrophoblast and concentrations increase on maternal plasma during pregnancy, reaching peak values between 26th to 33th gestational weeks. Pregnancies complicated by PE follow the same pattern until 13th to 16th week, when maternal plasma concentrations of PlGF become proportionally lower (Figure 4). A significant fall can be detected between 11 to 9 weeks previous to clinical presentation of PE. (Levine et al., 2004) PlGF is associated with an

inflammatory state in the presence of placenta, reason why it is believed to be a possible marker associated with disease severity. Differences between PlGF plasma concentrations are particularly higher before the 35th GA. Measurement of PlGF at this point of pregnancy could be of particular aid to diagnosis and prognosis in a critical moment of concern for maternal health and the risks of preterm birth.

Diagnostic studies using PlGF alone or in association with sFLT-1 showed a good accuracy to predict PE development at second and third trimesters (Hassan et al., 2013; Rizos et al., 2013). Rana et al published a study with 600 women suspected to have PE, demonstrating that the increase on sFLT-1/ PlGF ratio associated to hypertension and proteinuria had good ability to identify women at risk of adverse outcomes, especially if presenting before 34 weeks of pregnancy (Rana, Powe, *et al.*, 2012).

The study by Chappell et al evaluated the use of PlGF concentrations to predict delivery within 14 days in women with PE. Maternal plasma concentrations of PlGF below the 5th percentile of normality, or <100 pg/mL, had elevated sensitivity and negative predictive value (NPV) to predict delivery within 14 days of admission in women with PE, especially if presenting before 35th week of pregnancy. Reduced concentrations of PlGF had better discriminative ability alone (AUC ROC 0,87; 95% CI 0,81-0,93) than in association with common variables used to evaluate severity of disease (SBP, DBP, proteinuria, ALT and uric acid) (Chappell et al., 2013).

PlGF, as shown on the previous studies, appears to be a promising biomarker to predict disease progression and adverse outcomes in the clinical practice. However, definitions of plasma values in HDP other than PE and the validity of these findings in our population still need to be addressed.

The proper distinction of women affected by HDP with higher risk for serious complications could help defining those who would benefit from intensive surveillance and an early interventionist approach, regardless of risks associated with preterm delivery.

1.3 OBJECTIVES

1.3.1 Main objective

To evaluate the accuracy of the fullPIERS model and the biomarker PlGF as predictors of adverse outcomes in pregnant women with hypertensive disorders of pregnancy.

1.3.2 Specific objectives

- To test the accuracy of the fullPIERS model and PlGF concentrations as predictors of adverse outcomes.
- To compare the accuracy of the fullPIERS model and PlGF concentrations as predictors of adverse events between the group of patients with preeclampsia and with other hypertensive disorders of pregnancy.

Development

Methodology

2 DEVELOPMENT

2.1 METHODOLOGY

2.1.1 Study design

This is a prospective cohort study.

2.1.2 Patient selection

Pregnant women admitted to the obstetric centre of Sao Lucas of Pontifical Catholic University of Rio Grande do Sul Hospital (HSL-PUCRS), who meet the inclusion criteria and sign a consent form were selected to participate in the study from March of 2015 to December of 2016.

2.1.3 Inclusion criteria

Pregnant women, from 20 weeks of gestation, admitted to the hospital with SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg at two different measurements.

2.1.4 Exclusion criteria

- Presence of disease with systemic alteration that, in the opinion of the investigators, could interfere in the outcomes independently of HDP (infections with systemic involvement, advanced neoplasia);
- Active labour at the moment of hospital admission;
- Presence of any component of the adverse outcome at the moment of hospital admission;
- Patient refusal to participate in the study.

2.1.5 Study definitions

2.1.5.1 Definition of hypertensive disorders of pregnancy

Hypertension: SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg at two different measurements with a minimum of 4 hours apart; or the use of anti-hypertensive medication.

Proteinuria: ≥ 300 mg of protein 24-h urine collection or ≥ 30 g/ g by random urinary sample protein: creatinine ratio.

Chronic hypertension: presence of hypertension, without proteinuria, or use of anti-hypertensive drug before the 20th gestational week.

Gestational hypertension: isolated hypertension from the 20th gestational week with absence of proteinuria.

Preeclampsia: presence, from the 20th gestational week, of either hypertension and proteinuria; or HELLP syndrome

Superimposed preeclampsia: new onset of proteinuria or the development of one additional clinical or biochemical feature of preeclampsia (e.g. abnormal liver function tests) on previous chronic hypertensive women.

2.1.5.2 Definition of combined adverse outcomes

1) Maternal mortality

2) Or, 1 or more of the following adverse outcomes:

a. Central neural system:
   i. Eclampsia: PE complicated by occurrence of seizures (1 or more) without an explainable;
   ii. Glasgow coma scale < 13 (Teasdale e Jennett, 1974);
   iii. Posterior reversible encephalopathy syndrome (PRES): presence of neurological symptoms associated with diagnostic imaging with evidence of encephalic white matter oedema, with a reversible characteristic (Hinchey *et al.*, 1996);
   iv. Stroke: acute neurological event with deficits lasting longer than 48 hours;
   v. Retinal detachment: separation of the inner layers of the retina from the underlying retinal pigment epithelium diagnosed by ophthalmological exam;
   vi. Cortical blindness: loss of visual acuity with intact papillary response to light;

b. Cardiovascular and respiratory systems:
   i. Use of positive inotropic drugs;

ii. Infusion of third parenteral anti-hypertensive drug;
iii. Myocardial ischemia/ infarction: electrocardiogram changes (ST segment elevation or depression, new Q pathological waves) with or without changes of biochemical markers of myocardial necrosis (troponin, creatine kinase-myocardial band);
iv. Pulmonary oedema: presence of acute and symptomatic pulmonary congestion with clinical and radiological evidence;
v. Requirement of fraction of inspired oxygen > 50% for longer than one hour;
vi. Need for advanced airway support: mechanical ventilation or continuous positive airway pressure, other than for caesarean section;
vii. Severe breathing difficulty: presence of dyspnoea and oxygen saturation by pulse oximetry < 90%;

c. Haematological alterations:
   i. Transfusion of any blood component;
   ii. Platelet count < 50.000 mm^3 without blood transfusion;
d. Hepatic alterations:
   i. Hepatic disfunction: international normalized ratio of the prothrombin time > 1.2, in the absence of disseminated intravascular coagulation or anticoagulant use;
   ii. Hepatic hematoma or rupture confirmed by diagnostic imaging or laparotomy;
e. Renal alterations:
   i. Acute renal insufficiency: defined as plasma creatinine > 1.7 mg/ dL, without underlying renal disease;
   ii. Acute renal failure: defined as plasma creatinine > 2.3 mg/ dL, without underlying renal disease;
   iii. Renal replacement therapy: need for haemodialysis or peritoneal dialysis;
f. Obstetric alterations:
   i. Postpartum haemorrhage requiring blood component transfusion or hysterectomy;
   ii. Placental abruption observed during labour or at anatomopathological examination.

2.1.6 Study procedures

A manual of procedures was developed and followed by the investigators (Appendice A). Demographic, clinical, and patient blood and urine samples were collected after signature of the Informed Consent Term (Appendice B), during the first 48 hours of admission. The study protocol (Appendice C) was fulfilled for each woman. An identification number was generated and used for database and samples storage.

Management of patients after admission was in accordance with the unit policies, without investigators interference. Measurement of blood pressure was performed according to the specific recommendations for standardized pregnant women in the HSL-PUCRS obstetric unit, which recommend sitting and left lateral decubitus measurements.

Patients were followed up for a period of 14 days after admission to observe maternal outcomes. A study flow-chart is presented at Figure 5.

2.1.6.1 Complementary laboratory tests

Laboratory tests were performed according to the standards established by the HSL / PUCRS Clinical Pathology Laboratory: blood flow cytometry (XE - 2100D, Sysmex corporation, Kobe, Japan); (Vitros 5.1 Fusion, NY, USA), with the exception of serum creatinine, using the colorimetric method (Vitros 5.1 Fusion Chemical System, NY).

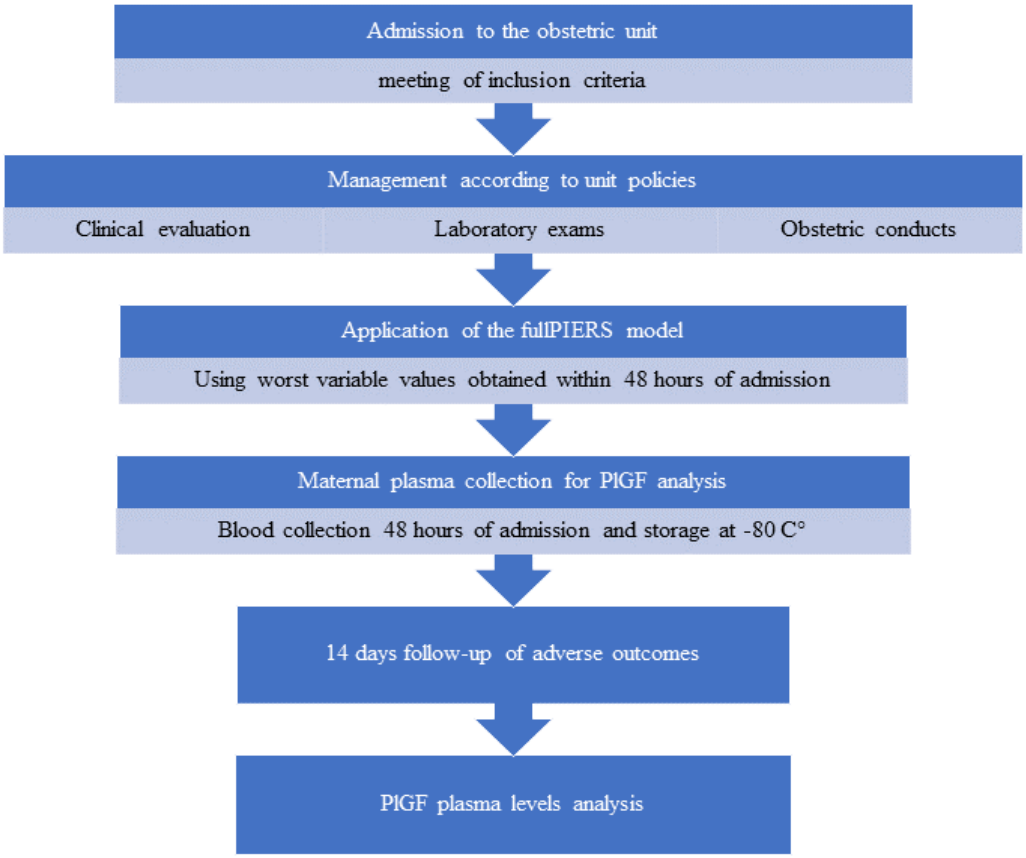
2.1.6.2 fullPIERS model application

The study database was completed based on direct questioning by the study team and review of electronic medical records. Biochemical variables were part of the medical management of pregnant women admitted with suspected and / or confirmed diagnosis of PE.

Oxygen saturation by pulse oximetry (SpO2), one of the studied variables, is not part of the usual evaluation of pregnant women. For women in whom the variable was not assessed, we used a pre-established SpO2 value of 97%. Assuming that the patients in whom this resource was not used presented better clinical status.

The application of the fullPIERS model was performed through the linear predictor provided by the main study, exemplified in Chart 2.

Figure 5 – Study procedures flow-chart



Source: Escouto (2018). fullPIERS, preeclampsia integrated estimate of risk. PlGF, placental growth factor.

Chart 2 – fullPIERS model linear predictor

$$\begin{aligned}\text{Logit}(pi) = {} & 2.68 + (-5.14 \times 10^{-2}\ \textbf{gestational age at admission}) \\ & + 1.23\ (\textbf{chest pain or dyspnea}) \\ & + (-2.71 \times 10^{-2}\ \textbf{creatinine}) + (2.07 \times 10^{-1}\ \textbf{platelets}) \\ & + (4.0 \times 10^{-5}\ \textbf{platelets}^2) + (1.01 \times 10^{-2}\ \textbf{AST}) \\ & + (-3.05 \times 10^{-6}\ \textbf{AST}^2) + (2.50 \times 10^{-4}\ \textbf{creatinine} \times \textbf{platelets}) \\ & + (-6.99 \times 10^{-5}\ \textbf{platelets} \times \textbf{AST}) + (2.53 \times 10^{-3}\ \textbf{platelets} \times \textbf{SpO}_2)\end{aligned}$$

Source: Escouto (2018). Modified from von Dadelszen *et al.*, 2011. fullPIERS, preeclampsia integrated estimate of risk score. AST, aspartate aminotransferase. SpO2, oxygen saturation by pulse oximetry.

2.1.6.3 Plasma collection

Within the first 48 hours after hospital admission, 5 mL of blood was collected from each woman in addition to collection of routine exams. The BD Vacutainer® blood specimen collection containing ethylenediaminetetraacetic acid was used. Samples were taken to the Laboratory of Nephrology / School of Medicine / PUCRS, where they were processed and centrifuged at 20,000 rpm for 10 minutes at room temperature. Plasma was separated into centrifuge microtubes containing 0.6 mL and stored at -80 ° C. Processing took no more than 60 minutes after collection (Appendice A).

2.1.6.4 PlGF plasma concentrations analysis

After inclusion of all study subjects, maternal plasma concentrations of PlGF were measured by investigators blinded to patients' clinical data. We performed ELISA (Enzyme-Linked Immunoabsorbent Assay) immunoenzymatic assay with the Human PlGF Quantikine Kit (R & D Systems Inc, Minneapolis, MN-EUA). The method detects PlGF quantitatively by the solid phase ELISA method.

Recombinant human PlGF, obtained through *E. coli*, is used for the formation of anti-PlGF human-specific antibodies. These antibodies are pre-inserted into microplates that received samples and controls. After washing any unbound substances, an enzyme-linked polyclonal antibody specific for human PlGF was added to the wells. Streptavidin conjugated to horseradish peroxidase was used as substrate to hydrogen peroxide in order to add colour for spectrophotometric reading. The ELISA method is described as sandwich because it detects the antigen, PlGF in this case, between two antibodies – capture and detection. Figure 6 illustrates schematically the ELISA assay.

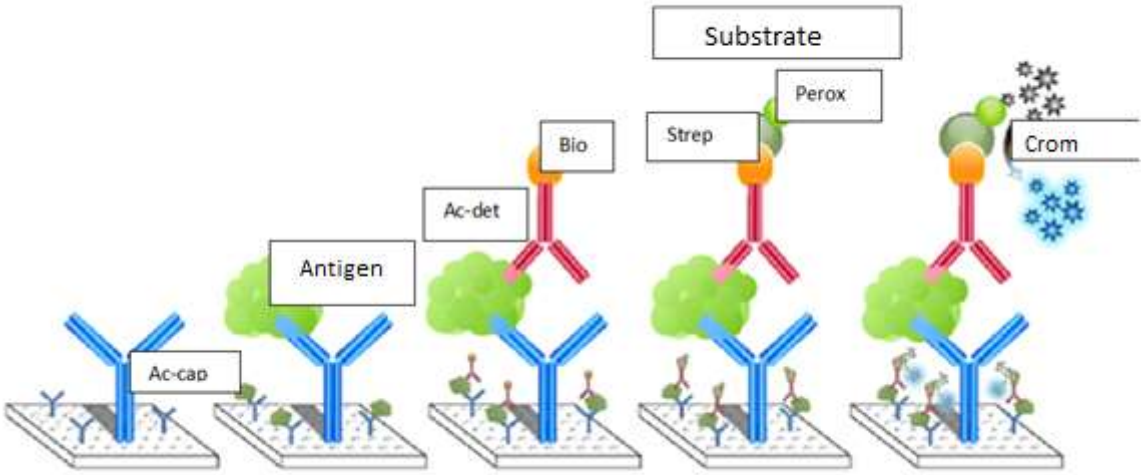
Assays were performed in accordance with manufacturer's recommendation. Protocol description is on Appendice D. Optic density of each sample was measured in 450 and 570 nm with ELISA plate reader (Spectramax®M2e, Molecular Devices, California, EUA). PlGF sample concentration was obtained by readings of the spectrophotometer and calibration curve values. Calibration curves had regression coefficients from 0.0018 to 0.0024. We performed calibration curves for all plates, even if assays were conducted at the same time.

Results are expressed as continuous values with minimum sensitivity of 7 pg / mL. Intra-assay and inter-assay coefficient of variation were below 10%. Concentration calculations considered the mean of duplicate readings applied to the formula:

$$y=ax+b$$

where **y** is the sample concentration, **a** is the R value related to calibration curve linearity and **b** is the start point of the calibration curve. All data were registered and analysed with Excel/ Microsoft Office 2016.

Figure 6 – ELISA sandwich assay scheme



Source: Escouto (2018) Adapted from EPITOMICS® Company Cat. 6112-1. Ac-cap, capture antibody; Ac-det, detection antibody; Bio, biotine; Strep, streptavidin; Perox, peroxidase; Crom, chromogen.

2.1.7 Statistical analysis

2.1.7.1 Sample size estimation

In order to evaluate the accuracy of the fullPIERS model and the PlGF values in the study population with an area under the ROC curve of 0.80, accepting a margin of error of less than 8%, at least 124 patients with HDP, of which at least 62 must present an adverse outcome. For comparison of 2 ROC curves, fullPIERS accuracy and PlGF accuracy, accepting a margin of error of less than 10% and a power of 80% to demonstrate a difference of 0.1, 111 patients with HDP were required. Sample size estimation was done using the Statstodo Online Program (Ltd) and with statistical-epidemiological supervision of Prof. Dr. Mario Wagner. After 150 patients included an interim analysis of adverse outcome was performed, due to low frequency of adverse outcomes we decided to increase the sample size to 400 women.

2.1.7.2 Data analysis

Quantitative variables were presented as mean ± standard deviation (SD) or median and interquartile range (IQR) as appropriate, after normality test. Differences between groups were evaluated using student's T test or rank sum test depending on the distribution of the data. For categorical variables, the Chi-square test or Fisher's exact test were applied.

Predicted probabilities of adverse outcomes within 48h, 7 days and up to 14 days were calculated with the fullPIERS model prediction equation. In order to assess the model capability to differentiate women at high risk of adverse outcomes, stratification capacity, calibration ability and classification accuracy were evaluated by the use of a risk stratification table. Likelihood ratios (LR) were calculated for multicategory diagnostic test, LR above 10 and below 0.1 were considered informative; LR between 0.1-0.2 or 5-10 were considered moderately informative; and non-informative if LR were between 0.2-0.5.

Discrimination was evaluated by the calculation of the AUC ROC with 95% confidence interval using consecutive cut-offs for the probability of combined adverse maternal outcomes within 48h, seven days and up to 14 days after admission.

Discrimination was interpreted as: non-informative (AUC<0.5); poor (0.5 >AUC ≤ 0.7); moderate (0.7 > AUC ≤ 0.9); high (> 0.9 AUC <1); and perfect (AUC=1).

A calibration plot was generated. Since adverse outcome is a dichotomous variable, the loess algorithm was used as a smoothing technique to estimate the observed probability (Austin e Steyerberg, 2014). Calibration was also assessed by evaluation of the linear predictor slope and intercept obtained after application of the fullPIERS model in our dataset. A well-calibrated model should have a slope equal to 1 (Debray *et al.*, 2015).

Bivariate analysis of candidate predictive outcome variables was carried out and variables associated with the outcome ($p<0.25$) and variables considered clinically important by the researchers were included in the multivariate analysis (Hosmer e Lemeshow, 2000). Non-linearity of continuous variables relationship with outcome was assessed and categorization or transformation were performed when appropriate. Stepwise backward elimination was used to build a model of adverse outcome prediction. Collinearity was checked and only the more clinically relevant variable between two highly correlate variables was kept. Clinically possible interactions were also evaluated. Model performance was measured by discrimination accuracy and calibration ability. We used the default 1,000 bootstrap replications to obtain confidence intervals for our parameters.

For PlGF plasma concentration analysis, women were classified in three groups according to GA at inclusion, <35, 35 to 36+6, and ≥37 weeks. The test results were classified as normal (≥5th percentile for GA), low (<5th percentile), and very low (<12 pg/mL). PlGF concentration <5th percentile for GA was calculated from a previous study that included 247 women with healthy pregnancies between 20 and 40 weeks (Saffer *et al.*, 2013).

Predicted probabilities of adverse outcomes within 48h, 7 days and up to 14 days were calculated and test performance was evaluated as sensitivity, specificity, positive and negative predictive values, positive and negative likelihood ratios, and receiver operating characteristics (ROC) areas. We also evaluated PlGF performance to predict delivery within 14 days and a newborn SGA.

Logistic regression was used to consider whether the utility of PlGF was limited to women delivering SGA children. Comparison of PlGF with the fullPIERS model and other tests (SBP, DBP, proteinuria, uric acid, ALT) was performed for combined adverse outcome by using unadjusted PlGF concentrations. The 4 tests, excluding

proteinuria, which adds to confirmation of diagnosis, were combined into a single predictor by using logistic regression, and ROC areas were compared for the prediction of the combined adverse outcome.

Analysis were performed using Stata 12 (StataCorp. 2011.Stata Statistical Software: Release 12. College Station, TX: StataCorp LP) and MS Excel (Microsoft Excel 2016 for Windows, released 2016, Redmond, WA, USA: Microsoft).

2.1.8 ETHICS

Study protocol was approved by Research Ethics Committee of Hospital São Lucas, number 1.143.057 (ANNEX A). The selection of patients was restricted to inclusion and exclusion criteria. The patients' approach and obtaining the consent term was performed by trained personnel, ensuring participants understanding of the study and their willingness to participate.

Possible risks and discomforts caused by the study (interview period, use of medical records, risks of blood collection) were explained to the participant. We also offered follow-up at the postpartum outpatient clinic for women who experienced HDP at HSL- PUCRS.

The authors agreed to keep the revised data confidential.

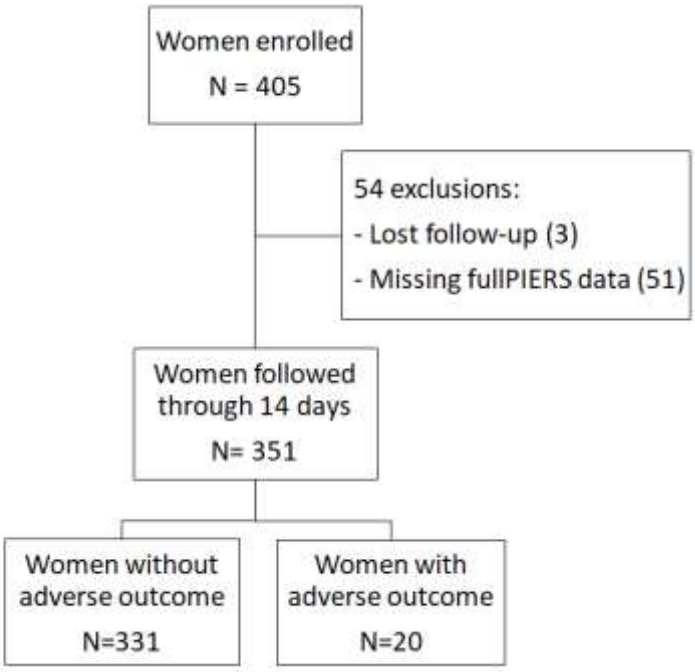
Results

2.2 RESULTS

2.2.1 fullPIERS model

A total of 405 women were included in the study between March of 2015 to December of 2016. Three participants were lost to follow-up and 51 (13%) women did not have all the necessary data to apply the fullPIERS model. In total 351 women participated in the study and 20 (5%) developed at least one event of the maternal adverse outcomes defined (Figure 7). Table 1 shows demographic, clinical and laboratory characteristics of women with and without adverse outcomes.

Figure 7 – Study flow chart



Source: Escouto (2018). fullPIERS indicates preeclampsia integrated estimate of risk score

Table 1 - Characteristics of women with and without adverse outcome within 14 days of admission

	Total 351	Without n=331	With n=20	*P*
Maternal age at admission (years), median (IQR)	351	29.0 (22-34)	28.0 (22-32)	0.407
Gestational age at admission (weeks), mean (SD)	351	35.8 (3.8)	32.9 (5.5)	0.002
Gestational age at admission <34 weeks, n (%)	351	80 (24)	9 (45)	0.038
Race, white, n (%)	348	193 (59)	11 (55)	0.735
Medical visits during pregnancy, median (IQR)	349	8 (6-10)	6 (5-8)	0.034
Smoking, n (%)	351	42 (13)	2 (13)	0.999
Parity ≥1, n (%)	350	187 (57)	15 (75)	0.161
Previous history of PE, n (%)	350	40 (12)	5 (25)	0.157
Gestational diabetes mellitus, n (%)	350	14 (4)	0	-
Multiple pregnancy, n (%)	351	14 (4)	1 (5)	0.606
Highest pregnancy SBP (mmHg), mean (SD)	340	143.5 (18.5)	137.2 (29.6)	0.384
Anti-hypertensive drug use at admission	351	97 (29)	7 (35)	0.588
HDP classification				
Gestational hypertension	77	76 (23)	1 (5)	0.090
Preeclampsia	170	157 (47)	13 (65)	0.127
Chronic hypertension	43	41 (12)	2 (10)	0.999
Superimposed preeclampsia	61	57 (17)	4 (20)	0.761
Clinical data within 48 hours of admission				
Systolic blood pressure (mmHg), mean (SD)	351	151.0 (18.2)	151.6 (19.0)	0.892
Diastolic blood pressure (mmHg), mean (SD)	351	91.9 (14.1)	93.9 (14.0)	0.537
Severe hypertension*, n (%)	351	84 (25)	6 (30)	0.646
Pulse oximetry (%), mean (SD)	351	97.3 (1.6)	97.9 (1.5)	0.174
Haemoglobin (mg/dL), mean (SD)	351	11.9 (1.2)	11.2 (1.6)	0.044
Platelet count (10^9/L), median (IQR)	351	209.5 (65.0-223.6)	149.8 (66.2-221.9)	0.057
International normalized ratio, median (IQR)	320	1.0 (0.9-1.0)	1.0 (0.9-1.0)	0.937
Fibrinogen (mmol/L), mean (SD)	308	488.8 (95.6)	443.4 (120.0)	0.069
Activated partial thromboplastin time (s), median (IQR)	319	27.2 (25.6-29.0)	27.2 (26.0-28.9)	0.846

Creatinine(mg/dL), mean (SD)	351	0.78 (0.18)	0.93 (0.38)	0.100
	Total 351	Without n=331	With n=20	*P*
Uric acid(mg/dL), mean (SD)	351	5.02 (1.39)	6.00 (1.46)	0.002
Total bilirubin (mg/dL), mean (SD)	311	0.65 (0.27)	0.75 (0.29)	0.171
Aspartate transaminase (U/L), mean (SD)	351	22.0 (18.0-28.0)	27.5 (23.0-49.5)	0.004
Alanine transaminase (U/L), mean (SD)	335	24 (19-29)	27.5 (23-61.5)	0.015
Lactate dehydrogenase (U/L), mean (SD)	346	503 (435-592)	663 (496-747)	<0.001
Urinary protein: creatinine ratio, median (IQR)	341	0.39 (0.16-1.09)	1.37 (0.40-4.33)	0.009
Interventions				
Corticosteroid administration, n (%)	346	54 (17)	10 (53)	<0.001
Magnesium sulphate administration, n (%)	347	67 (20)	13 (65)	<0.001
Gestational outcomes				
Admission-to-delivery interval (days) median (IQR)	347	2 (1-7)	1 (0-5.5)	0.184
Gestational age at delivery (weeks), mean (SD)	348	37.0 (3.0)	33.7 (4.9)	0.008
Gestational age at delivery < 37 weeks, n (%)	348	108 (33)	13 (65)	0.003
Hospital stay (days), median (IQR)	351	4 (3-8)	5.5 (3-13)	0.296
Caesarean section delivery, n (%)	347	160 (49)	18 (90)	<0.001
Caesarean section by maternal condition, n (%)	177	55 (34)	13 (76)	0.001
Birth weight (g), median (IQR)	347	2915 (2395-3340)	2208 (1273-2908)	0.003
Small for gestational age, n (%)	344	48 (15)	5 (26)	0.189
Perinatal death, n (%)	347	7 (2)	2 (10)	0.089

Source: Escouto (2018). Student's T test, Wilcoxon rank sum test and Chi-squared test were used to calculate differences between groups according to variable characteristic and distribution. IQR, interquartile range. PE, preeclampsia. SD, standard deviation. * Severe hypertension, systolic blood pressure >160 and/or diastolic blood pressure > 110.

Mean GA at admission was lower in women with adverse outcomes than in women without adverse outcomes. No differences were disclosed in the incidence of adverse outcomes between groups of HDP. The use of anti-hypertensive treatment at

admission was similar between groups that subsequently developed adverse outcomes. There were no differences between systolic and diastolic blood pressures at admission between groups. Severe hypertension was present at admission in one third of women, and there was no significant difference among groups (Table 1).

Uric acid was higher in women that presented adverse outcomes (6.00 ±1.46 md/dL) than in women without (5.02 ± 1.39 mg/dL) (P=0.002). AST had higher concentrations in the adverse outcome group, 27.5 (23-50) U/L, versus 22.0 (18-28) U/L (P=0.004). Also, urinary protein to creatinine ratio was higher amongst women who developed adverse outcome (0.39 [0.16-1.09] versus 1.37 [0.40-4.33], P=0.009).

Admission-to-delivery interval was similar between groups. Ninety percent of women in the adverse outcome group underwent a caesarean section, while almost 50% of women without adverse outcome also had caesarean section. Indication for the surgical delivery was due to maternal health status in 55 (34%) women without adverse outcome and 13 (76%) of women with adverse outcome (P<0.001). Although the proportion of perinatal deaths was higher among pregnancies with adverse outcome (10%) than in those without adverse outcomes (2%), no statistical significant difference was found.

Median eligibility-to-outcome interval was less than one day after admission (0-2 days). Table 2 shows maternal adverse outcomes according to time after admission. The most common outcome was transfusion of any blood component, 6 cases (24%), followed by 4 (16%) cases of placental abruption. No cases of maternal mortality occurred. Eclampsia occurred in 3 women, only 1% of women who experienced PE and superimposed preeclampsia. HELLP syndrome was not part of combined adverse maternal outcome, 10 (3%) women had a diagnosis of HELLP syndrome, four (20%) were on the adverse outcome group whereas 6 (2%) were on the no adverse outcome group (P<0.001).

