The trend of VEGF-A and PlGF in pregnant patients: a perspective case-control study on 214 women

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Summary

Objective: The aim of this study was to measure plasmatic concentrations of vascular endothelial growth factor-A (VEGF-A) and placental growth factor (PIGF) in pregnant women, and to evaluate their relationship with age, hormonal status, gestational age, and different diseases of pregnancy. *Methods:* We selected a control group of 163 patients (96 fertile and 67 in menopause) and a group of 214 pregnant patients during the whole gestational period. VEGF-A and PlGF were assayed by ELISA and EIA methods, respectively. Statistical analysis was performed using the Mann-Whitney test. *Results:* The control group showed mean VEGF-A and PlGF values of 89.87 pg/ml and 10.22 pg/ml, respectively; PlGF showed the highest values in menopausal patients. The group of pregnant patients showed VEGF-A values of 27.05 pg/ml and PlGF values of 231.36 pg/ml respectively, with lower (for the VEGF-A) and higher (for the PlGF) statistical significance. These values were not influenced by biological age, but were related to gestational age: VEGF-A showed a decrease and PlGF an increase particularly after the 20th gestational week. PlGF showed a statistically significant decrease compared to physiological gestation in spontaneous and threatened abortions ($p < 0.0001$) and in ectopic pregnancies ($p < 0.0001$), an increase in ultrasound and CTG alterations ($p < 0.05$), and threatened premature delivery and uterine hypercontractility ($p < 0.01$); on the other hand VEGF-A showed a statistically significant increase in ectopic pregnancies ($p < 0.05$). *Conclusions:* VEGF-A and PlGF may play a diagnostic and prognostic role in pregnancy. Further studies are required to better understand the meaning of variability of their values.

Key words: VEGF; PLGF; Pregnancy; Twin pregnancies; Preeclampsia; IUGR; Preterm delivery; Ectopic pregnancy; Abortion.

Introduction

Vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) belong to the family of vascular endothelial growth factors, which includes VEGF-A, -B, -C, -D, -E, PlGF, and endocrine gland-derived growth factor. All these growth factors carry out their activities by binding to specific receptors belonging to the family of tyrosine kinases [1-3].

VEGF-A is a dimerous glycoprotein which is coded by a gene located in the short arm of chromosome 6 – precisely at p21.3 according to some authors or at p12 according to other authors [1-3]. PlGF is a homodimerous glycoprotein coded by a gene located in the long arm of chromosome 14 (at q24.3).

The expression of gene coding for VEGF-A is minutely up-regulated by hypoxia through inducible transcription factors, such as hypoxia-inducible factor-1 (HIF-1) and hypoxia-inducible factor-2 (HIF-2), which bind to responsive elements located near the VEGF-A promoter [4, 5]. Additional mechanisms regulating VEGF-A transcription include several other growth factors, in particular epidermal growth factor (EGF), platelet-derived growth factor (PDGF), insulin-like growth factor-I (IGF-1), tumor necrosis factor-α (TNF-α), and transforming growth factor-β (TGF-β and a series of inflammatory cytokines, such as interleukin (IL)-1α, IL-1β, IL-6, IL-10, IL-13 [6].

VEGF-A specifically binds to vascular endothelial growth factor receptor (VEGFR)-1, also called fms-like tyrosine-kinase (sFlt1), to VEGFR-2, also called fetal liver kinase (flk-1), and to neuropilins NRP-1 and NRP-2. VEGFR-1 and VEGFR-2 are both made up of seven extracellular domains similar to immunoglobulins, a transmembrane region, and an intracellular domain of a tyrosine kinase [7, 8].

PlGF binds exclusively to VEGFR-1 and Nrp-1, but it can also form a heterodimer with VEGF-A, which is capable of binding to VEGFR-2 and the heterodimerous VEGFR-1/VEGFR-2 as well [9, 10].

VEGFR-1 is expressed by endothelial cells, osteoblasts, macrophages, trophoblasts, renal mesangial cells, and other hematopoietic cells. Its expression is increased by hypoxia and during angiogenesis. VEGFR-1 is first expressed by endothelial angioblasts during embryonic development and decreases at the end of it [11].

VEGFR-2 is expressed by endothelial, neuronal and pancreatic ductal cells, osteoblasts, megacariocytes, and ancestor retinal cells [12].

