transactions

Is inflammation the cause of pre-eclampsia?

Wenda Ramma and Asif Ahmed[1]

University/BHF Centre for Cardiovascular Sciences, Queen's Medical Research Institute, College of Medicine and Veterinary Medicine, University of Edinburgh, 47 Little France Crescent, Edinburgh EH16 4TJ, U.K.

Abstract

It has been proposed that either excessive inflammation or an imbalance in angiogenic factors cause pre-eclampsia. In the present review, the arguments for and against the role of inflammation and/or angiogenic imbalance as the cause of pre-eclampsia are discussed on the basis of the Bradford–Hill criteria for disease causation. Although both angiogenic imbalance and systemic inflammation are implicated in pre-eclampsia, the absence of temporality of inflammatory markers with pre-eclampsia challenges the concept that excessive inflammation is the cause of pre-eclampsia. In contrast, the elevation of anti-angiogenic factors that precede the clinical signs of pre-eclampsia fulfils the criterion of temporality. The second most important criterion is the dose–response relationship. Although such a relationship has not been proven between pro-inflammatory cytokines and pre-eclampsia, high levels of anti-angiogenic factors have been shown to correlate with increased incidence and disease severity, hence satisfying this condition. Finally, as the removal of circulating sFlt-1 (soluble Fms-like tyrosine kinase receptor-1) from pre-eclamptic patients significantly improves the clinical outcome, it fulfils the Hill's experiment principle, which states that removal of the cause by an appropriate experimental regimen should ameliorate the condition. In contrast, treatment with high doses of corticosteroid fails to improve maternal outcome in pre-eclampsia, despite suppressing inflammation. Inflammation may enhance the pathology induced by the imbalance in the angiogenic factors, but does not by itself cause pre-eclampsia. Development of therapies based on the angiogenic and cytoprotective mechanisms seems more promising.

Background

Pre-eclampsia is an important cause of maternal and perinatal mortality affecting 5–7% of pregnant women [1]. Clinically, it is defined as the *de novo* onset of hypertension (systolic blood pressure $\geqslant 140$ mmHg or diastolic blood pressure $\geqslant 90$ mmHg) and proteinuria ($\geqslant 300$ mg/24 h) after 20 weeks of gestation. In extreme cases, serious complications of pre-eclampsia can include acute renal failure, seizures (eclampsia), pulmonary oedema, acute liver injury, haemolysis and/or thrombocytopenia. The last three signs occur together as part of the HELLP (haemolysis, elevated liver enzymes and low platelets) syndrome, a severe variant of pre-eclampsia. Apart from the hypertension and proteinuria, the central nervous system also plays a role, such as headache and hyperrelexia.

Pre-eclampsia has been cited as "the disease of the-ories" because of its unknown aetiology [2]. In recent years, endothelial dysfunction has emerged as the leading phenomenon responsible for the clinical signs of the disorder [3]. Endothelial dysfunction is commonly linked to the impairment of the NO (nitric oxide) pathway and decrease in NO bioavailability [4,5]. Factors proposed to cause endothelial dysfunction in pre-eclampsia include poor placental vascular remodelling and placental ischaemia, oxidative stress [6,7], excessive inflammation [8], imbalance in angiogenic factors [9–12] and the loss of endogenous protective regulators [3,13–15].

Poor placentation and reduction in uterine blood flow (placental ischaemia) has for a long time been proposed to be the leading cause for the increase in oxidative and endoplasmic reticulum stress, production of potent pro-inflammatory mediators and anti-angiogenic factors in pre-eclampsia [16,17]. However, the role of inadequate placental vascular remodelling due to defective trophoblast invasion as the leading cause of pre-eclampsia has been disputed on the basis of evidence that failure to remodel the uterine arteries is also associated with intrauterine growth restriction, where no signs of hypertension and proteinuria are observed [18].

More recently, the 'excessive inflammation' and the 'angiogenic imbalance' theories were highlighted as the cause of pre-eclampsia. Although a generalized systemic inflammation is common to all pregnancies [19], Redman et al. [8] proposed that pre-eclampsia is not intrinsically different from normal pregnancy, but it is at the extreme end of a continuous spectrum of inflammatory responses that are a feature of pregnancy itself. In parallel, Ahmed [9] had proposed that

Key words: cytokine, inflammation, pre-eclampsia, soluble endoglin (sEng), soluble Fms-like tyrosine kinase receptor-1 (sFlt-1), vascular endothelial growth factor (VEGF).

Abbreviations used: eNOS, endothelial nitric oxide synthase; ET-1, endothelin-1; HELLP, haemolysis, elevated liver enzymes and low platelets; IFNγ, interferon γ; IL, interleukin; PlGF, placenta growth factor; sEng, soluble endoglin; sFlt-1, soluble Fms-like tyrosine kinase receptor-1; TNFα, tumour necrosis factor α; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor.

[1]To whom correspondence should be addressed (email a.s.ahmed@ed.ac.uk).

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Table 1 | **Bradford–Hill criteria of causation**

Criterion	Explanation
Temporality	Exposure always precedes the outcome
Dose-response	Increasing amount of exposure increases the risk
Experiment	The condition can be altered (prevented or ameliorated) by an appropriate experimental regimen
Strength	The stronger the association, the more likely it is that the relationship of 'A' to 'B' is causal
Consistency	The association is consistent when results are replicated in studies in different settings using different methods
Specificity	When a single putative cause produces a specific effect
Coherence	The association should be compatible with existing theory and knowledge
Plausibility	There needs to be some theoretical basis for positing an association between a vector and disease

it is the imbalance in angiogenic factors that cause pre-eclampsia; in particular, the increase in sFlt-1 (soluble Fms-like tyrosine kinase receptor-1) and sEng (soluble endoglin) and the decrease in PlGF (placenta growth factor). In the present review, the arguments for the role of inflammation and angiogenic imbalance as the cause of pre-eclampsia are discussed on the basis of the Bradford–Hill criteria for disease causation (Table 1).

Evidence of the role of angiogenic factors in pre-eclampsia

In 1997, the angiogenic imbalance hypothesis proposed that pre-eclampsia arises due to loss of VEGF (vascular endoethlial growth factor) activity as a result of rise in sFlt-1 [9]. This gained support when it was shown that sFlt-1 levels increased in the amniotic fluid [20] and placenta [21] of pre-eclamptic women. The role of sFlt-1 in the aetiology of pre-eclampsia acquired serious recognition when it was shown that adenoviral overexpression of sFlt-1 in pregnant rats mimicked the clinical manifestations of pre-eclampsia [10]. Indeed, anti-VEGF therapy also promotes hypertension and proteinuria in cancer patients [22]. Furthermore, the circulating level of maternal sFlt-1 is significantly elevated before the onset of the disorder and persisted until delivery [23]. High levels of sFlt-1 causes endothelial dysfunction [10,24,25] and inhibits angiogenesis [26,27] by binding to and antagonizing the function of both VEGF and PlGF [28]. In addition, the circulating levels of VEGF and PlGF are decreased before the onset of pre-eclampsia [12,29]. Both VEGF [30,31] and PlGF [32] have been shown to stimulate NO release [33], indicating that their loss would lead to a reduction in NO production. Like sFlt-1, sEng is elevated early in pre-eclampsia [11] and limits the activity of TGFβ1 (transforming growth factor β1) signalling and eNOS (endothelial nitric oxide synthase) [34,35], hence promoting vascular dysfunction and inhibiting angiogenesis [11].

