UNIVERSIDADE ESTADUAL DE CAMPINAS

FACULDADE DE CIÊNCIAS MÉDICAS

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PRÉ-ECLÂMPSIA ENTRE NULÍPARAS DE BAIXO RISCO: UM DESAFIO A SER SUPERADO COMBINANDO-SE ESTRATÉGIAS JÁ CONSAGRADAS E NOVAS TECNOLOGIAS

PREECLAMPSIA AMONG NULLIPAROUS LOW-RISK PREGNANT WOMEN: A CHALLENGE TO BE OVERCOME BY COMBINING ALREADY ESTABLISHED STRATEGIES AND NEW TECHNOLOGIES

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Tese apresentada à Faculdade de Ciências Médicas da Universidade Estadual de Campinas, Programa de Tocoginecologia, como parte dos requisitos exigidos para a obtenção do título de Doutora em Ciências da Saúde, na Área de Concentração de Saúde Materna e Perinatal

Thesis presented to the School of Medical Sciences from the University of Campinas, program of Obstetrics and Gynecology, as part of the requirements for obtaining the title of Doctor in Health Science, in the concentration area of Maternal and Perinatal Health

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ESTE EXEMPLAR CORRESPONDE À VERSÃO FINAL DISSERTAÇÃO/TESE DEFENDIDA PELA ALUNA JUSSARA MAYRINK E ORIENTADA PELO PROF. DR. JOSÉ GUILHERME CECATTI

CAMPINAS

2018

Agência(s) de fomento e nº(s) de processo(s): Não se aplica.

Ficha catalográfica
Universidade Estadual de Campinas
Biblioteca da Faculdade de Ciências Médicas
Maristella Soares dos Santos - CRB 8/8402

M455p

Mayrink, Jussara, 1983-
Pré-eclâmpsia entre nulíparas de baixo risco : um desafio a ser superado combinando-se estratégias já consagradas e novas tecnologias / Jussara de Souza Mayrink Novais. – Campinas, SP : [s.n.], 2018.

Orientador: José Guilherme Cecatti.
Coorientador: Maria Laura Costa do Nascimento.
Tese (doutorado) – Universidade Estadual de Campinas, Faculdade de Ciências Médicas.

1. Pré-eclâmpsia. 2. Fatores de risco. 3. Predição. I. Cecatti, José Guilherme. II. Costa, Maria Laura. III. Universidade Estadual de Campinas. Faculdade de Ciências Médicas. IV. Título.

Informações para Biblioteca Digital

Título em outro idioma: Preeclampsia among nulliparous low-risk pregnant women : a challenge to be overcome by combining already established strategies and new technologies
Palavras-chave em inglês:
preeclampsia
Risk factors
Prediction
Área de concentração: Saúde Materna e Perinatal
Titulação: Doutora em Ciências da Saúde
Banca examinadora:
José Guilherme Cecatti [Orientador]
Mary Ângela Parpinelli
Giuliane de Jesus Lajos
José Carlos Peraçoli
Leandro Gustavo de Oliveira
Data de defesa: 13-12-2018
Programa de Pós-Graduação: Tocoginecologia

BANCA EXAMINADORA DA DEFESA DE DOUTORADO

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Data da defesa: 13/12/2018

DEDICATÓRIA

Dedico esse trabalho à minha Manuela, por ter me transformado num ser humano de verdade.

AGRADECIMENTOS

Em primeiro lugar agradeço a Deus, por ter me enchido de ânimo, coragem e saúde para trabalhar.

Meu querido Ju, filósofo social, pela parceria na vida! Obrigada por todos esses anos ao seu lado, marcados por muita filosofia e amor! Obrigada pelas várias e valorosas contribuições para a conclusão desse trabalho!

Grace, por ter se esforçado acima, muito acima, de suas possibilidades, permitindo dessa maneira que eu chegasse até aqui. Mãe, obrigada por todo seu empenho!

Aos meus irmãos Naná e Marquinho, por serem a minha história de vida. Minha avó Aparecida, mulher à frente de seu tempo, grande fonte de inspiração sempre!

Dr. Guilherme, pela empatia e pela imensurável experiência dividida (ressalto que ainda não consegui aprender tudo o que poderia). Obrigada pela generosidade com que algumas vezes transitou em meu mundo para conseguir melhor me orientar.

Laurinha, por ter se empenhado em minha formação como obstetra desde 2008, pela co-orientação, pelo apoio de sempre nas mais diferentes jornadas, mais ainda, obrigada pelo entusiasmo com o qual mantém seus alunos motivados!

Débora, que com sua alma amadurecida tanto dividiu comigo...

Aos colegas do SAMBA, sem os quais a finalização desse trabalho não teria sido possível!

Aos queridos e inesquecíveis professores dessa Universidade que me formou e da qual sinto tanto orgulho de pertencer! Aos professores da pós-graduação de ouro, com agradecimento especial à chiquérrima dra. Sophie, por me fazer pensar, pensar muito...

À dra. Cássia, por tudo o que me ensinou, mas, principalmente, por me ensinar a ser leve e otimista!

Aos meus queridos amigos, por serem suporte nas adversidades.

Por fim, agradeço às pacientes que de uma forma ou de outra dividiram comigo histórias, sofrimentos, angústias e alegrias. Não tenho dúvida de que o ofício de ser médica é o mais delicioso dos ofícios e se fosse para escolher de novo, escolheria a mesma coisa.

RESUMO

Objetivo: realizar uma revisão narrativa sobre os diversos aspectos da pré-eclâmpsia, incluindo impacto mundial, predição, prevalência e aspectos fisiopatológicos. No que se refere à predição, fazer uma revisão sistemática sobre a capacidade da metabolômica em predizer distúrbios hipertensivos da gestação; estudar pré-eclâmpsia sob os aspectos de sua incidência, fatores de risco e resultados associados numa coorte constituída por gestantes nulíparas brasileiras de baixo risco. Além disso, investigar o papel da pressão arterial como preditor da ocorrência de pré-eclâmpsia e seus diferentes subtipos nessa população. **Sujeitos e métodos:** foram realizadas duas revisões da literatura científica, sendo uma revisão narrativa e uma revisão sistemática, com consulta das principais bases eletrônicas: PubMed, EMBASE, Web of Knowledge, Latin America and Caribbean Health Sciences Literature (LILACS), Scientific Electronic Library Online (Scielo), Health Technology Assessment (HTA), Database of Abstracts of Reviews of Effects (DARE). Além disso, da coorte brasileira de nulíparas de baixo risco construída a partir do estudo *Preterm Screening and Metabolomics in Brazil and Auckland (Preterm SAMBA)*, derivou um estudo de caso-controle aninhado em que foram investigados a incidência, fatores sociodemográficos e clínicos associados à pré-eclâmpsia, bem como de resultados maternos e perinatais. Foram estimadas as razões de risco e seus respectivos intervalos de confiança a 95% para a ocorrência de pré-eclâmpsia. Também nessa coorte analisamos o desempenho da pressão arterial média aferida em três momentos distintos da gestação (20, 27 e 37 semanas) em predizer a ocorrência de pré-eclâmpsia por meio de curvas ROC e análise de área sob a curva; **Resultados:** foram discutidos conceitos, aspectos fisiopatológicos e de prevenção e predição da pré-eclâmpsia, e a revisão sistemática abordou a capacidade da metabolômica em predizer a ocorrência de pré-eclâmpsia. A incidência de pré-eclâmpsia foi de 7,5%, das quais 16,1% foram pré-eclâmpsia de início precoce. A obesidade, o ganho de peso por semana e a pressão arterial diastólica acima de 75mmHg às 20 semanas foram os fatores correlacionados com a ocorrência de pré-eclâmpsia. Os casos de pré-eclâmpsia mostraram mais resultados adversos comparados ao grupo controle com mais indicações de cesárea (3,5 vezes) e maior permanência hospitalar acima de 5 dias (5,8 vezes) comparado aos controles. Eles

também tiveram um menor peso ao nascimento (média de 379g a menos), mais crianças pequenas para a idade gestacional (PIG) com uma razão de risco de 2,45, Apgar de 5º minuto menor que 7 com uma razão de risco de 2,11, mais admissões em UTI neonatal com uma razão de risco de 3,34 e Near Miss Neonatal com uma razão de risco de 3,65. Na análise da pressão arterial média em três momentos distintos da gestação, o grupo com pré-eclâmpsia de manifestação precoce apresentou valores mais elevados de pressão arterial média às 20 semanas. O grupo com pré-eclâmpsia de manifestação tardia apresentou maior incremento da pressão arterial média entre 20 e 37 semanas. Quanto à acurácia, é às 37 semanas que esse marcador apresenta melhor capacidade de predição de pré-eclâmpsia. **Conclusões:** paralelamente ao conhecimento sobre o tema compilado nas duas revisões realizadas, nesta amostra apenas a obesidade, ganho de peso por semana e a pressão arterial diastólica acima de 75 mmHg às 20 semanas mostraram estar associadas à pré-eclâmpsia que determinou também mais cesáreas e tempo prolongado de admissão hospitalar, além de resultados neonatais piores. Pressão arterial média às 20 semanas é superior em mulheres que apresentarão pré-eclâmpsia de manifestação precoce. Pressão arterial média tem seu melhor desempenho como preditor da ocorrência de pré-eclâmpsia às 37 semanas.

Palavras-chave: pré-eclâmpsia, incidência, fatores de risco maternos, pré-eclâmpsia de início precoce, pré-eclâmpsia de início tardio, predição.

ABSTRACT

Objective: to perform a review about several aspects of preeclampsia, including its global impact, prediction, prevalence and physiopathological aspects. Regarding prediction, to perform a systematic review on the capacity of metabolomics to predict hypertensive disorders of Pregnancy; to assess preeclampsia with its incidence, risk factors and associated outcomes in a cohort of Brazilian nulliparous low-risk pregnant women. Besides that, to investigate the role of blood pressure as predictor for the occurrence of preeclampsia and its different subtypes in this population. **Subjects and methods:** two reviews of scientific literature were performed, one narrative review and one systematic review, with search of information in the main electronic databases: PubMed, EMBASE, Web of Knowledge, Latin America and Caribbean Health Sciences Literature (LILACS), Scientific Electronic Library Online (Scielo), Health Technology Assessment (HTA), Database of Abstracts of Reviews of Effects (DARE). In addition, from the Brazilian cohort of nulliparous low-risk pregnant women built with the *Preterm Screening and Metabolomics in Brazil and Auckland (Preterm SAMBA)* study, a secondary nested case control came up, which investigated the incidence, sociodemographic and clinical factors associated to preeclampsia, and also its maternal and perinatal outcomes. The risk ratios and their respective 95% confidence intervals for the occurrence of preeclampsia were estimated. Still in this cohort we assessed the performance of the mean blood pressure measured in three different periods of pregnancy (20, 27 and 37 weeks) in predicting the occurrence of preeclampsia by constructing ROC curves and estimating their area under the curve (AUC). **Results:** concepts, physiopathological aspects and prevention and prediction of preeclampsia were discussed; the systematic review approached the capacity of metabolomics to predict the occurrence of preeclampsia. The incidence of preeclampsia was 7.5% of whom 14 (16.1%) had early-onset preeclampsia. Obesity, weight gain rate per week and diastolic blood pressure above 75 mmHg at 20 weeks were the factors correlated to the occurrence of preeclampsia. Cases of preeclampsia showed more adverse results compared to the control group: they had more C-sections (3.58 fold) and hospital stay above 5 days (5.8 fold) than controls. They also had lower birthweight (a mean of 379g lower), small for gestational age (SGA) babies with a risk ratio of 2.45

(1.52-3.95), 5th minute Apgar score below 7 with a risk ratio of 2.11 (1.03-4.29), NICU admission with a risk ratio of 3.34 (1.61-6.90) and Neonatal Near Miss with a risk ratio of 3.65 (1.78-7.49). Mean arterial blood pressure at 20 weeks of gestation was higher in the early-onset preeclampsia group. The increment of blood pressure between 20 and 37 weeks of gestation was higher in the late-onset preeclampsia group. The accuracy of mean arterial blood pressure as a preeclampsia predictor was modest, exhibiting the best values at 37 weeks of gestation. **Conclusion:** in parallel to the knowledge on the topic that was compiled in two reviews performed, in this sample only obesity, weight gain rate per week and diastolic blood pressure above 75mmHg at 20 weeks showed to be associated with preeclampsia. Preeclampsia determined also more C-sections and prolonged hospital admission, besides poorer neonatal outcomes. The mean arterial blood pressure at 20 weeks of gestation was higher among early-onset preeclampsia cases and the accuracy of this marker as a preeclampsia predictor was modest.

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Key words: preeclampsia, incidence, maternal risk factors, early-onset preeclampsia, late-onset preeclampsia, prediction

LISTA DE ABREVIATURAS E SIGLAS

ACOG	*American College of Obstetricians and Gynecologists*
AGA	*Adequate for Gestational Age*
ANOVA	*Analysis of variance*
AUC	*Area under the curve*
BMI	*Body Mass Index*
CAISM	Centro de Atenção Integral à Saúde da Mulher
CCN	Comprimento cabeça-nádega
CEP	Comitê de Ética em Pesquisa
CRL	*Crown-rump length*
DARE	*Database of Abstracts of Reviews of Effects*
DBP	*Diastolic Blood Pressure*
DIC	*Disseminated Intravascular Coagulation*
IRB	*Institutional Review Board*
GA	*Gestational Age*
HELLP	*Homolyses, elevated liver enzymes, low platelet*
HMDB	*Human Metabolome Database*
HSROC	*Hierarchical summary receiver characteristic operating curve*
HTA	*Health Technology Assessment*
IG	Idade Gestacional
IMC	Índice de Massa Corpórea
ITU	Infecção do trato urinário

LGA *Large for Gestational Age*

MAP *Mean Arterial Pressure*

MS Mass spectrometry

NICE *National Institute for Health and Clinical Excellence*

NICU *Neonatal Intensive Care Unit*

OMS Organização Mundial da Saúde

PAM Pressão arterial média

PAPP-A *Pregnancy-associated plasma protein-A*

PE *Preeclampsia*

PlGF *Placental Growth Factor*

PLTC *Potentially Life-Threatening Condition*

POS *Polycystic Ovarian Syndrome*

PRISMA *Preferred Reporting Items for Systematic Reviews and Meta-Analysis*

PSU *Primary Sampling Unit*

QUADAS *Quality Assessment of Diagnostic Accuracy Studies*

R^2 *Coeficiente de correlação*

ROC *Receiver Operator Characteristic*

RR *Risk Ratio*

SBP *Systolic Blood Pressure*

SGA *Small for Gestational Age*

UTI Unidade de Terapia Intensiva

WHO *World Health Organization*

Sumário

1. Introdução:

1.1- Introdução

De acordo com alguns autores germânicos, data de 2200 aC os primeiros relatos referentes à eclâmpsia, observados em papiros no Egito antigo (1). A palavra eclâmpsia origina-se do grego, *éklampsis,* e significa "luz brilhante" (1). Por cerca de 2000 anos, eclâmpsia foi entendida como uma doença caracterizada por crises convulsivas, típicas do final da gestação, e que se resolviam com o parto. Data do fim do século 19 quando cientistas da época - verdadeiros entusiastas do empirismo cuidador- reconheceram a semelhança entre o aspecto edemaciado das mulheres que apresentavam convulsão e o edema da doença de Bright, uma glomerulonefrite de início abrupto, caracterizada por proteinúria. A partir daí, houve uma procura por alterações urinárias nas gestantes com crises convulsivas, o que culminou com o achado de proteinúria nas mesmas. Com o advento da possibilidade de aferição da pressão arterial de forma não invasiva, percebeu-se que essas mulheres apresentavam aumento de seus níveis pressóricos. Não tardou para que viesse o entendimento de que proteinúria e hipertensão arterial *PREcediam* o aparecimento das crises convulsivas. Definia-se assim, a condição hipertensiva "pré-eclâmpsia", já na época compreendida em seu caráter progressivo de gravidade e que poderia conduzir - e que conduzia de maneira nefasta - a graves consequências para as vidas materna e fetal (1, 2).

Ainda hoje, as condições hipertensivas figuram como importantes complicadores da gestação, com uma incidência que varia conforme as particularidades das populações estudadas, podendo ultrapassar a marca de 10% em algumas regiões (3). Pré-eclâmpsia e eclâmpsia ocupam o segundo ou terceiro lugar no ranking mundial das causas de morbidade e mortalidade materna (4). Numa análise implementada pela Organização Mundial de Saúde, para avaliação de causas de morte materna ocorridas entre os anos de 2003 e 2009, as causas hipertensivas aparecem em segundo lugar, ocorrendo em 14% das vezes, precedidas apenas por causa hemorrágica, responsável por 27,1% das mortes maternas (5).

Recentemente, Abalos *et al*. demonstraram, numa revisão sistemática envolvendo 40 países com 39 milhões de mulheres, uma taxa estimada de pré-

eclâmpsia e eclâmpsia de 4,6% e 1,4%, respectivamente, sendo que no tocante ao Brasil, esses números foram de 1,5% e 0,6%. Essa revisão faz menção aos países em que sequer se conhecem esses números por inexistência de registros oficiais, o que em muito dificulta a recomendação de estratégias para intervenções que possam contribuir para melhores resultados maternos e perinatais (6). A despeito dos números apresentados, os dados brasileiros ainda são subestimados, já que há diferenças regionais inquestionáveis num país de tamanho continental, como bem demonstrou Giordano et.al, ao encontrar para regiões Sul e Sudeste, prevalência de eclâmpsia de 0,2% e para regiões Norte, Nordeste e Centro-Oeste, de 8,1% (7).

Ainda sobre o Brasil, de acordo com dados publicados em 2016, numa análise em que mais de 80 mil mulheres provenientes das cinco regiões do país foram monitorizadas quanto à morbidade materna grave, observou-se que a principal causa de admissão hospitalar foi a hipertensão arterial, perfazendo 73% dos quadros classificados como ameaçadores à vida materna, sendo eclâmpsia a principal causa de morte materna no país. O índice de mortalidade (calculado pela razão entre casos de morte materna por near miss materno + morte materna) encontrado nessa ocasião foi de 15,4%, o que é considerado um valor aceitável (abaixo de 20%), mas que ainda está muito longe do observado em países desenvolvidos (<2%) (8). Na análise sobre os casos de eclâmpsia, considerando o mesmo estudo de morbidade materna grave, a prevalência de eventos de quase morte (*Near Miss*, forma mais extrema de morbidade materna grave) incluindo eclâmpsia, foi de 4,2 casos por cada 1000 nascidos vivos ou ainda de 8,3 casos por cada morte materna (9). Destaque para a importância da utilização do indicador *Near Miss* Materno ou morbidade materna severa aguda que reúne os casos de mulheres que quase morreram por alguma Condição Potencialmente Ameaçadora da Vida durante o período gravídico-puerperal, mas que sobreviveram. Essa análise é capaz de refletir de forma mais fidedigna a qualidade do cuidado obstétrico se comparada ao número absoluto de mortes maternas, que por si só já é um evento raro. Por esse motivo, na última década houve um aumento do interesse por esse indicador, que culminou com a iniciativa da Organização Mundial da Saúde em uniformizar critérios de identificação de casos de *Near Miss* Materno, objetivando facilitar a monitorização e programar melhorias no cuidado obstétrico (10).

Nos últimos 50 anos tem-se observado uma tendência decrescente da ocorrência desses agravos em países de alta renda, paralelamente a um movimento contrário nos países de média e baixa renda, o que se deve basicamente ao acesso ao cuidado pré-natal de qualidade, bem como ao manejo adequado dos casos de pré-eclâmpsia e eclâmpsia, com melhores resultados maternos e perinatais (6, 11). Ampliando-se a análise de forma generalizada para outros maus desfechos obstétricos, a fim de exemplificarmos essa discrepância em números, em países de baixa e média renda, o risco de morte por causa materna chega a ser 33 vezes superior ao observado em países de alta renda (12). Como já ficou demonstrado, a realização de um número superior a 8 consultas de pré-natal atua como fator de proteção para ocorrência de pré-eclâmpsia (13). Um inquérito realizado na Califórnia em 2011, a fim de se investigar o aumento do número de mortes maternas observado naquela região no início dos anos 2000, revelou que 79% das mortes relacionadas à pré-eclâmpsia deviam-se a falhas em seu manejo (14, 15). Em outras palavras, a melhoria dos índices de saúde materna e fetal está absolutamente associada ao amplo acesso aos serviços e ao acolhimento e manejo de qualidade dos agravos, traduzindo-se em melhores resultados perinatais.

1.2- Conceitos e Classificação

As condições hipertensivas na gestação podem ser classificadas em hipertensão arterial prévia à gestação ou de manifestação antes das 20 semanas e hipertensão arterial com início às 20 semanas ou depois disso. No primeiro grupo incluem-se:

- ✓ Hipertensão arterial crônica essencial ou secundária;
- ✓ Hipertensão do jaleco branco;
- ✓ Hipertensão “mascarada”.

No grupo da hipertensão arterial que aparece às 20 semanas ou mais, incluem-se:

- ✓ Hipertensão gestacional transitória;
- ✓ Hipertensão gestacional;
- ✓ Pré-eclâmpsia, que pode ser isolada ou sobreposta à hipertensão arterial crônica. Nesse grupo, hipertensão arterial é definida como pressão arterial sistólica igual ou superior a 140 mmHg e/ou pressão arterial diastólica igual ou superior a 90

mmHg que devem ser aferidas em duas ocasiões distantes em pelo menos 4-6 horas, em aparelho calibrado e adequado para o biótipo da mulher em avaliação e por profissional capacitado (16). Em se tratando de pré-eclâmpsia, é necessário que coexista alguma das seguintes condições:

- Proteinúria (demonstrada pela relação proteinúria/creatininúria acima de 0,3 mg/mg ou em fita urinária igual ou acima 1+ ou ainda através de proteinúria de 24 horas superior a 300mg/24h);
- Disfunções de órgãos maternos que podem ser: insuficiência renal, caracterizada por creatinina acima de 1,02 mg/dL; acometimento hepático, caracterizada por elevação de transaminases duas vezes acima da normalidade ou dor em hipocôndrio direito ou epigastralgia; complicações neurológicas - caracterizadas por escotomas ou cefaleia persistente acompanhada por hiperreflexia ou estados confusionais ou eclâmpsia ou acidente vascular cerebral ou amaurose; complicações hematológicas que consistem em plaquetopenia ou hemólise;
- Disfunções uteroplacentárias: restrição de crescimento fetal; alterações de estudo Dopplervelocimétrico de artérias umbilicais, em especial se combinado a alterações em artérias uterinas (16, 17).

Conforme se pode notar, proteinúria não é condição *sine qua non* para caracterizar pré-eclâmpsia, conforme ocorria anteriormente (18, 19). De acordo com o conceito proposto pela Sociedade Internacional para Estudos em Hipertensão Gestacional, publicado em 2014 e reforçado em 2018 (20), toda gestante hipertensa deve ser investigada quanto ao acometimento de múltiplos órgãos, ainda que apresente proteinúria negativa, a fim de se descartar a hipótese de pré-eclâmpsia. Essa abordagem é inovadora e tende, de forma mais abrangente, englobar casos de certa forma negligenciados pela ausência de proteinúria.

A Pré-eclâmpsia pode apresentar sinais de gravidade, quando os níveis de pressão arterial sistólica forem superiores a 160mmHg e/ou os níveis de pressão arterial diastólica forem superiores a 110mmHg, ou quando houver a coexistência de eclâmpsia ou síndrome HELLP. Essa última define-se como hemólise, plaquetopenia com contagem de plaquetas inferior a 150 mil e elevação de transaminases hepáticas

duas vezes acima do limite superior da normalidade (17). A proteinúria maciça (acima de 5 gramas em 24 horas) deixou de ser considerada como critério isolado de gravidade com as modificações conceituais propostas pela Sociedade Internacional para Estudos em Hipertensão Gestacional, de 2014 (21).

No que se refere ao momento de manifestação, a pré-eclâmpsia é chamada de precoce, quando se dá antes das 34 semanas completas de gestação, e tardia após essa idade gestacional. Pode ainda ser pré-termo, quando o aparecimento acontece entre 34 semanas e 1 dia e 37 semanas e pré-eclâmpsia no termo, quando acontece a partir de 37 semanas e 1 dia (17, 22). A necessidade de classificar pré-eclâmpsia impõe-se na medida em que a análise histológica placentária de mulheres com o agravo mostrou que nas de manifestação precoce as lesões vasculares eram predominantes, bem como um evidente menor volume placentário, ao passo que maiores volumes placentários foram mais comuns nos casos de manifestação após 37 semanas, com sinais de resposta inflamatória crônica (23). Nesse sentido, os casos de aparecimento precoce de pré-eclâmpsia estariam mais associados à insuficiência placentária, e, portanto, à restrição de crescimento fetal. Por outro lado, os casos mais tardios apresentariam quadros clínicos mais brandos. Em suma: acometimentos placentários distintos, fenótipos distintos (2, 24-26).

1.3- Fisiopatologia

A pré-eclâmpsia ainda possui fisiopatologia não completamente esclarecida. É provável que decorra de um modelo composto por dois estágios interligados: placentação anormal e resposta materna inflamatória (27, 28).

O processo conhecido como placentação é minuciosamente coordenado e de sua efetiva ocorrência dependerá a manutenção do equilíbrio da unidade feto placentária. Numa gestação normal, há considerável aumento do débito sanguíneo uterino, a fim de garantir suplemento adequado para o espaço interviloso e, por extensão, desenvolvimento fetal adequado. Para que se atinja esse resultado, artérias espiraladas passam por um processo de remodelamento composto por 4 passos sequenciais promovido por invasão trofoblástica de suas paredes. Inicialmente, há o estágio de invasão da decídua, seguido por migração trofoblástica intra-arterial, com posterior invasão intramural dos vasos, quando há perda da camada média (muscular),

substituída por material fibrinoide e tecido conjuntivo. O último passo é o da reendotelização dos vasos e outras adaptações maternas induzidas (26, 29). Esses vasos passam a apresentar diâmetro médio muito superior ao observado em úteros de mulheres não gestantes, com baixa resistência ao fluxo de sangue e podem assim fornecer ao espaço interviloso suplemento sanguíneo adequado para a manutenção da gestação de forma efetiva (29, 30). Por outro lado, as artérias radiais e arqueadas terão aumento da pressão sanguínea em suas paredes, resultante do maior débito sanguíneo, o que agirá como produtor de estresse, e, em última análise, gerará secreção de óxido nítrico pelo endotélio, determinando vasodilatação dos vasos uterinos de forma global (30). O remodelamento das artérias espiraladas ocorre mais na parte central do leito placentário, reduzindo-se progressivamente em direção à periferia (26).

Esse processo de adaptação das artérias espiraladas pode não ocorrer de forma ideal, quando aproximadamente 90% dos vasos sofrem as transformações esperadas. Nos casos patológicos, o remodelamento pode ser parcial, completamente ausente, ou ainda ausente com lesões obstrutivas dos vasos. Nos casos de pré-eclâmpsia, a proporção de vasos remodelados encontra-se consideravelmente reduzida, em especial na região central do leito placentário. Quando está associada a restrição de crescimento fetal, há lesões obstrutivas. Nesses casos, as artérias sofrem um processo de aterose, com resultados muito semelhantes à formação de placas ateromatosas, com seus lúmens invadidos por macrófagos ricos em lipídeos, infiltrado inflamatório mononuclear perivascular e necrose fibrinoide das paredes dos vasos, com consequente isquemia uteroplacentária. Há então a instalação de um círculo vicioso de isquemia e reperfusão no espaço interviloso, com estresse metabólico dos retículos endoplasmáticos das células trofoblásticas, estruturas responsáveis pela homeostase celular e, em última instância, apoptose das mesmas. Esse processo liberará nanomoléculas na circulação materna, capazes de desencadear ampla resposta inflamatória intravascular, etapa essencial para o desenvolvimento da pré-eclâmpsia (31), bem como radicais livres, em decorrência do amplo estresse oxidativo e do colapso de mecanismos placentários e enzimas antioxidantes (32).

Pode-se dizer que tal processo é imuno-mediado, envolve resposta inflamatória sistêmica e predisposição genética materna (29, 32, 33).

O estresse oxidativo supracitado bem como a apoptose celular seriam determinantes para um desequilíbrio entre fatores pró-angiogênicos e anti-angiogênicos, com predomínio desses últimos (33). O aumento das concentrações de VEGFR-1 (capaz de bloquear a ação angiogênica do VEGF) e de endoglina solúvel e a síntese diminuída do fator de crescimento placentário (PlGF) estão associadas ao predomínio de elementos anti-angiogênicos próprios da pré-eclâmpsia (33).

Por fim, o entendimento ainda que parcial da fisiopatologia da pré-eclâmpsia, inclui a ativação e consequente consumo plaquetário, em níveis acima dos observados em gestações normais, vasoespasmo e deficiência de prostaciclina que tem ação vasodilatadora e que inibe agregação plaquetária. Do contrário, a síntese de tromboxano A2 está aumentada em placentas de mulheres com pré-eclâmpsia, o que determina predomínio de vasoconstrição e maior agregação plaquetária (34), bem como a de trombina, que tem sua expressão máxima na coagulação intravascular disseminada, traduzida clinicamente em descolamento prematuro placentário (35). A maior síntese de trombina faz parte de uma resposta inflamatória mais vigorosa característica da pré-eclâmpsia, conforme já exposto acima e determina depósito de fibrina em múltiplos órgãos o que reforça o caráter sistêmico da condição patológica.

1.4- Repercussões a médio e longo prazo e prevenção

O diagnóstico de pré-eclâmpsia ou eclâmpsia em uma gestante faz-se seguir por uma concentração de esforços em torno das possíveis implicações agudas desses agravos (trombocitopenia grave, coagulação intravascular disseminada, descolamento prematuro de placenta, entre outros). Entretanto, é fundamental que haja um olhar também sobre suas implicações em longo prazo, que são inúmeras e por vezes, irreparáveis. Após gestação complicada por pré-eclâmpsia, cerca de 20% das mulheres evoluirão com hipertensão ou microalbuminúria em até sete anos, sendo que o mesmo ocorre com apenas 2% das mulheres que tiveram gestações sem intercorrências (4). De forma semelhante, o risco de infarto agudo do miocárdio, acidente vascular cerebral e tromboembolismo venoso é substancialmente superior em mulheres com antecedente pessoal de pré-eclâmpsia, conforme já demonstrado em metanálise publicada em 2007 (36).

Em relação aos recém-nascidos, a complexidade reside na decisão pelo momento em que os riscos no ambiente intra-útero superam os do ambiente extrauterino. Nesse sentido, prematuridade e suas inúmeras consequências como síndrome respiratória aguda, hemorragia intraventricular, sepse, displasia bronco pulmonar e déficits de desenvolvimento neuropsicomotor são alguns dos cenários com os quais o bebê da mãe com pré-eclâmpsia (em geral a de ocorrência precoce, antes de 34 semanas) terá que se deparar e, por muitas vezes, contra os quais terá que lutar (16). Alguns estudos já demonstraram impacto negativo sobre o desenvolvimento neurocognitivo desses bebês avaliados nos primeiros dois anos de vida (37, 38).

Em última análise, mães que vivenciaram uma experiência de quase morte (near miss, a forma mais extrema de morbidade materna grave) em decorrência de pré-eclâmpsia também podem apresentar consequências psicológicas, com impactos emocionais que envolvem ansiedade, isolamento, dificuldades de amamentação, transtornos depressivos, e comprometimento da capacidade reprodutiva dentre outras. Estadias prolongadas em unidades de terapia intensiva, seja pela própria mulher, seja pelo recém-nascido, e limitações de ordem física ou mental podem interromper a ordem natural de simbiose entre mãe e bebê (39).

Nesse contexto de múltiplas e devastadoras consequências da pré-eclâmpsia, surge a necessidade de sua prevenção. Já foi demonstrado por metanálise que o emprego precoce (anterior às 16 semanas de gestação) de baixa dose de aspirina reduz a ocorrência de pré-eclâmpsia, em especial de suas formas mais graves (antes de 34 semanas) (40). Em um ensaio clínico recente envolvendo cerca de 2000 gestantes, comparando-se uso de aspirina e placebo, ficou demonstrada redução em 62% da ocorrência de casos precoces de pré-eclâmpsia no grupo que fez uso diário de 150 mg de aspirina (41). Dessa forma, delineia-se a necessidade premente de se discernir, o mais precocemente possível, a gestante que esteja sob maior risco de apresentar pré-eclâmpsia, idealmente em sua fase subclínica, a fim de que medidas preventivas sejam implementadas. Semelhante ao que ocorreu nos últimos 20 anos com o rastreamento e detecção de aneuploidias, em que modelos preditores passaram a ser aplicados no primeiro trimestre de forma muito mais efetiva, atualmente busca-se incessantemente um modelo preditor eficiente e precoce de pré-eclâmpsia, dentro da proposta da nova pirâmide invertida de cuidados pré-natais, em que esforços são concentrados no

primeiro trimestre, de forma a termos diagnósticos mais precoces e propostas terapêuticas estabelecidas em fases subclínicas dos agravos (42-44).

1.5- Predição (Fatores preditores)

Considerando a amplitude do impacto social e econômico da pré-eclâmpsia, além é claro das evidentes repercussões clínicas, surge a necessidade de predição desse agravo. Ao longo dos anos, houve a identificação de marcadores biofísicos e bioquímicos que foram propostos como possíveis indicadores precoces de algum erro no complexo processo de placentação, que levaria à pré-eclâmpsia. O primeiro passo seria a identificação dos fatores de risco para a ocorrência dessa condição.

São apontados como fatores de risco para pré-eclâmpsia: ter apresentado pré-eclâmpsia em gestação anterior, ser nulípara, estar em algum dos extremos de idade (abaixo de 20 anos ou acima de 40 anos), e ter descendência afro-americana. Condições patológicas pré-existentes como hipertensão arterial crônica, diabetes mellitus, nefropatia, síndrome do anticorpo antifosfolípide também fazem parte da lista (17, 45), bem como IMC acima de 35 e uso de tecnologias de reprodução assistida (46, 47). Essa última associa-se a maior incidência de gestações múltiplas e a um aumento da idade média da mulher em sua primeira gestação que em conjunto atuam para maior ocorrência de pré-eclâmpsia (48, 49).

