

## Les marqueurs sériques de la pré-éclampsie: le point sur les molécules à déterminer au premier, deuxième et troisième trimestre

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### RÉSUMÉ

*La pré-éclampsie est une pathologie gestationnelle qui touche jusqu'à 10% de grossesses et qui contribue substantiellement à la morbidité et la mortalité périnatale de la mère et du nouveau-né. Plusieurs molécules ont été évaluées comme marqueurs sériques potentielles pour la présence et la confirmation de la maladie dans le dernier trimestre et par la suite pour sa prédiction au cours du deuxième trimestre. Avec les sensibilités augmentées des méthodes immuno-analytiques courants, ces efforts sont de plus en plus focalisés sur le premier trimestre. Une invasion trophoblastique compromise est supposée être à l'origine des événements menant à la pré-éclampsie, ce qui suggère qu'il devrait être possible de détecter, aux premiers stades de la grossesse par le biais de niveaux sériques anormaux de certaines molécules, un risque augmenté de pré-éclampsie ultérieure. Entrent particulièrement en ligne les marqueurs apparentés à l'activité trophoblastique. Cet article essaie de résumer, avec les données de la littérature de même que d'une étude interne, l'utilité de divers marqueurs sériques maternels pour la prédiction précoce d'une pré-éclampsie arrivant tard dans la gestation. Ce dépistage de pré-éclampsie tardive (late onset) au premier trimestre reste difficile, et aucun marqueur «miracle», associé spécifiquement à cette pathologie, a pu être identifié à ce jour. L'application de formules combinant plusieurs marqueurs, qui doivent être indépendants les uns des autres, pourrait augmenter le taux de détection. Des concentrations élevées d'inhibine A, d'activine A, de l'endogliline soluble, ou des niveaux réduits de Placenta Growth Factor (PLGF), de la PAPP-A ou de la protéine placentaire 13 (PP13) indiquent, avec une bonne sensibilité mais une spécificité assez basse, un risque augmenté d'une pathologie gestationnelle ultérieure et devrait alerter le praticien.*

**Mots-Clés:** Grossesse; Pré-éclampsie; Marqueurs sériques; Dépistage; Premier trimestre

### RIASSUNTO

**Marcatori serici della pre-eclampsia: il punto sugli analiti da determinare nel primo, secondo e terzo trimestre di gravidanza.** La pre-eclampsia è una specifica patologia gestazionale che colpisce circa il 10% delle gravidanze e che contribuisce in maniera sostanziale alla morbidità ed alla mortalità perinatale sia della madre sia del neonato. Molte molecole sono già state valutate come potenziali marcatori serici per la presenza della malattia e la diagnosi nel terzo trimestre di gravidanza e in seguito per la sua predizione nel secondo trimestre. In ragione delle sensibilità sempre più elevate degli attuali metodi immunochimici la ricerca è attualmente rivolta al primo trimestre. Un' invasione trofoblastica inadeguata nelle fasi precoci della gravidanza è ritenuta essere all'origine degli eventi che conducono alla pre-eclampsia, suggerendo che dovrebbe essere possibile individuare livelli anomali di alcune molecole o marcatori, in particolare quelli legati alla attività trofoblastica, nelle fasi molto precoci della gravidanza, in grado di prevedere un rischio aumentato di evoluzione della pre-eclampsia. Lo scopo del presente lavoro è quello di rivedere, con i dati della letteratura e con studi e casistica personale, l'utilità dei diversi marcatori individuabili nel siero materno, con particolare attenzione a quelli di origine placentare, nella individuazione precoce di una pre-eclampsia che si svilupperà nelle fasi più tardive della gravidanza. E' ancora difficile predire l'esordio della pre-eclampsia fin dalla fasi precoci della gravidanza e, al momento, non è ancora stato trovato il marcatore "miracoloso" del primo trimestre che possa essere specificamente associato a questa patologia. L'utilizzo di formule che associno le concentrazioni di gruppi di marcatori, che devono essere indipendenti tra loro, può aumentare la individuazione delle gravidanze a rischio. Elevate concentrazioni di inibina A, di attivina A e di endoglina solubile (sEng) oppure ridotti livelli di fattore di crescita placentare (PLGF), di proteina plasmatica A associata alla gravidanza (PAPP-A) o di proteina placentare 13 (PP13) indicano, con elevata sensibilità ma con bassa specificità l'aumentato rischio di una patologia gestazionale che si svilupperà più tardivamente e dovrebbero quindi allertare il clinico.

**Parole chiave:** Gravidanza; Pre-eclampsia; Marcatori serici; Screening; Primo trimestre

**ABSTRACT****Serum markers for pre-eclampsia: An update on the analytes to be determined in the first, second, and third trimester.**

*Pre-eclampsia is a pregnancy-specific disorder which affects up to 10% of pregnancies and which contributes substantially to perinatal morbidity and mortality of both mother and newborn. Many molecules have been evaluated as potential serum markers for the presence and confirmation of the disease in the third trimester, and subsequently for its prediction in the second trimester. With the increasing sensitivities of the laboratory assays, these efforts are now focused to the first trimester. Impaired early trophoblast invasion is supposed to be at the origin of the events leading to pre-eclampsia, suggesting that it should be possible to detect abnormal levels of certain molecules or markers, in particular those related to trophoblastic activity, at the very early stages of pregnancy. The scope of this paper is to review, with data from the literature as well as from an in-house study, the usefulness of various maternal serum markers, with a special focus on those of placental origin, for the early prediction of pre-eclampsia occurring later during gestation. It remains difficult to predict late-onset preeclampsia in early pregnancy, and no "miracle" first trimester serum marker has so far been found to be specifically associated to this pathology. The use of formulae combining concentrations of a set of markers, which must be regulated independently from each other, may increase the detection rate. Elevated concentrations of Inhibin A, Activin A and soluble endoglin (sEng), or reduced levels of Placenta Growth Factor (PLGF), Pregnancy-associated Plasma Protein A (PAPP-A), or Placental Protein-13 (PP13) indicate, with high sensitivity but low specificity, an increased risk of a later occurring gestational pathology and should alert the clinician.*