Table 2 - Maternal adverse outcomes in women admitted in the obstetric unit, according to time of occurrence

Adverse outcome	48 hours	7 days	14 days
Total	17	24	25
Maternal mortality	0	0	0
CNS			
Eclampsia, n (%)	2 (12)	2 (8)	3 (12)
PRES, n (%)	0	1 (4)	1 (4)
Cardiovascular			
Use of inotropic agents, n (%)	1 (6)	3 (13)	3 (12)
Third parenteral antihypertensive, n (%)	1 (6)	2 (8)	2 (8)
Acute pulmonary edema, n (%)	1 (6)	1 (4)	1 (4)
Hematological			
Transfusion of any blood component, n (%)	5 (29)	6 (25)	6 (24)
Hepatic			
Hepatic dysfunction, n (%)	1 (6)	1 (4)	1 (4)
Liver hematoma, n (%)	0	1 (4)	1 (0)
Renal			
Acute renal injury (creatinine 1.7-2.3 mg/dL), n (%)	2 (12)	2 (8)	2 (8)
Obstetric			
Placental abruption, n (%)	3 (18)	4 (17)	4 (16)
Uterine rupture, n (%)	1 (6)	1 (4)	1 (4)

Source: Escouto (2018). CNS, central nervous system; PRES, posterior reversible encephalopathy syndrome.

2.2.1.1 Accuracy of the fullPIERS model as predictor of maternal adverse outcomes

Within 48h of admission, the fullPIERS model predicted adverse maternal outcome with poor discrimination, AUC 0.639 (0.458-0.819 95% CI). Seven days after admission, the model predicted adverse outcomes with lower accuracy, AUC 0.612 (0.440-0.783 95% CI). Within 14 days after inclusion, the discriminative ability of the model was similar to previous time points, AUC 0.637 (0.491-0.783 95%CI)

Table 3 shows the risk stratification of fullPIERS model according to risk strata using the predictive probability intervals employed at the fullPIERS study. For prediction of adverse outcomes after 48h of admission, 231 (66%) women were

categorized in the low-risk group (predicted probability < 10%), and only 6 (3%) had an adverse maternal outcome, NPV 0.93. Five (1%) women were categorized in the high-risk group (predicted probability ≥30%), and one (20%) had an adverse maternal outcome, PPV 0.2. For prediction of adverse outcomes within 7 and 14 days of admission, the model maintained a NPV above 90% for low-risk cut-off and a PPV of 0.2 for women with a predictive risk ≥30%.

fullPIERS model risk stratification LR at 10-20% predicted probability stratum was useful to predict adverse outcomes from seven to 14 days after admission. Most women with adverse outcomes felt into that stratum, LR for adverse outcome were 16.6 (15-18) and 12.4 (11-14) for 7 and 14 days after admission, respectively. They provide strong evidence to predict the occurrence of adverse maternal outcomes. The evidence is not as strong with predicting adverse outcomes within 48h, however it moderately informs that women with predicted probability of 10-20% are 6 to 10 times more likely to have an adverse event within 48 hours.

The calibration performance of the fullPIERS model applied to the studied sample was poor, with a slope 0.35 (95% CI 0.08-0.62) and an intercept -1.13 (95%CI -2.4-0.14), representing inconsistency of estimation on extreme values.

In order to evaluate differences of regression coefficient between the study sample and the fullPIERS model development sample, we performed a logistic regression using the same predictor variables and interactions as the original model and compared both AUC ROC (Figure 7). When using coefficients from the studied sample the AUC ROC for adverse outcomes within 14 days of admission improved to 0.784 (95% CI 0.684-0.884) (P=0.031). Using a predicted probability cut-off of 0.05, the model correctly classified 70% of women at risk of adverse outcome within 14 days of admission. We also performed the analysis to predict adverse outcome at 48h (AUC ROC 0.746, 95% CI 0.630-0.863) and 7 days (AUC ROC 0.793, 95% CI 0.700-0.887) after admission.

When evaluating only women who developed PE and superimposed PE (n=231), the fullPIERS model maintained a poor discriminatory ability (AUC ROC 0.635, 95% CI 0.479-0.792). This ability increased after using study sample coefficients of fullPIERS model variables (AUC ROC 0.741, 95% CI 0.616-0.867), but there was no significant difference between both curves (*P*=0.120) (Figure 8).

Post-hoc analysis was conducted to evaluate this study's power for comparison to the development study sample. This study was underpowered to compare discriminatory ability to the development cohort. In order to have power of 80% with an alpha error of 0.05, we needed the occurrence of 29 adverse maternal outcomes. It was not possible to evaluate the power of the present study to compare calibration performance, because information on calibration was missing from the development cohort study.

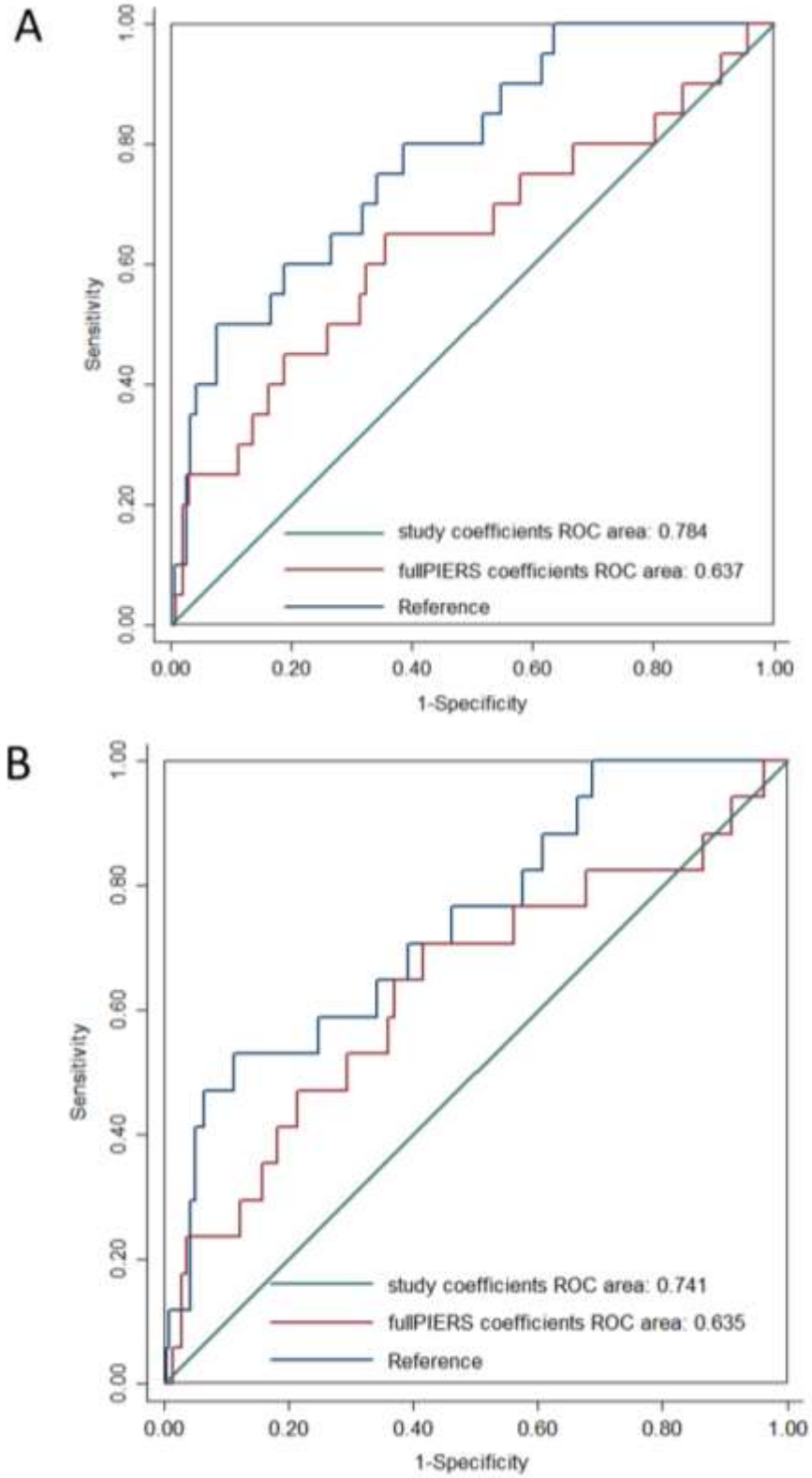
After bivariate analysis (Table 4), we developed a model of prediction of adverse outcomes within 14 days after admission using the studied sample data from 343 women. Variables included at the final model were GA at admission, race and worst LDH within 48 hours. The AUC ROC for adverse outcome within 14 days of admission, after bootstrap replications was 0.770 (95% CI 0.643-0.896). Classification accuracy of the model was good. Using a predicted probability cut-off of 0.058, the model correctly classified 74% of women at risk of adverse outcome within 14 days of admission. We also performed the analysis to predict adverse outcome at 48h (AUC ROC 0.710, 95% CI 0.541-0.880) and 7 days (AUC ROC 0.759, 95% CI 0.612-0.907) after admission. When evaluating only women with PE, the model lost discriminatory ability within 48h of admission (AUC ROC 0.675, 95% CI 0.460-0.890) and after 7 days (AUC ROC 0.734, 95% CI 0.549-0.919). The comparison of AUC ROC between local model and fullPIERS model applied to the studied sample, did not achieve statistical significant difference at any time point.

Table 3 – Risk stratification: fullPIERS performance by predicted probability of adverse outcome within 14 days

Predicted probability	N (%)	With n(%)	Without n(%)	LR (95% CI)	PPV (95% CI)	NPV (95% CI)
Adverse maternal outcome within 48h						
0.00-0.0099	231 (66)	6 (3)	225 (97)	0.6 (0.1-1.2)	0.03 (0.01-0.06)	0.93(0.9-0.97)
0.01-0.024	69 (20)	4 (6)	65 (94)	1.5 (0.6-2.3)	0.06 (0.02-0.15)	0.96(0.9-0.98)
0.025-0.049	30 (9)	0	30 (100)	-	-	-
0.050-0.099	8 (2)	1 (13)	7 (87)	3.4 (1.4-5.4)	0.1 (0.01-0.5)	0.99(0.98-0.99)
0.10-0.19	7 (1.7)	2 (29)	5 (71)	9.6 (8-11)	0.3 (0.05-0.7)	0.97(0.9-0.98)
0.20-0.29	1 (0.3)	0	1 (100)	-	-	-
≥0.30	5 (1)	1 (20)	4 (80)	6.0(3.8-8)	0.2 (0.01-0.7)	0.96(0.9-0.98)
Total	351	14	334			
Adverse maternal outcome within 7 days						
0.00-0.0099	231 (66)	7 (3)	224 (97)	0.7 (0.1-1.2)	0.03 (0.01-0.06)	0.93(0.9-0.99)
0.01-0.024	69 (20)	4 (6)	65 (94)	1.4 (0.5-2.2)	0.06 (0.02-0.1)	0.96(0.9-0.98)
0.025-0.049	30 (9)	0	30 (100)	-	-	-
0.050-0.099	8 (2)	1 (13)	7 (87)	3.2 (1.1-5.2)	0.1 (0.01-0.5)	0.96(0.9-0.98)
0.10-0.19	7 (1.7)	3 (43)	4 (57)	16.6 (15.2-18)	0.4 (0.1-0.8)	0.97(0.9-0.98)
0.20-0.29	1 (0.3)	0	1 (100)	-	-	-
≥0.30	5 (1)	1	4 (100)	0.2 (0-9)	0.2 (0.01-0.7)	0.96(0.9-0.98)
Total	351	16	336			
Adverse maternal outcome within 14 days						
0.00-0.0099	231 (66)	8 (3)	223 (97)	0.6 (0.05-1.1)	0.03 (0.02-0.07)	0.9 (0.8-0.94)
0.01-0.024	69 (20)	6 (9)	63 (91)	1.6 (0.9-2.3)	0.09 (0.04-0.2)	0.95(0.9-0.97)
0.025-0.049	30 (9)	1 (3)	29 (97)	0.6 (0-2.5)	0.03 (0.002-0.2)	0.94(0.9-0.96)
0.050-0.099	8 (2)	1 (13)	7 (87)	2.4 (0.3-4.4)	0.13 (0.01-0.53)	0.94(0.9-0.97)
0.10-0.19	7 (1.7)	3 (43)	4 (57)	12.4 (11-13.8)	0.43 (0.12-0.8)	0.95(0.9-0.97)
0.20-0.29	1 (0.3)	0	1 (100)	-	-	-
≥0.30	5 (1)	1 (20)	4 (80)	4.1 (2-6.3)	0.2 (0.01-0.7)	0.95(0.9-0.97)
Total	351	20	331			

Source: Escouto (2018). CI indicates confidence interval. fullPIERS preeclampsia integrated estimate of risk. LR, likelihood ratio; NPV. Negative predictive value. PPV, positive predictive value.

Figure 8 – ROC curves for differences of regression coefficients between the studied sample and the fullPIERS model development sample



Source: Escouto (2018). A. ROC curves for all women. B. Roc curves for women with PE and SPE. fullPIERS indicates preeclampsia integrated estimate of risk. ROC receiving operator characteristics.

Table 4 - Bivariate analysis

	n	OR (95% CI)	*P*	LR test
Ethnicity*				
White	348	1.44 (0.6-10.7)	0.19	1.92
Others		2.58 (0.4-5.3)	0.58	
Primigravida, n (%)	350	0.44 (0.15-1.23)	0.12	2.75
Previous history of PE, n (%)	350	2.12 (0.83-7.0)	0.10	2.31
Preeclampsia	351	2.06 (0.8-5.3)	0.13	2.36
Nausea/vomiting	351	2.60 (1.05-6.45)	0.04	4.12
Headache	350	2.14 (0.80-5.71)	0.13	2.5
Scotomas	351	2.36 (0.93-5.98)	0.07	3.03
	n	Coefficient	SE	*P*	LR test
Maternal age at admission (years), log scale	351	-0.72	0.82	0.37	0.79
Gestational age at admission (weeks), cubic scale	351	-0.01	0.01	0.01	7.67
Systolic blood pressure (mmHg)	351	0.002	0.01	0.89	0.02
Diastolic blood pressure (mmHg)	351	0.010	0.02	0.54	0.38
Pulse oximetry (%)	351	0.23	0.17	0.16	2.14
Hemoglobin (mg/dL), mean (SD)	351	-0.36	0,18	0.04	3.98
Platelet count (10^9/L), n/□L	351	-0.23	0.10	0.03	5.16
Fibrinogen (mmol/L)	308	-0.006	0.003	0.07	3.65
Creatinine (mg/dL)	351	-4.28	1.78	0.02	5.64
Uric acid (mg/dL)	344	0.48	0.16	<0.01	8.86
Total bilirubin (mg/dL)	311	2.11	1.39	0.13	2.24
Aspartate transaminase (U/L)	351	-44.75	14.29	0.002	10.10
Alanine transaminase (U/L)	335	-39.35	15.25	<0.01	7.02
Lactate dehydrogenase (U/L)	346	-1571.2	437.7	<0.01	12.86
Urinary protein: creatinine ratio	341	0.35	0.14	0.013	6.15

Source: Escouto (2018). Bivariate analysis was obtained through Logistic regression. * Black is the reference category; OR: odds ratio; LR: likelihood ratio; PE: preeclampsia; SD: standard deviation; SE: standard error. Creatinine, aspartate and alanine transaminases and lactate dehydrogenase are expressed in inverse scale. Total bilirubin is expressed in square root scale. Urinary protein: creatinine ratio is expressed in log scale.

2.2.1.2 Accuracy of the fullPIERS model as predictor of neonatal adverse outcomes

A secondary analysis of fullPIERS model ability to predict neonatal adverse outcomes within 14 days of admission was performed. Perinatal outcome was defined as presence of one or more of the following: 1) perinatal death; 2) SGA infant; or 3) gestational age (GA) at birth <34 weeks. Eighty-one (23%) cases of combined perinatal adverse outcomes occurred. SGA was the commonest event, 53 (66%) cases, followed by GA at delivery lower than 34 weeks, 44 (54%) events. One stillbirth and eight neonatal deaths were observed. Table 5 presents perinatal adverse outcomes distribution.

Table 5 - Perinatal adverse outcomes in women admitted with HDP in the obstetric unit, divided by presence of adverse maternal outcomes

	Total 347	Without n=327	With n=20	*P*
Transient tachypnea of the newborn, n (%)	17 (4.8)	13 (3.9)	4 (20)	0.012
Pulmonary haemorrhage, n (%)	1 (0.3)	0	1 (5)	
Respiratory distress syndrome of the newborn, n (%)	5 (1.4)	5 (1.5)	0	
Necrotizing enterocolitis, n (%)	1 (0.3)	1 (5)	0	
Sepsis, n (%)	4 (1.1)	3 (0.9)	1 (5)	0.212
Perinatal death				
Neonatal death, n (%)	8 (2.3)	6 (1.8)	2 (10)	0.072
Stillbirth, n (%)	1 (0.3)	1 (0.3)	0	

Source: Escouto (2018).

Risk stratification table for fullPIERS's ability to predict perinatal adverse outcomes by predicted probability thresholds was built (Table 6). Overall, LR for multicategory diagnostic test was non-informative. However, from a predicted probability of 5%, LR were moderate informative. In other words, women who

experienced perinatal adverse outcomes were 5 to 10 times more likely to have ≥5% predicted risk than women without outcome. PPV and NPV were also more informative from 5% probability threshold (Table xx). Discriminative ability of the fullPIERS model to predict perinatal adverse outcomes was also moderate, AUC ROC was 0.753 (95% CI, 0.687-0.819) after bootstrap replications.

Table 6 – Risk stratification: fullPIERS performance by predicted probability of perinatal adverse outcomes within 14 days

Predicted probability	N (%)	With (%)	Without (%)	LR (95% CI)	PPV (95% CI)	NPV (95% CI)
0.00-0.0099	228 (66)	37 (46)	191 (72)	0.63 (0.4-0.9)	0.16 (0.1-0.2)	0.62 (0.5-0.7)
0.01-0.024	67 (19)	21 (26)	46 (17)	1.49 (1.0-1.9)	0.31 (0.2-0.4)	0.78 (0.7-0.8)
0.025-0.049	30 (9)	10 (12)	20 (8)	1.63 (0.1-2.3)	0.33 (0.2-0.5)	0.77 (0.7-0.8)
0.050-0.099	8 (2)	6 (7)	2 (1)	9.78 (8.2-11.4)	0.75 (0.4-0.96)	0.78 (0.7-0.8)
0.10-0.19	6 (2)	4 (5)	2 (1)	6.52 (4.8-8.2)	0.67 (0.2-0.9)	0.77 (0.7-0.8)
0.20-0.29	1 (0.3)	0	1 (0.4)	-	-	-
≥0.30	5 (1.7)	3 (4)	2 (0.6)	4.89 (3.1-6.7)	0.60 (0.2-0.9)	0.77 (0.7-0.8)
Total	345	81	264			

Source: Escouto (2018). CI indicates confidence interval. fullPIERS, preeclampsia integrated estimate of risk. LR, likelihood ratio. NPV. Negative predictive value. PPV, positive predictive value.

2.2.2 Placental growth factor

From 405 women that entered the study, 392 (97%) patients were included in the PlGF analysis. Demographical and clinical characteristics are displayed at Table 7 according to GA at inclusion. Maternal age at admission was higher in women with GA lower than 37 weeks.

Women presenting before 35 weeks significantly differed from women presenting after 37 weeks on characteristics associated with disease severity. Higher levels of creatinine, ALT, AST, LDH, random urine protein-to-creatinine ratio, as well as higher values of the fullPIERS predictive scores (Table 7). Previous history of PE was also higher among women presenting before the 37th week of pregnancy. Surprisingly, blood pressure levels and presence of severe hypertension was similar between groups of GA at inclusion (Table 7).

Seventy-one percent of women were previously normotensive. The majority (45% of included women) had a diagnosis of PE. Most women with GH presented after 37 weeks of pregnancy. Distribution of PE women was similar between GA groups. PE superimposed to previous hypertensive women, however was higher among women included before GA of 37 weeks (Table 7).

Figure 9 displays PlGF mean concentrations according to classification of HDP. Median PlGF plasma concentrations were lower in women with PE (89.6 pg/mL; IQR: 41.6-203.1) when compared to GH (155.6 pg/mL; IQR: 82.9-237.5) and CH (158.1 pg/mL; IQR: 69.5-331.4) groups. Difference persisted after adjustment for GA (P<0.001 for GH and P=0.009 for CH). There were no differences in PlGF values between women with PE and SPE (126.5 pg/mL; IQR: 43.2-314.7; P=0.07). Also, SPE women showed similar PlGF plasma concentrations in comparison to women with GH (P=0.52) and CH (P=0.54). However, when evaluating only women included before 35 weeks of pregnancy, women with SPE had significant lower plasma concentration of PlGF, in comparison to women with CH (P=0.046) (Figure 10).

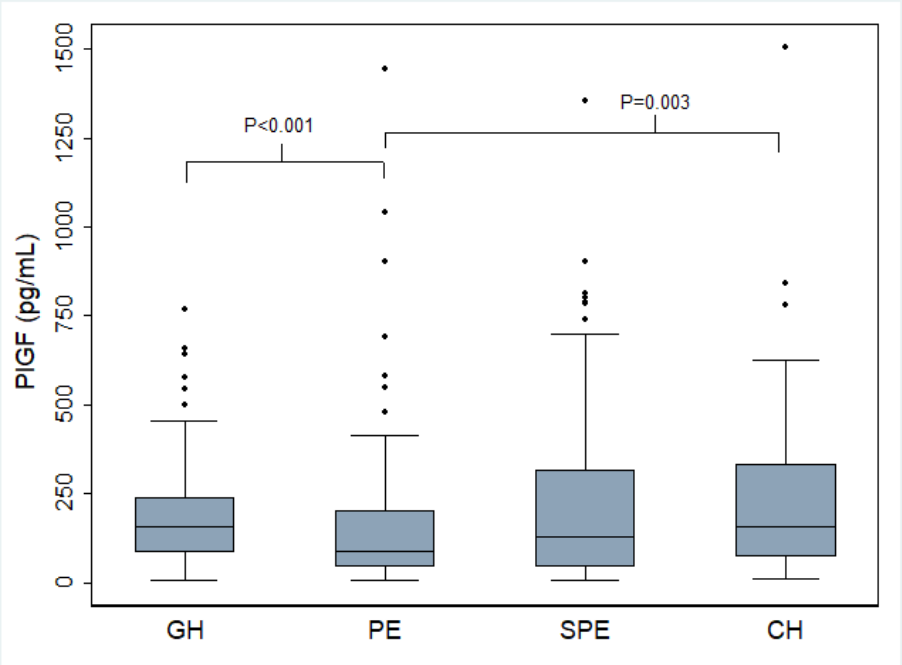
Table 7 – Characteristics of women with hypertensive disorders of pregnancy, according to gestational age at admission

	All N=192	<35 weeks N=119	35-36.6 weeks N=77	≥37 weeks N=196	*P*
Maternal age at admission (years), median (IQR)	29.0 (22-34)	31.0 (25-35) *	31.0 (23-38) §	27.0 (22-33)	<0.001
Race, white, n (%)	225 (58)	79 (66)	40 (52)	106 (55)	0.221
Smoking, n (%)	48 (12)	17 (14)	10 (13)	21 (11)	0.629
Parity ≥1, n (%)	167 (43)	39 (33) §	28 (36) ¶	100 (51)	0.003
Previous history of PE, n (%)	51 (13)	22 (18) §	15 (19) §	14 (7)	0.003
Gestational diabetes mellitus, n (%)	17 (4)	7 (6)	4 (5)	6 (3)	0.457
Multiple pregnancy, n (%)	17 (5)	14 (12) † *	2 (3)	1 (1)	<0.001
Anti-hypertensive drug use at admission	106 (27)	46 (39) §	17 (22) ¶	43 (22)	0.002
HDP classification					
Gestational hypertension	101 (26)	14 (12) †	12 (16) †	75 (38)	< 0.005
Preeclampsia	175 (45)	58 (49)	40 (52)	77 (39)	0.093
Chronic hypertension	56 (14)	19 (16)	9 (12)	28 (14)	0.705
Superimposed preeclampsia	60 (15)	28 (24) †	16 (21) §	16 (8)	<0.001
Clinical measures within 48 hours of admission					
Systolic blood pressure (mmHg), mean (SD)	149.8 (18.0)	154.8 (19.5)	148.4 (20.6)	148.6 (15.7)	0.099
Diastolic blood pressure (mmHg), mean (SD)	91.2 (11.8)	92.8 (89.0)	89.0 (15.1)	91.1 (11.7)	0.170
Severe hypertension, n (%)	154 (39)	53 (45)	32 (42)	69 (35)	0.233
Pulse oximetry (%), mean (SD)	98 (97-98)	98 (96-98)	98 (97-98)	97.5 (97-98)	0.464
Platelet count (10^9/L), median (IQR)	211.0 (169-255)	217.5 (169-260)	203.5 (159-258)	213.1 (174-253)	0.891
Fibrinogen (mmol/L), median (IQR)	471.0 (420-550)	458.0 (409-515)	496.0 (434-560)	472.5 (427-552)	0.226
Creatinine (mg/dL), mean (SD)	0.79 (0.2)	0.83 (0.2) ¶	0.79 (0.2)	0.77 (0.2)	0.035
Uric acid (mg/dL), mean (SD)	5.1 (1.5)	5.3 (1.6)	5.1 (1.4)	4.9 (1.2)	0.157
Total bilirubin (mg/dL), mean (SD)	0.65 (0.3)	0.63 (0.3)	0.64 (0.3)	0.67 (0.3)	0.500
Aspartate transaminase (U/L), median (IQR)	23 (18-29)	24 (19-33) § ‡	21 (18-28)	22 (17-27)	0.004
Alanine transaminase (U/L), median (IQR)	24 (19-27)	26 (21-35) §	24 (19-28)	23 (18-27)	0.011
Lactate dehydrogenase (U/L), median (IQR)	503.5 (434-574)	535 (441-647) ¶	495 (444-572)	535 (441-647)	0.048
Urinary protein: creatinine ratio, median (IQR)	0.38 (0.12-1.09)	0.55 (0.22-2.58) †	0.51 (0.22-1.09) §	0.27 (0.9-0.73)	<0.001

	All N=192	<35 weeks N=119	35-36.6 weeks N=77	≥37 weeks N=196	*P*
fullPIERS probability, median (IQR)	0.006 (0.002-0.014)	0.008 (0.003-0.019) *	0.006 (0.003-0.013)	0.004 (0.002-0.010)	0.010
Interventions					
Corticosteroid administration, n (%)	69 (18)	66 (57) # †	1 (1)	2 (1)	<0.001
Magnesium sulphate administration, n (%)	83 (21)	55 (47) † *	17 (23) †	11 (6)	<0.001
Gestational outcomes					
Admission-to-delivery interval (days) median (IQR)	1 (1-1)	8 (2-33) #, †	4 (1-7) †	1 (0-1)	<0.001
Gestational age at delivery (weeks), mean (SD)	37.0 (3.2)	33.8 (3.9) #, †	36.9 (1.0) †	38.9 (1.2)	<0.001
Hospital stay (days), median (IQR)	4 (1-8)	7 (4-11) †	7 (4-10) †	4 (3-5)	<0.001
Caesarean section delivery, n (%)	199 (51)	80 (68) ‡ †	40 (53)	79 (41)	<0.001
Caesarean section due to maternal condition, n (%)	70 (36)	43 (54) †	16 (40) ‡	11 (14)	<0.001
Birthweight (g), median (IQR)	2942.5 (2355-3380)	1947.5 (1353-2823) #, †	2810.0 (2415-3055) †	3225.0 (2915-3585)	<0.001
Small for gestational age, n (%)	56 (15)	32 (28) † ‡	10 (13)	14 (7)	<0.001
Perinatal death, n (%)	10 (3)	10 (9)	0	0	<0.001

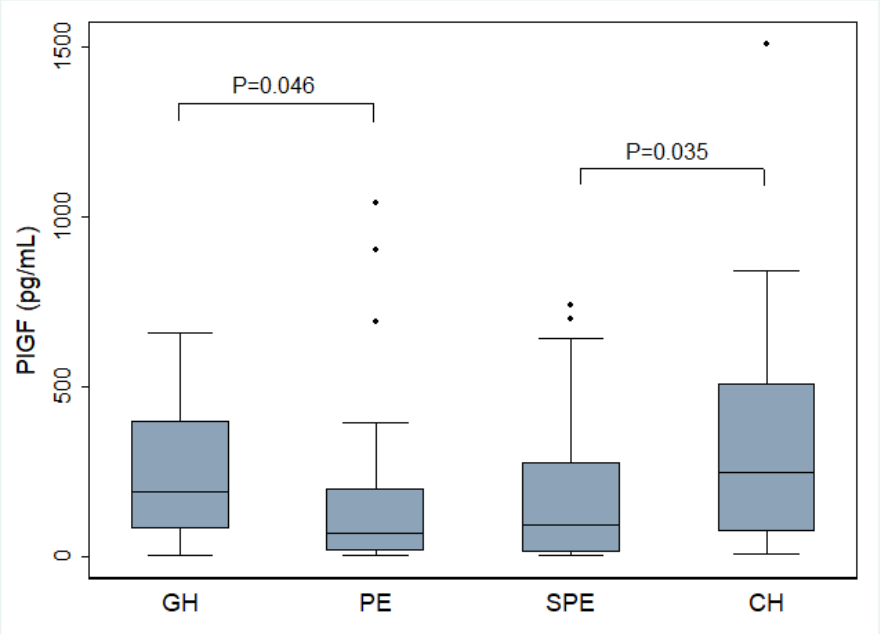
Source: Escouto (2018). ANOVA with Bonferroni corrections, Kruskal-wallis and Chi-squared tests were used to calculate differences between groups according to variable characteristic and distribution. fullPIERS: preeclampsia integrated estimate of risk (VON DADELDZEN, 2011). IQR, interquartile range. PE, preeclampsia. SD, standard deviation. Severe hypertension, systolic blood pressure >160 and/or diastolic blood pressure > 110. * $P < 0.01$ compared to 35 to 36+6 wk; § $P < 0.01$ compared to >37 wk; ¶ $P < 0.05$ compared to >37 wk; # $P < 0.001$ when compared to 35 to 36+6 wk; † $P < 0.001$ when compared to > 37 wk; ‡ $P < 0.05$ compared to 35 to 36+6 wk.

Figure 9 – Boxplot disclosing PlGF plasma concentrations according to Hypertensive Disorders of Pregnancy classification



Source: Escouto (2018). CH, chronic hypertension. GH, gestational hypertension. PE, preeclampsia. PlGF, placental growth factor. SPE, superimposed preeclampsia.

Figure 10 – Boxplot disclosing PlGF plasma concentrations according to Hypertensive Disorders of Pregnancy classification in women admitted to obstetric unit before 35 weeks of pregnancy



Source: Escouto (2018). CH, chronic hypertension. GH, gestational hypertension. PE, preeclampsia. PlGF, placental growth factor. SPE, superimposed preeclampsia.