O *National Institute for Health and Clinical Excellence* (NICE) propôs num documento elaborado em 2010 a classificação dos fatores de risco em "risco moderado" e "alto risco" para pré-eclâmpsia, numa tentativa de transformá-los em ferramentas capazes de definir o grupo para o qual estaria indicada a aplicação imediata de medidas profiláticas (50). Foram classificados como de alto risco os seguintes fatores:

- Histórico de algum distúrbio hipertensivo em gestações anteriores;
- Doença renal crônica;
- Doenças autoimunes como Lúpus Eritematoso Sistêmico ou Síndrome do Anticorpo Antifosfolípide;
- Diabetes tipo 1 ou 2;
- Hipertensão Arterial Crônica.

Foram considerados como fatores de risco moderado:

- Primigestas;
- Mulheres com 40 anos ou mais;
- Intervalo interpartal superior a 10 anos;
- Índice de Massa Corpórea (IMC) superior a 35 kg/m^2 no início do pré-natal;
- História familiar de pré-eclâmpsia;
- Gestação múltipla.

Para esse órgão (NICE), a presença de dois fatores de risco moderado ou de um único fator de alto risco elegeria a gestante para aplicação de medidas profiláticas (uso de aspirina antes de 16 semanas de gestação, cuidados pré-natais em serviço especializado) (41).

Para o *American College of Obstetricians and Gynecologists* (ACOG) os fatores de risco são os mesmos apontados pelo instituto NICE (51), à exceção do IMC, aqui considerado 30 kg/m2 como valor acima do qual a mulher seria considerada como alto risco para pré-eclâmpsia. Além disso, esse órgão não reconhece gradação para os fatores de risco, mas os engloba sob a mesma denominação de “alto risco” (52).

Um estudo prospectivo aplicando o conceito proposto pelo instituto NICE de utilizar fatores de risco como testes preditores para pré-eclâmpsia obteve taxa de detecção para casos de pré-eclâmpsia precoce e tardia de 37% e 28,9%, respectivamente, para uma taxa de 5% de falsos-positivos, considerados numa população heterogênea, composta por nulíparas e multíparas (53). Nesse mesmo estudo, ficou demonstrado que o fator clínico de maior poder preditor foi a história prévia de pré-eclâmpsia. Evidentemente, esse cenário não favorece a identificação de nulíparas em risco para pré-eclâmpsia, o que se constitui numa grande limitação, já que a incidência desse agravo é maior nesse grupo de gestantes. Especificamente para nulíparas, um estudo multicêntrico conduzido entre mais de 8 mil mulheres de baixo risco para complicações gestacionais demonstrou taxa de detecção de 37% para pré-eclâmpsia ao utilizar um modelo preditor composto exclusivamente por fatores clínicos (54), dentre os quais obesidade e primiparidade apareceram como principais elementos demográficos preditores de pré-eclâmpsia de instalação precoce.

A aferição da pressão arterial faz parte do cuidado pré-natal de rotina e pode ser o primeiro indicador clínico da ocorrência de alguma condição hipertensiva. Tendo em vista que os níveis de pressão arterial média encontram-se elevados em gestantes que desenvolverão pré-eclâmpsia já no primeiro ou segundo trimestre, ou muitas vezes até mesmo antes da gestação (55, 56), esse marcador deixa de representar uma ferramenta para o diagnóstico de condições hipertensivas e passa a atuar como importante elemento preditor da pré-eclâmpsia. Em revisão sistemática publicada em 2008, foi demonstrada a maior acurácia da Pressão Arterial Média (PAM) na predição de pré-eclâmpsia entre gestantes de baixo risco no segundo trimestre, quando comparada à aferição isolada de pressão arterial sistólica e diastólica; entre mulheres de alto risco para pré-eclâmpsia é a pressão arterial diastólica acima de 75 mmHg que exibe o maior poder preditor do agravo em questão (57). É necessário ressaltar que o aparelho utilizado nas aferições seja específico e validado para a obtenção da pressão arterial, bem como que seja utilizado por profissional treinado. Avaliada numa população heterogênea composta por nulíparas e multíparas, a taxa de predição da PAM para os casos precoces e tardios de pré-eclâmpsia são, respectivamente, 58 e 44%, para 5% de falsos-positivos (43).

O estudo dopplervelocimétrico das artérias uterinas proporciona uma avaliação não invasiva da circulação uteroplacentária (43, 58). Ao que os estudos indicam, esse recurso seria bastante útil para predição de casos de pré-eclâmpsia de manifestação precoce e quando aplicados entre gestantes consideradas de alto risco para desenvolvimento do agravo (59). Ao se detectar um aumento de resistência ao fluxo sanguíneo às 23 semanas de gestação nas artérias uterinas, numa população aleatória e heterogênea de gestantes, obteve-se uma sensibilidade de 77,8% e especificidade de 95% para predição de pré-eclâmpsia de manifestação precoce (60). Por outro lado, os números não são animadores quando o desfecho avaliado são casos de pré-eclâmpsia de forma geral (incluindo os de manifestação tardia, que são maioria). Nesses casos, a sensibilidade encontrada é de 42,8%, que não tem aplicabilidade clínica em se tratando de um teste de rastreamento (60). Ao se realizar o estudo Doppler nas artérias uterinas precocemente, entre 11 e 13 semanas, e analisá-lo de forma isolada, a taxa de detecção de pré-eclâmpsia precoce e tardia é de 59 e 40% respectivamente, com 5% de falsos-positivos. É necessário ressaltar que a realização do Doppler é

inteiramente dependente da disponibilidade do aparelho de ultrassom (o que nem sempre é uma realidade a depender do centro clínico considerado) do avaliador e de sua perícia e treinamento em obtê-lo, o que restringe ainda mais seu uso em larga escala (43). Considerando essas variáveis e a escassez de evidências científicas existentes sobre melhorias nos resultados perinatais e maternos, o estudo Doppler de artérias uterinas não é recomendado como teste de rastreamento para pré-eclâmpsia entre mulheres consideradas de baixo risco por seus antecedentes pessoais e história clínica (22, 61).

Recentemente, uma revisão sistemática avaliando marcadores inflamatórios e sua capacidade de predição de pré-eclâmpsia não encontrou um fator que isoladamente fosse suficiente para a predição do agravo (62). Inúmeros biomarcadores foram estudados, mas PAPP-A e PIGF demonstraram melhores resultados na identificação de casos de pré-eclâmpsia de manifestação precoce (antes de 34 semanas) (63). PAPP-A (pregnancy-associated plasma protein-A) é uma metaloprotease produzida pelo sinciciotrofoblasto, que está envolvida com o sistema composto pelos fatores de crescimento insulina-símile, fundamentais para o crescimento e desenvolvimento placentários (35). A redução dos níveis séricos de PAPP-A está relacionada à ocorrência de pré-eclâmpsia. PIGF (placental growth factor) é uma glicoproteína, membro da família dos fatores de crescimento endotelial, secretada pelo citotrofoblasto, com funções pró-angiogênicas importantes para o desenvolvimento adequado placentário, conforme já dito anteriormente. Postula-se que a redução de seus níveis esteja associada ao desenvolvimento de pré-eclâmpsia (64). A definição de parâmetros de normalidade para esses marcadores sofre influência da presença ou não de diabetes mellitus, da paridade (multíparas apresentam valores de PAPP-A inferiores às nulíparas), gemelaridade (que apresentam níveis superiores de PAPP-A e PIGF aos observados em gestações únicas), idade materna avançada (mulheres com mais de 35 anos exibem valores inferiores dos marcadores em questão), dentre outros elementos (65). Conforme já demonstrado, os biomarcadores não são bons preditores de casos de pré-eclâmpsia tardia (entre 34 e 37 semanas), uma vez que, aparentemente, a taxa de estresse oxidativo do sinciciotrofoblasto nesses casos não seria suficiente para que ocorressem alterações séricas dos mesmos (28). A taxa de detecção dos casos tardios de pré-eclâmpsia é de

43% quando são dosados conjuntamente PAPP-A e PIGF, considerando-se 5% de falsos-positivos. Para os casos de pré-eclâmpsia precoce (antes de 34 semanas), esse valor sobe para 60% (43). Num estudo de coorte entre nulíparas de baixo risco para complicações gestacionais em que o desempenho do PIGF foi testado isoladamente, ficou demonstrado que sua maior utilidade encontra-se restrita aos casos de pré-eclâmpsia de aparecimento precoce, apresentando níveis reduzidos entre 14-16 semanas de gestação nesses casos. Nessa mesma análise, a PAPP-A não acrescentou qualquer valor preditivo ao ser associada com outros fatores na predição de casos de pré-eclâmpsia precoce (54).

Estudos têm sido realizados no intuito de se concluir sobre um modelo preditor, cuja sensibilidade possa abranger os diversos fenótipos da pré-eclâmpsia entre os mais diferentes perfis de mulheres, sejam elas nulíparas ou multíparas. Em 2012, Myatt et al., numa análise multivariada entre gestantes nulíparas de muito baixo risco demonstraram, através de um modelo incluindo variáveis clínicas (descendência afro-americana, pressão arterial sistólica, índice de massa corpórea e nível de escolaridade) e biomarcadores (ADAM 12, PAPP-A e PIGF) dosados no primeiro trimestre, uma sensibilidade de 46% e especificidade de 80% (66). Em 2013, uma nova análise entre mulheres nulíparas de muito baixo risco para identificação de casos precoces de pré-eclâmpsia através de um modelo preditor incluindo PIGF dosado às 15 semanas de gestação, história familiar de pré-eclâmpsia, utilização de fertilização assistida e pressão arterial média obteve resultados muito semelhantes ao estudo supracitado (sensibilidade de 45% e 95% de especificidade), sendo que o acréscimo da análise Dopplervelocimétrica de artérias uterinas às 20 semanas em nada contribuiu para melhorar o desempenho desse modelo (67).

Valores de sensibilidade como as mostradas pelos referidos estudos não têm aplicação clínica, e refletem a complexidade de se encontrar um modelo preditor para um agravo como a pré-eclâmpsia, com fenótipos variáveis, além da dificuldade de se eleger pacientes de alto risco, em meio a uma população considerada de muito baixo risco. Provavelmente a predição dos casos de manifestação precoce é completamente distinta da predição dos casos de apresentação tardia, baseado no fato de que os biomarcadores dosados no primeiro trimestre na maioria dos estudos refletem adaptações e alterações placentárias que se instalam precocemente na gestação e que

estão associadas ao fenótipo da pré-eclâmpsia que se instala antes de 34 semanas. Ademais, além da complexidade inerente à pré-eclâmpsia, um modelo preditor que inclua a realização de estudo Doppler das artérias uterinas, biomarcadores e aspectos sociodemográficos está longe de se apresentar como um teste de fácil e ampla utilização clínica, considerando seu custo, efetividade e dependência de condições ideais de treinamento de seus implementadores.

Por esse motivo, a necessidade da busca por outras formas de obtenção de predição, que possam incluir os diferentes fenótipos da síndrome de pré-eclâmpsia, considerando inclusive o fato de que os casos de manifestação tardia são os mais frequentes (68), se mantém. Nesse cenário, as tecnologias ômicas têm sido apontadas como promissoras na identificação de modelos preditores precoces de pré-eclâmpsia, inclusive numa fase subclínica da doença. A metabolômica é uma dessas tecnologias e se destina à identificação de metabólitos, pequenas moléculas que representam a linha final da expressão gênica e uma assinatura fenotípica em alta resolução da doença que se pretende estudar (69).

Esforços têm sido feitos no intuito de se buscar um perfil metabolômico que possa associar-se à pré-eclâmpsia (68, 70-80), sem que até o momento tenham sido sugeridos modelos preditores que possam ter real aplicabilidade clínica ou que tenham sido validados em grandes populações.

Em 2010, Kenny et al., num estudo caso-controle elaborado em duas fases sequenciais de exploração e validação, envolvendo nulíparas com gestações sem complicações e nulíparas que desenvolveram pré-eclâmpsia, utilizaram análise de amostras de sangue coletadas entre 15 e 16 semanas para, através da metabolômica (espectrometria de massa e cromatografia líquida), avaliar numa fase exploratória se haveria alguma diferença de metabólitos entre os grupos estudados e, uma vez identificados, se esses seriam aplicáveis à população. Na fase exploratória, com 60 mulheres nulíparas sem comorbidades e 60 mulheres que desenvolveram pré-eclâmpsia (precoce e tardia), foram identificados 45 metabólitos pertencentes a 11 classes químicas distintas: aminoácidos, carboidratos, carnitinas, eicosanoides, ácidos graxos, fosfolípides, porfirinas, fosfatidilserina e esteroides. Nessa análise, o modelo preditor proposto mostrou índice de correlação R^2 de 0,58 e AUC de 0,96. Na fase de validação, com uma população distinta de 40 mulheres sem comorbidades e 40

mulheres que desenvolveram pré-eclâmpsia, 34 desses 45 metabólitos foram identificados, com índice de correlação R^2 de 0,57 e AUC 0,95. Num modelo envolvendo 14 metabólitos, extraídos do grupo encontrado nas fases exploratória e de validação, considerados como possuidores de maior potencial preditivo, a taxa de detecção para ambas as fases considerando uma taxa de falso-positivo de 10% foi de 77 e 73%, respectivamente para fases exploratória e de validação. Para uma taxa de detecção de 90%, o que é um número ideal em se tratando de um teste de rastreamento, as taxas de falso-positivo se tornam impraticáveis: 21 e 24% nas fases exploratória e de validação. Essas análises foram efetuadas numa população relativamente pequena e muito pouco heterogênea. Em especial a fase de validação, cujo estudo ideal se faz sobre uma coorte heterogênea e sob uma perspectiva prospectiva, preferencialmente. Portanto, novos estudos são necessários a fim de se validar um modelo preditor capaz de distinguir precocemente casos de pré-eclâmpsia e dentre esses, casos precoces e tardios (70).

Em 2012 foi publicado um estudo que avaliou especificamente casos de pré-eclâmpsia de manifestação precoce (antes das 34 semanas). Mulheres entre 11 e 13 semanas de gestação passaram por coletas de sangue, sendo 30 com pré-eclâmpsia e 60 controles, sem quaisquer intercorrências e submetidas à análise de espectrometria por ressonância nuclear magnética. Foram identificados 20 metabólitos em quantidades significativamente diferentes entre os dois grupos avaliados. Dois modelos foram propostos e analisados em relação à capacidade preditiva: o primeiro com 4 metabólitos (citrato, glicerol, hidroxiisovalerato, metionina) com características maternas (peso e presença de outras comorbidades); o segundo modelo com 3 metabólitos (citrato, glicerol e hidroxiisovalerato) com outras características maternas (paridade e presença de outras comorbidades), índice de pulsatilidade de artérias uterinas e CCN (comprimento cabeça-nádega) fetal. O primeiro modelo apresentou AUC de 0,904 com sensibilidade de 75,9% e taxa de falso positivo de 4,9%. O segundo modelo apresentou AUC de 0,98 com sensibilidade de 82,6% com taxa de falso positivo de 1,6%. É importante destacar que o resultado observado com o segundo modelo incluiu o estudo Doppler de artérias uterinas, que isoladamente tem sensibilidade de 40% (72).

A elucidação do perfil metabolômico da pré-eclâmpsia poderá atuar não apenas na predição, como também auxiliando para que haja um melhor entendimento do agravo no que se refere a mecanismos celulares e moleculares. Em última análise, poderá contribuir para um melhor entendimento da doença em todas as suas nuances: prevenção, diagnóstico e condução terapêutica.

Observação: essa introdução foi adaptada e deu origem a um artigo de revisão narrativa, correspondente ao primeiro produto dessa tese, já aceito para publicação.

2. Objetivos:

2.1- Objetivo geral

Estudar de forma abrangente a ocorrência de pré-eclâmpsia na atualidade, enfocando o conhecimento geral sobre essa condição através de revisões da literatura científica sobre o assunto, e mais especificamente a ocorrência e características da pré-eclâmpsia em uma coorte de nulíparas brasileiras de baixo risco.

2.2- Objetivos específicos

1. Desenvolver uma revisão narrativa atualizada sobre a ocorrência, conceito, fisiopatologia, repercussões, prevenção e predição da pré-eclâmpsia;
2. Desenvolver um método padronizado para revisar sistematicamente os estudos em predição de distúrbios hipertensivos na gestação através de marcadores metabolômicos;
3. Apresentar os resultados de uma revisão sistemática avaliando o uso de metabolômica na predição de condições hipertensivas na gestação;
4. Determinar a incidência de pré-eclâmpsia numa coorte brasileira de nulíparas de baixo risco e os fatores sociodemográficos e clínicos associados ao seu desenvolvimento;
5. Avaliar o desempenho da pressão arterial média aferida em três momentos distintos da gestação (20, 27 e 37 semanas) em predizer a ocorrência de pré-eclâmpsia;

3- Método

Para responder aos diferentes objetivos específicos foram utilizados métodos diferentes como descritos a seguir. As variáveis, entretanto, foram descritas conjuntamente.

3.1- Método para os objetivos 1 a 3

Partindo de uma ampla revisão de literatura, foi elaborada uma revisão narrativa sobre pré-eclâmpsia, a fim de termos um documento final que sirva de instrução para médicos e profissionais da saúde interessados no tema. Para este objetivo específico, foram considerados aspectos de prevalência, fatores de risco, impacto mundial, aspectos fisiopatológicos e predição. Esta revisão está inserida na introdução dessa tese e adaptada para um artigo científico de revisão já submetido a periódico científico em inglês.

Além disso, faremos uma revisão sistemática utilizando bases eletrônicas para busca dos artigos publicados nos últimos 20 anos a fim de respondermos a seguinte questão: qual é a efetividade da metabolômica em predizer a ocorrência de condições hipertensivas na gestação?

As bases eletrônicas a serem consultadas foram: PubMed, EMBASE, Scopus, Web of Knowledge, Latin America and Caribbean Health Sciences Literature (LILACS), Scientific Electronic Library Online (Scielo), Health Technology Assessment (HTA), Database of Abstracts of Reviews of Effects (DARE). Os artigos a serem incluídos serão os estudos de coorte e caso controle publicados na língua inglesa, referentes aos últimos 20 anos. Serão excluídos: cartas ao editor, estudos de corte transversal, relatos de caso, revisões, estudos experimentais com animais, opiniões de especialistas, comentários e editoriais.

As chaves de busca a serem utilizadas foram:

- Preeclampsia OR pre-eclampsia OR eclampsia OR gestational hypertension OR white coat hypertension OR severe preeclampsia OR late preeclampsia OR early preeclampsia OR pregnancy induced hypertension OR hypertensive syndromes of pregnancy;

  AND
- Metabolomic*OR metabonomic* OR metabolit* OR HNMR OR proton NMR OR proton nuclear magnetic resonance OR liquid chromatogra* OR gas chromatogra* OR UPLC OR HPLC OR high pressure liquid chromatograph* OR ultra-performance liquid chromatograph* OR ultra performance liquid chromatograph* OR lipidomic* OR mass spectrometr*;

  AND
- Screen*OR predict*OR profil*

O desfecho principal avaliado foi a ocorrência de pré-eclâmpsia. Como desfechos secundários consideraremos pré-eclâmpsia de manifestação precoce e tardia, hipertensão gestacional, pré-eclâmpsia superajuntada à hipertensão arterial crônica e hipertensão do jaleco branco. Dois revisores farão a análise independente de títulos e resumos dos artigos encontrados após checagem das bases eletrônicas. Um terceiro revisor atuará para decidir em caso de discordância na avaliação dos dois revisores principais. Faremos uma metanálise caso os dados sejam suficientes e assim permitam.

3.2- Método para objetivos 4 e 5

O estudo *Preterm Screening and Metabolomics in Brazil and Auckland (Preterm SAMBA)* (Anexo 1) ocorreu em duas fases: desenvolvimento e validação. A primeira foi um estudo caso-controle com mulheres que participaram do estudo SCOPE (Screening for Pregnancy Endpoints Study) (54). Em linhas gerais, esse estudo constituiu-se numa coorte composta por 5690 gestantes de baixo risco, recrutadas entre os anos de novembro de 2004 e agosto de 2008, provenientes da Nova Zelândia, Austrália, Irlanda e Reino Unido, que resultou na criação de um banco de dados e de material biológico (sangue e cabelo) das participantes. A fase de desenvolvimento do estudo Preterm SAMBA foi completamente realizada na Nova Zelândia para a criação do modelo de

prematuridade e na Irlanda para o correspondente da pré-eclâmpsia. Comparando dois grupos de gestantes, sendo um grupo sem complicações e outro grupo com algum dos agravos a serem estudados, pretendeu-se por meio da análise metabolômica das amostras de sangue encontrar possíveis metabólitos que pudessem estar correlacionados à ocorrência do agravo em estudo. O objetivo foi estudar os seguintes agravos: prematuridade, pré-eclâmpsia, diabetes gestacional e restrição de crescimento intrauterino. Na presente abordagem estamos enfocando apenas a pré-eclâmpsia como resultado dessa coorte.

A segunda fase do estudo Preterm SAMBA, a fase de validação, foi assim chamada por ter o objetivo de validar numa coorte brasileira de nulíparas de baixo risco o modelo metabolômico obtido na fase de desenvolvimento, para cada um dos resultados inicialmente previstos. Para isso, uma coorte prospectiva de 1200 gestantes provenientes de cinco unidades obstétricas participantes da Rede Brasileira de Estudos em Saúde Reprodutiva e Perinatal foi construída. Essas cinco unidades obstétricas estão localizadas em diferentes regiões geográficas brasileiras (Nordeste, Sudeste e Sul) e foram as seguintes:

- Maternidade do CAISM da Universidade Estadual de Campinas, UNICAMP, em Campinas, SP;
- Maternidade da Faculdade de Medicina de Botucatu, UNESP, em Botucatu, SP;
- Maternidade do Hospital das Clínicas, Universidade Federal do Rio Grande do Sul, UFRGS, em Porto Alegre, RS;
- Maternidade do Hospital das Clínicas, Universidade Federal de Pernambuco, UFPE, em Recife, PE;
- MEAC- Maternidade Escola da Universidade Federal do Ceará, UFCE, em Fortaleza, CE.

Participantes

Para serem incluídas, as mulheres deveriam ser nulíparas, com gestação de feto único e estarem entre 19 e 21 semanas de idade gestacional. Foram excluídas as mulheres que apresentassem:

- 3 ou mais abortos anteriores;
- Feto com malformações;
- Diagnóstico prévio de hipertensão arterial crônica em uso de medicação e/ou diabetes mellitus e/ou nefropatia;
- Pressão arterial superior a 160x100 mmHg no momento da admissão no estudo;
- Lúpus Eritematoso Sistêmico e/ou síndrome do anticorpo antifosfolípide;
- Doença falciforme;
- Retrovirose;
- Malformações uterinas (útero bicorno, septo uterino);
- Antecedente de cone a frio;
- Uso crônico de corticosteroides ou AAS ou cálcio em dose superior a 1g ao dia ou óleo de peixe em dose superior a 2,7g ao dia ou vitamina C acima de 1000mg ao dia ou vitamina E acima de 400UI ao dia;
- Uso de heparina em qualquer de suas apresentações e doses;
- Antecedente de cerclagem uterina;

Cálculo do tamanho amostral

O cálculo do tamanho amostral fez-se em função do desfecho prematuridade, o primeiro a ser analisado. Assumindo um erro tipo I de 5% e que a estimativa pela área sob a curva ROC seria de pelo menos 0,68 e, a fim de testarmos as hipóteses com poder adequado (80% do poder, β=0,2), o tamanho amostral precisaria se aproximar a 80 casos de parto prematuro. Tomando-se a prevalência mínima esperada desse desfecho no Brasil de 7%, o tamanho total da coorte calculado foi de 1150 mulheres.

Procedimentos

A coleta de dados foi realizada em três momentos do seguimento pré-natal das participantes. A primeira visita era realizada entre 19 e 21 semanas de gestação, momento da inclusão da participante, em que amostras de sangue e cabelo eram coletadas e armazenadas. Além disso, uma série de dados clínicos, antecedentes pessoais e familiares eram armazenados numa plataforma eletrônica (MedSciNet).

Essas gestantes passaram por avaliação de peso, altura, pressão arterial (aferida 3 vezes com intervalo de 5 minutos entre elas), pregas cutâneas (biciptal, triciptal, escapular e supra-ilíaca), perímetro cefálico e circunferência do braço. A mesma avaliação se repetiu entre 27 e 29 semanas e entre 37 e 39 semanas, exceto pela coleta de sangue e cabelo, reservada apenas para a primeira visita.

Definição operacional das variáveis:

Definição operacional das variáveis utilizadas nos estudos, em ordem alfabética:

- Adequação de peso para a idade gestacional: caracterização do peso do recém-nascido ao nascer em função da idade gestacional, conforme consta em prontuário médico, categorizado em pequeno para a idade gestacional, adequado para a idade gestacional e grande para a idade gestacional.
- Alcoolismo na gravidez: hábito de consumir bebida alcoólica durante a gravidez, auto referido pela mulher, categorizado em nunca tomou na gravidez, poucas vezes e muitas vezes.
- Altura: conforme consta em prontuário médico ou referido pela paciente ou medido pelo investigador com fita métrica graduada em milímetros, descrita em metros.
- Anemia: estado patológico caracterizado pela insuficiência de hemoglobina nos glóbulos sanguíneos, conforme auto referido pela paciente em ter tido diagnóstico ou tratamento de anemia antes da gestação ou conforme consta em prontuário médico valor de hemoglobina (Hb)< 10g/L , categorizado em sim e não.
- Antecedente de abortamento: história prévia de perda gestacional antes de 22 semanas ou com feto pesando menos de 500g, auto referida em entrevista, em número inteiro.
- Antecedente de gestação ectópica: história prévia de gestação ectópica, que consiste em gravidez na qual o embrião implantou-se fora da cavidade uterina, auto referida em entrevista, em número inteiro.

- Antecedente de Infertilidade: definida como ter tido um período de ausência de gravidez após 12 meses de tentativa com relações sexuais regulares, auto referido em entrevista, categorizado em sim e não.
- Antecedente familiar de complicações obstétricas: histórico da mãe da mulher participante em ter tido complicações obstétricas durante a sua vida reprodutiva, auto referida pela mulher entrevistada, categorizada em nenhuma, abortos, hipertensão gestacional, pré-eclâmpsia, eclâmpsia, recém-nascido com baixo peso ao nascimento, parto antes de 37 semanas, parto pré-termo com trabalho de parto espontâneo, óbito fetal e/ou morte neonatal.
- Antecedente mórbido: história prévia à gravidez de doenças/agravos à saúde, auto referida pela mulher, categorizado em nenhuma, asma, hipertensão na vigência de contraceptivo oral, hipertensão leve/moderada, síndrome do cólon irritável, incontinência urinária, depressão, doença intestinal inflamatória, doença celíaca, artrite reumatoide, epilepsia, tromboembolismo confirmado por exame de imagem com histórico de tratamento anticoagulante, tromboembolismo suspeito (relato de ter usado anticoagulação previamente à gravidez, mas não teve o diagnóstico confirmado) e outro.
- Apgar 1º minuto: escore de avaliação das condições recém-nascido ao nascimento, através da utilização do índice de APGAR no 1^{o} minuto, conforme consta em prontuário médico, em número inteiro de 0 a 10.
- Apgar 5º minuto: escore de avaliação das condições recém-nascido ao nascimento, através da utilização do índice de APGAR no 5^{o} minuto, conforme consta em prontuário médico, em número inteiro de 0 a 10.
- or da pele/etnia: cor da pele da mulher, auto referida em entrevista, categorizada em branca, parda, preta, amarela e outra.
- Doença da Tireoide: alteração do funcionamento da glândula tireoide comprometendo a normal produção e liberação de hormônios tireoidianos, auto referida pela mulher em entrevista, categorizado em nunca foi testada, hipotireoidismo diagnosticado no primeiro trimestre, hipotireoidismo prévio, tratamento adequado e bom controle clínico no primeiro trimestre, tireotoxicose

no primeiro trimestre (sem ou com medicação), antecedente de tireotoxicose e outras.

- Escolaridade: nível de formação escolar referido pela mulher, auto referida pela mulher em entrevista, em anos completos.
- Estado marital: estado civil da paciente, auto referida no momento em que entra no estudo, categorizada em solteira, amasiada, casada, divorciada ou viúva.
- Idade gestacional ao nascimento: número de semanas que a gestação alcançou até a resolução através do parto, conforme registro em prontuário.
- Idade gestacional na admissão ao estudo: tempo de gestação, em semanas, identificado através da diferença em semanas da data da última menstruação e/ou ultrassom obstétrico realizado até o segundo trimestre e a data de admissão no estudo. No caso de haver discordância entre as duas estimativas, deve se considerar como referência o primeiro ultrassom realizado. Variável obtida conforme consta em prontuário médico ou calculada pelo avaliador, descrita em semanas completas.
- Idade Materna: número de anos da mulher, auto referida em entrevista no início do estudo, em número inteiro.
- Índice de massa corpórea (IMC): quantidade de massa corpórea por metro quadrado que infere o grau de obesidade de um indivíduo, calculada através da divisão do peso, em kg, sobre o quadrado da altura, em metros, segundo constam em prontuário médico, em número decimal.
- Infecção do Trato Urinário (ITU): infecção do sistema urinário sintomático ou não, conforme prontuário médico constando antecedente de pelo menos 1 ITU, confirmada por urocultura, categorizado em sim e não.
- Internação em UTI neonatal: admissão do recém-nascido em unidade de terapia intensiva, conforme prontuário médico, categorizado em sim e não.
- Nível socioeconômico: síntese das características da mulher em relação à sua renda, ocupação e escolaridade, medido pelo investigador através do Índice de Privação Múltipla e pela classificação de ocupação do chefe de família usando o sistema NS-SEC que gerará um índice socioeconômico, descrito em número inteiro (ordinal).

- Número de dias de internação pós-parto: total de dias que a mulher ficou internada do dia do parto até a alta médica, conforme consta em prontuário médico, em número inteiro.
- Número de gestações: número de vezes que a mulher refere ter ficado grávida previamente à gestação atual, auto referida em entrevista, em número inteiro.
- Número de pessoas dependentes da renda mensal: quantidade de indivíduos que usufruem ou dependem de uma dada renda familiar incluindo os próprios geradores dessa renda, auto referido em entrevista, em número inteiro.
- Parto pré-termo: condição obstétrica que culminou com o nascimento que aconteceu antes das 37 semanas completas, conforme consta em prontuário médico, categorizado em parto pré-termo espontâneo, rotura prematura de membranas e parto prematuro terapêutico/eletivo (aquele que não foi precedido de contrações ou rotura prematura e é fruto de uma indicação médica).
- Perfil metabolômico: painel de metabólitos da mulher, obtido através da análise das amostras de plasma desproteinizadas colhidas às 20 semanas de gestação, utilizando as técnicas de GC-MS, LC-MS e lipidômica com base em CG-MS, através de esterificação metílica de ácidos graxos (FAME), de forma descritiva.
- Peso do recém-nascido: peso do recém-nascido, conforme consta em prontuário médico desde que registrado logo após seu nascimento, em gramas.
- Peso no início da gravidez: peso da mulher, conforme consta na anotação de primeira consulta do prontuário médico, desde que tenha sido até 16 semanas de gravidez, em kg.
- Peso no final da gravidez: peso de um indivíduo previamente ao parto, conforme consta na anotação do prontuário médico desde que sua mensuração tenha ocorrido até 1 semana antes do parto, em kg.
- Pré-eclâmpsia: desenvolvimento de hipertensão arterial após 20 semanas, que pode ou não estar associada à proteinúria (na urina de 24 horas, proteinúria acima de 300 mg ou 1+ ou mais em fita urinária ou relação proteinúria/creatininúria igual ou superior a 0,3 tomada numa amostra isolada de urina) ou à disfunção sistêmica ou à disfunção útero-placentária. As disfunções sistêmicas podem ser:
- Perda de função renal, com creatinina acima de 1,02 mg/dL;

- Disfunção hepática com elevação de transaminases hepáticas acima do dobro dos níveis normais;
- Alterações hematológicas como plaquetopenia abaixo de 100 mil ou coagulação intravascular disseminada ou hemólise;
- Complicações neurológicas como estado mental alterado, amausose, hiperreflexia com clônus, escotomas, turvamento visual, diplopia, Doppler de artéria oftálmica com IP superior a 0,78;
- Pressão arterial média: pressão arterial resultante da fórmula (2PD+PS)/3, onde PD é pressão diastólica e PS é pressão sistólica, aferidas com manguito adequado ao biótipo do indivíduo avaliado.
- Renda familiar: somatório da renda individual dos moradores do mesmo domicílio da mulher, auto referida em entrevista, expressa em reais.
- Tabagismo: Vício ou abuso do tabaco, auto referida pela mulher em entrevista com aproximadamente 20 semanas de gravidez, categorizada em não fumantes, aquelas que não fumaram em nenhum momento da gravidez; fumantes, aquelas que mantiveram o hábito de fumar após o primeiro trimestre; ex-fumantes, aquelas que fumaram só primeiro trimestre de gravidez.
- Tempo de internação: quantidade de dias de internação do recém-nascido desde o parto até a alta hospitalar, conforme consta em prontuário médico, em número inteiro.
- Tempo de tabagismo na gravidez: quantidade de meses que fumou durante a gravidez, auto referido, em quantidade de meses.
- Trabalho de parto: refere-se ao aparecimento ou não do trabalho de parto, que consiste no aparecimento espontâneo ou induzido de contrações uterinas com frequência de 2 a 3 em cada 10 minutos e com duração mínima de 30 segundos, segundo consta em prontuário medico, categorizado em espontâneo, induzido, cesárea sem trabalho de parto.
- Tratamento de fertilização na gravidez atual: ter realizado técnica de reprodução assistida para obter sucesso em engravidar referindo-se à gravidez atual, auto referido em entrevista, categorizado em não, hormonal, inseminação artificial, fertilização in vitro, ICSI e doação de oócito.

