STUDY PROTOCOL FOR A PROSPECTIVE, MULTICENTRE, COHORT STUDY: Preeclampsia sequential screening using angiogenic factors during 1st trimester of pregnancy (CRISP STUDY)

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ABSTRACT

Introduction. Preeclampsia (PE) affects from 2 to 8% of pregnant women. Recent studies show that prevention is the best strategy to improve perinatal outcomes. Therefore, the development of new strategies for preeclampsia screening becomes essential in order to determine the individual risk for each patient, and thus, to identify those who would be candidates for receiving prophylactic treatment with low-dose aspirin from the first trimester of pregnancy. The aim of our study is to determine prospectively, during clinical practice~~,~~ the predictive and preventive capacity of a model of preeclampsia sequential screening in the first trimester of pregnancy.

Methods and Analysis. This is a prospective, multicentre, cohort study, with the collaboration of *Hospital de la Santa Creu i Sant Pau* (Barcelona), *Hospital Universitario de Cruces* (Bilbao), *Hospital Son Llàtzer* (Mallorca) and *Hospital Clínico Universitario Lozano Blesa* (Zaragoza). Women with a singleton pregnancy attending to the 12-week ultrasound scan at one of the maternity hospitals participating in the study between March 1st 2021 and 30th October 2022 will be recruited. Patients who accept to participate in the study will be classified into three risk groups (low-risk, moderate-risk and high risk) based on medical history, Mean Arterial Pressure (MAP), Pregnancy-Associated Plasma Protein A (PAPP-A) and Uterine Artery Pulsatility Index (UTPI). Placental Growth Factor (PlGF) will only be determined in those patients classified as intermediate risk after this first step and then reclassified in high and low-risk patients depending on its values. The number of first-trimester scans performed by these hospitals is approximately 8200 patients annually. Due to PE prevalence in our environment is around 3% of the total population, a total of 246 cases of PE are to be expected. Therefore, based on similar previous experiences, we could assume that 80% of the patients will accept to participate in the study, meaning a total sample of 6560 pregnant women.

Ethics and dissemination. The study will be conducted in accordance with the principles of Good Clinical Practice. This study was approved by the Clinical Research Ethics Committee (CEIC) of Health Research Institute of Aragon (IIS Aragon), on 8th July 2020

INTRODUCTION

Background

Preeclampsia impact

The incidence of preeclampsia (PE) is about 2-8%, being one of the leading causes of perinatal morbidity and directly responsible for 10-15 % of the maternal deaths in the world (1). The severity of the disease is determined to a large extent on gestational age.

Early-onset preeclampsia (diagnosed before 32 weeks of gestation) is most uncommon but it has worse fetal and maternal outcomes than late-onset preeclampsia (2). Women with early-onset PE present a higher risk of cardiovascular morbidity, along with respiratory, liver and kidney complications and a higher mortality rate (42.1/100000 in early-onset PE vs. 11.2/100000 in late-onset PE and 4.2/100000 in non-PE pregnancies) (3). This higher rate of adverse outcomes is observed in the short and in the long term, associating an increment in the risk of suffering coronary disease and strokes (4). Early-onset PE is associated with Intrauterine Growth Restriction (IUGR) affecting perinatal outcomes and long-term neurological development (5). According to *Sociedad Española de Neonatología*, 20.8% of newborns who weighed less than 1500g were born from mothers who suffered hypertension during pregnancy (6). Conversely, late-onset PE though less severe, is far more frequent. It is a very important cause of maternal and neonatal morbidity and mortality (7) and entails neurodevelopmental consequences (8).

Pathogenesis of PE: angiogenesis and placenta

The placenta is the organ responsible for ensuring a correct exchange of oxygen and nutrients between mother and fetus. Thereby, disorders in its development can produce placental insufficiency or dysfunction. At a pathophysiological level, a reduction of the uteroplacental flow is produced, which can cause some alterations with clinical expressions on both the fetus with IUGR and the mother with PE.

