Serum Placental Growth Factor (PlGF) as a Biomarker of Ectopic Pregnancy

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Abstract: Objective: The aim of the current study was to investigate whether serum PlGF concentration has a role in differentiation between a normal intrauterine pregnancy, an ectopic pregnancy and a non-viable intrauterine pregnancy. **Methods**: The Study included three groups of women: group I, including women with viable intrauterine pregnancy; group II, including women with first-trimester missed abortion; and group III, including women with proven tubal ectopic pregnancy. All included women were at gestations between 6 and 13 weeks. All included women were subjected to serum assays for β-hCG and PlGF. **Results:** The median value of serum hCG was lower in women of group III than that in women of group II than that in women of group I; these differences were, however, statistically non-significant (p=0.190). The median value of serum PlGF was significantly lower in women of group III than that in women of group II than that in women of group I (p <0.001). Serum PlGF level was a significant predictor for differentiating an ectopic pregnancy from a normal intrauterine pregnancy [AUC = 0.948, 95% CI (0.891 to 1.005), p <0.001]. On the contrary, serum hCG concentration was not a significant predictor for differentiating an ectopic pregnancy from a normal intrauterine pregnancy [AUC = 0.571, 95% CI (0.413 to 0.729), p =0.380]. **Conclusion:** Serum PlGF assay seems to be a promising biomarker for differentiating ectopic pregnancy from both normal intrauterine pregnancy and non-viable intrauterine pregnancy. These features are probably unique to PlGF, which advantages it over the standard β-hCG assay.

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Key Words: Ectopic pregnancy – Placental growth factor (PlGF) – Pregnancy of unknown location – Human chorionic gonadotropin (hCG)

1.Introduction

Ectopic pregnancy is the leading cause of maternal mortality in early pregnancy. The overall prevalence of ectopic pregnancy is 1-2% [1].The gold standard tool for diagnosing ectopic pregnancy is laparoscopy, which is obviously disadvantaged by being invasive and relatively costly[2]. Non-invasive diagnosis of ectopic pregnancy is a clinical challenge in the majority of cases. Early and diagnosis of ectopic pregnancy decreases morbidity and mortality and allows for conservative management, preservation of the affected Fallopian tube, and, consequently, preservation of the patient's future fertility[3]. Other than finding an extrauterine typical gestational sac with an embryonic pole or a yolk sac, or finding a considerable pelvic collection suggestive of hem peritoneum, transvaginal sonographic scan (TVS), on its own, is neither sensitive nor specific for diagnosing ectopic pregnancy[4].Advances in the complementary role of TVS and serum human chorionic gonadotropin (hCG) assays over the last two decades have remarkably improved the sensitivity and specificity of early diagnosis of ectopic pregnancy. Nevertheless, several limitations facing both TVS and serum hCG assay remain, notably the need for serial rather than snap-shot hCG assay in integration with a skilled high-resolution TVS. This 'serial' assessment requires 48 hours up to one week for diagnosing an ectopic pregnancy, which raises the risk of tubal rupture and life-threatening hemorrhage[3]. Serum progesterone has also been proposed [5]. Low serum progesterone was associated with early pregnancy failure; it does not discriminate between an ectopic pregnancy and a non-viable intrauterine pregnancy, however[6]. Several authors have proposed other biomarkers including activin-A, pregnancy-specific beta-1-glycoprotein (SP1), pregnancy-associated plasma protein-A (PAPP-A), human placental lactogen (hPL), inhibin-A, estradiol, relaxin, renin, vascular endothelial growth factor (VEGF) and placental growth factor (PlGF)[7-8]. PlGF is a member of the VEGF family [9]. In normal pregnancy there is a steady increase in serum levels of PlGF during the first two trimesters [10]. Serum PlGF level has been associated with viability of early pregnancy [11].The aim of the current study was to investigate whether serum PlGF concentration has a role in differentiation between a normal intrauterine pregnancy, an ectopic pregnancy and a non-viable intrauterine pregnancy.