2.2.2.1 PlGF and pregnancy outcomes

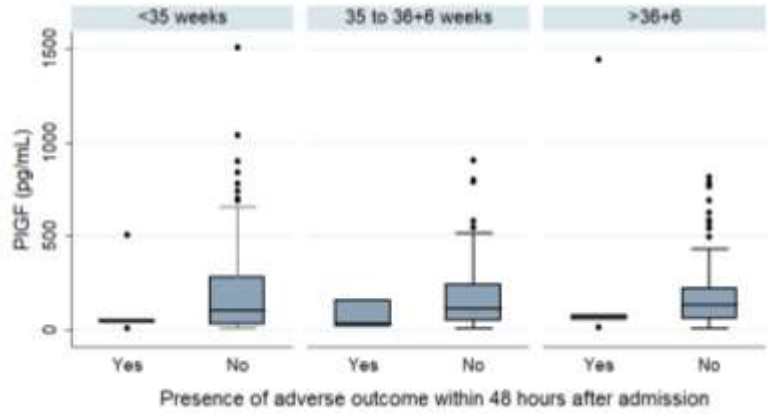
As expected, due to management of preterm pregnancies, corticosteroid administration, hospital stay and caesarean section deliveries were significantly higher in the group presenting before 35 weeks. Neonatal adverse outcomes, low median birthweight, SGA diagnosis and perinatal death, were associated with women with HDP presenting before 37 weeks (table xx). Median PlGF plasma concentrations were lower in pregnancies that delivered SGA infants (45.0 pg/mL; IQR: 12-108) *versus* pregnancies without SGA infants (132.3 pg/mL; IQR 58-265), $P<0.001$. Women presenting before GA of 35 weeks and with a SGA child had lower median PlGF plasma concentrations than those presenting at the same GA and without a SGA child (Figure 10). Concentrations of PlGF did not differ significantly in presence of SGA in women presenting ≥35 weeks of pregnancy.

Delivery within 14 days after inclusion was associated with lower PlGF concentrations (104.9 pg/mL; IQR: 46-205) *versus* delivery ≥14 days (234.1 pg/mL; IQR 93-438), $P<0.001$. Significant difference between PlGF plasma concentrations was present again only in women presenting before the 35th pregnancy week.

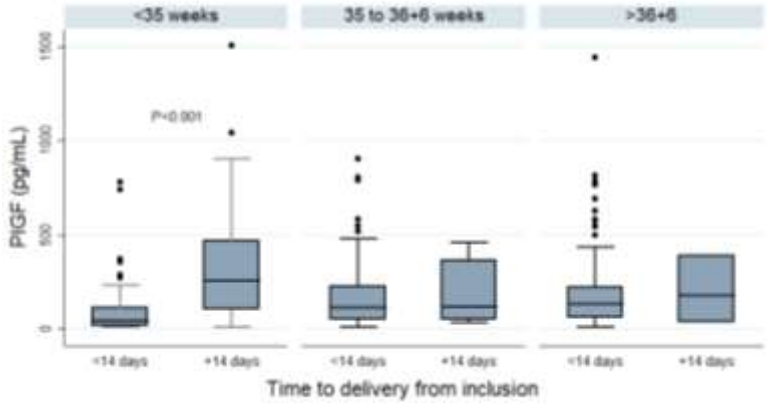
Presence of combined maternal adverse outcome occurred in 19 (5%) of women and 13 (68%)% events occurred within 48h of inclusion. Transfusion of any blood component was the commonest outcome (34% of events), followed by infusion of inotropic medication (26% of events). PlGF values was lower in women with presence of adverse outcomes, (46.8 pg/mL; IQR: 21-157) *versus* delivery ≥14 days (118.9 pg/mL; IQR 51-240), $P<0.014$. However, no significant statistical difference was found on PlGF values between presence or absence of adverse outcomes according to different groups of GA (Figure 11).

Figure 11 – Placental growth factor maternal concentrations at inclusion divided by gestational age categories, according to gestational outcomes

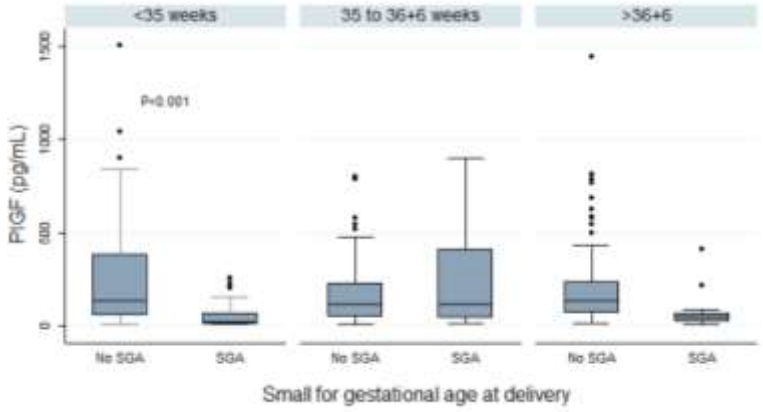
A



B



C



D		< 35 weeks	35-36.6 weeks	≥ 37 weeks
Delivery within 14 days of admission				
Yes		45.4 (12-114)	111.4 (46-227)	131.8 (58-222)
No		257.9 (102-466)	116.6 (49-366)	175.5 (31-391)
Maternal adverse outcome within 48h				
Yes		44.6 (42-53)	21.3 (21-57)	75.7 (54-76)
No		104.8 (29-285)	115.5 (51-241)	133.3 (58-222)
Small for gestational age	Yes	24.8 (8-71)	114.0 (51-119)	45.8 (20-72)
No		133.5 (55-382)	116.4 (43-412)	134.2 (66-235)

Source: Escouto (2018). A. Placental growth factor (PlGF) maternal concentrations by gestational age categories, according to presence of combined maternal adverse outcome. B PlGF according to time for delivery before or from 14 days after inclusion. C. PlGF according to presence of small for gestational age (SGA) newborn. D. PlGF plasma concentrations are

expressed as median (interquartile range) pg/mL.

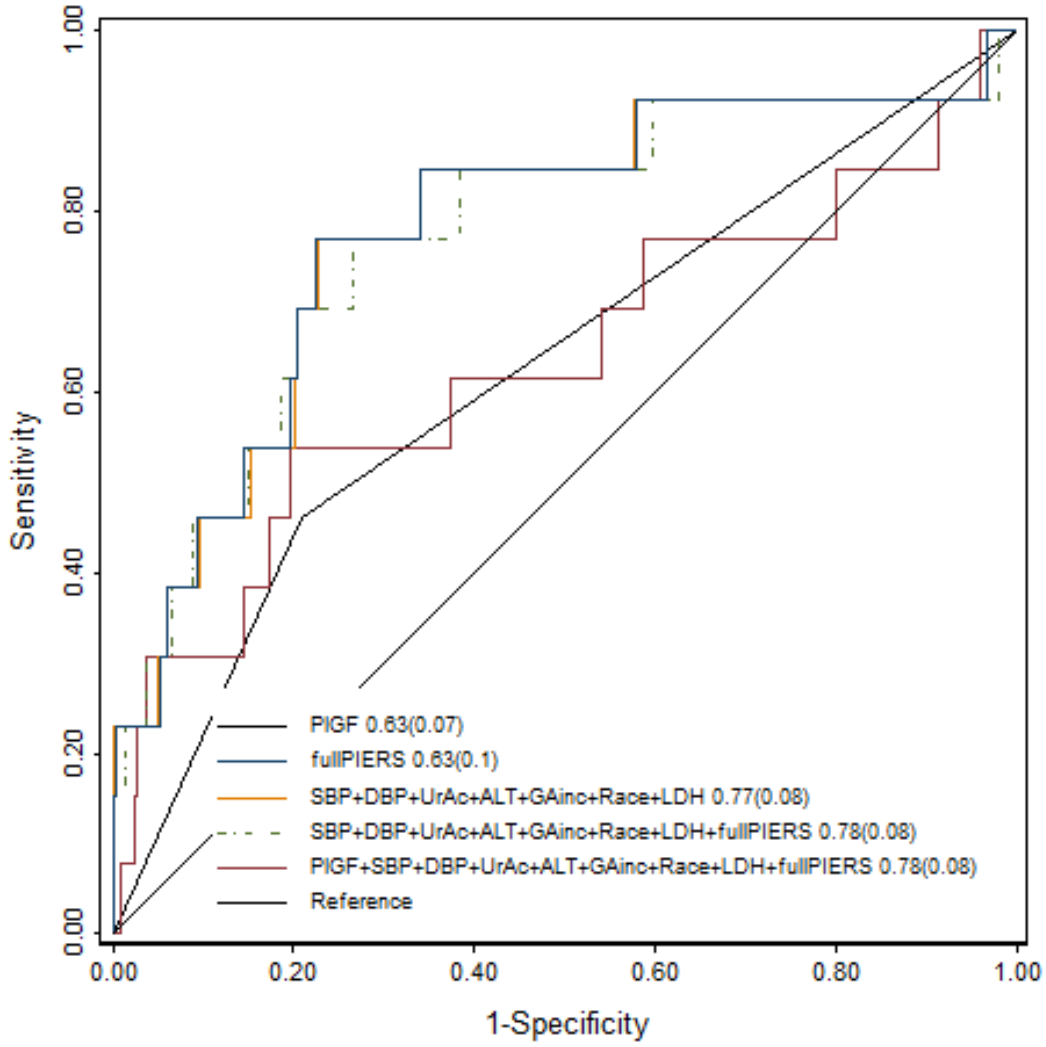
2.2.2.2 Accuracy of PlGF as predictor of adverse outcomes

Table 8 shows PlGF test performance to predict adverse outcomes at different cut-offs. Overall, PlGF's performance was modest. PlGF lower than the 5th percentile was the best threshold for prediction of maternal adverse outcomes within 48h in women before 35 weeks of pregnancy; with sensitivity of 0.80, NPV of 0.98 and AUC ROC of 0.672 (CI 95%0.5-0.9). Continuous values of PlGF, with a predicted probability of 5% had the best performance for prediction of maternal adverse outcomes at all GAs, with specificities from 74-99%, NPV from 0.97 to 0.98 and ROC areas between 0.629 to 0.747. Using the threshold of <100 pg/mL, best accuracy was obtained in women after 37 weeks of pregnancy, sensitivity of 0.8, specificity of 0.6, NPV of 0.99 and PPV of 0.04.

Logistic regression was performed and there was no indication that performance of PlGF <5th percentile to predict maternal adverse outcomes interacted with subsequent SGA newborns. Unadjusted OR 4.1 (CI 95% 1.3-12.5; *P* 0.01) and adjusted OR of 3.9 (CI 95% 1.1-13.9; *P* 0.03).

The area under the ROC curve for PlGF <5th percentile in predicting maternal adverse outcomes within 48h was moderate. When compared to standard tests used in HDP and independent predictors of maternal adverse outcomes resulted from our study, PlGF did not add to predictive accuracy. The fullPIERS model did not improve predictive ability as well, singly and in combination to other tests (Figure 12). Table 9 presents ROC areas for PlGF ability to discriminate maternal adverse outcomes within 48h in different threshold evaluated, singly and in comparison, to other tests. PlGF did not significantly improve predictive ability, singly or in combination to other tests in any of the determined thresholds. Table 9 also displays the comparison of AUC ROC of fullPIERS model plus PlGF.

Figure 12 – ROC areas of PlGF < 5th percentile compared with 8 other signs/ tests (fullPIERS model, systolic and diastolic blood pressure, uric acid, alanine transaminase, gestational age at inclusion, skin colour and lactate dehydrogenase) for prediction of maternal adverse outcomes within 48h in women with HDP



Source: Escouto (2018). ROC areas (standard error). Tests were measured singly or in combination. ALT indicates alanine transaminase. DBP, diastolic blood pressure. fullPIERS, fullPIERS model preeclampsia integrated estimate of risk (von Dadelszen, 2011). GAinc, gestational age at inclusion. HDP, hypertensive disorders of pregnancy. LDH, lactate dehydrogenase. PlGF, placental growth factor. ROC receiver operating characteristics. SBP, systolic blood pressure. UrAcid, uric acid.

Due to null values in some prediction tables cells, prediction of delivery within 14 days for continuous and below 5th percentile of PlGF could only be estimated for women included at GA below 35 weeks. PlGF had good performance to predict delivery at continuous and categorized values before 35 weeks. Continuous values of PlGF had higher AUC ROC for determination of delivery within 14 days than PlGF

categorized by 5th percentiles and <100 pg/mL in women before 35 weeks' gestation (Table 8). When logistic regression was performed, there was no indication of interaction between PlGF continuous values and small for GA to predict delivery within 14 days. Unadjusted OR 0.43 (CI 95% 0.32-0.58; *P*<0.001) and adjusted OR of 0.40 (CI 95% 0.29-0.55; *P*<0.001).

PlGF was also a good predictor of delivery of SGA new-born. PlGF <5th percentile predicted delivery of a SGA infant with sensitivity of 0.75, specificity 0.65, PPV of 0.45, NPV of 0.87, and AUC ROC 0.698, in women presenting before 35 weeks of pregnancy. Accuracy decreased at later GAs. PlGF threshold of <100 pg/mL predicted SGA newborn with sensitivity and NPV similar to diagnostic accuracy estimates obtained by using a <5th percentile cut off at 35 weeks. However, classification and discrimination abilities increased in women presenting after 37 weeks of pregnancy, with sensitivity of 0.86, specificity of 0.61, PPV of 0.15, NPV of 0.98, and AUC ROC 0.734 (Table 9).

Table 8 – Test performance of PlGF, according to gestational age at admission

	<35 weeks	35-36.6 weeks	≥37 weeks
	N=119	N=77	N=196
PlGF < 5th percentile	Maternal adverse outcomes within 48h		
Sensitivity	0.80 (0.4-0.96)	0.33 (0.1-0.8)	0.20 (0.04-0.6)
n/N	4/5	1/3	1/5
Specificity	0.54 (0.5-0.6)	0.91 (0.8-0.95)	0.96 (0.92-0.98)
n/N	62/114	67/74	184/191
Positive predictive value	0.07 (0.02-0.2)	0.13 (0.01-0.53)	0.13 (0.01-0.53)
n/N	4/56	1/8	1/8
Negative predictive value	0.98 (0.9-0.99)	0.97 (0.89-0.99)	0.98 (0.94-0.99)
n/N	62 (63)	67/69	184/188
Positive likelihood ratio	1.75 (1.1-2.8)	3.5 (0.6-20.3)	5.46 (0.8-36.4)
Negative likelihood ratio	0.37 (0.1-2.1)	0.74 (0.3-1.6)	0.83 (0.5-1.3)
AUC ROC	0.672 (0.47-0.87)	0.619 (0.29-0.95)	0.582 (0.39-0.78)
PlGF, continuous values (cut-off probability 0.05)	Maternal adverse outcomes within 48h		
Sensitivity	0.20 (0.03-0.62)	0.67 (0.2-0.9)	0.20 (0.04-0.62)
n/N	1/5	2/3	1/5
Specificity	0.75 (0.67-0.82)	0.74 (0.63-0.83)	0.99 (0.97-0.99)
n/N	86/114	55/74	190/191
Positive predictive value	0.03 (0.01-0.2)	0.10 (0.01-0.32)	0.5 (0.03-0.97)
n/N	1/29	2/21	1/2
Negative predictive value	0.97 (0.8-0.99)	0.98 (0.89-0.99)	0.98 (0.94-0.99)
n/N	86/90	55/77	190/194
Positive likelihood ratio	0.81 (0.14-4.84)	2.59 (1.0-6.3)	38.2 (0.5-1.2)
Negative likelihood ratio	1.06 (0.68-7.16)	0.45 (0.1-2.2)	0.8 (0.52-1.2)
AUC ROC	0.656 (0.48-0.83)	0.747 (0.39-1.0)	0.629 (0.30-0.96)
PlGF < 100 pg/mL	Maternal adverse outcomes within 48h		
Sensitivity	0.80 (0.4-0.96)	0.67 (0.2-0.9)	0.80 (0.4-0.96)
n/N	4/5	2/3	4/5
Specificity	0.52 (0.4-0.6)	0.54 (0.4-0.6)	0.59 (0.5-0.7)
n/N	59/114	34/74	113/191
Positive predictive value	0.07 (0.02-0.2)	0.06 (0.01-0.2)	0.04 (0.02-0.13)
n/N	4/59	2/36	4/82
Negative predictive value	0.98 (0.9-0.99)	0.97 (0.85-0.99)	0.99 (0.94-0.99)
n/N	59/60	40/41	113/114
Positive likelihood ratio	1.66 (1.0-2.7)	1.4 (0.6-3.4)	1.96 (1.2-3.1)
Negative likelihood ratio	0.39 (0.1-2.3)	0.62 (0.1-3.1)	0.34 (0.1-2.0)
AUC ROC	0.659 (0.46-0.86)	0.603 (0.27-0.93)	0.696 (0.50-0.89)

	<35 weeks N=119	35-36.6 weeks N=77 *	≥37 weeks N=196 *
PlGF <5th percentile		Delivery within 14 days	
Sensitivity	0.67 (0.5-0.8)	-	-
n/N	44/66		
Specificity	0.77 (0.6-0.9)	-	-
n/N	41/53		
Positive predictive value	0.79 (0.65-0.88)	-	-
n/N	44/56		
Negative predictive value	0.65 (0.52-0.76)	-	-
n/N	42/63		
Positive likelihood ratio	2.94 (1.7-5.0)	-	-
Negative likelihood ratio	0.43 (0.3-0.6)	-	-
AUC ROC	0.720 (0.64-0.80)	-	-
PlGF, continuous values (cut-off probability 0.5)		Delivery within 14 days	
Sensitivity	0.82 (0.7-0.9)	-	-
n/N	54/66		
Specificity	0.74 (0.6-0.8)	-	-
n/N	39/53		
Positive predictive value	0.79 (0.68-0.88)	-	-
n/N	54/68		
Negative predictive value	0.76 (0.62-0.87)	-	-
n/N	39/51		
Positive likelihood ratio	3.1 (1.9-4.9)	-	-
Negative likelihood ratio	0.25 (0.1-0.4)	-	-
AUC ROC	0.809 (0.72-0.89)	-	-
PlGF < 100 pg/mL		Delivery within 14 days	
Sensitivity	0.70 (0.6-0.8)	0.47 (0.35-0.6)	0.42 (0.35-0.49)
n/N	46/66	31/66	81/193
Specificity	0.76 (0.6-0.9)	0.55 (0.25-0.82)	0.67 (0.13-0.98)
n/N	40/53	6/11	2/3
Positive predictive value	0.78 (0.65-0.87)	0.86 (0.7-0.95)	0.99 (0.92-0.99)
n/N	46/59	31/36	81/82
Negative predictive value	0.67 (0.53-0.78)	0.15 (0.05-0.3)	0.02 (0.01-0.07)
n/N	40/60	5/41	2/114
Positive likelihood ratio	2.90 (1.8-4.8)	1.0 (0.5-2.1)	1.26 (0.3-6.3)
Negative likelihood ratio	0.40 (0.3-0.6)	0.97 (0.67-1.4)	0.87 (0.6-1.4)
AUC ROC	0.726 (0.64-0.81)	0.50	0.4

	<35 weeks N=119	35-36.6 weeks* N=77	≥37 weeks N=196
PlGF <5th percentile		Small for gestational age	
Sensitivity	0.75 (0.6-0.9)	0.1 (0.01-0.5)	0.29 (0.1-0.5)
n/N	24/32	1/10	4/14
Specificity	0.65 (0.5-0.7)	0.89 (0.78-0.95)	0.98 (0.94-0.99)
n/N	53/82	58/65	176/180
Positive predictive value	0.45 (0.3-0.6)	0.1 (0.01-0.5)	0.5 (0.2-0.8)
n/N	24/53	1/8	4/8
Negative predictive value	0.87 (0.75-0.94)	0.88 (0.5-0.99)	0.94 (0.9-0.97)
n/N	53/61	58/67	176/186
Positive likelihood ratio	2.12 (1.5-3.0)	0.93 (0.1-6.8)	12.86 (3.6-46.0)
Negative likelihood ratio	0.39 (0.2-0.7)	1.0 (0.8-1.2)	0.73 (0.5-1.0)
AUC ROC	0.698 (0.60-0.79)	0.5	0.632 (0.51-0.76)
PlGF, continuous values (cut-off probability 0.3)		Small for gestational age	
Sensitivity	0.66 (0.5-0.8)	-	0.30 (0.1-0.5)
n/N	21/32		4/14
Specificity	0.76 (0.7-0.8)	-	0.98 (0.95-0.99)
n/N	62/82		177/180
Positive predictive value	0.51 (0.35-0.67)	-	0.57 (0.2-0.88)
n/N	21/41		4/7
Negative predictive value	0.85 (0.74-0.92)	-	0.95 (0.9-0.97)
n/N	62/73		177/187
Positive likelihood ratio	2.69 (1.7-4.2)	-	17.14 (4.2-69.1)
Negative likelihood ratio	0.46 (0.3-0.7)	-	0.73 (0.5-1.0)
AUC ROC	0.782 (0.69-0.87)	-	0.779 (0.63-0.93)
PlGF < 100 pg/mL		Small for gestational age	
Sensitivity	0.78 (0.6-0.9)	0.5 (0.2-0.8)	0.86 (0.6-0.96)
n/N	25/32	5/10	12/14
Specificity	0.62 (0.5-0.7)	0.5 (0.4-0.6)	0.61 (0.5-0.7)
n/N	51/82	31/60	110/180
Positive predictive value	0.45 (0.3-0.6)	0.15 (0.1-0.3)	0.15 (0.1-0.2)
n/N	25/56	5/34	12/82
Negative predictive value	0.88 (0.76-0.95)	0.86 (0.7-0.9)	0.98 (0.93-0.99)
n/N	51/58	31/36	110/112
Positive likelihood ratio	2.1 (1.5-2.9)	1.12 (0.6-2.2)	2.20 (1.7-2.9)
Negative likelihood ratio	0.35 (2.3-15.2)	0.9 (0.5-1.7)	0.23 (0.1-0.8)
AUC ROC	0.702 (0.61-.79)	0.5	0.734 (0.63-0.84)

Source: Escouto (2018). AUC ROC, area under the receiver operator characteristic. PlGF, placental growth factor. * Test results were omitted because at least one of the values for prediction are null.

Table 9 – ROC areas for PlGF compared with 8 other signs/ tests for prediction of maternal adverse outcomes within 48h in 315 women presenting with HDP

Tests	PlGF continuous	PlGF <5th	PlGF <100 pg/ mL
PlGF	0.625 (0.09)	0.627 (0.07)	0.635 (0.06)
PlGF + SBP + DBP	0.620 (0.09)	0.660 (0.08)	0.657 (0.08)
PlGF + SBP + DBP + UrAcid+ ALT	0.719 (0.08)	0.730 (0.08)	0.732 (0.08)
PlGF + GAinc + Race + LDH	0.741 (0.08)	0.764 (0.08)	0.750 (0.09)
PlGF + fullPIERS	0.697 (0.09)	0.700 (0.09)	0.720 (0.07)
SBP + DBP + UrAcid + ALT + GAinc + Race + LDH	0.772 (0.08)	0.772 (0.08)	0.772 (0.08)
PlGF + SBP + DBP + UrAcid + ALT + GAinc + Race + LDH	0.770 (0.08)	0.779 (0.08)	0.773 (0.08)
PlGF + SBP + DBP + UrAcid + ALT + GAinc + Race + LDH + fullPIERS	0.632 (0.10)	0.779 (0.08)	0.774 (0.08)

Source: Escouto (2018). Tests were measured singly or in combination. ALT indicates alanine transaminase. DBP, diastolic blood pressure. fullPIERS, preeclampsia integrated estimate of risk (von Dadelszen, 2011). GAinc, gestational age at inclusion. HDP, hypertensive disorders of pregnancy; LDH, lactate dehydrogenase. PlGF, placental growth factor. ROC receiver operating characteristics. SBP, systolic blood pressure. UrAcid, uric acid.

Discussion

2.3 DISCUSSION

The present study evaluated the performance of the fullPIERS model and PlGF to predict adverse maternal and perinatal outcomes in a prospective cohort of women with HDP. It is important to stress that women with other HDP, besides PE were included. We intended to get closer to the actual challenge that clinicians face at obstetric centres, where pregnant women come to with elevated blood pressure and without previous health status information. The prospective nature of the study, also have to be pointed. Conducting such studies is costly and time-consuming. However, it improves evidence strength, and is especially valuable in obstetrics, where clinical trials are hampered by ethical concerns.

2.3.1 Study population and adverse outcomes

The studied sample consisted of women included with an average age of 28.7 years (SD 7.4), similar to other Brazilian reports of HDP (Gaio *et al.*, 2001; Madi *et al.*, 2012; Barbosa *et al.*, 2015; Almeida *et al.*, 2017). Almost 60% of women referred to be of white skin colour, a proportion that is particular to the southern states of Brazil due to a concentration of European immigrates to the area in the 19th century (Luvizotto, 2009).

Preeclampsia accounted for 44% of the included women, followed by GH (26%). Proportion of CH or SPE was close to 15%, each. This study HDP distribution was similar to the related by a nationwide USA study from Kuklina et al. In that survey, PE leaded HDP prevalence at 2006, with a rate of 112 per 1000 delivery hospitalizations, 46% of HDP cases (Kuklina *et al.*, 2009). Forty-two percent of our parous women with previous history of PE recurred in the index pregnancy. Even though our recurrence rate of PE was higher than other studies (14-25%) (Makkonen *et al.*, 2000; Hnat *et al.*, 2002; Van Rijn *et al.*, 2006; Cathelain-Soland *et al.*, 2010), OR for PE or SPE in the index pregnancy however, was not statistically significant (OR, 1.56; 95% CI: 0.8-3.0).

The rate of maternal adverse outcomes in our study was lower than the ones reported at other studies. While we had 5% of adverse maternal outcomes, other studies reporting severe maternal outcomes in women with HDP, showed proportions between 7-19% (Kuklina *et al.*, 2009; Payne *et al.*, 2014; Leaños-Miranda *et al.*, 2017). We

believe that one reason for the low rate of adverse maternal outcomes was the early interruption of pregnancies affected by HDP. Those actions might affect natural history of disease and the incidence of adverse maternal outcomes.

It is consensus that after 37 weeks of pregnancy induction of labour is the best strategy for mother and foetus (Koopmans, Bijlenga, *et al.*, 2009). In extreme preterm gestational ages (<34 weeks), the general consensus is to assess the severity of maternal and fetal disease for the definition of conduct. A systematic review of 2013(Churchill *et al.*, 2013) included four *interruption versus expectant* care studies in pregnancies affected by PE between 24 and 34 weeks and did not provide conclusions on preferential management. The MEXPRE study (Expectant Management of Preeclampsia) (Vigil-De Gracia *et al.*, 2013), a randomized clinical trial performed in several centres in Latin America, showed no benefit in expectant management in pregnant women with PE and GA between 28 and 33 weeks. In addition, there were more cases of placental detachment and SGA fetuses in the conservative treatment group.

Management of HDP presenting between 34 to 37 weeks is a subject of discussion. The HYPITAT-II study, compared induction of delivery within 24 hours versus expectant management for up to 37 weeks in pregnant women with non-severe HDP and GAs between 34 and 37 weeks. The study showed that induction reduced a small risk of maternal adverse outcomes in this population (RR 0.36, 95% CI: 0.12-1.11; *P*=0.07) (Broekhuijsen *et al.*, 2015).

It is difficult to access the relationship between time to interruption and development of adverse outcomes in an observational study. We found no differences in time-to-delivery interval between pregnancies with and without outcomes. Nonetheless, as expected, time-to-delivery was negatively correlated to GA at inclusion. Furthermore, despite of the positive effect of a possible reduction in maternal adverse outcomes rate, an early intervention might have negative effect on the proportion of surgical deliveries and perinatal adverse outcomes. (Kim *et al.*, 2010; Broekhuijsen *et al.*, 2015).

Induction failure rates and the need for cesarean section decrease with increasing GA. In Brazil, however, the indication of cesarean section as delivery method is the most common in patients with HDP. The indication of cesarean delivery is more often associated with maternal clinical severity than obstetric reasons such as cephalopelvic

disproportion or failure to induce labor (Reis *et al.*, 2014). Over fifty percent of deliveries in our study were cesarean sections. Thirty-four percent were indicated due to maternal conditions, 45% associated to obstetric reasons and 20% due to fetal status. Despite the high rate of cesarean sections, the proportion of maternal clinical status indication for cesarean section goes against the findings from Reis et al. This study had similar proportions of cesarean section indications to other studies conducted at high income countries as Japan and the Netherlands, where obstetrical causes were the commonest indications for cesarean sections (Koopmans, Bijlenga, *et al.*, 2009; Shibata *et al.*, 2016).

Interventional management is associated with threefold the risk of respiratory distress syndrome of the newborn (Broekhuijsen *et al.*, 2015). Barton et al, found that 25% of patients with stable mild GH without proteinuria had elective delivery at from 35 to 37 weeks of GA. Also, infants delivered at these GAs had increased neonatal complications and increased neonatal lengths of stay as compared with those delivered at >37weeks (Barton *et al.*, 2011). A North American trial with over 4000 pregnancies found that among women with induced labor or delivery by cesarean, both hypertensive and normotensive pregnancies had more adverse perinatal outcomes than pregnancies with corresponding hypertensive status and spontaneous labor. By comparison between neonatal outcomes in different GA at delivery, they also suggested that delivery between 35 and 37 weeks of gestation had greater impact on NICU admission and total neonatal stay than severity of the hypertensive disease (Habli *et al.*, 2007).

In this study, neonatal respiratory adverse outcomes alone outnumbered maternal outcomes. Five diagnosis of respiratory distress syndrome of the newborn occurred. And other 19 NICU admissions were due to respiratory dysfunction (17 cases of transient tachypnea of the newborn and 2 pulmonary hemorrhages). It is clear that, when managing a patient with HDP, the burden is on the clinician to weigh the possible benefit of prolonging pregnancy against the risks of developing adverse maternal and perinatal outcomes. Therefore, any management of those women must have well-defined maternal and fetal parameters.

2.3.2 The fullPIERS model and the prediction of adverse outcomes

The current study evaluated the performance of the fullPIERS model in a prospective cohort of women with HDP. The model performed with poor accuracy to discriminate risk of adverse maternal outcomes in our study. The AUC ROC curves for 48h, seven and 14 days, respectively, demonstrate poor predictive performance of the risk predictor applied to this particular cohort. At risk stratification table, over 90% of the patients were included at low risk categories, resulting in less than 0.1% of predicted probability. Likelihood ratios for prediction of adverse outcomes in 48 hours, seven, and 14 days provided scarce information on the presence or absence of adverse outcomes. The stratification table provided only strong evidence to *rule-in* the presence of adverse outcome up to 14 days at the risk category of 10-20% of predicted probability, where the high probability stratification group had high LR to adverse outcomes.

