We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900
Open access books available

186,000
International authors and editors

200M
Downloads

154
Countries delivered to

Our authors are among the

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS

BOOK CITATION INDEX

INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Biomarkers of Ectopic Pregnancy-Present and Future

Soundravally Rajendiren and Pooja Dhiman

Additional information is available at the end of the chapter

1. Introduction

One of the most common complications of pregnancy is early pregnancy failure, of which 25% result in miscarriages and 1-2% ends in ectopic pregnancy. Both these entities can present with similar symptoms of abdominal pain and/or vaginal bleeding [1]. The most common site of ectopic pregnancy is fallopian tube. As tubal ectopic pregnancy (EP) is associated with high morbidity and mortality, early and accurate diagnosis of this condition is warranted. Present protocols for diagnosis of ectopic pregnancy utilize serial serum human chorionic gonadotropin (β-hCG) levels and pelvic ultrasound [2]. However, distinction between an intrauterine or extrauterine pregnancy may not be possible in 8-31% of cases at the first visit, even with sophisticated transvaginal ultrasound [3]. As a result, multiple visits for serial β-hCG and ultrasound monitoring are required before a diagnosis can be established and management initiated. This interim period of uncertainty may lead to potential life threatening complications like intra-abdominal bleed and future infertility because of compromised tubal integrity. Therefore, early diagnosis of tubal ectopic not only prevents the added mortality in patients, but also plays an important role in preventing future infertility. Also, the optimal management strategies for ectopic pregnancy and other abnormal intrauterine pregnancy (IUP) like miscarriages differ, and these two distinct clinical entities need to be differentiated at the early stages of pregnancy. A circulating serum biomarker may aid in predicting early pregnancy outcome (viable intrauterine pregnancy, miscarriage or tubal ectopic pregnancy), and may guide in deciding the best management strategy (conservative, medical or surgical) for the patient. Numerous groups have focussed their attention on this issue, and have reported many potential candidate biomarkers. The present chapter discuss the recent status of these candidate biomarkers in diagnosing tubal ectopic pregnancy, along with our experience, and attempt to foresee the future diagnostic trend of this clinically significant entity.

1.1. Problem statement

Tubal ectopic pregnancy is an important cause of early pregnancy failure attributing significantly to morbidity and mortality, in absence of expeditious diagnosis. Currently, the diagnosis of ectopic pregnancy is based on combined ultrasonograhic findings and serial β-hCG levels. However, low positivity rates in early gestational age and a need for repeated testing are major limitations of these diagnostic procedures, leading to added risk of tubal rupture and intra-abdominal bleed. Therefore, a circulating serum based biomarker, which could differentiate tubal ectopic from viable intrauterine pregnancy and other abnormal gestation (miscarriages) at the first visit of the patient, is the need of the hour.

1.2. Application area

A circulating serum based biomarker which could accurately predict the outcome of pregnancy such as ectopic pregnancy, miscarriage and viable intrauterine pregnancy. This may be used to diagnose tubal ectopic, decide the course of management (medical or surgical), and may aid in monitoring the prognosis and response to treatment in patients. This advancement in clinical diagnostic may help in significantly bringing down the maternal morbidity and mortality associated with early pregnancy failure.

1.3. Research course

Due to paucity of suitable animal models of tubal ectopic pregnancy, biomarker researches have mainly been carried out in cohorts of women with tubal ectopic, miscarriages and viable intrauterine pregnancy (IUP).

1.4. Method used

Comprehensive literature search was carried out for identifying studies on women with gestational age of less than 12 weeks in which serum markers were used to predict the outcome of pregnancy. The databases used were Medline, Embase, CINAHIL and Cochrane library, using key work search ectopic pregnancy", "tubal pregnancy" alone and in combination with "diagnosis", "screening" and "biomarkers". Only the articles published in English were included and references of all selected articles were also thoroughly searched. All the studies that assessed diagnostic accuracy were included.

1.5. Status

The factors deciding the fate of first trimester pregnancy, and hence the potential biomarkers for tubal ectopic pregnancy can be systematically categorized into two groups-Markers related to implantation site & milieu and Markers related to embryo (Fig 1). Important biomarkers in both these categories are described
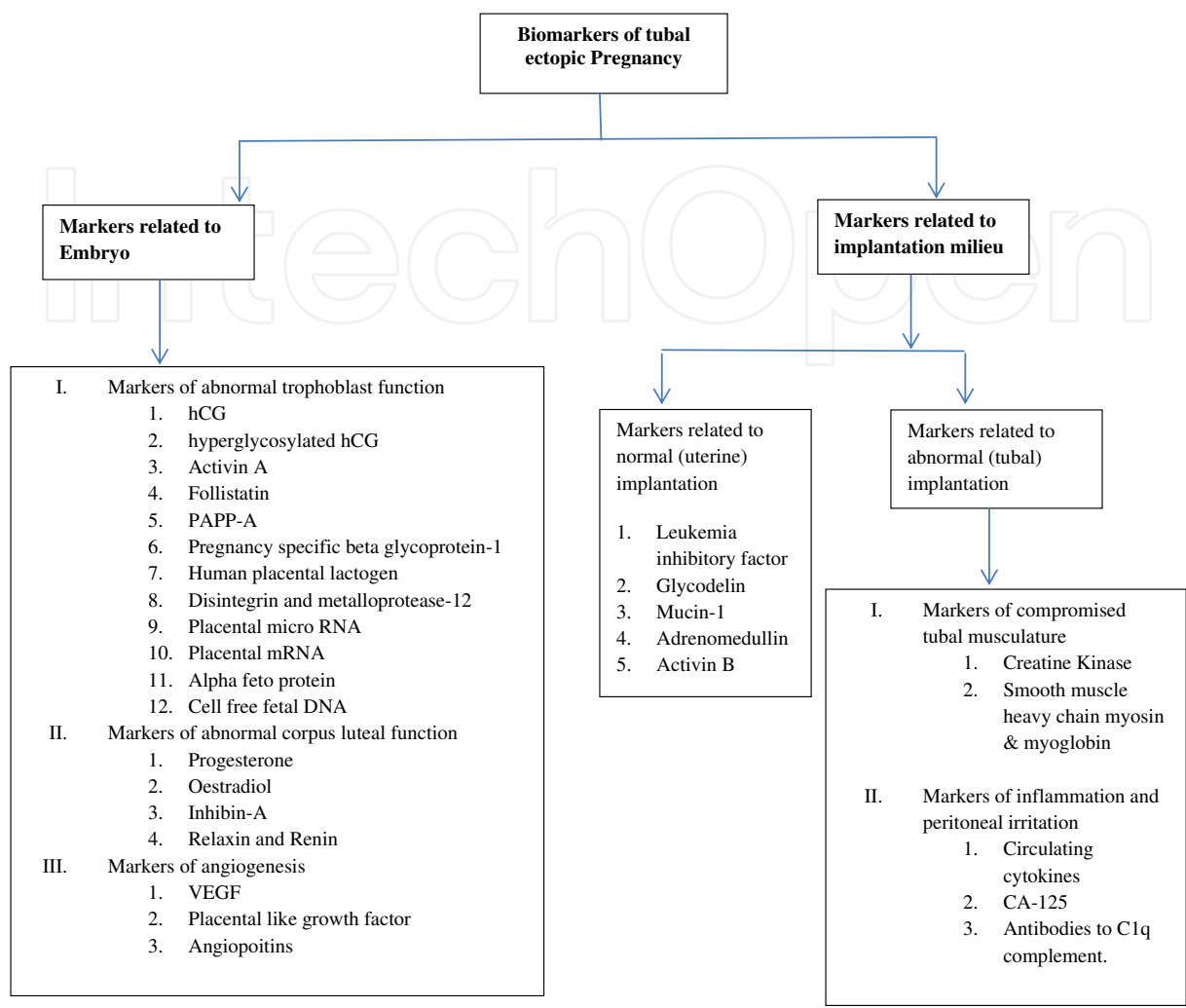
Figure 1. Candidate biomarkers for Ectopic Pregnancy

2. Markers related to embryo

2.1. Markers of abnormal trophoblast function

Theses are the molecules secreted from the conceptus. It would be probable that the pregnancy implanted in the ectopic environment will have abnormal growth kinetics that can be reflected in disarrayed trophoblast function. The following markers have been described in this section:

1.   Beta-human chorionic gonadotropin (β-hCG)

It is a glycoprotein hormone produced by trophoblast which maintains corpus luteum. Currently, β-hCG is the only biomarker used clinically in the diagnosis of ectopic pregnan-

cy[4]. A doubling of serum β-hCG over 48 hours is suggestive of viable intrauterine pregnancy [5]. If the levels remain static or fall, it is suggestive of early pregnancy failure, and the diagnosis need to be confirmed by more invasive procedures like endometrial curettage or laproscopy. To prevent unnecessary invasive procedures, studies suggested that the minimum rise of β-hCG over 48 hours to predict normal viable intrauterine pregnancy can be reduced to 53% [6] or 35% [7,8]. Another important concept in serum β-hCG estimation is the discriminatory zone, that is the minimum β-hCG concentration at which an intrauterine gestational sac could be reliably identified on ultrasound. The current discriminatory zone lies between 1500-3000 mIU/L [9]. Recently, a series of mathematical models incorporating β-hCG ratio (serum β-hCG at 48 hours/ serum β-hCG at 0 hours) have been developed to predict early pregnancy outcome, although it has not been sufficiently validated [10-13]. Another retrospective cohort study observed that in addition to the two values of serum β-hCG at 0 and 48 hours, third β-hCG evaluation on day 4 improved the accuracy of ectopic pregnancy prediction by 9.3% [14]. The role of β-hCG has also been studied to predict response to therapy. A cut-off value of 2121 mIU/L of the initial serum β-hCG, has shown a specificity of 76.4% and a sensitivity of 80.56% in predicting response to methotrexate therapy, with poor response below the cut-off [15]. Similarly, a cut-off of more than 1790mIU/ml was found to predict methotrexate treatment failure by another group [16]. Presently, serial monitoring of β-hCG is in use for the diagnostic triage of first trimester bleeding, but further studies are being conducted to use it in the prognostic protocol of EP.