VEGF-A strongly binds to VEGFR-1 and has a weaker

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affinity for VEGFR-2, even if the latter is the first receptor transmitting the VEGF signal to endothelial cells [13].

The VEGF/VEGFR-2 axis plays an important role both in physiological and pathological angiogenesis [14].

VEGF-A and PlGF are two pleiotropic growth factors that play distinct roles both in homoeostasis and development. Blood vessels are fundamental for normal development and growth because they supply oxygen and nourishment. Angiogenesis is a multistage process that depends on vascular growth factors.

The importance of VEGF-A is evidenced by the fact that its eventual decrease or damage, or damage to its receptors, causes an abnormal angiogenesis that could be lethal during embryo-fetal development [15]. On the other hand, PlGF plays a crucial role in pathological angiogenesis in adults, but an insufficient production of PlGF does not compromise the development of blood vessels, suggesting a less important role before birth [15].

VEGFR-1 and VEGFR-2 are also expressed by non-vascular tissues, which further suggests that they have other biological roles.

VEGF-A and its receptors are involved in the development and homoeostasis of many organs, such as the respiratory, skeletal, hematopoietic, nervous, renal, and reproductive systems, independently of their vascular role [16].

VEGF-A is produced by cytotrophoblastic cells and is poured in the syncytial lacunae, where it comes into contact with circulating maternal blood, in the same way as does human chorionic gonadotropin (hCG), whose detection in maternal blood is necessary for pregnancy tests.

Vasculogenesis and angiogenesis are essential for placental development and are both regulated by vascular growth factors [17]. Members of this family regulate angiogenesis, blood flow and vascular permeability, and coordinate various extracellular matrix bonds. VEGF-A and PlGF induce vasodilation by releasing nitric oxide (NO) and PGI-2 as a consequence of the stimulation of tyrosine-kinase receptors VEGFR-1 and VEGFR-2.

It has recently been discovered that PlGF is a powerful promoter of angiogenesis in vivo and is capable of stimulating the proliferation of microvascular endothelial cells in the human placenta at term [18].

The final configuration of the villous vascular beds is defined by the balance of VEGF-A, PlGF and their receptors. The predominance of VEGF-A promotes the formation of a rich arborization and of low-resistance capillary beds inside the mesenchymal and intermediate immature villi, which prevail in the first two trimesters of pregnancy. By contrast, the predominance of PlGF and its VEGFR-1 receptor in the last trimester is responsible for the absence of complex capillary beds, so that only poorly arborized capillary beds prevail [19].

Further studies are required to test the conflicting roles of VEGF-A, PlGF and their receptors in the genesis of villi [20, 21].

The balance between VEGF-A secretion and PlGF secretion can be regulated by partial oxygen pressure. VEGF-A and its receptors are up-regulated by conditions of reduced oxygenation both in the placenta and *in vitro,* while the opposite is true for PlGF.

As a consequence of these growth mechanisms, the villous vascular system differs from that of most human organs in two main respects. First of all, arteries and veins of this low-pressure system have a fairly thin middle tunic, and vasa vasorum are usually absent, except for some residual paravascular capillaries. Despite their intraluminal low pressure (10 and 20 mmHg, respectively), adequate compensation is obtained by the vascular walls because the surrounding intervillous space has a mean pO_2 exceeding 40 mmHg.

The bed of peripheral capillaries in terminal villi is not represented by richly arborized capillary nets, but rather by a great number of stretched capillaries, coiled in lightly arborized spirals [22, 23].

The purpose of our perspective randomized study was to measure the plasma concentrations of VEGF-A and PlGF and to evaluate their relationship with age, hormonal status, gestational age, and different pregnancy-related diseases.

The main objective was to detect variations of plasma concentrations (either increasing or decreasing) in order to introduce the test of these two indicators as a tool for monitoring pregnancy and for early diagnosis of pregnancy complications.

