Investigating Maternal Brain Alterations in Preeclampsia

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Investigating maternal brain alterations in preeclampsia: the need for a multidisciplinary effort

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Abbreviation list

Blood brain barrier (BBB); Blood pressure (BP); Cerebral blood flow (CBF); Cerebral vascular resistance (CVR); Cerebrospinal fluid (CSF); Gamma amino butyric acid (GABA); Human pluripotent stem cells (hPSCs); Junctional Adhesion Molecules (JAMs); Lipopolysaccharide (LPS); Magnesium sulphate ($MgSO_4$); Magnetic resonance imaging (MRI); Magnetic resonance spectroscopy (MRS); Magnetic resonance spectroscopy focused on hydrogen metabolites (H-MRS); Magnetic resonance spectroscopy focused on phosphorus metabolites (P-MRS); Neurofilament light chain (NfL); Neuron Specific Enolase (NSE); Pentylenetetrazole (PTZ); Posterior reversible encephalopathy syndrome (PRES); Reduced uteroplacental perfusion pressure (RUPP); Reduced uteroplacental perfusion pressure plus high cholesterol diet (RUPP+HC); S100 calcium-binding protein B (S100B); Transendothelial electrical resistance (TEER); White matter lesions (WML)

Abstract:

Porpuose of Review: To provide insight into the mechanisms underlying cerebral pathophysiology in preeclampsia and to highlight possible methods for evaluation, screening and surveillance of cerebral complications in the condition.

Recent Findings: Every 12 minutes, a woman dies as a consequence of preeclampsia with cerebral complications, such as eclampsia, among the most common causes. The incidence of eclampsia has been estimated to vary from one in 100 to one in 2000 deliveries, with the highest incidences in low-income countries. The pathophysiology of eclampsia remains enigmatic. The increased blood pressure cannot be the only underlying cause, since some cases of eclampsia arise without simultaneous hypertension. Evaluation of brain alterations in preeclampsia and eclampsia is challenging and demands a multidisciplinary collaboration, since no single method can accurately and fully describe how preeclampsia affects the brain.

Summary: Cerebral complications of preeclampsia are a significant factor in maternal morbidity and mortality worldwide. No single method can accurately describe the full picture of how preeclampsia affects the brain vasculature and parenchyma. We recommend a multinational effort to overcome, not only the issue of limited sample availability, but also, to optimize the quality of research.

Keywords: preeclampsia; eclampsia; brain complications; brain blood barrier; preclinical studies; biomarkers; brain imaging

Introduction

Preeclampsia has classically been characterized by new onset of hypertension and proteinuria after 20 weeks of gestation [1]. However, in several new guidelines, proteinuria is not mandatory for diagnosis if the onset of hypertension is accompanied by other signs of organ impairment including thrombocytopenia, renal insufficiency, impaired liver function, pulmonary edema, fetal growth restriction or seizures (eclampsia) [2, 3]. The majority of maternal deaths related to preeclampsia can be avoided by providing timely and effective care and delivery to high-risk women [4]. Thus, optimization of health care for women during pregnancy to prevent and treat preeclampsia, both in high and low-income countries, is a necessary step towards achievement of the Millennium Development Goals [5].

Eclampsia is defined as tonic-clonic seizures in the presence of preeclampsia and in the absence of other underlying causes of seizures [6]. In cases of recurrent seizures, there is a higher risk of severe hypoxia, aspiration pneumonia, maternal injury and status epilepticus. [7]. Although eclampsia has been defined as a severe complication of preeclampsia, it can occur even in the absence of hypertension [8]. Eclampsia occurs in about 1 in 2000 deliveries in high income countries, while in low and middle income countries it is estimated that the incidence of eclampsia varies from 1 in 100 to 1 in 1700 deliveries [9] (Figure 1).

Currently, there is a gap in knowledge regarding the underlying pathophysiology of preeclampsia and eclampsia. This gap in knowledge impairs the development of tools for the diagnosis and management of women with neurological impairment such as eclampsia or intracerebral edema. Research within the field has been hampered by; i) the low incidence of eclampsia in high income countries where most research is conducted; ii) the fact that preeclampsia is a uniquely human condition; iii) poor access to imaging techniques for evaluation of maternal brain function and iiii) the limited applicability of *in vitro* models.