- Uso de drogas ilícitas: uso de substâncias nocivas e ilegais de serem comercializadas e/ou consumidas, conforme relatado pela mulher, categorizado em não, anfetaminas, maconha, cocaína e outras.
- Via de parto - via de resolução ou de parturição da atual gestação, segundo consta no prontuário médico, categorizada em parto normal, cesárea ou parto com fórceps.

Aspectos Éticos

O estudo Preterm SAMBA foi avaliado e aprovado pelo CEP da UNICAMP (Anexo 2). Todas as mulheres foram informadas sobre os procedimentos do estudo na hora em que foram convidadas para participar e antes de assinarem o respectivo Termos de Consentimento Livre e Esclarecido. Além dos procedimentos de coleta de informações epidemiológicas e clínicas sobre cada caso, amostras biológicas de sangue e cabelo foram coletadas também às 20 semanas e passaram a fazer parte do biobanco da instituição para análise de marcadores metabolômicos.

4. Resultados

Os resultados estão apresentados na forma de artigos:

1. Mayrink J, Costa ML, Cecatti JG. Preeclampsia in 2018: revisiting concepts, physiopathology, and prediction. Accepted by *The Scientific World Journal*, 2018. (Anexo 3)

2. Mayrink J, Leite DF, Costa ML, Cecatti JG. Metabolomics for prediction of pregnancy hypertensive disorders: a systematic review and metanalysis protocol. Submitted to the *Systematic Reviews*, 2018. (Anexo 4)

3. Mayrink J, Leite DF, Costa ML, Cecatti JG. Prediction of hypertensive disorders in a look towards metabolomics technologies: a systematic review. Still to be submitted.

4. Mayrink J, Souza RT, Feitosa FE, Rocha Filho EA, Leite DF, Vettorazzi J, Calderon IM, Sousa MH, Costa ML, Cecatti JG, for the Preterm SAMBA study group. Incidence and risk factors for Preeclampsia in a cohort of healthy nulliparous pregnant women. Submitted to *BJOG*, 2018. (Anexo 5)

5. Mayrink J, Souza RT, Feitosa FE, Rocha Filho EA, Leite DF, Vettorazzi J, Calderon IM, Sousa MH, Costa ML, Cecatti JG, for the Preterm SAMBA study group. Mean arterial blood pressure: potential predictive tool for preeclampsia in a cohort of low-risk nulliparous pregnant women. Submitted to *Pregnancy Hypertension*, 2018. (Anexo 6)

4.1. Artigo 1

REVIEW ARTICLE

Preeclampsia in 2018: revisiting concepts, physiopathology, and prediction

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Abstract

Preeclampsia currently remains one of the leading causes of death and severe maternal morbidity. Although its prevalence is still underestimated in some places due to underreporting, preeclampsia is a disease that health professionals need to know how to deal with and take action. For this reason, the studies about the theme remain along with the advances in their understanding, that often implies improvement and change of concepts and conducts. The complexity of its etiology is a challenge and requires further studies for its full understanding. Apparently, poor adaptation of the maternal organism to the conceptus, marked by the non-occurrence of changes in the uterine spiral arteries, determines a series of systemic repercussions that compound the various forms of preeclampsia presentation. In recent years, the use of acetylsalicylic acid to prevent cases of early onset of the disease has been consolidated and, alongside, studies have advanced the development of accessible and effective methods of identifying women at risk of preeclampsia. The aim of this review is to discuss updates on the occurrence, concept, pathophysiology, repercussion, prevention, and prediction of preeclampsia.

Keywords: review, preeclampsia, gestational hypertension.

1. Introduction

According to some German authors, the first reports referring to eclampsia date from 2200 BC, observed in papyri of ancient Egypt [1]. The word eclampsia originates from the Greek, *éklampsis*, and means "bright light" [1]. For about 2000 years, eclampsia was understood as a disease characterized by convulsive seizures, typical of late gestation, that ended at the childbirth. Scientists from the late 19th century - true enthusiasts of caregiver empiricism - recognized the similarity between the swollen appearance of women who had seizures and the edema of Bright's disease, an abrupt glomerulonephritis onset characterized by proteinuria. Thereafter, urinary alterations in childbearing women with seizures were searched, which culminated in finding proteinuria in them. With the advent of non-invasive blood pressure measurement, it was observed that these women had increased blood pressure levels. It was not long before the understanding came that proteinuria and arterial hypertension preceded the onset of the seizures. Thus, it was defined the "preeclampsia" hypertensive condition, already understood at the time in its progressive character of severity and which could lead - and led in a grim way - to series of consequences for the maternal and fetal lives [1-3].

Even today, hypertensive conditions portray important complications during the gestation, with an incidence that varies according to the particularities of the population studied, and can exceed the mark of 10% in some regions **[4]**. Preeclampsia and eclampsia rank second or third in the world ranking of maternal morbidity and mortality causes [5]. In an analysis implemented by the World Health Organization, which evaluated the causes of maternal death occurred between 2003 and 2009, the hypertensive causes appear in the second place, occurring in 14% of the cases, preceded only by hemorrhagic causes, responsible for 27.1% of the maternal deaths [6].

Recently, *Abalos et al*., in a systematic review involving 40 countries with 39 million women, showed an estimated rate of preeclampsia and eclampsia of 4.6% and 1.4%, respectively; in Brazil, these numbers were 1.5% and 0.6%. The review mentioned countries where these numbers are not even known due to lack of official records, which makes it very difficult to recommend strategies for interventions that could contribute to better maternal and perinatal outcomes [7]. Despite the numbers presented, Brazilian data are still underestimated because there are unquestionable

regional differences in a continental sized country – as *Giordano et.al* have demonstrated in finding eclampsia prevalence of 0.2% in South and Southeast regions while in the North, Northeast, and Center-West regions, this proportion was of 8.1% [8].

Still regarding Brazil, according to data published in an analysis of 2016, in which more than 80,000 women (from the five regions of the country) were monitored for severe maternal morbidity, it was observed that the main cause of hospital admission was hypertension, making up 73% of that classified as maternal life-threatening condition, given the fact that the main cause of maternal death in the country is eclampsia. The mortality index (calculated by the ratio of maternal death cases to maternal near miss + maternal death) found at that time was 15.4%, which is considered an acceptable value (below 20%), but still very high, far from the ones observed in high-income countries (<2%) [9]. In the analysis of eclampsia cases, considering the same study of severe maternal morbidity, the prevalence of near-miss events (Near Miss, the most extreme form of severe maternal morbidity), including eclampsia, was of 4.2 cases per 1000 live births or 8.3 cases per maternal death [10]. It is important to highlight the use of the Near Miss indicator or severe acute maternal morbidity - which includes the cases of women who almost died from some Potentially Life-Threatening Condition (PLTC) during the pregnancy-puerperal period but, nevertheless, survived. This analysis is able to reflect more accurately on the quality of obstetric care if compared to the absolute number of maternal deaths, which by itself is already a rare event. For this reason, in the last decade, there was an increase in interest in this indicator, which culminated in the World Health Organization's initiative to standardize Maternal Near Miss case identification criteria, intending to facilitate the monitoring and planning of improvements in obstetric care [11].

In the last 50 years, there has been a decreasing trend in the incidence of these aggravations in high-income countries, alongside an opposite movement in middle and low-income countries, which is basically due to access to quality prenatal care as well as adequate management of cases of preeclampsia and eclampsia, with better maternal and perinatal outcomes [7, 12]. Broadening the analysis in a generalized way to other poor obstetric outcomes, in order to illustrate this discrepancy in numbers in middle and low-income countries, the risk of death due to maternal causes can reach up to 33 times higher than that observed in high-income countries [13]. As it has already been demonstrated, a number of more than 8 prenatal visits a protective factor for the

occurrence of preeclampsia [14]. An inquiry conducted in California in 2011, in order to investigate the increase in maternal deaths observed in that region in the early 2000s, revealed that 79% of preeclampsia related deaths were due to poor management [15, 16]. In other words, the improvement of maternal and fetal health outcomes is absolutely associated with the wide access to services and the quality care and management of the complications, translated into better perinatal outcomes.

2. Concepts and Classification

Hypertensive conditions during pregnancy can be classified as arterial hypertension prior to gestation or with manifestation before 20 weeks, and arterial hypertension starting at or after 20 weeks. The first group includes:

- ✓ Essential chronic or secondary arterial hypertension;
- ✓ White coat hypertension;
- ✓ "Masked" hypertension.

The hypertension group, that appears at 20 weeks or more, includes:

- ✓ Transient gestational hypertension;
- ✓ Gestational hypertension;
- ✓ Preeclampsia, which can be isolated or superposed on chronic hypertension**.** In this group, arterial hypertension is defined as systolic blood pressure equal to or greater than 140 mmHg and/or diastolic blood pressure equal to or greater than 90 mmHg, which should be measured on two distant occasions at least 4-6 hours apart, in a calibrated and adequate blood pressure monitor for the biotype of the woman under evaluation and managed by a trained professional [17]. When it comes to preeclampsia, one of the following conditions must coexist:

- Proteinuria (demonstrated by the ratio of proteinuria/creatininuria above 0.3 mg / mg, or by urine dipstick test equal to or above 1+, or by 24-hour proteinuria above 300mg / 24h);
- Dysfunctions of maternal organs which can be: renal insufficiency, characterized by creatinine above 1.02 mg / dL; hepatic impairment, characterized by an elevation of transaminases two times above normal levels, or pain in the right hypochondrium, or epigastralgia; neurological complications, characterized by

scotomas or persistent cephalgia accompanied by hyperreflexia or confusional states or eclampsia or cerebrovascular accident or amaurosis; and haematological complications consisting of thrombocytopenia or hemolysis;

- Uteroplacental dysfunctions: fetal growth restriction; changes in the Doppler velocimetry studies of the umbilical artery, especially if combined with alterations in uterine arteries [17, 18].

As it can be noticed, proteinuria is not a *sine qua non* condition to characterize preeclampsia, as previously ensued [19, 20]. According to the concept proposed by the International Society for Studies in Gestational Hypertension, published in 2014 and reinforced in 2018 [21], every hypertensive pregnant woman should be investigated for multiple organ involvement, even if presenting negative proteinuria, in order to discard the hypothesis of preeclampsia. This approach is innovative and tends more broadly to encompass cases that are somewhat neglected by the absence of proteinuria.

Preeclampsia may show signs of severity when systolic blood pressure levels are greater than 160mmHg and/or diastolic blood pressure levels are greater than 110mmHg, or when there is concomitance of eclampsia or HELLP syndrome. The last is defined as hemolysis, thrombocytopenia with a platelet count inferior to 150,000 and elevation of hepatic transaminases twice the upper limit of normality [18]. Massive proteinuria (above 5 grams in 24 hours) was no longer considered an isolated criterion of severity with the conceptual modifications proposed by the International Society for Studies on Gestational Hypertension, in 2014 [22], and should now be evaluated in consonance with the other clinical data and laboratory tests presented by the pregnant woman in question, mainly to decide the ideal moment of gestational interruption.

Regarding the moment of manifestation, preeclampsia is called early when it occurs before completed 34 weeks of gestation, and late after this gestational age. It may also be preterm when the onset occurs between 34 weeks and 1 day and 37 weeks, and at term preeclampsia when it occurs from 37 weeks and 1 day [18, 23]. The need of classifying preeclampsia is enforced as placental histological analysis of women with the aggravation showed that, in those with early onset, the vascular lesions were predominant and lower placental volume was evident, whereas larger placental volumes were more common in cases of manifestation after 37 weeks, with signs of chronic

inflammatory response [24]. In this sense, cases of early-onset preeclampsia would be more associated with placental insufficiency, and therefore, with fetal growth restriction. On the other hand, later-onset cases would present milder clinical conditions. In summary: different placental damages, distinct phenotypes [3, 25-27].

3. Pathophysiology

The pathophysiology of preeclampsia has not yet been fully elucidated. It is likely to be elapsed from a model composed of two interrelated stages: abnormal placentation and maternal inflammatory response [28, 29].

The process known as placentation is meticulously coordinated, and the equilibrium maintenance of the fetoplacental unit depends on its effective occurrence. In a normal pregnancy, there is a considerable increase in uterine blood flow in order to ensure adequate supplementation for the intervillous space and, by extension, adequate fetal development. In order to achieve this result, spiral arteries undergo a process of remodeling composed of 4 sequential steps promoted by a trophoblastic invasion of their walls. Initially, the decidua is invaded, followed by intra-arterial trophoblast migration, with a subsequent intramural invasion of the vessels, when there is loss of the middle (muscular) layer, replaced by fibrinoid material and connective tissue. The last step is that of vessel reendothelialization and other induced maternal adaptations [27, 30]. These vessels start to present a mean diameter - much higher than observed in uteri of non-pregnant women with low resistance to blood flow - and can thus provide intervillous space with adequate blood supply to maintain pregnancy effectively [31, 32] (Table 1). On the other hand, the radial and arched arteries will have increased blood pressure in their walls, resulting from the higher blood flow, which will act as a stress producer, and, ultimately, will generate nitric oxide secretion by the endothelium, determining an overall vasodilation of the uterine vessels [32]. The remodeling of the spiral arteries occurs more in the central part of the placental bed, reducing progressively towards the periphery [27].

This process of adaptation of the spiral arteries cannot occur ideally when approximately 90% of the vessels undergo the expected changes. In pathological cases, remodeling may be partial, completely absent, or even absent with obstructive vessel

lesions. In cases of preeclampsia, the proportion of remodeled vessels is found considerably reduced, especially in the central region of the placental bed. When associated with fetal growth restriction, there are obstructive lesions. In these cases, the arteries undergo a process of atherosis with very similar results to the formation of atheromatous plaques, with their lumens invaded by lipid-rich macrophages, perivascular mononuclear inflammatory infiltrate and fibrinoid necrosis of the vessel walls, with consequent utero-placental ischemia. Then, a vicious circle of ischemia and reperfusion in the intervillous space is installed, with the metabolic stress of the endoplasmic reticulum of the trophoblast cells, which are structures responsible for cellular homeostasis and, ultimately, for the apoptosis of the same. This process releases nanomolecules into the maternal circulation – which is capable of triggering a broad intravascular inflammatory response, an essential step for the development of preeclampsia [30] - as well as free radicals, in consequence of the extensive oxidative stress and the collapse of placental mechanisms and antioxidants enzymes [33]. It can be said that such a process is immuno-mediated**,** as it involves systemic inflammatory response and maternal genetic predisposition [31, 33, 34].

The aforementioned oxidative stress, as well as the cellular apoptosis, would be determinant for an imbalance between pro-angiogenic and anti-angiogenic factors, with a predominance of the latter [34]. Increased concentrations of VEGFR-1 (capable of blocking the angiogenic action of VEGF) and the soluble form of this vascular endothelial growth factor, sFlt-1(fms-like tyrosine kinase 1) – a potent antagonist of VEGF action - and decreased synthesis of placental growth factor (PlGF) are associated with the predominance of anti-angiogenic elements characteristic of preeclampsia [34, 35].

Finally, the understanding of the pathophysiology of preeclampsia - even though it is partial - includes the activation and consequent platelet consumption at levels above those observed in normal pregnancies, vasospasm and prostacyclin deficiency that have a vasodilatory action and inhibit platelet aggregation. Otherwise, the synthesis of thromboxane A2 is increased in placentas of women with preeclampsia, which determines the predominance of vasoconstriction and increased platelet aggregation [36], as well as thrombin, which has its maximum expression in disseminated intravascular coagulation, clinically translated to placental abruption [37]. Bigger synthesis of thrombin is part of a more vigorous inflammatory response characteristic of

preeclampsia, as already exposed above, and determines the deposition of fibrin in multiple organs, which reinforces the systemic character of the pathological condition.

4. Medium and long-term repercussions and prevention

The diagnosis of preeclampsia or eclampsia in a pregnant woman is followed by efforts involving the possible acute implications of these disorders (severe thrombocytopenia, disseminated intravascular coagulation, placental abruption, among others). However, it is also very important to look upon their long-term implications, which are innumerable and, sometimes, irreparable. After a pregnancy complicated by preeclampsia, about 20% of women will develop hypertension or microalbuminuria within seven years, and the same occurs with only 2% of women who have had pregnancies with no complications [5]. Similarly, the risk of acute myocardial infarction, stroke, and venous thromboembolism is substantially higher in women with a personal history of preeclampsia, as demonstrated in a meta-analysis published in 2007 [38].

In relation to newborns, the complexity lies in the decision of the moment when the risks in the intrauterine environment outweigh the risks of the extrauterine one. In this sense, prematurity and its innumerable consequences - such as acute respiratory syndrome, intraventricular hemorrhage, sepsis, bronchopulmonary dysplasia, and deficits in neuropsychomotor development - are some of the scenarios with which the infant born from a mother with preeclampsia (usually premature, before 34 weeks) will have to come across and, very frequently, against which they will have to fight [17]. Some studies have already shown a negative impact on the neurocognitive development of these infants evaluated in their first two years of life [39, 40].

Ultimately, mothers who have experienced near-death experience (near miss, the most extreme form of severe maternal morbidity) because of preeclampsia may also have psychological consequences with emotional impacts that involve anxiety, isolation, difficulties in breastfeeding, depressive disorders, and impairment of reproductive capacity, among others. The prolonged stays in intensive care units, either because of the woman herself or the newborn, and physical or mental limitations may interrupt the natural order of the symbiosis between mother and baby [41].

In this context of multiple and devastating consequences of preeclampsia, the need for its prevention emerges. It has already been demonstrated by a meta-analysis that early use (before the 16th week of pregnancy) of low-dose aspirin reduces the occurrence of preeclampsia, especially in its more severe forms (before 34 weeks) [42]. A recent clinical trial involving about 2,000 pregnant women compared aspirin and placebo use and observed a 62% reduction in the occurrence of early preeclampsia, in the group that daily took 150 mg aspirin [43]. Metformin has emerged as a target of studies in different groups of pregnant women, regarding its effects on preeclampsia risk [44]. Recently, a meta-analysis showed that compared to insulin, metformin decreased the risk of pregnancy-induced hypertension in a group of gestational diabetes women. On the other hand, when compared to placebo, metformin did not exhibit any beneficial effect regarding to preeclampsia [45, 46]. In parallel, Pravastatin has been pointed as a good option to prevent preeclampsia, although larger studies must be done with dose escalation to confirm its effectiveness [47, 48]. Thus, it is outlined the urgent need to discern, as early as possible, the pregnant woman who is at greater risk of presenting preeclampsia, ideally in its subclinical phase, so that it is possible to implement preventive measures. Similar to what has occurred in the last 20 years with the tracing and detection of aneuploidies, in which predictive models were applied in the first trimester in a much more effective way, an efficient and early predictor of preeclampsia is currently being searched within the new inverted pyramid proposal of prenatal care. This proposal suggests that efforts should be concentrated in the first trimester, in order to have an earlier diagnosis and therapeutic proposals established in the subclinical stages of the aggravations [49-51].

5. Prediction (Predicting Factors)

Considering the magnitude of the social and economic impact of preeclampsia, in addition to the evident clinical repercussions, there is a need to foresee this condition. Regarding to the time when it is possible to predict preeclampsia, although the tendency around the first trimester, some models have been proposed in later gestational ages, based on the fact that a huge proportion of pregnant women need to be reassessed in a late-second or early-third trimester. This proportion of pregnant women may develop preeclampsia after 32 weeks of gestation [52-54]. However, there is an undeniable

concentration of efforts in the first trimester. Over the years, biophysical and biochemical markers were identified as possible early indicators of failures in the complex placentation process, which would lead to preeclampsia. The first step would be to identify the risk factors for the occurrence of this condition.

The risk factors for preeclampsia have been listed as: having had preeclampsia in a previous gestation, being nulliparous, being at some age extreme (below 20 years old or above 40), and having African-American descent. Pre-existing pathological conditions such as chronic hypertension, diabetes mellitus, nephropathy, antiphospholipid antibody syndrome are also included in the list [18, 55], as well as BMI above 35 and use of assisted reproductive technologies [56, 57]. The latter is associated with a higher incidence of multiple pregnancies and with an increase in the average age of women in their first pregnancy, which together acts to increase the occurrence of preeclampsia [58, 59].

The *National Institute for Health and Clinical Excellence (NICE)* proposed, in a document published in 2010, a classification of the risk factors for preeclampsia as "moderate risk" and "high risk", so that it would make them tools capable of defining the group for which the immediate application of prophylactic measures would be indicated [60]. The following factors were classified as high risks:

- History of any hypertensive disorder in previous pregnancies;
- Chronic kidney disease;
- Autoimmune diseases such as Systemic Lupus Erythematosus or Antiphospholipid Antibody Syndrome;
- Diabetes type 1 or 2;
- Chronic Arterial Hypertension.

The following factors were considered as moderate risk factors:

- Primiparity;
- Women aged 40 years or older;
- Inter delivery interval greater than 10 years;
- Body Mass Index (BMI) greater than 35 kg / m^2 at the beginning of prenatal care;

- A family history of preeclampsia;
- Multiple gestations.

According to this institute (NICE), the presence of two moderate risk factors or a single high-risk factor would admit pregnant women to prophylactic measures (aspirin use before the 16th week of gestation and prenatal care in a specialized service) [43].

For the *American College of Obstetricians and Gynecologists* (ACOG), the risk factors are the same as those reported by the NICE institute [61], with exception of the BMI, here considered 30 kg / m^2 the value above which a woman would be at a high risk for preeclampsia. In addition, this institution does not recognize different scales for risk factors, thus they categorize them all under the same denomination of "high risk" [62].

A prospective study applying the concept proposed by the NICE institute to use risk factors as test predictors for preeclampsia obtained a detection rate of 37% and 28.9% of cases in early and late preeclampsia, respectively. It had a rate of 5% of false-positive and it studied a heterogeneous population, composed of nulliparous and multiparous [63]. In this same study, it was demonstrated that the clinical factor of greater predictive capacity was the previous history of preeclampsia. Evidently, this scenario does not favor the identification of nulliparous women at risk for preeclampsia, which constitutes a major limitation, since the incidence of this complication is higher in this group of pregnant women. Specifically for nulliparous women, a multicenter study conducted among more than 8,000 women with low-risk pregnancy demonstrated a 37% detection rate for preeclampsia using a predictor model composed exclusively by clinical factors [64], obesity and primiparity appeared as the main demographic predictor elements of early preeclampsia.

Blood pressure monitoring is part of a prenatal routine and may be the first clinical occurrence indicator of any hypertensive condition. Considering that the average blood pressure levels are high in pregnant women who will develop preeclampsia in the first or second trimester, or even who had already had it elevated before pregnancy [65, 66], this marker ceases to represent a tool for the diagnosis of hypertensive conditions and starts to act as an important predictor of preeclampsia. In a systematic review published in 2008, it has been demonstrated a higher accuracy of the

Mean Arterial Pressure (MAP) in the prediction of pre-eclampsia among low-risk pregnant women in the second trimester when compared to the isolated measurement of systolic and diastolic blood pressure. Among women at high risk for preeclampsia, it is the diastolic blood pressure above 75 mmHg that exhibits the greatest predictive capacity of the disease in question [67]. It is necessary to emphasize that the device used in the measurements must be specialized and validated to obtain blood pressure values, as well as it must be managed by a trained professional. Evaluated in a heterogeneous population composed by nulliparous and multiparous, the prediction rate of the MAP in early and late preeclampsia cases is 58% and 44%, respectively, with 5% of false positives [50].

The Doppler velocimetry study of the uterine arteries provides a non-invasive evaluation of the utero-placental circulation [50, 68]. As indicated by studies, this resource would be very useful to predict cases of early preeclampsia and, when applied in pregnant women who are considered to be at high risk, to evaluate the development of the disease [69]. When an increase in blood flow resistance in the uterine arteries at 23 weeks of gestation is detected, in a random and heterogeneous population of pregnant women, the sensitivity obtained was of 77.8% and the specificity was of 95% for early preeclampsia prediction [70]. On the other hand, the numbers are not encouraging when the cases of preeclampsia, in general, are evaluated (including those of late manifestation, which are the majority). In these cases, the sensitivity found was of 42.8%, which has no clinical applicability in the case of a screening test [70]. When the Doppler study was early performed in the uterine arteries, between 11 and 13 weeks, and analyzed in an isolated way, the detection rate of early and late preeclampsia is 59% and 40% respectively, with 5% of false-positive. It is necessary to emphasize that Doppler performance is entirely dependent on the availability of the ultrasound device (which is not always a reality depending on the considered clinical center), on the examiner and on his or her expertise and training, which restricts its use on a large scale [50]. Considering these variables and the scarcity of existing scientific evidence that showed the improvement in perinatal and maternal outcomes, the Doppler uterine artery study is not recommended as a screening test for preeclampsia among women considered at low risk due to their personal and clinical history [71, 72].

Innumerous biomarkers have been studied, as indicated in a recent systematic review that evaluates inflammatory evidence and their capacity to predict preeclampsia.

However, it is still not possible to elect one isolated factor that is sufficient for a prediction of this condition [73]. Table 1 lists some of the most important biomarkers and their accuracy in predicting preeclampsia occurrence, expressed in the area under the ROC curve (AUC). These biomarkers were evaluated before the 16th week of gestation.

It is noteworthy that, when analyzed specifically for cases of early manifestation (before 34 weeks), PAPP-A and PlGF demonstrated better results [74, 75], which draws our attention to the challenge that the preeclampsia prediction may represent, considering its phenotypes variety. Furthermore, the definition of normality parameters for these markers is influenced by the presence or absence of diabetes mellitus, parity (multiparous patients have lower PAPP-A values than nulliparous), twinhood (who have higher levels of PAPP-A and PIGF than those observed in single pregnancies), advanced maternal age (women over 35 years old presented lower values of the markers in question), among other elements [76].

Considering the complexity of the preeclampsia etiology, it is unlike that an isolated maternal factor will be able to predict this disease. Thus, the tendency worldwide is building algorithms, combining multiple factors. The results are sometimes robust. For instance, a model combining uterine artery Dopplervelocimetry, mean arterial blood pressure and PlGF reached a detection rate of 90% for early-onset cases of preeclampsia [77]. However, when the outcome analyzed is the late-onset cases – which is the vast majority of cases – these numbers keep modest.

For this reason, the need to search for other prediction ways that include different preeclampsia syndrome phenotypes, considering the fact that the cases of late manifestation are the most frequent [78], remains. In this scenario, omics technologies have been pointed as promising for the identification of early preeclampsia predictors, including a subclinical stage of the disease. Metabolomics is one of these technologies used for the metabolites´ identification, small molecules that represent the final line of gene expression and a phenotypic signature in high resolution of the disease desired to be studied [79, 80]. There have been efforts to seek a metabolic profile that may be associated with preeclampsia [78, 81-91], but no predictive models have been suggested so far that may have real clinical applicability or that have been validated in large populations. The elucidation of the metabolic profile of preeclampsia will be able to act

not only for prediction but also to provide a better understanding of the aggravation with regard to cellular and molecular mechanisms. Ultimately, it may contribute to a better understanding of the disease in all its nuances: prevention, diagnosis, and therapeutic conduction.

6. Conclusion

As has been said, preeclampsia is still one of the main causes of death and severe maternal morbidity. The complexity of its pathophysiology is a challenge for future studies and it may help with prevention measures and with the conduction of cases already defined as preeclampsia. Identifying risk groups for preeclampsia through accessible and effective technology, especially in developing countries, can result in better maternal and perinatal public health outcomes since prenatal care would be implemented prior to the establishment of the grievance. To that end, omics technologies have been further studied so that they can broaden the preeclampsia understanding as a whole and, particularly, its prediction.

Conflicts of interest

The authors have no conflicts of interest.

Funding Statement

This research was supported by Brazilian National Research Council (grant number 401636/2013-5) and Bill and Melinda Gates Foundation (grant number OPP1107597- Grand Challenges Brazil: Reducing the burden of preterm birth), which provided funding to PRETERM-SAMBA project (www.medscinet.com/samba).

Authors´ contributions

The idea of this review arose from a discussion among JM, MLC and JGC. JM drafted the first draft of the manuscript that was then amended and commented by MLC and JGC. All the three read and agreed on the content of the final version of the manuscript.

Ethical approval

This is a review article with no need for an ethical approval.

Consent

Not applicable.

9. References

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Table 1: Preeclampsia prediction potential of biomarkers and maternal characteristics

Biomarker	AUC (95%CI)	Reference
Maternal characteristics*	0.78 (0.71-085)	Goetzinger et al. [63]
PAPP-A	0.64 (0.57-0.72)	Goetzinger et al.
ADAM-12	0,58 (0.50-0.67)	Goetzinger et al.
Maternal characteristics +PAPP-A+ADAM-12	0.79 (0.71-0.86)	Goetzinger et al.
PlGF	0.61 (0.56-0.66)	Myatt et al. [54, 64]
PAPP-A	0.54 (0.49-0.59)	Myatt et al.
ADAM-12	0.58 (0.53-0.63)	Myatt et al.
Maternal characteristics+PlGF+PAPP-A+ADAM-12**	0.73 (0.69-0.77)	Myatt et al.
sFlt-1	0.54 (0.48-0.59)	Myatt et al.
PP13	0.51 (0.46-0.56)	Myatt et al.

*Considered maternal characteristics: African-American ethnicity, body mass index, pre-gestational diabetes mellitus; ** Considered maternal characteristics: African-American ethnicity, body mass index, systolic blood pressure and educational level; PAPP-A, pregnancy-associated plasma protein A; ADAM-12, A Disintegrin and Metalloprotease 12; PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase 1; PP13, placental protein 13; AUC, area under the curve.

4.2. Artigo 2

SYSTEMATIC REVIEW PROTOCOL

Metabolomics for prediction of pregnancy hypertensive disorders: a systematic review and metanalysis protocol

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Abstract

Background: hypertensive disorders are a very important cause of maternal morbidity and mortality worldwide, despite efforts on prevention. The lack of a tool to provide effective and early prediction of a high-risk group to develop hypertensive disorders may contribute to adverse maternal and fetal outcomes. Metabolomics has figured out as a promised technology to contribute to the improvement on pregnancy hypertensive disorders prediction. **Methods**: our primary outcome is hypertensive disorders of pregnancy. A detailed systematic literature search will be performed in electronic databases, using controlled terms 'preeclampsia', 'hypertensive disorders', 'metabolomics' and 'prediction' (and their variations). Studies from the latest twenty years will be included, except case reports, reviews, cross-sectional studies, letter to editors, expert opinions, commentaries papers or non-human research. If possible, we will perform a metanalysis. Two peer reviewers will independently perform the search and in cases of discordance a third reviewer will be consulted. **Discussion:** the results of this review will present the current use and performance of metabolomics for predicting gestational hypertensive disorders. Such data could potentially guide future studies and interventions to improve existing prediction models.

Key words

Preeclampsia, pregnancy, hypertension, hypertensive disorders, hypertensive syndromes, metabolomics, metabolome

Prospero register number: CRD42018097409

Background

Hypertensive disorders in pregnancy consist of a group of disorders that include preeclampsia, gestational hypertension, preeclampsia superimposed to chronic hypertension, white coat hypertension, masked hypertension and transient hypertension [1, 2] and appear as the second cause of maternal death in the world according to a study performed by the World Health Organization between 2003 and 2009 [3]. Preeclampsia is the leading cause of maternal morbidity and mortality in Brazil and in several low-and middle-income countries [4, 5]. Its prevalence can vary according to the set of analyses, but the number ranges from 2 to 10% of all pregnancies [4]. Every year, around 80 thousand women die because of preeclampsia and its complications [6], despite potential prevention implemented by low-dose aspirin [7]. This intervention can represent a reduction rate of around 50% in the incidence of the early-onset preeclampsia cases, which developed preeclampsia before 34 weeks of gestation [7, 8]. In this scenario, prediction of pregnant women under high-risk to develop preeclampsia is a key topic.

Some biomarkers have been proposed as earlier predictors (placental growth factor - PIGF, pregnancy-associated plasma protein A-PAPP-A) combined with clinical factors (pulsatility index of uterine arteries at Dopplervelocimetry exam, mean arterial blood pressure), showing different and sometimes conflicting detection rates [9-12]. These studies present limitations regarding the number of participants enrolled and heterogeneity to assess the prediction performance of those factors. Furthermore, the proposed prediction models from combining those factors outline better detection rates for early-onset preeclampsia cases compared to late-onset cases [13-15].

In the last decade, with the broad application of omics technologies, metabolomics has been identified as a promising tool for the identification of early predictors of preeclampsia. Through metabolomics, it would be possible to identify metabolites involved in the final line of gene expression and a phenotypic signature in high resolution of the disease to be studied [16-18]. Studies have provided some insights about preeclampsia prediction through metabolites, belonging to different chemical classes and showing different performances [16-19]. In 2010, Kenny et al provided the initial knowledge on the topic, identifying 14 metabolites belonging to different chemical classes. When combined in an algorithm they showed a very good performance, with an Area under the Curve (AUC) of 0.94 in a discovery phase of the study and a detection rate of 77% considering a false-positive rate of 10% [16]. It represents a very important tool option for prediction, especially with regard to cases of late-onset preeclampsia, which are the majority and the most difficult cases to predict [13-15]. Thus, the main objective of this systematic review is to determine the accuracy of metabolomics for predicting hypertensive disorders.

Methods and Analysis

Question formulation

In view of the social and economic implication of hypertensive disorders, their consequences to maternal and fetal lives worldwide and the lack of a useful screening test, in parallel to the increase of applicability of omics technologies, this systematic review will be guided by this question: what is the performance of metabolomics for predicting gestational hypertensive disorders?

Search Strategy

Electronic searches of literature will be carried out with these following databases: PubMed, EMBASE, Web of Knowledge, Latin America and Caribbean Health Sciences Literature (LILACS), Scientific Electronic Library Online (Scielo), Health Technology Assessment (HTA), Database of Abstracts of Reviews of Effects (DARE). We will include studies from the latest twenty years. Our search strategy will combine terms with Boolean connectors related to the following categories: 1) hypertensive disorders, preeclampsia, pregnancy; 2) metabolomics, metabolome; and 3) screening, prediction. In addition, we will search reference list of included articles. There will be no language restrictions. Before final publication, we will perform a new search in the databases in order to check if any study was published during the period of the systematic review elaboration.