2.   Hyperglycosylated hCG

Hyperglycosylated hCG (hCG-H) is a variant of hCG with four instead of two oligosaccharides. The site of production is first trimester invading extravillous cytotrophoblast, where it prevents trophoblast cell apoptosis [17,18]. Low proportions of hCG-H (< 50% of total hCG) was shown to predict failed pregnancy [19,20]. Another study identified a cut-off of 13μg/L for identifying failed pregnancy with 73% sensitivity and 98% specificity [21]. On the other hand, Butler et al did not find a role of hCG-H in the diagnostic algorithm for EP [22]. Recent study explored the role of hCG-H in predicting ongoing pregnancies after in vitro fertilization and found that day 9 level of hCG-H > 110pg/ml was 96% specific for ongoing pregnancy [23]. Conflicting results regarding the use of hCG-H as a marker of preeclampsia have also been reported [24,25]. Since, there is paucity of data on use of either concentration or proportion in distinguishing ectopic pregnancy from miscarriage, further studies are required to validate its usefulness as a biomarker for EP.

3.   Activin A

Activin A is a dimeric glycoprotein of TGF-β superfamily, with a role in cytotrophoblast invasion [26]. A study by Florio et al found Activin A level at a cut-off of ≤0.37ng/mL to have 100% sensitivity and 99.6% specificity for the diagnosis of EP [27]. Rausch et al found Activin A to have 80% sensitivity and 72% specificity as a single marker in a cohort of patients with EP [28]. Daponte et al also observed activin A concentrations to be significantly lower in women with EP and women with missed abortion, compared to IUP, and reported that at a threshold value of 505 pg/mL, activin A had 87.9% sensitivity and 100% specificity for

discriminating an ectopic pregnancy from viable pregnancies [29]. On the contrary, Activin-A was not found useful in diagnosing EP [30, 31]. Similarly, Elito et al demonstrated that serum activin A levels could not discriminate between an EP from a normal intrauterine pregnancy when an adnexal mass was found by transvaginal scan [32]. Therefore, A validation of this biomarker in a larger cohort would be encouraged before makes it a tool for diagnostics.

4. Follistatin (FS)

Follistatin is a circulating protein produced from human placenta, with rising serum concentration throughout pregnancy [33, 34]. Follistatin is primarily involved in modulating the biological activity of activin by binding to activin by high-affinity, which can neutralize the majority of the actions of activin [35]. Daponte et al reported that the concentration of FS was significantly lower in EP and MA compared to women with a viable IUP, and found that FS was able to discriminate IUP from EP, But not miscarriage from EP [29].

5. Pregnancy associated plasma protein-A (PAPPA)

PAPPA is a glycoprotein produced by trophoblast. Normally, PAPPA is up-regulated by progesterone, which promotes the adhesion and proliferation potential of trophoblastic cells [36]. It has been extensively studied and used as a marker of screening for first trimester aneuploidy [37]. Mueller et al found decreased levels of PAPPA in patients with ectopic pregnancy when compared to normal viable intrauterine pregnancy [38]. Dumps et al found a cut-off of <14.3 ng/ml to have a sensitivity of 64.5% with a 99% specificity for pregnancy failure [39] But the levels were found to be lower in miscarriages making the discrimination between EP and other abnormal intrauterine pregnancy dissatisfactory. On the other hand Ugurlu et al and Daponte et al observed that PAPPA can differentiate abnormal pregnancy from the viable normal IUP [40, 41]. Inconsistency in these results prompts us to study this marker in greater details.

6. Pregnancy specific beta glycoprotein-1 (SP-1)

Pregnancy specific beta glycoprotein-1 (SP-1) is a secreted protein produced from syncytiotrophoblast, thought to be involved in immunomodulation [42]. In addition, recently it has been observed that SP-1 induce transforming growth factor beta 1 (TGFB1), which among its other diverse functions inhibits T-cell function and has proangiogenic properties [43]. SP1 has also been reported as a first trimester maternal serum marker of small for gestational age (SGA) and preterm delivery [44]. For early pregnancy failure, Tornehave et al found lower levels of SP-1 to be suggestive of ectopic pregnancy [45]. Witt et al found a sensitivity of 65 % and a specificity of 74 % by taking a cut-off of 103.3μg/ml [46]. Further studies are needed to validate the use of SP-1 in EP.

7. Human placental lactogen (hPL)

Human placental lactogen is a circulating protein produced by the trophoblasts. Mueller et al found hPL levels to be decreased in ectopic pregnancy [38], but Rausch et al and Daponte et al found no difference in hPL levels between Ectopic pregnancy, abnormal IUP and normal viable IUP [28,41]. hPL as a biomarker of EP has to be evaluated in larger cohort of patients.

8.   A disintegrin and metalloprotease-12 (ADAM-12)

ADAM-12 is a glycoprotein primarily produced by syncytiotrophoblast, with a role in syncytial fusion [47]. ADAM-12 was found to be significantly decreased in patients with EP when compared to viable IUP in a cohort of 199 patients [48]. Horne et al observed that when measured in isolation, ADAM-12 levels had limited value as a diagnostic biomarker for EP [49], whereas Yang et al observed low levels of ADAM12 in complete spontaneous abortion and ectopic pregnancy compared to normal pregnancies [50]. Overall, this promising new marker still requires large prospective cohorts for validation.

9.   Placental micro-RNA

At least 31micro-RNA expressed from placenta have been isolated with various functions in gene regulation[51,52]. Zhao et al found that microRNA miR-323-3p was found to be lower in patients with EP as compared to those with normal IUP, yielding a sensitivity of 30% and a specificity of 90% in the diagnosis of ectopic pregnancy [53]. This is another promising area of biomarker discovery with further studies being conducted.

10.   Placental mRNA

Placental mRNAs secreted by trophoblasts can be altered in early pregnancy failure, and therefore have been studied recently for their diagnostic potential in EP. A recent case-control study conducted by Takacs et al demonstrated that patients with EP have significantly lower copy numbers of hCG and hPL mRNA in plasma compared to viable IUP [54]. Placental mRNA have also been studied for viability and chromosomal aneuploidy [55,56]. Evaluation of changes in placental mRNA expression may serve as a potential biomarker in future for detecting early pregnancy failures.

11.   Alpha feto protein (AFP)

AFP is a produced from both yolk sac and fetal liver with a role analogous to adult albumin [57]. Grosskinsky et al found AFP to be elevated in EP, whereas Kuscu et al study contradicted these findings [58, 59]. In our experience, we observed AFP concentration to be elevated in women with miscarriages when compared to normal IUP, but found it to be decreased in women with tubal ectopic compared to both IUP and miscarriages. ROC analysis in our study revealed that AFP was able to discriminate between miscarriage and ectopic, as well as between IUP and ectopic [Unpublished data].

12.   Cell free fetal DNA

Cell free fetal DNA escaping into the maternal circulation has also been evaluated as a marker of early pregnancy failure. Lazar et al estimated the cell-free foetal DNA of the Sry gene, and observed its concentration to be significantly higher in those with a tubal ectopic pregnancy. At a cut-off of more than 80 GE/ml, cell free fetal DNA was able to differentiate a tubal ectopic from an intrauterine pregnancy with sensitivity of 84%, a specificity of 76% [60]. As the technology at present is cumbersome, its utility as a useful serum biomarker in a clinical setup has been limited.

2.2. Markers of abnormal corpus luteal function

These are the markers for the evaluation of luteal function by measuring secreted products from the ovary. As the continuous function of the corpus luteum is essential for the mainte‐nance of early pregnancy, these functions may be suboptimal in ectopic pregnancy, and can be measured for diagnostic usage.