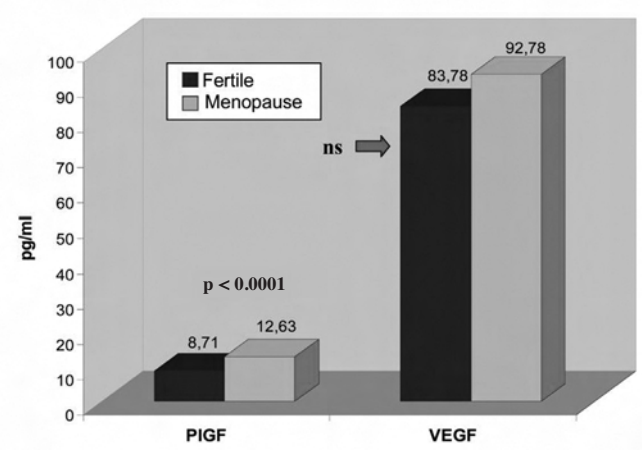
Materials and Methods

This study, perspective case-control, evaluated a study group and a control group of pregnant women admitted to the Department of Gynaecology and Obstetrics of the Italian Universities of Padua, Parma and Sassari between September 2005 and May 2007. The study was approved by the Review Boards of all centres.

In the control group, which consisted of 163 subjects (96 fertile women and 67 menopausal women), we included 13 healthy volunteers and 150 patients hospitalized for different diseases (uterine prolapse, urinary incontinence, cystocele, ovarian cysts, pelvic colics).

The study group consisted of 214 pregnant women in the first, second and third trimester of pregnancy. These women were divided into ten sub-groups based on pregnancy complications: sub-group 1 (10 patients with spontaneous and internal abortions); sub-group 2 (19 patients with threatened abortion, bleeding and placenta previa); sub-group 3: (10 patients with ectopic pregnancies); sub-group 4: (12 patients with intrauterine growth restriction); sub-group 5 (20 patients with non-reassuring cardiotocography/umbilical artery Doppler patterns); sub-group 6: (6 patients with gestational diabetes); sub-group 7: (33 patients with preeclampsia); sub-group 8 (60 patients with threatened preterm delivery/premature rupture of membranes); sub-group 9 (7 patients with multiple pregnancies); and sub-group 10 (37 patients with uncomplicated pregnancies)

We obtained the patients' informed consent to take peripheral venous blood samples and to determine the concentrations of VEGF-A and PlGF. Blood samples were introduced into two different Vacutainers: one with an ochre-yellow cap (capacity



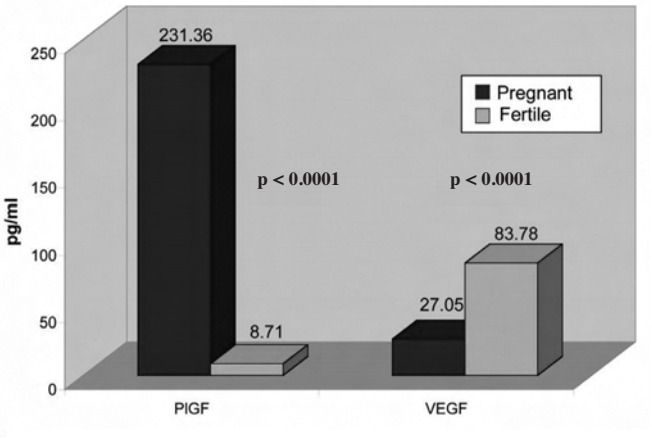


Figure 1. — Mean PlGF and VEGF-A levels in control subjects, fertile versus in menopause.
Figure 2. — Mean PlGF and VEGF-A levels in pregnant patients versus fertile subjects.

8.5 ml, containing a separating gel), and the other with a dark purple cap (capacity 4.0 ml, containing dipotassium EDTA as an anticoagulant). The samples were then taken to the Molecular Biology Laboratory of Padua Hospital, centrifuged at 1,500 rev/min for 5 min, frozen in liquid nitrogen, and stored.

VEGF-A was tested using the ELISA method (Bender Med System). An anti-VEGF polyclonal coating antibody is adsorbed onto microwells. The VEGF-A present in the standard or sample binds to antibodies adsorbed in the microwells. A biotin-conjugated polyclonal VEGF-A antibody is added and binds to the VEGF-A captured by the receptors. Following incubation, unbound biotin-conjugated VEGF-A is removed during a wash step. Horseradish peroxidase (HRP)-conjugated streptavidin (streptavidin-HRP) is added and binds to the biotin-conjugated VEGF-A. Following incubation, unbound streptavidin-HRP is removed during a wash step, and a substrate solution reactive with HRP is added to the wells. A colored product is formed in proportion to the amount of VEGF-A present in the sample. The reaction is terminated by the addition of acid and the absorbance is measured at 450 nm. For this test we prepared a standard curve from seven VEGF-A standard dilutions and then determined the VEGF-A concentration in the sample. The test is capable of determining values up to 0.26 pg/ml.