**Key words:** Pregnancy; Pre-eclampsia; Serum markers; Screening; First trimester

**INTRODUCTION**

Pre-eclampsia, a pregnancy-specific disorder affecting 8–10% of gestations<sup>1,2</sup> and defined by gestational hypertension and proteinuria, contributes substantially to the perinatal morbidity and mortality of both mother and newborn. It is thought to be an early placental dysfunction<sup>3</sup>, characterised by insufficient invasion of the spiral arteries by the trophoblast, placental ischaemia<sup>4</sup>, and impaired perfusion of the uteroplacental unit which can lead to fetal growth restriction (FGR). In spite of a large number of investigations, the underlying aetiology of the disease remains unclear. The release of substances of trophoblastic origin such as placental debris, growth factors, placental hormones, and pro-inflammatory cytokines lead to an excessive maternal inflammatory response<sup>5,6</sup>. The maternal symptoms (hypertension, proteinuria) appear later in pregnancy, and are the consequences of endothelial activation and dysfunction<sup>7,8</sup>. Currently, the delivery of the placenta is the only effective therapy of pre-eclampsia, but this carries for the baby the sequelae of an induced pre-term delivery.

The assessment and the prediction of pre-eclampsia has traditionally been on the basis of risk factors in the maternal history and the examination for the presence of hypertension, proteinuria and oedema. More recently, measurable manifestations of abnormal placentation and reduced placental perfusion associated with the disorder have been added to the panel of investigations. Research initially focused on non biochemical markers such as mid-trimester blood pressure measurements<sup>9,10</sup> or an increased sensitivity to vasoactive substances<sup>11</sup>, but none of these has so far been shown to be of clinical value. The exception to this fact is Doppler sonography of the uterine artery, which is currently under intense investigation but which is not within the scope of this paper on biochemical pre-eclampsia markers.

In the mid-nineties, the focus shifted towards biochemical markers. The purpose of this article is to review, in terms of laboratory predictive performance rather than biological function and pathogenetic role, those of these sub-

stances with a potential clinical usefulness for the assessment and the prediction of pre-eclampsia. Given the large and ever increasing number of them, this can only be done in a non exhaustive way.

**THIRD-TRIMESTER ASSESSMENT USING SERUM MARKERS**

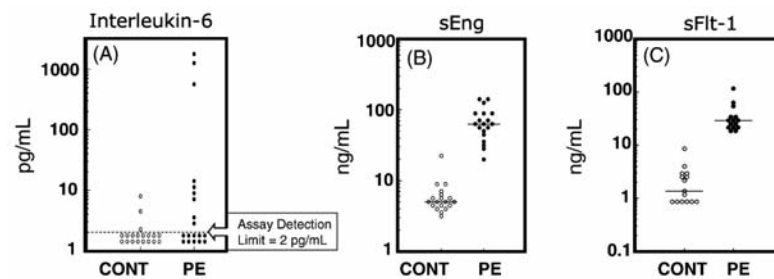
Before reviewing the serum markers playing a potential clinical role in the second and first trimester screening, we are taking a brief look on the information available on the molecules showing an abnormal circulating concentration pattern in the third trimester or after the onset of the maternal symptoms of pre-eclampsia. We have previously confirmed the presence of elevated serum levels of the three placental products pregnancy-associated plasma protein A (PAPP-A), inhibin A, activin A, and the non placental soluble endothelial (sE-) selectin<sup>12</sup>. Other adhesion molecules circulating in soluble form (ICAM-1, VCAM-1) show similarly elevated serum levels<sup>13</sup>, and the same was suggested to be the case for some inflammatory cytokines such as interleukin-6 (IL-6) and tumour necrosis factor  $\alpha$  (TNF- $\alpha$ )<sup>14</sup>. There are, however, a few serum marker proteins on which the literature is inconsistent in terms of the deviation of their serum levels in women with pre-eclampsia in comparison to healthy pregnant controls of identical gestational age. While significantly reduced serum concentrations of placenta growth factor (PLGF) have been confirmed<sup>15</sup>, those for the structurally related vascular endothelial growth factor (VEGF) have first been reported to be elevated in a number of earlier studies<sup>16-18</sup>. More recently, however, reports on reduced VEGF levels in pre-eclamptic pregnancies seem to dominate<sup>19,20</sup>. Similarly, reduced or at least less strongly increasing levels of macrophage colonystimulating factor (M-CSF)<sup>21</sup> as well as increased serum levels of this marker<sup>22</sup> had been linked with the presence of pre-eclampsia. Insulin-like growth factor binding protein-1 (IGF-BP1) was found to be elevated in pre-eclampsia as a function of the severity of the disease in a couple of reports<sup>23,24</sup>, while in another study no such effect was observed<sup>25</sup>. Lastly for leptin, a product

of the adipose tissue compartment but for which placental synthesis<sup>26</sup> and an involvement in inflammatory processes<sup>27</sup> had been suggested, increased<sup>28,29,30</sup>, unchanged<sup>31</sup>, and reduced<sup>32</sup> serum levels in pre-eclamptic women in comparison to healthy controls had been reported. These discrepancies could not be attributed with certainty to the different assay methodologies (RIA, ELISA) used for these tests.