Evidence of inflammation in pre-eclampsia

Both observational and experimental studies have demonstrated an association between inflammation and endothelial dysfunction [36,37]. Redman et al. [8] first proposed that pre-eclampsia arises as a result of an excessive maternal intravascular inflammatory response to pregnancy, which may occur because either the stimulus or the maternal response is too strong and involves both the innate and the adaptive immune system. Two decades ago, Greer et al. [38] first showed that neutrophil activation is confined to the maternal circulation in pregnancy-induced hypertension, where it may contribute to vascular damage. Soluble markers of neutrophil activation, released in the circulation from the degranulation of activated neutrophils, are also increased in pre-eclamptic patients. [39–42]. Further evidence of enhanced inflammation in pre-eclampsia has been demonstrated through the uncontrolled increased activation of the complement system compared with normal pregnancy. Activation of the complement system amplifies inflammation, promotes chemotaxis of inflammatory cells and generates proteolytic fragments that enhance phagocytosis by neutrophils and monocytes [43].

Another important feature of systemic inflammation in pre-eclampsia is the predominance of Th1-type immunity and the absence of Th2 tendency. T-cells may be classified as Th1 cells, which synthesize IL (interleukin)-2, IL-12, IFNγ (interferon γ) and TNFα (tumour necrosis factor α), and induce cellular immunity, or Th2 cells, which synthesize IL-4, IL-5, IL-6 and IL-10, and induce antibody production [44]. Normal pregnancy is characterized by a shift towards Th2-type immunity and the inhibition of cytotoxic Th1 immune responses, which could be harmful to the fetus [45]. In pre-eclamptic patients, the decidual lymphocytes and peripheral blood mononuclear cells are generally primed to synthesize high levels of Th1 cytokines. In contrast, the same patients exhibited low spontaneous or phytohaemagglutinin-induced expression of the Th2 cytokines [46–48]. In addition, circulating levels of pro-inflammatory cytokines such as IL-6, TNFα and the chemokines IL-8, IP-10 (IFNγ-inducible protein 10) and MCP-1 (monocyte chemoattractant protein 1) are also elevated in pre-eclampsia [49].

Inflammation compared with angiogenic imbalance hypothesis

Temporal relationship

According to Hill [50], causation can be determined using the temporal relationship, which implies that the cause of a disorder must precede its clinical signs. Numerous studies have shown that maternal circulatory sFlt-1 is elevated as early as 5–10 weeks before the onset of pre-eclampsia [23,51,52] and sEng is elevated as early as 11–13 weeks of

gestation before the development of pre-eclampsia [53–58]. Furthermore, a decrease in urinary PlGF also precedes the onset of pre-eclampsia [12]. Collectively, these observations provide evidence of the cause and effect relationship between the angiogenic imbalance theory and pre-eclampsia.

In contrast, neutrophil activation [38,59] and elevation of Th1 pro-inflammatory cytokines [49,60–62] have been shown to occur at the time of diagnosis of pre-eclampsia. In addition, a prospective nested case control study and a longitudinal study revealed that, at 18 weeks of gestation, the levels of inflammatory parameters including TNFα, IL-6 and IFNγ were not elevated in women who later developed pre-eclampsia compared with healthy controls [63,64]. The lack of a temporal relationship between the inflammatory cytokines and the maternal syndrome of pre-eclampsia puts into question the long-held concept that pre-eclampsia is caused by 'excessive inflammation'.

Interestingly, the study by Lynch et al. [65] suggested that early activation of the complement system in pregnancy elevates the concentrations of circulating complement-activated fragment Bb that could be associated with the subsequent development of pre-eclampsia, independent of the release of anti-angiogenic factors [43]. However, given the heterogeneous nature of pre-eclampsia and the small number of pre-eclamptic patients in this study (32 patients who developed pre-eclampsia), additional studies are needed to evaluate the relative implications of complement activation in the pathogenesis of pre-eclampsia.

Strength of association and consistency

Compelling and consistent data from clinical studies conducted in various centres have confirmed that circulating levels of sFlt-1, sEng and PlGF gave the highest strength of association with the clinical manifestation of early-onset pre-eclampsia, satisfying the two criteria of causation: strength of association between the cause and the disease and the consistency of association in different settings [23,51,66,67].

In contrast, clinical data vary greatly with respect to differences in specific level of inflammatory cytokines between normal pregnant women and pre-eclamptic women. Jonsson et al. [68] examined serum levels of 20 different cytokines and found significant differences in only two, IL-6 and IL-8, between pre-eclamptic and normal pregnant women. Sharma et al. [69] observed similar increase in serum IL-6 and IL-8 levels, but also found a significant increase in serum TNFα and a decrease in IL-10 in pre-eclamptic pregnant women compared with normal pregnant women. Additional studies have found increases in placental and/or peripheral blood levels of the inflammatory cytokines IL-2, IL-12, IL-15, IL-18 and IFNγ in pre-eclamptic pregnant women [69–71]. In contrast, Freeman et al. [72] observed that the serum levels of IL-10 and TNFα are increased from the first to the third trimester in healthy control pregnancies to nearly the same degree as in women with pre-eclampsia [72]. The inconclusive and contradictory findings about the inflammatory cytokines challenge the role of inflammation as the main cause of pre-eclampsia. Furthermore, with the exception of a few studies [73,74], many studies have failed to find a strong and consistent strength of association between the increase in inflammatory status and the clinical signs of pre-eclampsia, weakening the role of inflammation as the cause of pre-eclampsia.

Dose–response relationship

To date, the only cure for pre-eclampsia is the delivery of the baby and the removal of the placenta. Pre-eclampsia also occurs in molar pregnancies (absence of fetus), indicating that it is the placenta and not the fetus that causes the condition. Increase in placental mass, as in singletons to twins, triplets and quadruplets, also increases the incidence of pre-eclampsia. In twin pregnancies, a 2-fold increase in the level of sFlt-1 and an increase in the sFlt-1/PlGF ratio were observed [75]. In a secondary analysis of the NICHD (National Institute of Child Health and Human Development) MFMU (Maternal–Fetal Medicine Unit) trial of aspirin to prevent pre-eclampsia in high-risk pregnancies, the odds of developing pre-eclampsia were significantly increased among women with multiple placenta for each 2-fold elevation in sFlt-1, sEng and the ratio of angiogenic factors, and significantly decreased for each 2-fold elevation in circulating PlGF between 7 and 26 weeks of gestation [76]. Importantly, the fractional excretion of angiogenic factors was significantly higher in women with severe pre-eclampsia compared with those with mild pre-eclampsia or pregnant controls [77], demonstrating that the increase in sFlt-1 is directly proportional to the severity of the disorder. It is also worth noting that sFlt-1 can only induce hypertension and proteinuria in mice when its levels reach above a certain critical threshold [24], supporting the theory that women with fetal growth restriction, despite having elevated levels of sFlt-1 compared with controls, do not exhibit signs of hypertension or proteinurea [78]. These studies clearly demonstrate the dose–response relationship between the level of anti-angiogenic factors and the incidence and severity of pre-eclampsia. To our knowledge, such a relationship has not been established between pro-inflammatory cytokines and pre-eclampsia.