Calibration of the fullPIERS model applied to the study population was poor. Calibration examines how close the predictive procedure remains valid in the validation cohort. Calibration slope was below one: a demonstration that the predicted probabilities have large variance, possibly due to inconsistency of predictor effects or overfitting of the model in the development cohort (Debray *et al.*, 2015). Differences between the development study population and the current one might be responsible for the model's lower performance - especially differences in cohort size, outcomes incidence and predictor distributions.

Differences in cohort size between this study and development study are extensive, however compatible with differences between a regional site and a multicentre multinational study (Von Dadelszen *et al.*, 2011). We lost 13% of included women due to lack of necessary data to apply the fullPIERS model, probably due to the observational design of our study. Most of the missing variables were laboratory tests (platelets, creatinine and AST maternal plasma levels**)** that normally enter in the evaluation of disease's severity (Nice, 2010; Gynecologists e Pregnancy, 2013; Magee *et al.*, 2014; Malachias *et al.*, 2016). We believe that women without those laboratory tests were considered of low risk of adverse outcomes by the clinical assisting team. And, indeed, none of the women excluded from the fullPIERS model analysis experienced a maternal adverse outcome (data not presented).

One limitation of this study is the small number of observed maternal adverse outcomes. Little is known about adequate sample sizes to study model performance in other populations. Nevertheless, the power to detect statistically significant difference in model performance of binary outcomes prediction is also determined by the number of events, and a small number may lead to nonsignificant results, while true differences do exist (Vergouwe, 2003).

A possible explanation for the limited number of maternal adverse outcomes is the variety of presentations of HDP included. The risk for complications in women with mild GH are generally lower than other HDP (Barton *et al.*, 2001). Near miss cases are 8 times more frequent among women with PE (Abalos *et al.*, 2014). Nevertheless, in this study, even when evaluating only women previously normotensive who developed PE or presenting with SPE, the fullPIERS model's performance was no better.

During the development of our study protocol, we originally thought of using a broader definition of adverse outcomes. We intended to include liver enzymatic dysfunction to hepatic complications (LDH levels >600 U/L and/or AST >80 U/L) and a thrombocytopenia definition of <100 $x10^9$/L. Those definitions of adverse outcomes were previously used by other studies (Rana, Powe, *et al.*, 2012; De Oliveira *et al.*, 2013; Palomaki *et al.*, 2015; Dave *et al.*, 2016). This strategy would have increased the number of maternal adverse outcome from 20 (5%) to 66 (19%). Nevertheless, we opted to use adverse outcomes as defined by the fullPIERS model development study. This decision was made in order to allow adequate comparison between both studies. We followed the strategy suggested by the *Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis* (TRIPOD) guidelines. TRIPOD guidelines are intended to improve the reporting of prediction modelling studies of all types (development, validation, updating or extending) (Collins *et al.*, 2015).

Sample size and low incidence of adverse outcome can also affect assessment of calibration. Small sample size increases the width of predicted probability range, and low incidence of outcomes increases variation of the fitted loess curve in the extreme upper tail of predicted probability distribution (Austin e Steyerberg, 2014). Two previous studies, with similar sample sizes, were published. Akkermans et al (Akkermans *et al.*, 2014) included 216 women with severe early-onset PE in a retrospective validation of the fullPIERS model with good performance. In that cohort, 34% of women experienced one of the combined adverse outcomes. Additionally, a

Brazilian study (Almeida *et al.*, 2017) evaluated external validation of the fullPIERS model in a retrospective cohort of 325 women with severe PE. With a prevalence of 17% adverse outcomes from admission to discharge, the AUC ROC of the fullPIERS model was 0.72 (95%CI 0.67-0.77). Adverse outcome definitions in that study differed from the fullPIERS development study and from the current one - it included HELLP syndrome and foetal complications to the composite of adverse outcomes. The fullPIERS and miniPIERS study group developed an external validation of the fullPIERS model using the miniPIERS development cohort as the validation sample (Ukah, Payne, *et al.*, 2017). The analysis included 757 women, and the rate of adverse outcomes within 48 hours of admission was 14%. Despite the larger sample size and high adverse outcomes rate, the predictive model lost discriminatory power [AUC ROC: 0.77 (95% CI, 0.72–0.82)] versus [AUC ROC: 0.88 (95% CI, 0.84–0.92)], respectively, with additionally poor calibration performance. This study also included women with HDP, besides PE. However, omission of the distribution of HDP classification limits comparison with the current study population.

In an effort to improve predictive performance, we applied the study coefficients of a logistic regression using variables and intersections of the fullPIERS model. When a prognostic model performs less well in another population, adjusting the model using the new data should be considered to determine whether it will improve the performance in that population. The adjusted model is then based on both the development and validation data, further improving its stability and generalisability (Moons *et al.*, 2009). In our study, this approach did not enhance calibration performance, yet, discrimination ability was improved. These findings are expected, since different distribution of predictor values is likely to affect mainly the discriminative ability (Vergouwe, 2003).

A logistic equation, obtained from the current study population, was developed. Its purpose was not to seek an alternative to the fullPIERS model, but to show that local models usually perform better than the imported model (d'Avila, 2002). Modification of setting visibly results in a different distribution of the outcome and predictive factors, which commonly affects the generalisability of prognostic models (Moons *et al.*, 2009). Local models are likely to be better designed for individual decision-making because they include same characteristics of the study population.

To address the possible impact of an elevated rate of perinatal complications in the predictive performance of the fullPIERS model, we performed an additional analysis

of fullPIERS model ability to predict perinatal adverse outcomes: perinatal death, SGA newborn and delivery with GA before 34 weeks of pregnancy. Stratification and discriminative performances of the fullPIERS model were slightly better than when predicting maternal adverse outcomes. To this date, we could not find studies that used the fullPIERS model to evaluate prediction of perinatal morbidity.

The current study evaluated the performance of the fullPIERS model, a proposed predictive model of adverse outcomes in women presenting with suspected PE. We presented data of the model's performance when applied to women presenting with four categories of HDP (GH, PE, CH and SPE). However, the fullPIERS model performed with poor accuracy to predict adverse maternal outcomes in this cohort. No significant difference in the model's predictive performance was found when evaluating only women with PE. This study was underpowered to compare discriminatory ability to the original development cohort. Regardless of our negative results, we believe that the fullPIERS model is a significant advance to the management of HDP and should be applied to patients with the same characteristic as those proposed in the original development sample.

2.3.3 Placental growth factor and the prediction of adverse outcomes

Evaluation of PlGF predictive performance had to be addressed differently from the fullPIERS's analysis. One important characteristic of PlGF that interfered in the analysis was its different maternal plasma concentrations throughout pregnancy (Saffer *et al.*, 2013). Analysis was then categorized by intervals of GA with similar reference concentrations of plasma PlGF. Women included before 37 weeks of pregnancy – before 35 in particular – were in greater use of anti-hypertensive treatment, had more previous history of PE, and presented worst values of laboratory tests associated with disease severity. Creatinine levels were higher among women included before 35th week. Liver transaminases and LDH were also higher in the lowest GA category when compared to pregnancies presenting at term. Proteinuria was higher before 37 weeks, even after adjustment for presence of PE/ PES.

As expected, corticosteroid administration was higher amongst pregnancies before 35 weeks. The use of glucocorticoids in preterm pregnancies at risk of delivery before 34 weeks is well established to prevent respiratory complications in the neonatal period (Mckinlay *et al.*, 2012). Administration of magnesium sulphate was more frequent in preterm pregnancies. Magnesium sulphate use in patients with severe PE is highly recommended. A meta-analysis of 2010, showed that magnesium sulphate reduced in up to 60% in risk of developing eclampsia, and a trend towards reduction in overall PE mortality. The same review demonstrated a superior effect of magnesium sulfate in the prevention and control of seizures when compared to nimodipine, phenytoin and diazepam (Duley *et al.*, 2010).

Gestational outcomes were worst on preterm pregnancies. Longer hospital stays, lower birthweights, and all perinatal deaths occurred on preterm pregnancies. SGA infants were more frequent in pregnancies below 35 weeks. We could use SGA as estimate variable of foetal growth restriction, and severity of HDP, since the frequency of FGR is likely to be higher in severe HDP with early-onset (Odegård *et al.*, 2000).

Median maternal plasma concentrations of PlGF adjusted by GA at inclusion were lower in women with PE in comparison to GH and CH. Women with SPE had similar PlGF plasma concentrations to PE. However, there were no differences in PlGF values between SPE and CH women. These findings are in disagreement with the study from Bramham et al, after adjustment for gestation at sampling, women with CKD or CH (or

both) who developed SPE had significantly lower PlGF concentrations than did women with CKD or CHT (or both) without SPE (Bramham *et al.*, 2016). In this study, when we evaluated only women with GA of inclusion <35 weeks, women who developed SPE had statistically significant lower concentrations of plasma PlGF in comparison with CH who did not develop SPE. CKD patients were not included in the present cohort. Perni et al also disclosed lower concentrations of PlGF at 28 weeks of pregnancy among women who developed SPE before 35 weeks when compared to CH women (Perni *et al.*, 2012).

PlGF maternal plasma concentrations were lower in women who experienced maternal adverse outcomes. Differences were not sustained when we categorized women by GA, possibly due to the small number of women in each stratum.

Maternal concentrations of PlGF were also lower in pregnancies that delivered a SGA infant or women that delivered within 14 days after admission, difference significant only before 35 weeks of pregnancy. We believe that those finding emphasize the association of PlGF to severity of disease, since plasma concentrations are further suppressed in women presenting before 35 weeks of pregnancy.

2.3.3.1 PlGF ability to predict adverse outcomes

This study showed that maternal plasma concentrations of PlGF below 5th percentile for GA of normal pregnancies values had good performance to predict maternal adverse outcomes in women presenting with HDP before the 35th week of pregnancy. High sensitivity and negative predictive values were obtained along with a moderate discriminative ability (AUC ROC 0.672; 95% CI 0.5-0.9). In HDP, a test of high sensitivity is a better attribute than specificity. When considering benefits, harms and use of resources there is a greater preference for minimizing false negatives than false positives (Duckworth *et al.*, 2016). The consequences of false negatives are far greater than false positive because of the potentially severe maternal and perinatal mortality and morbidity associated with the HDP false positive test result will falsely identify a woman at high risk of developing adverse outcome, leading to unnecessary surveillance and prophylactic treatments. However, when you minimize false negatives, it helps preventing patients from not receiving timely management (Cnossen *et al.*, 2009; Hadker *et al.*, 2013).

Detecting women presenting before 35 weeks of GA at higher risk for adverse outcomes is important because the earlier is the disease onset, higher is the risk of complications. A population-based American study demonstrated that early-onset PE (<34 weeks GA) is associated with almost 4-fold increase risk of severe maternal morbidity in comparison to 1.7-fold in late-onset, compared to normal pregnancies (Lisonkova *et al.*, 2014). The same study showed higher risk of cardiovascular, respiratory, CNS, renal and other morbidities in women affected by early-onset PE.

Costs associated with women presenting before 35 weeks of pregnancy are also higher than near to term (Shih *et al.*, 2016). Maternal costs related to PE and preterm pregnancies are due to antepartum hospitalizations, additional hospital stay, caesarean deliveries, and various intra-partum health care utilizations (Liu *et al.*, 2009). Although maternal costs are only marginally higher at early pregnancies, neonatal costs are almost 90-fold greater at 28 weeks of pregnancy when compared to term pregnancies (Gilbert *et al.*, 2003), suggesting that efforts to prevent or delay delivery could dramatically reduce neonatal costs.

PlGF <5th percentile had reduced performance to predict maternal adverse outcomes with increase in GA. One possible explanation for this finding is the normal decline of maternal PlGF values at the third trimester of pregnancy (Taylor *et al.*, 2003; Saffer *et al.*, 2013), reducing test performance beyond 35 weeks of pregnancy. Nonetheless, advantage in the use of a biomarker to predict complications near term is of discussion when interruption of pregnancy might be the more suitable choice.

Continuous values of PlGF were evaluated, as an exploratory analysis to evaluate possible negative consequences when dichotomizing test results as positive or negative according to a single threshold. These consequences include loss of information about individual differences and loss of effect size and power in the case of bivariate relationships (Maccallum *et al.*, 2002). However, performance of PlGF to predict maternal adverse outcomes in women presenting before 35 weeks of pregnancy was not improved by use of continuous values. An arguable benefit might be speculated after 35 weeks of GA, since discriminative ability is higher. Also, as exploratory analysis, a PlGF threshold of 100 pg/ml was used and the predictive performance was similar to the 5th percentile at <35 weeks. As happened with raw values, PlGF maternal plasma concentrations <100 pg/mL had increased performance up to 37 weeks. In this case negative likelihood ratio and AUC ROC where better. A small negative likelihood ratio

is of particular interest at term because a negative test can prevent time and resources spending of transferring women with low risk for complications to a tertiary centre.

We found no other study evaluating PlGF's ability alone to predict maternal adverse outcomes in HDP with comparisons of presentation from 20th weeks until after term. An Indian study, however, showed an association of maternal plasma PlGF <122pg/mL at 22-24weeks and increased risk of caesarean delivery (OR 9.0; 95% CI, 5-16) and of developing postpartum haemorrhage (OR 2.4; 95% CI, 1-4) (Ghosh *et al.*, 2012). Meler et al evaluated PE women presenting below 37 weeks of GA and did not observe association of PlGF concentrations <12 pg/mL to presence of maternal complications (Meler *et al.*, 2014).

Most studies testing the use of PlGF to predict maternal adverse outcomes do so as the sFlt-1/PlGF ratio, and most include only preeclamptic pregnancies. Leaños-Miranda et al., found on OR ≥2.7 any adverse maternal outcome women with PE and sFlt-1/PlGF ratios in the highest quartile (Leaños-Miranda *et al.*, 2013). Rana et al. published a study with twin pregnancies where sFlt1/PlGF ratio > 85 presented AUC ROC to predict maternal and foetal outcomes of 0.75 (0.6–0.9) at term and 0.81 (0.7–0.96) <34 weeks in pregnancies affected by PE (Rana, Hacker, *et al.*, 2012).

A recently published systematic review examined the ability of the PlGF, either independently or combined with other factors, to predict maternal and foetal complications resulting from the HDP. Seventeen studies were included and no clinically useful performance for the prediction of adverse maternal outcomes was found (Ukah, Hutcheon, *et al.*, 2017). They suggested that future studies should examine whether its use for predicting adverse maternal outcomes in women with HDPs can be improved.

We also evaluated a possible increase to performance of multivariable models with the addition of PlGF. When compared to standard tests used in HDP and independent predictors of maternal adverse outcomes resulted from our study, PlGF did not significantly improve predictive ability. Three previous studies have added sFlt-1/PlGF ratio to other variables. One study found no significant difference on the addition of sFlt-1/PlGF to SBP and proteinuria to predict perinatal and maternal adverse outcomes in women with suspected PE (Salahuddin *et al.*, 2016). A second study compared mean uterine artery pulsatility index and sFlt-1/PlGF ratio for maternal and perinatal outcomes risk prediction in early-onset PE without significant differences as

well (Gómez-Arriaga *et al.*, 2014). Moore et al evaluated sFlt-1/PlGF ratio addition to 11 clinical variables - race, gravidity, GA at presentation, diagnosis of PE, among others - to predict adverse outcomes among women presenting before 37 weeks. They found an increase in discrimination ability (AUC ROC) from 0.82 to 0.91 (Moore *et al.*, 2012). Perhaps, a comparison of those variables in our study sample might be worthwhile.

Additionally, we evaluated PlGF performance to predict endpoints that can be related to perinatal adverse outcomes. PlGF's ability to predict delivery within 14 days after admission was moderate to high among women presenting with HDP before 35 weeks PlGF <5th percentile had lower performance than the obtained for PlGF continuous values, a loss that is expected when making use of dichotomization. Similar findings were observed with a threshold of 100 pg/mL. This study findings were comparable to those of the PELICAN project, in which PlGF <5th percentile predicted delivered within 14 days in women with PE presenting before 35 weeks with sensitivity 0.96 (0.9–0.99), specificity 0.56 (0.5–0.6), PPV 0.44 (0.36–0.5) and NPV 0.98 (0.9–0.99) (Chappell *et al.*, 2013). Similarly, a study conducted in a low resource setting in Mozambique demonstrated that PlGF <100 pg/mL had a sensitivity of 0.28, specificity of 0.89PPV of 0.3, and NPV of 0.89, to predict delivery within 14 days among women with suspected PE (Ukah, Mbofana, *et al.*, 2017). Other comparison can be made with a North American study that included 751 women with suspected PE before 35 weeks of pregnancy. PlGF <100 pg/mL predicted delivery within 14 days whit sensitivity of 0.93, specificity of 0.64, PPV of 0.7, NPV of 0.9 and AUC ROC of 0.85.

As we previously discussed, delivery of a SGA infant is related severity of HDP (Odegård *et al.*, 2000). Sensitivity, negative predictive values and discriminatory ability were also moderate to high for prediction of a SGA infant. Similarly, for PlGF <5th percentile, <100 pg/mL and continuous values. Although diagnostic accuracy is greatest for women presenting before 35 weeks of GA, this study found good accuracy when testing women presenting ≥ 37 weeks (by the use of <100 pg/mL threshold). Molvarec et al in a retrospective study with 89 women with HDP showed sensitivity of 0.73 and NPV of 0.88 for PlGF <12 pg/mL prediction of SGA infant before 35 weeks of pregnancy (Molvarec *et al.*, 2013). Leaños-Miranda et al., found a sensitivity of 0.85 and NPV of 0.63 for SGF infant, amongst women with PE, for sFlt-1/PlGF ratios >871 (Leaños-Miranda *et al.*, 2013).

The use of different methods makes comparisons between diverse studies difficult. Some studies evaluating maternal and perinatal adverse outcomes adopted the same method of our study: ELISA immunoassay, from R&D Systems fabricant (Moore *et al.*, 2012; Leaños-Miranda *et al.*, 2013). On the other hand, automated methods were used by others, such as Roche Elecsys system (Roche, Penzberg, Germany) (Rana, Powe, *et al.*, 2012; Gómez-Arriaga *et al.*, 2014) or Triage PlGF Test (Alere, CA, EUA) (Chappell *et al.*, 2013; Molvarec *et al.*, 2013).

This study showed that PlGF may predict maternal and perinatal adverse outcomes in women presenting with HDP, specially before 35th week of pregnancy. Although its performance does not allow the use of PlGF alone to guide management, it can be useful as an additional tool for screening of women with HDP at higher risk for adverse outcomes due to good sensitivities and negative predictive values. Whereas predictive performance is lower after 37 weeks of pregnancy, PlGF can still be of use for surveillance in situations where the risks/benefits of delivery are uncertain.

Final considerations

3 FINAL CONSIDERATIONS

The main objective of this study was to evaluate the accuracy of the fullPIERS model and the biomarker PlGF as predictors of maternal adverse outcomes in pregnant women with HDP. We evaluated both predictors in the same cohort of pregnant women presenting with elevated blood pressure from the 20th week at the obstetric centre of a tertiary hospital in Southern Brazil, with different categories of HDP and not only PE.

In our sample, the fullPIERS model performed with poor accuracy to discriminate risk of adverse maternal outcomes within 48 hours, seven days and 14 days. Calibration of the fullPIERS model applied to the study population was also poor. Even when evaluating only women that developed PE, the model's predictive performance did not improve.

Post-hoc analysis revealed that this study was underpowered to compare fullPIERS discriminatory ability to the original development cohort. The logical explanation for the underpower was the reduced number of maternal adverse outcomes observed. Five percent rate of events is low in comparison to other studies based in high-income countries and further inferior in comparison to studies at low-income settings. This may be due to the inclusion of less severe categories of HDP than PE. Most studies only included women with suspected PE, a feature knowingly associated with higher maternal risk.

By adjusting the model with study sample coefficients on variables and intersections of the fullPIERS model, we modestly improved discriminatory ability, without significant difference between AUC ROC; furthermore, calibration was not enhanced. The local model obtained by logistic regression also did not significantly improve risk prediction performance.

A novel approach using fullPIERS model to predict perinatal adverse outcomes - showed moderate stratification and discriminative performances. To this date, we could not find others studies that used the fullPIERS model to evaluate prediction of perinatal outcomes.

Median maternal plasma concentrations of PlGF were lower in women with PE in comparison to GH and CH, and no differences were found between PE and SPE. Lower concentrations of PlGF in women with SPE in comparison to CH were only found when GA at inclusion was <35 weeks.

PlGF maternal plasma concentrations were lower in women who experienced adverse outcomes, in who delivered a SGA infant and that delivered within 14 days of

admission. PlGF concentrations were particularly useful when women were included before 35 gestational weeks. This emphasizes the association of PlGF with disease severity.

PlGF below 5th percentile for GA of normal pregnancies values had good performance to predict maternal adverse outcomes in women presenting with HDP before the 35th week of pregnancy. High sensitivity and negative predictive values were obtained with a moderate discriminative ability. Those features minimize false negative rate and help preventing women from not receiving timely management.

Continuous values of PlGF and a threshold of <100 pg/mL had good stratification performance from 37 weeks of pregnancy, with lower negative likelihood ratios and higher AUC ROC. Those findings are of particular interest at term, because a negative test can prevent time and resources spending of transferring women with low risk for complications to a tertiary centre.

The ability of PlGF <5th percentile to predict delivery within 14 days after admission was moderate to high among women presenting with HDP before 35 weeks. Similar findings were observed with a threshold of 100 pg/mL.

Sensitivity, negative predictive values and discrimination of PlGF <5th percentile were moderate to high for prediction of a SGA infant. Although diagnostic accuracy is greatest for women presenting before 35 weeks of GA, good accuracy was found when testing women presenting ≥ 37 weeks, by the use of <100 pg/mL threshold.

We also evaluated a possible increase to performance of multivariable models with the addition of PlGF. When compared to standard tests used in HDP and independent predictors of maternal adverse outcomes resulted from our study, PlGF did not significantly improve predictive ability. The specific evaluation of an increase in performance of PlGF $<5^{th}$ percentile with the addition of the fullPIERS model, apparently added to discriminatory ability, however no statistical significance was found in the comparison.

In conclusion, in our sample the fullPIERS model and PlGF were limited predictors of maternal adverse outcomes in pregnant women with HDP, including PE. The performance of the fullPIERS model in our sample was inferior to that of the original cohort. PlGF as a biomarker appears to be an additional tool to predict delivery within 14 days and SGA newborn in women before 35 weeks gestation.

3.1 FUTURE PERSPECTIVES

A manuscript of the fullPIERS model validation in our study sample, entitled "The fullPIERS predictive model in women with hypertensive disorders of pregnancy" was submitted to the Green Journal of Obstetrics & Gynecology (Appendice E) We believe that there are merits to this study, even though it may be underpowered. First, we followed the recommendations on methodological conduct and reporting of external validation of prediction models, quality absent in the majority of the studies alike. Second, this study provided additional information to external validation of the fullPIERS model, helping in generalizability and transportability of the model. Third, we added to the fullPIERS risk prediction model the evaluation of HDP other than just PE.

The next step is to write the manuscript with the PlGF analysis. Several studies have evaluated PlGF's predictive ability for PE diagnosis and for a combination of maternal and perinatal adverse outcomes in women with suspected PE. Few studies evaluated the performance of PlGF to predict only maternal events, especially with a Delphi consensus definition of combined maternal adverse outcomes, as we did in the present study.

We concluded this study with mostly negative findings. However, we prefer to think of them as temporary negative findings instead. One possibility is to continue the inclusion of patients in the cohort and conduct further analysis when the number of adverse outcomes enables an enhanced power to the study. We could also re-write the first manuscript adding the prediction of perinatal adverse outcomes, novel data that were possible never been published.

This study can be further expanded and local equations may be developed and eventually prove to have better performance that models developed in other populations.

A recently published systematic review suggested that studies should investigate whether PlGF is a better predictor as an independent marker or combined with sFlt-1 in predicting timing of delivery and adverse maternal outcomes. As further steps, we intend to evaluate the effects of the addition of sFlt-1/PlGF plasma ratio and detection of urinary PlGF to improve predictive ability for adverse maternal outcomes in women of this cohort.

From the period as a research student at KCL, I still have unfinished work to keep up with. We recently submitted a manuscript entitled "Postpartum evaluation of cardiovascular disease risk for women with pregnancies complicated by hypertension"

for the Pregnancy Hypertension Journal. Besides manuscripts with complete reports of the projects presented at ANNEX C are in preparation and should be submitted for peer review shortly. The possibility to keep collaborations with the talented group of researchers from the Women's Health Department of KCL is of additional value for the development of my work as a researcher and for the expansion of the Nephrology Laboratory of PUCRS, a group of which I am proudly part of.

As for my future as a researcher of hypertensive disorders and renal diseases in pregnancy, I aim to follow the steps of my inspiring mentors… Maintain a successful juggling between clinical work and academic duties. Dedicate my work to improve the awareness in Brazil of the importance of education and of the scientific community. But mostly, I intend to keep an enthusiastic interest on the science and the stories involving these women affected by such frightening conditions in a moment supposed to be of pure joy.

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Appendices

APPENDICES

APPENDICE A – Manual of procedures for participating investigators (in Portuguese)

PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
FACULDADE DE MEDICINA
PROGRAMA DE PÓS-GRADUAÇÃO EM MEDICINA E CIÊNCIAS DA SAÚDE
ÁREA DE CONCENTRAÇÃO EM NEFROLOGIA
LABORATÓRIO DE NEFROLOGIA – INSTITUTO DE PESQUISAS BIOMÉDICAS

Manual de Operações e Procedimentos:

Pesquisa: Avaliação do modelo fullPIERS e PlGF como preditores de desfechos adversos maternos e perinatais em mulheres com Doença Hipertensiva Gestacional.

AUTORA: DANIELE CRISTÓVÃO ESCOUTO

PORTO ALEGRE, 2015.

1 DEFINIÇÕES NO ESTUDO

- Hipertensão arterial: pressão arterial sistólica (PAS) ≥ 140 mmHg e/ou pressão arterial diastólica (PAD) ≥ 90 mmHg por 2 medições em momentos diferentes.
- Hipertensão gestacional: elevação de valores de pressão arterial a partir da 20ª semana de gestação, sem presença de proteinúria significativa.
- Proteinúria significativa: valor ≥ 300 mg/ dL de proteínas na urina de 24 horas, ou pela quantificação da relação proteínas/creatinina em amostra aleatória de urina ≥ 3.
- Pré-eclâmpsia (PE): início, a partir da 20ª semana gestacional de hipertensão arterial e proteinúria significativa.
- Pré-eclâmpsia sobreposta (PES): PE em paciente com hipertensão arterial crônica.
- Síndrome HELLP: síndrome gestacional de critérios laboratoriais, composta por hemólise (esfregaço periférico característico e elevação sérica de desidrogenase lática ≥ 600 U/L), elevação sérica da aspartato aminotransferase ≥70 U/L e plaquetopenia ≤ 100.000 mm^3.
- Trabalho de parto: pelo menos 3 contrações uterinas em intervalos de 10min, com intensidade suficientemente forte, regulares, rítmicas e com duração de pelo menos 30s cada; colo uterino com ≥ 3 cm; grau mínimo de apagamento do colo uterino.

1.1. Desfechos:

- Mortalidade materna
- Ou 1 ou mais dos seguintes eventos:
  - SNC:
    - Eclâmpsia: PE complicada por crises convulsivas (1 ou mais) não atribuídas a outras causas;
    - Escala de coma de Glasgow < 13;
    - Síndrome da encefalopatia posterior reversível (PRES): presença de sintomas neurológicos associados à exame de imagem com evidência de edema de substância branca encefálica, de característica reversível.
    - Acidente vascular encefálico;
    - Descolamento de retina;
    - Cegueira cortical: redução da acuidade visual na presença de resposta a luz adequada;
  - Cardiovascular e repiratórios:
    - Uso de drogas inotropicas;
    - Infusão do terceiro anti-hipertensivo parenteral;
    - Síndrome coronariana aguda: presença de dor torácica típica, de característica instável, acompanhada ou não de alterações eletrocardiográficas (elevação ou depressão de segmento ST, nova onda Q patológica) e elevação de enzimas miocárdicas (troponinas, CK-MB);
    - Edema agudo pulmonar: congestão pulmonar aguda e sintomática com evidência clínica e radiológica;
    - Necessidade de FiO2 > 50% por mais de 1 hora;
    - Necessidade de suporte ventilatório avançado, por motivo outro que anestesia para parto cesáreo;
    - Disfunção ventilatória severa: presença de dispnéia e SpO2 < 90%;
  - Complicações hematológicas:
    - Transfusão de quaisquer hemocomponentes
    - Plaquetas < 50.000 mm^3;
  - Complicações hepáticas:
    - RNI >1.2, na ausência de CIVD ou uso de anticoagulante;
    - Hematoma hepático ou ruptura hepatica confirmadas por imagem ou laparotomia;
  - Complicações renais:
    - Injúria renal aguda: creatinina >1.7 mg/ dl, sem doença renal prévia;
    - Falência renal aguda: creatinina >2.3 mg/dl, sem doença renal prévia;
    - Terapia de substituição renal: necessidade de hemodiálise ou diálise peritoneal.
  - Complicações obstétricas:

- Hemorragia pós-parto;
- Descolamento de placenta observado durante o parto ou no exame anatomopatológico.

2 RESUMO DO ESTUDO

As doenças hipertensivas gestacionais estão entre as principais causas de morbimortalidade materna e perinatal. A adequada distinção dos casos de alto risco para eventos graves ajudaria a definir com maior precisão as pacientes que se beneficiariam de uma abordagem intervencionista precoce, mesmo com os riscos associados ao parto pé-termo.

O modelo fullPIERS, desenvolvido e validado em centros do Canadá, Austrália Inglaterra e Nova Zelândia. O modelo utiliza variáveis clínicas e laboratoriais e desfechos compostos como uma ferramenta acurada para estratificar gestantes com pré-eclâmpsia e alto risco para eventos graves.

Placental grotth fator (PlGF) apresenta-se em níveis sanguíneos reduzidos em mulheres com pré-eclâmpsia e acredita-se que este possa ser um marcador diretamente associado à gravidade de doença.

O objetivo deste estudo é estimar a acurácia do modelo fullPIERS e do bi marcador PlGF como preditores de desfechos adversos maternos e fetais em gestantes com Doença Hipertensiva Gestacional.