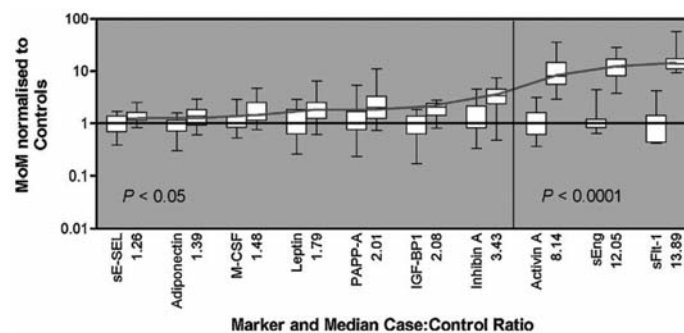
We have published a synopsis on fourteen thirdtrimester markers in this Journal<sup>33</sup>. More recently, we have enlarged the panel by the determination of interleukin-6 (IL-6), soluble endoglin (sEng), and soluble fms-like tyrosine kinase-1 (sFlt-1) in the same matched serum samples. The results are shown in Fig. 1A-C. Compared to the results from the other markers<sup>33</sup>, the effect is well-pronounced for sEng and sFlt-1, and this is illustrated in Fig. 2 which shows all analysed markers for which a significantly increased median serum concentration was observed as a function of the extent of the difference between cases and matched controls in our study<sup>33</sup>. These are — besides the mentioned IL-6, sEng and sFlt-1 — adiponectin<sup>34,35</sup>, leptin, soluble endothelial selectin, PAPP-A<sup>36,37</sup>, M-CSF, IGF-BP1, inhibin A<sup>38,40</sup>, and activin A<sup>39,40</sup> for which we found the strongest caseversus- control difference and the least overlap between the two populations in the previous study. sEng performed equally well or better (Fig. 2), confirming the findings of a recent study with a slightly larger sample size<sup>41</sup> with an even higher case: control median ratio in our sample. The same was the case for sFlt-1, where no overlap was seen and a very recent, large study

was confirmed<sup>42</sup>. IL-6 is not shown on Fig. 2 since the cytokine concentration was below the detection limit (2 pg/mL) in most controls and in many cases; a ratio between the medians of cases and controls could therefore not be calculated. Nevertheless, the increase was statistically significant ( $P = 0.0168$  by non parametric Mann-Whitney U test, Fig. 1A), which is in agreement with one recently published study<sup>43</sup>, but not with another<sup>44</sup>. It is possible that only severe cases of pre-eclampsia would show consistently elevated serum IL-6 levels; moreover, the laboratory tests with the highest possible sensitivities should be used for this cytokine.

We found both VEGF and PLGF to be reduced in the cases, but for VEGF the overlap between the two groups was too large for the marker standing a chance to be clinically useful. This overlap would explain the inconsistent data in the literature. For PLGF, on the other hand, we confirmed not only the previously reported reduction of serum levels in pre-eclampsia<sup>15</sup>, but we also observed the absence of an overlap between the case and control groups, a feature shared only with (the increased) activin A<sup>33</sup>, sEng, and sFlt-1. Pregnancy-specific glycoprotein 1 (SP1) and human placental lactogen (HPL) are two molecules solely produced by the placenta, and for this reason it is surprising that the literature on the clinical usefulness of these analytes for pre-eclampsia assessment and screening is comparatively poor. We have determined SP1 and HPL in our project<sup>33</sup>. For SP1 no difference was found, confirming an early study<sup>45</sup>. For HPL, however, we have observed a



**Figure 1** Interleukin-6, soluble endoglin, and fms-like tyrosine kinase-1 in women with pre-eclampsia and in controls matched for gestational age, maternal age and parity. The patient groups are the same as in<sup>12</sup>. Measurements were made using microplate enzyme immunoassays with the “matched pair” (IL-6) or “Quantikine®” (sEng and sFlt-1) methods (all from R&D Systems, Oxford, England). Open circles, controls; closed circles, women with pre-eclampsia after onset of maternal symptoms.



**Figure 2** Markers for which significantly increased serum concentrations were found in women with pre-eclampsia when compared to healthy, gestation-matched control pregnancies, as a function of the observed difference in multiple of the median (MoM) between the two groups. These are the same as in Fig. 1 and<sup>12</sup>. Boxes show the median, 25th and 75th centiles; whiskers show the range.

significant decrease for the case group in our study<sup>33</sup>, and this is not in full agreement with an early study where this marker was increased in primigravid pre-eclampsia patients, but not in multigravid pregnancies suffering from the same disease<sup>46</sup>. These findings are difficult to explain, but given the again massive overlap in HPL levels between the case and control group for HPL<sup>33</sup> we do not believe that this marker would become a clinical tool in relation to pre-eclampsia. It also has to be noted that the assay methods in these old studies were different from the current, highly sensitive immunoanalytical protocols.