2. Methods

The current cross-sectional study was conducted at Ain Shams University Maternity Hospital during

the period between May 2012 and December 2012. The study protocol was in agreement with the Helsinki Declaration for Ethical Medical Research, and was revised by the Ethical Research Committee of the Obstetrics and Gynecology Department, Ain Shams University. The Study included three groups of women: group I, including women with viable intrauterine pregnancy; group II, including women with first-trimester missed abortion; and group III, including women with proven tubal ectopic pregnancy. Diagnosis of a normal intrauterine pregnancy was made by transvaginal sonographic detection of an intrauterine gestational sac enclosing a yolk sac and an embryonic pole with detectable embryonic pulsations. Women of this group, in particular, were followed up till 13 weeks' gestation; those who had pregnancy loss before this gestation were excluded from the study. Diagnosis of missed abortion was made by sonographic detection of an intrauterine sac enclosing an embryonic pole with its crown-to-rump length (CRL) ≥ 7 mm with no detectable pulsations, absence of embryo with pulsations ≥ 2 weeks after a scan that showed a gestational sac without a yolk sac, or absence of embryo with pulsations ≥ 11 days weeks after a scan that showed a gestational sac with a yolk sac [12]. Diagnosis of ectopic pregnancy was made through sonographic detection of an extrauterine gestational sac with an embryonic pole and/or a yolk sac, or when a pregnancy of unknown location shows a plateauing (within 10%) quantitative serum β-hCG over 48 hours [12]. All included women were at gestations between 6 and 13 weeks. Women who were hemodynamically instable, having severe vaginal bleeding, and those who were known to have peripheral vascular disease or dyslipidemia were not included in the study. All included women were subjected to serum assays for β-hCG and PlGF. The purpose and procedures of the study were explained to all approached women by the main investigator. Women had to sign an informed written consent before participating in the study. Participating women were informed that they had the right to withdraw from the study at any phase without being adversely impacted regarding the medical service they had to receive.

Serum PlGF Assay

Five millimetres of blood were withdrawn from all recruited women. Samples were allowed to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Sera were stored at -20°C till the end of the study, when all samples were assayed at the same time. Samples were analyzed using the Human PlGF Qunatikine ELISA kit® [R&D Systems®, Abingdon, England, UK].

Sample Size Justification

Sample size was calculated using PASS version 11, setting thetype-1 error (α) at 0.05 and the power (1-β) at 0.8. Data from a previous study [13] showed that the mean serum PIGF was 15.2 ± 13.3pg/ml (in women who had viable intrauterine pregnancy), 4.2 ±2.8 (in women who had non-viable intrauterine pregnancy) and 0.2 ± 0.3 pg/ml (in women who had ectopic pregnancy). Calculation according to these values produced a minimal sample size of 26 women in each group.

Statistical Methods

Statistical analysis is to be performed using Statistical Package for Social Sciences (SPSS®) for Windows® version 15.0. Measured data will be described as range, mean and standard deviation (for parametric variables), range, median and interquartile range (for non-parametric variables), number and percentage (for categorical variables). Difference between two unrelated groups will be measured using unpaired student'st-test (for parametric variables), Mann-Whitney's U-test (for nonparametric variables) and Chi-squared test (for categorical variables).Association between variables will be assessed using Spearman's rank correlation coefficient (for non-parametric variables). Significance level will be set at 0.05.

3.Results

The study included 26 women in each group. There were no significant differences between women of the three groups regarding age, weight, BMI or gestational age (Table-1).

The median value of serum hCG was lower in women of group III than that in women of group II than that in women of group I; these differences were, however, statistically non-significant (p=0.190). The median value of serum PlGF was significantly lower in women of group III than that in women of group II than that in women of group I (p <0.001) (Table-2, Figure-1).

There was no significant correlation between serum PlGF and serum hCG concentrations [r_s=-0.051, p =0.659].

Receiver operator characteristics (ROC) curves were constructed for estimating the diagnostic value for both serum hCG and serum PlGF in differentiation between normal and abnormal pregnancy. Serum PlGF level was a significant predictor for differentiating a normal intrauterine pregnancy from abnormal pregnancy (missed abortion or ectopic pregnancy), through having a significantly large area under the curve (AUC) [AUC = 0.933, 95% CI (0.859 to 1.007), p<0.001]. On the contrary, serum hCG concentration was not a significant predictor for differentiating a normal from

abnormal pregnancy [AUC = 0.581, 95% CI (0.441 to 0.721), p =0.248] (Figure-2a).