3 REGISTRO DA EQUIPE

Nome	Função	Tarefas
Daniele Cristóvão Escouto	Aluna doutorado	A, B, C, E, G, H, I, J, K, L, M
Carlos Eduardo Poli de Figueiredo	Orientador	I, J, M
Bartira Ercília Pinheiro da Costa	Orientadora	I, J, K, M
Nathalia Paludo	Aluno (a) IC	A, B, C, E, G, K
Rayssa Amaral	Aluno (a) IC	A, B, C, E, G, K
Enfermagem do CO/ alojamento conjunto	Colaboradores	A, D, F, G
Júlia Motta	Téc. Laboratório Nefrologia	K

Legenda de tarefas:

A: Seleção de pacientes
B: Obtenção de consentimento
C: Preenchimento do protocolo de estudo
D: Medição de pressão arterial
E: Coleta de dados em prontuário
F: Coleta de sangue
G: Aferição de dosimetria de pulso
H: Avaliação de desfechos adversos
I: Avaliação de qualidade interna
J: Desenvolvimento de protocolos
K: Processar e armazenar de amostras
L: Alimentação de banco de dados
M: Treinamento da equipe
N:_____
O:_____
P:_____

4 TREINAMENTO

4.1 PROPRÓSITO

Definir os procedimentos a serem seguidos para treinamento inicial e continuado para pesquisadores e equipe. Permitir que a equipe de pesquisa receba introdução e educação continuada sobre suas funções e responsabilidades. Incluirá informações necessárias para condução adequada da pesquisa e conhecimentos sobre a estrutura, objetivos e hipóteses do estudo.

4.2 PÚBLICO-ALVO:

Todos os envolvidos no estudo.

4.3 RESPONSÁVEIS:

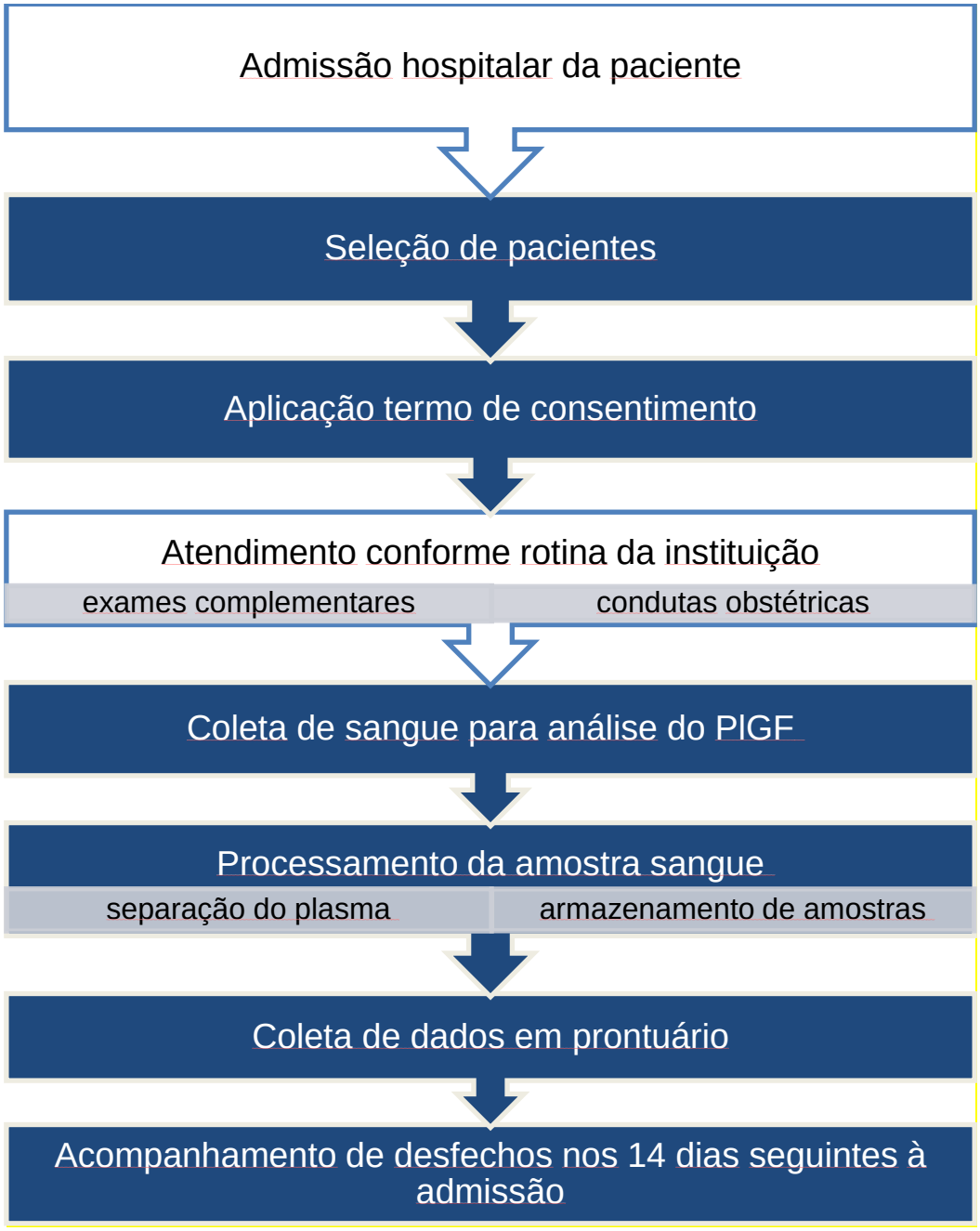
Investigadores principais.

4.4 PROCEDIMENTOS:

- Todos os envolvidos no estudo serão treinados para o protocolo de pesquisa, incluindo:

- Introdução à pesquisa clínica
- Siglas e definições do estudo
- Funções e responsabilidade da equipe
- Seleção de pacientes
- Obtenção de consentimento informado
- Treinamento de habilidades necessárias para o estudo

5 FLUXOGRAMA DO ESTUDO



6 SELEÇAO DE PACIENTES

6.1 PROPRÓSITO

Definir os procedimentos a serem seguidos para seleção de pacientes para o estudo. Incluirá informações necessárias para a distinção do público alvo da pesquisa e critérios de inclusão.

6.2 PÚBLICO-ALVO:

Membros da equipe envolvidos na identificação de pacientes e coleta de dados em prontuário.

6.3 RESPONSÁVEIS:

Investigadores principais.

6.4 PROCEDIMENTOS:

- Visitas diárias às unidades de internação obstétrica do HSL-PUCRS (CO e alojamento conjunto).
  - Busca de gestantes admitidas para internação hospitalar com idade gestacional ≥ semanas
  - Identificação das pacientes com PAS ≥ 140 mmHg e/ou PAD ≥ 90 mmHg
  - Seleção das pacientes que **não** estejam em trabalho de parto.
    - < 3 contrações uterinas adequadas em intervalo de 10 min;
    - colo uterino com dilatação inferior a 3 cm;
    - sem apagamento significativo do colo uterino.

7 EXCLUSÃO DE PACIENTES

7.1 PROPRÓSITO

Definir os procedimentos a serem seguidos para exclusão de pacientes que tenham sido selecionados para o estudo e apresentem critérios para retirada.

7.2 PÚBLICO-ALVO:

Investigadores principais.

7.3 RESPONSÁVEIS:

Investigadores principais.

7.4 PROCEDIMENTOS:

- Avaliação do protocolo de estudo e prontuário médico de cada indivíduo selecionado e exclusão dos seguintes casos:
  - gestantes não admitidas para internação hospitalar no HSL-PUCRS;
  - idade gestacional inferior a 20 semanas;
  - gestantes em trabalho de parto;
  - gestantes que não apresentem 2 aferições em momentos distintos de valores de pressão arterial compatíveis com Hipertensão Arterial;
  - presença de doenças com comprometimento sistêmico que, na opinião do pesquisador, possam interferir nos desfechos independentemente da DHG – esta observação deve, obrigatoriamente, ser registrada no protocolo de estudo e mantida entre os documentos da pesquisa.
- Avaliação do termo de consentimento de cada indivíduo e exclusão dos seguintes casos:
  - preenchimento e assinaturas inadequadas do termo de consentimento;
  - manifestação de vontade de retirada de consentimento para o estudo pelo participante ou responsável legal em qualquer momento do estudo.

8 APLICAÇÃO TERMO DE CONSENTIMENTO

8.1 PROPRÓSITO

Definir os procedimentos a serem seguidos para aplicação de termo de consentimento em pacientes que tenham sido selecionadas para participar do estudo.

8.2 PÚBLICO-ALVO:

Investigadores principais, bolsistas de iniciação científica.

8.3 RESPONSÁVEIS: INVESTIGADORES PRINCIPAIS.

8.4 PROCEDIMENTOS:

- Aplicação do termo de consentimento:
    - apresentar-se como investigador do estudo;
    - convidar a paciente para participar da pesquisa;
    - resumir a motivação do estudo;
    - resumir a participação da paciente no estudo;
    - descrever riscos da participação no estudo;
    - oferecer participação no ambulatório de Hipertensão Gestacional;
    - oferecer o termo de consentimento à paciente para leitura;
    - caso a paciente recuse-se a ler o termo, realizar leitura dinâmica do documento, enfatizando as principais informações de cada item;
    - questionar sobre dúvidas e esclarecê-las.
- Obtenção de assinaturas do termo de consentimento:
    - preencher duas vias de termo de consentimento com os dados da paciente e responsável, se for o caso;
    - solicitar à paciente ou responsável que assine duas vias de termo;
    - orientar que o consentimento pode ser retirado a qualquer momento conforme a vontade da participante;
    - o investigador deve assinar as duas vias do termo de consentimento;
    - entregar uma via para a paciente;
    - arquivar a segunda via no Laboratório de Nefrologia.

9 COLETA DE AMOSTRA DE SANGUE

9.1 PROPRÓSITO

Definir os procedimentos a serem seguidos para venóclise e coleta de sangue de pacientes que tenham sido selecionadas para participar do estudo.

9.2 PÚBLICO-ALVO:

Colaboradores e equipe de enfermagem.

9.3 RESPONSÁVEIS:

Investigadores principais.

9.4 PROCEDIMENTOS:

- Selecionar materiais para procedimento:
    - tubos vaccutainer estéreis com EDTA, agulha 21 ou menos, seringa de volume suficiente para coleta;
    - torniquete;
    - material para assepsia: álcool ou clorexidine líquida, algodão ou gazes;
    - rótulos;
    - luvas de procedimento;
    - recipiente para descarte de materiais pérfuro/cortantes.
- Seleção de veia:

  - a seleção de veia é por preferência individual, porém a veia cubital mediana, localizada na linha média da fossa antecubital é uma ótima escolha;
  - se as veias cubital mediana ou braquiocefálica não forem utilizáveis, as veias dorsais da mão podem ser usadas;
  - não realizar venóclise em áreas infectadas ou de membro recebendo terapia endovenosa; evitar áreas com escoriações, hematomas ou edema.
- Venóclise e coleta de sangue:
  - verificar nome da paciente/sujeito em estudo e rótulas em material para coleta;
  - lavar as mãos;
  - posicionar torniquete 7,5 a 10 cm acima do local de punção; o torniquete deve estar apertado o suficiente para distender a veia sem que obstrua o fluxo arterial;
  - instruir a paciente a abrir e fechar o punho diversas vezes para represar o sangue na veia e distende-la para punção;
  - colocar luvas de procedimento;
  - realizar assepsia da região de punção;
  - remover capa protetora da agulha; com a mão livre tensionar a pele e imobilizar a veia; alinhar agulha e veia e puncionar a pele em ângulo de 15 a 30 graus; reduzir o ângulo até a agulha estar quase paralela a pele para puncionar a parede da veia;
  - deixar a seringa encher-se livremente; caso o fluxo de sangue pare, girar, avançar ou retirar levemente a agulha;
  - remover torniquete quando estiver próximo do volume total de coleta; este procedimento previne a formação de hematomas;
  - quando a coleta de sangue estiver completa, retirar a agulha, puxar o embolo delicadamente para reestabelecer vácuo;
  - aplicar firme pressão no local de punção e solicitar que a paciente mantenha a pressão até o sangramento parar e eleve o membro para evitar formação de hematoma;
  - transferir sangue imediatamente no tubo vaccutainer com EDTA com a identificação da paciente; permitir que o vácuo do tubo transfira o sangue da seringa ao tubo;
  - inverter o tubo gentilmente 10 vezes;
  - descartar seringa e luvas em recipientes próprios;
  - lavar as mãos;
  - documentar data e hora da coleta.

10 PROCESSAMENTO DE AMOSTRA DE SANGUE

10.1 PROPRÓSITO

Definir os procedimentos a serem seguidos para processamento de amostras de sangue desde o final da coleta ao seu armazenamento.

10.2 PÚBLICO-ALVO:

Investigadores, bolsistas de IC, técnicos de laboratório.

10.3 RESPONSÁVEIS:

Investigadores principais.

10.4 PROCEDIMENTOS:

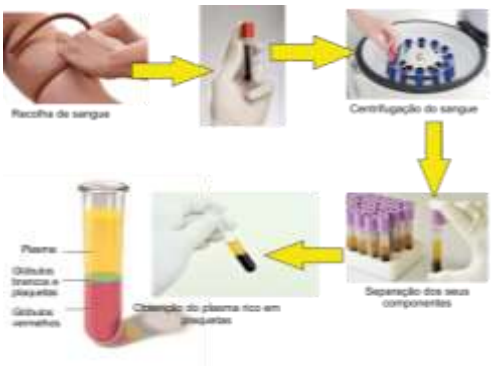
- Transporte para o laboratório de nefrologia:
  - Para transporte rápido, de curta distância, os tubos com amostra de sangue total podem vir em estantes em temperatura ambiente.
- Preparo da amostra:
  - Selecionar materiais para procedimento:
    - tubo vaccutainer com amostra de sangue devidamente rotulada;
    - estante para tubos;
    - luvas de procedimento;

- centrífuga;
- material para assepsia: álcool e guardanapos de papel;
- pipeta Pasteur estéril 3 ml;
- pinça de procedimento;
- tubos Eppendorf® 0,5 mL para armazenamento de amostras;
- caneta para identificação de amostras;
- recipiente para descarte de materiais pérfuro/cortantes.

o Separação do plasma:
- calçar luvas de procedimento;
- abrir a centrífuga, colocar o tubo com sangue e tubo como contrapeso;
- fechar a tampa da centrífuga, marcar 2000 RPM por 10 minutos;
- recomenda-se não abrir a tampa da centrífuga até alguns minutos após esta parar, pode ocorrer formação de aerossóis que podem ser infectantes, devendo-se aguardar sua sedimentação;
- retirar tubos da centrífuga com auxílio de pinça e colocar em estante própria;
- avaliar o aspecto da amostra: o soro deve estar livre de hemácias; amostras hemolisadas (plasma tinto de vermelho) ou lipêmicas (plasma de coloração branca e turva) devem ser descartadas e solicitada nova coleta;
- coletar a porção plasmática (superior) da amostra com pipeta Pasteur e transferir para tubos Eppendorf® de 0,5 ml – quantos forem necessários;
- manter no tubo de sangue cerca de 0,5 cm de plasma acima do *buffy coat* para preservar leucócitos;
- desprezar material utilizado em recipientes próprios;

o Identificação das amostras:
- verificar na listagem da porta o próximo número para identificação de amostras: 1 número para plasma e 1 número para DNA;
- com caneta própria para identificação, identificar cada Eppendorf® com número da amostra de plasma do paciente no corpo e tampa;
- identificar tubo com sangue restante com número da amostra de DNA.

o Armazenamento das amostras:
- armazenar as amostras de plasma, devidamente identificadas, em caixa do estudo, no freezer -80º C do Laboratório de Nefrologia;
- armazenar o tubo com sangue restante, devidamente identificado com o número da amostra de DNA, em estante na geladeira do Laboratório de Nefrologia;
- registrar em livro de coletas: nome, número de identificação da paciente, número de identificação das amostras, número de amostras, número da caixa de armazenamento no freezer -80°C e data de armazenamento.



11 COLETA DE DADOS EM PRONTUÁRIO

11.1 PROPRÓSITO

Definir os procedimentos a serem seguidos para coletar dados em prontuário dos pacientes para o estudo. Incluirá informações necessárias para a avaliação de variáreis clínicas e de desfecho.

11.2 PÚBLICO-ALVO

Membros da equipe envolvidos na identificação de pacientes e coleta de dados em prontuário.

11.3 RESPONSÁVEIS

Investigadores principais.

11.4 MATERIAIS NECESSÁRIOS

- Lista de pacientes incluídas no estudo;
- Protocolos de coleta de dados do estudo em branco;
- Termos de consentimento em branco;
- Oxímetro portátil.

11.5 PROCEDIMENTOS

- Visitas diárias às unidades de internação obstétrica do HSL-PUCRS (CO e alojamento conjunto);
- Busca de gestantes selecionadas para o estudo;
- Checar se a paciente assinou termo de consentimento do estudo;
- Checar se a paciente já possui protocolo do estudo preenchido, com identificação;
    - caso a paciente já tenha protocolo, pode-se preencher novo protocolo com nome, número de identificação, data e novos dados coletados.
- Coletar dados em prontuário impresso e eletrônico, selecionando aos piores valores das variáveis em análise.
- Coletar oximetria de pulso da paciente.
- Assinar protocolo de coleta.
- Guardar protocolo no Laboratório de Nefrologia, em arquivo próprio da paciente.

12 ACOMPANHAMENTO DE PACIENTES

12.1 PROPRÓSITO

Definir os procedimentos a serem seguidos para acompanhamento das pacientes em estudo. Incluirá informações necessárias para a avaliação de variáreis de desfecho.

12.2 PÚBLICO-ALVO

Membros da equipe envolvidos na e coleta de dados de pacientes em prontuário.

12.3 RESPONSÁVEIS

Investigadores principais.

12.4 MATERIAIS NECESSÁRIOS

- Lista de pacientes incluídas no estudo;
- Protocolos das pacientes em acompanhamento;
- Protocolos de coleta de dados do estudo em branco.

12.5 PROCEDIMENTOS

- Visitas diárias às unidades de internação obstétrica do HSL-PUCRS (CO e alojamento conjunto);
- Busca de gestantes em acompanhamento no estudo;
- Checar se a paciente assinou termo de consentimento do estudo;

- Checar se a paciente já possui protocolo do estudo preenchido, com identificação;
    - caso o protocolo da paciente não esteja em mãos, pode-se preencher novo protocolo com nome, número de identificação, data e novos dados coletados.
- Coletar dados em prontuário impresso e eletrônico, selecionando aos piores valores das variáveis em análise.
- Assinar protocolo de coleta.

Guardar protocolo no Laboratório de Nefrologia, em arquivo próprio da paciente.

APENDICE B– Informed consent form (in Portuguese)

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

LINHA DE PESQUISA EM NEFROLOGIA: ENFOQUE NA GESTAÇÃO E PRESSÃO ARTERIAL

TCLE aprovado pelo CEP em 31/05/2005 (OF N° 440/05) - CEP e aprovado pelo CONEP registro 11972

Pesquisadores Responsáveis: Bartira Ercíla Pinheiro da Costa, Carlos Eduardo Poli de Figueiredo

Entrevistador da Equipe de Pesquisa1: _____

Nome da paciente: _____

SOBRE A PESQUISA: A presente linha de pesquisa avalia aspectos da gravidez, como pressão sanguínea e pressão alta na busca do aumento do conhecimento, alívio do sofrimento e melhora da saúde de mulheres e crianças. Esta Linha de Pesquisa é parte do Programa de Pós-graduação em Medicina e Ciências da Saúde da Faculdade de Medicina e do Laboratório de Nefrologia do Instituto de Pesquisas Biomédicas da PUCRS.

Nos estudos serão avaliados diversos aspectos que podem influenciar na doença, tais como: marcadores presentes no sangue, na urina, na placenta ou em tecidos; função dos vasos sanguíneos; função das células; função de órgãos, como os rins; sensibilidade gustativa ao sal; e fatores genéticos.

A ideia é estudar fatores que possam ser importantes para a ocorrência da doença pré-eclâmpsia, que é a elevação da pressão arterial na gestação com perda de proteína na urina. Estes testes poderão ajudar a diagnosticar as pessoas em risco ou com esta condição, ou eventualmente auxiliar na formulação de novos tratamentos.

O QUE SERÁ FEITO: Você será convidada para uma entrevista com um dos membros da equipe de pesquisa. O pesquisador lhe dirá de que se trata a linha de pesquisa e o estudo que está sendo oferecido. Então será perguntado se deseja participar da pesquisa.

Caso concorde, após assinar este Termo de Consentimento Livre e Esclarecido, serão perguntados dados de sua história médica, coletado um volume de sangue venoso e/ou urina antes e depois do parto, além das coletas dos exames de rotina. Alguns dos estudos desta linha de pesquisa avaliam outros aspectos e também poderá ser coletado amostra de sangue do cordão umbilical após o parto e amostra da placenta, e/ou avaliação da função dos vasos por ecografia, e/ou medida da sensibilidade gustativa ao sal. Em alguns estudos, são avaliados a presença de marcadores genéticos. Os genes a serem estudados são extraídos do sangue ou da placenta, tentando identificar especificamente os possíveis causadores desta doença. Após o parto você poderá ser convidada a realizar acompanhamento clínico com o grupo no ambulatório Nefrologia. Este grupo atende e acompanha pacientes com hipertensão arterial sistêmica,

[1] Rúbrica Entrevistador:_____ Rúbrica Paciente/ Responsável:_____

doença hipertensiva da gestação (entre elas pré-eclâmpsia). As mulheres que desenvolvem complicação durante a gestação, têm um maior risco de doenças vasculares no futuro. A ideia do grupo é de acompanhar estas mulheres, a longo prazo, com a finalidade de observar a evolução, detectar fatores de risco ou sinais de doença, encaminhando a prevenção e/ou tratamento destes. Meses após o parto, poderá ser solicitado um exame de cintilografia renal que visa detectar a presença de cicatrizes no rim de mulheres em risco (cicatrizes são mais comuns em mulheres que desenvolveram hipertensão na gestação). Estas avaliações não interferirão nas suas avaliações e cuidados rotineiros.

O material biológico da pesquisa será coletado e congelado até a análise pelos colaboradores do Laboratório de Nefrologia da PUCRS. Os resultados serão publicados em revistas de circulação no meio médico e em congressos.

Para que os estudos possam ser realizados, é necessário que você faça a opção autorizando ou não a coleta dos diferentes materiais ou realização dos exames:

Acompanhamento ambulatorial: _____ AUTORIZO (Favor escrever SIM ou NÃO).

Urina: _____AUTORIZO (Favor escrever SIM ou NÃO).

Placenta_____AUTORIZO (Favor escrever SIM ou NÃO).

Sangue: _____AUTORIZO (Favor escrever SIM ou NÃO).

Sangue do Cordão Umbilical: _____AUTORIZO (Favor escrever SIM ou NÃO).

Ecografia dos vasos: _____AUTORIZO (Favor escrever SIM ou NÃO).

Análise genética: _____AUTORIZO (Favor escrever SIM ou NÃO).

Cintilografia renal: _____AUTORIZO (Favor escrever SIM ou NÃO).

Sensibilidade Gustativa ao Sal: _____ AUTORIZO (Favor escrever SIM ou NÃO).

*OBS.: Nem todos os testes acima serão necessariamente realizados.

CONFIDENCIALIDADE: Os registros serão mantidos em segredo.

MATERIAL EM ESTUDO E ARMAZENAMENTO: O material poderá ser utilizado apenas para esta pesquisa, ou também ser armazenado para emprego em futuros estudos. É necessário que você faça a opção autorizando ou não o armazenamento para emprego futuro: _____ AUTORIZO (Favor escrever SIM ou NÃO).

Se houver possibilidade de fazermos novas análises com o material coletado, será novamente solicitada a aprovação das Comissões de Ética em Pesquisa para realizar a avaliação adicional. Os estudos são desenvolvidos de forma anônima. Os resultados da pesquisa estarão disponíveis a você em qualquer momento por qualquer motivo. Questionamos se você gostaria de ser comunicada sobre o resultado do estudo. É necessário que você faça a opção escrevendo SIM ou NÃO: _____ QUERO SABER DO RESULTADO DA PESQUISA.

RISCOS E BENEFÍCIOS: Os riscos ou desconfortos dessa pesquisa são considerados mínimos. Este estudo não lhe trará nenhum tipo de discriminação individual ou coletiva. A presente pesquisa se propõe a colaborar com o conhecimento sobre a gestação e suas doenças relacionadas com o controle da pressão arterial, não trazendo benefícios diretos para as pacientes participantes.

LIBERDADE: A sua participação na pesquisa é totalmente voluntária e você pode desistir a qualquer momento, sem prejuízo do tratamento e sem a necessidade de explicar o motivo.

Eu, _____fui informada pelo(a) _____2 dos objetivos e justificativas dessa pesquisa de forma bem clara e detalhada. Recebi informações sobre cada passo que estarei envolvida. Todas as minhas dúvidas foram respondidas com clareza, e sei que poderei solicitar novos esclarecimentos a qualquer momento. Estou ciente que as informações por mim fornecidas serão mantidas em segredo e usadas somente conforme opção acima. Fui informada que se existirem danos a minha saúde, causados diretamente pela pesquisa, terei direito a tratamento médico e indenização, conforme estabelece a lei. Também sei que não terei nenhum custo que seja relacionado à pesquisa.

Caso tiver novas perguntas sobre este trabalho, posso chamar os pesquisadores pelos seguintes telefones (051) 33367700, 33369599, ou 3320 3000 - Ramais 3174 ou 2344, para qualquer dúvida como participante deste estudo.

Esta pesquisa tem aprovação do Comitê de Ética em Pesquisa da PUCRS. Sob as condições acima mencionadas, concordo em participar do presente estudo. Declaro que recebi cópia do presente Termo de Consentimento Livre e Esclarecido, aprovando-o e assinando-o após lê-lo com todo o cuidado possível.

Porto Alegre, ____ de _____ de _____.

_____ _____

Paciente ou Responsável Investigador CI CI/CRM

*EQUIPE PARTICIPANTE: Bartira Ercília P. da Costa, Carlos Eduardo Poli de Figueiredo, Domingos d'Ávila, Giovani Gadonski, Ivan Antonello, João Steibel, Daniele Cristóvão Escouto, Letícia G Paula, Breno J Acauan Filho (obs.: A lista dos participantes é periodicamente atualizada com nome dos participantes, alunos de PG, iniciação científica e equipe assistente).

[2] Rúbrica Entrevistador:_____ Rúbrica Paciente/ Responsável:_____

APPENDICE C – Data collection protocol (in Portuguese)

Estudo em Hipertensão Gestacional
Laboratório de Nefrologia
Responsáveis: Daniele Escouto, Bartira Pinheiro-Machado, Carlos Eduardo Poli de Figueiredo

Protocolo de Coleta de Dados

ID estudo	Registro HSL	Nome		
Endereço		Cidade	Telefones	
Dados demográficos				
DN	Idade:	cor	Escolaridade	
HMP				
HAS prévia	Comorbidades	Descrição de comorbidade		
Uso de medicamentos	Descrição medicamentos			
Hx Familiar				
Hx HAS	Parentesco	Hx DHG	Parentesco	
Hx Obstétrica				
Gestações	Partos	Abortos	Causa	
DHG prévia	Dça gestacional prévia	Descrever		
Gestação atual - dados admissão				
DUM	IG(SEM)DUM	IG(DIA)DUM	IG(SEM)ECO	IG(DIA)ECO
n. CPN	n. Fetos	Peso inicial	Peso inclus.	Altura
PAS Mínima	PAS máxima	PAD mínima	PAD máxima	
Anti-HIV	TSM / TSF	Intercorrências	Medicações uso	
Exame clínico				
PAS	PAD	PAM	SpO2	
Dispnéia	Dor torácica	Sintomas premonitórios	Qual?	
Exames laboratoriais – piores 48 horas				
Hematócrito	Hemoglobina	Leucócitos	Bastonados	Segmentados
Basófilos	Eosinófilos	Monócitos	Linfócitos	Plaquetas
Creatinina	Acido Urico	Glicemia	TP (RNI)	TP atividade
KTTP	Fibrinogênio	LDH	BD	BI
TGO	TGP	Prot. amostra	Crea amostra	P/C Calc.
ProtU de 24h	CreaU de 24h	pH	Densidade	
Proteínas	Hemoglobina(pc)	Leucócitos(pc)	Hemácias(pc)	Cel. Epite. (pc)
Dados do parto				
Data parto	IGP(SEM)	IGP(DIA)	Tipo parto	Causa PC
Descrição parto				
Inotrópicos	anti-HAS EV	Quais	Uso de CE	Uso MgSO4
FETO/RN				
Sexo	Apgar 1	Apgar 5	Peso RN	IGPed(SEM)
IGPed(DIA)	Class. RN	Peso placenta	UTINeo	Mortalidade fetal
Desfecho materno				
Mortalidade	SNC	CV	Hematológicos	Hepático
Renais	Obstétrico	SOFA	PlGF1	PlGF2

Observações:

Data da Coleta: _____
Responsável pela coleta: _____

APPENDICE D – Protocol for PlGF measurement (in Portuguese)

PROCEDIMENTO PARA ELISA PlGF SORO

1 - MATERIAIS

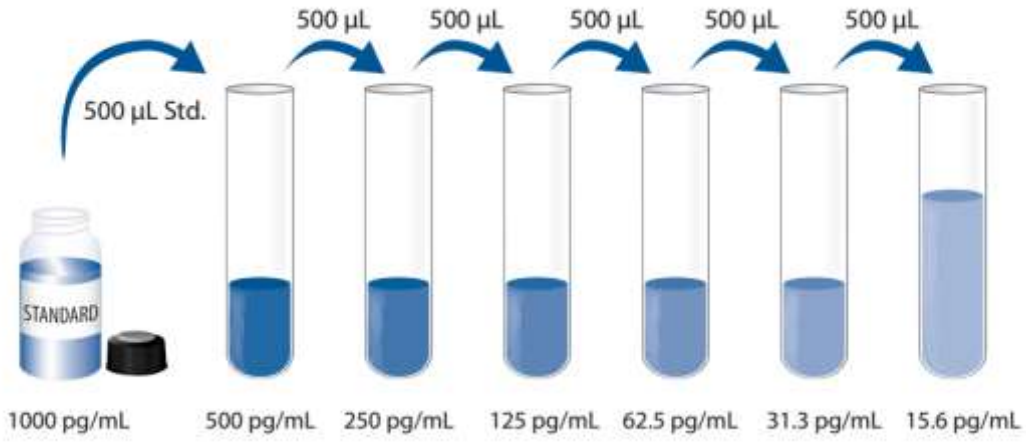
1. Microplaca com H. PlGF (descartar após 1 mês)
2. H. PlGF Standard (descartar após 4 horas)
3. H. PLGF Conjugate (1 mês)
4. Diluente RD1-22 - ressuspender pré-uso (1 mês)
5. Calibrador de diluente RD6-11 (1 mês)
6. Wash buffer concentrate (1 mês)
7. Color reagent A (1 mês)
8. Color reagent B (1 mês)
9. Stop solution (1 mês)
10. Adesivos para selar placas
11. Leitor de microplacas
    Capaz de ler 450nm com correção 540 ou 570nm
12. Pipetas e pontas
13. Água destilada
14. Material para lavagem de placas
15. Cilindro graduado de 500mL
16. Tubos para diluição padrão

2 - PREPARO DE REAGENTES

1. Todos os reagentes devem estar em temperatura ambiente
2. Wash buffer:
   a) se houver formação de cristais, agitar delicadamente até dissolver delicadamente
   b) adicionar 20mL do concentrado de Wash buffer a 480mL de água destilada para produzir Wash buffer 500mL total
3. Solução de Substrato
   a) reagentes A e B devem ser misturados em volumes iguais dentro de 15 minutos do uso
   b) proteger da luz
   c) 200µL da solução é necessário por poço
4. Human PlGF padrão
   a) observar etiqueta do frasco para volume de reconstituição
   b) reconstituir calibrador diluente RD6-11 - produção de solução padrão na concentração 1000µg/mL
   c) manter a solução em repouso por ≥ 15 minutos
   d) agite delicadamente antes do uso

3 - CURVA DE CONCENTRAÇÕES

1. Pipete 500µL do calibrador de diluente RD6-11 em cada tubo (6)
2. Use a solução padrão para produzir a série de diluições
   a) misture a solução em cada tubo antes da próxima transferência
   b) a solução padrão corresponde a concentração 1000pg/mL
   c) a solução calibradora de diluente corresponde a concentração 0pg/mL



4 - PROCEDIMENTOS DO ENSAIO

A) Todos os reagentes e amostras devem estar em temperatura ambiente e agitados para evitar cristais.
B) Standards, amostras e controles devem estar em duplicata.
1. Prepare reagentes, amostras e soluções padrão conforme previamente orientado.
2. remova o excesso placas e coloque na bolsa de alumínio contendo "desiccant pack" e sele.
3. Adicione 100µL de diluente RD1-22 em cada poço
4. Adicione 100µL de H. PlGF Standard, controles ou amostra por poço
5. Cubra a placa com adesivo e mantenha em temperatura ambiente por 2 horas
6. Aspire cada poço e lave:
    a) preencha cada poço com 400µL de wash buffer
    b) remova completamente o líquido dos poços
    c) repita o processo 3 vezes - total de 4 lavagens
    d) após a última lavagem, remova qualquer resto por aspiração
    e) inverta a placa e seque em papel toalha
7. Adicione 200µL de H. PlGF Conjugate em cada poço
8. Cubra a placa com um novo adesivo e mantenha em temperatura ambiente por 2 horas
9. Repita o procedimento 6
10. Adicione 200µL de solução de Substrato em cada poço
11. Mantenha em temperatura ambiente por 30min protegido da luz
12. Adicione 50µL de Stop Solution em cada poço
    a) a cor dos poços deve modificar de azul para amarelo
    b) se ficar verde ou não uniforme, dê pequenos tapas na placa para uniformizar
13. Determine a densidade óptica de cada poço dentro de 30 minutos
    - use leitor setado em 450nm
    - se disponível, sete correção para 540nm ou 570nm
    - se indisponível: subtraia leituras em 540 ou 570 das leituras em 450

APPENDICE E – Articles submitted as first author

ARTICLE 1 – Original article submitted to the Green Journal of Obstetrics & Gynecology entitled "The fullPIERS predictive model in women with hypertensive disorders of pregnancy". Authors: **Daniele Cristovao Escouto**, MD; Rayssa Russowsky; Natalia Paludo; Bartira E Pinheiro da Costa, PhD; Carlos Eduardo Poli-de–Figueiredo, MD, PhD.