PlGF was tested using the EIA method (R & D Systems). This test employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for PlGF is precoated onto a microplate. Standards and samples are pipetted into the wells and any PlGF present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for PlGF is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and colour develops in proportion to the amount of PlGF bound in the initial step. Colour development is stopped and the intensity of colour is measured. In this case, too, we prepared a standard curve from standard dilutions and analyzed the samples by spectrophotometry. The test is capable of determining values up to 0.26 pg/ml.

Statistical analysis was performed using the non-parametric Mann-Whitney test, since data distribution was log-normal. A simple linear correlation test was also used. Statistical significance was reached for $p < 0.05$.

Results

The control group was composed of 163 women, with mean age 47.4 years (range 15-90 yrs). In this group, the mean VEGF-A concentration was 89.87 ± 100.31 pg/ml (range 0.26-538.8) and the mean PlGF concentration was 10.22 ± 6.21 pg/ml (range 0.26-31.26).

A total of 96 women were fertile and 67 were menopausal, with mean age of 35.2 and 65.6 years, respectively. Descriptive data for the two indicators concerning these two sub-groups are as follows: in the first sub-group (fertile women), mean VEGF-A values were 83.78 ± 92.50 pg/ml (range 0.26-474) and PlGF values were 8.71 ± 5.24 pg/ml (range 0.26 ± 27.30); in the second sub-group (menopausal women), mean VEGF-A and PlGF values were 94.2 ± 100.79 pg/ml (range 0.26-538.80) and 12.63 ± 12.7 pg/ml (range 0.26-31.20), respectively.

VEGF-A values were higher in the second sub-group than in the first one (92.78 vs 83.78 pg/ml), but they did not reach statistical significance. By contrast, mean PlGF values showed a statistical significant difference ($p < 0.0001$) with higher concentrations reported in menopausal women (12.63 pg/ml vs 8.71 pg/ml) (Figure 1).

We evaluated the presence of a correlation between the levels of the two indicators and the age of patients in the whole control group. VEGF-A levels showed no correlation with age, whereas PlGF levels showed a moderate but statistically significant increase with aging ($r = 0.24$; $p = 0.0019$).

We then carried out the same evaluation in the two subgroups of the control group, but did not find any statistically significant correlation.

Descriptive data for the second group of pregnant patients are: mean age, 32 years (range 16-48); mean VEGF-A values, 27.05 ± 87.29 pg/ml (range 0.26-851.90); and mean PlGF values, 231.36 ± 275.78 pg/ml (range 3.50-1,000.00).

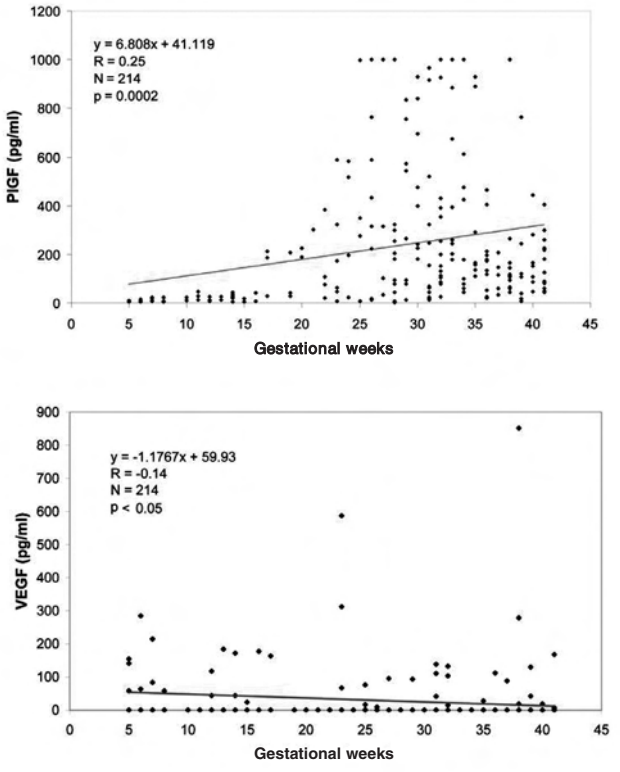


Figure 3. — Correlation between PlGF and VEGF-A levels in pregnant subjects according to gestational week.