This review aims to present the current knowledge of preeclampsia and its neurological effects and to highlight the need for a multidisciplinary approach, including

utility of preclinical models, clinical tools for evaluation and analysing the impact of findings from clinical studies, to better understand the pathophysiology of the condition.

Diagnosis and treatment of eclampsia

In pregnant women, there are no imaging criteria or circulating biomarkers to aid in the prediction of eclampsia, and even clinical predictors may have poor predictive ability for eclampsia. The diagnosis can be challenging if the woman is not diagnosed with preeclampsia before onset of seizures or if there are other underlying disorders that can possibly contribute to seizures. In addition, seizures are often unexpected, even in the cases where women present with preeclampsia before onset of eclampsia.

The cornerstones in the treatment of eclampsia are prevention of maternal injury, support of respiratory and cardiovascular function, prevention of recurrent convulsions and reduction of blood pressure (BP) to a safe range [10]. Systolic BP control is essential in avoiding hemorrhagic stroke and should be kept below 160 mmHg [11, 12]. Treatment with magnesium sulphate ($MgSO_4$) is indicated as clinical management for manifest eclampsia to avoid recurrent seizures [13]. In women with eclampsia, $MgSO_4$ treatment reduces the risk of recurrent fits and maternal death by 59% and 38%, respectively [10]. Furthermore, treatment with $MgSO_4$ has a protective effect against developing eclampsia, in particular in women with severe preeclampsia or with imminent signs of eclampsia (blurred vision, severe epigastric pain or headache) [13]. Despite being the drug of choice for preventing eclampsia and maternal death, the mechanism of action of $MgSO_4$ is not well understood. Suggested mechanisms include reducing blood brain barrier (BBB) permeability, reducing neuroinflammation, or modulation of gamma amino butyric acid (GABA) receptor activation [14].

Cerebral alterations after preeclampsia and eclampsia

Cerebral imaging of women with eclampsia often shows a condition defined as posterior reversible encephalopathy syndrome (PRES), a type of cerebral edema thought to be reversible without longterm effects. However, lately, there have been questions about

the actual reversibility of the cerebral effects of eclampsia, or severe preeclampsia, since women who have experienced preeclampsia previously are at higher risk of stroke, vascular dementia, epilepsy and cognitive failure months to years after pregnancy [15-17]. Magnetic resonance imaging (MRI) studies have revealed changes in both morphology and function of the brain in women with preeclampsia and eclampsia. For example, women with a previous pregnancy complicated by eclampsia or severe preeclampsia have an increased number of white matter lesions (WML) several years after the event [18, 19]. These lesions are areas of hyperintensity on T2 weighted MRI brain scans that are known to correlate with cognitive decline and dementia [20, 21]. The distribution of WML does not appear to correspond to areas most often affected in PRES, a finding which argues against the notion of a direct causal pathway [22]. A recent study did, however, show reduced gray matter volume in posterior localizations in the brain in women with a history of preeclampsia and current hypertension [23]. In addition, PRES is not always restricted to the posterior regions of the brain but can also be located in the temporal or frontal regions [24].

Others also report reduced cortical volume or total brain volume in women with a history of preeclampsia [25, 26]. In the study by Mielke et al [27], women with a history of pregnancy hypertensive disease performed worse in a cognitive test measuring processing speed and had greater brain atrophy compared to women with previous normal pregnancies. These recordings were made decades after the affected pregnancy, and differences remained statistically significant even after adjustment for traditional cardiovascular risk factors [25]. More recently, a meta-analysis of cognitive function after preeclampsia, found a correlation between preeclampsia and later subjective, but not objective, impairment of cognition. However the authors also stated that high quality studies are lacking in this field and that most studies did not correct for confounders [28].

Cerebrovascular blood flow in preeclampsia and eclampsia

The ethiology of eclampsia and cerebral complications in relation to preeclampsia is not well understood, but it is thought to be the result of impaired regulation of cerebrovascular function resulting in mainly vasogenic edema, but also partly cytotoxic edema, predominately in the parieto-occipital regions of the brain. The underlying

166 mechanism might be cerebral vasoconstriction, impaired autoregulation with forced dilatation of cerebral arteries and/or endothelial dysfunction [8, 29, 30].