Specificity and experiment

Animal studies provide strong evidence linking sFlt-1 and sEng to the pathogenesis of pre-eclampsia [10,11,25,79–86]. In pregnant rats, sEng acts synergistically with sFlt-1 to induce endothelial dysfunction, hypertension, severe proteinuria and HELLP syndrome [11]. Furthermore, neutralization of sFlt-1 below a critical threshold using VEGF eliminates the signs of pre-eclampsia in mice [24,84]. In addition, Ahmad and Ahmed [26] showed previously that immunoprecipitation of sFlt-1 from placental conditioned medium of pre-eclamptic women restored angiogenesis as demonstrated by *in vitro* tube formation. Following on from this concept, recently, an elegant study by Thadhani et al. [87] showed that the extractions of sFlt-1 from the plasma of pre-eclamptic patients by apheresis (using a negatively charged dextran sulfate cellulose column to which positively

charged sFlt-1 is adsorbed), resulted in an approximate 35% reduction in circulating sFlt-1. As a consequence, this was accompanied by a reduction and stabilization of maternal blood pressure leading to an increase in gestational age in two patients by 16 days (for singleton pregnancy) and 19 days (twin pregnancy) respectively. Collectively, these separate studies fulfil the criteria of specificity and strengthen evidence for the role of anti-angiogenic factors as the likely candidates for the pre-eclampsia.

Several studies have attempted to show that inflammation itself causes the clinical signs of pre-eclampsia. For example, Faas et al. [88] showed that infusion of a single dose of endotoxin to pregnant rats caused glomerular damage in the kidney, as well as hypertension and proteinuria. In addition, Hayakawa et al. [62], who developed an animal model of immune-mediated abortion and pre-eclampsia due to excessive activation of Th1/Th2 lymphocyte, showed that *in vitro*-activated splenocytes with IL-12 and IL-4 injected into pregnant mice induced hypertension, proteinuria and histological renal changes, but not in controls. In a more recent study, Zenclussen et al. [89] showed that activated Th1-like cells, but not Th2-like cells, injected into mice led to elevated blood pressure, proteinuria and kidney damage exclusively in pregnant mice [89]. In support of these studies, other groups have reported that immunosuppression or inhibition of the inflammatory network improves the aforementioned pathological conditions.

Indeed, in a rat model of pregnancy-induced hypertension, as described previously [90], pharmacological immunosuppression of lymphocytes improved blood pressure and endothelial function [91,92], indicating that prevention of T-cell proliferation may decrease hypertension. In a more recent study, Zhou et al. [93] showed that injection of angiotensin-II type I receptor-agonistic autoantibodies induced pre-eclampsia-like features in mice and the inhibition of IL-6 in these mice inhibited these signs. Despite these studies, the proposal that pre-eclampsia is primarily an inflammatory disorder is somewhat at odds with clinical experience of the use of corticosteroids used to accelerate fetal lung maturation. A prospective double-blind randomized clinical trial of 24 mg of betamethasone given as two 12 mg doses 24 h apart and repeated weekly confirmed the known beneficial effects of this treatment on neonatal outcome, but failed to show any beneficial effect to the mother [94]. This dose of betamethasone is equivalent to 160 mg of prednisolone, which would be regarded as a huge dose of corticosteroid and which would be expected to have major effects in other inflammatory conditions. Moreover, in a similar clinical trial, dexamethasone failed to improve the outcome of women with HELLP syndrome [95].

Coherence and plausibility criteria

According to the Hill's criteria of causation, the association needs to be compatible with existing knowledge. The biology of VEGF helps to validate the role of the anti-angiogenic factor sFlt-1 as a cause of pre-eclampsia. VEGF is critical for endothelial cells homoeostasis [96] and plays a crucial role in the health of fenestrated and sinusoidal endothelium found in the renal glomerulus, brain and liver [97], organs that are severely compromised in pre-eclampsia. VEGF activates both VEGFR (VEGF receptor)-1 and VEGFR-2 to stimulate NO required for angiogenesis [30,31,98–102], which suggests its beneficial role in the regulation of vascular tone and blood pressure [103]. Indeed, exogenously administered VEGF was shown to ameliorate post-cyclosporine-mediated hypertension, endothelial dysfunction and nephropathy [104,105]. Beneficial effects of VEGF have also been observed in animal models of kidney disease [106–108]. Furthermore, cancer patients receiving anti-VEGF therapy [22] or rats treated with VEGF-neutralizing antibody or sFlt-1 [109] exhibit pre-eclampsia-like symptoms, indicating that reduction in VEGF bioavailability is the likely initiator of vascular damage responsible for the main clinical signs of pre-eclampsia.

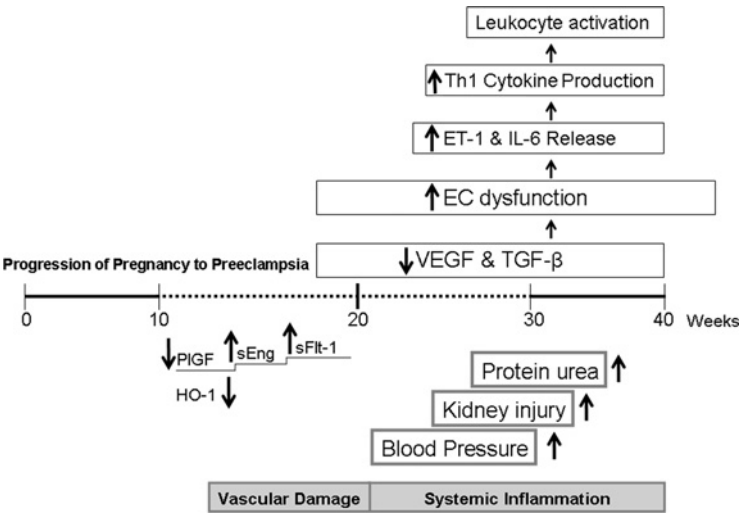
Increases in inflammatory cytokine production have been associated with endothelial dysfunction, increased placenta apoptosis, decreased angiogenesis and kidney abnormalities that are relevant to the pathophysiology of pre-eclampsia [110]. Endothelial cell damage induces the production of ET-1 (endothelin-1) [111,112]. In pregnant mice, ET-1 was shown to cause hypertension, proteinuria and renal damage. Furthermore, IL-6 acts downstream of TNFα to induce the release of ET-1 in mice. In addition, in the presence of autoantibodies from pre-eclamptic women, IL-6 mediates the release of ET-1 from human placental villous explants [93]. These studies validate the original *in vitro* observations of Collino et al. [113], which showed that pre-eclamptic sera induces nephrin shedding from podocytes through ET-1 release by endothelial glomerular cells. These separate studies show an association between inflammation and ET-1 in rodents, and ET-1 may play an important role in pre-eclampsia, but pregnant women with excessive inflammation and high levels of inflammatory cytokines, such as those with infections, do not always develop pre-eclampsia.

Conclusion

Collectively, the studies are important in elucidating the sequence of events involved in the progression of pre-eclampsia and its clinical signs (Figure 1). There is no doubt that both angiogenic imbalance and systemic inflammation are elevated in pre-eclampsia. However, on the basis of the evidence and arguments made above, we advocate that inflammation is a consequence rather than a cause of pre-eclampsia. The most essential criterion of Bradford–Hill is the principle of temporality, which states that when a factor is believed to cause a disease, the factor must necessarily always precede the occurrence of the disease. The absence of temporality between the elevation in the circulating inflammatory markers and the development of the maternal syndrome of pre-eclampsia challenges the concept that excessive inflammation is the cause of pre-eclampsia. In contrast, the increase in circulating anti-angiogenic factors, which precedes the clinical signs of pre-eclampsia, fulfils

Figure 1 | **Progression of pregnancy to pre-eclampsia**

A schematic diagram of the sequence of events along gestational age leading to the clinical signs of pre-eclampsia. EC, endothelial cell; HO-1, haem oxygenase 1; TGF-β, transforming growth factor β.



the condition of temporality. Another crucial criterion is the dose–response relationship, where increasing the level of exposure to a factor increases the incidence and severity of the disease. Although such a relationship has not been established between pro-inflammatory cytokines and pre-eclampsia, high levels of anti-angiogenic factors have been shown to correlate with increased incidence of the disorder and disease severity, hence satisfying this condition. Furthermore, Hill's experiment criterion states that removal of the cause by an appropriate experimental regimen should ameliorate the condition. Indeed, compelling evidence supporting a role for anti-angiogenic factors as the leading cause of pre-eclampsia has recently been shown in a clinical study, where removal of circulating sFlt-1 from pre-eclamptic patients significantly improved the clinical outcome [87]. In contrast, treatment with high doses of corticosteroids failed to ameliorate the maternal outcome in pre-eclampsia despite suppressing inflammation.