We compared mean PlGF and VEGF-A values between pregnant women and fertile women in the control group. Both indicators showed statistically significant differences ($p < 0.0001$), with mean PlGF values higher in pregnant patients (231.36 pg/ml vs 8.71 pg/ml) and mean VEGF-A values higher in fertile control subjects (27.05 pg/ml vs 83.78 pg/ml) (Figure 2).

No correlation was found between VEGF-A and PlGF levels and age of pregnant patients. On the other hand, a statistically significant correlation was found between VEGF-A/PlGF levels and gestational age (r = -0.14, $p < 0.05$ for VEGF-A; r = 0.25, p = 0.0002 for PlGF), showing an increasing trend for PlGF and a decreasing trend for VEGF-A (Figure 3).

For descriptive data purposes, the group of pregnant patients was divided into two sub-groups according to the gestational week: one sub-group with pregnant women before the 20th gestational week (n = 46), and another sub-group with women after the 20th gestational week (n = 168). Their descriptive data are as follows: pregnant patients before the 20th gestational week had mean plasma VEGF-A and PlGF concentrations of 47.99 ± 77.31 pg/ml (range 0.26-284.80) and 38.83 ± 59.76 pg/ml (range 4.90-226.60), respectively; pregnant patients after the 20th gestational week had mean plasma VEGF-A and PlGF concentrations of 21.31 ± 89.18 pg/ml (range 0.26-851.90) and 284.08 ± 288.16 pg/ml (range 3.50-1000.00), respectively. Differences in the indicator levels between the two sub-groups reached statistical significance, with a clear increase in PlGF after the 20th gestational week ($p < 0.0001$) and a decrease in VEGF-A (p = 0.0006).

Descriptive data distribution for the ten pregnant patient sub-groups was as follows: the sub-group with spontaneous and internal abortions (n = 10) had a mean PlGF value of 44.11 ± 98.55 pg/ml (range 5.7-324.00) and a mean VEGF-A value of 84.12 ± 179.73 pg/ml (range 0.26-587.10); the sub-group with threatened abortion and vaginal bleeding (n = 19) had a mean PlGF value of167.14 ± 264.72 pg/ml (range 6.10-1,000.00) and a mean VEGF-A value of 25.64 ± 56.17 pg/ml (range 0.26-183.90); the sub-group with ectopic pregnancies (n = 10) had a mean PlGF value of 10.12 ± 5.19 pg/ml (range 4.90-22.30) and a mean VEGF-A value of 74.99 ± 103.84 pg/ml (range 0.26-284.80); the sub-group with intrauterine growth restriction (n = 12) had a mean P1GF value of 178.51 ± 184.28 pg/ml (range 14.30-611.00) and a mean VEGF-A value of 20.37 ± 47.56 pg/ml (range 0.26-138.60); the sub-group with cardiotocography/umbilical artery Doppler alterations (n = 20) had a mean PlGF value of 206.51 ± 131.78 pg/ml (range 25.80-464.20) and a mean VEGF-A value of 14.18 ± 62.26 pg/ml (range 0.26-278.70); the sub-group with gestational diabetes (n = 6) had a mean PlGF value of 241.18 ± 182.42 pg/ml (range 60.70-589.30) and a mean VEGF-A value of 14.9 ± 35.86 pg/ml (range 0.26-88.10); the sub-group with toxemia of pregnancy and hypertension (n = 33) had a mean PlGF value of 188.78 ± 267.03 pg/ml (range 3.50-1000.00) and a mean VEGF-A value of 23.72 ± 56.22 pg/ml (range 0.26-214.40); the sub-group with threatened preterm delivery and hypercontractility (n = 60) had a mean PlGF value of 374.11 ± 319.84 (range 5.80-1000.00) and a mean VEGF-A value of 15.39 ± 50.76 pg/ml (range 0.26-312.30); the sub-group with multiple pregnancies (n = 7) had a mean PlGF value of 275.41 ± 285.07 pg/ml (range 8.30-886.00) and a mean VEGF-A value of 2.58 ± 6.14 pg/ml (range 0.26-16.50); and the sub-group with uncomplicated pregnancies (n = 37) had a mean PlGF value of 201.88 ± 282.00 pg/ml (range 9.00-1000.00) and a mean VEGF-A value of 36.98 ± 143.69 pg/ml (range 0.26-851.90).