In recent yers, the study and characterization of new ways of angiogenesis have been particularly important in the comprehension of PE, allowing us to focus our efforts on the prevention and treatment of PE. (9) It is know that for a correct placental development and

function, a balance between the production of angiogenic (Placental Growth Factor, PLGF) and antiangiogenic factors (Soluble Fms-like tyrosine kinase-1, sFlt-1) is required.

Therefore, PE is ultimately, a placental disease caused by a syncytiotrophoblastic disfunction, and due to PIGF and sFlt-1 are produced, at least partially, in the syncytiotrophoblast, both are biomarkers with a proved predictive potential of PE and its adverse outcomes (10)

Prevention

An early prophylactic administration (before 16 weeks of pregnancy) of Acetyl Salicylic Acid (ASA) every night reduces the incidence of preterm preeclampsia (<37 weeks) by 62%, and by 90% in case of early-onset PE (before 32 weeks) (11, 12), which represents a reduction of 70% in the average stay in Intensive Care Units (ICU) of these newborns(13). Moreover, some authors have reported some benefits of the ASA on IUGR, with a reduction of the incidence between 50 and 89% (14,15).

Actual status, purpose and justification of the project

It is essential to develop universal PE screening strategies during the first trimester of pregnancy in order to determine the individual risk of each patient, selecting those with a higher risk, who are candidates for early prophylactic treatment with low-dose aspirin (16,17). Universal preeclampsia screening has been proved to be cost-effective (18). Different systems of screening have been described with different detection rates. Some scientific societies (19) propose a screening system based on demographic characteristics of the mother and risk factors in her medical history. This strategy is simple but, however, presents a very low detection rate with a high false-positive rate, and does not quantify the individual risk of each patient (20).

For this reason, different multivariant models have been developed, similar to those which are already used in other pregnancy diseases. Until now, the best PE early detection rates are obtained using an approach of competitive risks at 11-13 weeks of pregnancy by means of a combination of maternal characteristics (age, parity, medical history as thrombophilia, kidney disease and chronic hypertension), maternal biophysical variables (Mean Arterial Pressure (MAP), Doppler of Uterine Arteries (UTPI)) and biochemical variables (PlGF) that predicts approximately a 90% of early-onset PE (<32 weeks) and 75% of late-onset PE (<37 weeks) with a 10% false-positive rate (21, 22). Moreover, other observational retrospective studies have

demonstrated that the incorporation of antiangiogenic biomarkers as sFlt-1 can increase the predictive ability of the screening of PE during the first trimester (23).

Unfortunately, developing an universal screening system including angiogenic and antiangiogenic markers (PlGF and sFlt-1) has a high economical cost, which is hardly affordable for many Healthcare systems. For that reason, different PE-screening systems have been created at a lower cost. Maternal characteristics, arterial pressure and Uterine Arteries Pulsatility Index combined with Pregnancy-Associated Plasma Protein A levels (PAPP-A) presents a detection rate of early-onset PE of 80.8%, and for late-onset PE of 39.6%, with a 10% false-positive rate. This strategy does not increase the economic cost significantly, because PAPP-A is already used in the screening of chromosome disorders routinely (24).

However, recent retrospective studies with large sample sizes (25, 26) suggest a two-step screening. According to these analyses, similar detection rates are achieved if applying low-economic cost strategies to the entire population (12), but selecting a small group of patients with intermediate or high risk to determine angiogenic markers in only this group, being 30% of all pregnant women. Until now, these analyses have been performed in retrospective cohorts submitted to universal screening, with a high risk of methodological biases.

The aim of this study is to evaluate prospectively, for the first time and in a clinical scenario, a population sequential screening of preeclampsia, saving the determination of angiogenic factors (PlGF, sFlt-1) to a subgroup of patients with an intermediate risk after the first step of the screening, maintaining a high detection rate but with a lower economic cost.