Although inflammation may not be the cause of pre-eclampsia, it may enhance the pathology of the disorder in the presence of the anti-angiogenic factors. A recent study demonstrated that sFlt-1 act synergistically with pro-inflammatory mediators to activate endothelial cells compared with endothelial cells treated with TNFα alone [114]. Treatment with sFlt-1 or VEGF-neutralizing antibodies or blockade of VEGFR-1 and VEGFR-2 reduced the phosphorylation of Akt and eNOS leading to reduced NO bioavailability and increased endothelial dysfunction [114]. Furthermore, a recent study demonstrated that adenoviral overexpression of sFlt-1 in endothelial cells resulted in substantial inhibition of eNOS phosphorylation and NO production as well as a decrease in VEGFR-2 phosphorylation in pre-eclamptic placenta [27]. These separate results provide additional support for the original hypothesis put forward by Ahmed in 1997 [9] that lack of VEGF and NO bioavailability is responsible for the endothelial dysfunction in pre-eclampsia.

Whereas inflammation itself may not be the leading cause of pre-eclampsia, it should also be recognized that pregnant women with risk factors associated with chronic inflammation (obesity, pre-pregnancy hypertension, diabetes mellitus and dyslipidaemia) are more likely to develop pre-eclampsia [115–118]. Furthermore, patients with autoimmune diseases, particularly systemic lupus erythaematosus and antiphospholipid syndrome, have increased risks of developing pre-eclampsia [119,120]. The possibility that inflammatory mediators may have local autocrine or paracrine effects, which can lead to amplification of the anti-angiogenic factors' effects, cannot be ruled out at this stage.

In conclusion, we hypothesize that inflammation is not a major contributor in the development of pre-eclampsia. In the interest of finding an effective treatment, our focus should be to target development of therapies based on the angiogenic [28,121] and cytoprotective [3] theories, which seem more promising.