168 Studies employing animal models of preeclampsia have demonstrated that cerebral vasogenic edema is the result of impaired autoregulation of cerebral blood flow and 170 increased BBB permeability [31]. However, since preeclampsia is a condition unique to humans, findings from animal studies with induced preeclampsia can be difficult to 172 interpret and translate to humans.

The original basic concept of the underlying cause of PRES, cerebral vasoconstriction, 174 is based on findings where angiography had shown caliber-changes in the cerebral arteries where vasoconstriction was thought to occur in response to hypertension with 176 autoregulatory compensation, resulting in hypoxia, endothelial dysfunction and subsequent vasogenic and cytotoxic edema [32, 33]. This theory has become less popular in favour for 178 failure of cerebral autoregulation and hyperperfusion, but not abandoned.

Cerebral vascular autoregulation is a physiological mechanism that maintains a 180 relatively constant cerebral blood flow despite changes in cerebral perfusion pressure. The theory proposed to explain the loss of cerebral vascular autoregulation is based on dilation 182 of brain vessels (decrease in cerebral vascular resistance, CVR) instead of vessel constriction, in response to increasing systemic blood pressure. This causes increased 184 cerebral blood flow (CBF), increased pressure on the vessel wall which would in turn causes intracerebral vasogenic edema [34]. However, this process can only occur when the 186 upper limit of the cerebral autoregulation is reached, which is not the case in many women with eclampsia and preeclampsia with PRES [35, 36]. Consequently, the reason why 188 pregnant women, with blood pressures within the normal range of cerebral autoregulation, develop neurological symptoms and/or eclampsia remains unexplained [37]. Some studies 190 have shown that it might be the dynamic cerebral autoregulation (i.e continuous rapid changes in vessel diameter in response to small changes in systemic blood pressure) that is 192 impaired in preeclampsia. This might be one of the explanations as to why eclampsia can occur at blood pressure levels that are lower than the upper range of cerebral autoregulation 194 [38].

Thus, at present, our knowledge of the pathophysiology of edema and seizures in 196 preeclampsia is incomplete, and further research is required.

Endothelial dysfunction in preeclampsia

Endothelial dysfunction is a systemic pathological state characterized by the loss of the physiological response of the endothelium to mediators of vasodilation and vasoconstriction derived from both endothelial and non-endothelial origin [39]. This condition has been reported in women with preeclampsia [40] and is thought to be a generalized condition that targets multiple organs, including the brain. In this review we focus on the importance of brain endothelial cells, a key component of the BBB, in relation to preeclampsia pathophysiology.

The BBB is a complex structure primarily composed of capillary endothelial cells in conjunction with astrocytes, pericytes and basement membrane. In the BBB, adjacent endothelial cells are tightly associated via intercellular tight junction complexes that restrict paracellular transport, thereby regulating the cerebral environment [41]. Consequently, disruption of tight junction function results in loss of BBB integrity and subsequent increased permeability.

The key components of intercellular tight junctions are the transmembrane proteins occludin, claudin and Junctional Adhesion Molecules (JAMs), which form complex strands that govern the permeability characteristics of the paracellular route [42, 43]. In order to maintain this restricted diffusion pathway, tight junctions are linked to the cytoplasmic zonula occludens proteins that provide a structural bridge to the actin cytoskeleton. Furthermore, the phenotype of BBB endothelial cells differs from that of peripheral endothelial cells, since they express higher levels of tight junction proteins, membrane transporters belonging to the ATP-binding cassette and Solute Carrier families, and metabolic enzymes [44-46]. It is this phenotype that is fundamentally responsible for defining the highly restrictive vascular permeability characteristics of the BBB.

Since preeclampsia with cerebral complications may occur in pregnant women with mild hypertension, or even without diagnosis of hypertension [47], it is accepted that circulating factors, probably released from the placenta, may target brain endothelial cells and increase BBB permeability, potentially facilitating the onset of edema and seizures (Figure 2). In support of this hypothesis, Warrington et al [48] reported that pregnant rats

with induced placental ischemia demonstrated increased permeability of the cerebral vasculature. Thus, factors released from the ischemic placenta in preeclampsia might sensitize the cerebral vasculature to changes in blood pressure, which might enhance BBB permeability.