1. Progesterone

Maternal progesterone is initially produced by corpus luteum followed by placenta, and ensures appropriate development of the endometrium, uterine growth, adequate uterine blood supply, and preparation of the uterus for labor [60]. Progesterone levels are found to be low in both ectopic pregnancy and other abnormal IUP, when compared to viable IUP [62]. Extensive studies have been performed for the use of progesterone both as a single marker and in multiple marker settings to diagnose EP. A recent meta-analysis of 26 cohort studies, including 9436 pregnant women observed that at a cut-off values from 3.2 to 6 ng/mL, progesterone predicted a non-viable pregnancy with sensitivity of 74.6% and a specificity of 98.4%, although it was not able to distinguish EP from other abnormal IUP [63]. Similar results were observed by the meta analysis performed by Mol et al with a progesterone cut-off of <5ng/ml [64]. In our experience of the cohort of women, progesterone concentration was significantly lower in woman with ectopic pregnancy and women with miscarriages compared to patients with normal IUP. We observed a cut-off of <4.6ng/ml, to have a sensitivity of 93.3% and a specificity of 96.7% in differentiating EP from viable IUP, and a a cut-off of <12ng/ml, to have a sensitivity of 92.8% and a specificity of 90% in differentiating miscarriages from viable IUP, but found progesterone to be unable to discriminate between EP and other abnormal IUP [Unpublished data]. Also, high progesterone does not rule out EP in a patient with inconclusive ultrasound findings. Therefore, further large scale studies are required to study the utility of progesterone as a single marker or as a part of multiple markers to diagnose EP with acceptable sensitivity and specificity.

2. Oestradiol

Oestradiol (E2) is another important steroid secreted from the corpus luteum of pregnancy in response to hCG and could serve as a marker of pregnancy dynamics. Guillaume at al observed in his preliminary study that all of the EP patients had an E2 level of less than 650 pg/ml giving a sensitivity of 100% and specificity of 99% [65]. Witt et al however, did not find any difference in serum E2 when women with EP were compared with those with nonviable intrauterine pregnancies [46]. Mantzavinos et al on the other hand reported that E2 concentrations rose continuously in viable pregnancies, whereas in EP the values plateaued after the sixth week and declined after the eighth week of gestation [66]. However, as the concentrations of serum oestradiol have a considerable overlap among various clinical studies, this marker has not been put into clinical use.

3. Inhibin A

Inhibin A is a dimeric protein produced from corpus luteum [67]. Seifer et al observed lower levels of inhibin-A in EP compared to normal viable IUP [68]. Further, Segel et reported that

at a cut-off of 50 pg/mL, Inhibin-A had 100% sensitivity and specificity for diagnosing EP compared to viable IUPs, but found decrease in both sensitivity and specificity when patients of EP were compared with those of other abnormal IUP [69]. Similarly, Rausch et al found Inhibin A to have a sensitivity of 83% and a sensitivity of 79% at a lower cut-off of 23.67pg/ml in discriminating EP from viable IUP [28]. In contrast to the above findings, Chetty et al found Inhibin-A to be unable to discriminate EP from viable IUP in their cohort of 109 patients [70]. It can be concluded that although Inhibin-A is a promising marker for the early pregnancy viability, further studies need to be carried out to use it as a biomarker in the detection of EP.

4.   Relaxin and renin

Relaxin is a peptide hormone produced by the corpus luteum of pregnancy, which is elevated shortly after conception and remain steady until the 15th week of gestation [71]. Garcia et al observed a lower levels of serum relaxin concentrations in patients with EP than that in those with a viable IUP [72]. On the other hand, Witt et al found relaxin to be poorly discriminatory as a biomarker of ectopic pregnancy [46].

Renin is another peptide produced by ovaries and the production rises after pregnancy [73]. Meunier et al reported that active renin was significantly decreased in women with EP when compared with those with an ongoing IUP or spontaneous miscarriage, however because of its low specificity and sensitivity, the use of renin in the clinical setting has been limited [74].

2.3. Markers of angiogenesis

1.   Vascular Endothelial Growth Factor (VEGF)

VEGF plays an important role in the angiogenesis of the fetomaternal unit [75]. It has been reported that maternal serum VEGF concentrations are associated with depth of trophoblastic penetration into the tubal wall [76]. A case control study observed a cut-off of 200pg/ml had a sensitivity of 60% and a specificity of 90 % in predicting EP [77]. Similar findings were observed by Felemban et al [78]. Daponte et al observed a sensitivity of 78% and a specificity of 100 % in predicting EP at a cut-off of 174.5pg/ml. [41]. Recently Fernandes et al reported that serum level of VEGF was significantly higher in women with EP, and when threshold concentrations of serum VEGF level > 200 pg/ml were used, an EP could be distinguished from a normal pregnancy with a sensitivity of 51.4%, a specificity of 90.9% [79]. On the contrary, Rausch et al found no difference in the levels of VEGF between EP, abnormal IUP and viable IUP [28]. The variations in the study results of VEGF shows that further investigations are required to ascertain its suitability as a biomarker for EP.

2.   Placental like growth factor (PlGF)

PIGF is a pro-angiogenic growth factor produced by the trophoblast at the site of implantation [80]. Horne et al reported the expression of PIGF m-RNA to be lower in trophoblastic cells in patients with EP as compared to those with viable IUP [81]. Daponte et al later observed that at a PIGF level of more than 15.73pg/ml could differentiate viable pregnancy from early pregnancy failures including EP and abnormal IUP with reasonable sensitivity and specificity, but could not differentiate among them [82]. Recent report by Martínez-Ruiz et al also didn't

find significant differences for PlGF between EP and viable IUP [83]. Therefore, there is a need of validation of this marker in a larger cohort.

3.   Angiopoietins

Angiopoietins are proteins which belong to the family of angiogenic proteins, which has been shown to be critically involved in the process of placental maturation and growth from early pregnancy. Angiopoietin-1 (Ang-1) and Ang-2 are two critical regulators with different functions of vascular development and angiogenesis [84, 85]. Daponte et al reported Ang-1 and Ang-2 concentrations and their ratio to be lower in EP compared to IUP. They also found the trophoblastic Ang-1 mRNA expression levels to be lower in EP compared to IUP, while Ang-2 mRNA was found to be higher in EP than in IUP [86]. Schneuer et al in their recent study suggested that the lower Ang-1/Ang-2 ratio in first trimester is associated with most adverse pregnancy outcomes, but do not predict outcomes any better than clinical and maternal risk factor information [87].

2.4. Markers related to normal uterine implantation

These biomarkers are released into the circulation as a result of normal interaction between the pregnancy and the uterine decidua. As the normal process is disrupted in EP, these surrogate markers can be used to diagnose EP.

1.   Leukemia inhibitory factor (LIF)

LIF is a cytokine of interleukin-6 family with a key role in implantation [88]. Wegner et al observed that women with ectopic pregnancy had low serum concentrations of LIF and could diagnose it with moderate sensitivity and specificity [89]. However, Daponte et al failed to find any difference in LIF concentrations in patients with ectopic pregnancy and other abnormal intrauterine pregnancy [41]. A further attempt in validation of LIF has yielded conflicting results. Mueller et al found LIF levels to be undetectable in serum of patients with ectopic pregnancy and viable intrauterine pregnancy [38], whereas Iyibozkurt AC et al found increased levels of LIF in patients with EP compared to IUP [90]. Increased immunohisto-chemical expression of LIF in fallopian tube was found to be increased in EP compared to non –pregnant and healthy pregnant controls, indicating its role in ectopic implantation of embryo [91]. Similarly it was observed that LIF expression was increased in inflamed fallopian tube and might be one of the reasons of increased susceptibility of salphingitis patients to EP [92].

2.   Glycodelin (Placental protein 14)

Glycodelin is secreted from endometrium and fallopian tube, with immunomodulatory role in implantation. Its serum concentration increases during early first trimester of pregnancy, till 8-10 weeks of gestation and then progressively declines [93, 94]. Foth et al found signifi-cantly lower levels of serum glycodelin in patients with ectopic pregnancy compared to IUP and incomplete abortion with a study population of 169 subjects [95]. Out of the three groups, who studied glycodelin in a multiple marker setting, two had found glycodelin levels to be decreased in EP, while one observed no significant difference between EP and abnormal IUP [28, 38, 41]. Further studies are required to validate the use of this marker in EP.

3.   Mucin-1 (Muc-1)

Muc-1 is a glycoprotein expressed by endometrium and fallopian tube epithelium involved in implantation [96]. Muc-1 expression was observed to be lower in luminal epithelial of tubes with ectopic pregnancies [97]. Similar findings were observed by Reefaat et al [98]. However, the role of serum Muc-1 as a diagnostic modality has not been studied in much detail.

4.   Adrenomedullin

Adrenomedullin is a peptide hormone belonging to calcitonin/calcitonin gene related peptide, and is thought be involved in endometrial angiogenesis. Liao et al found plasma and oviductal tissue adrenomedullin to be lower in EP and suggested that this decreases ciliary beating and muscle contraction leading to retained embryo and its implantation in the oviduct [99]. Similar findings were observed in nasal epithelium in patients of tEP by the same group [100]. Further studies are required to explore the possible role of serum adrenomedullin as a diagnostic marker for EP.