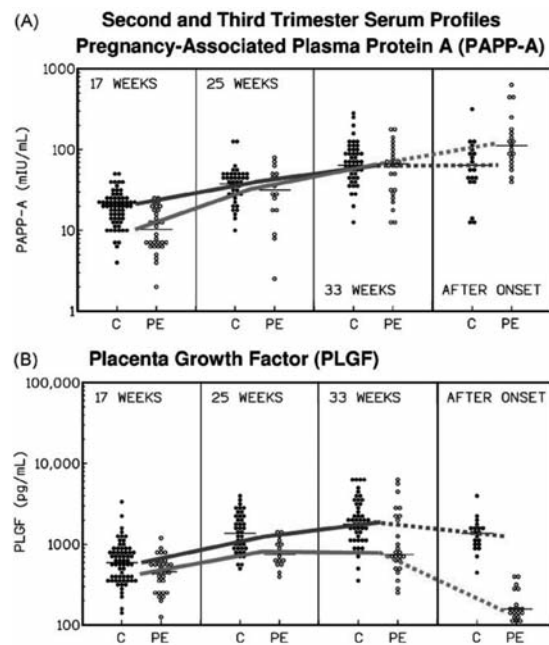
Controversial results for some markers are likely to be the consequence of the varying severity of the disease and the fact that many of them are produced in more than one tissue and at different rates depending on source and gestational age. Variations between methods, on the other hand, become less and less accountable for these variations since we are observing a shift towards better controlled, commercially available assays. Although it is not the major clinical goal to perform these serum determinations in the third trimester and after onset of the first maternal symptoms of pre-eclampsia, the results provide useful information on the correlation with these symptoms and on which markers will stand the highest chance to become a useful laboratory tool for early pre-eclampsia risk detection. In conclusion, reduced serum PLGF and increased activin A, sEng and sFlt-1 concentrations seem to be the best indicators of preeclampsia in the third trimester, and therefore could carry the potential to be useful, alone or in combination, at earlier gestational stages.

## SECOND-TRIMESTER SCREENING

In current antenatal care, it is attempted to predict any abnormal or pathological development of the pregnancy as early as possible — this both in the interest of the patient and the clinician. Biochemical risk assessment using the concentrations of various serum protein markers in the second (and in the first, see below) trimester of pregnancy has been proposed and introduced for several gestational pathologies, amongst them fetal trisomy 21 (Down syndrome) and preeclampsia. The parameter that most strongly influences the final screening result is the gestational age, which therefore has to be precisely determined. Other factors which may have, depending on the selected marker, an influence are the maternal weight (or BMI), ethnicity, and smoking. The risk for the pathology to be present or to occur later is calculated from the concentration of one or several markers, normalised to multiples of the median, and correction factors established from the above characteristics or conditions. In pre-eclampsia screening, the result is strongly influenced by the length of the interval between the time of the testing (blood collection) and the onset of the maternal symptoms; the screening efficiency increases with the reduction of this time interval and hence with the severity of the disease.

Inhibin A<sup>47-49</sup> and activin A<sup>39,49</sup> were amongst the first biochemical serum markers to be found of interest in second-trimester pre-eclampsia screening. As it was the case after onset of the maternal symptoms, their levels were found to be increased in comparison to healthy controls. PLGF, on the other hand, was found to be reduced

not only in the third, but also in the second trimester in women subsequently developing pre-eclampsia<sup>50</sup>. The situation is different for PAPP-A. Serum concentrations of this large glycoprotein were found to be increased after the onset of the disease in the third trimester<sup>12,36,37</sup>, but reduced in early pregnancy<sup>51</sup>. We were subsequently able to confirm this “biphasic” pattern (i.e. a steeper increase, as a function of gestational age, of PAPP-A levels in subsequently pre-eclamptic pregnancies than in healthy control gestations): at 17 weeks, i.e. in the early second trimester, we observed reduced concentrations for this marker, while at 25 and 33 weeks no significant differences between cases and controls were found<sup>52</sup>. This is illustrated in Fig. 3A. PLGF, on the other hand, was confirmed to remain reduced in the case group throughout the examined gestational range starting at 17 weeks<sup>52</sup> and shown in Fig. 3B. At 17 weeks but not later, SP1 and HPL levels were also found to be decreased in subsequently pre-eclamptic pregnancies, as it was the case for PAPP-A<sup>52</sup>. When comparing several markers of placental and non placental origin in the same women (25 cases of mild, late-onset pre-eclampsia and 64 controls) at 17 and 33 weeks, we found significantly lower serum levels for PAPP-A, SP1, and HPL at 17 and for PLGF at 17 as well as at 33 weeks. Inhibin A and activin A were reduced at 17 and increased at 33 weeks<sup>53</sup>. Statistical significance was not reached for these two markers, but the trend clearly follows the placental compensation (steeper increase in pathological situations) hypothesis<sup>54</sup> outlined above. For IGF-BP1, a protein not



**Figure 3** Development of serum concentration differences for PAPP-A (A) and PLGF (B) between pre-eclampsia patients (red line) and healthy pregnancies (blue line): PAPP-A levels change from decreased levels in the early second trimester to increased concentrations after the onset of the maternal symptoms, while PLGF in pre-eclampsia patients always remains below the normal medians. Note that the groups of patients and controls are different in the 17/25/33-week group<sup>52</sup> and in the post-onset study<sup>12</sup>.

derived from the placenta, no such compensation was observed in our longitudinal study: at 33 weeks (but which was before the onset of symptoms in our group of mild PE patients) its median serum level was still below the one of the control group<sup>53</sup>. However, it has to be noted that serum IGF-BP1 was increased, in different groups of patients, once the maternal symptoms of the disease became apparent<sup>12,23,24</sup>. Precisely, in another longitudinal study covering the second and the third trimester, the serum concentrations of IGFBP-1 were measured at six time points between 16 and 36 weeks in normotensive and subsequently pre-eclamptic pregnancies: at 16 weeks, they were lower in the cases than in the controls, at 28 and 32 weeks no alteration was found, whereas at 36 weeks the IGF-BP1 levels were increased in comparison to the controls<sup>55</sup>.