Both Cipolla et al [31] and Johnson et al 2014 [14] have reported a hypertensive pregnancy-dependent significant increase in BBB permeability to sodium fluorescein (376 Da) in a rat model of severe preeclampsia, whilst Cipolla and Kraig [31], but not Johnson et al [14], also showed increased permeability of Texas red dextran (70 kDa). The increased BBB permeability in this model may be associated with tight junction disruption, but no direct evidence has yet been reported. Furthermore, rat studies investigating the effect of preeclampsia on brain physiology postpartum report that decreased expression of the tight junction protein occludin in the posterior cortex was associated with edema [49].

Several reports have described a change in circulating concentrations of cytokines in women who developed preeclampsia, i.e. higher proinflammatory and lower anti-inflammatory concentrations compared to normal pregnancies [50, 51]. However, little is known about circulating concentrations of these cytokines in women who experienced brain complications such as eclampsia, altough circulating TNF-α may contribute to cerebral edema by increasing BBB permeability [52]. Furthermore, in *in vitro* studies the increase in BBB permeability by plasma from women with preeclampsia could be prevented by inhibition of vascular endothelial growth factor signaling [53]. Future studies should focus on the impact of the increased inflammatory state on the brain vasculature in preeclampsia.

Evaluation of brain function in women with preeclampsia or eclampsia

Preclinical models

Animal models

Cerebral complications in preeclampsia are difficult to simulate in *in vivo* models. Classical models for preeclampsia, such as the reduced uteroplacental perfusion pressure (RUPP) model have been combined with high cholesterol diet (RUPP+HC) in order to

mimic severe preeclampsia [14]. This RUPP+HC model results in high BP and reduced placental and pup weights. To evaluate eclampsia in this RUPP+HC model, seizures are induced by administration of the neuroexcitatory agent pentylenetetrazole (PTZ). In this study, seizure threshold to PTZ was decreased, indicating that preeclampsia had a harmful effect on the brain. Furthermore, in the same RUPP+HC model, a lower percentage water content in the posterior cerebral cortex, high *in vivo* BBB permeability to sodium fluorescein and high microglia activation were demonstrated RUPP+HC rats compared to late pregnant controls [14]. The latter study also showed administration of $MgSO_4$ reversed the above effects.

More recently, another rat model of eclampsia, based on administration of lipopolysaccharide (LPS) plus PTZ to pregnant rats, has been validated by different groups [54, 14, 55]. This model demonstrates not only preeclampsia-like syndrome plus seizures, but neuroinflammation, brain edema and high levels of proinflammatory cytokines in peripheral blood and in cerebrospinal fluid. These abnormalities can be prevented by $MgSO_4$. Interestingly, placental ischemia hastened the onset of seizures compared to pregnant controls but had no effect on seizure duration. Other available rat models of hypertension in pregnancy have not been used in studies of maternal brain alteration.

Despite validation of available eclampsia models, rat models have limited applicability in terms of defining whether the brain regions affected in rodents correspond to the affected areas in human. In addition, since preeclampsia is a uniquely human condition, an animal model can never truly reflect the effects of preeclampsia on the human brain. Consequently, there is a need for more studies to better understand how cerebral complications arise during severe preeclampsia [31].

In vitro models

Since the preeclampsia-associated modulation of BBB permeability is a multifactorial phenomenon involving alterations in the restrictive characteristics of tight junction complexes and intracellular signaling events, *in vitro* models of the BBB can prove useful in characterizing these alterations. Ideally, the models should be functionally reproducible, retaining key characteristics of the *in vivo* BBB, including high

290 transendothelial electrical resistance (TEER), low permeability, and expression of functional receptors and associated signaling and endocytotic pathways, ATP-binding
292 cassette, Solute Carrier transporters, and tight junction proteins. A summary of the available models is presented in Table 1.