Study selection process

After searching all sources of databases cited above, all the citations will be exported into EndNote® software. Firstly, two reviewers (JM and DFBL) will independently assess titles and abstracts. Only papers considered potentially relevant according to the inclusion criteria will be retrieved for further consideration. Cases of divergence will be analyzed by a third reviewer (MLC) who will do the final decision. A fourth reviewer (JGC) will check all procedures before approving the data extraction.

Study inclusion criteria

Hypertensive disorders developed at any gestational age will be considered the domain studied. Original studies including pregnant women are the inclusion criteria, and congenital malformation is the exclusion criteria.

Interventions/exposure

Prediction of hypertensive disorders through metabolomics technologies is the intervention to be studied. The biomarker analysis should have been performed on samples taken before the hypertensive disorder diagnosis.

Design

Our systematic review will include original studies (cohort or case control studies), including single or multiple pregnancies, as the studied population, and hypertensive disorders developed at any time of pregnancy, as the outcome of interest. We will exclude any studies that are: cross-sectional studies, case reports, editorials, letter to editors, commentaries, expert opinions, any type of reviews, and experimental studies with animals, and when it is not possible to extract the data about the outcomes of interest.

Outcomes

We will include studies reporting outcomes of any hypertensive disorder developed during pregnancy. Our primary outcome is preeclampsia, defined as the onset of hypertension (a systolic blood pressure of 140 mmHg or more and/or a diastolic blood pressure of 90 mmHg or more) after 20 weeks of gestation, measured at least in two different occasions, combined with: (1) proteinuria (300 mg/day or at least 1g/L [1+] on dipstick testing or spot urine protein/creatinine>30mg/mmol [0.3mg/mg]) or (2) systemic complications or (3) uteroplacental dysfunction (fetal growth restriction) [1]. By systemic complications, we will consider:

- Hematological complications (thrombocytopenia- platelet count below 150,000/dL, disseminated intravascular coagulation, hemolysis);
- Hepatic disfunction (elevated transaminases – at least twice upper limit of normal +- right upper quadrant or epigastric abdominal pain);

- Neurological dysfunction (examples include eclampsia, altered mental status, blindness, stroke or more commonly hyperreflexia when accompanied by clonus, severe headaches when accompanied by hyperreflexia, persistent visual scotomata;
- Renal dysfunction (creatinine > 1.2mg/dL);

Secondary outcomes include:

- Early-onset preeclampsia: when occurs before or at 33 weeks of gestation [20];
- Late-onset preeclampsia: when occurs at or after 34 weeks of gestation [20].
- Gestational hypertension: de novo development of high blood pressure after 20 weeks of gestation (a systolic blood pressure of 140 mmHg or more and/or a diastolic blood pressure of 90 mmHg or more), without any of the abnormalities that define preeclampsia as discussed above [1]
- White coat hypertension: it is demonstrated when a normal blood pressure is registered during 24 hours ambulatory monitoring in the first half of pregnancy [1].
- Preeclampsia superimposed on chronic hypertension: in a patient with high blood pressure predating the pregnancy, it is registered the occurrence of preeclampsia [1].
- Masked hypertension: is characterized by blood pressure that is normal at office or clinic but elevated at other times, most typically diagnosed by 24 hours ambulatory blood pressure monitoring [2].

- Transient gestational hypertension: is hypertension that arises in the second or third trimester. The hypertension is detected in the clinic but then settles with repeated blood pressure readings [2].

Data extraction

Data will be extracted through a standardized data compilation form in duplicate to avoid errors. The variables of interest from each included study are: authors, country, year of publication, study design, number of participants, preeclampsia prevalence, gestational age of recruitment, biological samples utilized, laboratory methods, metabolomics technology applied and metabolites. The metabolites will be matched with the Human Metabolome Database (HMDB) in order to check their biological function and chemical subclass. Missing data will be requested from study authors. Pairs of data-extraction forms will be checked for discrepancies.

Study quality assessment

The same two reviewers (JM and DFBL) who judged eligibility of papers will independently assess the risk of bias in included studies, but this time rating the methodological quality of the primary research. A third reviewer (MLC) will solve divergences when needed. Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) is the standard scale to be applied to access internal validity [21]. This tool is composed by four domains: patient selection, index test (metabolomics technique), reference standard (arterial blood pressure) and flow and timing of patient inclusion and follow up. Each domain is assessed in terms of risk of bias and the first three are assessed in terms of concerns regarding applicability. For each domain, every study will be labelled as “low”, “high” or “unclear” risk of bias.

Strategy for data synthesis

In accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA), a flow diagram will be drawn [22]. Tables will show data regarding studies characteristics and risk of bias assessment. Narrative data will be analyzed and structured according to the outcomes: preeclampsia, gestational hypertension, transient gestational hypertension, white coat hypertension, masked hypertension. If possible, we are going to perform a subgroup analysis according to the metabolomics methods applied: gas or liquid chromatography, coupled with mass spectrometry, or proton nuclear magnetic resonance. A meta-analysis will be performed (hierarchical summary receiver characteristic operating curve, HSROC) and accuracy measures will be calculated depending on data availability. Heterogeneity will also be assessed, through l-square test.

Discussion

Prediction of hypertensive disorders has been studied over the years with specific challenges. Among nulliparous for example, there is no history of previous events and a previous history of preeclampsia, is considered the most consistent predictive risk factor [23]. Another challenge to overcome is regarding to late-onset preeclampsia cases, which represent the majority of them. As cited above, the algorithms composed by biochemical and clinical factors showed better results with early onset cases of preeclampsia [13, 14].

Metabolomics is a very complex technology and it has emerged as a possibility for prediction of adverse pregnancy outcomes [24-26]. The techniques employed are nuclear magnetic resonance spectroscopy, gas or liquid chromatography-mass

spectrometry, Fourier transform infrared spectrometry and capillary electrophoresis [26]. Because of this complexity, results may be different concerning to the metabolites found. Consequently, generalizing results is also a challenge to overcome. This systematic review will contribute to optimize the knowledge about the metabolites found in the studies and perhaps classify them according to HMDB, enabling quality translational research.

In addition, this systematic review will contribute to establish the current state of knowledge concerning the capacity of metabolomics to predict the occurrence of preeclampsia. Taking into account that this outcome involves relevant consequences for maternal and neonatal lives, the development of a tool that would predict preeclampsia is essential. Furthermore, the results of this systematic review could be used to guide future studies in this field.

List of abbreviations

PlGF- placental growth factor

PAPP-A- pregnancy-associated plasma protein A

AUC- area under the curve

LILACS- Latin America and Caribbean Health Sciences Literature

Scielo- Scientific Electronic Library Online

HTA- Health Technology Assessment

DARE- Database of Abstracts of Reviews of Effects

HMDB- Human Metabolome Database

QUADAS-2- Quality Assessment of Diagnostic Accuracy Studies

PRISMA- Preferred Reporting Items for Systematic Reviews and Meta-Analysis

HSROC- Hierarchical summary receiver characteristic operating curve

Declarations

Acknowledgements

This study is financed by Bill and Melinda Gates Foundation.

Competing interests

The author(s) declare that they have not competing interests.

Author's contributions

JM worked out the protocol, developed searches and data management, will participate in selection, inclusion, quality assessment and data extraction of papers. DFBL helped working out the protocol and will participate in selection, inclusion, quality assessment and data extraction as well. MLC and JGC helped working out the protocol, and MLC will solve any disagreement concerning to the selected papers. JGC will supervise all the development of the systematic review. All authors read and approved this final manuscript.

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4.3. Artigo 3

The systematic review, to be submitted for publication.

SYSTEMATIC REVIEW

Prediction of pregnancy-related hypertensive disorders in a look towards metabolomics technologies: a systematic review and meta-analysis

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Abstract

Objective: to determine the accuracy of metabolomics in predicting hypertensive disorders.

Design: systematic review of data on test accuracy.

Data sources: PubMed, EMBASE, Scopus, Web of Knowledge, Latin America and Caribbean Health Sciences Literature (LILACS), Scientific Electronic Library Online (Scielo), Health Technology Assessment (HTA) and Database of Abstracts of Reviews of Effects (DARE).

Review method: two independent reviewers selected English articles on metabolomics techniques applied to predict hypertensive disorders during pregnancy. 2x2 tables were constructed with data on study characteristics, main results, and accuracy of metabolite models.

Results: Among 4597 potentially relevant citations on metabolomics applied to predict hypertensive disorders during pregnancy, 22 articles were selected to compound this review: 15 case control studies and 7 observational studies. Metabolomics was applied in the first half of pregnancy, and identified remarkable differences in metabolome profile between hypertensive (usually preeclampsia) patients and a normotensive group. These differences are related to amino acids, lipids, vitamin D, hydrogen peroxide, endocrine disruptors, among others.

Conclusion: metabolomics, the newest omics technology, seems to represent an efficient tool to predict preeclampsia. Nevertheless, it is mandatory to validate the method in larger studies with a heterogeneous population.

Introduction

Preeclampsia was defined as a harmful outcome in pregnant women more than 2,000 years before Christ. It is a potentially-life threatening condition to the mother and baby (1). A high prevalence rate of preeclampsia persists, ranging from 3 to 10%, according to the region studied. As a result, the race is on to identify the group of pregnant women at high-risk for the disease, candidates for prophylactic measures.

Basically, clinicians now manage clinical features, such as new onset of hypertension and end-organ dysfunction including proteinuria, leading to the cascade of events culminating in preeclampsia. Several biomarkers have been studied as predictive tools for preeclampsia, e.g. soluble fms-like tyrosine kinase-1, soluble endoglin, markers of apoptosis and inflammation, placental protein 13, C-reactive protein, and markers of placental hypoxia and distress (2). However, none are sufficiently sensitive or specific to predict preeclampsia in early pregnancy. Research aimed at identifying the group of pregnant women at high risk of developing preeclampsia is essential to increase surveillance and promote preventive interventions for these women.

Recently, novel technologies, such as Metabolomics have been applied to predict preeclampsia. Metabolomics is known as the newest member of the “omics” family. The technology has been applied to improve disease biomarkers and understand the pathogenesis of preeclampsia. Metabolome - a collection of metabolites – is defined and used in research to pursue the phenotypic signature of a disease of interest. Metabolites are low-molecular-weight chemicals (<1500 Da) resulting from changes in gene and protein expression (3). Metabolomics methods include nuclear magnetic resonance spectroscopy (NMR) and gas- and liquid- chromatography-mass spectrometry (GC- and LC-MS) (3). Preeclampsia has a complex etiology and is considered a multifactorial disease. Metabolomics, therefore, a high throughput technique seems perfectly adequate, since it can simultaneously encompass a wide range of metabolic pathways. The objective of this systematic review was to determine the accuracy of metabolomics in predicting hypertensive disorders.

Methods

This systematic review was based on our previously published protocol (Mayrink et al., 2018), included in the international prospective register of systematic reviews (PROSPERO number CRD42018097409). The research question in our review was: how is the performance of metabolomics in predicting gestational hypertensive disorders? This systematic review was conducted, in accordance with the preferred reported items for systematic reviews and meta-analysis protocols (PRISMA-P) (4).

Search strategy

We performed an electronic search in PubMed, EMBASE, Scopus, Web of Knowledge, Latin America and Caribbean Health Sciences Literature (LILACS), Scientific Electronic Library Online (Scielo), Health Technology Assessment (HTA), and Database of Abstracts of Reviews of Effects (DARE). Search strategy consists of terms related to preeclampsia, metabolomics and prediction, along with their variations and combined with Boolean operators (AND and OR). **Table 1** summarizes the terms applied in our search. Articles were restricted to the English language and only case control and cohort studies were included. Letters to the editor, editorials, comments, expert opinions, any type of review, experimental animal studies, Congress supplements case reports, random trials, and cross-sectional studies were all excluded.

Trained reviewers screened titles and abstracts for relevance (JM and DFL). Full papers were analyzed to be included or excluded (JM and DFL) when needed. Disagreements were solved by a third reviewer (MLC). Article quality was assessed by QUADAS (Quality Assessment of Diagnostic Accuracy Studies) criteria (5).

The domain of interest was any form of hypertensive disorder developed during pregnancy, in single or multiple gestations.

Outcomes

Preeclampsia was the primary outcome. Reference standards for preeclampsia included hypertension established after 20 weeks of gestation (systolic blood pressure of 140 mmHg or higher and/or diastolic blood pressure of 90 mmHg or higher, at two

different occasions at least 4 hours apart), combined with proteinuria or systemic complications or uteroplacental dysfunction (6, 7).

Proteinuria is defined as 300 mg or more of protein excreted in urine per day, or at least 1+ (1g/L) on dipstick testing, or spot urine protein/creatinine>30mg/mmol (0.3mg/mg) (6, 7). Systemic complications include hematological, hepatic or neurological or renal dysfunction (detailed in our previously published protocol) (Mayrink et al., 2018) or even uteroplacental dysfunction indicating the presence of fetal growth restriction (6, 7).

Secondary outcomes were early-onset preeclampsia, characterized by the onset of preeclampsia at or before 34 weeks of gestation; late-onset preeclampsia, after 34+1 weeks of gestation; gestational hypertension (hypertension is the only clinical finding); preeclampsia superimposed on chronic hypertension (arterial hypertension diagnosed before pregnancy); white coat hypertension (normal blood pressure recorded in a 24h ambulatory monitoring); masked hypertension (characterized as normal blood pressure in the office or clinic and altered under other circumstances) and transient gestational hypertension (higher blood pressure at levels during a certain period that is later maintained after repeated blood pressure readings) (6, 7).

Interventions

Metabolomics was the intervention studied. Biomarker analyses of samples drawn prior to diagnosis of hypertensive disorder were included in the study. We excluded articles in which blood samples were collected after diagnosis of hypertension was established.

Results

Figure 1 summarizes the flow chart of studies included in the review. Overall, there were 22 studies: 15 case control studies and 7 observational cohort studies. One study described gestational hypertension. There are no data on multiple pregnancies. Two studies included only nulliparous pregnant women. Thirteen studies reported lipidomic analysis and/or amino acid analysis. Six studies described vitamin D analysis. One was a report on caffeine and two studied BPA (bisphenol A). Twenty-one studies analyzed

blood samples and two studies analyzed urine samples (one study analyzed both, urine and blood). Table 2 summarizes studies that were included, containing the following information: name of the first author, year of publication, country where study was performed, number of participants, biological specimen, parity, type of metabolomics method, list of metabolites and direction of metabolites compared with control. **Table 3** additional information on accuracy of the metabolomics model.

Study summary

Bahado-Singh et al. applied metabolomics to predict both types of preeclampsia, early- and late-onset cases (8, 9). Using blood sample analysis during the first trimester of pregnancy, basically between 11 and 13+6 weeks, those authors reinforced the existence of multiple phenotypes for preeclampsia, already cited by other authors (8, 10). In 2015, arginine and 2-hydroxybutyrate were identified as predictors of early-onset preeclampsia, which is plausible, given their relation with oxidative stress and strong association with preeclampsia (11). In a previous study of part of the same population, the authors identified four metabolites with distinct concentrations between cases and controls: citrate, hydroxyvalerate, methionine and glycerol. In a model with these metabolites, accuracy in the Area Under the Curve was 0.904 (0.828-0.98) (9). Then, a population of pregnant women was assessed for late-onset preeclampsia by those authors. In 2016, carnitine, pyruvate and acetone resulted in an accuracy of 0.629 (Area under the Curve). When combined in a model with uterine Doppler velocimetry and maternal weight, this value increased to 0.734 (12). Years before, with part of the same participants enrolled in this study, those authors had identified 17 metabolites capable of discriminating cases of late-onset preeclampsia from controls (8). Finally, the group conducted a two-step evaluation in the first and third trimester. It was a recent attempt to predict onset of preeclampsia after 37 weeks, which accounts for the vast majority of cases. A combination of metabolomics and proteomics technologies, in a serial model of metabolomics from the first and third trimester, yielded an AUC of 0.817 (13).

Studies on vitamin D deficiency in preeclampsia cases have demonstrated conflicting results. Baker et al. observed vitamin D deficiency in a group of preeclampsia patients,

with about 4-fold odds of developing severe preeclampsia, in comparison to a group with sufficient vitamin D (14). In a nested case-control study Wetta et al. (15) found no difference between vitamin D status, when comparing preeclampsia cases with onset of disease before 37 weeks of gestation and controls. Two years later, Ates et al observed similar results, this time in a prospective study, demonstrating a lack of relationship between vitamin D deficiency and adverse pregnancy outcomes, such as preeclampsia (16). On the other hand, a study from 2011 showed lower 25OH vitamin D levels in women with severe preeclampsia, measured at the beginning of the second trimester. In this study, a model combining 25OHD, VEGF and sFLT-1/PlGF ratio showed an Area under the curve of 0.851, a higher value than either marker alone (17). Recently, vitamin D levels were evaluated in a group of nulliparous pregnant women, analyzed by a urine and blood metabolomic approach. There were no differences in metabolite concentrations, when serum samples from preeclampsia patients were compared to those of normotensive women. In contrast, changes in urine metabolome concentrations were observed. Hypertensive pregnant women had lower concentrations of urinary 25(OH)D3 and 24,25(OH)2D3 (18).

A longitudinal study of multiple variables involving different methods used metabolomics to measure 8-epi prostaglandin F2aphfa (PGF2alpha), a marker of lipid peroxidation. According to the authors, the accuracy of a marker in predicting preeclampsia at 20 or 24 weeks of gestation was modest (19), although in the preeclampsia group, levels of the marker tended to increase.

In a study by Rijvers et al, a consistent decrease in L-arginine was found between 16 and 20 weeks of gestation in a preeclampsia group, compared to gestational hypertension cases. This can be clinically relevant, since L-arginine supplementation is possible as highlighted by some authors. Nevertheless, further large randomized control trials are still necessary (20). Khalil et al found lower levels of L-arginine and L-homoarginine in a group of women with earlier development of preeclampsia and unchanged levels in late-onset preeclampsia patients, compared to normotensive pregnant women. Even after the exclusion of women with chronic hypertension, findings were similar (21). The difference identified in this study is relevant and reinforces the complex etiology of preeclampsia. Rijvers et al. showed lower levels of

SMDA and L- citrulline in a pre-pregnancy period. This can indicate that a vasodilator endothelial function–or dysfunction--may be established before pregnancy.

Endocrine disrupting chemicals (EDC) have been studied as possible contributors to the pathogenesis of preeclampsia (22). Ye Y et al. described bisphenol A (BPA) as a potential candidate. According to these authors, high BPA levels (> 4.4 microg/L) exhibited an OR of 17.12 (CI 5.87-49.9). The accuracy of this metabolite was 0.73 (area under the curve) (23). Another analysis of the external factors that may contribute to the occurrence of preeclampsia was made by Eichelberger et al. who studied caffeine and its main by-product, paraxanthine (24). Those authors found no difference in concentration levels of paraxanthine and caffeine between severe preeclampsia and normotensive pregnant groups. The ratio of both groups, however, showed a statistically significant difference, with higher values observed in the normotensive group.

Another study that used metabolomics, conducted a target investigation separately in early-onset and late-onset cases of preeclampsia (25). Lower levels of taurine and asparagine in early-onset preeclampsia cases were found, compared to controls and lower glycylglycine levels in late-onset preeclampsia cases (25).

In a study carried out by Koster et al., the metabolite stearoylcarnitine was shown to be a common biomarker for both early-onset and late-onset preeclampsia. Added to the model, it improved the detection rate by 45% and 21% for early and late-onset cases of preeclampsia, respectively (26). A model composed of prior risk, MAP and stearoylcarnitine reached higher detection rates than a model that combined prior risk, MAP, PlGF and PAPP-A for the detection of late-onset preeclampsia. It is important to find a common marker between early-onset and late-onset preeclampsia, considering the feasibility of general screening. Nevertheless, throughout the study the difference between subtypes could be reaffirmed by the identification of different metabolites.

In 2010, Kenny et al conducted an untargeted study of nulliparous pregnant women exclusively, identifying a list of different metabolite levels between cases and controls (27). A study by Odibo et al. showed that 4 metabolites were able to discriminate

between preeclampsia cases and controls, with an accuracy of 0.82, after adjusting for potential confounders (28).

A study by Austdal et al retrieved from search in the database for this systematic review was the only one conducting an isolated investigation of gestational hypertension (29). Furthermore, it was one of the two studies that analyzed urine. Metabolites identified in cases of preeclampsia and gestational hypertension were virtually the same. Urine citrate was decreased in cases that later developed gestational hypertension. Austdal et al. carried out an analysis during the first trimester. A study by Diaz et al. was the second study analyzing urine metabolomes during the second trimester (30). This might explain the differences found. The study by Diaz indicated decreased levels of acetate, formate, fumarate (all involved with the Krebs cycle), succinate, 2-Oxoglutarate and isoleucine (30)). Both studies showed increased levels of 4- Deoxythreonic acid, a degradation product of 3-hydroxybutyrate.

Discussion

Preeclampsia is a complex syndrome involving multiple biological pathways. It is currently possible to hypothesize that preeclampsia is a result of a confluence of disturbances, some preceding the clinical manifestation of the disease (31). Spiral artery remodeling process, angiogenic and anti-angiogenic factors, hypoxia-reoxygenation balance, and endothelial dysfunction are some integrating elements of the sequence resulting in a complex and life-threatening syndrome (32). It is reasonable, therefore, to apply a holistic approach to systems biology such as metabolomics to identify the metabolic signature of this disease and predict its subsequent development (3).

<u>Vitamin D</u>

In the last decade, many studies have been conducted to clarify the relationship between maternal vitamin D status and pregnancy outcomes, achieving contradictory results (33, 34). Metabolomics technology – liquid chromatography-mass spectrometry method -- is very reliable and considered the gold standard for the measurement of this metabolite. Traditionally, this represents an advantage. The deficiency of its active

metabolite 1, 25-dihydroxyvitamin D, according to some hypothesis, exhibits diverse functions in the pathophysiology of preeclampsia: abnormal placental implantation, angiogenesis, excessive inflammation, immune dysfunction (35). According to some studies, there is an increase in inflammatory cytokine levels, such as TNF-alpha, and a tendency towards T-cell activity (36). It is fundamental to clarify the relationship between vitamin D levels and the occurrence of preeclampsia, considering that it is a directly modifiable behavioral factor.

Lipids

Maternal serum lipids are markedly elevated in healthy pregnancies, probably a hormone- induced increase. Preeclampsia patients may have even higher levels (37, 38). Lipidome – the group of lipid metabolites - contains key mediators of vascular tone (sphingosine phosphatase), inflammation (prostaglandins), insulin sensitivity (free fatty acids), and liver function (lipoproteins). Lipids are definitely involved in the etiology of preeclampsia either indirectly, as a substrate for another key factor for the disease, or directly, as a disease mediator (38).

Sphingolipids and sphingolipid-related proteins have been related to important factors involved in preeclampsia. Sphingosine-1-phosphate and ceramide are two major sphingolipids; low levels of the former (angiogenic) protein and high levels of the latter (pro-apoptotic) protein were found in serum analyses of third-semester preeclampsia cases. When fatty acid oxidation is defective, increased levels of acylcarnitine can be observed. It has been proposed that oxidized cholesterol contributes to atherosclerosis and vascular disease, as well as atherosclerotic disease. Oxidized cholesterol results from the exposure of phosphocoline to reactive oxygen species, which are increased in preeclampsia cases (39).

Glycerophosphocholines resulted from cell lysis, probably correlated with apoptosis described in preeclampsia. For the propanoate pathway, cases of late-onset preeclampsia showed higher concentrations of L- valine, 2-hydroxybutyric acid, lactic acid, acetone and propan-2-ol (12). Glycerol has been regarded as an important biomarker of late-onset preeclampsia. The relationship of glycerol with abnormal lipid metabolism and maternal obesity has been reinforced (12, 40). Decreased glycerol

levels in cases of late-onset preeclampsia may be due to its conversion to triglycerides (38, 41).

Amino acids

Changes in amino acid concentrations were shown. Preeclampsia patients had decreased concentrations of taurine, asparagine and glycylglycine. Taurine can be found in mammalian tissues. Its function is associated with cytoprotection, involving the placentation process, including the mechanism controlling spiral artery remodeling. Nevertheless, a reduction in taurine activity may be associated with impaired placentation, which is correlated with the pathophysiology of preeclampsia (25). Glycylglycine is closely associated with homocysteine levels and participates in the metabolic pathway that antagonizes homocysteine. Homocysteine is linked to cardiovascular risk and endothelial dysfunction. Lower glycine levels promote higher homocysteine levels. Interestingly, this metabolite was reduced in the late-onset preeclampsia group, included in the metabolic changes that are closely associated with the pathology of preeclampsia (25).

Changes in concentrations of glutamate and alanine have been shown to be related to ischemia, a feature of preeclampsia. Ischemia is responsible for increasing glutamate uptake and alanine release (42). Valine, leucine, isoleucine and pyruvic acid were increased among late-onset cases of preeclampsia (12). Leucine plays an important role in fetal nutrition. Impaired placentation contributes to reduce amino acid transport across the placenta. Indeed, a reduction in L-arginine and L-homoarginine was observed (21) among preeclampsia patients. These are substrates for oxide nitric synthesis, a potent vasodilator with a major function in endothelial cells. Asymmetric dimethylarginine (ADMA) is an inhibitor of the enzyme responsible for NO production. NO is an important regulator of trophoblast implantation, differentiation, motility, invasion and apoptosis (21). Decreased levels of arginine and homoarginine in blood samples of preeclampsia patients investigated by metabolomics analysis, reinforces the involvement of NO molecule in the etiology of the disorder (11, 21). Furthermore, there is evidence that elevated ADMA levels are associated with endothelial dysfunction, and consequently, preeclampsia (20, 43).

Carnitine is a non-essential amino acid that is closely associated with lipid metabolism. Its function is to bind to fatty acid, form acyl-carnitine and shuttle it to mitochondria, to integrate the mitochondrial oxidative metabolism (12).

Hydrogen Peroxide (H2O2)

Hydrogen peroxide is a member of the reactive oxygen species family (44). We hypothesize that this metabolite contributes to vasoconstriction observed in preeclampsia cases and to a decrease in vasodilator factors such as nitric oxide. Arginases are responsible for nitric oxide metabolism. Thus, factors that improve the activity of arginase may contribute to reduce NO. H2O2 appears to have a similar action (44).

BPA and phthalates

BPA and phtalates are widely present in many products of daily life. BPA is found in water bottles, metal coating, flooring materials and thermal papers (23). Phthalates are used as plasticizers, including medical material and food processing equipment (22, 45). It is hypothesized that BPA can cross the placenta and induce degeneration and necrosis of placental cells, disturbing the process of angiogenesis (45). Phthalates can affect placental gene expression determining decreased placental growth (45). Furthermore, phthalates may also cause a pro-inflammatory response and increase oxidative stress (22). All those processes have been associated with the etiology of preeclampsia. It was possible to show a higher BPA concentration in urine samples and placental tissues of pregnant women diagnosed with preeclampsia at the time of delivery, applying metabolomics. BPA had a very good accuracy as a predictor of disease, when measured between 16 and 20 weeks of gestation (23, 45). Knowledge of the actual contribution of BPA, phthalates and other endocrine disruptors to the etiology of preeclampsia is absolutely relevant, given that behavioural attitude can change maternal exposure to these risk factors.

Caffeine

Paraxanthine (1,7 dimethylxanthine) is the primary metabolite of dietary caffeine that represents almost 80% of caffeine by-product. Caffeine metabolism occurs in the

cytochrome P450 1A2 system, producing paraxanthine. The activity of this cytochrome can be demonstrated by the ratio of paraxanthine/caffeine. A study by Eichelberger showed a decreased risk of severe preeclampsia with increasing paraxanthine/caffeine ratio in a logistic regression analysis. However, the absolute concentration levels of caffeine and paraxanthine did not exhibit any difference. These findings suggested that faster metabolism rather than absolute caffeine consumption may be associated with a lower risk of severe preeclampsia. This is not exactly in accordance with others studies, which indicated a direct influence of caffeine levels on the risk of preeclampsia and failed to perform analysis of caffeine metabolism (14). The mechanism by which the metabolic rate may influence the risk for preeclampsia remains unknown.

Urine Metabolome

Due to non-invasiveness of the technique, the development of study research focused on urine metabolome has been encouraged (46). Two studies in this field have been selected. One study implemented during the first trimester, identified major metabolic differences: decreased urinary hippurate and increased urinary creatinine (47). Hippurate is produced in the gut microflora and its excretion may increase with fruit intake. Thus, decreased urinary hippurate in pregnant women with preeclampsia may be associated with a healthier diet in a normotensive group. Another study also confirmed the protective role of a healthier diet related to the occurrence of preeclampsia (48). This is very meaningful, since dietary habits can be modified. Dimethylamine is another metabolite that is increased in urine samples of pregnant women with preeclampsia. It is derived from asymmetric dimethylarginine, a biomarker of cardiovascular risk, indicated since the last decade (49).

Conclusion

As it is possible to conclude, scientific literature provides researches of a list of various different metabolites. An explanation for this wide variety can be the active state flux exhibited by samples, and also the use of different platforms (nuclear resonance magnetic versus mass spectrometry) (28, 52). When metabolomics models are compared with previously established methods, for instance, PAA-P and PP-13 measured in the first trimester of pregnancy, the accuracy is even better: a 14-

metabolite model approach exhibited 0.92 of Area Under the Curve, whereas the comparative value for placental hormones was 0.878 (50, 51). Furthermore, metabolomics results illustrate an important attribute of this technology: the capacity to generate credible hypotheses on the mechanism and causation of complex disorders. The complexity of preeclampsia is advantageous to metabolomics. However, this technology is far from achieving feasibility. On this purpose, it is mandatory to validate the method in larger studies and with a heterogeneous population.