The effect of smoking on the concentration of a given serum marker may have to be accounted for in the risk evaluation. Inhibin A levels were reported to be elevated in smokers<sup>56</sup>, but no studies on a possible effect on activin A and PLGF levels are available in the literature. This is surprising since it is precisely these two serum markers which show significant differences between pregnancies subsequently affected by pre-eclampsia (elevation for the former and reduction for the latter) and healthy ones. We have therefore determined these and other markers in a pre-existing sample of smokers (light or heavy) and non smokers in the second trimester<sup>57</sup>. Pregnancies with subsequent mild (late onset) pre-eclampsia had been included, but those with FGR were excluded since these are themselves characterised by a strong association with smoking. We have found a substantial effect of smoking on the serum levels of PLGF and inhibin A; they were increased by approximately two-fold ( $P < 0.001$ )<sup>57</sup>. Precisely, for these two markers, the deviations between subsequently pre-eclamptic and healthy, gestation matched pregnancies were found to be amongst the largest from the second trimester and until the onset of symptoms<sup>33</sup>. On the other hand, we could not demonstrate an effect of maternal smoking on the serum levels of activin A, PAPP-A, SP1, HPL, leptin, sE-selectin, IGF-BP1, M-CSF, and VEGF at 17 weeks of gestation, and this was not influenced by the possible later development of pre-eclampsia<sup>57</sup>. For PAPP-A, there is a conflicting report of a slight but significant reduction (to 0.89 MoM) in the literature for smokers' levels in the first trimester<sup>58</sup>. The discrepancy could be explained by the different gestational age of the study populations together with the possible placental compensatory action mentioned above<sup>54</sup>. As a consequence, correction factors will have to be applied in second trimester pre-eclampsia risk evaluations based on PLGF and for inhibin A, but not for other markers which have so far been investigated and mentioned above.

In the literature, information on the presence of fetal growth restriction (FGR) is scarce, and many published studies suffer from a bias induced by a higher incidence of pregnancies with FGR amongst the case groups (pre-eclampsia) in comparison to the control groups. This may generally be responsible for any set of discordant results reported for the second trimester or later in pregnancy, when the findings become more specifically related to a given pathology (in the first trimester many markers are nonspecifically reduced, see next section). We have inve-

stigated the serum levels of several markers, in the early second trimester, in separate groups of pregnancies with pre-eclampsia but without FGR, and with FGR but without pre-eclampsia, and found PAPP-A, SP1, and HPL to be reduced in both pathologies<sup>59</sup>. However, the second trimester concentrations of PLGF and VEGF, two structurally closely related molecules, were specifically reduced only in pre-eclamptic or only in FGR pregnancies, respectively<sup>59</sup>. The same was the case in the early third trimester. HPL remained low throughout pregnancy in gestations affected by FGR, but fell within the range of normal, healthy pregnancies by 25 gestational weeks<sup>52</sup> in those with later developing pre-eclampsia.

### FIRST-TRIMESTER SCREENING

The early identification of women at risk for developing pre-eclampsia would highly improve their monitoring and surveillance, and therefore reduce maternal and fetal morbidity by the benefit from adequate therapeutic measures. A considerable number of first trimester serum markers has been investigated to date for their potential to become useful tools for pre-eclampsia screening; they have been reviewed recently<sup>60</sup> and the selection presented here is not exhaustive.

### INHIBIN A AND ACTIVIN A

These glycoprotein hormones are predominantly secreted by the placenta, but it is not known to which extent and by what mechanisms they contribute to the aetiology of pre-eclampsia. Besides being elevated later in gestations leading to pre-eclampsia, inhibin (and/or activin) A were also found to be increased in the early second<sup>48,49</sup> and the first trimester<sup>48,62</sup> in the case group when compared to gestation-matched controls. In a narrow gestational bracket (12–13 weeks), using 52 cases of mostly mild, late onset pre-eclampsia and 104 healthy controls matched not only for gestational age (by the day) but also for maternal age, maternal weight and storage time, we have crosssectionally compared the performance of ten serum markers<sup>63</sup>. In this study, significantly increased serum levels of both inhibin A and activin A ( $P = 0.01$  for both) in comparison to the controls were detected, thus confirming the earlier reports. As for other markers, the severity of the pathology (interval to symptom onset) influences the screening detection rate and, unfortunately, the assay method and antibody specificities have changed. The first tests, which date back 15 years or more, determined mostly the subunit of inhibin/activin and thus were less specific. The latest assay protocols have now also abandoned the denaturation step which was previously required, and we can hope that a move towards a more consistent and standardised protocol has been initiated. As a consequence of this and with the results above, inhibin A and activin A seem to be the most promising biochemical markers for early pregnancy pre-eclampsia screening.

### PREGNANCY-ASSOCIATED PLASMA PROTEIN A

This very large, predominantly placental glycoprotein

complex modulates IGF activity and has been suggested to play a role in human embryo implantation<sup>64</sup>. With circulating PAPP-A levels that are increased in the third and decreased in the early second trimester (see above and Fig. 3A), we expected even lower (in terms of MoM) serum PAPP-A concentrations at 12 weeks of gestation, but surprisingly a statistical significance was not reached in our study on 52 pre-eclampsia patients mentioned above<sup>63</sup>. First-trimester serum PAPP-A levels are very low, and even the most modern assay methods may still not be sensitive enough for this application.

### PLACENTA GROWTH FACTOR (PLGF)

This marker was found to be one of the best serum indicators from the early second trimester onwards (see above) but, as for PAPP-A, a statistically significant reduction could not be demonstrated by direct, non parametric analysis in our cross-sectional study mentioned above<sup>63</sup>. A similar result was reported in an earlier, large study together with the observation that PLGF serum concentrations were actually increased in pregnancies with fetal growth restriction<sup>65</sup>. PLGF therefore does not seem to be a good predictor for (at least mild) pre-eclampsia when analysed too early in pregnancy.