294 There is very rarely healthy human brain tissue available to generate BBB due to obvious ethical issues. However primary human brain endothelial cells are commercially
296 available. Immortalised cell lines are an alternative to primary cell cultures, although immortalization has a negative impact on normal cell physiology. Of all the human-based
298 models [56, 57] the hCMEC/d3 cell line has consistently proved to be the most reliable in terms of phenotype and relevance to studying BBB function [58-60], whilst in recent years,
300 the use of human pluripotent stem cells (hPSCs) for developing *in vitro* models of the human BBB appears to be extremely promising [61].

302 Animal-derived BBB models have obvious advantages over human-based models in terms of tissue availability and versatility, and brain tissue from rodents has been widely
304 employed. More robust BBB models, based on bovine and porcine primary endothelial cells, demonstrate high TEER [62-66] low permeability of small tracer molecules [64, 62,
306 63, 66], expression of phenotypical proteins [62, 67, 68, 65] and responsiveness to endogenous mediators and xenobiotics [69, 70]. *In vitro* BBB models have also been
308 generated using immortalized rodent, bovine and porcine endothelial cell lines, which can express tight junction proteins and functionally active BBB transporters [71-74, 57, 75].

310 All *in vitro* model systems have limitations (Table 1), and the major considerations when employing *in vitro* BBB models are optimization of culture conditions and the use of
312 more complex co-culture systems to maintain, or enhance, the *in vivo* BBB phenotype. These considerations will help create a reliable and reproducible model system in which to
314 study BBB function.

316 **Clinical studies**

Brain Imaging in Preeclampsia

318 Brain imaging is the gold standard for analysis of brain alterations during severe preeclampsia. These are summarized in Table 2.

320

Transcranial Doppler.

Transcranial Doppler ultrasound is a non-invasive technique that can be utilized to measure cerebral blood flow. The middle cerebral artery is the most commonly studied vessel due to the ease of access via the temporal window. Transcranial Doppler has been used for assessment of cerebrovascular function in clinical settings, including vasospasm in subarachnoid hemorrhage and stroke. In Transcranial Doppler studies of the middle cerebral artery, cerebral perfusion pressure has been shown to be increased in women with preeclampsia compared to normal pregnant women [76, 77, 38]. Consequently, Belfort et al proposed that elevated cerebral perfusion pressure may contribute to the pathophysiology of vasogenic edema and hypertensive encephalopathy in preeclampsia/eclampsia [78].

Dynamic cerebral autoregulation, the ability of vessels to respond to subtle changes in systemic blood pressure by contracting or dilating has been studied in normal pregnancies and in pregnancies complicated by hypertensive disorders, using a combination of Transcranial Doppler, continuous non-invasive blood pressure monitoring, and continuous end-tidal CO_2 monitoring [79]. Women with preeclampsia have been shown to have impaired dynamic autoregulation compared to women with a normal pregnancy and, interestingly, this impairment does not correlate to higher systemic blood pressure [38]. A possible mechanism is that elevated cerebral perfusion pressure and/or impaired dynamic autoregulation may lead to disruption of the endothelium and microstructure of the cerebral vasculature (barotrauma) and result in cerebral edema and hemorrhage.

Compared to the gold standard of angiography, Transcranial Doppler offers the advantage of decreased expense and lack of radiation exposure. Limitations of Transcranial Doppler include the dependence on the operator for the handheld technique and anatomic variations including inadequate acoustic windows. Most studies evaluating cerebral blood flow in preeclampsia/eclampsia have focused on sonation of the middle cerebral artery. However, since most cerebral pathology in preeclampsia/eclampsia involves the posterior cerebral circulation (visual disturbances, cortical blindness, and posterior reversible encephalopathy) future studies should evaluate the posterior cerebral circulation. Studies monitoring cerebral perfusion pressure and dynamic cerebral autoregulation in both

eclampsia and preeclampsia are lacking, and more efforts are required to determine the influence of these parameters on cerebral complications.

Magnetic Resonance Imaging (MRI)

Magnetic resonance imaging (MRI) produces very high contrast images of soft tissue, making it the method of choice for imaging the brain and spinal cord. In the context of preeclampsia, MRI can prove useful for detecting increases in water content, e.g. due to gliosis or vasogenic edema and has been used for estimation of white matter lesion burden, for detection of the PRES, and for cortical volume assessments (Figure 3).