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References

1 World Health Organization (2005) Make Every Mother and Child Count: the World Health Report 2005, p. 63, WHO, Geneva
2 Dekker, G.A. and Sibai, B.M. (1998) Etiology and pathogenesis of preeclampsia: current concepts. Am. J. Obstet. Gynecol. **179**, 1359–1375
3 Ahmed, A. (2011) New insights into the etiology of preeclampsia: identification of key elusive factors for the vascular complications. Thromb. Res. **127** (Suppl. 3), S72–S75
4 Cooke, J.P., Dzau, J. and Creager, A. (1991) Endothelial dysfunction in hypercholesterolemia is corrected by L-arginine. Basic Res. Cardiol. **86** (Suppl. 2), 173–181
5 Bivalacqua, T.J., Usta, M.F., Champion, H.C., Kadowitz, P.J. and Hellstrom, W.J. (2003) Endothelial dysfunction in erectile dysfunction: role of the endothelium in erectile physiology and disease. J. Androl. **24**, S17–S37
6 Roberts, J.M. and Redman, C.W. (1993) Pre-eclampsia: more than pregnancy-induced hypertension. Lancet **341**, 1447–1451
7 Roberts, J.M., Taylor, R.N., Musci, T.J., Rodgers, G.M., Hubel, C.A. and McLaughlin, M.K. (1989) Preeclampsia: an endothelial cell disorder. Am. J. Obstet. Gynecol. **161**, 1200–1204
8 Redman, C.W., Sacks, G.P. and Sargent, I.L. (1999) Preeclampsia: an excessive maternal inflammatory response to pregnancy. Am. J. Obstet. Gynecol. **180**, 499–506
9 Ahmed, A. (1997) Heparin-binding angiogenic growth factors in pregnancy. Trophoblast Res. **10**, 215–258
10 Maynard, S.E., Min, J.Y., Merchan, J., Lim, K.H., Li, J., Mondal, S., Libermann, T.A., Morgan, J.P., Sellke, F.W., Stillman, I.E. et al. (2003) Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. J. Clin. Invest. **111**, 649–658
11 Venkatesha, S., Toporsian, M., Lam, C., Hanai, J., Mammoto, T., Kim, Y.M., Bdolah, Y., Lim, K.H., Yuan, H.T., Libermann, T.A. et al. (2006) Soluble endoglin contributes to the pathogenesis of preeclampsia. Nat. Med. **12**, 642–649
12 Levine, R.J., Thadhani, R., Qian, C., Lam, C., Lim, K.H., Yu, K.F., Blink, A.L., Sachs, B.P., Epstein, F.H., Sibai, B.M. et al. (2005) Urinary placental growth factor and risk of preeclampsia. JAMA, J. Am. Med. Assoc. **293**, 77–85
13 Ahmed, A., Rahman, M., Zhang, X., Acevedo, C.H., Nijjar, S., Rushton, I., Bussolati, B. and St John, J. (2000) Induction of placental heme oxygenase-1 is protective against TNFα-induced cytotoxicity and promotes vessel relaxation. Mol. Med. **6**, 391–409
14 Cudmore, M., Ahmad, S., Al-Ani, B., Fujisawa, T., Coxall, H., Chudasama, K., Devey, L.R., Wigmore, S.J., Abbas, A., Hewett, P.W. and Ahmed, A. (2007) Negative regulation of soluble Flt-1 and soluble endoglin release by heme oxygenase-1. Circulation **115**, 1789–1797
15 Cudmore, M.J., Ahmad, S., Sissaoui, S., Ramma, W., Ma, B., Fujisawa, T., Al-Ani, B., Wang, K., Cai, M., Crispi, F. et al. (2011) Loss of Akt activity increases circulating soluble endoglin release in preeclampsia: identification of inter-dependency between Akt-1 and heme oxygenase-1. Eur. Heart J., doi:10.1093/eurheartj/ehr065
16 Burton, G.J., Woods, A.W., Jauniaux, E. and Kingdom, J.C. (2009) Rheological and physiological consequences of conversion of the maternal spiral arteries for uteroplacental blood flow during human pregnancy. Placenta **30**, 473–482
17 Redman, C.W. and Sargent, I.L. (2010) Immunology of pre-eclampsia. Am. J. Reprod. Immunol. **63**, 534–543
18 Ayuk, P.T. and Matijevic, R. (2006) Placental ischaemia is a consequence rather than a cause of pre-eclampsia. Med. Hypotheses **67**, 792–795
19 Willis, C., Morris, J.M., Danis, V. and Gallery, E.D. (2003) Cytokine production by peripheral blood monocytes during the normal human ovulatory menstrual cycle. Hum. Reprod. **18**, 1173–1178
20 Vuorela, P., Helske, S., Hornig, C., Alitalo, K., Weich, H. and Halmesmaki, E. (2000) Amniotic fluid–soluble vascular endothelial growth factor receptor-1 in preeclampsia. Obstet. Gynecol. **95**, 353–357
21 Ahmad, S. and Ahmed, A. (2001) Regulation of soluble VEGFR-1 by VEGF and oxygen and its elevation in pre-eclampsia and fetal growth restriction. Placenta **22**, A7
22 Kabbinavar, F., Hurwitz, H.I., Fehrenbacher, L., Meropol, N.J., Novotny, W.F., Lieberman, G., Griffing, S. and Bergsland, E. (2003) Phase II, randomized trial comparing bevacizumab plus fluorouracil (FU)/leucovorin (LV) with FU/LV alone in patients with metastatic colorectal cancer. J. Clin. Oncol. **21**, 60–65
23 Levine, R.J., Maynard, S.E., Qian, C., Lim, K.H., England, L.J., Yu, K.F., Schisterman, E.F., Thadhani, R., Sachs, B.P., Epstein, F.H. et al. (2004) Circulating angiogenic factors and the risk of preeclampsia. N. Engl. J. Med. **350**, 672–683
24 Bergmann, A., Ahmad, S., Cudmore, M., Gruber, A.D., Wittschen, P., Lindenmaier, W., Christofori, G., Gross, V., Gonzalves, A., Grone, H.J. et al. (2010) Reduction of circulating soluble Flt-1 alleviates preeclampsia-like symptoms in a mouse model. J. Cell. Mol. Med. **14**, 1857–1867
25 Costantine, M.M., Tamayo, E., Lu, F., Bytautiene, E., Longo, M., Hankins, G.D. and Saade, G.R. (2010) Using pravastatin to improve the vascular reactivity in a mouse model of soluble fms-like tyrosine kinase-1-induced preeclampsia. Obstet. Gynecol. **116**, 114–120
26 Ahmad, S. and Ahmed, A. (2004) Elevated placental soluble vascular endothelial growth factor receptor-1 inhibits angiogenesis in preeclampsia. Circ. Res. **95**, 884–891
27 Ahmad, S., Hewett, P.W., Al-Ani, B., Sissaoui, S., Fujisawa, T., Cudmore, M.J. and Ahmed, A. (2011) Autocrine activity of soluble Flt-1 controls endothelial cell function and angiogenesis. Vasc. Cell **3**, 15
28 Ahmed, A. and Cudmore, M.J. (2009) Can the biology of VEGF and haem oxygenases help solve pre-eclampsia? Biochem. Soc. Trans. **37**, 1237–1242
29 Foidart, J.M., Schaaps, J.P., Chantraine, F., Munaut, C. and Lorquet, S. (2009) Dysregulation of anti-angiogenic agents (sFlt-1, PLGF, and sEndoglin) in preeclampsia: a step forward but not the definitive answer. J. Reprod. Immunol. **82**, 106–111
30 Ahmed, A., Dunk, C., Kniss, D. and Wilkes, M. (1997) Role of VEGF receptor-1 (Flt-1) in mediating calcium-dependent nitric oxide release and limiting DNA synthesis in human trophoblast cells. Lab. Invest. **76**, 779–791
31 Papapetropoulos, A., Garcia-Cardena, G., Madri, J.A. and Sessa, W.C. (1997) Nitric oxide production contributes to the angiogenic properties of vascular endothelial growth factor in human endothelial cells. J. Clin. Invest. **100**, 3131–3139
32 Khaliq, A., Dunk, C., Jiang, J., Shams, M., Li, X.F., Acevedo, C., Weich, H., Whittle, M. and Ahmed, A. (1999) Hypoxia down-regulates placenta growth factor, whereas fetal growth restriction up-regulates placenta growth factor expression: molecular evidence for "placental hyperoxia" in intrauterine growth restriction. Lab. Invest. **79**, 151–170
33 Bussolati, B., Dunk, C., Grohman, M., Kontos, C.D., Mason, J. and Ahmed, A. (2001) Vascular endothelial growth factor receptor-1 modulates vascular endothelial growth factor-mediated angiogenesis via nitric oxide. Am. J. Pathol. **159**, 993–1008
34 Jerkic, M., Rivas-Elena, J.V., Prieto, M., Carron, R., Sanz-Rodriguez, F., Perez-Barriocanal, F., Rodriguez-Barbero, A., Bernabeu, C. and Lopez-Novoa, J.M. (2004) Endoglin regulates nitric oxide-dependent vasodilatation. FASEB J. **18**, 609–611
35 Toporsian, M., Gros, R., Kabir, M.G., Vera, S., Govindaraju, K., Eidelman, D.H., Husain, M. and Letarte, M. (2005) A role for endoglin in coupling eNOS activity and regulating vascular tone revealed in hereditary hemorrhagic telangiectasia. Circ. Res. **96**, 684–692
36 Zimmerman, G.A., Prescott, S.M. and McIntyre, T.M. (1992) Endothelial cell interactions with granulocytes: tethering and signaling molecules. Immunol. Today **13**, 93–100
37 Mantovani, A. and Dejana, E. (1989) Cytokines as communication signals between leukocytes and endothelial cells. Immunol. Today **10**, 370–375
38 Greer, I.A., Dawes, J., Johnston, T.A. and Calder, A.A. (1991) Neutrophil activation is confined to the maternal circulation in pregnancy-induced hypertension. Obstet. Gynecol. **78**, 28–32
39 Prieto, J.A., Panyutich, A.V. and Heine, R.P. (1997) Neutrophil activation in preeclampsia. Are defensins and lactoferrin elevated in preeclamptic patients? J. Reprod. Med. **42**, 29–32

40 Holthe, M.R., Staff, A.C., Berge, L.N., Fagerhol, M.K. and Lyberg, T. (2005) Calprotectin plasma level is elevated in preeclampsia. Acta Obstet. Gynecol. Scand. **84**, 151–154

41 Braekke, K., Holthe, M.R., Harsem, N.K., Fagerhol, M.K. and Staff, A.C. (2005) Calprotectin, a marker of inflammation, is elevated in the maternal but not in the fetal circulation in preeclampsia. Am. J. Obstet. Gynecol. **193**, 227–233

42 Belo, L., Santos-Silva, A., Caslake, M., Cooney, J., Pereira-Leite, L., Quintanilha, A. and Rebelo, I. (2003) Neutrophil activation and C-reactive protein concentration in preeclampsia. Hypertens. Pregnancy **22**, 129–141

43 Lynch, A.M., Murphy, J.R., Gibbs, R.S., Levine, R.J., Giclas, P.C., Salmon, J.E. and Holers, V.M. (2010) The interrelationship of complement-activation fragments and angiogenesis-related factors in early pregnancy and their association with pre-eclampsia. BJOG **117**, 456–462

44 Mosmann, T.R. and Sad, S. (1996) The expanding universe of T-cell subsets: Th1, Th2 and more. Immunol. Today **17**, 138–146

45 Wegmann, T.G., Lin, H., Guilbert, L. and Mosmann, T.R. (1993) Bidirectional cytokine interactions in the maternal–fetal relationship: is successful pregnancy a TH2 phenomenon? Immunol. Today **14**, 353–356

46 Saito, S., Sakai, M., Sasaki, Y., Tanebe, K., Tsuda, H. and Michimata, T. (1999) Quantitative analysis of peripheral blood Th0, Th1, Th2 and the Th1:Th2 cell ratio during normal human pregnancy and preeclampsia. Clin. Exp. Immunol. **117**, 550–555