In our narrow-interval first-trimester serum study involving 52 cases and 104 matched controls<sup>63</sup>, we were able to confirm, using descriptive non parametric statistical analysis, significantly increased first trimester serum concentrations of inhibin A and activin A in women subsequently developing pre-eclampsia, together with a trend towards reduced levels for PAPP-A but for which statistical significance was not reached by this method (Mann-

Whitney U test, Table 1). Univariable analysis also yielded an association between an increased risk for pre-eclampsia and reduced PAPP-A as well as HPL levels.

### LEPTIN, IL-8, CRP

These essentially non placental markers have been studied in the context of pre-eclampsia due to their association with inflammation, and hence to their possible involvement in the pathogenesis of the disease. A cross-sectional study reported elevated serum leptin levels in established pre-eclampsia<sup>66</sup> while in another investigation<sup>31</sup>, no difference between pre-eclamptic and normotensive, pregnant women was found. Information in the literature on serum leptin levels prior to the onset of pre-eclampsia are scarce. A longitudinal study reported elevated serum leptin concentrations between 20 and 36 weeks of gestation in women with subsequent pre-eclampsia<sup>67</sup>. Another report is in agreement<sup>66</sup>, but there are also second<sup>68</sup> and first-trimester studies where no difference in leptin levels between women with and without subsequent pre-eclampsia could be observed<sup>61</sup>, including our own<sup>63</sup>. For the other inflammation-associated markers IL-8 and CRP, no changed serum levels were observed as a function of later occurring pre-eclampsia<sup>63</sup>.

In our 2007 study<sup>63</sup>, the most potent first trimester serum markers associated with the risk to later develop preeclampsia were inhibin A and activin A, followed by PAPP-A. It is interesting to note that these are largely, but not exclusively produced by the placenta. Leptin, for which we did not observe an association with subsequent pre-eclampsia, would also fall into the category of proteins partially produced by the placenta, but the non placental pro-

**Table 1** Median and range for the serum concentrations of 15 marker proteins in the first trimester (11+2 to 13+6 weeks of gestation) in pregnancies later developing mild, late-onset pre-eclampsia (cases) and in healthy pregnancies (controls) matched for gestational age, maternal age, maternal weight, and sample storage time.

| A. Clinical parameters             | Cases Mean ± S.D. |              | Controls Mean ± S.D. |              | P             |
|------------------------------------|-------------------|--------------|----------------------|--------------|---------------|
| Maternal age, years                | 30.9 ± 4.9        |              | 31.3 ± 4.5           |              | n.s. (match)  |
| Maternal weight, kg                | 74.6 ± 17.0       |              | 74.2 ± 15.0          |              | n.s. (match)  |
| Gestational age at delivery, weeks | 38.9 ± 1.6        |              | N/A                  |              | —             |
| Birthweight, g                     | 3190 ± 592        |              | N/A                  |              | —             |
| B. Marker proteins unit            | Cases (n = 40)    |              | Controls (n = 80)    |              | P             |
|                                    | Median            | Range        | Median               | Range        |               |
| PAPP-A, mIU/mL                     | 2.59              | (0.51–11.1)  | 3.02                 | (0.54–7.96)  | n.s.          |
| SP1, mg/mL                         | 8.90              | (3.26–40.9)  | 9.70                 | (1.93–28.1)  | n.s.          |
| HPL, mg/mL                         | 0.647             | (0.127–1.20) | 0.703                | (0.162–1.42) | n.s.          |
| Free β-hCG, mIU/mL                 | 34.4              | (11.7–104)   | 35.1                 | (10.4–111)   | n.s.          |
| Inhibin A, pg/mL                   | 191               | (85–360)     | 141                  | (26–584)     | <b>0.0092</b> |
| Activin A, pg/mL                   | 2489              | (1200–9759)  | 2109                 | (929–5518)   | <b>0.0015</b> |
| PLGF, pg/mL                        | 149               | (68–309)     | 144                  | (56–233)     | n.s.          |
| Leptin, ng/mL                      | 37.9              | (6.39–119)   | 31.4                 | (7.26–122)   | n.s.          |
| Adiponectin, μg/mL                 | 10.4              | (2.06–21.4)  | 9.80                 | (2.88–22.9)  | n.s.          |
| sE-Selectin, ng/mL                 | 666               | (497–793)    | 680                  | (568–872)    | n.s.          |
| CRP, μg/mL                         | 6.92              | (0.55–31.4)  | 7.21                 | (1.02–127)   | n.s.          |
| IL-8, pg/mL                        | 7.82              | (1.92–43.2)  | 6.64                 | (1.92–28.0)  | <b>0.0463</b> |
| Midkine, ng/mL                     | 4.03              | (0.17–101)   | 3.05                 | (0.30–181)   | n.s.          |
| sEng, ng/mL                        | 5.35              | (3.77–8.70)  | 4.78                 | (3.08–7.53)  | <b>0.0230</b> |
| sFlt-1, pg/mL                      | 1537              | (659–3654)   | 1354                 | (525–3082)   | <b>0.0414</b> |

Three pregnancies affected by fetal growth restriction (in addition to pre-eclampsia) have been excluded from this analysis. P values were obtained with the Mann-Whitney U test (Student t test for maternal age and weight); statistically significant differences ( $P < 0.05$ ) are marked in bold. N/A: not available (results of normal births not reported back by clinicians).

duction, by the adipose tissue, is predominant and correlated with body mass. Production of a small fraction by the placenta is similarly occurring for CRP, and this is also the case for cytokines such as IL-8 with their ubiquitous production and multiple action. Leptin, CRP and IL-8 are related to inflammatory processes<sup>27</sup> such as pre-eclampsia, but this may play a role only later in gestation. Interestingly the markers SP1 and free -hCG, which can be placed on the opposite side of the production distribution scale, i.e. which are synthesised and released exclusively by the placenta, are no indicators for an increased risk of pre-eclampsia. These combined observations illustrate that the pathogenetic mechanisms of this disease are complex, and that is unlikely that a single "miracle" serum marker for early pregnancy pre-eclampsia screening would be identified in the near future.

