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SERUM BIOMARKERS FOR DETECTING ECTOPIC PREGNANCY

Mary E Rausch, MD MSCE[1] and **Kurt Barnhart, MD MSCE**[2]

[1]Department of Obstetrics and Gynecology, Center for Human Reproduction, North Shore University Hospital, Manhasset, NY

[2] Department of Obstetrics and Gynecology, Penn Fertility Care, Perelman School of Medicine, University of Pennsylvania, Philadelphia PA.

Abstract

Unless an ectopic pregnancy is visible by ultrasound, diagnosis can be a challenge. Differentiating ectopic pregnancies from intrauterine pregnancies can be impossible without intervention or follow-up. This poses a clinical dilemma to the practitioner given the inherent danger to the mother of tubal rupture of an ectopic pregnancy versus the fear of intervening in the case of a desired pregnancy without certainty of diagnosis. Early diagnostic modalities are clearly lacking, and serum biomarkers are currently being investigated as a solution to need for a rapid and accurate test for ectopic pregnancy.

Keywords

ectopic pregnancy; serum test; biomarkers; proteomics; multiple markers

Introduction

Ectopic pregnancy (EP) is a major cause of maternal morbidity and is responsible for pregnancy-related deaths in the first trimester.[1] Diagnosing an EP is a challenge to the clinician because there is no definitive non-surgical diagnostic test when the diagnosis is unclear by routine blood tests and ultrasound, and diagnosis often requires following patients over multiple visits. A rapid and accurate serum test to detect the presence of an EP would permit early treatments to prevent mortality and morbidity of this condition with preservation of fallopian tube function and fertility.[1] Currently, research is underway to both identify novel biomarkers and combine new and existing markers into a multiple marker test with the goal of accurately identifying ectopic pregnancies. The following discussion will describe the current use of biomarkers in clinical practice and the present state of serum biomarker research, including the markers being investigated and the methods which are being used to discover novel candidates.

Current use of serum biomarkers for the diagnosis of ectopic pregnancy

A serum biomarker is a molecule that an affected individual produces that indicates the diseased state and is detectable in the serum. A biomarker for EP will ideally allow early

Correspondence: Dr Mary E. Rausch Center for Human Reproduction North Shore University Hospital 300 Community Drive Manhasset, NY 11030 Mrausch@nshs.edu Fax: 516-562-1710 Telephone: 516-562-2229.

diagnosis or predict prognosis. [2] Currently, the only biomarker used routinely in clinical practice is human chorionic gonadotropion (hCG), but it is not diagnostic and can only assist diagnosis in combination with ultrasound use. A single value is useful clinically in determining whether a gestational sac should reliably be visible on ultrasound (the "discriminatory zone") in an intrauterine pregnancy. Below this level, following serial hCG levels can help to distinguish a viable intrauterine pregnancy from an EP or nonviable intrauterine pregnancy. However, even observing serial levels has limitations in that the expected minimum rate of rise in 48 hours in a viable pregnancy varies in reports from 35% to 66%. [3] Given that one value of hCG below the discriminatory zone is nondiagnostic, clinicians must also follow patients over several days to a week, which increases the risk of tubal rupture and life-threatening hemorrhage.[1] Despite its limitations, serial hCG levels in combination with transvaginal ultrasound is the most commonly used clinical method for determining which patients are at highest risk for EP and warrant surgical or medical treatment.

Progesterone has also been studied extensively and has been used in some clinical centers as an adjunct to transvaginal ultrasound and hCG levels. Early in pregnancy prior to placental production, progesterone is secreted by the corpus luteum and is a critical hormone for the establishment of normal pregnancy.[4] A systematic review of progesterone as a serum marker for EP found that a single value did have good discrimination for a nonviable pregnancy, and only 0.3% of patients with a viable intrauterine pregnancy in the combined studies had serum progesterone value less than 5ng/ml. [5] However, a low value could not discriminate between a nonviable intrauterine pregnancy and an EP.[5] Further, in the studies examined, 2.6% of patients with a serum progesterone level greater than 20ng/ml had an EP, and high values do not definitively rule out an EP. [5] In summary, a low single serum progesterone level can aid in identifying patients at higher risk for an EP who need to be followed vigilantly, but a high value should be interpreted cautiously given the continued possibility of an EP.