47 Orange, S., Horvath, J. and Hennessy, A. (2003) Preeclampsia is associated with a reduced interleukin-10 production from peripheral blood mononuclear cells. Hypertens. Pregnancy **22**, 1–8

48 Holthe, M.R., Staff, A.C., Berge, L.N. and Lyberg, T. (2004) Leukocyte adhesion molecules and reactive oxygen species in preeclampsia. Obstet. Gynecol. **103**, 913–922

49 Szarka, A., Rigo, J., Jr., Lazar, L., Beko, G. and Molvarec, A. (2010) Circulating cytokines, chemokines and adhesion molecules in normal pregnancy and preeclampsia determined by multiplex suspension array. BMC Immunol. **11**, 59

50 Hill, A.B. (1965) The environment and disease: association or causation? Proc. R. Soc. Med. **58**, 295–300

51 Levine, R.J., Lam, C., Qian, C., Yu, K.F., Maynard, S.E., Sachs, B.P., Sibai, B.M., Epstein, F.H., Romero, R., Thadhani, R. and Karumanchi, S.A. (2006) Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. N. Engl. J. Med. **355**, 992–1005

52 Chaiworapongsa, T., Romero, R., Kim, Y.M., Kim, G.J., Kim, M.R., Espinoza, J., Bujold, E., Gonçalves, L., Gomez, R., Edwin, S. and Mazor, M. (2005) Plasma soluble vascular endothelial growth factor receptor-1 concentration is elevated prior to the clinical diagnosis of pre-eclampsia. J. Matern. Fetal Neonatal Med. **17**, 3–18

53 Rana, S., Karumanchi, S.A., Levine, R.J., Venkatesha, S., Rauh-Hain, J.A., Tamez, H. and Thadhani, R. (2007) Sequential changes in antiangiogenic factors in early pregnancy and risk of developing preeclampsia. Hypertension **50**, 137–142

54 Romero, R., Nien, J.K., Espinoza, J., Todem, D., Fu, W., Chung, H., Kusanovic, J.P., Gotsch, F., Erez, O., Mazaki-Tovi, S. et al. (2008) A longitudinal study of angiogenic (placental growth factor) and anti-angiogenic (soluble endoglin and soluble vascular endothelial growth factor receptor-1) factors in normal pregnancy and patients destined to develop preeclampsia and deliver a small for gestational age neonate. J. Matern. Fetal Neonatal Med. **21**, 9–23

55 Erez, O., Romero, R., Espinoza, J., Fu, W., Todem, D., Kusanovic, J.P., Gotsch, F., Edwin, S., Nien, J.K., Chaiworapongsa, T. et al. (2008) The change in concentrations of angiogenic and anti-angiogenic factors in maternal plasma between the first and second trimesters in risk assessment for the subsequent development of preeclampsia and small-for-gestational age. J. Matern. Fetal Neonatal Med. **21**, 279–287

56 Baumann, M.U., Bersinger, N.A., Mohaupt, M.G., Raio, L., Gerber, S. and Surbek, D.V. (2008) First-trimester serum levels of soluble endoglin and soluble fms-like tyrosine kinase-1 as first-trimester markers for late-onset preeclampsia. Am. J. Obstet. Gynecol. **199**, 266.e1–266.e6

57 Lim, J.H., Kim, S.Y., Park, S.Y., Lee, M.H., Yang, J.H., Kim, M.Y., Chung, J.H., Lee, S.W. and Ryu, H.M. (2009) Soluble endoglin and transforming growth factor-β1 in women who subsequently developed preeclampsia. Prenatal Diagn. **29**, 471–476

58 Foidart, J.M., Munaut, C., Chantraine, F., Akolekar, R. and Nicolaides, K.H. (2010) Maternal plasma soluble endoglin at 11–13 weeks' gestation in pre-eclampsia. Ultrasound Obstet. Gynecol. **35**, 680–687

59 Barden, A., Graham, D., Beilin, L.J., Ritchie, J., Baker, R., Walters, B.N. and Michael, C.A. (1997) Neutrophil CD11B expression and neutrophil activation in pre-eclampsia. Clin. Sci. **92**, 37–44

60 Peracoli, J.C., Rudge, M.V. and Peracoli, M.T. (2007) Tumor necrosis factor-α in gestation and puerperium of women with gestational hypertension and pre-eclampsia. Am. J. Reprod. Immunol. **57**, 177–185

61 Greer, I.A., Lyall, F., Perera, T., Boswell, F. and Macara, L.M. (1994) Increased concentrations of cytokines interleukin-6 and interleukin-1 receptor antagonist in plasma of women with preeclampsia: a mechanism for endothelial dysfunction? Obstet. Gynecol. **84**, 937–940

62 Hayakawa, S., Fujikawa, T., Fukuoka, H., Chisima, F., Karasaki-Suzuki, M., Ohkoshi, E., Ohi, H., Kiyoshi Fujii, T., Tochigi, M., Satoh, K. et al. (2000) Murine fetal resorption and experimental pre-eclampsia are induced by both excessive Th1 and Th2 activation. J. Reprod. Immunol. **47**, 121–138

63 Djurovic, S., Clausen, T., Wergeland, R., Brosstad, F., Berg, K. and Henriksen, T. (2002) Absence of enhanced systemic inflammatory response at 18 weeks of gestation in women with subsequent pre-eclampsia. BJOG **109**, 759–764

64 Kronborg, C.S., Gjedsted, J., Vittinghus, E., Hansen, T.K., Allen, J. and Knudsen, U.B. (2011) Longitudinal measurement of cytokines in pre-eclamptic and normotensive pregnancies. Acta Obstet. Gynecol. Scand. **90**, 791–796

65 Lynch, A.M., Murphy, J.R., Byers, T., Gibbs, R.S., Neville, M.C., Giclas, P.C., Salmon, J.E. and Holers, V.M. (2008) Alternative complement pathway activation fragment Bb in early pregnancy as a predictor of preeclampsia. Am. J. Obstet. Gynecol. **198**, 385.e1–385.e9

66 Noori, M., Donald, A.E., Angelakopoulou, A., Hingorani, A.D. and Williams, D.J. (2010) Prospective study of placental angiogenic factors and maternal vascular function before and after preeclampsia and gestational hypertension. Circulation **122**, 478–487

67 Buhimschi, C.S., Baumbusch, M.A., Dulay, A.T., Lee, S., Wehrum, M., Zhao, G., Bahtiyar, M.O., Pettker, C.M., Ali, U.A., Funai, E.F. and Buhimschi, I.A. (2010) The role of urinary soluble endoglin in the diagnosis of pre-eclampsia: comparison with soluble fms-like tyrosine kinase 1 to placental growth factor ratio. BJOG **117**, 321–330

68 Jonsson, Y., Ruber, M., Matthiesen, L., Berg, G., Nieminen, K., Sharma, S., Ernerudh, J. and Ekerfelt, C. (2006) Cytokine mapping of sera from women with preeclampsia and normal pregnancies. J. Reprod. Immunol. **70**, 83–91

69 Sharma, A., Satyam, A. and Sharma, J.B. (2007) Leptin, IL-10 and inflammatory markers (TNF-α, IL-6 and IL-8) in pre-eclamptic, normotensive pregnant and healthy non-pregnant women. Am. J. Reprod. Immunol. **58**, 21–30

70 Kaleli, I., Kaleli, B., Demir, M., Yildirim, B., Cevahir, N. and Demir, S. (2005) Serum levels of neopterin and interleukin-2 receptor in women with severe preeclampsia. J. Clin. Lab. Anal. **19**, 36–39

