Diagnostic Biomarkers for Predicting Adverse Early Pregnancy Outcomes

1. Background

The World Health Organization defines a biomarker as ‘any substance, structure or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease’. In early pregnancy, the most commonly used biomarkers to predict outcome have been maternal serum human chorionic gonadotrophin (hCG) and progesterone.

Transvaginal scanning (TVS) has revolutionised the diagnosis of early pregnancy complications and is now considered the diagnostic test of choice. However, ultrasound imaging is operator-dependent and the quality of the diagnosis depends on their skill and experience. A biomarker that helps accurately determine the location or viability of an early gestation could be used to reduce the clinical burden of ‘pregnancy of unknown location’ (PUL) cases. The term ‘PUL’ describes a clinically stable woman who presents with a positive pregnancy test, but no TVS evidence of intra- or extraterine pregnancy. A biomarker may also distinguish those women who need to be treated urgently, either surgically, medically or expectantly. The ideal biomarker should be consistent, accurate, inexpensive, and could be used at the point of care.

Biomarker development for clinical use is generally divided into four phases:

1. preclinical exploration,
2. clinical assay development,
3. assessment of predictive ability in a retrospective study, and
4. validation in a prospective setting.

Despite extensive research, the role of several novel biomarkers for the diagnosis of early pregnancy complications has been explored with mixed results.

This Scientific Impact Paper will discuss the controversies surrounding the current use of biomarkers and their potential future uses.

2. Human chorionic gonadotrophin (hCG)

Maternal serum hCG and in particular its subunit β-hCG is the most widely available and used biomarker in routine clinical practice for the assessment of women with suspected early pregnancy complications. β-hCG level is directly related to the amount of active villous trophoblast and it doubles every 1.4–1.6 days from the time of first detection to day 35 of pregnancy, and then for every 2.0–2.7 days until day 42 of pregnancy. β-hCG levels are routinely measured in cases where the ultrasound findings are nondiagnostic. However, a single measurement of maternal serum β-hCG is of limited value due to the wide range of levels in normal early pregnancy. As a result, it has not been possible to define a cut-off level below which a miscarriage could be reliably diagnosed. It had been proposed that in women for whom an intrauterine pregnancy cannot be confirmed during TVS, a single measurement of serum β-hCG above 1000–2000 IU/l could be used to diagnose an ectopic pregnancy. However, it has since been shown that in as many as 78% of women with ectopic pregnancies visible on ultrasound, serum β-hCG values were below 1000 IU/l. By contrast, in a number of women with normal intrauterine pregnancies, the pregnancy could not be detected on ultrasound despite initial serum β-hCG levels...
greater than 1000 IU/l. This scenario is most likely to occur in women with multiple pregnancies. Noncritical adoption of β-hCG cut-off levels in these cases could lead to the unintended medical or surgical termination of wanted intrauterine pregnancies.

Serial β-hCG measurements are more useful. Slower doubling times of β-hCG levels have been shown to be associated with miscarriage. It has been established that in the majority of ectopic pregnancies, there is a suboptimal rise or fall (i.e. less than 66%) in β-hCG over 48 hours. However, in 15–20% of ectopic pregnancies and in 8% of miscarriages, the β-hCG profile mimics that of a viable intrauterine pregnancy. As a result, it is not possible to accurately determine the location and viability of pregnancy based on the pattern of β-hCG changes.

Serum β-hCG has been widely used in the management of ectopic pregnancy. The recent advances in ultrasound technology and the high sensitivity of the latest urine pregnancy tests have led to an increase in the diagnosis of small ectopic pregnancies that are usually self-limiting. As a result, expectant management has been advocated and serum β-hCG levels at initial presentation are used in patient selection, as well as in monitoring the progress until complete resolution. Similarly, in women who opt for medical management, and in those diagnosed with an ectopic pregnancy who have undergone a salpingotomy, serum β-hCG levels are used to monitor the reabsorption of any residual trophoblast.

Measurements of total and β-hCG in serum and urine have been extensively used since the 1970s in the follow-up of complete hydatiform moles after surgical evacuation. The gold standard for the diagnosis of a molar pregnancy is the presence of trophoblastic hyperplasia on histology. In the UK, women diagnosed with a complete or partial hydatidiform mole are registered with one of three regional centres for monitoring of hCG urine levels to screen for developing persisting gestational trophoblastic disease. Both complete and partial hydatidiform moles have been increasingly diagnosed with ultrasound in early pregnancy, with the median gestational age for diagnosis of complete mole falling from 12 to 9 weeks of gestation over the past two decades. The ultrasound diagnosis of complete moles is accurate, but the diagnosis of partial moles has always been more difficult as the hydatidiform changes are less pronounced and there is often a fetus or fetal remnants. The differential diagnosis between partial mole and missed miscarriage presenting with villous oedema, secondary to prolonged retention of the placenta tissue after embryonic demise, is particularly difficult as partial mole may not present with abnormally high maternal serum β-hCG.