Phases of biomarker research

Despite the important clinical need for biomarkers of EP, the currently used biomarkers are limited. Research is currently underway to identify and develop novel biomarkers which may have improved diagnostic accuracy. This discovery process has distinct phases: 1) preclinical exploration to identify promising markers, 2) establishment of a clinical assay to be used on a larger scale, 3) testing the utility of the biomarker typically with a longitudinal or retrospective cohort, and 4) validation of the marker to determine is clinical value, usually in a prospective screening.[2, 6-7] Although the preliminary stages have been pursued for the diagnosis of EP and are described in the following sections, no studies have progressed to Phase IV.

Proposed biomarkers for ectopic pregnancy

A number of EP biomarkers have been proposed, although with limited validation (Table 1).[3, 8-9] Biomarkers have been evaluated based on the different biological functions theorized to be altered in the abnormal growth of an EP in the fallopian tube. These include 1) markers of abnormal trophoblast, corpus luteum and endometrial function, 2) markers of implantation and growth in the fallopian tube, such as angiogenesis and muscle cell damage, and 3) inflammatory markers.

Once a pregnancy is established, the rise of human chorionic gonadotropin (hCG) produced by the trophoblasts is one indicator of the viability of the pregnancy. Trophoblasts also produce a number of other proteins which have also been examined for the ability to differentiate normal versus abnormal pregnancies. Such proteins include activin A,

pregnancy-specific beta-1-glycoprotein (SP1), pregnancy-associated plasma protein-A (PAPP-A), and human placental lactogen (hPL).[3, 9-11] In addition, the corpus luteum is maintained in pregnancy by the trophoblast secretion of hCG, and normal corpus luteal function is necessary for the continued progression of a pregnancy. Given the abnormal hCG dynamics of an EP, researchers have looked to an alteration in luteal proteins as a possible marker of EP, including not only progesterone, but inhibin A, estradiol, relaxin, and renin.[3, 9-11] Endometrial proteins, such as glycodelin, activin B, leukemia inhibitory factor, which are released into the maternal circulation with normal implantation have also been studied.[3, 9-11] As expected, these proteins which are involved in the normal implantation and progression of pregnancy are frequently found to be decreased in ectopic pregnancies, but as a single marker, they do dot demonstrate consistently good discriminatory values. [3, 9-11]

The markers which reflect the viability of a pregnancy may be higher in normal intrauterine pregnancies, but may not differentiate an abnormal pregnancy in the uterus (miscarriage) versus an abnormal pregnancy in the fallopian tube (EP). Markers reflecting the location of the pregnancy, rather than viability, may therefore be able to differentiate between the two types of nonviable conceptuses: a miscarriage and an EP. One such marker is that of smooth muscle damage. As the EP grows and invades the muscular layer of the fallopian tube, it is possible that markers of muscle cell damage may also rise. Myoglobin and smooth muscle heavy-chain myosin have been studied but were found not be useful in screening for EP.[12] Creatine kinase (CK) has been studied extensively, and is often statistically elevated in ectopic pregnancies, especially if ruptured, but with overall poor discriminatory values. [3, 9-11]In contrast to implantation in the well-vascularized endometrium, tubal implantation may involve other factors that are induced under hypoxic conditions and vascular endothelial growth factor (VEGF), a marker of angiogenesis, has also been examined.[13] There is increased expression of VEGF and its receptor at the implantation site of an EP, suggesting it may be involved in the implantation of the pregnancy in the fallopian tube.[14] Serum VEGF and beta-HCG levels also positively correlate with the depth of trophoblastic penetration into the wall of the oviduct.[13] Indeed, VEGF levels have been found to elevated in EP, but as a single marker, do not have adequate discrimination.[3,9-11]

Markers of inflammation and peritoneal irritation, such as interleukin-8, interleukin-6, tumor necrosis factor-alpha, CA-125, have also been investigated with conflicting results and no consistent proven utility for discrimination of EP. [3, 9-11]

Biomarkers used in combination

Given the ramifications of a false positive or negative test, namely interruption of a desired, normal pregnancy or serious morbidity and possibly mortality, respectively, only a test with superior sensitivity and specificity would be clinically applicable. As none of the currently discovered biomarkers has consistently differentiated ectopic pregnancies, several researchers have attempted to combine several markers into one test with better diagnostics than individual proteins (Table 2).

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%. [15] Combining markers with multiple biologic functions has proven more successful. One 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.[16]

More recently, 12 markers previously associated in the literature with EP that spanned a number of possible mechanisms were assessed individually and in combination. As single markers, inhibin A, progesterone, activin A, VEGF, SP1, and PAPP-A were differentially expressed in patients with EP and IUP ($p < 0.0001$) with fair diagnostic properties (AUC >

0.6). TNF-alpha, IL-6, IL-8, glycodelin, CK, and hPL had limited value.[9] A diagnostic algorithm was developed to maximize both sensitivity and specificity with four markers (progesterone, VEGF, inhibin A, activin A) which achieved 100% specificity and 98% (93-100%) sensitivity, only in those that could be characterized (42% of the sample).[9] Overall, a single EP was misclassified which corresponds with 99% (96-100%) accuracy.[9] Of interest was that these models demonstrated perfect discrimination in the subgroup of patients where ultrasound is usually non-diagnostic.[9] Although they demonstrate promising results, such multiple marker tests need external validation before they can be put into practice.

