Vascular endothelial growth factor (VEGF) and discrimination between abnormal intrauterine and ectopic pregnancy

Elisabeth Kucera-Sliutz 1,4, Ingrid Schiebel 1, Franz König 2, Sepp Leodolter 1, Gerhard Sliutz 1 and Heinz Koelbl 3

1 Department of Gynaecology and Obstetrics, University of Vienna, Waehringer-Guertel 18–20, 2 Department of Medical Statistics, University of Vienna, Schwarzenbrunnerstrasse 17, 1090 Vienna, Austria and 3 Department of Gynaecology, Martin-Luther University, 06097 Halle/Saale, Germany

BACKGROUND: This study evaluated serum vascular endothelial growth factor (VEGF) levels in women with abnormal intrauterine and ectopic pregnancies (EP) at 6 weeks gestation. METHODS: We conducted a prospective case–control study comparing serum VEGF concentrations among 84 women with abnormal intrauterine and EP matched for gestational age (42 women in each group). We analysed whether serum VEGF levels >200 pg/ml would discriminate between abnormal intrauterine pregnancies and EP at 6 weeks gestation, and we calculated sensitivity, specificity and positive predictive values. RESULTS: Serum VEGF concentrations did not show statistically significant differences between women with abnormal intrauterine pregnancies (median, 198.5 pg/ml; range, 0–701.6) and EP (median, 211.2 pg/ml; range 0–628.8). When threshold concentrations of a serum VEGF level >200 pg/ml were used, abnormal intrauterine pregnancy could be distinguished from EP with a sensitivity of 56%, a specificity of 51%, and a positive predictive value of 53%. CONCLUSIONS: VEGF does not discriminate ectopic from abnormal intrauterine pregnancies at 6 weeks gestation, and thus should not be used in clinical management.

Key words: ectopic/pregnancy/VEGF

Introduction
It is a well known fact that the incidence of ectopic pregnancy (EP) has dramatically increased over the past three decades, although in the recent past it has finally levelled out to an incidence of ~2% of all pregnancies (Tulandi and Sammour, 2000). As management of EP has changed over the years towards a conservative approach, early diagnosis is important in order to allow conservative treatment options (Tulandi and Sammour, 2000; Felemban et al., 2001).

EP might be diagnosed as early as 4–5 weeks gestation, although at first presentation nearly 40–50% of EP cases are initially misdiagnosed despite high-resolution vaginal ultrasonography and sensitive quantitative β-hCG assays (Abbott et al., 1990). For this reason, several markers, in addition to non-invasive algorithms, have been under investigation for the diagnosis of EP, including maternal serum creatine kinase levels (Saha et al., 1999), fetal fibronectin levels from cervico-vaginal swabs (Nowacek et al., 1999), and serum vascular endothelial growth factor (VEGF) levels (Daniel et al., 1999, Felemban et al., 2001). Although maternal serum creatine kinase levels and fetal fibronectin levels from cervico-vaginal swabs were reported to be elevated in EP as compared with intrauterine pregnancy, their usefulness for distinguishing EP from intrauterine pregnancy has not yet been proven. On the contrary, an elevated serum VEGF level with a threshold of >200 pg/ml was proposed as a promising novel marker for a diagnosis of EP at 7.5 and 5 weeks gestation in two earlier studies (Daniel et al., 1999; Felemban et al., 2001).

For the establishment of a viable pregnancy, implantation and placentation are the early and crucial processes, both being accompanied by angiogenesis, for which VEGF is mainly accountable and plays a key role (Nowacek et al., 1999). VEGF is a potent angiogenic factor which serves as a major modulator of vascular growth, remodelling and permeability in endometrium, decidua, trophoblast, and of the vascular development of the embryo (Carmeliet et al., 1996; Clark et al., 1996; Shifren et al., 1996; Torry et al., 1996; Torry and Torry, 1997). Possible sites of VEGF production include the corpus luteum, the endometrium, and the placenta (Evans et al., 1998; Wheeler et al., 1999; Jelkman, 2001). VEGF shows an increase during the first 10 weeks of pregnancy, although the source of this increase is unknown. Secretion and expression of VEGF are induced by growth factors and cytokines and depend on local conditions such as hypoxia (Leung et al., 1989; Ladoux and Frelin, 1993; Torry and Torry, 1997; Daniel et al., 1999; Neufeld et al., 1999). The low
basal expression of VEGF messenger RNA under normoxic conditions in the cytotrophoblast and the syncytiotrophoblast (in vitro) support the lower levels of VEGF in patients with intrauterine pregnancy as compared with EP (Shore et al., 1997). As the implantation environment in the oviduct is different from the well-vascularized endometrium, secretion and production of VEGF may be affected in EP (Shifren et al., 1996; Torry et al., 1996; Torry and Torry, 1997).

In an earlier study, other authors hypothesized that implantation of the conceptus within the oviduct might trigger increased VEGF production as a form of accommodation to the unfavourable environment (Daniel et al., 1999).