National and international groups working on preeclampsia

Preeclampsia is one of the most important research topics in maternal-fetal medicine, being the screening of this disease the most important point of interest due to its high clinical impact. Dr. Llurba (PI of Hospital de la Santa Creu I Sant Pau research group) is an international leader in preeclampsia. All the Hospitals participating in this study have worked, in the last years, on different projects focused on preeclampsia and placental insufficiency, some of them collaborating between them (PI16/00375) (MSC EC10-205). Besides, these investigation groups share research lines with the most important international groups working on PE at this moment: Kings College (London), Oxford University, Johns Hopkins University and Charité University Medicine (Berlin).

HYPOTHESIS

Primary hypothesis

It is possible to perform a sequential population screening of PE, saving the determination of angiogenic markers (PlGF+/- SFlt-1) for the subgroup of patients with an intermediate risk of having PE, maintaining a high rate of detection ~~of PE~~ but with a lower economic cost.

Secondary hypothesis

sFlt-1 measurement increases the PE detection rate during the first trimester of pregnancy if determined at subgroups at risk.

Universal PE screening during the first trimester without angiogenic factors (medical history + MAP + UTPI + PAPP-A) selects effectively those patients with a high or intermediate risk of having PE without increasing the cost of the assistance. Sequential population screening of PE presents a similar detection rate of IUGR but with a lower economic cost.

OBJECTIVES

Primary objectives

To determine prospectively, in a clinical scenario, the predictive and preventive capacity of a sequential screening of PE in the first trimester of pregnancy; determining Placental Growth Factor (PlGF) only in those patients classified as intermediate risk after the universal screening without angiogenic factors: medical history, Mean Arterial Pressure (MAP), Pregnancy-Associated Plasma Protein-A (PAPP-A), Uterine Artery Pulsatility Index (UTPI).

Secondary objectives

To analyze the different risk subgroups of PE during the first trimester of pregnancy in which could be efficient the sequential determination of sFLt-1.

To analyze the diagnostic and economic yield of the universal screening in the first trimester without angiogenic factors (maternal medical history + MAP + UTPI + PAPP-A), used as a tool to identify those patients at high and intermediate risk of having PE.

To determine the predictive and preventive capacity of a PE sequential screening model in the first trimester of pregnancy for IUGR.

To assess the impact of a PE sequential screening in the incidence of the pathology associated with placental insufficiency (PE, IUGR) in our population.

METHODS

Study design

A multicentric prospective cohort study will be conducted within four Spanish hospitals: *Hospital de la Santa Creu i Sant Pau* (Barcelona), *Hospital Universitario de Cruces* (Bilbao), *Hospital Son Llàtzer* (Mallorca) and *Hospital Clínico Universitario Lozano Blesa* (Zaragoza).

ELIGIBILITY CRITERIA

All singleton pregnancies that present to the 12-week scan in the Obstetrics Unit of the participant hospitals, between March 1st 2021 and 30th October 2022, will be included

Inclusion criteria: Singleton pregnancies; Gestational age less than 14 weeks, estimated according to Crown-Rump Length (CRL); Blood sample between 8 and 14 weeks of pregnancy; Patients who accept to participate in the study and sign the informed consent.

Exclusion criteria: Fetus with chromosomal disorders, major congenital malformations or congenital infections diagnosed in the first-trimester ultrasound; Multiple pregnancies; Non-acceptance of participation in the study.

CONTROL VARIABLES

An assigned study code and the inclusion date will be collected in all patients.

Predictive variables

Medical history

- Filiation and demographic data: age, race, socioeconomic status
- Personal history: tobacco consumption, personal history of diabetes, arterial hypertension, renal diseases, autoimmune diseases, genetic or acquired thrombophilia, and the mean arterial pressure the day of the 12-week ultrasound scan.
- Obstetric history: last menstrual period, parity, previous history of PE, use of assisted reproduction techniques in the current pregnancy.