Discovery of novel biomarkers

Testing for differences between normal and abnormal pregnancy based on a hypothesis that any one maker may be beneficial is the traditional method to search for new markers. More recently, genomics [17-18] and proteomics [19-20] have been utilized for a more unbiased approach to biomarker discovery. Quantitatively comparing the proteome of biological fluids such as serum from patients and normal controls has great potential for detecting novel biomarkers not dependent on the imagination and hypotheses of the researchers. However, such studies are very challenging due to the high complexity of the serum proteomes, a wide protein abundance range with very low concentrations of most clinically useful biomarkers, patient-to-patient variability, and potential variations in sample collection and processing.

A recent study that screened the proteome of a small group of women with EP and controls re-identified several proteins previously associated with EP with either high (CGB and CGA) or low (PAPPA, CSH1, and PAEP) significance, confirming their possible utility as biomarkers for EP. [19] However, this unbiased approach also discovered potential novel biomarkers, including ADAM-12 and ISM2 (Isthmin 2) as well as five specific isoforms of the pregnancy-specific beta-1-glycoprotein family multiple proteins.[19]

One of the most promising, novel candidate biomarkers was ADAM-12 (a disintegrin and metalloprotease-12). It was selected for further validation testing in a larger group of 199 patients using a commercial DELFIA assay which showed significantly lower levels of ADAM-12 in the EP group [mean 11.7ng/ml±48.2] compared to the IUP group [mean 115.4ng/ml±214.1; $p < 0.0001$)] with good discrimination between the groups (AUC=0.82). [21] The discoveries of novel biomarkers, such as these, may improve the diagnostic ability of existing multiple marker tests even if they do not have sufficient diagnostic capability as single markers.

Future directions

Given the present lack of a clinically useful test for the accurate diagnosis of EP, there are several goals for the future biomarker research if a robust and validated test is to be obtained. Identifying novel candidates via unbiased techniques is currently underway, and as the list of potential candidates grows, such biomarkers need to be triaged to select out those with the most promise. Multiple marker tests, which can take advantage of different biologic mechanisms of a panel of biomarkers rather than a single protein, will more likely be able to differentiate amongst normal and abnormal pregnancies. The final key step is the validation of candidates in independent cohorts, which is lacking in all of the studies up to date. Further, the best clinical use of such a test needs to be clearly defined and honed as they are being developed. For instance, should such tests be optimized for detecting EP from intrauterine pregnancies, distinguishing a nonviable pregnancy (EP or miscarriage) from a normal, viable pregnancy, or assist in prognosis (determine the most threatening pregnancies

at risk of rupture). A test developed with any of these goals could be of significant assistance to clinicians in deciding how to triage and treat their patients.

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Table 1

Proposed markers for ectopic pregnancy based on biologic function

Abormal implantation	
Trophoblast function	human chorionic gonadotropin (hCG)
	Activin A
	Pregnancy-specific beta-1-glycoprotein (SP1)
	Pregnancy-associated plasma protein-A (PAPP-A)
	Human placental lactogen (hPL)
Corpus luteal function	Progesterone
	Inhibin A
	Estradiol
	Relaxin
	Renin
Endometrial function	Glycodelin
	Activin B
	Leukemia inhibitory factor
Growth in fallopian tube	
Angiogenesis	Vascular endothelial growth factor (VEGF)
Muscle cell damage	Myoglobin
	Smooth muscle heavy-chain myosin \ Creatine kinase (CK)
Inflammation and peritoneal irritation	Interleukin-8
	Interleukin-6
	Tumor necrosis factor-alpha
	CA-125

Table 2

Diagnostic properties of multiple marker tests in predicting ectopic pregnancy

Source	Biomarkers	Sensitivity	Specificity
Soriano et al 2003[15]	IL-6, IL-8, and TNF-alpha	52.9%	100%
Mueller et al 2004[16]	[VEGF/(PAPP-A X P)]	97.7%	92.4%
Rausch et al 2011[9]	progesterone, VEGF, inhibin A, activin A	98%	100%*

* only in those that could be characterized (42% of the sample)