71 Bachmayer, N., Rafik Hamad, R., Liszka, L., Bremme, K. and Sverremark-Ekstrom, E. (2006) Aberrant uterine natural killer (NK)-cell expression and altered placental and serum levels of the NK-cell promoting cytokine interleukin-12 in pre-eclampsia. Am. J. Reprod. Immunol. **56**, 292–301

72 Freeman, D.J., McManus, F., Brown, E.A., Cherry, L., Norrie, J., Ramsay, J.E., Clark, P., Walker, I.D., Sattar, N. and Greer, I.A. (2004) Short- and long-term changes in plasma inflammatory markers associated with preeclampsia. Hypertension **44**, 708–714

73 Mihu, D., Costin, N., Mihu, C.M., Blaga, L.D. and Pop, R.B. (2008) C-reactive protein, marker for evaluation of systemic inflammatory response in preeclampsia. Rev. Med. Chir. Soc. Med. Nat. Iasi **112**, 1019–1025

74 D'Anna, R., Baviera, G., Giordano, D., Todarello, G., Corrado, F. and Buemi, M. (2008) Second trimester neutrophil gelatinase-associated lipocalin as a potential prediagnostic marker of preeclampsia. Acta Obstet. Gynecol. Scand. **87**, 1370–1373

75 Bdolah, Y., Lam, C., Rajakumar, A., Shivalingappa, V., Mutter, W., Sachs, B.P., Lim, K.H., Bdolah-Abram, T., Epstein, F.H. and Karumanchi, S.A. (2008) Twin pregnancy and the risk of preeclampsia: bigger placenta or relative ischemia? Am. J. Obstet. Gynecol. **198**, 428.e1–428.e6

76 Powers, R.W., Jeyabalan, A., Clifton, R.G., Van Dorsten, P., Hauth, J.C., Klebanoff, M.A., Lindheimer, M.D., Sibai, B., Landon, M. and Miodovnik, M. (2010) Soluble fms-Like tyrosine kinase 1 (sFlt1), endoglin and placental growth factor (PlGF) in preeclampsia among high risk pregnancies. PLoS ONE **5**, e13263

77 Buhimschi, C.S., Magloire, L., Funai, E., Norwitz, E.R., Kuczynski, E., Martin, R., Richman, S., Guller, S., Lockwood, C.J. and Buhimschi, I.A. (2006) Fractional excretion of angiogenic factors in women with severe preeclampsia. Obstet. Gynecol. **107**, 1103–1113

78 Wallner, W., Sengenberger, R., Strick, R., Strissel, P.L., Meurer, B., Beckmann, M.W. and Schlembach, D. (2007) Angiogenic growth factors in maternal and fetal serum in pregnancies complicated by intrauterine growth restriction. Clin. Sci. **112**, 51–57

79 Makris, A., Thornton, C., Thompson, J., Thomson, S., Martin, R., Ogle, R., Waugh, R., McKenzie, P., Kirwan, P. and Hennessy, A. (2007) Uteroplacental ischemia results in proteinuric hypertension and elevated sFLT-1. Kidney Int. **71**, 977–984

80 Roberts, L., LaMarca, B.B., Fournier, L., Bain, J., Cockrell, K. and Granger, J.P. (2006) Enhanced endothelin synthesis by endothelial cells exposed to sera from pregnant rats with decreased uterine perfusion. Hypertension **47**, 615–618

81 Agunanne, E.E., Uddin, M.N., Horvat, D. and Puschett, J.B. (2010) Contribution of angiogenic factors in a rat model of pre-eclampsia. Am. J. Nephrol. **32**, 332–339

82 Lu, F., Longo, M., Tamayo, E., Maner, W., Al-Hendy, A., Anderson, G.D., Hankins, G.D. and Saade, G.R. (2007) The effect of over-expression of sFlt-1 on blood pressure and the occurrence of other manifestations of preeclampsia in unrestrained conscious pregnant mice. Am. J. Obstet. Gynecol. **196**, 396.e1–396.e7

83 Gilbert, J.S., Babcock, S.A. and Granger, J.P. (2007) Hypertension produced by reduced uterine perfusion in pregnant rats is associated with increased soluble fms-like tyrosine kinase-1 expression. Hypertension **50**, 1142–1147

84 Li, Z., Zhang, Y., Ying Ma, J., Kapoun, A.M., Shao, Q., Kerr, I., Lam, A., O'Young, G., Sannajust, F., Stathis, P. et al. (2007) Recombinant vascular endothelial growth factor 121 attenuates hypertension and improves kidney damage in a rat model of preeclampsia. Hypertension **50**, 686–692

85 Bytautiene, E., Lu, F., Tamayo, E.H., Hankins, G.D., Longo, M., Kublickiene, K. and Saade, G.R. (2010) Long-term maternal cardiovascular function in a mouse model of sFlt-1-induced preeclampsia. Am. J. Physiol. Heart Circ. Physiol. **298**, H189–H193

86 Suzuki, H., Ohkuchi, A., Matsubara, S., Takei, Y., Murakami, M., Shibuya, M., Suzuki, M. and Sato, Y. (2009) Effect of recombinant placental growth factor 2 on hypertension induced by full-length mouse soluble fms-like tyrosine kinase 1 adenoviral vector in pregnant mice. Hypertension **54**, 1129–1135

87 Thadhani, R., Kisner, T., Hagmann, H., Bossung, V., Noack, S., Schaarschmidt, W., Jank, A., Kribs, A., Cornely, O.A., Kreyssig, C. et al. (2011) Pilot study of extracorporeal removal of soluble fms-like tyrosine kinase 1 in preeclampsia. Circulation **124**, 940–950

88 Faas, M.M., Schuiling, G.A., Baller, J.F., Visscher, C.A. and Bakker, W.W. (1994) A new animal model for human preeclampsia: ultra-low-dose endotoxin infusion in pregnant rats. Am. J. Obstet. Gynecol. **171**, 158–164

89 Zenclussen, A.C., Fest, S., Joachim, R., Klapp, B.F. and Arck, P.C. (2004) Introducing a mouse model for pre-eclampsia: adoptive transfer of activated Th1 cells leads to pre-eclampsia-like symptoms exclusively in pregnant mice. Eur. J. Immunol. **34**, 377–387

90 Elmarakby, A.A., Quigley, J.E., Imig, J.D., Pollock, J.S. and Pollock, D.M. (2008) TNF-α inhibition reduces renal injury in DOCA-salt hypertensive rats. Am. J. Physiol. Regul. Integr. Comp. Physiol. **294**, R76–R83

91 Guzik, T.J., Hoch, N.E., Brown, K.A., McCann, L.A., Rahman, A., Dikalov, S., Goronzy, J., Weyand, C. and Harrison, D.G. (2007) Role of the T cell in the genesis of angiotensin II induced hypertension and vascular dysfunction. J. Exp. Med. **204**, 2449–2460

92 Tinsley, J.H., Chiasson, V.L., South, S., Mahajan, A. and Mitchell, B.M. (2009) Immunosuppression improves blood pressure and endothelial function in a rat model of pregnancy-induced hypertension. Am. J. Hypertens. **22**, 1107–1114

93 Zhou, C.C., Irani, R.A., Dai, Y., Blackwell, S.C., Hicks, M.J., Ramin, S.M., Kellems, R.E. and Xia, Y. (2011) Autoantibody-mediated IL-6-dependent endothelin-1 elevation underlies pathogenesis in a mouse model of preeclampsia. J. Immunol. **186**, 6024–6034