5.   Activin B

Activins, dimeric proteins of transforming growth factor-beta (TGF-b), have recently been found in gonadal fluid with growth factor like actions [101]. It is produced by many organs including pituitary gland, gonads, placenta etc. It has been shown to promote decidualization of the endometrium during pregnancy [102]. Consequently low serum activin levels have been associated with ectopic pregnancy. Although recent studies have mainly focussed the role of serum activin A as a potential marker of ectopic pregnancy, limited literature is available regarding the role of activin B in-spite of the experimental evidence of differential effect of activin B on decidua. In our population, we observed that the concentration of activin B in maternal serum to be significantly lower in patients with tEP compared to those with normal IUP [Unpublished data]. Similar results were observed by Horne et al, who found decreased expression of activin B in endometrium along with decreased serum levels of activin B with normal levels of progesterone in 11 women with tEP [103]. Activin B is a new, though promising marker for EP diagnostic triage.

3. Markers related to tubal implantation

3.1. Markers of compromised tubal musculature markers

These markers reflect the disruption of the integrity of the tubal circular smooth muscle layer, which can happen in an ectopic implantation. These markers of muscle damage have been investigated as biomarkers for diagnosing EP.

1.   Creatine Kinase (CK)

Creatine Kinase is the enzyme released from damaged muscles, which is currently used in the diagnosis of myocardial infarction. Lavie et al found serum CK levels to be significantly higher in patients with tEP as compared to those with missed abortion or normal IUP [104]. Similar

findings were observed by Birkhahn et al and Duncan et al [105,106]. In a study conducted in our population, we found that the optimal cut-off for total CK, CK-MM and CPK-MB% as predictors of ruptured EP were 147 IU/L, 135 IU/L and 10%, respectively, with the former two having higher specificity, and latter high sensitivity [107]. On the contrary, several independent study groups found CK to be inadequate in diagnosing EP [108-112]. Recently, Safdarian et al studied the role of CK-1 as an indicator for differentiating between the successful and unsuccessful treatment groups in EP, but failed to find any relation between initial CPK serum levels [113].

2.   Smooth muscle heavy-chain myosin (SMHC) & Myoglobin

Smooth muscle heavy chain myosin and myoglobin are markers of smooth muscle damage. As the ectopic pregnancy grows to invade the muscular layer of the fallopian tube, markers of muscle damage should also rise. Birkhahn et al studied serum myoglobin and smooth muscle heavy-chain myosin and observed a statistically significant elevation in the serum levels of SMHC, but did not find it useful in the screening for EP [105,114]. As there is paucity of data regarding the usefulness of these markers in the screening of EP, their clinical utility is limited.

3.2. Markers of inflammation and peritoneal irritation

EP can lead to inflammation and peritoneal irritation and the following biomarkers have been investigated as an potential biomarkers of the same process.

1.   Circulating cytokines

Several cytokines as marker of peritoneal inflammation has been reported including IL-2R, IL-6, IL-8, IL-10, IL-11, IL-15 and TNF-α. Soriano et al observed increased concentration of IL-6, IL-8 and TNF-α in patients with EP compared with normal and abnormal IUP. IL-8 at a cut-off of >40 pg/ml was shown to have a sensitivity and specificity of 82.4% and 81.8 % respectively in diagnosing EP [115]. Experience in our population regarding IL-6 & IL-8 levels, we observed that the level of IL-6 shows a significant increase in the women with tubal ectopic pregnancy in comparison to intrauterine abortion and normal pregnancy. It was also seen that IL-8 levels decrease significantly in the tubal ectopic pregnancy cases and in intrauterine abortion patient when compared to the normal pregnancy group. ROC analysis revealed that at the cut-off of 26.48pg/ml of IL-6 level predict the probability of tubal ectopic pregnancy with 53.57% sensitivity, 80%specificity [Unpublished paper]. Similar observations were made by Rausch et al, who observed lower values of IL-8 and TNF-α in women with EP, whereas no significant difference was observed in the IL-6 levels between EP and viable IUP [28]. No difference has been observed in the levels of IL-10 and IL-11between EP and viable IUP [90]. IL-15 has also been studied as it is expressed by human placental tissue culture and it is maximally expressed during the implantation period in the deciduas. Daponte et al reported that IL-15 concentrations were significantly higher in women with EP compared to patients with IUP, and found IL-15 to have high diagnostic accuracy for the discrimination of a viable IUP from an EP with an area under the curve of 0.818 [116].

2. CA-125

Conflicting results have been reported by several groups regarding the status of circulating CA-125 in EP with some groups reporting an increase, some decrease and few found no difference between viable IUP and EP [117-121]. In our experience in the cohort of patients, we found CA125 concentration to be significantly higher in woman with miscarriages compared to patients with normal IUP, but not in women with ectopic compared to IUP [Unpublished paper]. Women with **IU** abortion were found to have significantly higher CA-125 levels, compared to the other two groups. Katsikis et al also reported that when using CA-125 concentration of more than 41.9 U/ml as a threshold for the diagnosis of IU abortive pregnancy, sensitivity was 80% and specificity was 87% for discriminating it from EP [122].

3. Antibodies to C1q complement

C1q complement has been shown to promote trophoblast invasion of deciduas, a crucial step in normal placental development. Animal models have demonstrated that the lack of C1q is characterized by poor trophoblast invasion and pregnancy failure [123,124]. Studies based on these observations have measured the levels of antibodies to C1q complement in early pregnancy failure have been conducted. Daponte et al failed to observe any difference between normal viable IUP, EP and other abnormal IUP [116]. As new studies would be undertaken by different groups, more information regarding this marker is likely to emerge.

The biomarkers studied in ectopic pregnancy with their current status are summarized in table 1.

Sl No	Biomarker	Cut-off	Sensitivity	Specificity	Reference
1	B-hCG				
	a. Single serum levels	<2000mIU/ml	87%	39%	Shaunik et al 2011
	b. 48 hours rise	<53% rise	91%	66.6%	Barnhart 2004
		<35% rise	83.2%	70.8%	Morse 2012
	c. Mathematical models				
	M1	0.21	83%	88%	Condous 2004
	M4	-	92%	91%	Condous 2007
			31%	98%	Kirk 2006
			81%	89%	Barnhart 2010
2	Hyperglycosylated hCG	13μg/L	73%	98.1%	Sutton-Riley 2006
3	Pregnancy associated plasma protein-A (PAPPA)	0.53 ng/ml	81%	54%	Rausch 2011
4	Pregnancy specific beta glycoprotein-1 (SP-1)	103.3μg/ml	65%	74%	Witt 1990

Sl No	Biomarker	Cut-off	Sensitivity	Specificity	Reference
5	ADAM-12	48.49ng/ml	97%	37%	Rausch 2011
6	Activin A	0.37ng/ml	100%	99.6%	Florio 2007
		0.38 ng/ml	80%	72%	Rausch 2011
		0.26 ng/ml	59.6%	69%	Warrick 2012
		505 pg/ml	87.9%	100%	Daponte 2013
7	Progesterone	3.2-6 ng/ml	74.6%	98.4%	Verhaegen 2012
		5ng/ml	95%	40%	Mol 1998
		10.75 ng/ml	85%	85%	Katsikis 2006
8	Oestradiol	650 pg/ml	100%	90%	Guillaume 1990
9	Inhibin A	50 pg/ml	100%	100%	Segel 2008
		28.67 pg/ml	83%	79%	Rausch 2011
10	VEGF	200 pg/ml	60%	90%	Daniel 1999
		174.5 pg/ml	78%	100%	Daponte 2005
		200 pg/ml	88%	100%	Felemban 2002
		28.24 pg/ml	95%	50%	Rausch 2011
11	PIGF	15.7 pg/ml	86%	73%	Daponte 2011
12	LIF	6.2pg/ml	73%	72%	Wegener 2001
13	Interleukin-8	40 pg/ml	82.4%	81.8%	Soriano 2003
14	Interleukin-15	16.1 pg/ml	92%	68%	Daponte 2013

Table 1. Current status of biomarkers of tubal Ectopic pregnancy

4. Further research

Since the factors and their interplay involved in maintenance of normal viable pregnancy are not completely understood, various approaches have been explored in biomarker discovery including unbiased proteomics, shotgun proteomics [125,126]. The challenges in biomarker discovery include the variability of biomarkers with gestational age, impact of coexistent morbidities like hypertension, diabetes, chromosomal anomalies on biomarker levels and their interpretation. Other emerging markers studied in ectopic pregnancy include: Endocannibi-noid system abnormalities in the form of high anandamide levels and reduced receptor expression have been implicated, in women with ectopic pregnancy [127,128].

Brown et al conducted a proteomics study and identified fibronectin has ability to discriminate EP from other pregnancy outcomes suggesting its diagnostic potential and its use as an adjunct to future multiplex EP diagnostic tests [129].

Serum Macrophage Inhibitory Cytokine-1 levels were found to be lower in women with histologically confirmed EP compared to women with definite viable intra-uterine pregnancy by Skubisz et al [130].