Serum PlGF level was a significant predictor for differentiating an ectopic pregnancy from a normal intrauterine pregnancy, through having a significantly large area under the curve (AUC) [AUC = 0.948, 95% CI (0.891 to 1.005), p <0.001]. On the contrary, serum hCG concentration was not a significant predictor for differentiating an ectopic pregnancy from a normal intrauterine pregnancy [AUC = 0.571, 95% CI (0.413 to 0.729), p =0.380] (Figure-2b).

Serum PlGF level was a significant predictor for differentiating an ectopic pregnancy from missed abortion, through having a significantly large area under the curve (AUC) [AUC = 0.880, 95% CI (0.771 to 0.989), p <0.001]. On the contrary, serum hCG concentration was not a significant predictor for differentiating an ectopic pregnancy from missed abortion [AUC = 0.490, 95% CI (0.326 to 0.653), p =0.898] (Figure-2c).

Table-3 shows the accuracy of both serum PlGF and hCG concentrations in differentiation between normal and abnormal pregnancy, between ectopic pregnancy and normal intrauterine pregnancy, and between ectopic pregnancy and missed abortion.

Table-1Difference between Groups regarding Age, Weight and BMI

	Group I [Viable Pregnancy Group] (n=26)	**Group II [Missed Abortion Group] (n=26)**	**Group III [Ectopic Pregnancy Group] (n=26)**	***P****
Age (Years)				
Range	20 – 31	19 – 35	19 – 35	0.066
Mean ± SD	25.35 ± 3.43	27.92 ± 4.29	26.65 ± 3.99	NS
Weight (Kg)				
Range	60 – 80	59 – 86	59 – 77	0.054
Mean ± SD	70.69 ± 7.31	70.88 ± 6.51	67 ± 5.23	NS
BMI (Kg/m^2)				
Range	23.44 – 31.64	21.16 – 30.47	23.05 – 29.71	0.113
Mean ± SD	26.62 ± 2.71	26.08 ± 1.99	25.36 ± 1.62	NS
Gestational Age (Weeks)				
Range	6 – 13	6 – 13	6 – 12	0.429
Mean ± SD	8.22 ± 2.11	8.31 ± 2.37	7.61 ± 1.88	NS

SD standard deviation BMI body mass index [calculated as weight (kg) divided by squared height (m^2)]
* Analysis using one-way ANOVA test NS non-significant

Table-2Difference between Groups regarding Serum hCG and Serum PlGF

	Group I [Viable Pregnancy Group] (n=26)	**Group II [Missed Abortion Group] (n=26)**	**Group III [Ectopic Pregnancy Group] (n=26)**	***P****
Serum hCG (IU/ml)				
Range	1287 – 14059	1119 – 10123	1276 – 10400	0.190
Median (IQR)	3591 (2040 – 7143)	3057 (2036 – 4211)	2750 (1758 – 6000)	NS
Serum PlGF (pg/ml)				
Range	1.82 – 26.16	1.82 – 8.19	0.39 – 16.72	<0.001
Median (IQR)	17.8 (13.8 – 24.5)	4.7 (3.9 – 6.3)	1.8 (1.2 – 3.2)	HS

IQR interquartile range [central 50% of ascending-ordered set of data] hCG human chorionic gonadotropin
PlGF placental growth factor * Analysis using Kurskal-Wallis test
NS non-significant – HS highly significant