94 Amorim, M.M., Santos, L.C. and Faundes, A. (1999) Corticosteroid therapy for prevention of respiratory distress syndrome in severe preeclampsia. Am. J. Obstet. Gynecol. **180**, 1283–1288

95 Fonseca, J.E., Mendez, F., Catano, C. and Arias, F. (2005) Dexamethasone treatment does not improve the outcome of women with HELLP syndrome: a double-blind, placebo-controlled, randomized clinical trial. Am. J. Obstet. Gynecol. **193**, 1591–1598

96 Ferrara, N. (2004) Vascular endothelial growth factor: basic science and clinical progress. Endocr. Rev. **25**, 581–611

97 Esser, S., Wolburg, K., Wolburg, H., Breier, G., Kurzchalia, T. and Risau, W. (1998) Vascular endothelial growth factor induces endothelial fenestrations *in vitro*. J. Cell Biol. **140**, 947–959

98 Murohara, T., Asahara, T., Silver, M., Bauters, C., Masuda, H., Kalka, C., Kearney, M., Chen, D., Symes, J.F., Fishman, M.C. et al. (1998) Nitric oxide synthase modulates angiogenesis in response to tissue ischemia. J. Clin. Invest. **101**, 2567–2578

99 Bussolati, B., Dunk, C., Grohman, M., Kontos, C.D., Mason, J. and Ahmed, A. (2001) Vascular endothelial growth factor receptor-1 modulates vascular endothelial growth factor-mediated angiogenesis via nitric oxide. Am. J. Pathol. **159**, 993–1008

100 Kroll, J. and Waltenberger, J. (1999) A novel function of VEGF receptor-2 (KDR): rapid release of nitric oxide in response to VEGF-A stimulation in endothelial cells. Biochem. Biophys. Res. Commun. **265**, 636–639

101 Ahmad, S., Hewett, P.W., Wang, P., Al-Ani, B., Cudmore, M., Fujisawa, T., Haigh, J.J., le Noble, F., Wang, L., Mukhopadhyay, D. and Ahmed, A. (2006) Direct evidence for endothelial vascular endothelial growth factor receptor-1 function in nitric oxide-mediated angiogenesis. Circ. Res. **99**, 715–722

102 Cudmore, M., Ahmad, S., Al-Ani, B., Hewett, P., Ahmed, S. and Ahmed, A. (2006) VEGF-E activates endothelial nitric oxide synthase to induce angiogenesis via cGMP and PKG-independent pathways. Biochem. Biophys. Res. Commun. **345**, 1275–1282

103 He, H., Venema, V.J., Gu, X., Venema, R.C., Marrero, M.B. and Caldwell, R.B. (1999) Vascular endothelial growth factor signals endothelial cell production of nitric oxide and prostacyclin through flk-1/KDR activation of c-Src. J. Biol. Chem. **274**, 25130–25135

104 Kang, D.H., Kim, Y.G., Andoh, T.F., Gordon, K.L., Suga, S., Mazzali, M., Jefferson, J.A., Hughes, J., Bennett, W., Schreiner, G.F. and Johnson, R.J. (2001) Post-cyclosporine-mediated hypertension and nephropathy: amelioration by vascular endothelial growth factor. Am. J. Physiol. Renal Physiol. **280**, F727–F736

105 Eremina, V. and Quaggin, S.E. (2004) The role of VEGF-A in glomerular development and function. Curr. Opin. Nephrol. Hypertens. **13**, 9–15

106 Ostendorf, T., Kunter, U., Eitner, F., Loos, A., Regele, H., Kerjaschki, D., Henninger, D.D., Janjic, N. and Floege, J. (1999) $VEGF_{165}$ mediates glomerular endothelial repair. J. Clin. Invest. **104**, 913–923

107 Masuda, Y., Shimizu, A., Mori, T., Ishiwata, T., Kitamura, H., Ohashi, R., Ishizaki, M., Asano, G., Sugisaki, Y. and Yamanaka, N. (2001) Vascular endothelial growth factor enhances glomerular capillary repair and accelerates resolution of experimentally induced glomerulonephritis. Am. J. Pathol. **159**, 599–608

108 Kim, Y.G., Suga, S.I., Kang, D.H., Jefferson, J.A., Mazzali, M., Gordon, K.L., Matsui, K., Breiteneder-Geleff, S., Shankland, S.J., Hughes, J. et al. (2000) Vascular endothelial growth factor accelerates renal recovery in experimental thrombotic microangiopathy. Kidney Int. **58**, 2390–2399

109 Sugimoto, H., Hamano, Y., Charytan, D., Cosgrove, D., Kieran, M., Sudhakar, A. and Kalluri, R. (2003) Neutralization of circulating vascular endothelial growth factor (VEGF) by anti-VEGF antibodies and soluble VEGF receptor 1 (sFlt-1) induces proteinuria. J. Biol. Chem. **278**, 12605–12608

110 Saito, S., Shiozaki, A., Nakashima, A., Sakai, M. and Sasaki, Y. (2007) The role of the immune system in preeclampsia. Mol. Aspects Med. **28**, 192–209

111 Poston, L. (2006) Endothelial dysfunction in pre-eclampsia. Pharmacol. Rep. **58** (Suppl.), 69–74

112 LaMarca, B.B., Cockrell, K., Sullivan, E., Bennett, W. and Granger, J.P. (2005) Role of endothelin in mediating tumor necrosis factor-induced hypertension in pregnant rats. Hypertension **46**, 82–86

113 Collino, F., Bussolati, B., Gerbaudo, E., Marozio, L., Pelissetto, S., Benedetto, C. and Camussi, G. (2008) Preeclamptic sera induce nephrin shedding from podocytes through endothelin-1 release by endothelial glomerular cells. Am. J. Physiol. Renal Physiol. **294**, F1185–F1194

114 Cindrova-Davies, T., Sanders, D.A., Burton, G.J. and Charnock-Jones, D.S. (2011) Soluble FLT1 sensitizes endothelial cells to inflammatory cytokines by antagonizing VEGF receptor-mediated signalling. Cardiovasc. Res. **89**, 671–679

115 Sibai, B., Dekker, G. and Kupferminc, M. (2005) Pre-eclampsia. Lancet **365**, 785–799

116 Sibai, B.M., Ewell, M., Levine, R.J., Klebanoff, M.A., Esterlitz, J., Catalano, P.M., Goldenberg, R.L. and Joffe, G. (1997) Risk factors associated with preeclampsia in healthy nulliparous women: the Calcium for Preeclampsia Prevention (CPEP) Study Group. Am. J. Obstet. Gynecol. **177**, 1003–1010
117 Duckitt, K. and Harrington, D. (2005) Risk factors for pre-eclampsia at antenatal booking: systematic review of controlled studies. Br. Med. J. **330**, 565
118 Catov, J.M., Bodnar, L.M., Ness, R.B., Barron, S.J. and Roberts, J.M. (2007) Inflammation and dyslipidemia related to risk of spontaneous preterm birth. Am. J. Epidemiol. **166**, 1312–1319
119 Giannakopoulos, B., Passam, F., Ioannou, Y. and Krilis, S.A. (2009) How we diagnose the antiphospholipid syndrome. Blood **113**, 985–994
120 Hanly, J.G. (2003) Antiphospholipid syndrome: an overview. Can. Med. Assoc. J. **168**, 1675–1682
121 Maynard, S.E. and Karumanchi, S.A. (2011) Angiogenic factors and preeclampsia. Semin. Nephrol. **31**, 33–46

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