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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'.[1] 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 extrauterine pregnancy.[2] 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 usable at the point of care.[3] A reliable biomarker should reduce not just the clinical burden, but the emotional burden of uncertainty in pregnancy for the woman and her family.

Biomarker development for clinical use is generally divided into four phases:[3]

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

Extensive research into several novel biomarkers has yielded mixed results in the diagnosis of early pregnancy complications. The biomarkers described in the last decade are not particularly useful and much work has still to be done before a new biomarker could be used independently at clinical presentation.

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

2. Human chorionic gonadotrophin (hCG)

Maternal serum hCG and in particular its subunit β-hCG is the most widely available biomarker used 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, which doubles every 1.4–1.6 days from the time of first detection to day 35 of pregnancy, and then every 2.0–2.7 days until day 42 of pregnancy.[4]

β-hCG levels are routinely measured in cases where the ultrasound findings are non-diagnostic. 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 indicative of an ectopic pregnancy.[5] 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.[6,7] 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.[6] 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 in diagnosis. Slower doubling times of β-hCG levels have been shown to be associated with miscarriage,[8] where it has been established that in 66% of ectopic pregnancies there is a suboptimal rise or fall 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.[9] As a result, it is not possible to determine accurately the location and viability of pregnancy based on changes in the pattern of β-hCG.

Serum β-hCG measurement is still widely used in the management of ectopic pregnancy. Recent advances in ultrasound technology and the high sensitivity of the latest urine pregnancy tests have led to an increase in the diagnosis of ectopic pregnancies at an earlier stage of development.[10] As a result, expectant management has been advocated and serum β-hCG levels at initial presentation are used in patient selection for expectant versus surgical management, as well as in monitoring progress until complete resolution.[10] 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 hCG or of its β-subunit (β-hCG) in maternal serum and/or urine have been extensively used since the 1970s in the follow-up of complete hydatiform moles after surgical evacuation.[11] 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 over the past two decades from 12 to 9 weeks of gestation.[12] 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.[13] 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 a partial mole may not present with abnormally high maternal serum β-hCG.[14] In the UK, women with a histologically-confirmed diagnosis of complete or partial hydatidiform mole are registered with one of three regional centres for monitoring of hCG urine levels to screen for the development of persisting gestational trophoblastic disease.[15]

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 the corpus luteum. It has been shown that the likelihood of a spontaneous pregnancy failure decreases with increasing maternal serum progesterone levels.[16-18] Overall, levels below 20 nmol/l (6 ng/mL) have a high positive predictive value for the diagnosis of a failing pregnancy whereas levels over 60 nmol/l (19 ng/mL) are 'strongly' associated with a viable pregnancy. 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.[16] In particular, in women with a spontaneous pregnancy, clinical symptoms (pain and/or bleeding) and inconclusive ultrasound examination, a serum progesterone level $\leq$ 6 ng/mL, predicts a non-viable pregnancy with a pooled sensitivity of 75.6%. As a result, a single serum progesterone measurement at the initial visit 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 examined in early pregnancy can be categorised according to their biological origin.[19]

Fallopian tube dysfunction markers

Markers include creatine kinase (CK), an enzyme released following muscle damage; myoglobin; smooth muscle heavy chain myosin; and adrenomedullin (ADM), a peptide hormone thought to be involved in ciliary beat activity in the fallopian tube.[19-22] CK was previously used in clinical practice to diagnose a myocardial infarction but it has also been shown that serum CK concentrations are significantly higher in women with ectopic pregnancy compared with women with missed miscarriage or viable intrauterine pregnancy.[19] However, the results of subsequent studies have been conflicting[20,21] and further research is necessary.

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 placental lactogen (HPL); activin A; A disintegrin; soluble vascular endothelial growth factor receptor 1 (sFlt-1); placental growth factor (PlGF); and metalloprotease-12 (ADAM-12).[20,21,23,24] These markers are mainly produced by the trophoblast/placenta and their concentrations are lower in women with an ectopic pregnancy or in those with a threatened miscarriage who will subsequently miscarry compared to those with a viable intrauterine pregnancy.[23,24] However, these biomarkers are primarily produced after 7 weeks of gestation and their clinical applicability is limited.[20] Very recently plasma concentrations of cell-free pregnancy-associated microRNAs (miRNAs) have been evaluated in ectopic pregnancy and found to show a different distribution pattern compared to viable intrauterine pregnancy.[25,26]

Abnormal corpus luteum function markers

Estradiol and inhibin A are produced by the corpus luteum in response to hCG, and serum concentrations are lower in women with an ectopic pregnancy. However, the suitability of these biomarkers has been questioned due to considerable overlap in concentrations between groups and conflicting data.[20]

Inflammation markers

The use 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.[20] In threatened miscarriage, maternal serum CA125 has high predictive value in identifying pregnancies that are 'likely to continue' compared with hCG and progesterone,[27] whereas no difference is found in high-sensitivity C-reactive protein (HSCRP) levels.[28]

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.[20]

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.[20]

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 care of women with PUL, and in planning and monitoring the management of women with ectopic or molar pregnancies.
- Prospective studies on the use of hCG are needed to evaluate the incidence of complete or partial hydatidiform mole in women presenting with missed miscarriage to allow them to opt for a conservative management if a hydatidiform mole is unlikely, since histology may not be available in such cases.
- Single progesterone measurement is useful to identify women with PUL who are at low risk of complications and therefore may not require a close follow-up.
- None of the novel biomarkers are sufficiently accurate to be used in clinical practice for the diagnosis and management of early pregnancy complications.

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