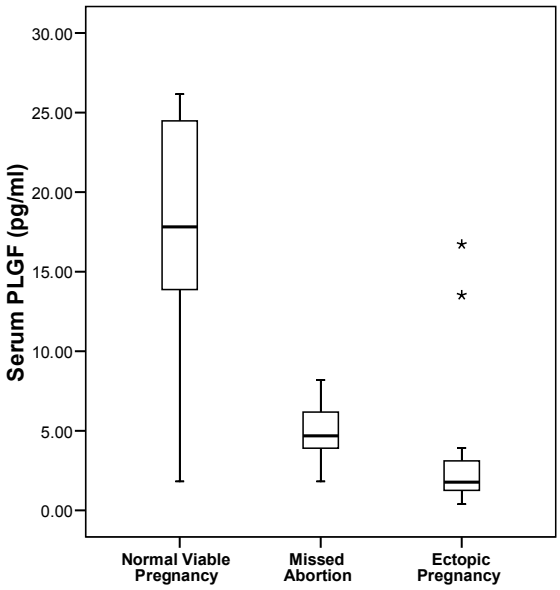
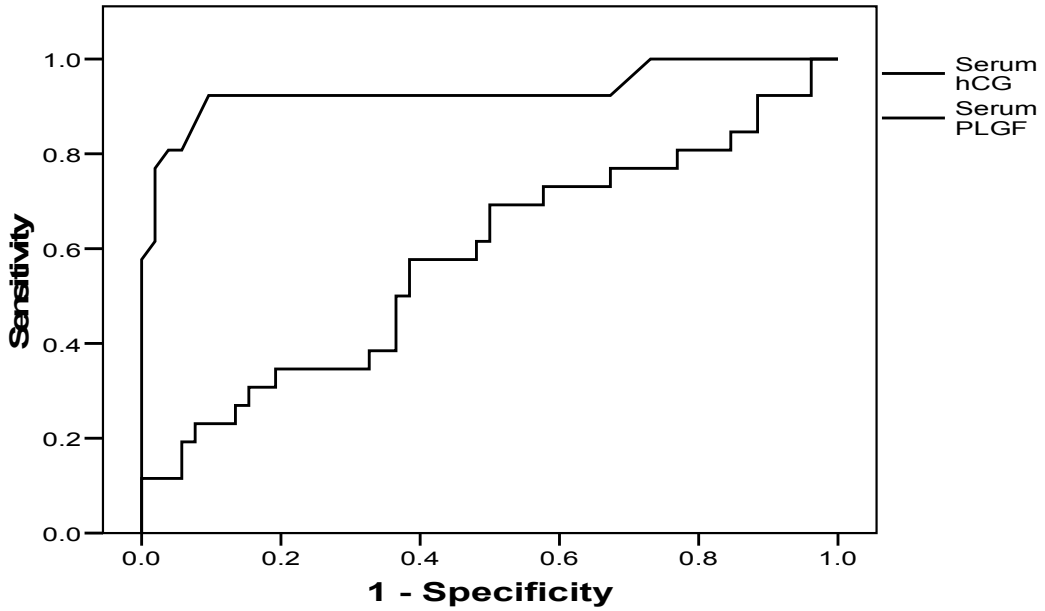