- Treatment with aspirin, heparin or antihypertensives during the ongoing pregnancy.

Physical examination

Mean arterial pressure (MAP) using the formula:

$$MAP = \frac{(\text{Diastolic arterial pressure x } 2) \ + \ \text{systolic arterial pressure}}{3}$$

Weight, height and Maternal Body Mass Index (BMI)

Ultrasound examination

- Crown-Rump Length (CRL) (27)
- Mean Uterine arteries Pulsatility Index (UtAPI).
- Umbilical artery pulsatility index (UA-PI), Middle Cerebral Artery Pulsatility Index (MCA-PI) and Cerebro-Placental ratio (CPR).
- Estimated fetal weight centile in 2nd and 3rd trimester, (28)

Blood test samples

PAPP-A will be determine using fluoroinmunoessay in the autoanalyzer DELFIA_Xpress, expressed in multiples of median (MoMs). PlGF will be determine using immunoanalysis electroquimioluminscent automatized in the analyzer Cobas e601 (Roche Diagnostics). sFlt-1 will be determine using immunoanalysis electroquimioluminscent automatized in the analyzer Cobas e601 (Roche Diagnostics).

OUTCOMES

Primary outcomes

Diagnosis of preeclampsia during pregnancy following the definition of the *International Society for the Study of Hypertension in Pregnancy*, (ISSHP)

Secondary outcomes

- Early-onset Preeclampsia: diagnosos before 34 weeks of pregnancy
- Severe preeclampsia (ISSHP)
- Pregnancy-induced hypertension
- Small for gestational age: birth weight below the 10th percentile (29)

- Intrauterine Growth Restriction (IUGR, Delphi procedure Consensus. Gordijn SJ, 2016)
- Perinatal mortality (>22 weeks of pregnancy - < 28 days postpartum).
- Neonatal acidosis (arterial pH <7.10 + base excess >12mEq/L
- Days of admission in neonatal Intensive Care Unit
- Significant neonatal morbidity: convulsions, intraventricular haemorrhage > III grade, periventricular leukomalacia, hypoxic-ischemic encephalopathy, abnormal electroencephalogram, necrotizing enterocolitis, acute renal failure (serum creatinine >1.5mg/dL) or heart failure (requiring inotropic agents).
- Gestational age at birth
- Type of delivery (vaginal, spontaneous or instrumental, cesarean section)
- The economic cost of the analysis of angiogenic and antiangiogenic factors in euros (PlGF and sFlt-1).

SAMPLE SIZE

Considering the number of first-trimester ultrasounds that are conducted in the participant hospitals, we estimate that 8200 patients will be potential candidates to participate in the study. Due to PE prevalence in our environment is around 3% of the total population, which means that approximately 246 patients of our sample will develop PE and based on similar previous experiences we could assume that around 80% of the patients will consent to participate in the study, meaning a total sample of 6560 pregnant women.

Based on a pilot study previously performed, we estimate that a 30% of the patients (which means approximately 1968 pregnancies) will be classified as intermediate or high risk after the PE universal screening. This sample size assures enough statistic power and the possibility of extrapolating the results to the clinical practice.

DATA COLLECTION

All the data required to carry out this study, including those derived from sonographies and blood tests, will be collected during the normal pregnancy control, without further appointments. This is one of the most important aspects of this project: developing an efficient

screening of preeclampsia during the first trimester using the explorations and visits that are already included in the pregnancy control.

The data will be collected during normal pregnancy control as follows:

A blood test will be requested in all pregnant women in the first appointment of pregnancy control between 9 and 13 weeks, to determine risk of chromosomal disorders according to the national protocol (*Sociedad Española de Ginecología y Obstetricia*, SEGO). This blood sample will be collected preferentially between 11 and 13 weeks, and it will be used this same sample to analyze angiogenic factors without requiring new blood extractions.