Besides morphology, MRI can be used for investigations of physiology and metabolism. With a diffusion weighted sequence (diffusion weighted imaging), it is possible to differentiate vasogenic and cytotoxic edema. Blood flow and flow velocity can be measured with phase contrast MR technique [80]. Although various contrast agent-based techniques are used for assessment of tissue perfusion, they cannot be used in pregnancy. There are however newer MR perfusion techniques that do not employ contrast agents, namely arterial spin labeling [81] and intravoxel incoherent motion [82], and intravoxel incoherent motion studies report reduced perfusion in a part of the basal ganglia in women with preeclampsia [83].

Tissue metabolism can be investigated with MR spectroscopy, most often focusing on hydrogen metabolites (H-MRS)[84], but also on phosphorus metabolites (P-MRS) [85] and a P-MRS study reports a reduction in cerebral magnesium levels of the brain in women with preeclampsia [86]. Using H-MRS, changes in osmolytes were detected in the brains of women with preeclampsia [87, 88], suggesting that brain and plasma osmolality may play a role in the cerebral edema associated with preeclampsia and eclampsia. To date, the MR spectroscopy technique has rarely been used in women with eclampsia.[89]

Disadvantages with MRI include that it is not as available as ultrasound or CT, examination times are longer, cost is higher, thereby making it difficult to examine critically ill patients.

Cerebral biomarkers

In preeclampsia, four cerebral biomarkers have been investigated in the setting of brain alterations: 1) S100B (10.7 kDa monomer; 21 kDa homodimer) from astroglial cells, 2) Neuron Specific Enolase (NSE) (47 kDa) from neurons 3) Neurofilament light chain (NfL) (68 kDa) and 4) tau (six isoforms, 36.8 - 45.9 kDa) from axons. Amongst these, S100B has been most studied. If these centrally-derived biomarkers, when detected peripherally, are proven to have a high accuracy in diagnosing cerebral complications at onset or before onset, they could be used to predict or diagnose cerebral complications in preeclampsia.

However, in terms of applicability in the clinical setting, there is still a large gap in knowledge about the utility of circulating cerebral biomarkers in the prediction and diagnosis of cerebral complications in preeclampsia. Most studies have evaluated women with preeclampsia without cerebral complications. Prospective studies investigating women with cerebral complications in preeclampsia, such as cerebral edema and/or eclampsia, are needed to verify the accuracy of these biomarkers.

The possible advantage of analyzing circulating biomarkers is that women with cerebral complications, or manifest cerebral signs, could be evaluated and treated according to their risk of complications. This would facilitate allocation of treatment such as magnesium sulphate, decision about delivery and allocation to the right level of care. Briefly, we will analyze current available biomarkers in the next sections.

S100B in preeclampsia

Since S100B is produced by astroglial cells, and in particular in the end-feet surrounding the neurovascular unit, and it is thought that S100B might enter the blood stream after an isolated BBB injury, even without injury to the brain parenchyma [90]. This is supported by studies examining the loss of BBB integrity in pharmacological studies of drugs directed to the brain [91].

Increased plasma levels of S100B have been reported in women with eclampsia [92], whilst women with severe preeclampsia had higher plasma levels of S100B compared to women with mild disease [93]. Also, increased plasma concentrations of S100B were

correlated with visual disturbances among women with preeclampsia [94] or with cerebral symptoms [95]. Therefore, S100B seems to be a promising blood-based biomarker for cerebral impairment in preeclampsia.

Neuron Specific Enolase in preeclampsia

Neuron Specific Enolase (NSE) is found in neurons, red blood cells and in the neuroendocrine system. NSE in combination with clinical parameters has been recommended as neurologic prognostication in patients with cardiac arrest and hypoxic ischemic encephalopathy [96].

Little information is available on these markers in preeclampsia. Two studies report that plasma concentrations of NSE were increased in late pregnancy in women developing preeclampsia [97] and that plasma concentrations of NSE were still increased one year after delivery in women who had preeclampsia compared to healthy controls [98].