Another recent study by Beer et al that screened the proteome of a small group of women with EP and controls identified potential novel biomarkers, including ADAM-12 and ISM2 (Isthmin 2) as well as five specific isoforms of pregnancy-specific beta-1-glycoprotein 131].

5. Use of multiple biomarkers in ectopic pregnancy

As maintenance of a viable pregnancy requires an interplay of multiple factors, no single marker has been used successfully as a biomarker for EP. It seems prudent, therefore, to combine these markers and use them in the multiple marker setting. Rausch et al demonstrated that a four-marker test including Progesterone, VEGF, Inhibin A, and Activin A could predict EP with 100% accuracy in those with an hCG<1500 mIU/mL [28]. Further studies are necessary to fully assess the discriminatory capacity of such a test. Similarly, Feng et al found a combination of $\Delta\beta$-hCG, Progesterone and Oestradiol to be helpful in distinguishing EPs and normal IUPs, facilitating earlier diagnosis and the timely implementation of medical treatment to prevent tubal rupture [132].

Soriano et al found that the combination of inflammatory cytokines IL-6, IL-8, and TNF-alpha was able to predict EP with specificity of 100%, but sensitivity of 52.9% [115].

Another group in Switzerland developed a multiple marker test, the "triple marker analysis" [VEGF/(PAPP-A X P)] had a sensitivity of 97.7% with a specificity of 92.4% in diagnosing EP [38].

In another study, investigators studied serum levels of 17β-estradiol (E2), progesterone (P4), testosterone (T), beta-human chorionic gonadotropin (β-hCG), vascular endothelial growth factor-A (VEGF-A), placental growth factor (PIGF), and a distintegrin and metalloprotease protein 12 (ADAM12) in different patient groups with no definite results [133].

6. Conclusion

Novel biomarker of ectopic pregnancy with adequate sensitivity and specificity could assist in early diagnosis and hence timely intervention, thereby dramatically reducing the morbidity and mortality. There are number of potential molecules for use as biomarkers in women at risk for EP. As no single biomarker is ready for use in clinical setting, more prospective cohorts including ectopic pregnancy, normal and abnormal IUPs are required to validate these markers. Also, it would be prudent to concentrate the efforts on developing a panel of markers which include markers of viability, location of implantation and fetal milieu. Recent times have witnessed positive developments in this field, but lot of validation is required before a marker can be used independently in clinical setting.

Author details

Soundravally Rajendiren[*] and Pooja Dhiman

*Address all correspondence to: soundy27@yahoo.co.in

Department of Biochemistry, JIPMER, Puducherry, India

References

[1] Hasan R, Baird DD, Herring AH, Olshan AF, Johnsson Funk ML, Hartmann KE. Pattern and predictors of vaginal bleeding in the first trimester of pregnancy. Ann Epidemiol 2010;20 (7):524-31.

[2] Barnhart KT. Clinical practice-Ectopic pregnancy. N Engl J Med 2009; 361:379-87.

[3] Condous G, Okaro E, Bourne T. The conservative management of early pregnancy complication: a review of literature. Ultrasound Obstet Gynecol 2003; 22: 420-30.

[4] Shaunik A, Kulp J, Appleby DH, Sammel MD, Barnhart KT. Utility of dilation and curettage in the diagnosis of pregnancy of unknown location. Am J Obstet Gynecol 2011;204:130–136.

[5] Lenton EA, Neal LM, Sulaiman R. Plasma concentrations of human chorionic gonadotropin from the time of implantation until the second week of pregnancy. Fertil Steril 1982;37(6):773-8.

[6] Barnhart KT, Sammel MD, Rinaudo PF, Zhou L, Hummel AC, Guo W. Symptomatic patients with an early viable intrauterine pregnancy: hCG curves redefined. Obstetrics and Gynecology 2004;104:50-55.

[7] Seeber BE, Sammel MD, Guo W, Zhou L, Hummel A, Barnhart KT. Application of redefined of human chorionic gonadotropin curves for the diagnosis of women at risk for ectopic pregnancy. Fertility and sterility 2006; 86:454-9.

[8] Morse CB, Sammel MD, Shaukina A, Allen-Taylor L, Oberfoell NL, Takacas P, Chung K et al. Performance of human chorionic gonadotropin curves in women at risk for ectopic pregnancy: exceptions to the rules. Fertili Steril 2012;97(1):101-6.

[9] Desai D, Lu J, Wyness SP, Greene DN, Olson KN, Wiley CL, Grenache DG. Human chorionic gonadotropin discriminatory zone in ectopic pregnancy: does assay harmonization matter? Fertil Steril. 2014;101(6):1671-4.

[10] Condous G, Okaro E, Khalid A, Timmerman D, Lu C, Zhou Y et al. The use of a new logistic regression model for predicting the outcome of pregnancies of unknown location. Hum Reprod 2004; 19: 1900-10.

[11] Kirk E, Condous G, Haid Z, Lu C, Van Huffel S, Timmerman D, Bourne T. The practical application of a mathematical model to predict the outcome of pregnancies of unknown location. Ultrasound Obstet Gynecol 2006; 27(3): 311-5.

[12] Barnhart KT, Sammel MD, Appleby D, Rausch M, Molinaro T, Van Calster B et al. Does a prediction model for pregnancy of unknown location developed in the UK validate on a US population? Hum Reprod 2010; 25:2434-40.

[13] Condous G, Van Calster B, Kirk E, Haider Z, Timmerman D, Van Huffel S, Bourne T. Prediction of ectopic pregnancy in women with a pregnancy of unknown location. Ultrasound Obstet Gynecol 2007b;29:680–687.

[14] Zee J, Sammel MD, Chung K, Takacs P, Bourne T, Barnhart KT. Ectopic pregnancy prediction in women with a pregnancy of unknown location: data beyond 48 hour are necessary. Hum Reprod 2014;29(3):441-7.

[15] Helmy S, Bader Y, Pablik E, Tiringer D, Pils S, Lami T, Koblik H et al. Cut-off value of initial serum β-hCG level predicting a successful methotrexate therapy in tubal ectopic pregnancy: a retrospective cohort study. Eur J Obstet Gynecol Reprod Biol 2014;179C:175-80.

[16] Nowak-Markwitz E, Michalak M, Olejnik M, Spaczynski M. Cut-off value of human chorionic gonadotropin in relation to the number of methotrexate cycles in the successful treatment of ectopic pregnancy. Fertil Steril 2009;92(4):1203-7.

[17] Cole LA, Butler SA. Hyperglycosylated human chorionic gonadotropin and human chorionic gonadotropin free beta-subunit: tumor markers and tumor promoters. J Reprod Med 2008;53:499-512.

[18] Guibourdenche J, Handschuh K, Tsatsaris V, Gerbaud P, Leguy MC, Muller F, Brion DE, Fournier T. Hyperglycosylated hCG is a marker of early human trophoblast invasion. J Clin Endocrinol Metab 2010; 95:E240-244.

[19] Sasaki Y, Ladner DG, Cole LA. Hyperglycosylated human chorionic gonadotropin and the source of pregnancy failures. Fertil Steril 2008;89(6):1781-6.

[20] Cole LA. Hyperglycosylated hCG and pregnancy failures. J Reprod Immunol 2012;93(2):119-22.

[21] Sutton-Riley JM, Khanlian SA, Byrn FW, Cole LA. A single serum test for measuring early pregnancy outcome with high predictive value. Clin Biochem 2006;39:682-7.

[22] Butler SA, Abban TK, Borrelli PT, Luttoo JM, Kemp B, Iles RK. Single point biochemical measurement algorithm for early diagnosis of ectopic pregnancy. Clin Biochem. 2013 ;46(13-14):1257-63.

[23] Chuan S, Homer M, Pandian R, Conway D, Garzo G, Yeo L, Su HI. Hyperglycosylated human chorionic gonadotropin as an early predictor of pregnancy outcomes after in vitro fertilization. Fertil Steril. 2014;101(2):392-8.

[24] Keikkala E, Ranta JK, Vuorela P, Leinonen R, Laivuori H, Väisänen S, Marttala J, Romppanen J, Pulkki K, Stenman UH, Heinonen S. Serum hyperglycosylated human chorionic gonadotrophin at 14-17 weeks of gestation does not predict preeclampsia. Prenat Diagn. 2014;34(7):699-705.

[25] Keikkala E, Vuorela P, Laivuori H, Romppanen J, Heinonen S, Stenman UH. First tri-mester hyperglycosylated human chorionic gonadotrophin in serum-a marker of ear-ly-onset preeclampsia. Placenta. 2013;34(11):1059-65.

[26] Bearfield C, Jauniaux E, Groome N, Sargent IL, Muttukrishna S. The secretion and ef-fect of inhibin A, activin A and follistatin on first-trimester trophoblasts in vitro. Eur J Endocrinol. 2005; 152(6):909-16.

[27] Florio P, Severi FM, Bocchi C, Luisi S, Mazzini M, Danero S, Torricelli M, Petraglia F. Single serum activin a testing to predict ectopic pregnancy. J Clin Endocrinol Metab. 2007; 92(5):1748-53.