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Figure 1: Flow chart of study selection

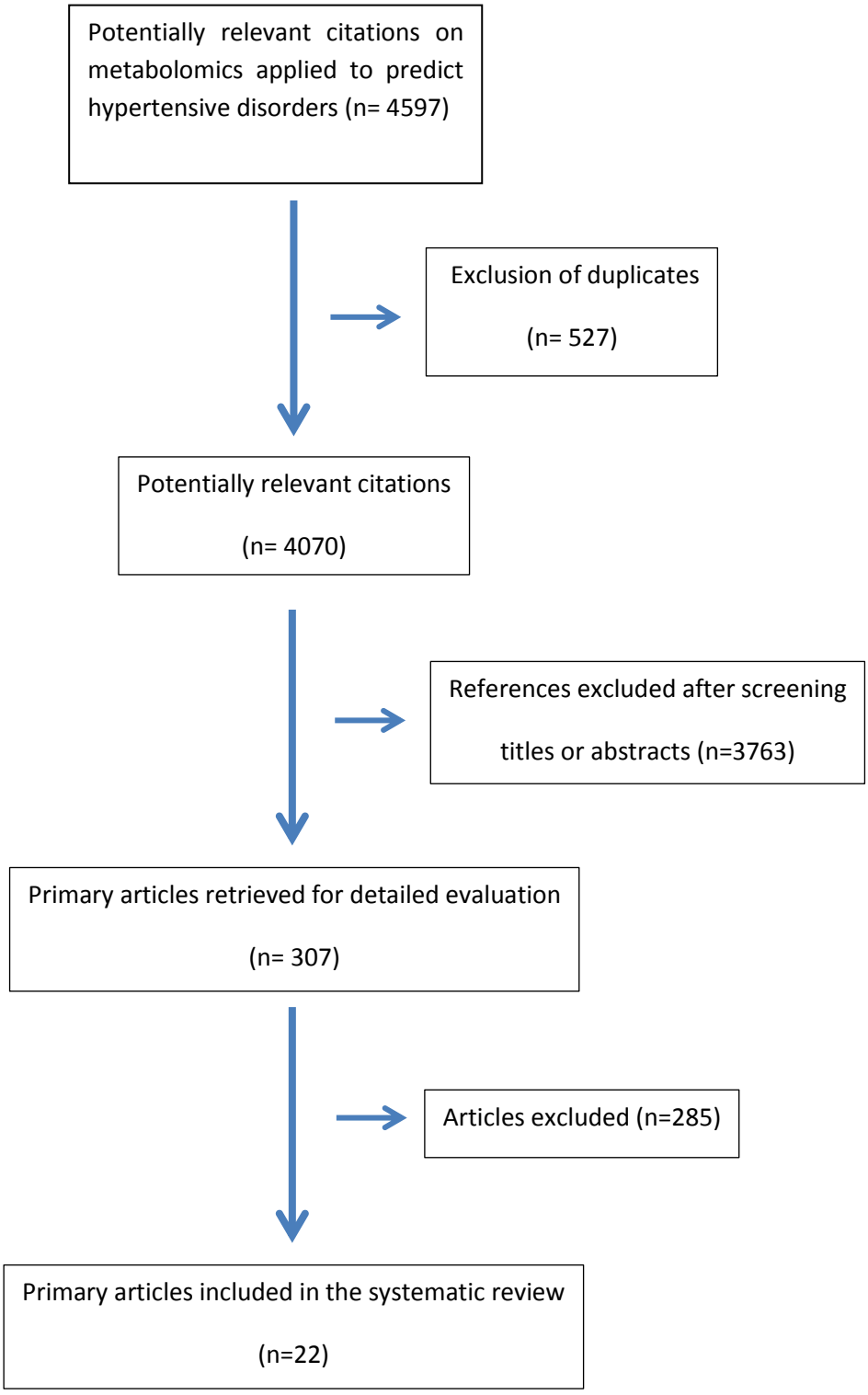

Table 1: Search strategy

Preeclampsia	Preeclampsia OR pre-eclampsia OR eclampsia OR gestational hypertension OR white coat hypertension OR severe preeclampsia OR late preeclampsia OR early preeclampsia OR pregnancy-induced hypertension OR hypertensive syndromes of pregnancy
AND	
Metabolomics	Metabolomic*OR metabonomic* OR metabolit* OR HNMR OR proton NMR OR proton nuclear magnetic resonance OR liquid chromatogra* OR gas chromatogra* OR UPLC OR HPLC OR high pressure liquid chromatograph* OR ultra-performance liquid chromatograph* OR ultra performance liquid chromatograph* OR lipidomic* OR mass spectrometr*
AND	
Prediction	Screen*OR predict*OR profil*

Table 2. Studies of metabolomics included for prediction of hypertensive diseases of pregnancy with their respective characteristics and findings

Study	Author, year, country	Type of study Parity	Sample taken (weeks)	Body fluid	List of Metabolites	Change X control	Number of Participants
Study 1	Kuc S. et al, 2014, Netherlands	Nested case-control UPLC-MS/MS LC-MS	8-13+6 N+M	Blood (serum)	Taurine EO Asparagine EO Glycylglycine LO Oxylipins	Decreased Decreased Decreased No changes	Controls- 500 EO PE- 68 LO PE- 99
Study 2	Eichelberger K. Y. et al, 2015, USA	Nested case-control LC-MS	15-20 N+M	Blood (serum)	Caffeine Paraxanthine Paraxanthine/caffeine	No changes No changes Decreased	Controls- 87 PE (severe)- 25
Study 3	Koster M. P. H. et al, 2015, Netherlands	Nested case-control UPLC-MS/MS	8-13+6 N+M	Blood (serum)	**EO-PE:** Stearoylcarnitine Isobutyrylcarnitine Hexanoylcarnitine Octanoylcarnitine Decenoylcarnitine Decanoylcarnitine Dodecenoylcarnitine Lauroylcarnitine Tetradecenoylcarnitine Hexadecenoylcarnitine Octenoylcarnitine Linoleylcarnitine **LO-PE** Palmitoylcarnitine Oleylcarnitine Stearoylcarnitine Linoleylcarnitine Tetradecenoylcarnitine Dodecenoylcarnitine	 Decreased Decreased Increased Increased Increased Increased Increased Increased Increased Increased Increased Decreased Decreased Decreased Decreased Decreased Increased Increased	Controls- 500 EO-PE- 68 LO-PE- 99

					Decenoylcarnitine Decanoylcarnitine Octanoylcarnitine Octenoylcarnitine Hexanoylcarnitine	Increased Increased Increased Increased Increased	
Study 4	Kenny, L. C. et al, 2010, Australia, New Zealand	Nested case-control UPLC-MS	14-16 Nulliparous Late-onset cases	Blood (plasma)	5-Hydroxytryptophan Monosaccharide(s) Decanoylcarnitine Methylglutaric acid/or adipic acid Oleic acid Docosahexaenoic acid/or docosatriynoic Acid γ-Butyrolactone and/or oxolan-3-one 2-Oxovaleric acid/or oxomethylbutanoic acid Acetoacetic acid Hexadecenoyleicosatetraenoylsnglycerol Di-(octadecadienoyl)-snglycerol Sphingosine 1-phosphate Sphinganine 1-phosphate Vitamin D3 derivatives	Decreased Increased Increased Decreased Increased Increased Increased Increased Increased Increased Increased Increased Increased Increased	<u>Discovery</u> Controls 60 PE- 60 <u>Validation</u> Controls- 40 PE- 39
Study 5	Baker A. M. et al., 2010, USA	Nested case-control LC-MS	15-20 N+M	Blood (serum)	25 (OH) D	Decreased	Controls- 198 PE- 43
Study 6	Odibo A. O. et al, 2011, USA	Nested case-control LC-MS	11-14 N+M	Blood (serum)	Hydroxyisovalerylcarnitine Hydroxymethylbutyrylcarnitine Arginine Hydroxyproline	Increased Increased Increased Increased	Controls- 41 Preeclampsia -41
Study 7	Khalil A. A. et al, 2013 UK	Case-control GC-MS GC-MS/MS	11-13+6 N+M	Blood (plasma)	ADMA (asymmetric dimethylarginine) L-arginine L-homoarginine	No changes/ No changes Decreased / NC Decreased /NC	Controls: 300 EO-PE: 25 LO-PE: 50
Study 8	Austdal M. et al, 2015 Norwey	Prospective H- NMR	11-13+6 N+M	Blood (serum) Urine	<u>Blood</u> <u>*PE*</u> Triglycerides 3-hydroxybutyrate Pyruvate Lactate Phosphatidylcholine <u>*Gestational Hypertension*</u> Triglycerides HDL Lactate N-acetyl glycoproteins Glucose Phosphatidylcholine	 Increased Increased Decreased Decreased Decreased Increased Decreased Decreased Decreased Decreased Decreased	Controls- 552 PE- 26 GH- 21
					<u>Urine</u> <u>*PE:*</u> Creatinine Glycine 4-deoxythreonic acid α-hydroxyisobutyrate Histidine Dimethylamine Hippurate Lactate Proline betaine <u>*Gestational Hypertension*</u> Creatinine	 Increased Increased Increased Increased Increased Increased Decreased Decreased Decreased Increased	

					Glycine 4-deoxythreonic acid α-hydroxyisobutyrate Histidine Dimethylamine Hippurate Lactate Proline betaine Urinary citrate excretion	Increased Increased Increased Increased Increased Decreased Decreased Decreased Decreased	
Study 9	Bahado-Singh et al, 2015, UK	Prospective NMR spectrometry	11-13+6 N+M	Blood (serum)	2hydroxybutyrate 3hydroxyisovalerate acetone Citrate Glycerol	Decreased Increased Decreased Increased	EO- 50 Controls- 108 Discovery (60%)—30x65 Validation (40%)—20x43
Study 10	Ates S et al, 2016, Turkey	Prospective LC-MS/MS	11-14 N+M	Blood (serum)	25OHD2 25OHD3	Decreased No changes	229 participants 4 PE
Study 11	Bahado-Singh et al, 2017, UK	Prospective NMR spectrometry	11-13+6 N+M	Blood (serum)	Carnitine Pyruvate Acetone Citrate Dimethylamine Valine Leucine Methylhistidine 3- Hydroxybutyrate 2-hydroxybutyric acid Lactic acid Propan-2-ol	Increased Increased Increased Increased Decreased Increased Increased Increased Increased Increased Increased Increased	Controls- 115 PE-LO- 59
Study 12	Tamblyn J. A et al, 2018 UK	Prospective LC-MS/MS	15 N	Blood (serum) and urine	<u>Blood:</u> 25(OH)D3 25(OH)D2 3-epi-25OHD3 1,25(OH)2D3 24,25(OH)2D3 <u>Urine:</u> 25(OH)D3 24,25(OH)2D3	 No changes No changes No changes No changes No changes Decreased Decreased	Controls-25 PE-25
Study 13	Diaz S. O. et al, 2013 Portugal	Prospective H-NMR	14-26 weeks N+M	Urine	Acetate Formate Fumarate Succinate 2-Oxoglutarate Isoleucine	Decreased Decreased Decreased Decreased Decreased Decreased	Controls= 84 Pre-PE (31-37)=9
Study 14	Bahado-Singh et al., 2017 UK	Case-control NMR spectrometry LC-MS/MS	11-13+6 and 32-33+6 N+M	Blood (serum)	<u>First trimester:</u> Glucose Putrescine Urea PCaaC40:6 Dimethyl sulfone <u>Third trimester:</u> Serotonin T4-OH-proline Hexose Acetic Acid Dimethyl sulfone	 Decreased Decreased Decreased Increased Decreased Decreased Decreased Decreased Increased Increased	Controls- 63 PE (>=37 weeks)- 35
Study	Bodnar LM et	Case-cohort	≤26	Blood	25OHD2	No	Controls-

15	al, 2014 USA	LC-MS	N+M	(serum)	25OHD3	difference, after controlling for confundiers	3068 PE- 717
Study 16	Dobierzewska, A. et al., 2017 Santiago, Chile	Prospective HPLC-MS	11-14 22-24 32-36 N+M	Blood (plasma)	Sphingosine-1-phosphate Ceramide Sphingomyelin	No changes across gestation	Controls- 7 PE- 7
Study 17	Wetta et al., 2014 USA	Nested case-control LC-MS	15-21 N+M	Blood (serum)	25OHD2 25OHD3	In general, no difference	Controls- 177 PE<37- 89
Study 18	Woodham PC et al, 2011, USA	Nested case-control LC-MS	15-20 N+M	Blood (serum)	25OHD2 25OHD3	Decreased	Controls- 123 Severe PE&- 41
Study 19	Chappell L et al, 2012, UK	Case-control GC-MS	20-36 N+M	Blood (serum)	8-epi prostaglandin F2alpha	Increased	Controls-21 PE-17
Study 20	Rijvers, CAH et al, 2013 Netherlands	Retrospective longitudinal LC-MS/MS	prepregnancy, 12, 16 and 20 N+M	Blood (plasma)	ADMA (asymmetric dimethylarginine) SDMA (an inert ADMA isomer) L-arginine L-citrulline L-arginine/ADMA	No change Decreased (PP) No change Decreased (PP) Increased (12) + decreased (20)	Controls-18 PE/GH- 17 (6 PE)
Study 21	Ye Y et al, 2017 China	Nested Case control LC-MS	**entre 13 e 20 semanas** 16-20 N+M	Blood (serum)	Bisphenol A (BPA)	Increased	Controls- 99 PE - 74
Study 22	Cantowine DE et al, 2016 USA	Nested case-control Chromatography and MS	Visits 1: 10wks 2: 18wks 3:26 wks 4: 35 wks N+M	Urine	Bisphenol A (BPA) 9 Phathalates (MEHP)	Increased Increased	Controls- 432 PE-50

Table 3

Study	Metabolites	Results
Study 1	EO- Taurine, asparagine LO- glycylglycine	Model: EO-PE: prior risk+ MAP+ taurine AUC 0.93 DR: 88% FPR 10% LO-PE: none metabolite improved DR
Study 2	Paraxanthine Caffeine Paraxanthine/caffeine	• Paraxanthine cases X controls= 96.4ng/mL x 38.0 ng/mL (p=.12) • Ratio cases X controls=0.37 x 0.23 (p=.02)
Study 3	Stearoylcarnitine	Model: ***PAM + prior risk + stearoylcarnitine*** EO- DR 50% (25-70%) AUC 0.747 LO- DR 29% (8-46%) AUC 0.692
Study 4	Metabolite Classes Discovery: acyl glycines, amino acids, amino ketones, bile acids, carbohydrates, carnitines, discarboxylic acid, eicosanoids, fatty acids, keto or hydroxy FA, lipids, phosphatidylserines, phospholipids, porphyrins, steroids and steroids derivates Validation: amino acids, amino ketones, bile acids, carbohydrates, carnitines, discarboxylic acid, eicosanoids, fatty acids, keto or hydroxy FA, lipids, phosphatidylserines, phospholipids, steroids and steroids derivates.	Discovery: AUC .96 with DR 77% (45 metabolites) Validation: AUC .95 with DR 73% (34 metabolites) Discovery: AUC .94 with DR 71% (14 metabolites) Validation: AUC .92 with DR 68% (14 metabolites) FPR 5%
Study 5	25 (OH) D	Cases x controls: 26 x 10% (rate of vit D deficiency) OR 3.63 (1.52-8.65)
Study 6	Phenylalanine + Alanine + Glutamate + C6OH (hydroxyhexanoylcarnitine) Phenylalanine + Alanine + Glutamate	DR 50% FPR 10% ; AUC 0.82 (all cases) AUC 0.81
Study 7	L- arginine L- homoarginine ADMA (Asymmetric Dimethylarginine)	EO-PE: L-arginine, L-hormoaginine: levels were lower compared to normal (p 0.02 and 0.006); the ratios of ADMA/L-arginine and ADMA/ L- homoarginine were higher (p 0.003 0.012); LO-PE: there were no differences
Study 8	Model for urine: ***PE*** Creatinine Glycine 4-deoxythreonic acid α-hydroxyisobutyrate Histidine Dimethylamine Hippurate Lactate Proline betaine	***PE x no-PE*** S: 67.5% Sp: 74.2% AUC: 70.8%

	Gestational Hypertension Creatinine Glycine 4-deoxythreonic acid α-hydroxyisobutyrate Histidine Dimethylamine Hippurate Lactate Proline betaine Urinary citrate excretion Model for blood ***PE*** Triglycerides 3-hydroxybutyrate Pyruvate Lactate Phosphatidylcholine ***Gestational Hypertension*** Triglycerides HDL Lactate N-acetyl glycoproteins Glucose Phosphatidylcholine	***Gestational Hypertension x no-Gestational Hypertension*** S: 38.3% Sp: 89.3% AUC: 63.8% ***PE x no-PE*** S: 63.8% Sp: 65.4% AUC: 64.6% ***Gestational Hypertension x no-Gestational Hypertension*** S: 76.9% Sp: 55.0% AUC: 66.1%
Study 9	Model 1: 2-hydroxybutyrate, 3-hydroxyisovalerate, acetone, citrate, and glycerol. Model 2: 3-hydroxyisovalerate, arginine, glycerol+ UtPI	<u>Discovery:</u> Model 1: S: 82.5% Spec: 82.3% AUC: 0.896 (0.862-0.929) Model 2: S: 90.8% Spec: 90.8% AUC: 0.956 (0.938-0.975) <u>Validation:</u> Model 1: S: 75% Spec: 74.4% AUC: 0.835 (0.769-0.941) Model 2: S: 90% Spec: 88.4% AUC: 0.916 (0.836-0.996)
Study 10	25OHD2 + 25OHD3= total	229→4 PE (1.7%) No difference regarding to vitamin D status p=1 229→ 11 GH (4.8%) No difference regarding to vitamin D status p=1
Study 11	Model with carnitine, pyruvate,acetone	AUC 0.629 (0.49-0.767) S: 30,4% Spec: 80,4%
Study 14	<u>First trimester model:</u> Carnitine Putrescine Urea <u>Third trimester model:</u> Methylhistidine Serotonin Citrate Hexose Propylene Glycol	AUC: 0.701 (0.589-0.814) S: 72.7% Spec:57.4% AUC: 0.761 (0.648-0.875) S: 74.2% Spec: 72.3% Combined first and third: AUC:0.817 (0.732-0.902)
Study 15	25OHD	IncPE: 2.6% No difference after controlling for confunders
Study	Sphingosine-1-phosphate (S1P)	Did Not Change across gestation

16	Ceramide Sphingomyelin	Elevated through gestation in control/ Cer 14 was significantly decreased in PE group at 1 tri (p 0.009) SM 16 and 18 were lower in PE group at 1 trimester (p 0.007 and 0.002)
Study 17	25OHD	No difference regarding to vitamin D status p 0.46
Study 18	25OHD sFLT-1/PlGF ratio VEGF 25OHD + VEGF 25OHD + sFLT-1/PLGF 25OHD + VEGF + sFLT-1/PlGF	AUC: 0.745 AUC: 0.669 AUC: 0.677 AUC: 0.787 AUC: 0.834 AUC: 0.851
Study 19	8-epi prostaglandin F2alpha	AUC: 0.62 (0.44-0.81) (at 20 weeks) AUC: 0.55 (0.35-0.75) (at 24 weeks)
Study 21	BPA	PE (incidence) = 2.66% Cases x Controls 3.4 x 1.5microg/L p<0.01 AUC: 0.73 (0.65-0.81)
Study 22	BPA MEHP	HR 1.58 (1.20-2.08) HR 1.55 (1.14-212)

4.4. Artigo 4

CLINICAL ARTICLE

Incidence and risk factors for Preeclampsia in a cohort of healthy nulliparous pregnant women: a nested case-control study

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Running title: clinical risk factors for predicting preeclampsia

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Abstract

Objective: to determine the incidence, socio-demographic and clinical risk factors for preeclampsia and associated maternal and perinatal adverse outcomes.

Design: nested case-control derived from the multicentre cohort study Preterm SAMBA.

Setting: five different centres in Brazil

Population: nulliparous low-risk pregnant women

Methods: clinical data were prospectively collected, and risk factors were assessed comparatively between PE cases and controls using RR (95%CI) plus multivariate analysis.

Main Outcome Measures: Preeclampsia incidence, risk factors, maternal and fetal outcomes

Results: complete data were available for 1,165 participants. The incidence of preeclampsia was 7.5%. Body mass index during first medical visit and diastolic blood pressure over 75 mmHg at 20 weeks of gestation were independently associated with the occurrence of preeclampsia. Women with preeclampsia presented a higher incidence of adverse maternal outcomes, including C-section (3.5 fold), preterm birth below 34 weeks of gestation (3.9 fold) and hospital stay longer than 5 days (5.8 fold) than controls. They also had worse perinatal outcomes, including lower birthweight (a mean 379g lower), small for gestational age babies (RR 2.45 [1.52-3.95]), 5-minute Apgar score less than 7 (RR 2.11 [1.03-4.29]), NICU admission (RR 3.34 [1.61-6.9]) and Neonatal Near Miss (3.65 [1.78-7.49]).

Conclusions: The incidence of PE in this study was 7.5%. Weight gain rate per week, obesity and diastolic blood pressure equal to or higher than 75mmHg at 20 weeks of gestation was shown to be associated with preeclampsia. Preeclampsia also led to a higher number of C-sections and prolonged hospital admission, in addition to worse neonatal outcomes.

Funding: Bill and Melinda Gates Foundation (grant **OPP1107597**) and CNPq (grant 401636/2013-5).

Keywords: preeclampsia: clinical research; risk management; epidemiology: general obstetric.

Tweetable abstract: preeclampsia in nulliparous women is associated with maternal weight and diastolic blood pressure at 20 weeks

Introduction

Preeclampsia is considered an important cause of maternal mortality and severe maternal morbidity. [1] For every woman who dies, it is estimated that around 20 other women suffer from severe morbidity and disability. [2,3] In view of the social and economic implications of this condition, great effort has been made to expeditiously prevent, diagnose and treat preeclampsia. [4-7]

The magnitude of the problem in some places across the world is still not fully known, especially in low and middle-income countries. In particular, the actual incidence of preeclampsia remains largely unknown (8). There is usually suboptimal reporting of the disease, leading to constraints on public health applicability (9). Another important aspect is identifying pregnant women at risk of developing preeclampsia, especially in the nulliparous group.[3] From clinical risk factors to omics technology, there is still currently no single good predictor of preeclampsia. [10-15]

Therefore, clinical factors remain an inexpensive and rapid way to predict the occurrence of preeclampsia. Nevertheless, the lack of data from nulliparous women generates the need to take a careful look at this particular group of women. This current study intends to evaluate the incidence of preeclampsia and its sub-phenotypes (early-onset and late-onset), socio-demographic and clinical risk factors for preeclampsia, as well as assess their ability to predict this disorder in a cohort of healthy nulliparous Brazilian pregnant women.

Methods

This is a nested case-control study derived from a secondary analysis of the Preterm-SAMBA study (Preterm Screening and Metabolomics in Brazil and Auckland), a

prospective multicentre cohort study conducted in five Brazilian centres between July 2015 and March 2018. The research protocol was previously published elsewhere. [16] Briefly, the original study design was based on the primary goal of developing a predictive model for preterm birth. The study was developed in two phases: a discovery phase and a validation phase. The first phase was a case-control study, involving participants from the previously described SCOPE study.[3] In the validation phase, this model was validated in the Preterm SAMBA Brazilian cohort. Other maternal and perinatal outcomes of great interest were considered in the secondary objectives such as preeclampsia (currently addressed), gestational diabetes mellitus and fetal growth restriction. For this nested case-control approach, cases were women who developed preeclampsia and controls were all the remaining women free from the disorder. Preterm-SAMBA study was conducted according to Declaration of Helsinki guidelines. Appropriate approval was obtained from the five centres involved in the study. All recruited participants gave their written informed consent.

Participants

The study enrolled low-risk nulliparous pregnant women between 19 and 20+6 weeks of gestation, with a singleton pregnancy, from five different centres in Brazil (from Campinas, Botucatu, Recife, Fortaleza and Porto Alegre). Exclusion criteria were: 3 or more previous abortions; cervical suture; fetal malformation; chronic hypertension requiring antihypertensive drugs and/or diabetes and/or renal disease; arterial blood pressure higher than 160x100 mmHg at the time of enrolment; Systemic Lupus Erythematosus and/or antiphospholipid syndrome; sickle cell disease; HIV infection; congenital uterine anomalies (bicornuate uterus, septate uterus); previous cervical knife

cone biopsy; chronic exposure to corticosteroids or calcium at a dosage above 1g or fish oil at a dosage above 2.7g per day or vitamin C above 1000mg per day or vitamin E above 400UI per day; heparin or aspirin use (any dosage or presentation form).

Sample size estimation

Sample size was calculated according to the primary outcome - preterm birth. Assuming a type I error of 5% and accuracy of the test of at least 0.68 according to the area under the ROC curve, and to test the hypotheses with adequate power (80% of power, $\beta = 0.2$), the sample size would need to approach 80 cases of preterm delivery. The minimum expected prevalence of this outcome was presumed to be 7% in Brazil, therefore the sample size was calculated at 1150 women. In addition, considering that the mean prevalence of preeclampsia is about 8-9%[17] among Brazilian nulliparous women, this sample would be able to identify around 92 to 103 cases of preeclampsia.

Procedures

All steps of the main study have been previously described [16]. Data were collected at three different set points (visits) during follow-up. On the first visit, between 19 and 21 weeks of gestation, a full assessment was performed to gather information on sociodemographic characteristics, reproductive family history, current or previous diseases, personal habits, with a complete follow-up until delivery and immediate postpartum period. During the interview, data were entered into a central database with internet access and complete audit trail (MedSciNet). Anthropometric measurements plus nutritional assessment were also performed. The same evaluation was conducted on both subsequent visits, at 27-29 weeks of gestation and at 37-39 weeks of gestation.

Outcome

The outcome of interest for the current analyses is preeclampsia. In this study, preeclampsia was defined as the occurrence of hypertension (SBP≥140 and/or DBP>90mmHg) in at least two different time periods, combined with proteinuria (300 mg/24hour or at least 1g/L [2+] on dipstick testing or spot urine protein/creatinine >30mg/mmol [0.3mg/mg]). Preeclampsia was also classified as early-onset when diagnosed before 34 weeks of gestation and as late-onset otherwise. In the absence of proteinuria, the disorder was also defined as the occurrence of any systemic complications/organ dysfunction [18] including:

- Haematological complications (thrombocytopenia - platelet count below 100,000/dL, DIC, haemolysis);
- Hepatic dysfunction (elevated liver enzymes – at least twice the upper limit of normal + right upper quadrant or epigastric abdominal pain);
- Neurological dysfunction (eclampsia, altered mental status, blindness, stroke, hyperreflexia with clonus, severe headaches, visual scotomata when persistent);
- Renal dysfunction (creatinine > 1.2mg/dL);

Statistical analysis

We determined the general incidence of preeclampsia and early-onset and late-onset preeclampsia. Several socio-demographic, clinical factors and lifestyle habits were regarded as potential risk factors. Furthermore, maternal and neonatal outcomes associated with preeclampsia were addressed. Bivariate analysis was performed, estimating the Risk Ratios (RR) and their respective 95% Confidence Intervals, using

Student's t, qui-square or Fisher's exact tests accordingly. Finally, a multivariate analysis with a Poisson regression model was performed to identify which factors were independently associated with preeclampsia in this sample, estimating the adjusted RR for those identified. Each centre/hospital was considered as a Primary Sampling Unit (PSU) in every analysis. SPSS software version 20.0 and Stata software version 7.0 were used for analysis.

Funding

This is study was jointly funded by the Bill and Melinda Gates Foundation (grant **OPP1107597**) and CNPq (grant 401636/2013-5). The funders played no role in study development, data collection, data analysis or data interpretation.

Results:

Among 1,373 participants screened for eligibility in the Preterm SAMBA study, complete pregnancy outcome data were available for 1,165 women (Figure 1). Preeclampsia developed in 87 (7.5%) participants of whom 14 (16.1%) had early-onset preeclampsia while the remaining 73 were late-onset. The socio-demographic characteristics of women who developed preeclampsia and controls are shown in Table 1. Among patient characteristics, the rate of weight gain per week equal to or more than 0.75 kg, obesity (BMI > 30.9 Kg/m^2) and diastolic blood pressure equal to or higher than 75 mmHg at 20 weeks of gestation were shown to increase more than twice the risk of preeclampsia (Table 1).

Maternal and neonatal outcomes in preeclampsia were worse for both the mothers and their neonates (Table 2). The threshold for caesarean section among women with preeclampsia was 3.58, while hospital admission for 5 days or more was almost 6-fold

higher. Women with preeclampsia had more preterm births at less than 34 weeks of gestation (3.97 fold) than controls. Neonates of women with preeclampsia had a significantly lower birthweight (a mean of 379g lower), and there was a twofold to threefold higher occurrence of small for gestational age (SGA) babies, 5-minute Apgar scores less than 7, NICU admission and Neonatal Near Miss events. There was only one case of fetal death, occurring in a 26-year old woman, at 26 weeks of gestation. She was admitted to hospital, complaining of a headache. Arterial blood pressure was 170 x 110 mmHg, protein in dipstick urinalysis was +3 and no fetal heart beats were identified. The induction of labour lasted 24 hours, resulting in vaginal delivery of a baby weighing 620g.

On multivariate analysis, diastolic blood pressure at 20 weeks of gestation and BMI at enrolment were independently associated with the occurrence of preeclampsia, with an adjusted risk ratio of 1.04 (Table 3).

Discussion

Main Findings

Our study revealed that the incidence of preeclampsia was 7.5% in a nulliparous group of low-risk pregnant women from three different Brazilian regions, which is higher than values obtained from other cohorts of nulliparous pregnant women.[19-21] Current analysis was able to identify only three factors significantly associated with the development of preeclampsia: weight gain rate per week, obesity and value of diastolic blood pressure measured at 20 weeks of gestation equal to or higher than 75 mmHg. The low number of preeclampsia cases in this sample probably prevented us from identifying additional factors, limiting the capacity to predict preeclampsia by using a composition of factors. Not surprisingly, our findings on perinatal outcomes added support to other studies,

showing more frequently preterm births, neonatal near miss, 5-minute Apgar score less than 7 and low birth weight in obstetric complications such as preeclampsia. [22, 23]

Strengths and Limitations

To the best of our knowledge, this was the first time that a Brazilian cohort of low-risk nulliparous pregnant women received follow-up with data acquisition on preeclampsia incidence. The manner of calculating the rate of weight gain was a limitation of our analysis. Patients were recruited from 19 to 21 weeks of gestation to the last measurement at the end of prenatal care. Potential bias – reverse causality – may occur, since after preeclampsia is diagnosed, weight is influenced by oedema, typical for this disease. Another limiting characteristic is that the database does not have information on the precise time when antihypertensive drugs were initiated if used.

Interpretation

In our cohort, data on the actual incidence of preeclampsia was totally distinct from findings of a systematic review published in 2008 showing a prevalence of 1.5% for preeclampsia and 0.6% for eclampsia. According to those authors, their numbers were underestimated in some regions due to lack of information. [8] Almost 10 years later, a study implemented in Brazil showed that the prevalence of preeclampsia was 8.1% in specific regions. [24] Our study currently revealed that the incidence of preeclampsia is 7.5% in a nulliparous group of low-risk pregnant women, which is higher than values obtained from other cohorts. [19-21] The high prevalence of obesity in our population may explain the incidence of preeclampsia. Despite the lack of data available on this topic in our country, most recently a cross-sectional study involving 1,279 pregnant women

showed that the prevalence of overweight or obese women was almost 40% during the first prenatal visit. [25]

Owing to the high incidence of preeclampsia and considering its impact, [2, 26, 27] it is essential to find an effective tool that provides early identification of pregnant women at high risk for this disease. The aim is to implement prophylactic measures and avoid harmful consequences. Thus, a permanent search for a model with widespread global application has begun, considering the results achieved by studies using low-dose aspirin as a prophylactic measure. [28, 29] However, the prediction of preeclampsia is challenging, in view of the complexity of its aetiology. [30] It is unlikely that a single risk factor is able to predict the occurrence of this condition.

Maternal clinical factors have emerged as an interesting screening alternative. In 2010, a guideline of the National Collaborating Centre for Women's and Children's Health recommended the applicability of maternal clinical factors as screening tests**.** According to the guideline, a previous history of gestational hypertensive disorder, autoimmune disease (systemic erythematous lupus or antiphospholipid syndrome), chronic renal disease, diabetes and chronic hypertension are considered high-risk factors. Once any of these factors are present, prophylactic measures must be initiated. [31] The NICE screening proposal was assessed in a prospective study involving a heterogeneous population composed of nulliparous and multiparous pregnant women. Detection rates of 37% and 28.9% were obtained for early-onset (before 34 weeks of gestation) and late-onset (at or after 34 weeks of gestation) preeclampsia cases, respectively. [32.] These numbers were confirmed in another study applying the NICE criteria with a third of preeclampsia cases identified. [33]

Our cohort pointed to only three factors related to increased risk of preeclampsia: weight gain rate per week, obesity and value of diastolic blood pressure measured at 20 weeks of gestation equal to or higher than 75 mmHg. A cohort of more than 62,000 nulliparous pregnant women obtained the same result concerning the influence of weight gain per week on preeclampsia risk.[21] We also reinforced the concept that obesity predisposes to the occurrence of preeclampsia, especially in late-onset cases. This is probably associated with the inflammatory property of adipose tissue and its effects on endothelial function. [34] Considering that both BMI and weight gain rate are modifiable risk factors, consolidated knowledge of their predictive function attaches importance to antenatal counselling and prenatal care follow-up. [35, 36]

Our cohort of nulliparous low risk pregnant women also indicated that a diastolic blood pressure higher than 75 mmHg was correlated to the occurrence of preeclampsia. This finding is not in agreement with another study [37], which showed that mean arterial blood pressure was a better predictor of preeclampsia in a low-risk group of pregnant women. Our study was implemented among nulliparous pregnant women and identified only three risk factors correlated to preeclampsia. This provided us with an interesting insight into the challenge posed by this specific group of women, since nulliparity is known to increase the prevalence of preeclampsia. [10, 38]

The modest predictive power achieved through models with only maternal clinical factors have prompted prospective studies among heterogeneous populations. These studies used multivariate analysis combining maternal clinical factors to other elements such as uterine artery Doppler and biomarkers, in order to develop algorithms of preeclampsia prediction. Despite the high detection rate of these elements, these studies were implemented among a heterogeneous population of pregnant women at high risk of

developing preeclampsia. [32, 33] Furthermore, these studies did not segregate nulliparous women, which is a limitation. It is well-known that the most consistent predictive clinical factor for preeclampsia, which is a previous history of preeclampsia, cannot be applied to first-time mothers. [3]

Biochemical factors have been studied with modest results in terms of prediction potential.[39,40] Furthermore, the potential costs incurred and technologies available for biomarker processing represent a limiting factor for use on a large scale, especially in low and middle-income countries. Thus, clinical risk factors play a crucial role as a cheap and tangible screening instrument for preeclampsia even nowadays.

To date, no single screening test has shown sufficiently accurate specificity and sensitivity to predict preeclampsia cases [41]. The value of clinical risk factors, biochemical markers, uterine Doppler as predictive markers addressed separately remains modest at best for all women destined to develop preeclampsia.[42] This is probably due to the multifactorial aetiology of the condition. Genetic, immunological, environmental and maternal factors have all made their contributions and remain to be fully elucidated. Therefore, preeclampsia is a heterogeneous disease concerning clinical presentation, pathology and outcomes. Notwithstanding decades of research, an enigma still persists surrounding a useful and accurate screening test model to identify early on pregnant women, primarily in the nulliparous group, at high risk for preeclampsia. Research into this field is of considerable importance.

Conclusion

In this study, the incidence of PE was 7.5% in this sample of low-risk nulliparous pregnant women. The rate of Gestational Weight Gain per week, obesity and diastolic blood

pressure equal to or higher than 75mmHg at 20 weeks of gestation was shown to be associated with preeclampsia. Preeclampsia also resulted in more C-sections and prolonged hospital admission, in addition to worse neonatal outcomes.

Acknowledgements

#The Preterm SAMBA study group also included: Mary A. Parpinelli, Karayna G Fernandes, José P Guida, Danielly Santana, Ricardo M Barbosa and Rafael B F Galvao, School of Medical Sciences, University of Campinas, Brazil; Bianca F. Cassettari, School of Medicine of Botucatu, UNESP, Brazil; Lucia Pfitscher, School of Medicine, Federal University of Rio Grande do Sul, Porto Alegre, Brazil; Daisy Lucena de Feitosa, School of Medicine, Federal University of Ceará, Fortaleza, Brazil; Elias de Melo Ferreira Júnior, Danilo Anacleto, School of Medicine, Federal University of Pernambuco, Recife, Brazil; Vilma Zotareli and Marcia Alice Silva, from Cemicamp.

In addition, we acknowledge the contribution of all institutions involved in the entire study, including the funders and also the participants, who kindly agreed to take part in the full study.

Disclosure of interests

The author(s) deny any conflicts of interests.

Contribution to authorship

The idea for the study arose from JGC and MLC. During development of the research proposal, important input was provided by FEF, EARF, JV and IMC from participating centres. After approval, implementation was performed by RTS, JM, DFL, JGC, FEF, EARF, JV and IMC. During the course of the study JPG, KGF and DSS were added to the study and played an important role in patient follow-up and data collection. JM, JGC and MLC planned the analysis which was performed by MHS. The first draft of the manuscript was prepared by JM. All authors discussed the results, gave suggestions and agreed to the final version of the manuscript.

Details of Ethics Approval

The current study is an ancillary analysis (preeclampsia) of the outcome from a Brazilian cohort of low-risk nulliparous women entitled “Preterm SAMBA” which was financially supported by the Bill and Melinda Gates Foundation and the Brazilian CNPq. The Preterm SAMBA study has been reviewed and approved by the Brazilian National Committee for Ethics in Research (CONEP) and by the Institutional Review Board (IRB) of the coordinating centre (Letter of approval 1.048.565 issued on 28th April 2015) and of all other Brazilian participating centres.