NfL and tau in preeclampsia

Neurofilament light chain (NfL) and tau are axonal proteins used as biomarkers for neurodegenerative disease. NfL is released into the cerebrospinal fluid (CSF) and subsequently peripheral blood and might be useful to rule out intracranial pathology in patients with traumatic brain injury [99]. Serum concentrations of tau have been proven to predict 6 months cerebral outcome after cardiac arrest in a pilot study [100]. Two studies have shown that concentrations of NfL are increased at the end of pregnancy but before onset of disease in women developing preeclampsia [101, 102] and one of these studies also showed that tau was increased before onset of disease [102]

Concluding remarks

Evaluation of brain alterations in preeclampsia and eclampsia is challenging and demands collaboration between experts in the laboratory, imaging specialties and in the clinical setting. No single method can accurately describe the full picture of how

preeclampsia affects the brain vasculature and parenchyma since there are sources of error no matter which method is employed.

Also, different mechanisms of pathophysiology have been reported, and evidence has been hard to pool since studies usually report findings from only one modality. If a combination of peripheral biomarkers, cerebral imaging and *in vitro* studies support the same findings, this will strengthen the validity of the results (Figure 4). In future research in preeclampsia and eclampsia, the above methods of evaluation of cerebrovascular alterations should be combined and outcomes should be focused on both short- and long-term cerebral complications such as eclampsia, PRES, impaired cognitive function and stroke. If women could be objectively identified as low or high risk of cerebral complications, treatment and support could be directed to the women at highest risk. In terms of prevention, identification of a biomarker that accurately reflects the risks of eclampsia and other preeclampsia-associated severe cerebral complications, as well as brain alterations later in life after a pregnancy complicated by preeclampsia, is highly desirable. However, eclampsia is a severe, but rare condition. We recommend a multinational effort to overcome the issue of limited sample availability, and a translational research approach to optimize the quality of research. Cerebral complications of preeclampsia are a significant factor in maternal morbidity and mortality worldwide. To reach the 5th WHO Millenium goal for decreased maternal mortality, there is undoubtedly a need for a multidisciplinary effort to gain increased knowledge of brain vascular alterations in preeclampsia.

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Author's roles

CE and LB: Designed and wrote the manuscript. All co-authors included their respective sections according to expertise. JMR, AKW and JP contributed to the writing of the manuscript and provided a critical revision of its contents. All coauthors approval the final version of this manuscript.

Figure Legends

Figure 1: Incidence of eclampsia worldwide. Figure indicates calculated incidence from literature described in supplementary Table S1. Calculated incidence per 100.000 deliveries.

Figure 2: Current concept of the pathophysiology of brain alterations in preeclampsia and eclampsia. A) We propose a communication between placental and maternal brain. **B)** The ischemic placenta releases harmuful substances, including exosomes, into the maternal circulation, which in turn reach brain endothelial cells generating damage in the blood brain barrier. **C)** Increased brain endothelial permeability will impair the function of brain parenchyma.

Figure 3. Magnetic resonance images of woman with eclampsia. FLAIR sequence **A)** shows high signal intensity changes compatible with posterior encephalopathy syndrome (PRES) in bilateral parietooccipital areas as well as in bilateral basal ganglia. Intravoxel incoherent motion (IVIM) images show reduced fast diffusion (D*), indicating reduced blood flow velocity **B)** and reduced perfusion fraction (f) indicating reduced blood volume **C).** Changes are more obvious in D* image. The findings support hypoperfusion in areas corresponding to the PRES findings.

Figure 4: Approach to the study of brain alterations in preeclampsia and eclampsia from bench to bedside. There is a need to develop multidisciplinary approaches to better understand the pathophysiology of brain alterations associated with preeclamspia and eclampsia. Among others, we have summarized the necessity for further improvements in: clinical management, epidemiological studies, brain imaging tools, development of prevention strategies such as use of biomarkers; and further preclinical research, including *in vitro* and animal model studies. This multidisciplinary approach will result in an iterative process leading to advancement of knowledge in the preeclampsia cerebral vascular field and will help reduce fetomaternal complications due to brain alterations.

508

510

Table Legends

512 **Table 1. Features of *in vitro* blood-brain barrier models for studies in preeclampsia***

*References within the manuscript

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Table 2: Features of clinical approaches for studing cerebral complications in
516 **preeclampsia.**

*References within the manuscript

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858