We compared the mean levels of the two indicators between the sub-group of women with uncomplicated pregnancies and the other nine sub-groups of women with complicated pregnancies. PlGF showed a statistically significant decrease in the sub-group with spontaneous and internal abortions ($p < 0.0001$) and in that with ectopic pregnancies ($p < 0.0001$), whereas it increased in the sub-group with ultrasound and cardiotocographic alterations ($p < 0.05$) and in that with threatened preterm delivery and uterine hypercontractility ($p < 0.001$). VEGF-A showed a statistically significant increase in the sub-group with ectopic pregnancies ($p < 0.05$).

Finally, we assessed the patterns of mean values of the two indicators among the ten sub-groups according to the gestational period (< 10, between 11 and 20, between 21 and 30, > 30 gestational weeks). Only PlGF showed sta-

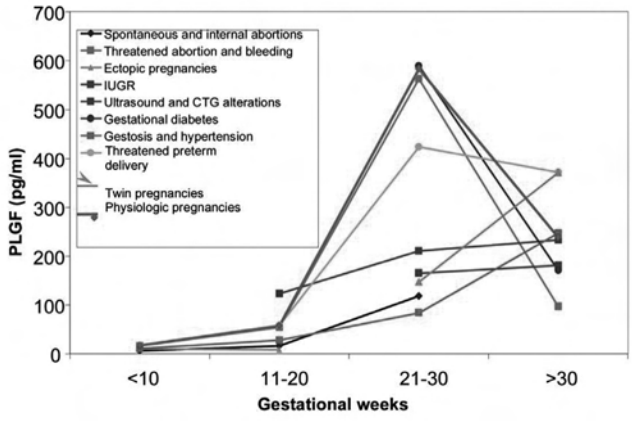


Figure 4. — PlGF pattern associated to different diagnoses, according to gestational week.

tistically significant differences over uncomplicated pregnancies, with a decrease in the spontaneous and internal abortion sub-group between 11-20 gestational weeks (57.3 pg/ml vs 16.47 pg/ml; $p < 0.005$) and an increase in the threatened preterm delivery and hypercontractility group after the 30th gestational week (238.93 pg/ml vs 371.70 pg/ml; $p < 0.05$) (Figure 4).

Discussion

Many studies in the last 15 years have examined the role of VEGF-A and PlGF both in uncomplicated and in complicated pregnancies. Nevertheless, this role is not completely clear yet. So far, no cut-off values have been proposed for diagnostic purposes, nor are such indicators commonly used in clinical practice, probably because of their high variability.

In our study, VEGF-A appeared to be more expressed than PlGF in the control group, with mean values of 89.87 pg/ml (median 68.00 pg/ml) and of 10.22 pg/ml (median 10.1 pg/ml), respectively. The control group was made up of patients hospitalized for gynecological (not oncological) problems or diseases, which according to the literature are unable to influence the values of the examined parameters. On the other hand, the PlGF and VEGF-A values observed in these patients did not substantially differ from those of a small group of healthy volunteers we were able to examine in our study.

The two sub-groups were not only largely different in size, but also had non-comparable mean ages (30.0 years for healthy volunteers vs 48.9 years for patients). Nevertheless, we observed that age did not influence the levels of the two indicators.

In fact, although a first evaluation of the control group demonstrated a weak but statistically significant correlation between age and PlGF (whereas no correlation was observed between age and VEGF-A), this fact can essentially be attributed to the subjects' hormonal conditions.

Consistently with the literature reports and physiology of embryogenesis, we observed an increase in PlGF and a decrease in VEGF-A in pregnant patients. This result has a great statistical significance if compared with the indicator values in fertile control subjects.

Confirming our findings in the control group, even in pregnant patients there was no statistically significant correlation between age and PlGF and VEGF-A, which were influenced by the gestational period: in particular, there was a positive statistically significant correlation between PlGF and increasing gestational weeks ($p = 0.0002$) and we observed that this factor started to increase clearly after the 20th gestational week. VEGF-A, too, was statistically correlated to the gestational period, though in a different way.