Funding

This study was jointly funded by the Bill and Melinda Gates Foundation (grant **OPP1107597**) and CNPq (grant 401636/2013-5). The funders played no role whatsoever in study development, data collection, data analysis or data interpretation.

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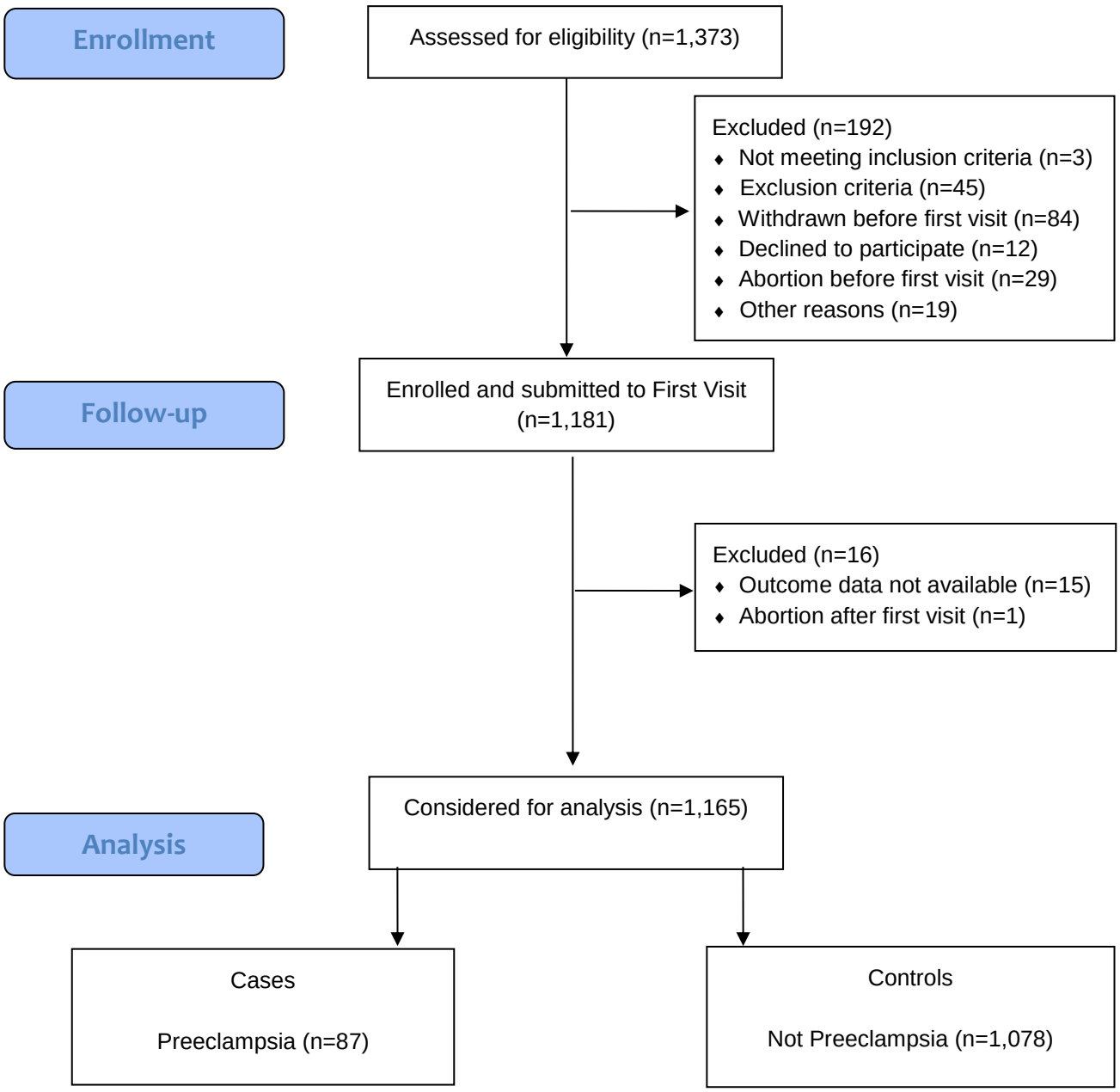

Figure 1. Flowchart of women participating in the study

Table 1: Estimated risk of selected socio-demographic and some medical history and personal characteristics in preeclampsia

Characteristics	Preeclampsia	Controls	RR (95%CI)
Maternal age (years)	**n** (%)	**n** (%)	
<20	20 (23)	271 (25.1)	0.91 [0.48-1.74]
20-34	60 (69)	736 (68.2)	Ref.
>34	7 (8)	71 (6.7)	1.19 [0.50-2.85]
Ethnicity			
White	27 (31)	435 (40.3)	Ref.
Others	60 (69)	643 (59.7)	1.46 [0.84-2.55]
Marital status [a]			
With partner	63 (72.4)	777 (72.0)	Ref.
Without partner	24 (27.6)	296 (27.4)	1.00 [0.47-2.12]
Schooling (years)			
< 12	58 (66.6)	733 (68.0)	Ref.
≥ 12	29 (33.4)	345 (32.0)	1.06 [0.45-2.51]
Annual Family Income (US$)			
Up to 3000	24 (27.6)	280 (25.9)	1.18 [0.63-2.23]
3000 to 6000	31 (35.6)	350 (32.4)	1.22 [0.64-2.34]
Above 6000	32 (36.8)	448 (41.7)	Ref.
Source of prenatal care			
Entirely public	81 (93.1)	927 (85.9)	2.10 [0.50-8.93]
Private/insurance/mixed	6 (6.9)	151 (14.1)	Ref.
Family history of hypertensive disease			
Any hypertensive disorder [b]	16 (18.4)	141 (13.0)	1.47 [0.83-2.60]
Pregnancy of participant´s mother	4 (4.6)	43 (3.9)	1.15 [0.12-10.62]
Smoking			
No smoking	81 (93.1)	997 (92.4)	Ref.
Stopped during pregnancy/ current smoker	6 (6.9)	81 (7.5)	0.92 [0.18-4.58]
Use of illicit drugs [c]			
Non-user	68 (78.2)	873 (80.9)	Ref.
Ceased during pregnancy/current user	2 (2.3)	52 (4.8)	0.51 [0.22-1.22]
Weight gain rate per week (kg) [d]			
<0.25	14 (16.1)	127 (11.7)	1.67 [0.42-6.59]
0.25-0.49	23 (26.4)	364 (33.7)	Ref.
0.50-0.74	26 (29.9)	367 (34.0)	1.11 [0.70-1.77]
≥0.75	15 (17.2)	109 (10.1)	**2.04 [1.12-3.69]**
Body Mass Index at enrolment [e,&]			
Underweight (<21.5 kg/m^2)	9 (10.3)	190 (17.6)	0.74 [0.38-1.45]
Normal weight (21.5-26.2)	28 (32.2)	433 (40.1)	Ref.
Overweight (26.3-30.9)	22 (25.3)	280 (25.9)	1.20 [0.75-1.92]
Obesity (>30.9)	28 (32.2)	174 (16.1)	**2.28 [1.39-3.74]**
Any previous maternal conditions (anaemia, thyroid, asthma, previous			

hypertensive disorder*, depression, POS)			
No	53 (60.9)	730 (67.7)	Ref.
Yes	34 (39.1)	348 (32.3)	1.31 [0.66-2.60]
Diastolic pressure at 20 weeks' gestation [e]			
< 75 mmHg	64 (73.6)	937 (86.9)	Ref.
≥ 75mmHg	23 (26.4)	140 (12.9)	**2.21 [1.30-3.74]**
Total	**87**	**1078**	

Missing information for a: 5; b:100; c: 170; d: 120; e: 1 case;
RR and 95%CI not presented due to small numbers.
* without using medication.
& reference: (43)

Table 2: Maternal and neonatal outcomes associated with preeclampsia

Characteristics	Preeclampsia n (%)	Controls n (%)	RR (95%CI)
Mode of delivery			
Vaginal	21 (24.1)	599 (55.5)	Ref.
C-section	66 (75.9)	479 (44.5)	**3.58 [1.57-8.12]**
Onset of labour			
Spontaneous	18 (20.7)	664 (61.5)	Ref.
Induced	36 (41.4)	211 (19.5)	**5.52 [2.21-13.83]**
Elective C-section	33 (37.9)	203 (19.0)	**5.30 [1.25-22.38]**
Gestational age at birth (weeks)			
<34	11 (12.6)	32 (3.0)	**3.97 [1.55-10.20]**
34-36	9 (10.3)	73 (6.7)	1.70 [0.57-5.06]
≥37	67 (77.1)	973 (90.3)	Ref.
Length of postpartum hospitalization			
1-4 days	67 (77.0)	1041 (96.5)	Ref.
≥ 5 days	20 (23.0)	37 (3.5)	**5.80 [2.12-15.91]**
Thromboembolic event before or after delivery			
No	87 (100.0)	1072 (99.4)	Ref.
Yes	0	4 (0.4)	#
Mean (SD) birthweight (g)	2779.4 (±843.1)	3158.8 (±558.9)	WMD= **-379.4 (-644.5 to -114.4)**
Adequacy of birthweight to GA [a]			
SGA (p< 10)	22 (25.3)	124 (11.5)	**2.45 [1.52-3.95]**
AGA (10 <p< 90)	54 (62.0)	824 (76.4)	Ref.
LGA (p>90)	10 (11.5)	118 (10.9)	1.27 [0.76-2.13]
Fetal death	1 (1.1)	6 (0.5)	1.92 [0.09-39.42]
Apgar score – at 5 minutes <7 [b]	3 (3.4)	16 (1.5)	**2.11 [1.03-4.29]**
Intubation required after birth	7 (8.0)	19 (1.7)	3.89 [0.41-36.95]
NICU admission	32 (36.7)	141 (13.1)	**3.34 [1.61-6.90]**
Neonatal Near Miss (Apgar 5<7 OR Birthweight < 1750g OR GA < 33) [c]	13 (14.9)	39 (3.6)	**3.65 [1.78-7.49]**
Total	**87**	**1078**	

Missing information for a: 13 cases; b: 65 cases; c: 62 cases.

RR and 95%CI not presented due to small numbers.

Table 3: Factors independently associated with preeclampsia on multivariate analysis [n=1164]

Characteristics	**RR_{adj} (95%CI)**
Diastolic blood pressure at 20 weeks' gestation (mmHg)	**1.04 [<1.01-1.06]**
Body Mass Index at enrolment (kg/m^2)	**1.04 [1.01-1.09]**

Variables included in the model (14): Maternal age (years); Ethnicity (White: 0/ other: 1); Marital status (with partner: 0/ without partner: 1); Schooling (<12 years: 0/ ≥12 years: 1); Annual Family Income (Up to US$6000: 1/ >US$6000: 0); Source of prenatal care (entirely public: 1/ other: 0); Family history of hypertensive disease: Any hypertensive disorder (yes: 1/ no: 0); Pregnancy of participant´s mother (yes: 1/ no: 0); Smoking (yes: 1/ no smoking: 0); Use of illicit drugs (yes: 1/ non-user:0); Weight gain rate per week (kg); Body Mass Index at enrolment (kg/m^2); Any previous maternal conditions (yes: 1/ no: 0); Diastolic blood pressure at 20 weeks' gestation (mmHg).

4.5. Artigo 5

ORIGINAL RESEARCH

Mean arterial blood pressure: potential predictive tool for preeclampsia in a cohort of low-risk nulliparous pregnant women

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Abstract

Objective: to assess mean arterial blood pressure (MAP) levels at 19-21, 27-29 and 37-39 weeks of gestation and performance of screening by MAP for the prediction of preeclampsia in a Brazilian cohort of low-risk nulliparous pregnant women.

Study design: this was a cohort approach to a secondary analysis of the Preterm SAMBA study. Mean arterial blood pressure was evaluated at three different time periods during pregnancy. Groups with early-onset preeclampsia, late-onset preeclampsia and normotension were compared. Increments in mean arterial blood pressure between 20 and 27 weeks and 20 and 37 weeks of gestation were also calculated for the three groups studied. The accuracy of mean arterial blood pressure in the prediction of preeclampsia was determined by ROC curves.

Main outcome measures: preeclampsia

Results: of the 1,373 participants enrolled, complete data were available for 1,165. The incidence of preeclampsia was 7.5%. Women with early-onset preeclampsia had higher mean arterial blood pressure levels at 20 weeks of gestation, compared to the normotensive group. Women with late-onset preeclampsia had higher mean arterial blood pressure levels at 37 weeks of gestation, than the normotensive groups and higher increases in this marker between 20 and 37 weeks of gestation. Based on ROC curves, the predictive performance of mean arterial blood pressure was higher at 37 weeks of gestation, with an area under the curve of 0.771.

Conclusion: as an isolated marker for the prediction of preeclampsia, the performance of mean arterial blood pressure was low in a low-risk nulliparous pregnant women group. Considering that early-onset preeclampsia cases had higher mean arterial blood pressure levels at 20 weeks of gestation, future studies with larger cohorts that combine multiple markers are needed for the development of a preeclampsia prediction model.

Keywords: preeclampsia, blood pressure, hypertension, prenatal screening, second trimester, third trimester.

1. Introduction

The prediction of preeclampsia is challenging. It is a complex syndrome, with multiple phenotypes, each with its own particularities of pathophysiology and clinical manifestations [1]. Effective prediction of the condition would represent an important strategy against adverse outcomes of maternal and perinatal health.

The majority of prospective studies in large general obstetric populations have demonstrated a modest capacity to predict preeclampsia by clinical risk factors, and approximately only a third of these cases are identified [2]. A prediction model using a combination of biomarkers and uterine artery Doppler has improved the diagnostic rate in early-onset cases, where disease manifestation occurs before 34 weeks of gestation. However, late-onset cases of preeclampsia (with manifestation occurring at or after 34 weeks) are the majority of cases in most clinical settings. The application of such an expensive technological screening model in low and middle-income countries is unfeasible, exactly the locations where there is a higher prevalence of preeclampsia [2-4].

Blood pressure measurement is part of routine surveillance during antenatal care. High blood pressure may be the first sign of a hypertensive disorder and is a diagnostic tool. Oscillations in BP measurements in a pregnant woman may reflect a trend for hypertensive disorder, and is a predictive test [5]. Since the sixties, several second-trimester studies have been reported on the use of blood pressure measurement for preeclampsia screening. There have been contradictory results concerning detection rates, which range from 8% to 93%. These different results were due to distinct diagnostic concepts of preeclampsia, diverse methods of population screening and also cutoff values used to define a positive screening test [6]. Futhermore, very few studies that specifically enroll nulliparous pregnant women have been carried out. There is actually a lack of information on this group, which is considered to be at high risk for preeclampsia [7]. The strongest known risk factor, which is a personal history of preeclampsia cannot be applied to this particular group of nulliparous women [3].

Thus, to assess the accuracy of mean arterial blood pressure (MAP) in a group of nulliparous pregnant women, this study showed the distribution of MAP levels at 19-21, 27-29 and 37-39 weeks of gestation and evaluated the accuracy of MAP at these three

different time periods (basically second and third semesters) as a predictor of preeclampsia.

2. Methods

This was a secondary analysis of the Preterm SAMBA study, a multicenter cohort study performed in 5 different centers in Brazil. From July 2015 to March 2018, 1,200 low-risk nulliparous pregnant women were enrolled and received follow-up during prenatal care, including only singleton pregnancies, without any fetal malformations or previous chronic maternal disease [8]. Ethical approval for the study was obtained from relevant institutional review boards and competent authorities of each center where the study was conducted. More detailed information on the study design and methods used in this study have already been previously published (9).

Participants and procedures

Criteria for participant enrollment were nulliparous women with singleton pregnancies between 19 and 21 weeks of gestation. Exclusion criteria included: previous history of chronic hypertension, use of medication, fetal malformations, diabetes mellitus, nephropathy, autoimmune diseases (systemic erythematous lupus or antiphospholipid syndrome), sickle cell disease, uterine malformations, previous cervical surgery, previous cerclage, history of 3 or more abortions, HIV infection, chronic use of corticosteroids or aspirin or calcium above 1g/day or fish oil above 2.7g/day or vitamin C above 1000mg/day or vitamin E above 400 UI/day or heparin.

At least three routine hospital visits were scheduled. Systolic and diastolic blood pressure of the women were measured, according to standard clinical procedure on the 3 occasions: at 19-21 weeks, 27-29 weeks and 37-39 weeks of gestation, using a manual sphygmomanometer. During the first visit, maternal characteristics and medical history were recorded. In addition, blood and hair samples were collected and stored appropriately in a biobank for subsequent analysis by metabolomics technology. Gestational age was estimated from the date of the last menstrual period and confirmed by an early ultrasonography performed before 20 weeks. For each scheduled visit, blood pressure was measured 3 times. Women were allowed to rest for 15 minutes before the first blood pressure measurement was performed. Between blood pressure

measurements, the investigator waited for at least 2 minutes. During the examination, participants remained in a sitting position, with their right arm supported at the level of their heart. An adult blood pressure cuff was used, selecting the proper size for each participant. Pressure reading at phase V of Korotkoff sounds corresponded to diastolic pressure. Mean arterial blood pressure was obtained by the equation (2DBP + SBP)/3. The mean blood pressure at each gestational age for the three measurements was obtained by the average of three mean blood pressure measurements [BP_m= ($BP1_m$ + $BP2_m$ + $BP3_m$)/3]. We also calculated the difference in mean blood pressure with measurements at 19-21 weeks and 27-29 weeks and measurements at 19-21 weeks and 37-39 weeks. Calculation was made in two steps: first, the difference was determined for each woman; and second, the mean difference was calculated.

Outcome

Preeclampsia was the main outcome of this analysis. It was defined as the onset of hypertension (systolic blood pressure of 140 mmHg or more and/or diastolic blood pressure of 90 mmHg or more) after 20 weeks of gestation, measured on at least two different occasions, in conjunction with proteinuria (≥300 mg/day or at least 1g/L [1+] on dipstick testing or spot urine protein/creatinine >30mg/mmol [0.3mg/mg]) or any signs of organ dysfunction [9]. Systemic complications were defined as: hematological complications (thrombocytopenia, disseminated intravascular coagulation or hemolysis); hepatic dysfunction (elevated transaminases); neurological dysfunction (examples include eclampsia, altered mental status, blindness, stroke or more commonly hyperreflexia when accompanied by clonus, severe headache when accompanied by hyperreflexia, persistent visual scotomata); renal dysfunction (creatinine > 1.2mg/dL) (10).

After delivery, each woman was classified as having a normal pregnancy (control group) or preeclampsia (case group). Cases were categorized into early-onset preeclampsia (women who developed preeclampsia before 34 weeks of gestation) and late-onset preeclampsia (women who developed preeclampsia after 34+1 weeks of gestation) [10] .

Statistical Analysis

Initially, the three groups were compared regarding sociodemographic characteristics of women using a Chi-square design-based test. Mean arterial blood pressure was then compared among the three groups (early-onset preeclampsia, late-onset preeclampsia and normotensive) using Student's t-test. The mean difference in MBP measured at 27 and 37 weeks was estimated and compared to values at 20 weeks. Finally, we checked to see whether mean arterial blood pressure had any predictive power at three time periods (20, 27 or 37 weeks of gestation) by comparing the area under the receiver-operating characteristic curves (AUROC). Analyses were performed using SPSS and Stata software.

3. Results

Of the 1,373 participants recruited for Preterm SAMBA study, follow-up of 1165 women was provided (Figure 1). In our cohort, the incidence of preeclampsia was 7.5% (87 cases) of whom 14 (16.1%) had early-onset preeclampsia (data not shown). The sociodemographic characteristics of women who developed preeclampsia and were analyzed according to subtypes and controls are shown in Table 1. There were no differences between preeclampsia and control groups. Throughout the gestation period, we observed that MAP showed an increasing trend in the three participating groups (Figure 2). MAP in the early-onset preeclampsia group showed the highest value at 20 weeks of gestation, compared to the control group (p value= 0.02) (Table 2). Specifically, the increment was higher in the late-onset preeclampsia group compared to the control group, in both stages of gestation: from 20 to 27 weeks and from 20 to 37 weeks of gestation, with a p value of 0.012 and 0.003, respectively (Table 2).

When compared to the early-onset preeclampsia group, there was no difference in increment. The predictive power of mean arterial blood pressure was assessed through ROC curves, and this marker showed the highest accuracy at 37 weeks of gestation with an area under the curve of 0.771 (Table 3). In our cohort, there were 2 cases of eclampsia and 6 cases of HELLP syndrome, characterized by hemolysis, low platelet count and elevated hepatic transaminases (data not shown).

4. Discussion

Mean arterial blood pressure (MAP) has remained the target of scientific research in quest for the prediction of preeclampsia over time. It is a feasible tool and part of antenatal surveillance. Few studies have shown blood pressure patterns among low- risk nulliparous pregnant women [11-14]. Our cohort study of nulliparous low-risk pregnant women showed patterns of mean arterial blood pressure measured during the second half of pregnancy. The observation of arterial blood pressure distribution throughout pregnancy is an essential component of antenatal care strategy. This marker can be obtained from medical records usually gathered from prenatal cards that are easily available in health care services around the world.

In uncomplicated pregnancies, arterial blood pressure pattern usually consists of a steady decrease in blood pressure during the first half of pregnancy, then an increase until the time of delivery [15]. In contrast, in women with hypertensive disorder (gestational hypertension or preeclampsia) blood pressure is generally stable during the first half of pregnancy, increasing until delivery. In 2001, a study analyzing more than 2,000 series of blood pressure systematically sampled by ambulatory monitoring showed that while diastolic blood pressure increases 7% between the middle of gestation and delivery in the normotensive group, this increment is around 12% and 15% in the hypertensive group [16]. Similarly, in our study there was an increment of 5.2% in mean arterial blood pressure in the normotensive group, compared to 13.3% in the late-onset preeclampsia group, with a significant difference between both ($p = 0.003$). In our study, the increment observed among late-onset preeclampsia cases was superior to the value seen among the early-onset preeclampsia group. We hypothesized that it was possible that the latter group had already started antihypertensive medication at 27 and 37 weeks of gestation. The lack of information about the exact time when antihypertensive medication was initiated was a weakness of our study.

Although we demonstrated that women with early-onset preeclampsia had a higher MAP at 20 weeks of gestation than the remaining participants, MAP performance was modest as a predictor, with an Area Under the Curve of only 0.619. This is not in accordance with a systematic review from 2008, which demonstrated that mean arterial blood pressure measured during the first or second trimester of gestation in a general

low-risk pregnant population was a better predictor of preeclampsia, with an Area Under the Curve of 0.76. For the high-risk pregnant women group, this review indicated that diastolic blood pressure is the best predictor of preeclampsia, when measured between 13 and 20 weeks of gestation [17]. The very few cases of preeclampsia may possibly explain the modest accuracy found in our cohort. In our analysis, the highest accuracy was achieved at 37 weeks of gestation, with an AUC of 0.771. Despite this number, prophylactic measures are totally unfeasible at this gestational age, considering the pathophysiology of preeclampsia. Regardless of the time period when preeclampsia is clinically established, it may be triggered in earlier stages of gestation [18, 19].

Furthermore, some studies have demonstrated a higher predictive power when MAP is measured during the first trimester of pregnancy [6, 14, 20], particularly when combined with other maternal factors. Participants were enrolled during the second trimester of gestation. Therefore, such data is not available, representing a weakness of our study. However, a cohort of more than 70,000 pregnant women found a similar detection rate of preeclampsia , with MAP measurement at 11-13 weeks or 19-24 weeks of gestation [21]. It is already known that the introduction of low-dose aspirin before 16 weeks of gestation in a group at high-risk for preeclampsia can reduce the incidence of EOP by almost 62% [22, 23]. This reinforces the importance of a proposed pregnancy care model in early pregnancy to identify possible life-threatening maternal and fetal health conditions [24]. Nevertheless, this should not reduce the significance attributed to a “second-look” prediction in the middle of gestation - in the second trimester - considering that it is still possible to redefine pregnancy management at this time period, including the frequency of visits, addressing content, time, method and place of delivery [21, 25].

Our study has many strengths. First, this was a prospective examination of a large population of low-risk nulliparous pregnant women. Second, there was data recording of maternal characteristics and medical history to identify risk factors associated with preeclampsia. Furthermore, we measured arterial blood pressure with a mercury sphygmomanometer which remains the gold standard for noninvasive blood pressure monitoring [6]. On the other hand, it is known that there is concern about the clinical performance and safety of these instruments. Although BMI had been registered at enrolment, a weakness of our study was that participants were not segregated by weight.

This can add a significant bias, despite the exclusion of women with comorbidities. Obesity was twofold higher among women with preeclampsia than among controls. Nevertheless, weight was not an exclusion factor. MAP is known to be dependent on weight [26]. However, a cuff of the appropriate size for each patient weight was selected for blood pressure measurement.

5. Conclusion

In the last 20 years, improvement in diagnostic tools has led to the prevention and prediction of different conditions, including obstetric health issues. Therefore, considerable effort has been devoted to identifying and modifying individual risk factors during the first half of pregnancy [24, 25]. Close surveillance of the mother and offspring may help to decide the best time for delivery. Furthermore, owing to the complexity of preeclampsia and its multifactorial etiology, the predictive power seems to be correlated with the association of distinct factors in a prediction model, and is not related to a unique isolated risk factor or biomarker [27]. Therefore, future studies should be conducted to analyze mean arterial blood pressure in combination with other strategies, to obtain a predictive algorithm for preeclampsia, particularly in the challenging nulliparous group.

Acknowledgements

#The Preterm SAMBA study group also included: Mary A. Parpinelli, Karayna G Fernandes, José P Guida, Danielly Santana, Ricardo M Barbosa and Rafael B F Galvao, School of Medical Sciences, University of Campinas, Brazil; Bianca F. Cassettari, School of Medicine of Botucatu, UNESP, Brazil; Lucia Pfitscher, School of Medicine, Federal University of Rio Grande do Sul, Porto Alegre, Brazil; Daisy Lucena de Feitosa, School of Medicine, Federal University of Ceará, Fortaleza, Brazil; Elias de Melo Ferreira Júnior, Danilo Anacleto, School of Medicine, Federal University of Pernambuco, Recife, Brazil; Vilma Zotareli and Marcia Alice Silva, from Cemicamp.

In addition, we acknowledge the contribution of all institutions involved in the whole study, including the funders and also the participants who kindly agreed to take part in the full study.

Declaration of interests

The author(s) deny any conflicts of interest.

Detail of ethics approval

This is a study derived from the Preterm SAMBA study which obtained ethical approval from the National Committee for Ethics in Research of Brazil (CONEP) and the Institutional Review Board (IRB) of the coordinating center (Letter of approval 1.048.565 issued on 28th April 2015) and all other Brazilian participating centers. Each woman signed an informed consent form before enrolment in the study.

Funding

This study was funded by the Bill and Melinda Gates Foundation, Seattle, WA (grant OPP1107597) and CNPq, Brazil (grant 401636/2013-5). The funders did not influence the study, including development, data collection, data analysis or data interpretation.

Author´s Contributions

The idea for the study arose from JGC, JM and MLC. During development of the research proposal, important input was provided by FEF, EARF, JV and IMC from participating centres. After approval, implementation was performed by RTS, JM, DFL, JGC, FEF, EARF, JV and IMC. JM, JGC and MLC planned the analysis. The first draft of the manuscript was prepared by JM. All authors discussed the results, gave suggestions and agreed to the final version of the manuscript.

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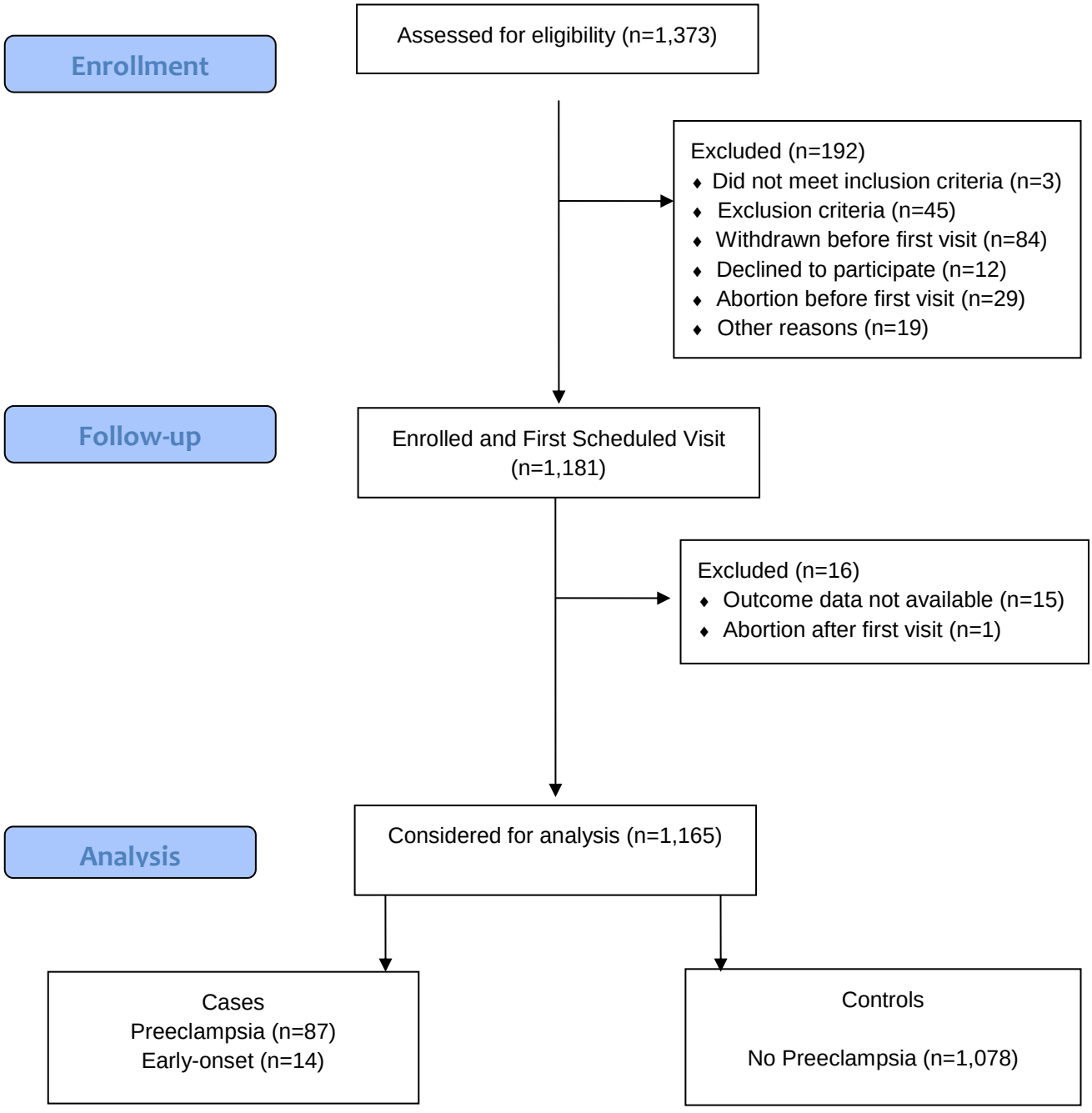

Figure 1. Flowchart of women participating in the study

Figure 2. Patterns of Mean Arterial Pressure (MAP) throughout gestation in the three groups: early-onset (EO), late-onset (LO) preeclampsia (PE) groups and control group.