The purpose of our study was to confirm whether or not serum VEGF levels >200 pg/ml would allow us to distinguish between abnormal intrauterine pregnancy and EP at 6 weeks gestation and thus reduce the high rate of initially misdiagnosed cases of early EP and, as a consequence, reduce the delay in initiation of treatment.

Patients and methods
Blood samples from pregnant Caucasian women were taken before treatment at the University Clinic of Vienna. Patients came either for previously-presumed miscarriage or EP from a gynaecological practice outside the hospital. Patients were matched for gestational age in days. The matching was achieved by selecting in each case 42 patients with abnormal intrauterine pregnancy and EP at the same gestational age in days with a maximum margin of one day; thus 84 patients remained for final analysis. Abnormal intrauterine pregnancies were diagnosed by means of serial β-hCG measurements and transvaginal sonography and defined as ultrasound evidence of miscarriage while EP was additionally confirmed by laparoscopy in all cases. All pregnancies were singletons and had been conceived spontaneously. There were no significant differences with regard to height and weight between the subjects. The study was carried out in accordance with the 1975 Helsinki Declaration on Human Experimentation and approved by the local ethics committee. Informed consent was given by subjects before the blood samples were collected.

Serum assay
Blood samples were obtained before treatment by peripheral venous puncture and immediately centrifuged at 1400 g for 15 min. Serum was withdrawn and frozen at −80°C until examination. For the measurement of serum VEGF, a commercially available enzyme-linked immunosorbant assay (ELISA) was used (Quantikine Human VEGF Immunoassay; R&D Systems, Minneapolis, MN, USA). All VEGF serum analyses were performed at the same time and in the same batch and were measured in duplicate. The intra-assay variability was 5.1% at a VEGF concentration of 512 pg/ml. Immunoassays were performed as described previously (Obermair et al., 1997). According to the manufacturer, the assay measures the isoform VEGF165 containing 165 amino acid residues. The assay recognizes both natural human VEGF and recombinant VEGF and does not exhibit any cross-reactivity with a series of cytokines and growth factors. The manufacturer claims a sensitivity of <5.0 pg/ml. Serum β-hCG was measured by the Abbott Axsym System in accordance with the 3rd International Reference Preparation.

Statistical analysis
Differences regarding patient specific characteristics such as age, gestational age in days, VEGF, progesterone and β-hCG between abnormal intrauterine pregnancy and EP were compared using the Wilcoxon 2-sample test. In accordance with others who suggested threshold levels of >200 pg/ml for serum VEGF to distinguish between intrauterine pregnancy and EP, sensitivity, specificity, and positive predictive values (PPV) were calculated and compared with the results of earlier studies (Daniel et al., 1999; Felemban et al., 2001). For sensitivity, specificity and PPV, 95% confidence intervals (CI) were calculated. For correlations analyses between age, gestational age, and serum concentrations of VEGF, and β-hCG Spearman’s correlation coefficients were performed. We used the SAS statistical software system (Version 8.1, SAS Institute Inc., Cary, NC) to carry out the calculations. P-values of <0.05 were considered to be statistically significant.

Results
Patient characteristics for the two groups are shown in Table I. Except for patients’ age (P = 0.02) no statistically significant differences were found between patients with abnormal intrauterine pregnancies and EP.

In our study, the median VEGF level among women with EP (211.2 pg/ml; range, 0–628.8) was higher than in those with abnormal intrauterine pregnancies (198.5 pg/ml; range, 0–701.6), but this difference did not reach statistical significance (Figure 1).

When threshold concentrations of a serum VEGF level >200 pg/ml were used, abnormal intrauterine pregnancy could be distinguished from EP with a sensitivity of 56% (95% CI: 39.7–71.5), a specificity of 51% (95% CI: 35.1–67.1), and a positive predictive value of 53% (95% CI: 37.7–68.8).

Spearman’s correlation coefficients revealed statistically significant negative correlations between VEGF and gestational age (P = 0.02, r = −0.24), VEGF and β-hCG (P < 0.0001, r = −0.47), and a positive correlation for β-hCG and gestational age (P = 0.01, r = 0.27).

Discussion
An earlier study tested the hypothesis that serum VEGF levels were specific for the discrimination between intrauterine pregnancy and EP and, based on the results obtained, introduced a serum VEGF level >200 pg/ml as a novel diagnostic marker for EP at an average gestational age of 50–53 days i.e. 7.5 weeks gestation (Daniel et al., 1999). Under the hypothesis that the implantation of the conceptus within the oviduct might trigger its increased production as an accommodation to the unfavourable environment, a VEGF level >200 pg/ml was introduced as a potential marker for diagnosis of EP also at 5 weeks gestation in a more recent study (Felemban et al., 2001). Our rationale for conducting this study was following the demand for larger prospective studies to confirm previous data.