Figure-1Box-Plot Chart showing Difference between Groups regarding Serum PLGF

a. Differentiation between Normal Intrauterine Pregnancy and Abnormal Pregnancy



b. Differentiation between Ectopic and Normal Intrauterine Pregnancy

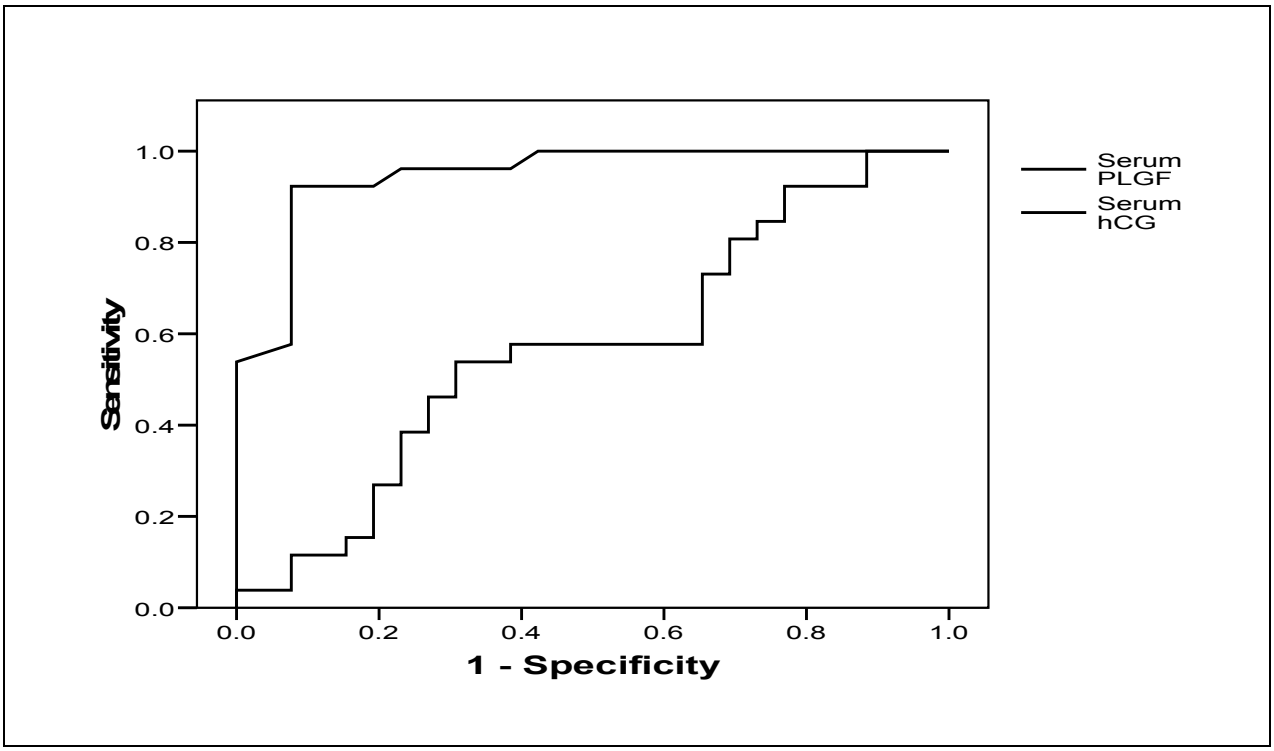


Figure-2ROC Curves for Serum hCG and PlGF for Differentiation between Normal and Abnormal Early Pregnancy

c. Differentiation between Ectopic Pregnancy and Missed Abortion

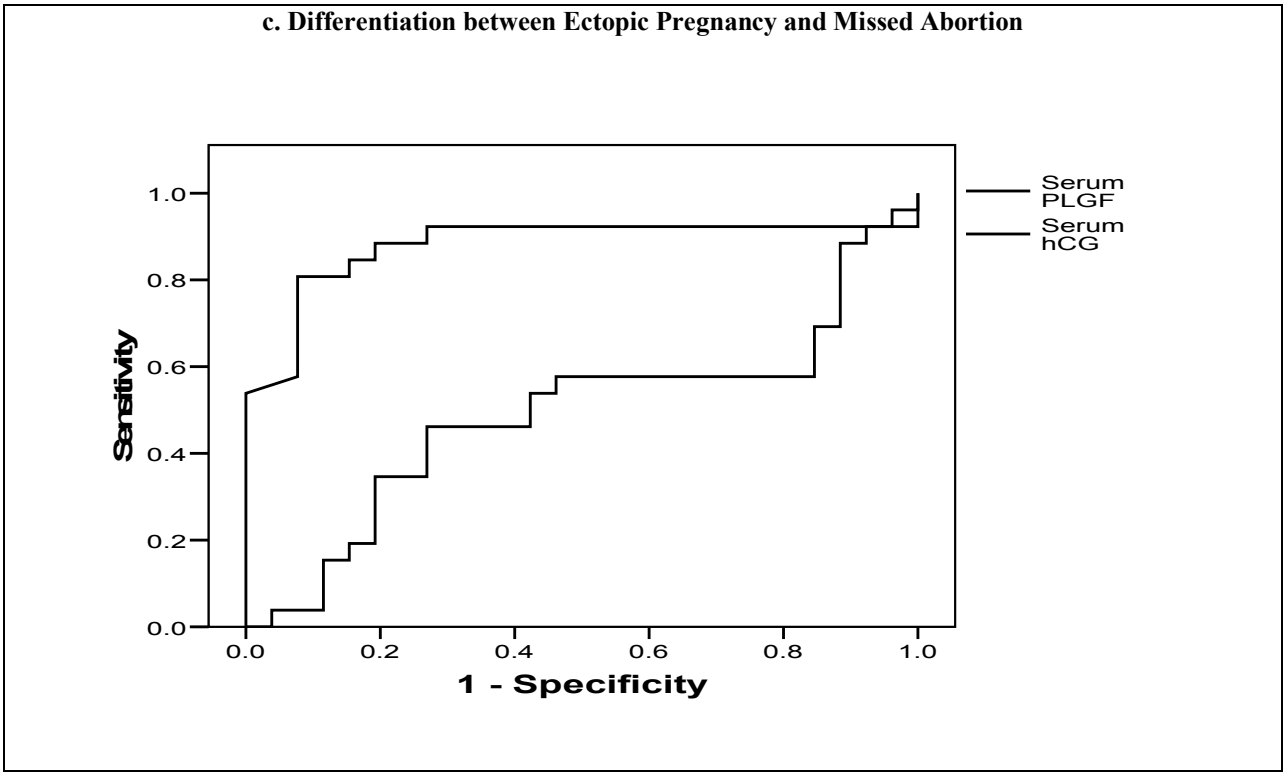


Figure-2ROC Curves for Serum hCG and PlGF for Differentiation between Normal and Abnormal Early Pregnancy (cont'd)