### INSULIN-LIKE GROWTH FACTOR-1 AND IGFBP-1

IGF-1 plays a role in human embryo implantation and trophoblast invasion<sup>69</sup>, and thus may indirectly influence the development of pre-eclampsia. A cross-sectional study suggested an association between the risk to develop preeclampsia and lowered serum levels of both IGF-1 and IGFBP-1 at 13 weeks<sup>70</sup>. This observation suggests that a difference in these markers may also be present earlier in the first trimester. However, information to date is scarce and further studies are needed to evaluate IGF-1 and IGFBP-1 levels as a possible marker of predicting pre-eclampsia.

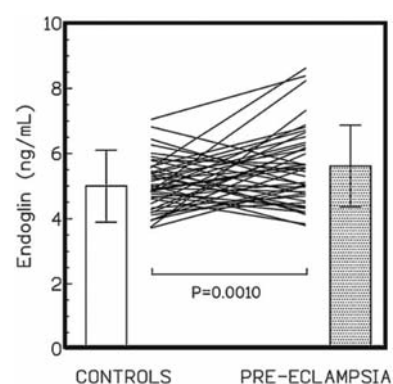
### SOLUBLE ENDOGLIN (SENG)

Endoglin is a co-receptor for transforming growth factor  $\beta$ 1 and  $\beta$ 3 expressed on trophoblast cells. A soluble, circulating form of this molecule has been identified<sup>71</sup> and suggested to be a possible factor causing the development of pre-eclampsia<sup>72</sup>. In healthy pregnancies, serum sEng levels increase particularly during the last two months, and in those with subsequent pre-eclampsia this rise was even more pronounced<sup>73</sup>, with a further increase for cases of the early-onset, severe form of the disease. We have recently determined sEng in our study group (11 + 2 to 13 + 6 weeks) mentioned above, and found a small but significant increase in the cases compared to the matched controls<sup>74</sup>. Paired analysis between carefully matched groups (one case and two controls, matched for gestational age by the day, maternal age and weight per group<sup>63</sup>) shows an overall increase in the cases but a large variation (Fig. 4). Receiver-operator curve (ROC) analysis yielded and area under the curve (AUC) of 0.628 (Fig. 5).

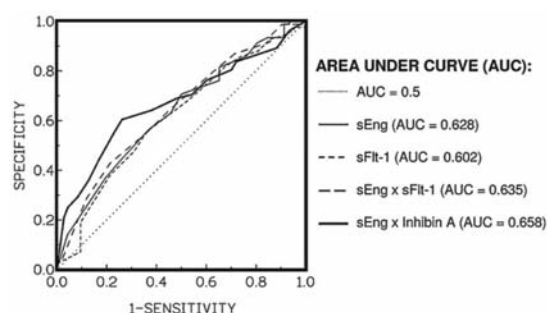
### SOLUBLE FMS-LIKE TYROSINE KINASE-1 (SFLT-1) OR SOLUBLE VEGF RECEPTOR-1 (SVEGFR-1)

PLGF and VEGF share close amino acid homology and are inhibited in their function by the circulating, soluble form of their receptor sVEGFR-1, known as sFlt-1. This impairs the binding of PLGF and VEGF with membrane-bound, functional Flt-1 in vascular tissues and thereby leads to endothelial dysfunction<sup>75</sup>. Intact Flt-1, soluble Flt-1 as well as their ligands PLGF and VEGF-A are expres-

sed by trophoblasts. Elevated sFlt-1 and, consecutively, lowered VEGF and PLGF levels may play a pathogenic role in the development of pre-eclampsia. A nested, longitudinal case control study measured sFlt-1, free PLGF, and free VEGF in monthly intervals and, in normal pregnancies, while the serum concentrations of PLGF decreased the levels of sFlt-1 increased constantly into the last trimester. In pregnancies with subsequent pre-eclampsia, these changes were detectable earlier and were more pronounced, allowing the prediction of the onset of the disease approximately five weeks prior to the appearance of the clinical symptoms<sup>76</sup>. In another, prospective study sFlt-1 and PLGF serum levels at or before 12 weeks of gestation were determined<sup>77</sup>, but no difference in sFlt-1 between cases and controls was observed while PLGF serum levels were lower in the later pre-eclamptic pregnancies. Yet another first trimester investigation, however, found no alteration in first trimester PLGF serum concentrations when pregnancies with and without subsequent pre-eclampsia were compared<sup>65</sup>, and this was in agreement with our own results<sup>63</sup>. In the analysis mentio-



**Figure 4** First trimester serum concentrations of soluble endoglin (sEng) in pregnancies later developing pre-eclampsia (hatched bar, n = 46) and healthy controls (open bar, n = 92, mean $\pm$ S.D.) matched for gestational age, maternal age and maternal weight<sup>63</sup>. The individual lines illustrate the paired analysis for the matching groups (one case and two controls each). The case population included three pregnancies with FGR (exclusion of these did not influence the result and statistical analysis).