[28] Rausch ME, Sammel MD, Takacs P, Chung K, Shaunik A, Barnhart KT. Development of a multiple marker test for ectopic pregnancy. Obstet Gynecol. 2011; 117(3):573-82.

[29] Daponte A, Deligeoroglou E, Garas A, Pournaras S, Hadjichristodoulou C, Messinis IE. Activin A and follistatin as biomarkers for ectopic pregnancy and missed abor-tion. Dis Markers. 2013;35(5):497-503.

[30] Warrick J, Gronowski A, Moffett C, Zhao Q, Bishop E, Woodworth A. Serum activin A does not predict ectopic pregnancy as a single measurement test, alone or as part of a multi-marker panel including progesterone and hCG. Clin Chim Acta. 2012; 413(7-8):707-11.

[31] Kirk E, Papageorghiou AT, Van Calster B, Condous G, Cowans N, Van Huffel S, Timmerman D, Spencer K, Bourne T. The use of serum inhibin A and activin A levels in predicting the outcome of 'pregnancies of unknown location'. Hum Reprod. 2009; 24(10):2451-6.

[32] Elito Júnior J, Gustavo Oliveira L, Octávio Fernandes Silva M, Araujo Júnior E, Ca-mano L. Serum activin A levels and tubal ectopic pregnancy. Iran J Reprod Med. 2014;12(3):227-8.

[33] de Kretser, DM, Foulds, LM, Hancock, M, Robertson DM. Partial characterization of inhibin, activin, and follistatin in the term human placenta. J. Clin. Endocrinol. Metab 1994;79:502–507.

[34] Woodruff, T.K., Sluss, P., Wang, E, Janssen I, Mersol-Barg MS. Activin A and follista-tin are dynamically regulated during human pregnancy. J. Endocrinol 1997;152:167–175.

[35] Nakamura, T, Takio K., Eto Y, Shibai H, Titani K, Sugino H. Activin-binding protein from rat ovary is follistatin. Science 1990;247:836–838.

[36] Wang J, Liu S, Qin HM, Zhao Y, Wang XQ, Yan Q. Pregnancy-associated plasma protein A up-regulated by progesterone promotes adhesion and proliferation of trophoblastic cells. Int J Clin Exp Pathol. 2014;7(4):1427-37.

[37] Nicolaides KH, Spencer K, Avgidou K, Faiola S, Falcon O. Multicenter study of first-trimester screening for trisomy 21 in 75 821 pregnancies: results and estimation of the potential impact of individual risk-orientated two-stage first-trimester screening. Ultrasound Obstet Gynecol. 2005; 25(3):221-6.

[38] Mueller MD, Raio L, Spoerri S, Ghezzi F, Dreher E, Bersinger NA. Novel placental and non-placental serum markers in ectopic versus normal intrauterine pregnancy. Fertil Steril 2004;81(4):1106-11.

[39] Dumps P, Meisser A, Pons D, Morales MA, Anguenot JL, Campana A, Bischof P. Accuracy of single measurements of pregnancy-associated plasma protein-A, human chorionic gonadotropin and progesterone in the diagnosis of early pregnancy failure. Eur J Obstet Gynecol Reprod Biol. 2002; 100(2):174-80.

[40] Ugurlu EN, Ozaksit G, Karaer A, Zulfikaroglu E, Atalay A, Ugur M. The value of vascular endothelial growth factor, pregnancy-associated plasma protein-A, and progesterone for early differentiation of ectopic pregnancies, normal intrauterine pregnancies, and spontaneous miscarriages. Fertil Steril. 2009;91:1657–61.

[41] Daponte A, Pournaras S, Zintzaras E, Kallitsaris A, Lialios G, Maniatis AN, et al. The value of a single combined measurement of VEGF, glycodelin, progesterone, PAPP-A, HPL and LIF for differentiating between ectopic and abnormal intrauterine pregnancy. Hum Reprod. 2005;20:3163–6.

[42] Horne CH, Towler CM, Pugh-Humphreys RG, Thomson AW, Bohn H. Pregnancy specific beta1-glycoprotein--a product of the syncytiotrophoblast. Experientia. 1976; 32(9):1197.

[43] Ha CT, Wu JA, Irmak S, Lisboa FA, Dizon AM, Warren JW, Ergun S, Dveksler GS. Human pregnancy specific beta-1-glycoprotein 1 (PSG1) has a potential role in placental vascular morphogenesis. Biol Reprod. 2010 ;83(1):27-35.

[44] Pihl K, Larsen T, Laursen I, Krebs L, Christiansen M. First trimester maternal serum pregnancy-specific beta-1-glycoprotein (SP1) as a marker of adverse pregnancy outcome. Prenat Diagn. 2009;29(13):1256-61.

[45] Tornehave D, Chemnitz J, Westergaard JG, Teisner B, Poulsen HK, Bolton AE, Grudzinskas JG. Placental proteins in peripheral blood and tissues of ectopic pregnancies. Gynecol Obstet Invest. 1987; 23(2):97-102.

[46] Witt BR, Wolf GC, Wainwright CJ, Johnston PD, Thorneycroft IH. Relaxin, CA-125, progesterone, estradiol, Schwangerschaft protein, and human chorionic gonadotropin as predictors of outcome in threatened and non-threatened pregnancies. Fertil Steril. 1990; 53(6):1029-36.

[47] Huppertz B, Bartz C, Kokozidou M. Trophoblast fusion: fusogenic proteins, syncytins and ADAMs, and other prerequisites for syncytial fusion. Micron. 2006; 37(6): 509-17.

[48] Rausch ME, Beer L, Sammel MD, Takacs P, Chung, Shaunik A, Speicher D, Barnhart KT. A disintegrin and metalloprotease protein-12 as a novel marker for the diagnosis of ectopic pregnancy. Fertil Steril. 2011;95(4):1373-8.

[49] Horne AW, Brown JK, Tong S, Kaitu'u-Lino T. Evaluation of ADAM-12 as a diagnostic biomarker of ectopic pregnancy in women with a pregnancy of unknown location. PLoS One. 2012;7(8):e41442.

[50] Yang J, Wu J, Guo F, Wang D, Chen K, Li J, Du L, Yin A. Maternal serum disintegrin and metalloprotease protein-12 in early pregnancy as a potential marker of adverse pregnancy outcomes. PLoS One. 2014;9(5):e97284.

[51] Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T. Identification of novel genes coding for small expressed RNAs. Science. 2001; 294:853–8.

[52] Lee RC, Ambros V. An extensive class of small RNAs in Caenorhabditis elegans. Science. 2001;294:862–4.

[53] Zhao Z, Zhao Q, Warrick J, Lockwood CM, Woodworth A, Moley KH, Gronowski AM. Circulating microRNA miR-323-3p as a biomarker of ectopic pregnancy. Clin Chem. 2012; 58:896–905.

[54] Takacs P, Jaramillo S, Datar R, Williams A, Olczyk J, Barnhart K. Placental mRNA in maternal plasma as a predictor of ectopic pregnancy. Int J Gynaecol Obstet. 2012;117:131–3.

[55] Lo YM, Tsui NB, Chiu RW, Lau TK, Leung TN, Heung MM, Gerovassili A et al. Plasma placental RNA allelic ratio permits noninvasive prenatal chromosomal aneuploidy detection. Nat Med.2007;13:218–23.

[56] Ng EK, Tsui NB, Lau TK, Leung TN, Chiu RW, Panesar NS, Lit LC et al. mRNA of placental origin is readily detectable in maternal plasma. Proc Natl Acad Sci U S A. 2003;100:4748–53.

[57] Jones EA, Clement-Jones M, James OF & Wilson DI. Differences between human and mouse alpha-fetoprotein expression during early development. Journal of Anatomy 2001;198:555–559.

[58] Grosskinsky CM, Hage ML, Tyrey L, Christakos AC & Hughes CL. hCG, progesterone, alpha-fetoprotein, and estradiol in the identification of ectopic pregnancy. Obstetrics and Gynecology 1993; 81:705–709.

[59] Kuscu E, Vicdan K, Turhan NO, Oguz S, Zorlu G &Gokmen O. The hormonal profile in ectopic pregnancies. Materia Medica Polona 1993; 25:149–152.

[60] Lazar L, Nagy B, Ban Z, Nagy GR & Papp Z. Presence of cell-free fetal DNA in plasma of women with ectopic pregnancies. Clinical Chemistry 2006;52: 1599–1601.

[61] Arck P, Hansen PJ, Mulac Jericevic B, Piccinni MP, Szekeres-Bartho J. Progesterone during pregnancy: endocrine-immune cross talk in mammalian species and the role of stress. Am J Reprod Immunol. 2007; 58(3):268-79.

[62] Hinshaw K, Fayyad A, Munjuluri P. The management of early pregnancy loss. Royal College of Obstetricians and Gynaecologists, 2006. (Green-top guideline No 25).