Table-3Accuracy of Serum PlGF and hCG Concentrations for Differentiation between Normal and Abnormal Pregnancy

			Sensitivity	Specificity	PPV	NPV
Serum PlGF	Normal vs. Abnormal Pregnancy	≥ 7.68 pg/ml	92.3%	88.5%	88.9%	92%
	Ectopic vs. Normal Pregnancy	≤ 5.84 pg/ml	92.3%	92.3%	92.3%	92.3%
	Ectopic vs. Missed Abortion	≤ 3.34 pg/ml	80.8%	84.6%	84%	81.5%
Serum hCG	Normal vs. Abnormal Pregnancy	≥ 3322 IU/ml	57.7%	61.5%	60%	59.3%
	Ectopic vs. Normal Pregnancy	≤ 3024 IU/ml	57.7%	61.5%	60%	59.3%
	Ectopic vs. Missed Abortion	≤ 3025 IU/ml	57.7%	53.8%	55.6%	56%

PPV positive predictive value NPV negative predictive value

4. Discussion

Intrauterine implantation has been associated with the activity of PlGF[14]. PlGF is a secreted proangiogenic protein with similarities to vascular endothelial growth factor. It has been identified at the implantation site and acts on neighbouring cells, notably endothelial cells, through the receptors flt-1(VEGF) recptor1 and FK1-1/KDR VEGF reptors2) to facilitate the development of local blood supply [14].The role of PlGF in the development of an intrauterine vascular network is highlighted by its relationship to preeclampsia, which is associated with reduced placental vascularisation [15]. Lower maternal serum levels of PlGF in early pregnancy correlate with a greater risk of developing preeclampsia in the third trimester [16].To grow, and cause harm ectopic pregnancy needs to develop a supportive blood supply, and angiogenesis also occurs at tubal implantation sites. It is not known whether PlGF is involved in increasing the vascularization of the fallopian tube in ectopic implantation. However, it is known that another proangiogenic growth factor; VEGF is involved. VEGF and its receptors are up regulated at the tubal implantation site in ectopic pregnancy compared with elsewhere in the fallopian tube [17]. In addition, serum VEFG is increased in women with ectopic compared with intrauterine pregnancies [18].The normal response to implantation is an augmented secretion of PlGF, and this increase is reflected systemically, so that it can be measured in serum [19].

The current study showed a significantly lower serum PlGF in women who had ectopic pregnancy when compared to both normal and non-viable intrauterine pregnancy. These significant differences were not shown in concomitant β-hCG assays. In agreement, Daponte *et al.* found that PlGF concentration was significantly lower in women with ectopic pregnancy (14.60±3.42 pg/ml) and women with a missed abortion (16.25±4.73 pg/ml) compared with patients with viable intrauterine pregnancy (21.64±5.68 pg/ml; p=0.001)[20]. Patrelli *et al.* found that PlGF showed statistically significant lower concentrations in ectopic pregnancies compared to physiological gestation and threatened abortions [21]. Horne *et al.* found that PlGF is practically undetectable in women with tubal ectopic pregnancy and reduced, or undetectable, in miscarriage compared with viable intra-uterine pregnancy [13]. Muttukrishna *et al.* reported that maternal serum PlGF level is increased several fold in early pregnancy and that PlGF is markedly decreased in threatened miscarriage patients who subsequently have a miscarriage[22].Similar to the results of the current study, Daponte *et al.* found that PlGF showed a high accuracy in the diagnosis of ectopic pregnancy with cut-off point 15.7 (AUC =0.822)[20].Patrelli *et al.* concluded in his relevant study that PlGF may play a diagnostic and prognostic role in ectopic pregnancy[21].

In conclusion, serum PlGF assay seems to be a promising biomarker for differentiating ectopic pregnancy from both normal intrauterine pregnancy and non-viable intrauterine pregnancy. These features are probably unique to PlGF, which advantages it over the standard β-hCG assay.

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