In the first-trimester scan, the mean uterine arteries pulsatility index will be determined, as well as mean arterial pressure. In this visit, the patient will be asked about her medical history. We will offer the patients the inclusion to our study in that moment; those who agree to participate in the study will sign the informed consent.

In patients who do not agree to participate in the sequential screening study, will be classified according to the algorithm without angiogenic factors, in:

Low Risk: (Preeclampsia Risk <1/250)

High Risk: (Preeclampsia risk> 1/250

In the participants, the initial risk of PE will be calculated using maternal medical history, MAP, UTPI and PAPP-A (already used in aneuploidies screening), using the software validated to each laboratory. Patients will be classified into 3 groups:

- Low risk of PE (<1/500)
- Intermediate risk of PE (between 1/50 and 1/500)
- High risk of PE (>1/50)

In those patients classified as intermediate and high-risk, PlGF and s-Flt-1 will be determined from the blood samples kept in biobanks of each hospital according to the current legislation. SFlt-1 results will be analyzed at the end of the study in order to decrease the cost because there will not be used to make clinical decisions. PlGF in high-risk patients will not be considered either to reclassify those patients. In both cases, the diagnostic efficiency will be analyzed at the end of the study.

Patients in the intermediate-risk group (1/50-1/500) will be reclassified after adding PlGF levels in the predictive algorithm in 2 groups: Low risk of PE (<1/160) and High risk of PE (>1/160).

To the patients classified as high risk at any of the steps, will be offered prophylactic treatment with AcetylSalicylic acid (ASA) (150mg/24h) until 36 weeks of pregnancy, if there is not a contraindication.

The cut-off point of high risk after combined PE screening without using angiogenic factors had been obtained from a pilot study performed in *Hospital de la Santa Creu i Sant Pau* and *Hospital Universitario de Cruces*. According to this initial estimate with the selected cut-off point we expect to find 10% of high-risk patients and 20% of intermediate-risk after the first step of the screening. These results are in agreement with those published by other authors (25).

STATISTICAL ANALYSIS

All data from eligible patients, both those who participate in the study and those who reject it and therefore a one-step screening will be performed, will be analyzed.

The entire research team will participate in the recruitment and every team member will be responsible for the data that will be included in the electronic database, predictive variable and outcomes. This database will be anonymous and codified.

A bivariant statistical analysis will be performed in order to assess the relationship between dependent variables (diagnose of PE and/or IUGR) and the other variables included in this study. This association will be analyzed using: Continuous variables: Student's t-test; Categorical variables: Contingence tables. The inference will be estimated by means of X2 of Exact Fisher's test; Ordinal variables: Mann-Whitney U. Finally, a multivariate analysis will be performed using binomial logistic regression.

Different models will be tested, and two validity indices will be calculated on the final model: goodness of fit (Howmer-Lemeshow test) and discrimination (the area under the ROC curve).

All statistical analyzes will be performed with the IBM SPSS version 20.0 program, and the usual statistical significance level of 0.05 will be established.

ETHICS AND DISSEMINATION

The study will be conducted in accordance with the principles of Good Clinical Practice. The research team will guarantee the accuracy and trustworthiness of the data and reports that could be required, as well as its confidentiality. The investigator will keep all the documents of the study for, at least, 5 years after its conclusion, being available if they were requested by the monitor, auditor, ethics committee or Health Authority.

DISCUSSION

The main limitation of this study is that the data referring to the progress of the pregnancy and the delivery will be collected from the medical history of the patients; there could be errors on its transcription or interpretation and it is possible that some patients may decide to deliver in an another medical center. Contrary, prospective design in a clinical scenario with the participation of different Hospitals provides great extern validity.

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COMPETING INTERESTS: None declared

ETHICS APPROVAL: This study was approved by the Clinical Research Ethics Committee (CEIC) of Health Research Institute of Aragon (IIS Aragon), on 8th July 2020.

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