Title: The fullPIERS predictive model in women with hypertensive disorders of pregnancy

Authors: Daniele Cristovao Escouto, MD; Rayssa Russowsky; Natalia Paludo; Bartira E Pinheiro da Costa, PhD; Carlos Eduardo Poli-de–Figueiredo, MD, PhD.

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None of the authors report any conflict of interest, regarding the current study.

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Short Title: fullPIERS in pregnancy hypertension

Acknowledgments: Laboratory of Nephrology is supported by CNPq, FAPERGS, CAPES, São Lucas Hospital School of Medicine/PUCRS. Poli-de-Figueiredo is a CNPq researcher.

PRÉCIS: fullPIERS model presented poor discrimination regarding the risk of adverse maternal outcomes in women with Hypertensive Disorders of Pregnancy up to 14 days after admission.

ABSTRACT

Objectives - The aim of the study was to evaluate the performance of the fullPIERS model in women with Hypertensive Disorders of Pregnancy.

Methods - A prospective cohort study carried out at a teaching hospital in Porto Alegre, Brazil, enrolling pregnant women admitted to the hospital with a systolic blood pressure ≥ 140 and/or a diastolic blood pressure ≥ 90 mmHg from the 20th week of gestation on, and excluding women in active labour at admission. First 48 hours of admission worst clinical and laboratory data recorded; development of adverse maternal and perinatal outcomes scrutinized up to 14 days; post-partum classification into Hypertensive Disorders of Pregnancy categories.

Results – Of the 351 women enrolled, 20 (5%) developed at least one of the combined maternal adverse outcomes, within 48h of admission. The fullPIERS model presented poor outcomes discrimination [AUC 0.639 (95% CI 0.458-0.819)]. At the seventh admission day, the model's accuracy was even lower [AUC 0.612 (95% CI 0.440-0.783)]; remaining similar [AUC 0.637 (95% CI 0.491-0.783)] at 14 days. Calibration of the fullPIERS model was poor: slope - 0.35 (95% CI 0.08-0.62), intercept -1.13 (95%CI -2.4-0.14).

Conclusion - The fullPIERS model presented poor discrimination, regarding the risk of adverse maternal outcomes in women with Hypertensive Disorders of Pregnancy up to 14 days after admission.

Key words: Outcome Assessment; prognosis; gestational hypertension; pregnancy, high-risk.

INTRODUCTION

Hypertensive disorders of pregnancy develop in more than two percent of pregnancies, worldwide.[1] It has most certainly the major cause of maternal and neonatal morbimortality, being responsible for 15% of annual global maternal deaths. In Latin America, approximately 25% of maternal deaths are related with hypertensive complications of pregnancy.[2]

Classification of Hypertensive Disorders of Pregnancy varies in the literature and comprises a broad spectrum of severity. [3-6] Presence of hypertension previous to pregnancy characterizes chronic hypertension. Isolated elevation of blood pressure from the 20th gestational week is classified as gestational hypertension. Preeclampsia is the presence of increased blood pressure, accompanied by 24-hour urine protein ≥ 300 mg. High blood pressure, in a previously hypertensive woman, accompanied by the onset or worsening of proteinuria is classified as preeclampsia superimposed to chronic hypertension. The major challenge here, is to accurately determine the presence of

preeclampsia – by far the situation accompanied by the most ominous complications.[3] Yet the above classification, as well as others, has strict criteria and may not identify all cases of preeclampsia.[7] Nevertheless, elevated blood pressure during pregnancy is related to worst maternal and neonatal outcomes, regardless of the presence of proteinuria or other features that corroborate the diagnosis of preeclampsia.[8,9] Identifying high-risk situations among the Hypertensive Disorders of Pregnancy variety of presentations - and not only preeclampsia - represents a daily challenge for obstetricians.[10]

The fullPIERS model is a simple and low-cost evaluation instrument that uses clinical variables to stratify pregnant women with preeclampsia at high risk for adverse outcomes. [11] The model accurately classified pregnant women with preeclampsia at high risk of adverse outcomes, from 48 hours, (AUC ROC 0.88 [CI 95% 0.84-0.92]) up to seven days after their hospital admission (AUC ROC >0.7).[11] The use of the fullPIERS model in clinical practice, however, still needs validation in a broader population of women with Hypertensive Disorders of Pregnancy, beyond those already diagnosed as preeclampsia. The aim of this study is to evaluate the performance of the fullPIERS model as a predictor of maternal adverse outcomes in women with Hypertensive Disorders of Pregnancy in a Southern Brazilian population.

MATERIAL AND METHODS

This study was approved by the Ethics in Research Committee of Pontificia Universidade Católica do Rio Grande do Sul (PUCRS) (document number: CEP 1.143.057), was conducted in the Obstetric Center of the Obstetrics Department of São Lucas Hospital/PUCRS – a reference center for high risk pregnancies in Porto Alegre, RS, Brazil.

A prospective cohort study was conducted at the obstetric unit of a teaching hospital in Southern Brazil. Pregnant women admitted to the hospital with hypertension after the 20th gestational week, defined as a systolic blood pressure ≥ 140 mmHg and/or a diastolic blood pressure ≥ 90 mmHg, were included. Women in active labour at the moment of hospital admission were not included due to the short time for observation of outcomes. Also, women with any component of the combined outcome were not included.

Data were obtained from patient interview and medical records. Worst clinical and laboratory data within the first 48 hours post-admission were recorded. Blood counts,

coagulation tests, biochemical parameters, including serum creatinine, liver function test, uric acid, lipids profile, serum and urinary electrolytes, urine protein to creatinine ratio and urine dip were evaluated according to the Clinical Pathology Laboratory of the São Lucas Hospital/PUCRS usual procedures.

Classification into Hypertensive Disorders of Pregnancy categories was completed after delivery. Chronic hypertension was defined as the presence of hypertension, or use of any anti-hypertensive drug, before the 20th gestational week. Gestational hypertension was considered as the presence of hypertension from the 20th gestational week on, without proteinuria. Diagnostic of preeclampsia required the presence of hypertension starting at the 20th gestational week and proteinuria (≥ 300 mg of protein in a 24-h urine collection, ≥0.3 g protein/creatinine ratio at a random urine sample, or ≥ 2+ protein by dipstick). HELLP syndrome occurred whenever hemolysis, elevated liver enzymes, low platelets were present.7 Superimposed preeclampsia was defined as the new onset of proteinuria (if proteinuria previously absent), the new onset of hypertension; if both hypertension and proteinuria previously present, or the development of one additional clinical or biochemical feature of preeclampsia, e.g. abnormal liver function tests.

Development of maternal and perinatal outcomes was observed for 14 days. Adverse outcomes were defined in accordance to the fullPIERS model development study, as a composite of maternal mortality or one or more serious central nervous system, cardiovascular, respiratory, renal, hepatic, hematological or obstetrical complication. To account for missing values, re-evaluation of 40 medical records was undertaken, as well as re-evaluation of all adverse outcomes. A fullPIERS model predicted probability for combined adverse outcomes was calculated for each woman in the dataset, using the published fullPIERS model equation.11 It is accessible at https://pre-empt.bcchr.ca/monitoring/fullpiers electronic address.

Statistical analysis

Performance of the fullPIERS model was assessed by the use of the worst results for the available data of the predictor variables, within 48 hours of admission. Predicted probabilities of adverse outcomes within 48 hours, at seven days and up to 14 days were calculated with the fullPIERS model predictive equation. In order to assess the model's capability to differentiate women at high risk of adverse outcomes, stratification capacity, calibration ability and classification accuracy were evaluated by a risk stratification table. Likelihood ratio were calculated for multicategory diagnostic test:

above 10 and below 0.1 were considered informative; between 0.1-0.2, or 5-10 were considered moderately informative; and were non-informative if Likelihood Ratio was between 0.2-0.5. Discrimination was evaluated by calculation of the area under the curve (AUC) of the receiver operating characteristics curve (ROC), with 95% confidence interval using consecutive cut-offs for the probability of combined adverse maternal outcomes within 48h, seven days and up to 14 days after admission. Discrimination was interpreted as: non-informative (AUC=1); poor (0.5 >AUC ≤ 0.7); moderate (0.7 > AUC ≤ 0.9); high (> 0.9 AUC <1); and perfect (AUC=1). A calibration plot was generated. Since adverse outcome is a dichotomous variable, the loess algorithm was used as a smoothing technique to estimate the observed probability.[12] Calibration was also assessed by evaluation of the linear predictor slope and intercept, obtained after application of the fullPIERS model to our dataset. A well-calibrated model should have a slope equal to 1.[13] Bivariate analysis of candidate-predictive outcome variables was carried out. Variables associated with outcome ($p<0.25$) and variables considered clinically important by the authors were included in the multivariate analysis.[14] Non-linearity of continuous variables in relationship with outcome was assessed and categorization, or transformation, was performed when appropriate. Stepwise backward elimination was used to build a model of adverse outcome prediction. Collinearity was checked, and only the clinically more relevant variable between two highly correlate variables was maintained. Clinically possible interactions were also evaluated. Model performance was measured by discrimination accuracy and calibration ability. A default of 1,000 bootstrap replications to obtain confidence intervals for the used parameters was applied.

All the analysis was performed using Stata 12 (StataCorp. 2011. Stata Statistical Software: Release 12. College Station, TX: StataCorp LP) and Microsoft Excel 2016 for Windows, released 2016, Redmond, WA, USA: Microsoft) software.

RESULTS

Between March 1st, 2015 and December 31st, 2016, a total of 405 women were enrolled in the study. Three participants were lost to follow-up and 51 (13%) do not have enough data to allow applying the fullPIERS model. From the 351 women included, 20 (5%) developed at least one outcome of the combined maternal adverse outcomes (Figure1). Demographic, clinical and laboratory characteristics of women with and without adverse outcomes is presented at Table 1. Mean GA at admission was

lower among women with adverse outcomes, than in women without adverse outcomes. No difference in incidence of adverse outcomes was found among groups of Hypertensive Disorders of Pregnancy. The use of anti-hypertensive drugs at admission was similar among groups that, subsequently, developed adverse outcomes. There were no differences in systolic and diastolic blood pressures at admission between groups with and without adverse outcomes. Severe hypertension was present at admission in one-third of included women; there was no significant difference in the distribution between groups (Table 1). Uric acid was higher in women with adverse outcomes than in women without. AST had a higher mean level in the group with adverse outcomes. Also, urine protein to creatinine ratio was higher among women who developed adverse outcomes. Admission-to-delivery interval was similar between groups. Ninety percent of women in the group with adverse outcomes underwent a caesarean section, while almost 50% of women without adverse outcomes had a caesarean section. Indication for surgical delivery was based on maternal health status in 55 (34%) women without adverse outcome and in 13 (76%) of those with adverse outcomes (P <0.001). Although the proportion of perinatal death was higher among pregnancies with adverse outcomes (10%) than those without adverse outcomes (2%), no statistically significant difference could be found.

Median eligibility-to-outcome interval was less than one day post-admission (0-2 days). Table 2 depicts maternal adverse outcomes, according to time after admission. The most common adverse outcome was transfusion of blood components - six cases (24%), followed by four (16%) events of abruptio placenta. No maternal death occurred. Three women had evolved to develop eclampsia. HELLP syndrome was not part of the combined adverse maternal outcome in the study; 10 (3%) women had a diagnosis of HELLP syndrome, four (20%) were on the group with adverse outcomes, whereas 6 (2%) were on the group without (P =0.001).

The fullPIERS model predicted adverse maternal outcome with poor discrimination [AUC 0.639 (95% CI 0.458-0.819)] within 48 hours of admission, Again, seven days after admission the model predicted adverse outcomes with low accuracy [AUC 0.612 (95% CI 0.440-0.783)]. Within 14 days after inclusion, the discriminative ability of the model was similar [AUC 0.637 (95% CI 0.491-0.783)]. Table 3 shows the risk stratification of fullPIERS model according to the threshold of predicted probabilities provided by the development study. For prediction of adverse outcomes after 48 hours of admission, 231 (66%) women were categorized in the low-risk group (predicted

probability< 10%); only six (3%) had an adverse maternal outcome, negative predictive value of 0.93. Five (1%) women were categorized in the high-risk group (predicted probability ≥30%); one (20%) had an adverse maternal outcome, PPV of 0.2. For prediction of adverse outcomes within seven and 14 days of admission, the model maintained a negative predictive value of 0.9 for low-risk cut-off and a negative predictive value of 0.2 for women with a predictive probability ≥30%. Likelihood ratios for the use of fullPIERS model of risk stratification, cut-off values were only informative for the prediction of adverse outcomes from seven to 14 days after admission at the risk category of 10-20% of predicted probability. That was the category where most women with adverse outcomes fell into. Likelihood ratio for adverse outcome was 16.6 (15-18) and 12.4 (11-14) for seven and 14 days after admission, respectively. The calibration performance of the fullPIERS model applied to the study sample was poor, with 0.35 (95% CI 0.08-0.62) slope and 1.13 (95% CI -2.4-0.14) intercept, which means inconsistency of estimation at extreme values.

In order to evaluate differences of regression coefficient between the study sample and the fullPIERS model development sample, we performed a logistic regression using the same predictor variables and interactions as the original model and compared both areas under the ROC curve (Figure 2). Using coefficients from the study sample, the area under the ROC curve for adverse outcomes within 14 days of admission improved to 0.784 [95% CI 0.684-0.884 (P=0.031)]. Using a predicted probability cut-off of 0.05, the model correctly classified 70% of women at risk of adverse outcome within 14 days of admission. Analysis to predict adverse outcome at 48 hours was performed [AUC ROC 0.746 (95% CI 0.630-0.863)], as well as at seven days [AUC ROC 0.793 (95% CI 0.700-0.887)] after admission. Evaluating only women who developed preeclampsia and superimposed preeclampsia (n=231), the fullPIERS model maintained a poor discrimination ability [AUC ROC 0.635 (95% CI 0.479-0.792)]. This ability increased after using the study cohort coefficients of fullPIERS model variables [AUC ROC 0.741 (95% CI 0.616-0.867)], but there was no significant difference between both curves (P=0.120) (Figure 2).

After bivariate analysis (Table 4), a model of prediction of adverse outcomes within 14 days after admission was developed, using data from 343 women. Variables included in the final model were gestational age at admission, race and worst lactate dehydrogenase within 48 hours. The AUC ROC curve for adverse outcomes within 14 days of admission, after bootstrapping replications was 0.770 (95% CI 0.643-0.896).

Classification accuracy of the model was good. Using a predicted probability cut-off of 0.058, the model correctly classified 74% of women at risk of adverse outcomes within 14 days of admission. Analysis to predict adverse outcomes at 48 hours [AUC ROC 0.710 (95% CI 0.541-0.880)] was also performed, as well as at seven days [AUC ROC 0.759 (95% CI 0.612-0.907)] after admission. Evaluating only women with preeclampsia, the model lost its discriminatory ability for within 48 hours of admission [AUC ROC 0.675 (95% CI 0.460-0.890)] and for seven days [AUC ROC 0.734 (95% CI 0.549-0.919)] after admission.

DISCUSSION

The current study evaluated the performance of a fullPIERS model in a prospective cohort of women with Hypertensive Disorders of Pregnancy. It might be important to stress that women with other Hypertensive Disorders of Pregnancy, besides preeclampsia, were included. The model performed with poor accuracy to discriminate risk of adverse maternal outcomes in this cohort. The AUC ROC curves for 48h, seven and 14 days, respectively, demonstrate poor predictive performance of the risk predictor applied to this particular cohort. At risk stratification table, over 90% of the patients were included at low risk categories, < 0.1% of predicted probability. Likelihood ratios for prediction of adverse outcomes in 48 hours, seven, and 14 days provided scarce information on the presence or absence of adverse outcomes. The stratification table provided only strong evidence to *rule-in* the presence of adverse outcome up to 14 days at the risk category of 10-20% of predicted probability, where the high probability stratification group had high LR to adverse outcomes.

Calibration of the fullPIERS model applied to the study population was poor. Calibration examines how close the predictive procedure remains valid in the validation cohort. Calibration slope was below one: a demonstration that the predicted probabilities have large variance, possibly due to inconsistency of predictor effects or overfitting of the model in the development cohort.[13] Differences between the development study population and the current one might be responsible for the model's lower performance - especially differences in cohort size, outcomes incidence and predictor distributions.

One limitation to the study is the relative low number of observed adverse outcomes. One reason to explain that is the variety of presentations of Hypertensive Disorders of Pregnancy included. However, even when evaluating only women who developed

preeclampsia previously normotensive, or presenting with superimposed preeclampsia, the model's performance was no better. The admission-to-delivery short interval - median of two (1-7) days - may have influenced the incidence of adverse outcomes. Furthermore, 44 (13%) women had lower than 34 weeks gestational age on delivery, and nine (3%) perinatal deaths occurred. Possibly, elevated rate of neonatal adverse outcomes had a significant impact unaddressed by the fullPIERS model. A survey from the World Health Organization demonstrated that perinatal mortality is three to seven times higher in pregnancies complicated by PE and eclampsia.[1] The Prediction of complications in Early-onset Preeclampsia) study, addressed neonatal adverse outcomes in preeclampsia. The study included a large number of participants and 74% of neonates had, at least, one adverse outcome before discharge; 72% where admitted to an intensive care unit.[15] Sample size and low incidence of adverse outcomes may affect the assessment of calibration. Small sample size increases the width of predicted probability range, and low incidence of outcomes increases variation of the fitted loess curve in the extreme upper tail of predicted probability distribution.[12] Two previous studies, with similar sample sizes, have been published. Akkermans et al [16] included 216 women with severe early-onset preeclampsia in a retrospective validation of the fullPIERS model with good performance. In that cohort, 34% of women experienced one of the combined adverse outcomes. Additionally, a Brazilian study[17] evaluated external validation of the fullPIERS model in a retrospective cohort of 325 women with severe preeclampsia. With a prevalence of 17% adverse outcomes, from admission to discharge, the AUC ROC of the fullPIERS model was 0.72 (95%CI 0.67-0.77). Adverse outcome definition in that study differed from the fullPIERS development study and from the current one - it included HELLP syndrome and foetal complications to the composite of adverse outcomes.

The fullPIERS and miniPIERS study group developed an external validation of the fullPIERS model using the miniPIERS development cohort as validation sample.[18] Such analysis included 757 women, and the rate of adverse outcomes within 48 hours of admission was 14%. Despite the larger sample size and high adverse outcomes rate, the predictive model lost discriminatory power [AUC ROC: 0.77 (95% CI, 0.72–0.82)] versus [AUC ROC: 0.88 (95% CI, 0.84–0.92)], respectively, with additionally poor calibration performance. The study also enrolled women with Hypertensive Disorders of Pregnancy, besides PE. However, omission of the distribution of Hypertensive

Disorders of Pregnancy classification precludes comparison with the current study population.

In an effort to improve the predictive performance, the authors applied the study population's coefficients of a logistic regression, using variables and intersections of the fullPIERS model. This approach did not enhance calibration performance, yet discrimination ability was improved. A logistic equation, obtained from the current study population, was developed. Its purpose was not to seek an alternative to the fullPIERS model, but to show that local models usually perform better than the imported model. We believe that the fullPIERS model is a significant advance and should be applied to patients with the same characteristic as those proposed in the development sample, even though its predictive power may be reduced in some degree.

The current study evaluated the performance of a proposed predictive model of adverse outcomes in women presenting with elevated blood pressure from the 20th week of gestation. While a similar approach has been adopted previously,[18] data on the distribution of Hypertensive Disorders of Pregnancy classification and the model's discriminative performance applied to all participants enrolled and to women presenting with PE, separately, are presented. Although no significant difference in the model's predictive performance on women with or without PE could be found, it appears important to include all women with Hypertensive Disorders of Pregnancy in predictive studies. First, women present at obstetric units without information, concerning previous health. Second, diagnostic criteria of PE are still subject for discussion, and broader inclusion criteria might be a better approach.

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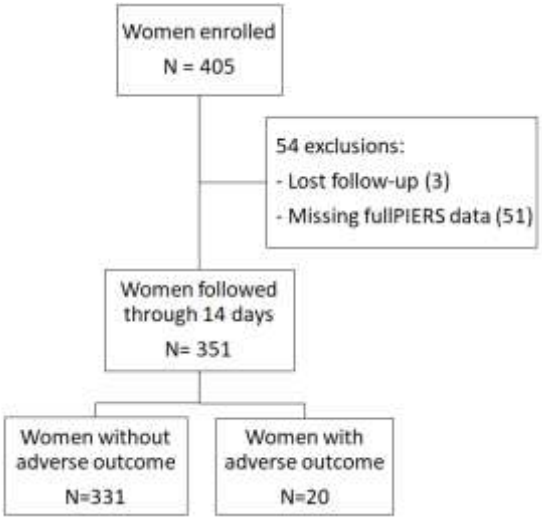

Figure 1- Study flow chart

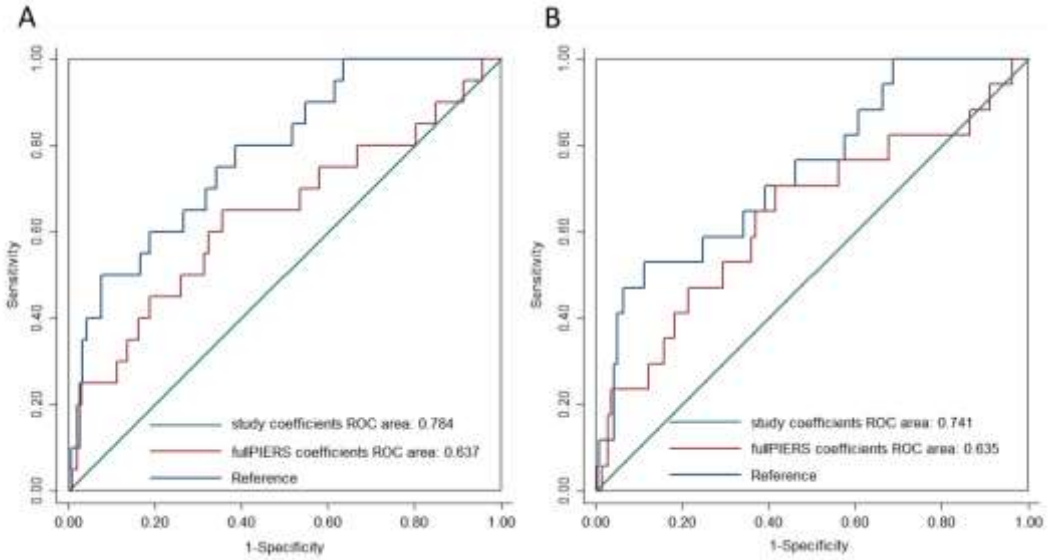

Figure 2 - Figure x. ROC curves for differences of regression coefficients between the study sample and the fullPIERS model development sample. A. ROC curves for all women. B. Roc curves for women with PE and SPE. ROC indicates receiving operator characteristics.

Table 1. Characteristics of women with and without adverse outcome within 14 days of admission.

	Total 351	Without n=331	With n=20	*P*
Maternal age at admission (years), median (IQR)	351	29.0 (22-34)	28.0 (22-32)	0.407
Gestational age at admission (weeks), mean (SD)	351	35.8 (3.8)	32.9 (5.5)	0.002
Gestational age at admission <34 weeks, n (%)	351	80 (24)	9 (45)	0.038
Race, white, n (%)	348	193 (59)	11 (55)	0.735

Medical visits during pregnancy, median (IQR)	349	8 (6-10)	6 (5-8)	0.034
Smoking, n (%)	351	42 (13)	2 (13)	0.999
Parity ≥1, n (%)	350	187 (57)	15 (75)	0.161
Previous history of PE, n (%)	350	40 (12)	5 (25)	0.157
Gestational diabetes mellitus, n (%)	350	14 (4)	0	-
Multiple pregnancy, n (%)	351	14 (4)	1 (5)	0.606
Highest pregnancy SBP (mmHg), mean (SD)	340	143.5 (18.5)	137.2 (29.6)	0.384
Anti-hypertensive drug use at admission	351	97 (29)	7 (35)	0.588
HDP classification				
Gestational hypertension	351	76 (23)	1 (5)	0.090
Preeclampsia	351	157 (47)	13 (65)	0.127
Chronic hypertension	351	41 (12)	2 (10)	0.999
Superimposed preeclampsia	351	57 (17)	4 (20)	0.761
Clinical data within 48 hours of admission				
Systolic blood pressure (mmHg), mean (SD)	351	151.0 (18.2)	151.6 (19.0)	0.892
Diastolic blood pressure (mmHg), mean (SD)	351	91.9 (14.1)	93.9 (14.0)	0.537
Severe hypertension*, n (%)	351	84 (25)	6 (30)	0.646
Pulse oximetry (%), mean (SD)	351	97.3 (1.6)	97.9 (1.5)	0.174
Haemoglobin (mg/dL), mean (SD)	351	11.9 (1.2)	11.3 (1.6)	0.044
Platelet count (10^9/L), median (IQR)	351	209.5 (65.0-223.6)	149.8 (66.2-221.9)	0.057
International normalized ratio, median (IQR)	320	1.0 (0.9-1.0)	1.0 (0.9-1.0)	0.937
Fibrinogen (mmol/L), mean (SD)	308	488.8 (95.6)	443.4 (120.0)	0.069
Activated partial thromboplastin time (s), median (IQR)	319	27.2 (25.6-29.0)	27.2 (26.0-28.9)	0.846
Creatinine(mg/dL), mean (SD)	351	0.78 (0.18)	0.93 (0.38)	0.100
Uric acid(mg/dL), mean (SD)	351	5.02 (1.39)	6.00 (1.46)	0.002
Total bilirubin (mg/dL), mean (SD)	311	0.65 (0.27)	0.75 (0.29)	0.171
Aspartate transaminase (U/L), mean (SD)	351	22.0 (18.0-28.0)	27.5 (23.0-49.5)	0.004
Alanine transaminase (U/L), mean (SD)	335	24 (19-29)	27.5 (23-61.5)	0.015

Lactate dehydrogenase (U/L), mean (SD)	346	503 (435-592)	663 (496-747)	<0.001
Urinary protein: creatinine ratio, median (IQR)	341	0.39 (0.16-1.09)	1.37 (0.40-4.33)	0.009
Interventions				
Corticosteroid administration, n (%)	346	54 (17)	10 (53)	<0.001
Magnesium sulphate administration, n (%)	347	67 (20)	13 (65)	<0.001
Gestational outcomes				
Admission-to-delivery interval (days) median (IQR)	347	2 (1-7)	1 (0-5.5)	0.184
Gestational age at delivery (weeks), mean (SD)	348	37.0 (3.0)	33.7 (4.9)	0.008
Gestational age at delivery < 37 weeks, n (%)	348	108 (33)	13 (65)	0.003
Hospital stay (days), median (IQR)	351	4 (3-8)	5.5 (3-13)	0.296
Caesarean section delivery, n (%)	347	160 (49)	18 (90)	<0.001
Caesarean section by maternal condition, n (%)	177	55 (34)	13 (76)	0.001
Birth weight (g), median (IQR)	347	2915 (2395-3340)	2208 (1273-2908)	0.003
Small for gestational age, n (%)	344	48 (15)	5 (26)	0.189
Perinatal death, n (%)	347	7 (2)	2 (10)	0.089

IQR, interquartile range; SD, standard deviation. * according to NHBPEPWGHBPP, 2000.