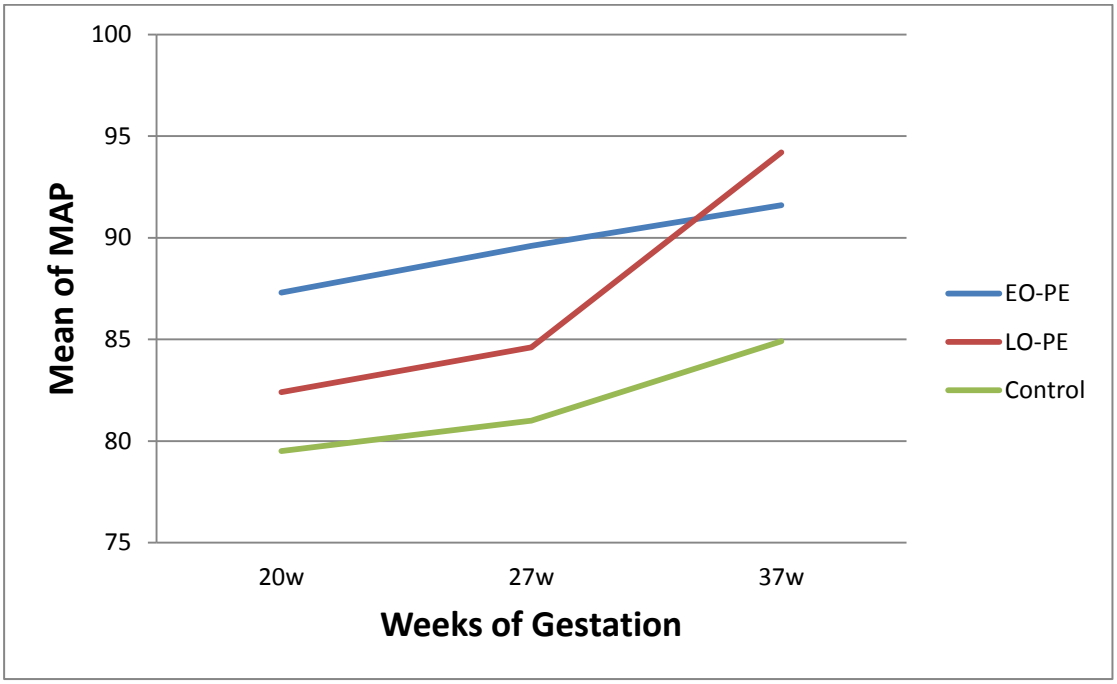

Table 1. Some sociodemographic characteristics of women included according to PE status

Characteristics	Early-onset PE n (%)	Late-onset PE n (%)	No PE n (%)	p value*
Maternal age				0.605
<20 years	1 (7.1)	19 (26.0)	271 (25.1)	
20-34 years	12 (85.8)	48 (65.8)	736 (68.3)	
>34 years	1 (7.1)	6 (8.2)	71 (6.6)	
Ethnicity				0.146
White	7 (50.0)	20 (27.4)	435 (40.4)	
Others	7 (50.0)	53 (72.6)	643 (59.6)	
Marital status [a]				0.975
With partner	10 (71.4)	53 (72.6)	777 (72.4)	
Without partner	4 (28.6)	20 (27.4)	296 (27.6)	
Schooling (years)				0.717
Up to 12	8 (57.1)	50 (68.5)	733 (68.0)	
≥ 12	6 (42.9)	23 (31.5)	345 (32.0)	
Annual family income				0.691
Up to 3000 US$	3 (21.4)	21 (28.8)	280 (26.0)	
3000-6000 US$	4 (28.6)	27 (37.0)	350 (32.5)	
>6000 US$	7 (50.0)	25 (34.2)	448 (41.5)	
Source of prenatal care				0.473
Entirely public	14 (100.0)	67 (91.8)	927 (86.0)	
Private/mixed	0 (-)	6 (8.2)	151 (14.0)	
Total	**14**	**73**	**1078**	

*p-value from Chi-square design-based
a: missing information for 5 cases

Table 2: Mean arterial blood pressure in the three time periods during pregnancy comparing preeclampsia groups and control

BP (mmHg)	Early-onset PE	Late-onset PE	No PE	p-value #	p-value @	p-value &
MBP at 20[h] weeks [a]	87.3	82.4	79.5	0.191	**0.024**	0.068
(95%CI)	(79.4-95.3)	(80.4-84.3)	(76.8-82.2)			
MBP at 27 weeks [b]	89.6	84.6	81.0	0.300	0.060	0.072
(95%CI)	(78.0-101.1)	(82.3-86.9)	(78.2-83.9)			
MBP_{27w}-MBP_{20w} y(z)	2.8 (3.2%)	4.2 (5.0%)	1.4 (1.7%)	0.100	**0.040**	**0.012**
MBP at 37 weeks [c]	91.6	94.2	84.9	0.581	0.218	**<0.001**
(95%CI)	(81.0-102.1)	(92.4-96.0)	(82.3-87.6)			
MBP_{37w}-MBP_{20w} y(z)	1.2 (1.3%)	13.3 (16.1%)	5.2 (6.5%)	**0.031**	0.229	**0.003**
Total	**14**	**73**	**1078**			

Missing information for: a: 1 case; b: 229 cases; c: 393 cases (125 already delivered)

y: mean difference atz: increment in percentage

Early-onset PE x Late-onset PE; @ Early-onset PE x No PE; & Late-onset PE x No PE

Table 3: Prediction of preeclampsia using mean blood pressure at different gestational ages among low-risk nullipara women

Gestational Age	Area Under the Curve	+/-
20 weeks	0.619	83/1048
27 weeks	0.630	59/857
37 weeks	0.771	43/707

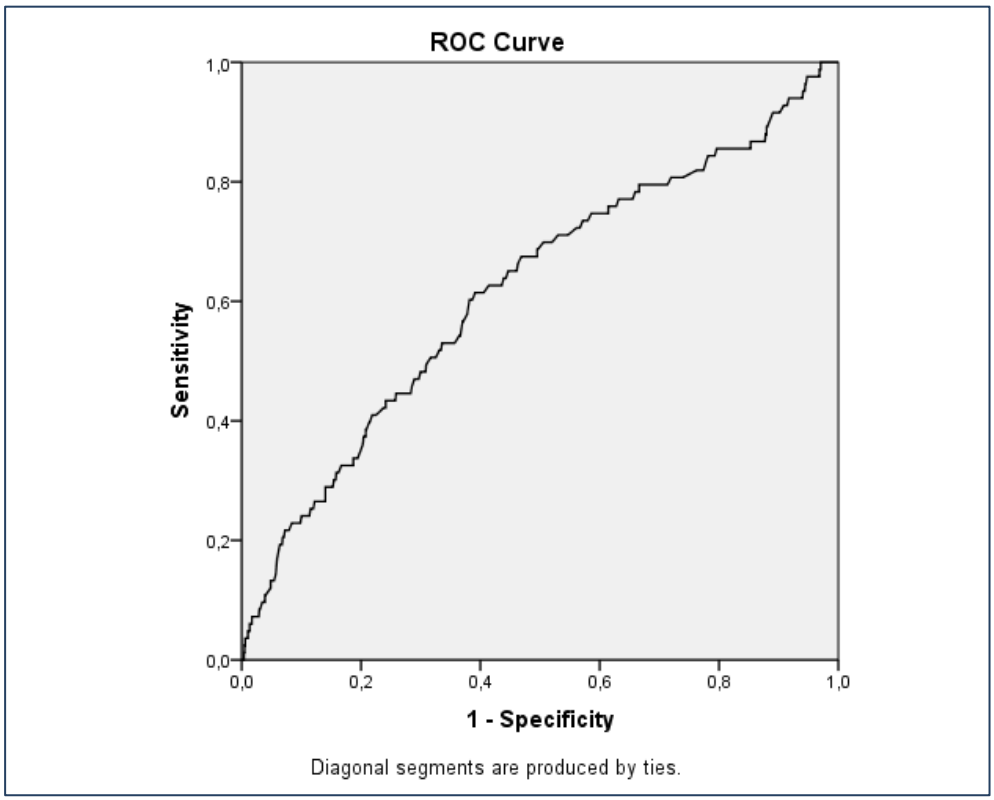

Figure 3. ROC curve for mean blood pressure at 20 weeks as a predictor of preeclampsia (AUC=0.619)

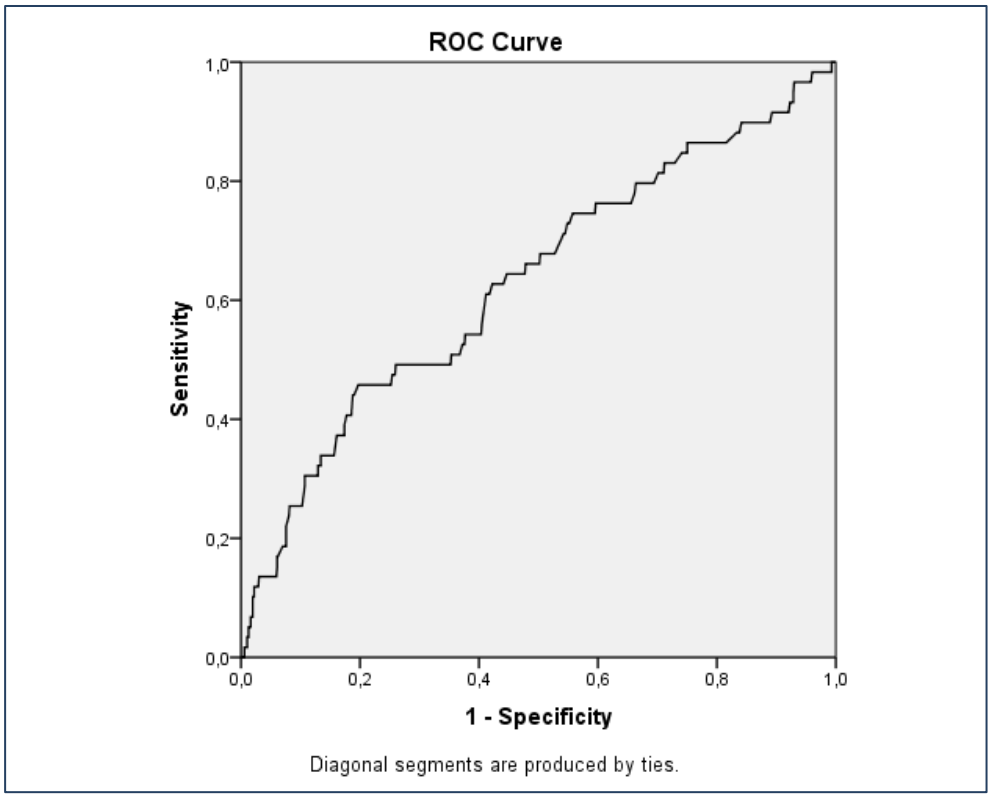

Figure 4. ROC curve for mean blood pressure at 27 weeks as a predictor of preeclampsia (AUC=0.630)

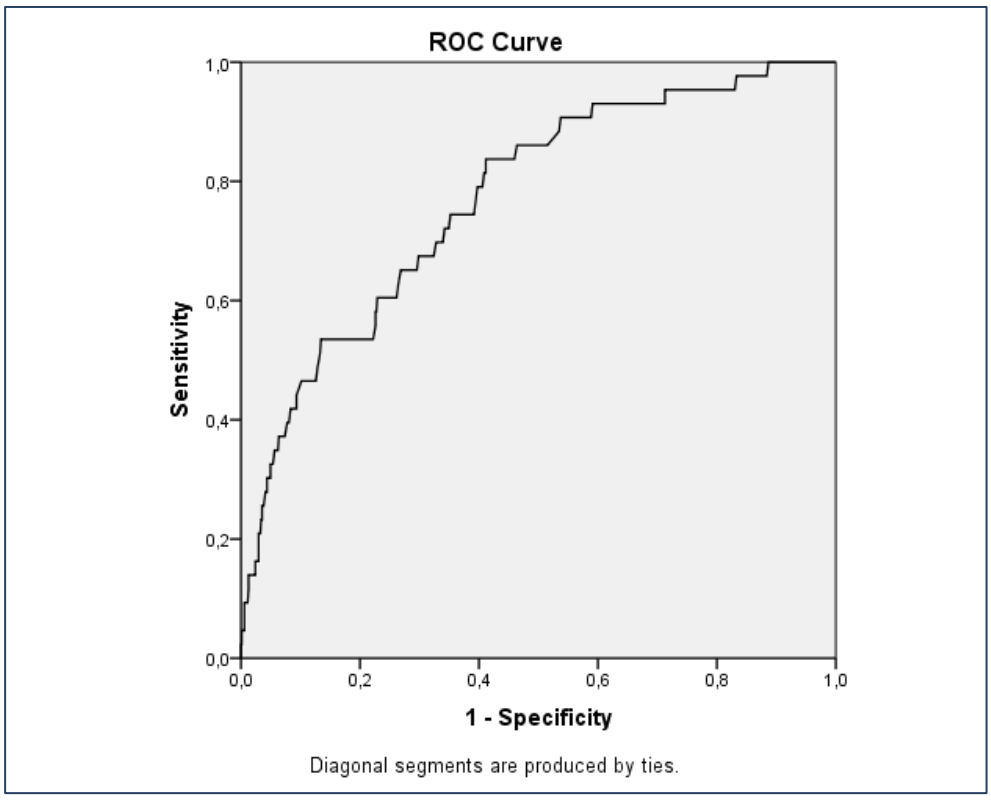

Figure 5. ROC curve for mean blood pressure at 37 weeks as a predictor of preeclampsia (AUC=0.771)

6. Discussão Geral

Pré-eclâmpsia tem sido por décadas tema de interesse entre estudiosos, já que apesar dos esforços mundiais em melhorar o entendimento sobre sua fisiopatologia, formas de prevenção e tratamento, esse agravo segue como importante causa de morbimortalidade materna e neonatal (3). Segundo dados da OMS, entre os anos de 2003 e 2009, distúrbios hipertensivos ocuparam o segundo lugar no ranking de causas de morte materna mundial (5).

A ideia central dessa tese é a discussão sobre as formas de predição da pré-eclâmpsia. Derivada do estudo Preterm SAMBA (Screening and Metabolomics in Brazil and Auckland) que construiu uma coorte multicêntrica de gestantes brasileiras nulíparas de baixo risco, planejada inicialmente para o desfecho "prematuridade"(81), essa análise de fatores preditores integra uma tendência universal de se concentrar esforços, pesquisas e muito estudo para predição e prevenção de agravos à saúde, consideradas prioridades em relação ao seu diagnóstico e tratamento. Essa iniciativa está em consonância com a proposta feita por Kypros Nicolaides em seu artigo sobre a inversão da pirâmide de cuidados pré-natais publicado em 2011 (44). Segundo ele, o século XXI está marcado por uma mudança de paradigma em relação ao primeiro e segundo trimestres gestacionais. Até então, esse período era visto como um momento em que ações de prevenção e condução de qualquer agravo eram incompatíveis com os recursos disponíveis, e por esse motivo os maiores investimentos e cuidados pré-natais eram concentrados na segunda metade da gestação. Nesse novo conceito de pirâmide invertida, a primeira metade da gestação seria o momento crucial para a aplicação de modelos preditores, capazes de direcionar os cuidados pré-natais conforme o perfil de risco de cada gestante e, a partir daí, implementar medidas profiláticas (44).

Embora não seja essa a realidade em muitos sistemas de saúde, o número de estudos com o olhar voltado para predição precoce de agravos gestacionais tem se multiplicado. A pré-eclâmpsia, com todo o seu impacto sobre saúde materna e neonatal, é alvo dessas análises. O estudo ASPRE (ASpirin for evidence–based PREeclampsia prevention) publicado recentemente (82) envolveu a impressionante marca de mais de 26 mil participantes que foram submetidas ao seguinte modelo para predição de pré-eclâmpsia entre 11 e 14 semanas de gestação: história obstétrica, estudo Doppler de

artérias uterinas com análise de índice de pulsatilidade, dosagem de PlGF e PAPP-A e medida de pressão arterial média. Através desse modelo, as participantes eram classificadas como de alto ou baixo risco para pré-eclâmpsia, sendo que para as primeiras prescrevia-se 150 mg de aspirina. Após o nascimento, os principais desfechos maternos e neonatais eram comparados entre os grupos expostos e não expostos ao uso do medicamento. Esse estudo foi capaz de demonstrar que dar aspirina para mulheres consideradas como de "alto risco" para pré-eclâmpsia reduziu em 62% a ocorrência de casos com manifestação anterior às 37 semanas (41). Embora ainda não demonstrado nessa análise em face da baixa incidência de pré-eclâmpsia nessa coorte, da ordem de 2,9%, conjetura-se se essa redução se traduzirá em melhores resultados maternos e neonatais. Além disso, o modelo preditor multifatorial proposto foi capaz de identificar a maioria das mulheres que desenvolveriam pré-eclâmpsia anteriormente às 37 semanas, indicando a efetividade do reconhecimento precoce das gestantes em risco para pré-eclâmpsia. Vale lembrar que esse modelo já havia sido testado anteriormente, com excelente performance em relação aos casos precoces de pré-eclâmpsia (43).

Entretanto, ao observarmos os resultados no que se refere aos casos de pré-eclâmpsia de manifestação tardia, que correspondem à maioria, encontramos resultados bem menos impressionantes: no estudo desenhado para teste do modelo preditor multifatorial (PAM, história obstétrica, dosagem sérica de PAPP-A e PlGF e estudo Doppler de artérias uterinas) a taxa de detecção para casos de manifestação após 37 semanas foi inferior a 50%, número confirmado pelo estudo ASPRE, que identificou menos de 40% desses casos (41, 43). Essa discrepância de detecção e prevenção entre os casos de manifestação precoce e tardia nos conduz a algumas reflexões.

Primeiramente, a hipótese de que casos precoces e tardios guardem evidentes diferenças em relação à sua fisiopatologia, embora reunidos sob a denominação de pré-eclâmpsia, é ratificada pelos achados do estudo ASPRE (23, 83). Conforme se pode perceber, um mesmo modelo preditor não encontra nos casos tardios alterações que são detectáveis nos casos precoces. Salienta-se com isso a necessidade de novas pesquisas para maiores esclarecimentos (2, 84). A aplicação de tecnologias ômicas, nas quais a metabolômica está incluída, pode representar um importante passo nesse sentido. O reconhecimento dos metabólitos envolvidos com a pré-eclâmpsia (e com seus diferentes fenótipos) pode funcionar como uma fotografia bastante útil de vias metabólicas

envolvidas no processo que resulta na manifestação clínica da pré-eclâmpsia (73, 75). Nosso estudo de validação da metabolômica para predição de pré-eclâmpsia numa coorte brasileira de nulíparas de baixo risco poderá auxiliar nesse sentido. Além disso, o fato de se dar numa população de nulíparas é bastante interessante, já que nesse grupo não é possível aplicar-se o principal fator clínico de predição de pré-eclâmpsia que é história pessoal desse agravo (54).

Secundariamente, o modelo preditor multifatorial supracitado envolve tecnologias diversas e dispendiosas, principalmente considerando-se que os países de maior ocorrência de pré-eclâmpsia são os de maior privação de recursos financeiros (6). Além disso, os casos de manifestação tardia são reconhecidamente mais prevalentes e, conforme demonstrado acima, menos sensíveis aos instrumentos de predição precoce (43, 85). O atual cenário socioeconômico brasileiro definitivamente não nos permite imaginar o uso rotineiro em larga escala de dosagem de biomarcadores, por exemplo, tampouco a realização do estudo Doppler de artérias uterinas, na medida em que não é incomum que em muitos locais haja deficiências de insumos considerados básicos para a assistência pré-natal(86-88). De qualquer forma, ainda não está claro que estas intervenções sejam efetivas para introdução em larga escala e não fazem parte de nenhum *guideline* internacional que trata do assunto.

Nesse sentido, diante da escassez de informação sobre incidência de pré-eclâmpsia no Brasil e de seus subtipos, buscamos nos aproximar desses números partindo de uma coorte multicêntrica. Concomitantemente, revisitamos estratégias mais simples e econômicas que possam auxiliar no processo de predição de pré-eclâmpsia. Além disso, o fizemos numa população de nulíparas assumindo ser esse o grupo de maior risco e sobre o qual o menor número de informações se possui (54, 89). Fatores de risco clínicos maternos foram apontados recentemente como importantes instrumentos para seleção e instituição de profilaxia em gestantes consideradas de alto risco para a ocorrência de pré-eclâmpsia, principalmente se considerarmos que essa é uma doença multifatorial (90). Em comparação ao algoritmo que utiliza biomarcadores e estudo Doppler, além de dados clínicos, esse método, baseado exclusivamente em fatores de risco, é mais sensível e menos específico, o que termina por indicar o uso de aspirina num maior número de gestantes e, por extensão, reduzir o número de casos de pré-eclâmpsia (91). Ademais,

essa é uma ferramenta que pode ser utilizada em qualquer lugar com emprego exclusivo de recursos humanos.

Por recursos humanos entenda-se: equipe treinada e apta a reconhecer, a partir da história clínica da gestante em questão, fatores de risco para pré-eclâmpsia que indiquem medidas profiláticas como introdução de aspirina e direcionamento dessa gestante para um serviço de atendimento especializado. A instrução de profissionais de saúde é considerada uma importante ferramenta no desafio de melhorar índices de saúde materna e neonatal. Nossa revisão sobre pré-eclâmpsia em seus aspectos de predição, impacto mundial e fisiopatologia procurou abordar de forma ampla o tema, servindo como fonte de estudo para os mais diversos profissionais de saúde. Nossa revisão sistemática direciona a atenção para o uso da metabolômica na predição de doenças hipertensivas que podem ocorrer durante a gestação. Pretende-se com uso dessa tecnologia ampliar o entendimento sobre vias metabólicas envolvidas na pré-eclâmpsia e servir de base para estudos futuros que possam elucidar métodos outros de prevenção desse agravo.

Experiência pessoal da participação no estudo SAMBA: caso 1232

Minha participação no estudo SAMBA teve início por ocasião de uma importante definição de área de interesse. O discernimento dessa área dentro do universo da Tocoginecologia se deu no mais clássico modelo “antes tarde do que nunca”. Após uma experiência fantástica no mestrado, a decisão pelo doutorado foi quase instantânea. Mas a mudança de tema se impunha e assim aconteceu meu mergulho pelos meandros da Obstetrícia, mergulho esse sem volta! E o estudo Preterm SAMBA estava para começar! Nunca havia visto de tão perto um estudo dessa magnitude. Não tardou para que eu percebesse que alcançar os resultados não era a parte mais interessante. O caminho até chegar lá (o modo de fazer pesquisa) certamente seria muito instigante, instrutivo, e por que não, desafiador. Uma coorte de nulíparas de baixo risco? O otimismo próprio de iniciante me fez imaginar que isso seria fácil! Ledo engano. O que se seguiu foram meses de busca ativa, na melhor definição do termo, perseguindo mulheres saudáveis que concordassem em fazer parte do estudo SAMBA. Para minha surpresa, o chamariz não era o ultrassom morfológico que oferecíamos, acostumados que estávamos à carência do sistema de saúde brasileiro. Era sim, o cuidado pré-natal, o seguimento, e mais que isso, o

relacionamento que se estabelecia com a gestante a partir do momento em que a mesma aceitava participar do estudo. O resultado: mais de 1200 mulheres incluídas e muitas histórias para guardar no coração e para contar também! Com a licença necessária do código de ética em pesquisa para revelar a identidade do caso 1232: eu mesma. Sim, em fevereiro de 2017 engravidei de minha doce Manuela. Gestação planejada e desejada. Claro que me incluí na coorte e por sorte incrementei nosso grupo controle, com uma gestação sem qualquer intercorrência. Manu nasceu no dia 7 de outubro, de parto normal, e é me divertindo com ela que esse trabalho está sendo escrito e finalizado.

7. Conclusões

1. Revisões sobre o tema pré-eclâmpsia, em seus diversos aspectos, representam uma importante estratégia para melhorar o conhecimento dos profissionais para o provimento de cuidado às gestantes e prevenir desfechos desfavoráveis, além de colaborar para a formulação de novas propostas de estudo pela identificação de lacunas do conhecimento no assunto.

2. A realização de uma revisão sistemática tem como base a elaboração de um protocolo no qual ficam claros a pergunta em que se fundamenta a revisão, estratégias de busca e desfechos e intervenções a serem avaliados.

3. A metabolômica é uma tecnologia que parece ter um papel auxiliar na predição da pré-eclâmpsia e os estudos têm sido planejados no intuito de promover a translação desse conhecimento para a prática clínica, a fim de viabilizar a predição de casos em grupos de particular interesse como o de nulíparas.

4. A incidência de pré-eclâmpsia numa coorte composta por nulíparas brasileiras de baixo risco foi de 7,5%. O risco de mulheres com obesidade e pressão arterial diastólica maior ou igual a 75 mmHg às 20 semanas de gestação de desenvolverem pré-eclâmpsia foi cerca de 2 vezes superior ao de mulheres nulíparas sem essas características. Mulheres com pré-eclâmpsia apresentaram maior risco de parto cesariano e internação prolongada; têm ainda maior risco de apresentarem piores resultados perinatais expressos por menor peso ao nascimento, maior proporção de prematuridade abaixo de 34 semanas, de Apgar baixo no quinto minuto de vida, de internação em unidades de terapia intensiva neonatal e de *near miss* neonatal.

5. A Pressão Arterial Média (PAM) aferida no terceiro trimestre entre 37 e 39 semanas apresenta modesta acurácia para predição de pré-eclâmpsia, superior à apresentada quando aferida entre 19 e 21 semanas e 27 e 29 semanas. Nos casos de pré-eclâmpsia de manifestação precoce, a PAM é superior às 20 semanas quando comparada a um grupo de mulheres gestantes normotensas.

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9. Anexos

9.5. Anexo 1. Protocolo do estudo

Cecatti *et al. BMC Pregnancy and Childbirth* (2016) 16:212
DOI 10.1186/s12884-016-1006-9

BMC Pregnancy and Childbirth

STUDY PROTOCOL Open Access

Use of metabolomics for the identification and validation of clinical biomarkers for preterm birth: Preterm SAMBA

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Abstract

Background: Spontaneous preterm birth is a complex syndrome with multiple pathways interactions determining its occurrence, including genetic, immunological, physiologic, biochemical and environmental factors. Despite great worldwide efforts in preterm birth prevention, there are no recent effective therapeutic strategies able to decrease spontaneous preterm birth rates or their consequent neonatal morbidity/mortality. The Preterm SAMBA study will associate metabolomics technologies to identify clinical and metabolite predictors for preterm birth. These innovative and unbiased techniques might be a strategic key to advance spontaneous preterm birth prediction.

Methods/design: Preterm SAMBA study consists of a discovery phase to identify biophysical and untargeted metabolomics from blood and hair samples associated with preterm birth, plus a validation phase to evaluate the performance of the predictive modelling. The first phase, a case–control study, will randomly select 100 women who had a spontaneous preterm birth (before 37 weeks) and 100 women who had term birth in the Cork Ireland and Auckland New Zealand cohorts within the SCOPE study, an international consortium aimed to identify potential metabolomic predictors using biophysical data and blood samples collected at 20 weeks of gestation. The validation phase will recruit 1150 Brazilian pregnant women from five participant centres and will collect blood and hair samples at 20 weeks of gestation to evaluate the performance of the algorithm model (sensitivity, specificity, predictive values and likelihood ratios) in predicting spontaneous preterm birth (before 34 weeks, with a secondary analysis of delivery before 37 weeks).

Discussion: The Preterm SAMBA study intends to step forward on preterm birth prediction using metabolomics techniques, and accurate protocols for sample collection among multi-ethnic populations. The use of metabolomics in medical science research is innovative and promises to provide solutions for disorders with multiple complex underlying determinants such as spontaneous preterm birth.

Keywords: Spontaneous preterm birth, Metabolomics, Prediction, Biological biomarker, Mass spectrometry

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Background

Despite improvements in antenatal and neonatal care, the number of premature newborns each year has not significantly decreased since the 1960s. Preterm birth (PTB) is the leading cause of neonatal morbidity and mortality and a major contributor to loss of life, long-term disability, and health care costs [1–4]. The associated morbidity, mortality and high health costs have been well documented with premature infants facing life-threatening short and long term complications [5–8].

Despite the enormity of the health economic burden of preterm birth, and many years of focused research, a common aetiology and/or predictive test have not yet been identified. Spontaneous preterm birth (sPTB) is considered one of the "Great Obstetrical Syndromes", which are conditions resulting from complex interactions between the maternal and fetal genome and the environment and which have a long preclinical period, fetal involvement and adaptive functioning in nature [9]. This reflects the multifactorial nature of this condition and the need to apply strategies that are capable of identifying multiple markers simultaneously in parallel with the assessment of clinical and biophysical risk factors.

There are many clinical [10–13] and biochemical risk factors [14–16] associated with sPTB and it is likely that these biochemical markers are present in the maternal blood long before the onset of a preterm labour. However, although certain candidate-driven approaches to studying these changes show promise, this has not resulted in effective predictive biomarkers for the general pregnant population. Due to these complex and dynamic characteristics of sPTB syndrome, it remains a difficult task to identify women and babies at risk.

Currently, the selection of women likely to deliver prematurely from clinical risk factors alone lacks the sensitivity required to effectively identify the majority of patients at risk of idiopathic sPTB [14]. Furthermore, parameters derived from previous obstetric history cannot be applied to nulliparous women. The association of biophysical predictors such as cervical length and/or vaginal biomarkers (fibronectin and phosphorylated insulin-like growth factor binding protein-1) enhances accuracy for prediction and enables more effective interventions for selected women. There are therapeutic interventions available for the prevention of sPTB, such as the use of progesterone [10, 17, 18] and cervical pessary [19]. Despite advances in selection of eligible women for such therapeutic interventions, the efficacy of cervical length or fetal fibronectin levels in asymptomatic women are still limited and seem to be more capable of discriminating women at lower risk than those at higher risk [20–22]. Owen et al. showed that almost 50 % of women with cervical length between 15 and 25 mm did not deliver before 35 weeks, as well as approximately 70 % with cervical length between 25–30 mm [23].

The development of a predictive test for spontaneous preterm birth would help to accurately identify a high-risk population. To be effective, therapies need to be commenced at a gestational age in which they are likely to be of benefit. A sensitive early pregnancy-screening test would facilitate the timely administration of prophylactic treatments to those women at highest risk. The development of physics, biology and medicine translational research can provide a comprehensive approach for biological processes with complex pathways and regulations. Metabolomics offers an unbiased hypothesis generating approach to identify and validate potential candidate metabolomic biomarkers [24, 25].

We propose a multi-strategy approach to biomarker discovery and validation through the establishment of a large early pregnancy biobank of appropriate samples, in conjunction with the application of analytical methods capable of quantifying multiple blood-borne species simultaneously, and using some clinical and epidemiological markers to identify women at highest risk of spontaneous preterm birth.

The development of predictive tests that translate into clinical care can be divided into two distinct phases; (i) hypothesis generation after acquisition of data, a non-biased process where no or limited biological knowledge is required and (ii) validation of generated hypotheses [26]. The Preterm SAMBA study goal spans both phases and aims to identify a clinically useful early pregnancy-screening test to ascertain which pregnancies are at risk of developing sPTB. Discovery-based methods will be applied to blood and hair samples taken from carefully matched phenotypes in both cohorts (preterm and term deliveries) to develop a predictive algorithm to identify those women at increased risk of sPTB and test the effectiveness of such an algorithm in a prospective cohort.

Methods/design

Preterm SAMBA, an international collaborative multicentre study for the development of predictive tests that translate into clinical care, can be divided into two distinct phases: The first component (Discovery phase) is a case–control study that aims to identify clinical and metabolomics biomarkers related to spontaneous preterm birth. For this initial phase, untargeted metabolomics techniques will be employed to identify and quantify potential predictor's metabolites that can be associated to potential clinical predictors. The second component (Validation phase) is a cohort study developed to validate the algorithm of prediction using the clinical and metabolomics biomarkers discovered in

the first component of the study. Thus, to evaluate the performance of the prediction model developed at the first phase, targeted metabolomics techniques will be employed to analyse participants' blood and hair samples to quantify those specific metabolites identified as potential predictors of preterm birth.

Discovery phase

The initial phase of the project consists of a case–control study utilizing data and samples collected for the SCOPE study (Screening for Pregnancy Endpoints study). The SCOPE consortium was an international effort to determine the causes and potential predictors for pregnancy complications and its methodology had already been previously published [27–29]. Briefly, the cohort comprised 5690 healthy pregnant women recruited between November 2004 and August 2008 in New Zealand, Australia, Ireland and United Kingdom. Inclusion and exclusion criteria for the SCOPE study are described in Tables 1 and 2, respectively. Exclusion criteria include major fetal anomaly, chronic hypertension, diabetes, renal disease, systemic lupus erythematous, and antiphospholipid syndrome. These will therefore be the same criteria for the current study.

Extensive sociodemographic and physical data will be collected including age, ethnicity, socio-economic status, dietary and lifestyle questionnaire, parity, BMI (body mass index) and cigarette smoking.

Plasma and serum samples will be collected at 20 weeks of gestation using stringent standard operating procedures designed for metabolomics studies, barcoded and stored at −80 °C within 2–4 h; the timing between collection and freezing will be known for all specimens.

Several Standard Operating Procedures (SOP) for sample preparation by removal of proteins via ultrafiltration were developed and validated. The analysis of deproteinized plasma samples will be performed employing gas chromatography and liquid chromatography mass spectrometry (GC-MS and LC-MS). GC-MS and LC-MS techniques will be performed as described previously [30, 31]. Quality control samples (acquired by pooling plasma from all subjects) will be interspersed in every 5th run to assess reproducibility and validity. It is envisaged that the socioeconomic/physical/biomarker discovery phase of the Preterm SAMBA study will identify several candidate markers and predictive multivariate models. Discriminatory metabolites will be translated to a targeted triple quad MS (QQQ-MS) platform, to be used in the validation phase.

Table 1 Inclusion criteria of Preterm SAMBA validation phase – Brazilian cohort

• Singleton pregnancy
• Nulliparous (no previous delivery ≥20 weeks)
• Up to 21 weeks of gestational age

Table 2 Exclusion criteria of Preterm SAMBA validation phase – Brazilian cohort

• Unsure LMP and unwilling to have dating US	• Major Uterine Anomaly
• ≥3 Miscarriages	• Cervical Suture
• Major Foetal Anomaly/Abnormal Karyotype	• Knife cone biopsy
• Essential Hypertension Treated Pre-pregnancy	• Ruptured membranes now
• Mod-Severe Hypertension at booking (≥160/100 mmHg)	• Long term Steroids
• Pre-pregnancy Diabetes	• Low-dose Aspirin
• Renal Disease	• Calcium (>1 g/24 h)
• Systemic Lupus Erythematosus	• Eicosapentaenoic acid (fish oil)
• Anti-phospholipid Syndrome	• Vit. C ≥1000 mg & Vit. E ≥400 UI
• Sickle Cell Disease	• Heparin/LMW Heparin
• HIV or Hep B or Hep C positive	

The proportion of pregnancies complicated by any preterm birth is approximately 10 %. Preterm SAMBA Discovery Phase will randomly select 100 women (n = 100) whose pregnancies reached term as compared to fifty randomly sampled pregnancies (n = 50) complicated by spontaneous preterm birth prior to 37 weeks gestation, in each of the Cork Ireland and Auckland New Zealand SCOPE cohorts (Fig. 1). The 20th week samples and data will be analysed to identify sPTB potential predictors. Considering there were no previous studies on this topic for preterm birth, these numbers were empirically estimated using a similar study performed for pre-eclampsia [31]. Using a type I error of 0.01, type II error of 0.10, a ratio between controls and cases of 1:1, an AUC of 0.9 and an OR of 10, the estimated sample of preterm birth is 49. We then anticipated around 50 preterm birth for each of the two centers.

Validation phase – the Brazilian multicentre cohort study

The Preterm SAMBA validation phase consists of a Brazilian multicentre cohort study with 1150 low-risk pregnant nulliparous women. Five of the 27 members of the Brazilian Network for Studies on Reproductive and Perinatal Health (BNSRPH), were chosen to participate in the Brazilian cohort (Table 3). Previous excellence performance in epidemiological and translational studies and diversity of cultural, ethnical and sociodemographic population characteristics were criteria for centre selection. Therefore, there are participating centres in three of the five regions of Brazil, which are the three most populated regions of the country: Northeast, Southeast and South.