**Figure 5** Receiver operator curve (ROC) analysis for inhibin A, soluble endoglin, sFlt-1 and their combinations in the first trimester. The groups of patients subsequently developing (mild) pre-eclampsia and matched healthy control pregnancies are the same as in<sup>63</sup>. Areas under the curve are (AUC) are given in the graph.

ned above for sEng, we have included the determination of sFlt-1 and similarly found increased serum levels for this molecule<sup>74</sup>; receiver-operator analysis yielded an AUC of 0.602 (Fig. 5).

**PLACENTAL PROTEIN 13 (PP13)**

The biological role of PP13, one of over 50 placenta-specific proteins, and its possible involvement in the pathogenesis of pre-eclampsia has not been investigated to date. In the second trimester, PP13 only detected early (severe) cases<sup>78</sup>. In the first trimester, however, more markedly decreased serum concentrations of this protein have been found<sup>79</sup> and recently confirmed<sup>80</sup>; ROC analysis yielded an AUC of 0.67. As it has been similarly demonstrated for PAPP-A, a recent, longitudinal study showed a steeper increase in the PP13 serum level as a function of gestational age for pregnancies with pre-eclampsia than for healthy controls, resulting in a crossover of the two curves around 20 pregnancy weeks<sup>81</sup>. In spite of the limited usefulness for mild, late-onset pre-eclampsia as it was seen for all other serum proteins described above, PP13 currently seems to be one of the more promising first-trimester markers. Further studies are however warranted and the assay is not yet widely available.

**CONCLUSIONS**

A growing number of biochemical serum markers has been tested for the prediction of pre-eclampsia but, unfortunately, they have not met the expectations. Longitudinal studies are scarce, and the sensitivities and predictive values are generally not satisfactory. Moreover, for many of these markers the limited results available are contradictory and thus do not provide sufficient justification for the use as screening tools in present-day clinical practice. Due to the timing of trophoblast invasion between 8 and 16 weeks of gestation, a preventive treatment and therefore a screening test should ideally be performed during the first or early second trimester. Due to placental compensation or other, so far unexplained mechanisms the list of candidate markers for pre-eclampsia screening varies as a function of gestational age. In the early second trimester, the best serum markers were found to be PLGF, PAPP-A, SP1 and HPL. With advancing pregnancy, of these only PLGF remains reduced in comparison to controls while the concentration curves of the other three “catch up” and reach or even overtake the normal medians. At this stage of gestation, care has to be taken to distinguish pre-eclampsia from other pathologies such as fetal growth restriction, for which different markers such as HPL or VEGF show a better and more specific screening efficiency. Inhibin A, activin A, sEng and sFlt-1 are potential pre-eclampsia screening markers for all trimesters, but the severity of the disease (time of onset of maternal symptoms) has a strong impact on the performance of them. In the first trimester, while PAPP-A and PLGF were surprisingly disappointing, PP13 and probably sEng, sFlt-1 and inhibin A seem to be the best markers for future clinical use. The usefulness of interleukin-8 may also deserve further investigation. Table 1 summarises the values for 15 markers from our first-trimester study.

PAPP-A, and probably other markers, show a comparatively high sensitivity but a low specificity in the first trimester; decreased serum concentrations are found in a number of pathologies differing in their aetiology (pre-eclampsia, FGR, fetal Down syndrome, etc.). Such a result should therefore alert the clinician and suggest a higher level of pregnancy surveillance. In the course of the second trimester, the screening results become more specific for a given pathology.

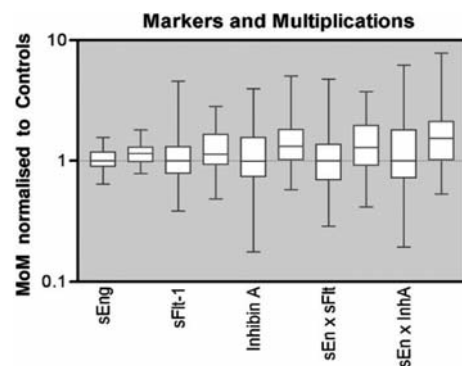
Combining the measurements of different markers in mathematical algorithms would theoretically improve the screening efficiency, but this only as long as their production and/or release is controlled by independent mechanisms. Unfortunately, and contrary to Down syndrome serum screening, this does generally not seem to be the case for pre-eclampsia in the first trimester. As a consequence, the multimarker formulae gave only marginally better results than did single marker determinations [74]. Fig. 6 illustrates some of these combinations from our study. For example, combining ROC analysis for sEng and sFlt-1 would yield an AUC of 0.852 if these two markers were totally independent from each other ( $1 - \{1 - 0.628\} \times \{1 - 0.602\}$ ), (Fig. 5). The same was shown to be the case for combinations involving PP13<sup>78</sup>. In conclusion, we can say that no single “miracle” marker has so far been identified, but some of them are more promising than others, and their usefulness depends on the gestational age at the assessment and the severity of the disease. Combining not several biochemical serum markers but one single, well-selected marker with uterine artery Doppler sonography may be the tool of choice for early pregnancy pre-eclampsia screening in the future.

**CONFLICTS OF INTEREST**

None

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**Figure 6** First-trimester pre-eclampsia screening performance with markers and their combinations by multiplication. The groups of patients subsequently developing (mild) preeclampsia (red boxes/whiskers) and matched healthy control (blue boxes/whiskers) pregnancies are the same as in<sup>63</sup>. Boxes show the median, 25th and 75th centiles; whiskers show the range.

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