Table 2. Maternal adverse outcomes in women admitted in the obstetric unit of a university hospital in Porto Alegre, according to time of occurrence.

Adverse outcome	48 hours	7 days	14 days
Total	17	24	25
Maternal mortality	0	0	0
CNS			
Eclampsia, n (%)	2 (12)	2 (8)	3 (12)
PRES, n (%)	0	1 (4)	1 (4)
Cardiovascular			
Use of inotropic agents, n (%)	1 (6)	3 (13)	3 (12)
Third parenteral antihypertensive, n (%)	1 (6)	2 (8)	2 (8)
Acute pulmonary edema, n (%)	1 (6)	1 (4)	1 (4)
Hematological			
Transfusion of any blood component, n (%)	5 (29)	6 (25)	6 (24)
Hepatic			
Hepatic dysfunction, n (%)	1 (6)	1 (4)	1 (4)
Liver hematoma, n (%)	0	1 (4)	1 (0)
Renal			
Acute renal injury (creatinine 1.7-2.3 mg/dL), n (%)	2 (12)	2 (8)	2 (8)
Obstetric			
Placental abruption, n (%)	3 (18)	4 (17)	4 (16)
Uterine rupture, n (%)	1 (6)	1 (4)	1 (4)

CNS, central nervous system; PRES, posterior reversible encephalopathy syndrome.

Table 3. Risk stratification: fullPIERS performance by predicted probability of adverse outcome within 14 days.

Predicted probability	N (%)	With (%)	Without (%)	LR (95% CI)	PPV (95% CI)	NPV (95% CI)
Adverse maternal outcome within 48h						
0.00-0.0099	231 (66)	6 (3)	225 (97)	0.6 (0.1-1.2)	0.03 (0.01-0.06)	0.93(0.9-0.97)
0.01-0.024	69 (20)	4 (6)	65 (94)	1.5 (0.6-2.3)	0.06 (0.02-0.15)	0.96(0.9-0.98)
0.025-0.049	30 (9)	0	30 (100)	-	-	-
0.050-0.099	8 (2)	1 (13)	7 (87)	3.4 (1.4-5.4) 9.6 (8-11)	0.1 (0.01-0.5)	0.99(0.98-0.99)
0.10-0.19	7 (1.7)	2 (29)	5 (71)	-	0.3 (0.05-0.7)	0.97(0.9-0.98)
0.20-0.29	1 (0.3)	0	1 (100)	6.0(3.8-8)	-	-
≥0.30	5 (1)	1 (20)	4 (80)		0.2 (0.01-0.7)	0.96(0.9-0.98)
Total	351	14	334			
Adverse maternal outcome within 7 days						

0.00-0.0099	231 (66)	7 (3)	224 (97)	0.7 (0.1-1.2)	0.03 (0.01-0.06)	0.93(0.9-0.99)
0.01-0.024	69 (20)	4 (6)	65 (94)	1.4 (0.5-2.2)	0.06 (0.02-0.1)	0.96(0.9-0.98)
0.025-0.049	30 (9)	0	30 (100)	-	-	-
0.050-0.099	8 (2)	1 (13)	7 (87)	3.2 (1.1-5.2)	0.1 (0.01-0.5)	0.96(0.9-0.98)
0.10-0.19	7 (1.7)	3 (43)	4 (57)	16.6 (15.2-18)	0.4 (0.1-0.8)	0.97(0.9-0.98)
0.20-0.29	1 (0.3)	0	1 (100)	-	-	-
≥0.30	5 (1)	1	4 (100)	0.2 (0-9)	0.2 (0.01-0.7)	0.96(0.9-0.98)
Total	351	16	336			
Adverse maternal outcome within 14 days						
0.00-0.0099	231 (66)	8 (3)	223 (97)	0.6 (0.05-1.1)	0.03 (0.02-0.07)	0.9 (0.8-0.94)
0.01-0.024	69 (20)	6 (9)	63 (91)	1.6 (0.9-2.3)	0.09 (0.04-0.2)	0.95(0.9-0.97)
0.025-0.049	30 (9)	1 (3)	29 (97)	0.6 (0-2.5)	0.03 (0.002-0.2)	0.94(0.9-0.96)
0.050-0.099	8 (2)	1 (13)	7 (87)	2.4 (0.3-4.4)	0.13 (0.01-0.53)	0.94(0.9-0.97)
0.10-0.19	7 (1.7)	3 (43)	4 (57)	12.4 (11-13.8)	0.43 (0.12-0.8)	0.95(0.9-0.97)
0.20-0.29	1 (0.3)	0	1 (100)	-	-	-
≥0.30	5 (1)	1 (20)	4 (80)	4.1 (2-6.3)	0.2 (0.01-0.7)	0.95(0.9-0.97)
Total	351	20	331			

CI indicates confidence interval; LR, likelihood ratio; NPV. Negative predictive value; PPV, positive predictive value.

Table 4. Bivariate analysis.

	n	OR (95% CI)	*P*	LR test
Ethnicity*				
White	348	1.44 (0.6-10.7)	0.19	1.92
Mixed		2.58 (0.4-5.3)	0.58	
Primigravida, n (%)	350	0.44 (0.15-1.23)	0.12	2.75
Previous history of PE, n (%)	350	2.12 (0.83-7.0)	0.10	2.31
Preeclampsia	351	2.06 (0.8-5.3)	0.13	2.36
Nausea/vomiting	351	2.60 (1.05-6.45)	0.04	4.12
Headache	350	2.14 (0.80-5.71)	0.13	2.5
Scotomas	351	2.36 (0.93-5.98)	0.07	3.03

	n	Coefficient	SE	*P*	LR test
Maternal age at admission (years), log scale	351	-0.72	0.82	0.37	0.79
Gestational age at admission (weeks), cubic scale	351	-0.01	0.01	0.01	7.67
Systolic blood pressure (mmHg)	351	0.002	0.01	0.89	0.02
Diastolic blood pressure (mmHg)	351	0.010	0.02	0.54	0.38
Pulse oximetry (%)	351	0.23	0.17	0.16	2.14
Hemoglobin (mg/dL), mean (SD)	351	-0.36	0,18	0.04	3.98

Platelet count (10^9/L), n/□L	351	-0.23	0.10	0.03	5.16
Fibrinogen (mmol/L)	308	-0.006	0.003	0.07	3.65
Creatinine (mg/dL)	351	-4.28	1.78	0.02	5.64
Uric acid (mg/dL)	344	0.48	0.16	<0.01	8.86
Total bilirubin (mg/dL)	311	2.11	1.39	0.13	2.24
Aspartate transaminase (U/L)	351	-44.75	14.29	0.002	10.10
Alanine transaminase (U/L)	335	-39.35	15.25	<0.01	7.02
Lactate dehydrogenase (U/L)	346	-1571.2	437.7	<0.01	12.86
Urinary protein: creatinine ratio	341	0.35	0.14	0.013	6.15

* Black is the reference category; OR: odds ratio; LR: likelihood ratio; PE: preeclampsia; SD: standard deviation; SE: standard error. Creatinine, aspartate and alanine transaminases and lactate dehydrogenase are expressed in inverse scale. Total bilirubin is expressed in square root scale. Urinary protein: creatinine ratio is expressed in log scale.

ARTICLE 2 – Original article submitted to Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health entitled "Postpartum evaluation of cardiovascular disease risk for women with pregnancies complicated by hypertension". Authors: **Daniele Cristovao Escouto**, Amanda Green, Lesia Kurlac, Pamela Loughna, Lucy Chappell, Kate Bramham.

Postpartum Evaluation of Cardiovascular Disease Risk for Women with Pregnancies Complicated by Hypertension

Authors:

Daniele Cristovao Escouto*[1,2], Amanda Green*[3], Anna Roberts[3], Lesia Kurlak[3], Kate Walker[3], Pamela Loughna[3], Lucy Chappell [1], Fiona Broughton Pipkin[3], Kate Bramham[1]

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[2]Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, Brazil.

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Email: kate.bramham@kcl.ac.uk

*Contributed equally to study

Abstract

Objectives: Postpartum stratification of cardiovascular risk for women with pregnancies complicated by pre-eclampsia is challenging. Our aim was to determine the best

performing clinical and biomarker predictors of future cardiovascular risk at six weeks postpartum in women with hypertensive pregnancies.

Study design: Prospective longitudinal cohort

Main outcome measures: Ten year- Framingham cardiovascular risk scores were calculated for 477 women (94 with gestational hypertension, 288 with pre-eclampsia, 30 with superimposed pre-eclampsia, 51 with chronic hypertension, 14 women with uncomplicated pregnancies). B-type natriuretic peptide (BNP), neutrophil gelatinase–associated lipocalin (NGAL) and placental growth factor (PlGF) were quantified at six weeks postpartum.

Results: Framingham cardiovascular risk scores were not higher in women with pregnancies complicated by pre-eclampsia than healthy controls. Nor were scores higher in women with pre-existing chronic hypertension complicated with superimposed pre-eclampsia compared with those without superimposed pre-eclampsia. Women with gestational hypertension also had higher risk scores than women with pre-eclampsia and healthy controls. Established risk factors of cardiovascular disease including diastolic blood pressure and previously diagnosed chronic hypertension were associated with higher scores, and African ethnicity, parity and estimated glomerular filtration rate also were independently associated with higher Framingham risk scores at six weeks postpartum. PlGF, BNP and NGAL concentrations were not associated with Framingham cardiovascular risk scores after adjustment for independent variables.

Conclusions: Established clinical predictors may enable risk stratification at six weeks postpartum, which are not enhanced by the biomarkers included in this study or a history of pre-eclampsia or superimposed pre-eclampsia in most recent pregnancy.

Key words: cardiovascular disease; blood pressure; pregnancy-induced hypertension; pre-eclampsia; postpartum; biomarkers; endothelium.

ARTICLE 3 – Original article submitted to Scientia Medica entitled “Using the RUPP Model of Preeclampsia to study the Blood Brain Barrier Permeability”. Authors: **Daniele Cristóvão Escouto**, Giovani Gadonski, Luiz Porcello-Marrone, Jaderson Costa da Costa, Nathália Paludo, Rayssa Ruszkowski do Amaral, Bartira Ercília Pinheiro da Costa, Carlos Eduardo Poli-de- Figueiredo.

Using the RUPP Model of Preeclampsia to study the Blood Brain Barrier Permeability

Daniele Cristóvão Escouto, Giovani Gadonski, Luiz Porcello-Marrone, Jaderson Costa da Costa, Nathália Paludo, Rayssa Ruszkowski do Amaral, Bartira Ercília Pinheiro da Costa, Carlos Eduardo Poli-de- Figueiredo

ABSTRACT

AIMS: We object to use the reduced uterine perfusion pressure (RUPP) model for preeclampsia to describe and evaluate the blood brain barrier altered permeability.

METHODS: Forty-one pregnant Wistar rats were divided into different intervention groups between 13 to 15 days of pregnancy: Pregnant-Control (PC; n=12), Reduced Uterine Perfusion Pressure (RUPP; n=15), Invasive Blood Pressure Control (IBP; n=7) and Reduced Uterine Perfusion Pressure and Invasive Blood Pressure (RUPP-IBP; n=7). Fourteen rats had mean arterial blood pressure (MAP) measured at day 21. All animals were then sacrificed, administered Evans blue dye and perfused with paraformaldehyde 4%. Brains were removed and evaluated by a blinded pathologist.

RESULTS: MAP was 85,4 ± 2.2mmHg in the IPB group and 102,5 ± 8.3mmHg in the RUPP-IPB group (*p* 0,002). None of the control rats had positive staining of brains. The RUPP rats had 82% of positive staining for at least one of brain hemispheres ($p < 0.001$).

CONCLUSION: We concluded that the RUPP model is a valid instrument to study BBB abnormalities.

KEY WORDS: animal models, eclampsia, hypertension, posterior leukoencephalopathy syndrome, pregnancy complications, pregnancy induced hypertension.

ARTICLE 4 – Original article, to be submitted for peer review entitled, "Alloimmunization in Rhesus D Negative Kidney Transplant Recipients". Authors: **Daniele Cristovao Escouto**, Sapna Shah, Christopher Callaghan, Olivia Shaw, Alexander M, Kate Bramham**.**

Alloimmunization in Rhesus D Negative Kidney Transplant Recipients

Daniele Cristovao Escouto[1]**, Sapna Shah**[2]**, Christopher Callaghan**[3]**, Olivia Shaw**[3]**, Alexander M, Kate Bramham**[2]

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[2]Department of Renal Sciences, Division of Transplantation and Mucosal Biology, King's College London and King's Health Partners, London, UK

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Abstract

Introduction: Alloimmunization may occur in a Rhesus D (Rh D) negative recipient after exposure to Rh D positive red blood cells present in the graft at time of transplantation. Haemolytic complications of the foetus and newborn in future pregnancies of female transplant recipients may occur in sensitised individuals. There is discrepancy amongst current practice in the UK regarding administration of anti Rh D antibody after transplantation to Rh D negative recipients of a Rh D positive kidney. One study, published 20 years ago, reported 2/42 (5%) of Rh D negative recipients had Rh D antibody after transplantation but development of anti-Rh D antibodies following renal transplantation with current surgical techniques is undetermined. The aim of this study is to determine the Rh D antibody status of Rh D negative recipients with Rh D positive grafts in order to inform the use of anti-D prophylaxis.

Methods: All Rh D negative renal transplant recipients receiving Rh D positive grafts from two London teaching hospitals between February 2000 until August 2015 were identified from hospital records. Recipient demographic data, nature of donor and Rh D antibody status before and after transplant were recorded. None of the patients received anti-Rh D prophylaxis after transplantation.

Results: A total of 125 Rh D negative patients underwent transplant during the time of observation. Of those, 78 (63%) received Rh D positive grafts. Deceased donors accounted for 73% of transplants and 29 (37%) of recipients were female. None of the recipients developed anti Rh D antibodies after transplantation.

Conclusion: The development of anti Rh D antibodies did not occur in Rh D negative recipients of Rh D positive kidney transplants; thus, anti-Rh D prophylaxis may not be necessary. The risk of haemolytic disease of the foetus and newborn in female transplant recipients is likely to be low. However, sensitisation to other red cell antigens (e.g. Kell) during transplantation may occur, and further assessment of another antibody development is needed.

Annexes

ANNEXES

ANNEX A – Research ethics committee approval (in Portuguese)

PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL - PUC/RS

Plataforma Brasil

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Avaliação do modelo fullPIERS e PIGF como preditores de desfechos adversos maternos e perinatais em mulheres com Doença Hipertensiva Gestacional.

Pesquisador: Carlos Eduardo Poli de Figueiredo

Área Temática:

Versão: 2

CAAE: 36211614.3.0000.5336

Instituição Proponente: UNIAO BRASILEIRA DE EDUCACAO E ASSISTENCIA

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 1.143.057

Data da Relatoria: 30/07/2015

Apresentação do Projeto:

Emenda sobre o projeto original.

Objetivo da Pesquisa:

A emenda requer a manutenção de amostras de DNA e urina para futuras pesquisas.

Avaliação dos Riscos e Benefícios:

Adequada no projeto original, não se modifica com essa emenda.

Comentários e Considerações sobre a Pesquisa:

A solicitação para a coleta de amostra e seu uso para pesquisas posteriores já estava clara no TCLE. Novas análises poderão trazer informações sem adicionar desgaste ou risco para as pacientes.

Considerações sobre os Termos de apresentação obrigatória:

A solicitação para a coleta de amostra e seu uso para pesquisas posteriores já estava clara no TCLE.

Recomendações:

Convém se manter informado sobre mudanças em andamento em relação ao biobanco da PUCRS.

Endereço: Av.Ipiranga, 6681, prédio 40, sala 505
Bairro: Partenon **CEP:** 90.619-900
UF: RS **Município:** PORTO ALEGRE
Telefone: (51)3320-3345 **Fax:** (51)3320-3345 **E-mail:** cep@pucrs.br

Conclusões ou Pendências e Lista de Inadequações:

Sugiro aprovação do adendo.

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

PORTO ALEGRE, 08 de Julho de 2015

Assinado por:
Rodolfo Herberto Schneider
(Coordenador)

This is the final approval conceded after an addendum to the original approval in November 2014.

ANNEX B – PlGF informations from fabricant

Product Details | Assay Procedure | Citations (52) | FAQs | Supplemental Products | Reviews

Assay Type	Solid Phase Sandwich ELISA
Format	96-well strip plate
Assay Length	3.5 hours or 4.5 hours
Sample Type & Volume Required Per Well	Cell Culture Supernates (100 uL), Serum (100 uL), EDTA Plasma (100 uL), Heparin Plasma (100 uL), Citrate Plasma (100 uL), Urine (100 uL)
Sensitivity	7 pg/mL
Assay Range	15.6 - 1,000 pg/mL (Cell Culture Supernates, Serum, EDTA Plasma, Heparin Plasma, Citrate Plasma, Urine)
Specificity	Natural and recombinant human PlGF
Cross-reactivity	Cross-reactivity observed with 1 or more available related molecules.Cross-species reactivity not tested.
Interference	Interference observed with 1 or more available related molecules.

Control Available

QC22 , Quantikine Immunoassay Control Group 7 - Please Inquire

Product Summary

The Quantikine Human PlGF Immunoassay is a 3.5 or 4.5 hour solid phase ELISA designed to measure human PlGF in cell culture supernates, serum, plasma, and urine. The immunoassay kit contains *E. coli*-expressed recombinant human PlGF and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate recombinant human PlGF accurately. Results obtained using natural human PlGF showed dose curves that were parallel to the standard curves obtained using the recombinant Quantikine kit standards. These results indicate that this kit can be used to determine relative mass values for natural human PlGF.

Precision

Intra-Assay Precision (Precision within an assay) Three samples of known concentration were tested on one plate to assess intra-assay precision

Inter-Assay Precision (Precision between assays) Three samples of known concentration were tested in separate assays to assess inter-assay precision

Cell Culture Supernates

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean	40.9	138	541	46.8	149	555
Standard Deviation	2.4	7.3	38.2	6.4	13.5	32.8
CV%	5.8	5.3	7.1	13.6	9.1	5.9

Serum, EDTA Plasma, Heparin Plasma, Citrate Plasma, Urine

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean	54.3	175	658	55	184	724
Standard Deviation	3.8	6.3	36.9	6.5	20.3	78.6
CV%	7	3.6	5.6	11.8	11	10.9

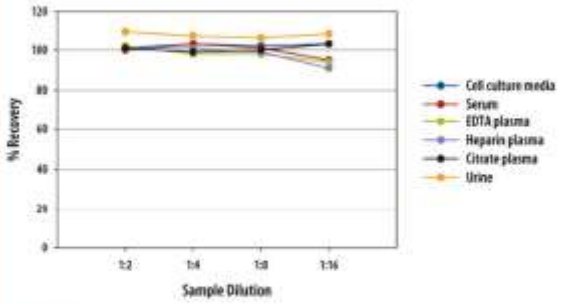
Recovery

The recovery of PlGF spiked to three different levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range %
Cell Culture Media (n=5)	97	82-107
Citrate Plasma (n=5)	99	93-120
EDTA Plasma (n=5)	102	92-117
Heparin Plasma (n=5)	94	81-113
Serum (n=5)	96	88-118
Urine (n=4)	97	85-112

Linearity

To assess the linearity of the assay, five samples were spiked with high concentrations of PlGF in various matrices and diluted with the appropriate Calibrator Diluent to produce samples with values within the dynamic range of the assay.



Product Datasheets

Product Datasheet | COA | SDS

Preparation and Storage

Storage	Store the unopened product at 2 - 8 °C. Do not use past expiration date.

ANNEX C – Abstracts published as first author

ARTICLE 1 – Abstract, entitled "Effect on renal graft function of switching from mycophenolate acid to azathioprine for pregnancy". Authors: **Daniele Cristovao Escouto**, Larissa Fonseca Borges, Kate Bramham. Abstract presented at the 28th Brazilian Congress of Nephrology and published at the Brazilian Journal of Nephrology, Vol.38 - Number 3 Suppl 1 / 2016.

março de 2015. Foram analisadas incidência de disfunção inicial do enxerto (DGF) e de rejeição aguda (RA), a taxa de filtração glomerular (TFG) do pela fórmula MDRD e as sobrevivas de pacientes e enxertos. **Resultados:** No período estudado 613 pacientes receberam TR de doador falecido, sendo 414 (67,5%) de doadores de critérios padrão (Grupo 1) e 199 (32,5%) de doadores de critérios expandidos (Grupo 2). Não foram observadas diferenças quanto à raça, gênero, cor do doador e receptor, doença de base, transplantes prévios, número de missmatches HLA e imunossupressão. A incidência de disfunção inicial do enxerto foi maior no grupo 2 (64,5% *vs.* 73,5%; $p = 0,033$), o que determinou uma diferença significativa no tempo de internação, que também foi maior no grupo 2 ($p < 0,02$). A comparação da função do enxerto mostrou que o grupo 1 apresentou TFG significativamente maior em 1 mês (45,5 ± 24,3mL/min *vs.* 34,4 ± 17,7mL/min), 3 meses (55,6 ± 27,3mL/min *vs.* 40,7 ± 16,2mL/min), 6 meses (55,0 ± 23,7mL/min *vs.* 40,6 ± 16,1mL/min) e 12 meses (60,5 ± 24,3mL/min *vs.* 44,3 ± 18,3mL/min); ($p < 0,01$). No período do estudo houve 78 perdas de enxerto sendo 49 (11,8%) no grupo 1 e 29 (14,6%) no grupo 2. Não houve diferença na sobrevida do enxerto em 1 ano (92,4% *vs.* 89,3%), 3 anos (89,2% *vs.* 87,9%) e 5 anos (85% *vs.* 78,9%); ($p = 0,268$) e na sobreviva do paciente em 1 ano (96,4% *vs.* 96,3%), 3 anos (91,2% *vs.* 94,3%) e 5 anos (86,4% *vs.* 92%); ($p = 0,264$). **Conclusão:** O uso de rins de DCE não impactou na sobreviva do enxerto e do receptor, mostrando ser, em médio prazo, uma estratégia segura para aumentar o número de transplantes renais.

AO: 49590

Effect on renal graft function of switching from mycophenolate acid to azathioprine for pregnancy

Autores: Daniele Cristovao Escouto*; Larissa Fonseca Borges Lopes; Kate Bramham

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PUCRS

Mycophenolate acid (MA) is teratogenic, hence cessation prior to conception is recommended for women with renal transplants. MA is frequently replaced with azathioprine (AZA); however the impact of replacing MA for pregnancy purposes on long term renal graft function is unknown. The aim of our study is to evaluate the effect of switching MA to AZA in order to conceive on long term graft function in women with renal transplants. **Methods:** Records of women attending renal pre-pregnancy counselling clinic in a London teaching hospital from 2004-2014 were examined. Inclusion criteria were: previous use of MA, switch to azathioprine and stable renal function for at least one year after transplantation. Outcomes included estimated glomerular filtration rate (eGFR) at 1 year after conversion from MA to AZA, renal graft biopsy and conversion back to MA. **Results:** We included 13 cases of conversion from MA to AZA. Prior to conversion, mean creatinine was 108.6 (32.5) mmol/dL and mean MDRD eGFR was 57.6 (16.7) ml/min/1.73m². Three women (23%) required renal graft biopsy after conversion, all were diagnosed with acute cellular rejection. Patients diagnosed with acute rejection tended to have shorter interval between transplant and switch, 2.0 (1.0-2.0) years versus 5.0 (3.0-7.3) years (p 0.08). Also, all patients with acute rejection were using 2g/day of MA prior to conversion, while others used a median of 1.0 (0.9-1.6) g/day (p 0.05). Five (39%) patients were converted back to MA, 3 empirically and 2 due to worsening of renal function. Within one year, there was no statistically significant change in creatinine, 4.0 (-2.5 to 12.5) mmol/dL (p 0.18), neither in eGFR, -2.0 (-5.0 to 3.0) ml/min/1.73m²/year (p 0.57). Nine pregnancies occurred: 6 live births and 3 miscarriages. Mean initial eGFR was higher in patients with successful pregnancies and live births, 71.2 (12.8) mmol/dL *versus* 49.1 (13.0) mmol/dL (p 0.01). Change in eGFR during 1 year after conversion was not affected by pregnancy (p 0.4). **Conclusions:** Most women with renal transplants of child-bearing age taking mycophenolate acid can be converted to azathioprine for pregnancy purposes, but with caution for those with shorter times after transplant, and on higher doses of MA.

AO: 51799

Estudo do efeito do polimorfismo K121Q na rejeição aguda em pacientes transplantados renais

Autores: Andrea Carla Bauer*; Denise Alves Sortica; Marjoriê Piuco Buffon; Bruna Bellicanta Nicoletto; Pamela Sachs Nique; Daisy Crispim; Roberto Ceratti Manfro; Luis Henrique Canani

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Introdução: Doença renal do diabetes (DRD) é uma complicação crônica microvascular que afeta aproximadamente 40% dos pacientes com diabetes mellitus (DM), sendo uma das principais causas de falência renal. Transplante renal é o tratamento de escolha para uma significativa proporção de pacientes em estágio final da doença renal crônica, incluindo pacientes com DM. Neste contexto, a rejeição aguda (RA) é uma importante complicação pós-transplante. O uso de biomarcadores como método de prognóstico, ou a detecção de eventos patológicos iniciais em transplantes renais é uma estratégia atrativa. Alguns estudos têm avaliado a relevância de

ARTICLE 2 – Abstract, entitled "The renin-angiotensin-aldosterone system and superimposed preeclampsia in women with chronic kidney disease and chronic hypertension". Authors: **Daniele Cristovao Escouto**, Lesia Kurlac, Carolyn Gill, Hiten Mistren, Lucy Chappell, Kate Bramham. Abstract presented at the International Society for the Study of Hypertension in Pregnancy World Congress 2016 (ISSHP 2016). This study competed for the *Zuspan Award*, a prize given to two young researchers who, according to the conference evaluators, developed and presented the most outstanding papers in the study on Hypertension.in Pregnancy. Published at congress proceedings in Pregnancy Hypertension, vol. 5, number 3, July 2016.

Clinical science

13 The renin–angiotensin–aldosterone system and superimposed pre-eclampsia in women with chronic kidney disease and chronic hypertension

Chronic hypertension

Daniele Cristovao Escouto [a], Lesia Kurlak [b], Carolyn Gill [c], Hiten Mistren [b], Lucy Chappel [c], Kate Bramham [c] ([a] PUCRS, Porto Alegre, RS, Brazil, [b] The University of Nottingham, Nottingham, United Kingdom, [c] King's College London, London, United Kingdom)

Introduction: Systemic renin–angiotensin–aldosterone system (RAAS) activity is augmented in non-pregnant women with chronic kidney disease (CKD) and chronic hypertension (CHT), but its activity in pregnancy and superimposed pre-eclampsia is poorly understood.

Objectives: (i) To compare longitudinal changes in plasma renin and angiotensinogen concentrations in pregnant women with CKD and CHT with healthy controls.

(ii) To compare differences in plasma renin and angiotensinogen concentrations in pregnant women with CKD and CHT with and without superimposed pre-eclampsia and women with pre-eclampsia without pre-existing disease.

Methods: Plasma renin activity and angiotensinogen were quantified in samples from women with CKD or CHT, women with pre-eclampsia without pre-existing disease and healthy controls recruited to a longitudinal prospective cohort study at two London Academic Health Centers. Demographics and pregnancy outcomes were extracted from patient records. RAAS component concentrations were compared across gestational ages using repeated measures analysis.

Results: One hundred and ninety-five women were recruited (CKD = 80; Primary CHT = 31; Pre-eclampsia without pre-existing disease = 19; Healthy controls = 65). Eighteen women with CKD (22.5%) and 10 women (32.3%) with primary CHT developed superimposed pre-eclampsia. Plasma renin activity was lower in women with CKD or CHT with compared to healthy controls at all gestational time points ($p < 0.006$). Women with CKD or CHT who later developed superimposed pre-eclampsia tended to have lower plasma renin activity adjusted for gestation age at sampling than women with CKD or CHT without superimposed pre-eclampsia. There were no differences in plasma angiotensinogen between groups. Women with superimposed pre-eclampsia had lower plasma renin activity compared to women with CKD or CHT without superimposed pre-eclampsia (p 0.003) or healthy controls ($p < 0.001$) with no differences in plasma angiotensinogen concentrations. There were no differences in plasma renin or angiotensinogen concentrations between women with superimposed pre-eclampsia and those with pre-eclampsia without pre-existing disease.

Conclusion: Women with CKD or CHT have suppressed plasma renin activity during pregnancy compared with healthy controls. After diagnosis of superimposed pre-eclampsia women with CKD or CHT have lower plasma renin than women without superimposed pre-eclampsia, but there are no differences between women with pre-eclampsia and superimposed pre-eclampsia, suggestive of a common pathophysiological pathway.

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Clinical science

14 CHIPS-Child: Testing the developmental programming hypothesis in the offspring of the CHIPS trial

Randomized trials

Laura Ann Magee [a], Anne Synnes [b], Peter Von Dadelszen [a], Anna Hutfield [b], On behalf of CHIPS-CHILD Working Grp ([a] St. George's, University of London, London, United Kingdom, [b] University of British Columbia, Vancouver, Canada)

Introduction: CHIPS-Child is a follow-up to the international CHIPS trial that showed that 'less tight' (vs. 'tight') control of maternal blood pressure (BP) was associated with no decrease (or increase) in either the primary perinatal outcome (pregnancy loss or high level neonatal care for >48 h) or measures of fetal growth (in utero).

Objectives: In CHIPS-Child, we tested the developmental programming hypothesis by determining if children born of mothers in 'less tight' (vs. 'tight') BP control arms of CHIPS showed differences in postnatal growth and health.

Methods: Follow-up was extended to 12 ± 2 months corrected post-gestational age for anthropometric measurements. For CHIPS subjects who consented to a CHIPS-Child study visit, we collected biological samples from the child (hair samples, buccal swabs) to evaluate hypothalamic–pituitary–adrenal axis (HPA) function (hair cortisol levels) and epigenetic change (DNA methylation analysis of buccal cells). The primary outcome was 'change in z-score for weight' (95% CI) between birth and 12 ± 2 months, compared between groups using linear regression adjusted for maternal pre-randomisation factors (hypertension type and centre, antihypertensive type, and maternal body mass index, body mass index [BMI]) (two-tailed $p > 0.05$) and any between-group differences at baseline among babies followed-up. Secondary outcomes were genome-wide and targeted DNA methylation status (from buccal swabs, measured using the Illumina infinium HumanMethylation450 array platform) and hair cortisol (as a measure of hypothalamic–pituitary activation).