### 3. Progesterone

Progesterone production in early pregnancy reflects the interaction between the trophoblast and corpus luteum. There is positive feedback between the rise in serum β-hCG and progesterone production by corpus luteum. It has been shown that the likelihood of a spontaneous pregnancy failure decreases with increasing maternal serum progesterone levels. A meta-analysis has shown that low serum progesterone is strongly associated with a failing pregnancy and can help to exclude a viable ongoing pregnancy. In particular, levels below 20 nmol/l have a high positive predictive value for the diagnosis of a failing pregnancy whereas levels over 60 nmol/l are ‘strongly’ associated with a viable pregnancy. As a result, the use of serum progesterone can reduce the number of follow-up visits and blood tests needed for women diagnosed with a PUL. Since the duration of administration of progesterone supplementation in IVF cycles can be variable, the use of progesterone assays may be influenced by exogenous progesterone administration and may therefore be unreliable.

### 4. Other biomarkers
Biomarkers that have been studied in early pregnancy can be categorised according to their biological origin.\textsuperscript{19}

**Fallopian tube dysfunction markers**

Markers include creatine kinase (CK), an enzyme released following muscle damage; myoglobin; smooth muscle heavy chain myosin; and adrenomedullin. CK is currently used in clinical practice to diagnose a myocardial infarction and it has been shown that serum CK concentrations are significantly higher in women with ectopic pregnancy compared with women with missed miscarriage or viable intrauterine pregnancy. However, the results of subsequent studies have been conflicting. Adrenomedullin is a peptide hormone thought to be involved in ciliary beat activity in the fallopian tube. Results from two small studies have shown that the plasma adrenomedullin levels are lower in women with ectopic pregnancy than in a viable intrauterine pregnancy, but further research is necessary.\textsuperscript{19}

**Abnormal embryo/trophoblast growth markers**

Markers include pregnancy-associated plasma protein A (PAPP-A); pregnancy-specific β-glycoprotein I (PSG-I or SP-I); human placent lactogen (HPL); activin A; and a disintegrin and metalloprotease-12 (ADAM-12). These markers are mainly produced by the trophoblast/placenta and their concentrations are lower in women with an ectopic pregnancy compared with a viable intrauterine pregnancy. However, these biomarkers are primarily produced after 7 weeks of gestation and their clinical applicability is limited.\textsuperscript{19}

**Abnormal corpus luteum function markers**

Oestradiol and inhibin A are produced by the corpus luteum in response to hCG, and serum concentrations are lower in women with an ectopic pregnancy. The suitability of these biomarkers has been questioned either due to considerable overlap in concentrations between groups or due to conflicting data.\textsuperscript{19}

**Inflammation markers**

The role of cancer antigen 125 (CA125) and several cytokines, such as interleukin (IL)-6, IL-8, IL-2 receptor and tumour necrosis factor-α (TNF-α), as markers of inflammation associated with ectopic pregnancy has been assessed, but the studies presented conflicting results regarding their potential clinical value.\textsuperscript{19}

**Uterine markers of abnormal implantation**

Lower serum concentrations of leukaemia inhibitory factor (LIF) and glycodelin are associated with the presence of an ectopic pregnancy; however, further research is needed to establish their clinical relevance.\textsuperscript{19}

**Abnormal angiogenic response markers**

Vascular endothelial growth factor (VEGF) is an angiogenic factor upregulated by tissue hypoxia and shown to play a vital role in implantation and placentation. Serum VEGF levels in women with ectopic pregnancy have been shown to be significantly higher compared with a viable intrauterine pregnancy; however, these results have not been replicated.\textsuperscript{19}
It is, therefore, evident from the above, that the novel biomarkers investigated at present are not particularly useful and much work is still to be done before a new biomarker could be used independently at clinical presentation.

5. Opinion

- Single measurement of β-hCG cannot be used to discriminate between intra- and extrauterine pregnancies.
- Serial β-hCG measurements can contribute to the management of women with PUL, and in planning and monitoring the management of women with ectopic pregnancies.
- Prospective studies on the use of hCG are needed to evaluate the incidence of hydatidiform mole in women presenting with missed miscarriage and who opt for a conservative management in which case histology may not be available.
- Single progesterone measurement is useful to identify women with PUL who are at low risk of complications and do not require follow-up.
- None of the novel biomarkers are accurate enough to be used in clinical practice for the diagnosis and management of early pregnancy complications.

References


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