Former study groups used a competitive immunoassay to measure total (both bound and free) immuno-reactive VEGF and, thus, for the first time described longitudinal changes in serum VEGF in early pregnancy (Anthony et al., 1994; Evans et al., 1998). Using the same commercially available ELISA (R&D systems) to measure the unbound isoform VEGF165, we also chose an identical VEGF detection and measuring method. We think that the problem in measuring unbound VEGF using
in our study we only included patients with abnormal intrauterine pregnancy, and EP is of major clinical importance. Therefore et al. (Jelkmann, 2001).

account when a novel diagnostic marker is under evaluation which has been previously described and must be taken into consideration (Anthony et al., 1992; Shweiki et al., 1992). When threshold concentrations of a serum VEGF level >200 pg/ml were used in the recently published study, abnormal intrauterine pregnancy could be distinguished from EP with a sensitivity of 87.5%, a specificity of 75% and a positive predictive value of 77.8%, while in our study corresponding values were 56.1, 51.2, and 53.5% respectively (Felemban et al., 2001). For the discrimination between abnormal intrauterine pregnancies and EP others found a sensitivity of 60%, predictive value of 77.8%, while in our study corresponding

terine pregnancies and EP, who, in most cases, consulted our outpatient department at 6 weeks gestation. According to previous results mean VEGF levels among women with EP were higher than in those with abnormal intrauterine pregnancies (Daniel et al., 1999; Felemban et al., 2001). Mean (± SEM) VEGF levels for abnormal intrauterine pregnancies (218.8 ± 27.9 pg/ml, n = 42) were higher than those reported by Felemban et al. (169.7 ± 16.6 pg/ml, n = 15), which might be due to the more advanced gestational age in our study (42 versus 35 days). In contrast with EP, we found mean (± SEM) VEGF levels (246.7 ± 23.6 pg/ml, n = 42) to be lower than those reported in the earlier study (306.1 ± 26.5 pg/ml, n = 15) (Felemban et al., 2001).

According to previous work we found highly significant correlations between β-hCG levels and gestational age, β-hCG and VEGF, and VEGF and gestational age, which might reflect the increase in tissue angiogenesis accompanying pregnancy (Evans et al., 1997). Today, we do not know whether the main difference between intrauterine pregnancy and EP is the viability of the trophoblast, or the site of implantation. Thus, up-regulated VEGF expression in cases of EP most obviously reflects a combination of both (Rodesch et al., 1992; Shweiki et al., 1992).

ELISA is that the amount of free unbound VEGF is likely to decrease as gestation advances due to increasing amounts of the binding protein, which is likely to be a truncated form of the soluble fms-like tyrosine kinase (flt) receptor produced by the placenta (Clark et al., 1998).

Some authors concluded that the concentrations of the VEGF binding protein were high in pregnancy, and showed that exogenous VEGF added to pregnancy serum was rapidly bound (Anthony et al., 1997). In this way high concentrations of the VEGF binding protein may account for those patients in our study who were found to have no measurable VEGF in the serum. While there were obviously no zero levels of VEGF in their study cohort (Daniel et al., 1999), we measured zero levels in some patients with abnormal intrauterine pregnancies and EP. Besides high concentrations of the VEGF binding protein the VEGF level may also be too low for detection, which has been previously described and must be taken into account when a novel diagnostic marker is under evaluation (Jelkmann, 2001).

In both previous studies VEGF levels were compared between patients with normal intrauterine pregnancy, abnormal intrauterine pregnancy, and EP (Daniel et al., 1999; Felemban et al., 2001). The discrimination between abnormal intrauterine pregnancies and EP is of major clinical importance. Therefore in our study we only included patients with abnormal intra-

### Table 1. Patient characteristics and median serum levels of β-hCG, progesterone and VEGF for patients with abnormal intrauterine (group 1) and ectopic pregnancies (group 2). Patients’ age in years and gestational age in days are reported as mean values ± standard deviations, while β-hCG, progesterone and VEGF are reported as median values with ranges in brackets.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Age (years)</th>
<th>Gestational age (days)</th>
<th>β-hCG (mIU/ml)</th>
<th>Progesterone (ng/ml)</th>
<th>VEGF (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>42</td>
<td>29 ± 7</td>
<td>43.7 ± 3.9</td>
<td>2304 (29-3522)</td>
<td>4.6 (0.7-35.1)</td>
<td>198.5</td>
</tr>
<tr>
<td>2</td>
<td>42</td>
<td>32 ± 5</td>
<td>43.4 ± 3.9</td>
<td>2345 (126-61855)</td>
<td>8.42 (0.9-28.1)</td>
<td>211.2</td>
</tr>
</tbody>
</table>

![Figure 1](image-url) **Figure 1.** Concentration of serum VEGF in patients with abnormal intrauterine and ectopic pregnancy (n = 84). The horizontal line represents the serum threshold level used for the calculation of sensitivity specificity and positive predictive value.
heterogeneity in patient profiles including clinical symptoms and previous exposure to risk indicators (such as history of EP, tubal surgery or pelvic inflammatory disease), still seem to be superior to currently available serological markers in diagnosing EP (Mol et al., 1999).

According to our data, we conclude that a VEGF level >200 pg/ml does not discriminate ectopic from abnormal intrauterine pregnancies at 6 weeks gestation and thus should not be used in clinical management.

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References

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