Results: Of 683 eligible CHIPS babies (59 sites), 500 (73.2%) were followed up in CHIPS-Child [244 (70.1%) in 'less tight' vs. 256 (75.7%) in 'tight' control] and 414 consented to participate [196, (80.3%) vs. 218 (85.2%)]. 272 (76.8%) of babies had anthropometry at 12 ± 2 months. Babies in 'less tight' (vs. 'tight') probably differed with regards to change in z-score for weight (primary outcome) [−0.27 (−0.54, 0.00); $p = 0.05$]; there was no difference in z-scores for length [0.14 (−0.20, 0.49); $p = 0.42$] or head circumference [0.19 (-0.89, 1.26); $p = 0.71$], or waist circumference (cm) [0.99 (-1.75, 3.72); $p = 0.45$]. Secondary outcomes were measurable only among the babies who were eligible for a study visit ($N = 92$), consented ($N = 45$), and supplied biological samples ($N = 41$). 16 DNA samples (9 vs. 7) passed quality control testing; no difference in DNA methylation was seen based on a false discovery rate >20%. In the 35 adequate hair samples, cortisol levels were significantly lower in 'less tight' (vs. 'tight')[−496 (−892, −100); $p = 0.02$].

Conclusions: The results of CHIPS-Child suggest that 'tight' BP control may be associated with both more rapid postnatal growth (birth to age 12 months) and developmental programming of the HPA axis. These results are limited by small sample size. Longer follow up would be necessary to determine if the findings are associated with important clinical outcomes.

(CHIPS-Child Working Group: JP Chanoine, AM Côté, A Devlin, A Gafni, W Ganzevoort, A Gruslin, M Helewa, E Hutton, G Koren, SK Lee, D McArthur, E Rey, WP Robinson, T Roseboom, J Singer, S Wilson, JM Moutquin).

doi:10.1016/j.preghy.2016.08.015

ARTICLE 3 – Abstract, entitled "Predictors of persistent hypertension after pregnancy disorders of pregnancy". Authors: **Daniele Cristovao Escouto**, Giovani Gadonski, Fernando Sontag, Luiza Vasconcelos Cunha, Ana Luiza Fonseca Siqueira, Nathalia Paludo, Rayssa Ruszkowski Amaral, Carlos Eduardo Poli-de-Figueiredo. Abstract presented at the International Society for the Study of Hypertension in Pregnancy World Congress 2016 (ISSHP 2016). Published at congress proceedings in Pregnancy Hypertension, vol. 5, number 3, July 2016.

Conclusions: Detection of relative FGR remains a clinical challenge. Our findings suggest that AC growth velocity in early third trimester determine eventual birth weight and that neonates at the lower end of the AGA spectrum have a reduced AC velocity and a worse neonatal outcome. Larger studies are needed to confirm these findings in prospective cohort.

doi:10.1016/j.preghy.2016.08.113

Clinical science

32 Predictors of persistent hypertension after pregnancies with hypertensive disorders of pregnancy

Chronic hypertension

Daniele Cristovao Escouto, Giovani Gadonski, Fernando Sontag, Luiza Vasconcelos Cunha, Ana Luiza Fonseca Siqueira, Nathalia Paludo, Rayssa Ruszkowski Amaral, Carlos Eduardo Poli-De-Figueiredo (Pucrs, Porto Alegre, RS, Brazil)

Introduction: Hypertensive disorders of pregnancy (HDP) occur in 6–10% of all pregnancies. A significant proportion of previously normotensive women persist with elevated blood pressure 12 weeks postpartum. Investigation on factors involved in the persistence of hypertension is of critical importance to provide quality health care for those women. The aim of our study is to identify factors involved in the persistence of hypertension at 12 weeks after pregnancies complicated by HDP.

Methods: We conducted a case-control study nested in a prospective cohort of outpatient women who had HDP at a teaching hospital in Porto Alegre, RS. At first visit, all women informed socio-demographic characteristics, medical and pregnancy histories. At follow-up visits we collected information on use of antihypertensive medication, blood pressure, urinary and serum analysis.

Results: We selected 99 women in the cohort with no preexisting hypertension and that were followed until 12 weeks postpartum. Hypertensive women after 12 weeks from delivery were considered cases ($n = 56$) and normotensive women after 12 weeks were labelled controls ($n = 43$). We developed a risk prediction model with independent variables for persistent hypertension after pregnancy. Our results showed that both blood pressure and use of antihypertensive medication were significantly different at first visit. Mean systolic and diastolic blood pressure were, respectively, 138.6 (20.6) mmHg and 91.9 (12.3) mmHg for the hypertensive group and 117.9 (12) mmHg and 78.4 (8.9) mmHg for controls ($p < 0.001$). Maternal age was significantly higher in the hypertensive group. Gestational outcomes and biochemical parameters were similar between groups. Gestational age at delivery, maternal age and both systolic and diastolic blood pressures at first visit were independent variables chosen to compose a risk model with an area under the ROC of 0.896 (95% CI 0.819-0.972) to predict persistent hypertension after pregnancy.

Conclusion: Gestational age at delivery, maternal age and blood pressure were identified as important factors involved in persistent hypertension 12 weeks after pregnancies complicated by HDP. We believe that prospective studies with larger population are needed to access the value of a risk model to predict, at first visit postpartum, which women have increased chance to become chronically hypertensive.

doi:10.1016/j.preghy.2016.08.114

Clinical science

33 Association between maternal cardiac function in gestational hypertension and pregnancy outcome

Gestational hypertension

Aleksandra Ilic[a], Snezana Stojsic[b], Djordje Ilic[b], Anastazija Stojsic-Milosavljevic[a], Maja Stefanovic[a], Snezana Tadic[a], Dragana Grkovic[a], Tatjana Miljkovic[a], Marija Bjelobrk[a], Katica Pavlovic[a], Jadranka Dejanovic[a] ([a]Institute of cardiovascular diseases of vojvodina, Novi Sad, Serbia, [b]Clinical Center of Vojvodina, Department of Obstetrics and GYnecology, Novi Sad, Serbia)

Introduction: It was well known that maternal cardiac function changed during pregnancy and that these changes were more pronounced in pregnancy complicated by hypertension. We were interested whether pregnancy outcome was more affected by changes in systolic or diastolic maternal function.

Objective: The purpose of this study was to determine the relationship of changes in function of the left ventricle (LV) with the intrauterine growth restriction (IUGR) and preterm delivery in pregnant women with gestational hypertension (GH), and reversibility of these changes after delivery.

Material and Methods: This study included 60 pregnant women with GH (defined as blood pressure ⩾140/90 mmHg that was appeared after 20th week of gestation (GW) and disappeared within six weeks postpartum). All participants underwent a complete two-dimensional, pulsed and tissue Doppler echocardiography that was used to assess parameters of diastolic function: IVRT , transmitral inflow velocities during diastole – E, A, E/A, DTE, the left ventricular filling index E/e'; parameters of systolic function: EF, cardiac output (CO), CO index (COi), IVCT, ET, stroke volume (SV), SVi, Ees, Vcf and transmitral inflow velocities during systole – velocity of septal and lateral mitral annulus: s's and s'l; and global cardiac function: Tei index. The echo was performed in the third trimester and 6 weeks after delivery, also as 24-h ambulatory blood pressure monitoring (ABPM).

Results: There weren't statistically significant differences between groups in the following parameters: age, heart rate, height, weight. Participants with IUGR had significant higher blood pressure. They also had more impaired parameters of the LV systolic and global function. Tei index was statistically significant higher in women whose children were IUGR ($0,50 \pm 0,05$ vs $0,47 \pm 0,05$; $p = 0,033$), also IVCT was statistically significant longer ($64,73 \pm 11,29$ vs $59,05 \pm 8,42$; $p = 0,031$). Univariate regression analysis revealed that CO had significant influence on IUGR ($p = 0,001$; OR 0,309; 95% CI 0,156 – 0,613), also as COi ($p = 0,011$; OR 0,255; 95% CI 0,089 – 0,733). Increasing CO for 1 l desceases risk of IUGR for 70%, while increasing COi for 1 l/m2 desceases risk of IUGR for 75%. Participants with preterm delivery also had significant higher blood pressure and significant more impaired parameters of the LV systolic function. SV was statistically significant lower in women with preterm delivery ($55,82 \pm 12,1$ vs $65,69 \pm 14,8$; $p = 0,018$), also as SVi ($28,61 \pm 5,8$ vs$32,64 \pm 7,3$; $p = 0,048$) and Vcf ($1,32 \pm 0,2$ vs $1,44 \pm 0,3$; $p = 0,042$). Univariate regression analysis revealed that EF had significant influence on preterm delivery ($p = 0,012$; OR 0,740; 95% CI 0,585 – 0,937). All changed echocardiographic parameters became improved six weeks after delivery.

Conclusion: Changes in left ventricle function in gestational hypertension predicts IUGR and preterm delivery. There was statistically significant relationship between impaired systolic and global cardiac function in gestational hypertension with IUGR, also as there was statistically significant association between impaired systolic function with preterm delivery, while there was no association

ARTICLE 4 – Abstract, entitled "Interval between blood pressure measurements in pregnant women with high blood pressure". Authors: **Daniele Cristovao Escouto**, M. R. Vieira, B. Pinheiro da Costa, Carlos Eduardo Poli-de-Figueiredo. Abstract presented at the 26th European Meeting on Hypertension and Cardiovascular Protection. Published at the Journal of Hypertension, 34: e264, September 2016.

rameter called preeclampsia risk score, with the formula: Score = –1.974*C6772–1.479*D1-1.335*D2. The risk score was normalized by summing all values calculated with K = –4.78. Thus, the score ranged in the interval: [0–4.79], negative values being eliminated. The reliability of the new prediction score was assessed by a ROC analysis. The area under the curve of 0.701 was calculated (95% CI = 0.640–0.756), the newly built parameter being validated as a highly reliable predictor of preeclampsia by a significance level $p < 0.001$. Thus, parameter levels over 1.34 (cut-off value) presented a Se = 3.2 (0.9–8.0) and a Sp = 100 (97.2–100) for association with preeclampsia. The analysis revealed that score values below 1.34 could be classified as high risk, between 1.34 and 3.45 as medium risk, and beyond this value as low risk. To use the newly constructed score, an interface was created which allows users to introduce the position status (1 = homozygous mutation, 0 = normal or heterozygous mutation) for each of the three mutations included in the previous score.

Conclusions: Of all the variables included in the model, MTHFR-C677T, D1-C/T and D2-Thr22Ala were independent predictors of preeclampsia. The preeclampsia risk score is calculated automatically, using the created interface, as a continuous value and is interpreted semiquantitatively in accordance with the values set.

PP.24.11 INTERVAL BETWEEN BLOOD PRESSURE MEASUREMENTS IN PREGNANT WOMEN WITH HIGH BLOOD PRESSURE

D. Escouto, M.R. Vieira, B. Pinheiro da Costa, C.E. Poli-de-Figueiredo. *Pontifical Catholic University of Rio Grande do Sul – Faculty of Medicine, Porto Alegre, BRAZIL.*

Objective: To analyze the behavior of blood pressure at the first 8 hours of emergency care in pregnant women who arrive with hypertension in an obstetric unit.

Design and method: Blood pressure was measured at an Obstetric Unit in a cohort of 415 pregnant women with high blood pressure at the initial evaluation. Data of the first 8 hours of blood pressure readings were analyzed by Generalized Estimated Equations test.

Results: At baseline the means(±SD) were 154.3 ± 16.5 mmHg and 98.0 ± 12.1 mmHg for systolic and diastolic blood pressure, respectively. There was a significant reduction in blood pressure during follow-up ($p < 0.001$). Blood pressure means (SD) were: 1st hour: 146.6+19.1 and 89.7+15.6, 2nd: 139.0+17.8 and 83.2+14.2, 3rd: 137.2+15.6 and 78.7+12.2, 4th: 136.9+14.7 and 78.8+14.5, 5th: 135.9+16.6 and 78.2+14.1, 6th: 135.6+16.3 and 77.9+13.5, 7th: 133.3+14.2 and 75.7+11.9, and 8th hour 133.8+15.6 and 76.9+12.9 mmHg for systolic and diastolic blood pressure, respectively. Blood pressure stabilized after the third hour.

Conclusions: The study provides evidence that an interval of at least three hours between measurements is adequate to establish the diagnosis of gestational hypertension in pregnant women presenting with high blood pressure at an obstetric unit.

PP.24.12 ECLAMPSIA VERSUS PREECLAMPSIA: INCREASED PROTEINURIA AND URIC ACID LEVELS ARE ASSOCIATED WITH ECLAMPTIC CRISIS

D. Escouto, L.G. Paula, B. Pinheiro da Costa, C.E. Poli-de-Figueiredo. *Pontifical Catholic University of Rio Grande do Sul – Faculty of Medicine, Porto Alegre, BRAZIL.*

Objective: Characterize a group of women that developed eclampsia and to compare it with a group that had preeclampsia focusing in maternal serum uric acid and proteinuria-to-creatininuria ratio.

Design and method: The records of patients delivered at Hospital São Lucas/ Pontifícia Universidade Católica do Rio Grande do Sul were reviewed retrospectively; 733 pregnant women with hypertension were analyzed; 424 had preeclampsia, and 52 eclampsia.

Results: Patients with eclampsia and preeclampsia were different in several clinical, laboratorial and demographic aspects. Patients with eclampsia had higher serum uric acid levels and protein excretion, systolic and diastolic blood pressure; were more likely to have cesarean section, and had worst perinatal outcomes. The combination of uric acid above 5.9 mg/dL and proteinuria/creatininuria ratio above 4.9 had a striking association with eclampsia.

Conclusions: Our data strongly suggest that the combination of increased levels of maternal serum uric acid especially above 5.9 mg/dL and proteinuria-to-creatininuria ratio above 4.9 may indicate special risk to develop eclamptic seizures and pregnancy interruption should be considered.

Results: Patients with eclampsia and preeclampsia were different in several clinical, laboratorial and demographic aspects. Patients with eclampsia had higher serum uric acid levels and protein excretion, systolic and diastolic blood pressure; were more likely to have cesarean section, and had worst perinatal outcomes. The combination of uric acid above 5.9 mg/dL and proteinuria/creatininuria ratio above 4.9 had a striking association with eclampsia.

Conclusions: Our data strongly suggest that the combination of increased levels of maternal serum uric acid especially above 5.9 mg/dL and proteinuria-to-creatininuria ratio above 4.9 may indicate special risk to develop eclamptic seizures and pregnancy interruption should be considered.

PP.24.13 HYPERTENSION IN PREGNANCY: FETAL OUTCOME. A SINGLE-CENTER 4-YEAR RETROSPECTIVE STUDY

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Objective: To assess the impact of studied maternal parameters (age, diabetes mellitus (DM), quantitative proteinuria, serum uric acid, number of antihypertensive drugs used, multiple pregnancy) on fetal outcome (birth weight, duration of pregnancy, Apgar score).

Design and method: 308 mothers with hypertension in pregnancy and their 339 fetuses were included. Pregnant women aged 32 (18–46; median, range) years were admitted to the St. Cyril and Methodius Hospital due to hypertension in pregnancy (4% with purely pre-existing hypertension) from June 2011 to June 2015. DM was present in 16% of women (type 1 DM in 7%, type 2 DM in 1.5% and gestational DM in 7.5%). Maternal antihypertensive treatment was evaluated until the day of delivery. In case of repeated measurement of maternal proteinuria and serum uric acid, the highest ante-partum value was used.

Results: Out of 339 fetuses, 335 were born alive, 84% per Caesarean section. Duration of gestation was 37 (24–42; median, range) weeks, birth weight 2600 (370–4820) g, proteinuria 0.580 (0.06–20.04) g/24 h, serum uric acid 344 (194–633) mmol/l. Most pregnant women (38%) were treated with 2 antihypertensive drugs, 32% with one drug, 23% with 3 drugs, 6% with 4 drugs and one woman with 5 drugs. The most frequently used antihypertensive drugs were methyldopa (97% women), isradipine (51%), i.v./oral urapidil (45%), metoprolol (8%) and amlodipine (7%).
Shorter duration of pregnancy and lower birth weight were associated with higher proteinuria and higher number of antihypertensives ($p < 0.0001$ for all correlations). Apgar score at 1 and 5 minutes was negatively correlated to proteinuria ($p < 0.01$), but not to the number of antihypertensives or other parameters.

Conclusions: In our study, only maternal proteinuria and number of antihypertensives needed to control blood pressure in pregnant hypertensive women had negative impact on fetal outcome. Proteinuria and number of antihypertensives were associated with shorter duration of pregnancy and lower birth weight. Proteinuria had negative influence on Apgar scores.

PP.24.14 PREDICTIVE VALUE OF ANEMIA AND HYPOCHROMIA FOR LOW BIRTH WEIGHT, GESTATIONAL AGE AND POOR APGAR SCORE IN PREGNANCY INDUCED HYPERTENSION

I. Muzos[1], R. Nitu[2], Z. Popa[2], M. Craina[2]. [1]*Victor Babes University of Medicine and Pharmacy, Department of Functional Sciences, Timisoara, ROMANIA,* [2]*Victor Babes University of Medicine and Pharmacy, 3rd Department of Obstetrics and Gynecology, Timisoara, ROMANIA*

Objective: Hypertension is a common complication of pregnancy, with adverse outcomes. Anemia is highly prevalent in pregnant women. It was the aim of the present study to assess the relationship between anemia and birth weight, gestational age and Apgar score in patients with pregnancy induced hypertension.

Design and method: Medical records of 77 women, aged 30 ± 5 years, with pregnancy induced hypertension were reviewed retrospectively. Data about complete blood count, Apgar score, birth weight and gestational age were collected.

Results: Hemoglobin (Hb) was: 12.39 ± 1.08 g/dl, packed cell volume (PCV): 38.18 ± 3.28 %, red blood cell count (RBC): 4.42 ± 0.41 millions/mm^3, mean corpuscular hemoglobin (MCH): 28.18 ± 2.06 pg, mean corpuscular hemoglobin concentration (MCHC): 32.46 ± 1.48 g%, Apgar score: 8.79 ± 1.69, gestational age: 37.92 ± 2.23 weeks and birth weight: 3.042 ± 678 g. Linear and multiple regression analysis revealed significant associations between RBC and gestational age, birth weight and Apgar score. The most sensitive predictors of a poor Apgar score (less than 8) were Hb less than 12 g/dl and MCHC less than 32 g%, and low

ARTICLE 5 – Abstract, entitled "Eclampsia versus Preeclampsia: increased proteinuria and uric acid are associated with eclamptic crisis". Authors: **Daniele Cristovao Escouto**, L. G. Paula, B. Pinheiro da Costa, Carlos Eduardo Poli-de-Figueiredo. Abstract presented at the 26th European Meeting on Hypertension and Cardiovascular Protection. Published at the Journal of Hypertension, 34: e264, September 2016.

rameter called preeclampsia risk score, with the formula: Score = –1.974*C6772–1.479*D1-1.335*D2. The risk score was normalized by summing all values calculated with K = –4.78. Thus, the score ranged in the interval: [0–4.79], negative values being eliminated. The reliability of the new prediction score was assessed by a ROC analysis. The area under the curve of 0.701 was calculated (95% CI = 0.640–0.756), the newly built parameter being validated as a highly reliable predictor of preeclampsia by a significance level $p < 0.001$. Thus, parameter levels over 1.34 (cut-off value) presented a Se = 3.2 (0.9–8.0) and a Sp = 100 (97.2–100) for association with preeclampsia. The analysis revealed that score values below 1.34 could be classified as high risk, between 1.34 and 3.45 as medium risk, and beyond this value as low risk. To use the newly constructed score, an interface was created which allows users to introduce the position status (1 = homozygous mutation, 0 = normal or heterozygous mutation) for each of the three mutations included in the previous score.

Conclusions: Of all the variables included in the model, MTHFR-C677T, D1-C/T and D2-Thr22Ala were independent predictors of preeclampsia. The preeclampsia risk score is calculated automatically, using the created interface, as a continuous value and is interpreted semiquantitatively in accordance with the values set.

PP.24.11 INTERVAL BETWEEN BLOOD PRESSURE MEASUREMENTS IN PREGNANT WOMEN WITH HIGH BLOOD PRESSURE

D. Escouto, M.R. Vieira, B. Pinheiro da Costa, C.E. Poli-de-Figueiredo. *Pontifical Catholic University of Rio Grande do Sul - Faculty of Medicine, Porto Alegre, BRAZIL.*

Objective: To analyze the behavior of blood pressure at the first 8 hours of emergency care in pregnant women who arrive with hypertension in an obstetric unit.

Design and method: Blood pressure was measured at an Obstetric Unit in a cohort of 415 pregnant women with high blood pressure at the initial evaluation. Data of the first 8 hours of blood pressure readings were analyzed by Generalized Estimated Equations test.

Results: At baseline the means(±SD) were 154.3 ± 16.5 mmHg and 98.0 ± 12.1 mmHg for systolic and diastolic blood pressure, respectively. There was a significant reduction in blood pressure during follow-up ($p < 0.001$). Blood pressure means (SD) were: 1st hour: 146.6±19.1 and 89.7±15.6, 2nd: 139.0±17.8 and 83.2±14.2, 3rd: 137.2±15.6 and 78.7±12.2, 4th: 136.9±14.7 and 78.8±14.5, 5th: 135.9±16.6 and 78.2±14.1, 6th: 135.6±16.3 and 77.9±13.5, 7th: 133.3±14.2 and 75.7±11.9, and 8th hour 133.8±15.6 and 76.9±12.9 mmHg for systolic and diastolic blood pressure, respectively. Blood pressure stabilized after the third hour.

Conclusions: The study provides evidence that an interval of at least three hours between measurements is adequate to establish the diagnosis of gestational hypertension in pregnant women presenting with high blood pressure at an obstetric unit.

PP.24.12 ECLAMPSIA VERSUS PREECLAMPSIA: INCREASED PROTEINURIA AND URIC ACID LEVELS ARE ASSOCIATED WITH ECLAMPTIC CRISIS

D. Escouto, L.G. Paula, B. Pinheiro da Costa, C.E. Poli-de-Figueiredo. *Pontifical Catholic University of Rio Grande do Sul - Faculty of Medicine, Porto Alegre, BRAZIL.*

Objective: Characterize a group of women that developed eclampsia and to compare it with a group that had preeclampsia focusing in maternal serum uric acid and proteinuria-to-creatininuria ratio.

Design and method: The records of patients delivered at Hospital São Lucas/ Pontifícia Universidade Católica do Rio Grande do Sul were reviewed retrospectively; 733 pregnant women with hypertension were analyzed: 424 had preeclampsia, and 52 eclampsia.

Results: Patients with eclampsia and preeclampsia were different in several clinical, laboratorial and demographic aspects. Patients with eclampsia had higher serum uric acid levels and protein excretion, systolic and diastolic blood pressure; were more likely to have cesarean section, and had worst perinatal outcomes. The combination of uric acid above 5.9 mg/dL and proteinuria/creatininuria ratio above 4.9 had a striking association with eclampsia.

Conclusions: Our data strongly suggest that the combination of increased levels of maternal serum uric acid especially above 5.9 mg/dL and proteinuria-to-creatininuria ratio above 4.9 may indicate special risk to develop eclamptic seizures and pregnancy interruption should be considered.

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PP.24.13 HYPERTENSION IN PREGNANCY: FETAL OUTCOME. A SINGLE-CENTER 4-YEAR RETROSPECTIVE STUDY

V. Pokorná[1], T. Pazmanová[2], J. Kaluzay[1], D. Skaltetyová[2], Z. Minarikova[1], P. Pontuch[1]. [1]*4th Department of Internal Medicine, Faculty of Medicine, Comenius University, St. Cyril and Methodius Hospital, Univers, Bratislava, SLOVAK REPUBLIC.* [2]*Department of Cardiology, National Institute of Cardiovascular Diseases, Bratislava, SLOVAK REPUBLIC*

Objective: To assess the impact of studied maternal parameters (age, diabetes mellitus (DM), quantitative proteinuria, serum uric acid, number of antihypertensive drugs used, multiple pregnancy) on fetal outcome (birth weight, duration of pregnancy, Apgar score).

Design and method: 308 mothers with hypertension in pregnancy and their 339 fetuses were included. Pregnant women aged 32 (18–46; median, range) years were admitted to the St. Cyril and Methodius Hospital due to hypertension in pregnancy (4% with purely pre-existing hypertension) from June 2011 to June 2015. DM was present in 16% of women (type 1 DM in 7%, type 2 DM in 1.5% and gestational DM in 7.5%). Maternal antihypertensive treatment was evaluated until the day of delivery. In case of repeated measurement of maternal proteinuria and serum uric acid, the highest ante-partum value was used.

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ANNEX D – Article published as co-author

ARTICLE 1 – Original article published, entitled "Phosphodiesterase and preeclampsia". Authors: Ann Brandolt Larré, Aline Parisoto, Bruna Fagundes Rockenbach, Debora Montenegro Pasin, Claudia Capellari, **Daniele Cristovao Escouto**, Bartira Ercilia Pinheiro da Costa, Carlos Eduardo Poli-de-Figueiredo. Published at *Medical Hypotheses*, 108 (2017) 94-100.

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Phosphodiesterases and preeclampsia

Anne Brandolt Larré, Aline Parisotto, Bruna Fagundes Rockenbach, Débora Montenegro Pasin, Cláudia Capellari, Daniele Cristovão Escouto, Bartira Ercília Pinheiro da Costa *, Carlos Eduardo Poli-de-Figueiredo

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ABSTRACT

Antagonizing vasodilation has been considered one of the potential mechanisms underlying the pathophysiology of preeclampsia. Phosphodiesterases hydrolyze cGMP, interfering with the action of nitric oxide on vascular smooth muscle, thus causing vasoconstriction. We hypothesize that the phosphodiesterases in maternal plasma, phosphodiesterase-5 in particular, may be linked to clinical manifestations in preeclampsia syndrome.

Introduction

Preeclampsia (PE) is a multisystemic syndrome [1,2] pregnancy-specific whose prevalence varies according to some authors, who suggest that the syndrome can complicate from 2% to 8% [3,4] of all pregnancies. It confers high morbidity and mortality to both mother and fetus [5,6]. It is characterized by elevated levels of blood pressure associated with the emergence of one or more maternal organ dysfunction after the 20th week of gestation [1,2]. The pathophysiology of the PE is complex, and it is still being elucidated. Many theories have proposed possible etiological mechanisms of such disease [7–13]. The main idea governing the understanding of PE is that the disease results from placental ischemia with the release of factors in the maternal circulation [11,14] and most studies suggest that the disease is triggered by a cascade combination of abnormal maternal inflammatory response and endothelial dysfunction associated with the angiogenic imbalance and abnormal vascular relaxation [9,13,15–18]. The vasoconstriction has been highlighted as one of the factors involved in the pathogenesis of PE. The release of nitric oxide by the endothelium promotes vasodilation, a major regulator of vascular tone that acts via the guanylate cyclase forming the cyclic guanosine monophosphate (cGMP) [19]. This second messenger, in its turn, is hydrolyzed by the phosphodiesterase (PDE), a cyclic nucleotide responsible for regulating the cellular levels of cGMP and 3′,5′-cyclic adenosine monophosphate (cAMP) [20].

Phosphodiesterase currently make up 11 structurally related families, but functionally variants. Some are specific for cAMP or cGMP, and others for both [21]. Phosphodiesterase that perform the hydrolysis of cGMP in vascular myocytes, in particular PDE-5, – due to its significant expression in this region – are responsible for the regulation of the muscle tone in the vessels. They regulate blood pressure by antagonizing the catalysis of the second messenger responsible for the degradation of nitric oxide; thus, these enzymes antagonize the vasodilation [22,23].

Phosphodiesterase activity from the plasma of women with PE is higher than in regular pregnancies [24]. Accordingly, it is believed that such enzymes are involved in the pathogenesis of PE [23,25]. Few studies relate the expression of variants of PDE with the syndrome, focusing, primarily, on PDE-5 expression, which appears to be at high levels in maternal placental tissue [23,25–29].

Hypothesis

Most human studies that establish a link between PE and PDE analyze the PDE-5 in the placental tissue and umbilical cord [23,25,30–32]. We believe in the hypothesis that the PDE enzymatic activity and expression, particularly the PDE-5, is increased in the plasma and placenta of women with PE. This alteration follows as a conceptual basis, which justifies the use of inhibitors of PDE in the prevention and treatment of PE.

Evaluation of the hypothesis

Preeclampsia

Preeclampsia is diagnosed by hypertension, i.e. systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg, pre-

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0306-9877/

ANNEX E – Final report from PhD Sandwich Program supervisors: Professor Carlos Eduardo Poli de Figueiredo and Professor Lucilla Poston

Pontifícia Universidade Católica do Rio Grande do Sul
FACULDADE DE MEDICINA
PÓS-GRADUAÇÃO EM MEDICINA E CIÊNCIAS DA SAÚDE

Porto Alegre, 01 de novembro de 2016.

À
Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
Coordenação de Bolsas no Exterior
Programa PDSE

Prezado (a) Senhor (a),

Venho, por meio deste, informar sobre as atividades desenvolvidas pela aluna Daniele Cristovao Escouto como parte de seu período de Doutorado Sanduíche no King's College London (KCL), sob orientação da Prof. Lucilla Poston.

Durante o período de novembro/2015 a agosto/2016, a aluna esteve envolvida em atividades de pesquisa na divisão de saúde da mulher do KCL. Lá desenvolveu atividades de acompanhamento clínico ambulatorial como observadora, coleta e análise estatística de dados, além de redação e apresentação de resultados em conferências internacionais.

Atenciosamente,

Carlos Eduardo Poli de Figueiredo, MD DPhil
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CAPES Foundation

To whom it may concern

Dear Sir/Madam,

INSTITUTIONAL PROGRAM OF ABROAD SANDWICH DOCTORATE - PDSE

This letter is to provide a short report on the research undertaken by Daniele Cristovao Escouto, a Sandwich PhD Student supported by CAPES (Brazil) under my supervision at the Division of Women's Health at St Thomas Hospital (King's College London) from November 2015 to August 2016.

During this period, Daniele was involved in studies in hypertension in pregnancy and renal diseases in pregnancy. She participated, as an observer, in obstetric and nephrology clinics, was involved in statistical analysis of data, undertook literature reviews and wrote scientific articles. She also presented some of her work developed during her time at King's College at international conferences.

We very much enjoyed having Daniele with us. She was delightful to work with.

Yours sincerely,

Professor Lucilla Poston

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