[63] Verhaegen J, Gallos ID, van Mello NM, Abdel-Aziz M, Takwoingi Y, Harb H, Deeks JJ, Mol BWJ, Coomarasamy A. Accuracy of single progesterone test to predict early pregnancy outcome in women with pain or bleeding: meta-analysis of cohort studies. BMJ 2012;345:e6077.

[64] Mol BW, Lijmer JG, Ankum WM, van der Veen F, Bossuyt PM. The accuracy of single serum progesterone measurement in the diagnosis of ectopic pregnancy: a meta-analysis. Hum Reprod. 1998; 13:3220–7.

[65] Guillaume J, Benjamin F, Sicuranza BJ, Deutsch S, Seltzer VL &Tores W. Serum estradiol as an aid in the diagnosis of ectopic pregnancy. Obstetrics and Gynecology 1990;76: 1126–1129.

[66] Mantzavinos T, Phocas I, Chrelias H, Sarandakou A&Zourlas PA.Serum levels of steroid and placental protein hormones in ectopic pregnancy. European Journal of Obstetrics, Gynecology, and Reproductive Biology 1991;39:117–122.

[67] Treetampinich C, O'Connor AE, MacLachlan V, Groome NP, de Kretser DM. Maternal serum inhibin A concentrations in early pregnancy after IVF and embryo transfer reflect the corpus luteum contribution and pregnancy outcome. Hum Reprod. 2000; 15:2028–32.

[68] Seifer DB, Lambert-Messerlian GM, Canick JA, Frishman GN, Schneyer AL. Serum inhibin levels are lower in ectopic than intrauterine spontaneously conceived pregnancies. Fertil Steril. 1996;65:667–9.

[69] Segal S, Gor H, Correa N, Mercado R, Veenstra K, Rivnay B. Inhibin A: marker for diagnosis of ectopic and early abnormal pregnancies. Reprod Biomed Online. 2008; 17:789–94.

[70] Chetty M, Sawyer E, Dew T, Chapman AJ, Elson J. The use of novel biochemical markers in predicting spontaneously resolving 'pregnancies of unknown location'. Hum Reprod. 2011;26:1318–23.

[71] Weiss G, O'Byrne EM &Steinetz BG. Relaxin: a product of the human corpus luteum in pregnancy. Science 1976;194:948–949.

[72] Garcia A, Skurnick JH, Goldsmith LT, Emmi A & Weiss G. Human chorionic gona-dotropin and relaxin concentrations in early ectopic and normal pregnancies. Obstet-rics and Gynecology 1990;75:779–783.

[73] Sealey JE, Cholst I, Glorioso N, Troffa C, Weintraub ID, James G &Laragh JH. Se-quential changes in plasma luteinising hormone and plasma prorenin during the menstrual cycle. Journal of Clinical Endocrinology and Metabolism1987;65: 1.

[74] Meunier K, Mignot TM, Maria B, Guichard A, Zorn JR &Cedard L. Predictive value of renin assay for the diagnosis of ectopic pregnancy. Fertility and Sterility 1991;55:432–435.

[75] Carmeliet P, Ferreira V, Breier G, Pollefeyt S, Kieckens L, Gertsenstein M, Fahriq M et al. Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. Nature 1996; 380:435–9.

[76] Cabar FR, Pereira PP, Schultz R, Francisco RP, Zugaib M. Vascular endothelial growth factor and β-human chorionic gonadotropin are associated with trophoblas-tic invasion into the tubal wall in ectopic pregnancy. Fertil Steril. 2010;94(5):1595-600.

[77] Daniel Y, Geva E, Lerner-Geva L, Eshed-Englender T, Gamzu R, Lessing JB, et al. Levels of vascular endothelial growth factor are elevated in patients with ectopic pregnancy: is this a novel marker? Fertil Steril. 1999; 72:1013–7.

[78] Felemban A, Sammour A, Tulandi T. Serum vascular endothelial growth factor as a possible marker for early ectopic pregnancy. Hum Reprod. 2002; 17:490–2.

[79] Fernandes da Silva MO, Elito J Jr, Daher S, Camano L, Fernandes Moron A. Associa-tion of serum levels of vascular endothelial growth factor and early ectopic pregnan-cy. Clin Exp Obstet Gynecol. 2013;40(4):489-91.

[80] Plaisier M, Rodrigues S, Willems F, Koolwijk P, van Hinsbergh VW, Helmerhorst FM. Different degrees of vascularization and their relationship to the expression of vascular endothelial growth factor, placental growth factor, angiopoietins, and their receptors in first-trimester decidual tissues. Fertil Steril. 2007; 88(1):176-87.

[81] Horne AW, Shaw JL, Murdoch A, McDonald SE, Williams AR, Jabbour HN, Duncan WC, Critchley HO. Placental growth factor: a promising diagnostic biomarker for tu-bal ectopic pregnancy. J Clin Endocrinol Metab. 2011; 96(1):E104-8.

[82] Daponte A, Pournaras S, Polyzos NP, Tsezou A, Skentou H, Anastasiadou F, Lialios G, Messinis IE. Soluble FMS-like tyrosine kinase-1 (sFlt-1) and serum placental growth factor (PlGF) as biomarkers for ectopic pregnancy and missed abortion. J Clin Endocrinol Metab. 2011; 96(9):E1444-51.

[83] Martínez-Ruiz A, Sarabia-Meseguer MD, Pérez-Fornieles J, Vílchez JA, Tovar-Zapata I, Noguera-Velasco JA. Placental growth factor, soluble fms-like tyrosine kinase 1 and progesterone as diagnostic biomarkers for ectopic pregnancy and missed abor-tion. Clin Biochem. 2014 ;47(9):844-7.

[84] Woolnough C, Wang Y, Kan CY, Morris JM, Tasevski V, Ashton AW. Source of angiopoietin-2 in the sera of women during pregnancy. Microvasc Res 2012;84: 367–374.

[85] Seval Y, Sati L, Celik-Ozenci C, Taskin O, Demir R. The distribution of angiopoietin-1, angiopoietin-2 and their receptors tie-1 and tie-2 in the very early human placenta. Placenta 2008;29: 809–815.

[86] Daponte A, Deligeoroglou E, Pournaras S, Tsezou A, Garas A, Anastasiadou F, Hadjichristodoulou C, Messinis IE. Angiopoietin-1 and angiopoietin-2 as serum biomarkers for ectopic pregnancy and missed abortion: a case-control study. Clin Chim Acta. 2013;415:145-51.

[87] Schneuer FJ, Roberts CL, Ashton AW, Guilbert C, Tasevski V, Morris JM, Nassar N. Angiopoietin 1 and 2 serum concentrations in first trimester of pregnancy as biomarkers of adverse pregnancy outcomes. Am J Obstet Gynecol. 2014;210(4):345.e1-9.

[88] Senturk LM, Arici A. Leukemia inhibitory factor in human reproduction. American journal of Reproductive Immunology 1998;39:137-143.

[89] Wegener NT, Mershon JL. Evaluation of leukemia inhibitory factors as a marker of ectopic pregnancy. American journal of Obstetrics and Gynaecology 2001;184:1074-76.

[90] Iyibozkurt AC, Kalelioglu I, Gursoy S, Corbacioglu A, Gurelpolat N, Karahan GE, Saygili H, Bengisu E. Evaluation of serum levels of IL-10, IL-11 and LIF in differentiation of eutopic and tubal ectopic pregnancy. Clin Exp Obstet Gynecol 2010;37(3): 217-20.

[91] Guney M, Erdemoglu E, Oral B, Karahan N, Mungan T. Leukemia inhibitory factor is immunohistochemically localized in tubal ectopic pregnancy. Acta Histochem 2008;110(4):319-23.

[92] Ji YF, Chen LY, Xu KH, Yao JF, Shi YF. Locally elevated leukemia inhibitory factor in the inflamed fallopian tube resembles that found in tubal pregnancy. Fertil Steril 2009;91(6):2308-14.

[93] Ruge S, Sorensen S, Vegtorp M, Vejerslev LO. The secretory endometrial protein, placental protein 14, in women with ectopic gestation. Fertility &Sterlity 1992;57:102-6.

[94] Vigne JL, Hornung D, Mueller MD, Taylor RN. Purification and characterization of an immunomodulatory endometrial protein glycodelin. J Biol Chem 2001;276:17101-5.

[95] Foth D, Romer T. Glycodelin serum levels in women with ectopic pregnancy. Eur J Obstet Gynecol Reprod Biol 2003;108(2):199-202.

[96] Aplin JD, Hey NA. MUC1, endometrium and embryo implantation. Biochem Soc Trans 1995;23:826-31.

[97] Savaris RF, da Silva LC, Moraes Gda S, Edelweiss MI. Expression of MUC1 in tubal pregnancy. Fertil Steril 2008;89(4):1015-17.

[98] Refaat B, Simpson H, Britton E, Biswas J, Wells M, Aplin JD, Ledger W. Why does the fallopian tube fail in ectopic pregnancy? The role of activins, inducible nitric oxide synthase and MUC 1 in ectopic implantation. Fertil Steril 2012;97(5):1115-23.