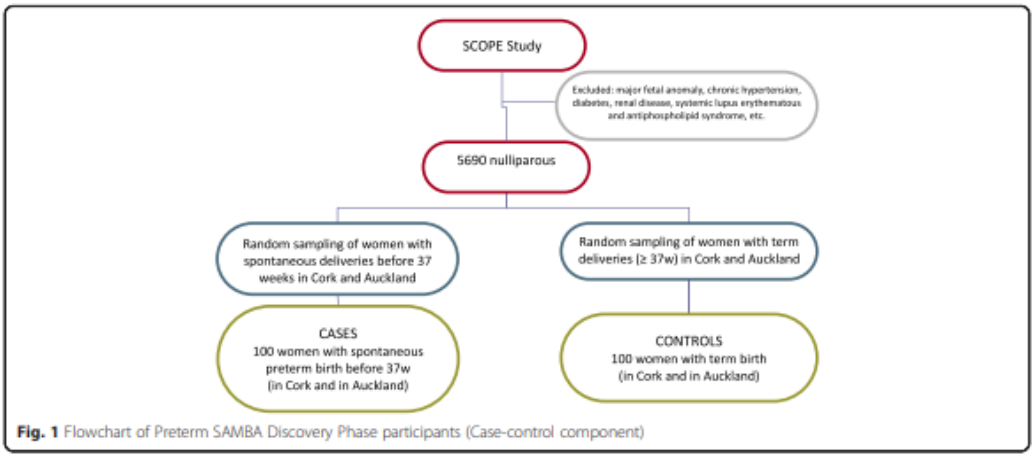

Fig. 1 Flowchart of Preterm SAMBA Discovery Phase participants (Case-control component)

Assuming a type I error rate, α, of 5 % and an estimated area under ROC curve of at least 0.68, then in order to test hypotheses to a suitable level of power (80 % power, $\beta = 0.2$), the sample size sufficient should approximate to 80 cases of spontaneous preterm birth (<34 weeks gestation), calculated using MEDCALC®. Based on a minimum expected preterm birth rate of 7 %, the total cohort size should therefore be of approximately 1150 subjects, around 230 women at each participating centres.

Recruitment and data collection

The recruitment strategies include approaching existing pregnant women in participating facilities during prenatal care visits and with website/internet, flyers and local community advertisings. After the identification of potential participants, the research assistant will invite women and obtain an informed consent form of those who meet the inclusion criteria and agree to participate. Maternal age and ethnicity will be recorded from all approached women to facilitate a comparison of those who are recruited and those who decline.

Table 3 Participating centres in the Preterm-SAMBA study validation phase – Brazilian cohort

Maternity of CAISM, University of Campinas, in Campinas, São Paulo.
Maternity of the School of Medicine from UNESP, in Botucatu, São Paulo.
Maternity of the Clinic Hospital, Federal University of Rio Grande do Sul, in Porto Alegre, Rio Grande do Sul.
Maternity of the Clinic Hospital, Federal University of Pernambuco, in Recife, Pernambuco.
MEAC – School Maternity of the Federal University of Ceará, in Fortaleza, Ceará.

All collected data will preferably be entered directly into the database, but printed forms will also be available in case of inability to access the internet-based database. In such cases, the data will be then entered later and completed printed forms will be stored, according to the required ethical principles.

Sociodemographic, physical data and pregnancy outcomes

First Visit (19–21 weeks): similarly to the SCOPE study, detailed information of sociodemographic characteristics (age, socioeconomic status, education, ethnicity, occupation and type of maternity care), maternal medical and obstetric history, infertility history, drugs and medications use, family medical and obstetric history and current pregnancy (occurrence and details of infection, vaginal bleeding, dipstick proteinuria, intercourse and hospital admission) will be collected.

Anthropometric measurements of maternal body mass index, height, weight, head circumference, arm circumference and triceps, biceps subscapular and suprailiac skinfolds will be performed according to standardized techniques. Height and weight of lightly clothed women will be measured to the nearest 0.1 mm and 0.1 kg respectively. Head and arm circumferences will be measured with an inelastic tape and skinfold thicknesses will be measured on the same side of the body to the nearest 0.2 mm using Harpenden (and/or Lange) skinfold calliper. The calliper is placed 1 cm distal to the firmly grasped skinfold, using the thumb and the index finger, at 90° to the skin. A single measurement is taken after 2 s.

Dietary intake will be assessed using a 24-h dietary recall administered by a trained professional who will query participants about food and beverage consumption

in the previous 24 h. A trained nutritionist will then estimate calories, macro and micronutrient intake using computer-based standard tables allowing for appropriate ethnic, social and regional variations.

Furthermore, three consecutive manual blood pressure measurements will be recorded, using an appropriate cuff size for different arm circumferences and using Korotkoff phase V for diastolic blood pressure.

Second and third Visit (27–29 weeks and 37–39 weeks; both optional): three consecutive manual blood pressure measurements, anthropometric parameters (weight, height, head and arm circumference and triceps, biceps, subscapular and suprailiac skinfolds) and occurrence and characteristics of infection, vaginal bleeding, dipstick proteinuria, intercourse and hospital admissions will be recorded.

Postpartum data: data will be collected from the participant's medical record, the prenatal chart and/or from a personal interview with the participant during hospital admission to minimize missing information. The main outcome is spontaneous preterm birth, defined as a birth before 34 weeks of gestational age due to preterm labour or premature rupture of membranes. Secondary outcomes will also be evaluated: spontaneous preterm birth alternatively defined as a birth before 37 weeks of gestational age due to preterm labour or premature rupture of membranes, provider-initiated preterm birth, defined as preterm birth due to medical indication on account of maternal or fetal conditions; pre-eclampsia, defined as having systolic blood pressure ≥ 140 or systolic blood pressure ≥ 90 mmHg after 20 weeks gestation on at least two occasions apart of 20 min, and/or proteinuria (24-h urinary protein ≥ 300 mg or urine dipstick ≥ ++) and/or severe maternal complications [32]; gestational diabetes mellitus according to ADA guidelines [33]; fetal growth restriction (FGR) defined as having birthweight below 10th percentile based on GROW customised birthweight centiles [34]. Clinical data will also be collected regarding the occurrence of preterm labour, cervical cerclage, deep vein thrombosis, infection, vaginal bleeding, dipstick proteinuria, intercourse, hospital admission, deep vein thrombosis during pregnancy and puerperium, and maternal mortality and the use of progesterone and/or pessary, tocolytic, antibiotic for preterm labour or pPROM, corticosteroids for fetal maturation, magnesium sulphate for neuroprotection during pregnancy. The occurrence of severe maternal morbidity and near miss will also be reported according to WHO guidelines [35]. Neonatal outcomes related to neonatal morbidity and mortality will be recorded until newborn discharge or death.

Sample collection, processing and storage

Non-fasting blood samples will be collected at 20 (between 19 and 21) weeks of gestational age (Fig. 2). All research assistants will be trained according to specific and detailed Standard Operation Protocols (SOPs) developed for sample collection, processing and storage. One of the study coordinators was trained by the SCOPE team, to guarantee adequate understanding of all necessary procedures. A maximum of 20 mL of blood will be collected to provide serum and plasma specimens. The blood specimens will be stored in 250 μL 2-D barcoded cryovials after one centrifugation cycle for plasma specimens (2000×g for 10 min at 4 °C) and two centrifugation cycles for serum (2000×g for 10 min and 2400×g for 10 min at 4 °C). The blood samples will be processed and frozen at −80 °C within 2–4 h. The time interval between collection and freezing will be recorded for all specimens.

Although the Preterm SAMBA strategy and workflow is focussed around the analysis of blood samples, we recently reported a proof-of-concept study, which highlighted the potential use of the hair metabolome in the prediction of pregnancy complications [36]. Hair samples will also be collected at 20 weeks' gestation. Samples (20–30 hair strands for each participant) will be collected from the occipital area, 0.5 cm away from the scalp, using blunt scissors. Then, hair will be packed in aluminium foil and stored at room temperature [36]. A unique linear barcode will be pasted on each hair package. All specimens and quality control information will be registered in the database.

Database

A specific database for the Preterm SAMBA Brazilian Cohort was developed together with MedSciNet, a Swedish based company specialized in the design and

Fig. 2 Visits of Preterm-SAMBA Validation Phase

development of online database systems linked with biobanks management systems, similarly to the database previously used for the SCOPE. The preterm SAMBA database will be centralized, secure, internet-based and FDA (United States Food and Drug Administration) and HIPAA (Health Insurance and Accountability Act of 1996, United States Security and Privacy Rules) compliant, which allows continuous data entry and monitoring of study progress. Completeness of clinical data and specimen collection will be constantly monitored, with incomplete fields 'flagged' for attention. The database allows several monitoring procedures with hierarchical access licenses and tracking system for all specimen aliquots stored. To comply with biobank regulatory issues, patients will only be identified by a unique study number. Pseudoanonymised metadata and interim data will be stored using our laboratory information management system. The identifying information about participants will be kept in a separate and secure local database.

Data and sample quality

Several procedures to enhance and assure data and sample quality will be adopted. All entered data will be prospectively and retrospectively monitored. During data entry, internal consistency of variables is performed and error messages are automatically flagged. After completing the collection of data from a participant, all information needs to be reviewed by a local monitor. Then, the final form has to be signed by the local principal investigator (PI) in order to be incorporated in the final database. The coordinating centre (Campinas, Brazil) will also perform a centralized monitoring of data and samples. An initial meeting with all researchers from Brazilian participating centres has been held to discuss the final protocol, procedures to be implemented, their particular characteristics and necessary approaches to be used to guarantee the implementation of the study. Another general meeting at the end of study is planned in order to discuss results, strategies for manuscripts' writing and submission and other related topics.

The coordinating centre will randomly select approximately 10 % of printed completed forms to carry out a check and validation of data from the forms and database entry during the first and second half of the study. This double-check procedure enhances data quality and decreases typing errors.

The record of information regarding sample collection, processing (precentrifugation and centrifugation) and storage processes will follow the Standard Preanalytical Coding for biospecimens (SPRECs) protocol, developed and recommended by the International Society for Biological and Environmental Repositories Biospecimen (ISBER) Science Working Group [37]. This protocol enables standardization of preanalytical information, using standard codes to refer to the techniques and conditions to which the samples were submitted.

Metabolomics analysis

The precise methodology to be used in the validation phase will depend on the ongoing discovery studies. As detailed above we anticipate that it will be based on a targeted triple quad MS (QQQ-MS) platform, as previously described [38]. We will subsequently describe details relating to metabolomics analysis techniques and metabolomics statistical analyses. We anticipate that data analysis will be integrated into the relational database such that decision rules may combine both clinical and spectrometric data.

The performance of the final algorithm developed in the discovery phase will be evaluated by its capacity to predict spontaneous preterm birth occurrence in women from the Brazilian cohort. The validation will be performed using the average squared difference between predicted and observed outcome (R^2), adjusted R^2 (same as R^2, but penalizes for the number of predictors), sensitivity, specificity, positive and negative predictive values, likelihood ratio and the area under the ROC curve.

Ancillary studies

The Preterm SAMBA Brazilian cohort study will collect additional data regarding other relevant maternal and fetal obstetric complications. Detailed clinical data related to the occurrence and severity of pre-eclampsia, fetal growth restriction and gestational diabetes mellitus will be recorded. Fetal growth restriction will be diagnosed if birthweight is below 10th customised percentile. The occurrence of severe maternal morbidity, maternal near miss and maternal mortality during pregnancy or up to discharge after delivery will also be recorded, according to WHO definitions [35].

Ethical aspects

The SCOPE study, whose data and samples will be analysed for the Preterm SAMBA discovery phase, was approved by local ethics committees in New Zealand and Ireland and registered in the Australian and New Zealand Clinical Trial Registry (ACTRN12607000551493) [28]. All women who participated in the SCOPE study provided written informed consent and agreed to have their data and samples used in other studies. The Preterm SAMBA study has been reviewed and approved by the National Committee for Ethics in Research of Brazil (CONEP) and by the Institutional Review Board (IRB) of the coordinating centre (Letter of approval 1.048.565 issued on 28th April 2015) and of all other Brazilian participating centres. All women who will be enrolled in the

Preterm SAMBA Brazilian cohort (Validation phase) will sign an informed consent form, also allowing for future additional studies with their biological samples without any additional consent.

The ethical principles stated in the Brazilian National Heath Council (Resolution CNS 466/12) will be respected in every stage of this study. The anonymity of the source of information will be guaranteed and the care for the women will be provided independent of her agreement to participate in the study. All ethical principles related to biobank storage and transport will be followed according to national and international rules related to research with human beings. The study also complies with the Declaration of Helsinki amended in Hong Kong in 1989. The methodological and ethical aspects of Preterm SAMBA study protocol were developed following STROBE guidelines [39].

Discussion

The *"Omics"* Science comprises genomics, transcriptomics, proteomics and metabolomics technologies, which each provide valuable translational surveys in biological processes. A metabolomics approach enables the evaluation of metabolic pathways and the correlation of biochemical changes related to pathophysiology of disease, providing a downstream result of gene expression and higher sensitivity to phenotype of disease [40–45]. Underlying conditions and factors related to the occurrence, severity or prognosis of diseases with complex determinants may be assessed, bringing to light the final product of organism metabolism: the metabolome [42].

The development of a two-phase metabolomics research program that includes two large cohorts of nulliparous women is not an easy task. The network collaboration is essential to develop, implement and analyse such complex data and, more importantly, to achieve reliable results. Precise protocols for sample collection, processing, storage and biobank management will be essential to assure high quality data and results.

Metabolomics profiling requires different techniques to address the detection and quantification of different classes of metabolites once there is no current method capable to identify all of them. Preterm SAMBA study will employ different untargeted techniques that require very carefully and standardized protocols for sample preparation [24]. Studying the metabolome in blood samples requires invasive collection and immediate processing. As an alternative, hair samples are non-invasive, do not need processing methods and can be stored at room temperature. Hair can, theoretically, reflect endogenous compounds and environmental exposures from many days/weeks ago. The determination of the hair metabolome is a possible approach to identify biomarkers for spontaneous preterm birth. It has already been explored in gestational diabetes and fetal growth restriction, revealing potential endogenous mechanisms involved in those pathologic conditions [36, 45].

The identification of spontaneous preterm birth predictors using multi-ethnic data/samples and the evaluation of performance in a culturally and ethnically different population is desirable and meaningful for external validation. The use of quality control records and SPREC protocol is another important recommendation for metabolomics studies due to the necessity to evaluate confounders for analytical measures such as the time between sample collection, storage and processing conditions and the occurrence of haemolysis, lipaemia and metabolic degradation on account of inadequate temperature or solar exposure [37].

In the context of translational research, metabolomics may enhance understanding of the underlying pathways, which lead to obstetric complications. Preterm SAMBA aims to identify and validate a predictive model for spontaneous preterm birth, but will also develop a biobank and database that will enable research on pre-eclampsia (PE), fetal growth restriction (FGR) and gestational diabetes mellitus (GDM). The possibility to combine biochemical, genetics and clinical information that can be large-scale and replicable empowers the development of knowledge for clinical practice in preterm birth prevention. This would be especially worthwhile and helpful for countries with a high proportion and high absolute number of preterm births as is the case of Brazil where around 12 % of all births occur prematurely [46].

A recent clustered designed study showed that 30 % of all spontaneous preterm births do not have any maternal, fetal or placental conditions identified that could be related to its occurrence [47]. The application of metabolomics techniques could be a promising approach for spontaneous preterm birth prediction, all the more in those cases of silent phenotype in which there are no known predictors. Metabolomics have been already described in other obstetric conditions as pre-eclampsia, gestational diabetes mellitus and fetal growth restriction [31, 40, 41, 45, 48, 49], showing excellent performance in terms of a discriminatory algorithm. Therefore, we believe metabolomics is a powerful and strategic key not only for preterm birth prediction, but hopefully also for its prevention. The detection of metabolic pathways related to PTB syndrome may enable the development of more accurate therapies for primary or secondary prevention of pregnant women identified as at high-risk.

At the end of the study, if we are successful in the identification of such an effective algorithm, certainly several other topics should be carefully considered.

Can this knowledge be really translated into a commercially available kit for screening purposes? Would the costs derived from this process be acceptable for low and middle-income countries? How will this be made available for populations in public sector? For discussing a future implementation of such a screening strategy, the following necessary points to be covered are to know if a concrete package of interventions to reduce preterm birth among those women identified as high-risk is available, and if it is cost-effective to be supported by the public health system. Finally, in this study we are planning to transfer the technology developed for the algorithm from New Zealand to Brazil, including lab technologies for assessing the biomarkers identified by metabolomics for preterm birth. Hopefully, if this is proved to be feasible, we believe that an important step for reducing the burden of preterm birth will have been achieved.

Abbreviations

ADA, American Diabetes Association; BMI, body mass index; CONEP, Brazilian National Committee for Ethics in Research; FAME, fatty acid methyl esterification; FDA, Food and Drug Administration; FGR, fetal growth restriction; GC, gas chromatography; GDM, gestational diabetes mellitus; LC, liquid chromatography; MS, mass spectrometry; PE, pre-eclampsia; PI, principal investigator; pPROM, preterm premature rupture of membranes; PTB, preterm birth; SAMBA, Screening and Metabolomics in Brazil and Auckland; SCOPE, Screening for Pregnancy Endpoints study; SOP, standard operation procedures; sPTB, spontaneous preterm birth; WHO, World Health Organization

Acknowledgements

The Preterm SAMBA study group also included: Mary A. Parpinelli, Jussara Mayrink, School of Medical Sciences, University of Campinas, Brazil; Kleber G. Franchini, LNBio, Campinas, Brazil; Iracema M. Calderon, Bianca F. Cassettari, School of Medicine of Botucatu, UNESP, Brazil; Janete Vetorazzi, Lucia Pfitscher, School of Medicine, Federal University of Rio Grande do Sul, Porto Alegre, Brazil; Edilberto P. Rocha Filho, Débora F Leite, School of Medicine, Federal University of Pernambuco, Recife, Brazil; Francisco E. Feitosa, Carolina L. Costa e Silva, School of Medicine, Federal University of Ceará, Fortaleza, Brazil.
The SCOPE study group also included: Lucilla Poston, King's College London and King's Health Partners, London, UK; Jenny E. Myers, Maternal & Fetal Health Research Centre, University of Manchester, UK; Nigel A.B. Simpson and James J Walker, University of Leeds, UK; Gus A. Dekker and Claire T. Roberts, University of Adelaide, South Australia.

Funding

This was one of the two big studies selected for sponsoring from the research call "Grand Challenges Brazil: Reducing the burden of preterm birth" number 05/2013 jointly issued by the Brazilian National Research Council (CNPq) and the Bill and Melinda Gates Foundation (Award 401636/2013-5). The funders played no role at all in the study design, writing the manuscript nor in the decision to submit the manuscript for publication. LC Kenny is supported by a Science Foundation Ireland Program Grant for INFANT (12/RC/2272). The SCOPE database is provided and maintained by MedSciNet AB (http://medscinet.com). The New Zealand SCOPE study was funded by the New Enterprise Research Fund, Foundation for Research Science and Technology; Health Research Council (04/198); Evelyn Bond Fund, Auckland District Health Board Charitable Trust. The Irish SCOPE study was funded by the Health Research Board of Ireland (CSA/2007/2; http://www.hrb.ie).

Authors' contributions

All authors contributed to the overall study design and specific methodologies. JGC, PNB, MLC and RCP conceived the study design. RTS, JGC, RCP and RP planned the implementation of the study. RTS and JGC drafted the manuscript. KS, LCK and SVB participated in the design of the methodomics methods for essays. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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Received: 5 March 2015 Accepted: 4 August 2016
Published online: 08 August 2016

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9.6. Anexo 2. Aprovação no CEP

INSTITUTIONAL REVIEW
BOARD OF UNICAMP
CAMPINAS CAMPUS

LETTER OF APPROVAL - IRB

DEATILS OF RESEARCH PROJECT

Research Title: Use of metabolomics for the identification and validation of clinical biomarkers for preterm birth

Investigator: Jose Guilherme Cecatti

Subject Area:

Version: 3

CAAE: 38522214.8.1001.5404

Proponent Institution: Hospital da Mulher Prof. Dr. José Aristodemo Pinotti - CAISM

Main Sponsor: Ministry of Science, Technology and Innovation

Bill & Melinda Gates Foundation

DETAILS OF THE LETTER OF APPROVAL

Letter of Approval Number: 1.048.565

Date of Rapporteurs: 28/04/2015

Project Introduction:

The presented amendment requests for the inclusion of two new centers in the study. It was necessary to modify the local investigators responsible for two of the participating centers UFPE – Recife and UFRGS – Porto Alegre.

The aim of this study is to develop a preterm birth screening test using biomarkers with relevant clinical use, comprising the development and validation phases of the study. The aim is to identify, early in pregnancy, women at higher risk for preterm birth, which could contribute to accurate and timely interventions to reduce the occurrence of adverse maternal and perinatal outcomes related to prematurity. This topic has increased in importance in the Brazilian and world scenarios because of the striking consequences of prematurity. Methods: The study will consist of two components: a development component, covered by a case-control study using women who participated in the SCOPE study, an international cohort that collected samples at 15 weeks of gestation of 5690 nulliparous women, analyzing two groups: Cases were those with data and samples from women who had spontaneous preterm birth before 34 weeks, and control group, composed of women who progressed to term delivery. The metabolomics profile will be analyzed along with sociodemographic data to develop a preterm birth predictive model. The other component of the study will be

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a five Brazilian' centers cohort. Blood and hair samples will be collected at 20 weeks of gestational age for metabolomics analyzes, as well as sociodemographic data, data related to current pregnancy, childbirth, postpartum and data related to maternal and neonatal outcomes. Both phases will occur simultaneously. Thus, the results of the development component will not be available before the end of the cohort study. Therefore, the evaluation of maternal and perinatal outcomes of cohort component using the predictive model generated by the development component (case-control study) will be retrospective. The metabolomics, high-tech samples analyses science, will initially be held at the University of Auckland, New Zealand. The study provides an international consortium involving the coordinating center, the University of Auckland and the Brazilian Laboratory LNBio for the transfer of technology to enable metabolomics analyses of study samples by Brazilian laboratory. 1150 low-risk nulliparous women will be included in the Brazilian cohort, approximately 230 in each participating center. Data Analysis: The analysis of the first component will be accomplished through sophisticated statistical processes using MetaboAnalyst® platform. The analysis of the second component is basically the diagnostic validation analysis of the predictive model using estimation of sensitivity, specificity, predictive values and likelihood ratios.

Objectives of the Research:

Primary Objective: To develop and validate a predictive algorithm to identify pregnant women at higher risk for preterm birth.

Secondary Objectives: 1. Identify a set of metabolites markers related to preterm birth in nulliparous women. 2. Develop a predictive algorithm for preterm birth including metabolomics, clinical and/or sociodemographic markers. 3. Validate the prediction obtained by the algorithm with maternal and neonatal outcomes in other group of nulliparous women.

Evaluation of risks and benefits:

Risks: It is noteworthy that the study will not perform any kind of intervention, advocating, in the cohort component, just biological sample collection (blood and hairs) at 20 weeks and collecting clinical and medical records information as established protocols. Potential minimal risks refer to collection of blood and hairs. It will be guaranteed the confidentiality of the source of information.

Benefits: The study does not bring immediate benefits to participants. However, the implementation of an effective algorithm preterm birth predictor in early gestational age would bring great benefits in the systematization of obstetric and neonatal care. The identification of higher risk population in the proposed gestational age (twenty weeks) provide a wide intervention window, starting early in the second trimester. New approach prospects in future studies may be generated if the results from this study are not able to effectively predict the populations at risk for preterm birth.

Comments and Considerations about the Research:

This is a multicenter research Project from the School of Medical Sciences of UNICAMP to be held at CAISM/UNICAMP. This study will have two components, one retrospective, which includes a case-control study using women who participated in the SCOPE study, an international cohort that collected samples at 15

weeks of gestational age of 5690 nulliparous women, in order to develop a predictive model for preterm birth, and one component for the predictive model validation, which will be a cohort of Brazilian women from five participating centers, where blood and hairs will be collected from 230 women. The project is sponsored by the Ministry of Science, Technology and Innovation and Bill and Melinda Gates Foundation. Potential minimal risks refer to blood and hair collection and the study does not bring direct benefits to participants. Biological samples will be sent to abroad for analysis. We consider that the research is pertinent, of great social relevance and supported by the literature.

Considerations of the Obligatory Terms:

The Research Project, the Consent Form, the Cover Page and the Research Committee approval by CAISM had already been submitted. The researcher answered the pending issues presented by this Institutional Review Board (IRB), namely: 1) Consent Form. 1.1) Researchers should be located not only by phone or email, but in his professional address, highlighting the department or unit where they can be located. PENDENCY ANSWERED. 1.2) Make it clear that the IRB contact is for any claims and/or complaints regarding the ethical aspects of research. PENDENCY ANSWERED. 1.3) Inform address and email of the IRB, not only the phone number. PENDENCY ANSWERED. 1.4) Make it clear that the participant will have no financial benefit. PENDENCY ANSWERED. 2) Letter of authorization and/or consent of the other participant centers. PENDENCY ANSWERED. 4) Append rules governing biobank for new samples. PENDENCY ANSWERED.

Reassessing of pending 3) placed in the previous Letter of Approval ("Should the project be sent to the Brazilian National Review Board (CONEP) since the study will send samples abroad"). According to Resolution 466, the need to apply the project to CONEP is when "sending genetic material abroad or any human biological material to obtain genetic material, except in cases where there is cooperation with the Brazilian government, including approved funding".

Recommendations:

No.

Conclusions or Pendencies and List of Inadequacies:

The amendment is justified by the need to modify two local investigators responsible for two of the participating center UFPE – Recife and UFRGS – Porto Alegre.

Situation of the Letter of Approval:

Approved.

Need for CONEP Evaluation:

No.

IRB Final Considerations:

INSTITUTIONAL REVIEW
BOARD OF UNICAMP
CAMPINAS CAMPUS

CAMPINAS, 5 May 2015.

_____

Signed by:

Renata Maria dos Santos Celeghini

(IRB Coordinator)

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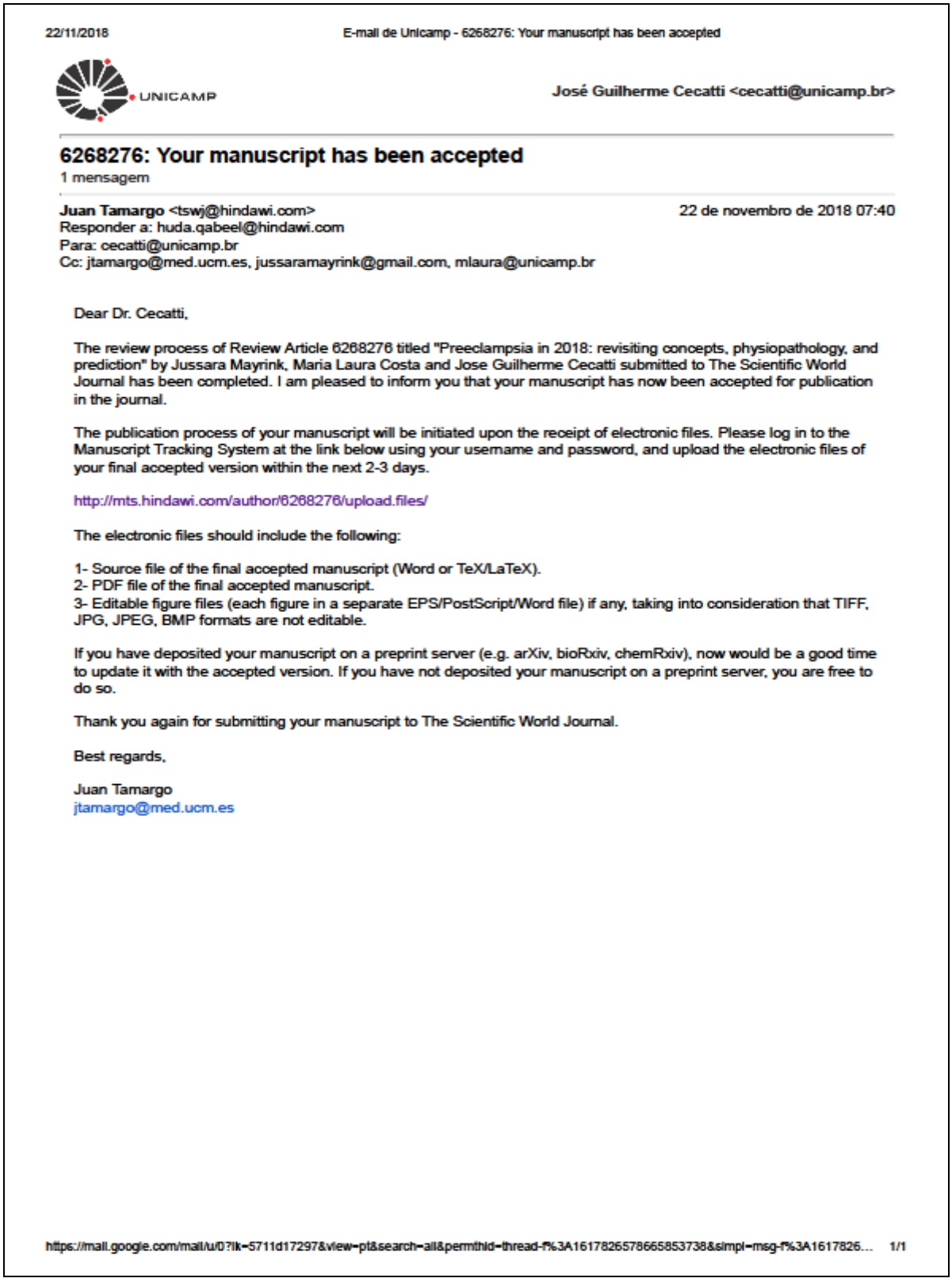
8.3. Anexo 3. Comprovante do envio do artigo 1



22/11/2018 E-mail de Unicamp - 6268276: Your manuscript has been accepted

UNICAMP

José Guilherme Cecatti <cecatti@unicamp.br>

6268276: Your manuscript has been accepted

1 mensagem

Juan Tamargo <tswj@hindawi.com> 22 de novembro de 2018 07:40
Responder a: huda.qabeel@hindawi.com
Para: cecatti@unicamp.br
Cc: jtamargo@med.ucm.es, jussaramayrink@gmail.com, mlaura@unicamp.br

Dear Dr. Cecatti,

The review process of Review Article 6268276 titled "Preeclampsia in 2018: revisiting concepts, physiopathology, and prediction" by Jussara Mayrink, Maria Laura Costa and Jose Guilherme Cecatti submitted to The Scientific World Journal has been completed. I am pleased to inform you that your manuscript has now been accepted for publication in the journal.

The publication process of your manuscript will be initiated upon the receipt of electronic files. Please log in to the Manuscript Tracking System at the link below using your username and password, and upload the electronic files of your final accepted version within the next 2-3 days.

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The electronic files should include the following:

1- Source file of the final accepted manuscript (Word or TeX/LaTeX).
2- PDF file of the final accepted manuscript.
3- Editable figure files (each figure in a separate EPS/PostScript/Word file) if any, taking into consideration that TIFF, JPG, JPEG, BMP formats are not editable.

If you have deposited your manuscript on a preprint server (e.g. arXiv, bioRxiv, chemRxiv), now would be a good time to update it with the accepted version. If you have not deposited your manuscript on a preprint server, you are free to do so.

Thank you again for submitting your manuscript to The Scientific World Journal.

Best regards,

Juan Tamargo
jtamargo@med.ucm.es

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8.4. Anexo 4. Comprovante do envio do artigo 2

UNICAMP

José Guilherme Cecatti <cecatti@unicamp.br>

Notification to co-authors of submission to Systematic Reviews SYSR-D-18-00442

1 mensagem

Systematic Reviews Editorial Office <em@editorialmanager.com> 2 de novembro de 2018 04:43
Responder a: Systematic Reviews Editorial Office <judy.maturan@springer.com>
Para: Jose Guilherme Cecatti <cecatti@unicamp.br>

SYSR-D-18-00442
Metabolomics for prediction of pregnancy hypertensive disorders: a systematic review and metanalysis protocol
Jussara Mayrink; Debora F Leite; Maria L Costa; Jose Guilherme Cecatti

Dear author:

You are receiving this email because you have been listed as an author on a manuscript recently submitted to Systematic Reviews. The manuscript details are below.

Title: Metabolomics for prediction of pregnancy hypertensive disorders: a systematic review and metanalysis protocol
Authors: Jussara Mayrink; Debora F Leite; Maria L Costa; Jose Guilherme Cecatti
Corresponding author: M.D Jussara Mayrink

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Kind regards,

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8.5. Anexo 5. Comprovante do envio do artigo 3

BJOG: An International Journal of Obstetrics & Gynaecology

Home

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Review

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Manuscript ID
BJOG-18-0192

Title
Incidence and risk factors for Preeclampsia in a cohort of healthy nulliparous pregnant women: a nested case-control study

Authors
Mayrink, Jussara
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Calderon, Iracema
Sousa, Maria
Costa, Maria
Cecatti, Jose
Preterm SAMBA , study group

Date Submitted
15-Nov-2018

8.6. Anexo 6. Comprovante do envio do artigo 4

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José Guilherme Cecatti <cecatti@unicamp.br>

Successfully received: submission Mean arterial blood pressure: potential predictive tool for preeclampsia in a cohort of low-risk nulliparous pregnant women for Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health

1 mensagem

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16 de novembro de 2018 13:06

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Ref: PREGHY_2018_259
Title: Mean arterial blood pressure: potential predictive tool for preeclampsia in a cohort of low-risk nulliparous pregnant women
Journal: Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health

Dear Professor Cecatti,

Thank you for submitting your manuscript for consideration for publication in Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health. Your submission was received in good order.

To track the status of your manuscript, please log into EVISE® at: http://www.evise.com/evise/faces/pages/navigation/NavController.jspx?JRNL_ACR=PREGHY and locate your submission under the header 'My Submissions with Journal' on your 'My Author Tasks' view.

Thank you for submitting your work to this journal.

Kind regards,

Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health

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