By dividing pregnant patients into two groups, before and after the 20th gestational week, we noted that in the latter group mean PlGF values were increased seven-fold and mean VEGF-A values were more than halved compared with the first group, with highly significant statistical differences ($p < 0.0001$ and $p = 0.0006$, respectively).

Furthermore, we examined the values of the two indicators in different pregnancy-related diseases, comparing them to those of uncomplicated pregnancy. This comparison was carried out by examining the values of the entire group of subjects in the different sub-groups and evaluating the pattern of data according to gestational weeks. However, this evaluation was not completely reliable due to the non-homogeneous distribution of our sub-groups in different classes of gestational weeks. Therefore, we observed some differences that had no statistical significance.

In the spontaneous and internal abortion sub-group, we found a statistically significant decrease in PlGF and in VEGF-A in the first 20 weeks of pregnancy, as reported in the literature [24]. Some studies have shown that an early decrease in NO and VEGF-A could be harmful to placental vascular growth and to endothelial regulation, leading to fetal death [25, 26].

A similar decrease in VEGF-A in the first ten weeks of pregnancy was also found in the threatened abortion sub-group.

In the ectopic pregnancy sub-group, too, there was a statistically significant decrease in PlGF in the first weeks of pregnancy, and an increase in VEGF-A, particularly between the 11th and the 20th gestational week.

The literature data concerning this condition show that due to the high sensitivity of serum hCG testing and to the increasing sensitivity of transvaginal ultrasound, the diagnosis of ectopic pregnancy has progressed dramatically in the last decade. The benefit of a single serum hCG measurement to confirm the absence of an ectopic pregnancy has been questioned, and serial measurements have been proposed [26, 27]. In fact, an abnormal pregnancy exists when serial serum hCG measurements decrease or do not increase in an appropriate way. Furthermore, serial hCG measurements are not easy to perform when the patient is in an emergency situation.

Serum VEGF-A levels are significantly increased in patients with ectopic pregnancies compared with controls (women with normal intrauterine pregnancies of match-

ing gestational age). The secretion and expression of VEGF-A could be induced by hypoxia, hormones, growth factors, and cytokines [28-30]. After 11 days from in vitro fertilization, VEGF-A levels higher than 700 pg/ml are strongly predictive of ectopic pregnancy [31]. Other authors have shown significantly increased VEGF-A levels in ectopic pregnancies, and have suggested the possibility of using a combination of VEGF-A and pregnancy-associated plasma protein A (PAPP-A) for early assessment of ectopic pregnancy [32]. This improvement could dramatically reduce the time required for diagnosis and the possibility of a tubal rupture and its sequelae.

Data for intrauterine growth restriction (IUGR) are extremely controversial in the literature. In our case series we did not find any statistically significant differences either for PlGF or for VEGF-A, but we observed that especially after the 20th gestational week, PlGF values tended to be lower than in uncomplicated pregnancy. Since reduced placental perfusion or a diminished density of villi is frequently observed in IUGR, studying placental vascular reactivity to VEGF-A and PlGF in IUGR is greatly useful [33]. The role of abnormal VEGF-A and PlGF expression in IUGR has been studied quite recently. When IUGR is caused by placental abnormalities or maternal diseases, growth retardation is usually a consequence of the inadequate presence of metabolic substrates and of reduced oxygen availability [34, 35]. Fetal ischemia is caused by placental failure: the foremost pathological factor associated with IUGR could be either the increase in vascular resistance or the upheaval of placental vascularization [36]. Data concerning VEGF-A and PlGF expression in IUGR are controversial. Several authors report both an increase and a decrease in VEGF-A and PlGF expression in the human placenta at the same stage of pregnancy [33]. In placental tissue, there are several potential sources of VEGF-A, but the contribution of mast cells to the VEGF-A pool could be significant, especially in pregnancies complicated by vascular abnormalities and hypoxia, like in IUGR [37].

It is believed that VEGFR-2 is more responsive to the vasodilating and hypotensive effects of VEGF-A. This greater reaction to VEGF-A rather than PlGF could be a consequence of the fact that the latter interacts only with VEGFR-1, while VEGF-A interacts both with VEGFR-1 and VEGFR-2 [21]. Relative deficiency of placental NO, or diminished sensitivity to VEGF-A and PlGF in pregnancies complicated by IUGR could strongly contribute to the development of a high impedance in fetal-placental circulation. By mainly interacting with VEGFR-2, VEGF-A is a powerful stimulus for increased vascular permeability. It may thus be inferred that abnormal placental transfer in IUGR could be related to pathological modifications of the placental vascular walls resulting from changes in the VEGF/VEGFR-2 system.