[99] Liao SB, Li HW, Ho JC, Yeung WS, Ng EH, Cheung AN, Tang F, O WS. Possible role of adrenomedullin in the pathogenesis of tubal ectopic pregnancy. J Clin Endocrinol Metab 2012;97(6):2105-12.

[100] O WS, Li HW, Liao SB, Cheung AN, Ng EH, Yeung WS, Ho JC, Tang F. Decreases in adrenomedullin expression and ciliary beat frequency in the nasal epithelium in tubal pregnancy. Fertil Steril. 2013;100(2):459-63.e1.

[101] Luisi S, Florio P, Reis FM, Petraglia F. Expression and secretion of activin A: Possible physiological and clinical implications. Eur J Endocrinol. 2001;145:225–36.

[102] Jones RL, Salamonsen LA, Zhao YC, Ethier JF, Drummond AE, Findlay JK. Expression of activin receptors, follistatin and betaglycan by human endometrial stromal cells; consistent with a role for activins during decidualization. Mol Hum Reprod 2002; 8:363–374.

[103] Horne AW, van den Driesche S, King AE, Burgess S, Myers M, Ludlow H, Lourenco P, Ghazal P, Williams AR, Critchley HO, Duncan WC. Endometrial inhibin/activin beta-B subunit expression is related to decidualization and is reduced in tubal ectopic pregnancy. J Clin Endocrinol Metab. 2008;93(6):2375-82.

[104] Lavie O, Beller U, Neuman M, Ben-Chetrit A, Gottcshalk-Sabag S and Diamant Y. Maternal serum CK: a possible predictor of tubal pregnancy. American Journal of Obstetrics and Gynaecology 1993;169:1149-50.

[105] Birkhahn RH, Gaeta TJ, Leo PJ, Bove JJ. The utility of maternal creatine kinase in the evaluation of ectopic pregnancy. Am J Emerg Med. 2000;18:695–7.

[106] Duncan WC, Sweeting VM, Cawood P, Illingworth PJ. Measurement of creatine kinase activity and diagnosis of ectopic pregnancy. Br J Obstet Gynaecol. 1995;102:233–7.

[107] Soundravally R, Krishna Latha T, Soundara Raghavan S, Ananthanarayanan PH, Srilatha K. Diagnostic significance of total creatine kinase and its isoform in tubal ectopic pregnancy. J Obstet Gynaecol Res. 2013;39(12):1587-91.

[108] Korhonen J, Alfthan H, Stenman UH, Ylostalo P. Failure of creatine kinase to predict ectopic pregnancy. Fertil Steril. 1996;65:922–4.

[109] Lincoln SR, Dockery JR, Long CA, Rock WA, Jr, Cowan BD. Maternal serum creatine kinase does not predict tubal pregnancy. J Assist Reprod Genet. 1996;13:702–4.

[110] Plewa MC, Ledrick D, Buderer NF, King RW. Serum creatine kinase is an unreliable predictor of ectopic pregnancy. Acad Emerg Med. 1998;5:300–3.

[111] Qasim SM, Trias A, Sachdev R, Kemmann E. Evaluation of serum creatine kinase levels in ectopic pregnancy. Fertil Steril. 1996;65:443–5.

[112] Vandermolen DT, Borzelleca JF. Serum creatine kinase does not predict ectopic pregnancy. Fertil Steril. 1996;65:916–21.

[113] Safdarian L, Aghahosseini M, Alleyassin A, Kohbodi M. Evaluation of Plasma Creatine Phosphokinase (CPK) Level Following a Single Injection of Methotrexate as a Predicator of Treatment Success in Ectopic Pregnancy. J Family Reprod Health. 2013;7(4):151-5.

[114] Birkhahn RH, Gaeta TJ, Paraschiv D, Bove JJ, Suzuki T, Katoh H, Nagai R. Serum levels of myoglobin, creatine phosphokinase, and smooth muscle heavy-chain myosin in patients with ectopic pregnancy. Ann Emerg Med. 2001; 38(6):628-32.

[115] Soriano D, Hugol D, Quang NT, Darai E. Serum concentrations of interleukin-2R (IL-2R), IL-6, IL-8, and tumor necrosis factor alpha in patients with ectopic pregnancy. Fertil Steril. 2003; 79(4):975-80.

[116] Daponte A, Deligeoroglou E, Pournaras S, Hadjichristodoulou C, Garas A, Anastasiadou F, Messinis IE. Interleukin-15 (IL-15) and anti-C1q antibodies as serum biomarkers for ectopic pregnancy and missed abortion. Clin Dev Immunol. 2013;2013:637513.

[117] Sadovsky Y, Pineda J, Collins JL. Serum CA-125 levels in women with ectopic and intrauterine pregnancies. J Reprod Med. 1999; 36(12):875-8.

[118] Brumsted JR, Nakajima ST, Badger G, Riddick DH, Gibson M. Serum concentration of CA-125 during the first trimester of normal and abnormal pregnancies. J Reprod Med. 1990; 35(5):499-502.

[119] Condous G, Kirk E, Syed A, Van Calster B, Van Huffel S, Timmerman D, et al. Do levels of serum cancer antigen 125 and creatine kinase predict the outcome in pregnancies of unknown location? Hum Reprod. 2005;20:3348–54.

[120] Kuscu E, Vicdan K, Turhan NO, Oguz S, Zorlu G, Gokmen O. The hormonal profile in ectopic pregnancies. Mater Med Pol. 1993;25:149–52.

[121] Schmidt T, Rein DT, Foth D, Eibach HW, Kurbacher CM, Mallmann P, et al. Prognostic value of repeated serum CA 125 measurements in first trimester pregnancy. Eur J Obstet Gynecol Reprod Biol. 2001;97:168–73.

[122] Katsikis I, Rousso D, Farmakiotis D, Kourtis A, Diamanti-Kandarakis E, Panidis D. Receiver operator characteristics and diagnostic value of progesterone and CA-125 in the prediction of ectopic and abortive intrauterine gestations. Eur J Obstet Gynecol Reprod Biol. 2006;125(2):226-32.

[123] Girardi G, Prohászka Z, Bulla R, Tedesco F, Scherjon S. Complement activation in animal and human pregnancies as a model for immunological recognition. Mol Immunol. 2011; 48(14):1621-30.

[124] Agostinis C, Bulla R, Tripodo C, Gismondi A, Stabile H, Bossi F, Guarnotta C, Garlanda C, De Seta F, Spessotto P, Santoni A, Ghebrehiwet B, Girardi G, Tedesco F. An alternative role of C1q in cell migration and tissue remodeling: contribution to trophoblast invasion and placental development. J Immunol. 2010; 185(7):4420-9.

[125] Echan LA, Tang HY, Ali-Khan N, Lee K, Speicher DW. Depletion of multiple high-abundance proteins improves protein profiling capacities of human serum and plasma. Proteomics. 2005;5:3292–303.

[126] Boschetti E, Righetti PG. The ProteoMiner in the proteomic arena: a non-depleting tool for discovering low-abundance species. J Proteomics. 2008;71:255–64.

[127] Gebeh AK, Willets JM, Marczylo EL, Taylor AH, Konje JC. Ectopic Pregnancy Is Associated with High Anandamide Levels and Aberrant Expression of FAAH and CB1 in Fallopian Tubes. J Clin Endocrinol Metab. 2012;97:2827–35.

[128] Horne AW, Phillips JA, 3rd, Kane N, Lourenco PC, McDonald SE, Williams AR, et al. CB1 expression is attenuated in Fallopian tube and decidua of women with ectopic pregnancy. PLoS One. 2008;3:e3969.

[129] Brown JK, Lauer KB, Ironmonger EL, Inglis NF, Bourne TH, Critchley HO, Horne AW. Shotgun proteomics identifies serum fibronectin as a candidate diagnostic biomarker for inclusion in future multiplex tests for ectopic pregnancy. PLoS One. 2013;8(6):e66974.

[130] Skubisz MM, Brown JK, Tong S, Kaitu'u-Lino T, Horne AW. Maternal Serum Macrophage Inhibitory Cytokine-1 as a Biomarker for Ectopic Pregnancy in Women with a Pregnancy of Unknown Location. PLoS One. 2013;8(6):e66339.

[131] Beer LA, Tang HY, Sriswasdi S, Barnhart KT, Speicher DW. Systematic discovery of ectopic pregnancy serum biomarkers using 3-D protein profiling coupled with label-free quantitation. J Proteome Res. 2011; 10(3):1126-38.

[132] Feng C, Chen ZY, Zhang J, Xu H, Zhang XM, Huang XF. Clinical utility of serum reproductive hormones for the early diagnosis of ectopic pregnancy in the first trimester. J Obstet Gynaecol Res. 2013 ;39(2):528-35.

[133] Zou S, Li X, Feng Y, Sun S, Li J, Egecioglu E, Billig H, Shao R. Comparison of the diagnostic values of circulating steroid hormones, VEGF-A, PIGF, and ADAM12 in women with ectopic pregnancy. J Transl Med. 2013;11:44.