On the other hand, an increase in PlGF (statistically significant in the first group) and a concomitant decrease in VEGF-A (non-statistically significant) were observed in the sub-groups with ultrasound and cardiotocographic alterations and with gestational diabetes.

No statistically significant differences were found in the preeclampsia sub-group. However, the indicator values patterns according to gestational weeks showed an increase in VEGF-A in the first 20 weeks and a simultaneous decrease in PlGF, in agreement with the literature.

Recent studies have shown an increased placental expression and secretion of soluble fms-like tyrosine kinase-1 (sFlt-1), a VEGF-A antagonist normally circulating in patients with preeclampsia. *In vitro* studies indicate that an excessive placental production of sFlt-1 induces an angiogenetic status in the serum of women with preeclampsia, which can be opposed by exogenous VEGF-A and PlGF [38].

It has been confirmed that sFlt-1 increases both in the placenta and blood of women affected by preeclampsia [39]. Serum concentrations in women with uncomplicated pregnancies or with pregnancies complicated by preeclampsia suddenly decrease after delivery, as could be expected if the majority of circulating sFlt-1 during pregnancy originated from the placenta, and circulating concentrations of free PlGF were reduced in preeclampsia [40].

The highest concentration of sFlt-1 and the lowest concentration of PlGF were detected in blood samples taken from patients between the 21st and the 32nd gestational week before the development of preeclampsia, grouped by severity.

Studies concerning VEGF-A concentrations in preeclampsia have reported controversial results. In those where free VEGF-A was measured, levels were lower in patients with preeclampsia than in controls of matching gestational age [41]. In others, where total VEGF-A was measured, including VEGF-A bound to proteins, levels were mildly higher in preeclampsia patients [42].

However, since only free VEGF-A is biologically active and capable of interacting with receptors on the cellular surface, only the studies that measure free VEGF-A are relevant for discussion concerning its angiogenetic activity. As free VEGF-A levels in preeclampsia are close to the limit of detection using immunoadsorption, it is difficult to evaluate changes in these levels.

Several studies have shown a great increase in circulating sFlt-1 starting about five weeks before onset of preeclampsia, along with a decrease in free circulating P1GF and VEGF. These findings support the hypothesis that circulating angiogenetic proteins could have a biological key role in preeclampsia [40].

Therapeutic strategies aimed at opposing endothelial dysfunction in preeclampsia with VEGF, P1GF and prostacyclins could be tested in patients with severe diseases, to evaluate if pregnancy can be continued safely.

In particular, these authors pointed out that women who tend to develop preeclampsia and hypertension have PlGF values lower than normotensive women, starting from the 10th to 11th gestational week; moreover, they tend to have lower VEGF-A levels in the last period of pregnancy, as we also found in our case series.

In the sub-groups of patients with threatened premature delivery and hypercontractility, we found a statistically

significant increase in PlGF, both when we evaluated the whole sub-group and when we considered in particular the values of the last gestational period (> 30 weeks), compared with those in uncomplicated pregnancies.

Finally, although our case series was limited, in women with multiple pregnancies we found an increase in PlGF particularly marked in the last gestational period (> 30 weeks).

Conclusions

Our study led us to believe that the two analyzed growth factors could have a diagnostic and monitoring role during pregnancy, revealing deviations from a physiological pattern. They were indeed greatly different during pregnancy compared with control subjects, and were influenced by hormonal status and gestational age.

It is clear that further studies are needed to better assess the meaning of the variability of these values and to define cut-off values. Nonetheless, they seem to be particularly promising in the monitoring of some pregnancy-related conditions, such as ectopic pregnancy, threatened abortion or premature delivery, preeclampsia and IUGR, which are currently monitored only by ultrasound. The new opportunities offered by proteomics could also be beneficial to early diagnosis, leading to routine use of plasma assays for these two growth factors in all pregnancies.

We believe that it would be useful to further investigate these opportunities, by examining other pregnant patients and taking serial samples instead of single samples during pregnancy. By so doing, we could obtain more detailed information about the patterns of plasma concentrations in specific pregnancy-related diseases.

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