Biomarkers for ectopic pregnancy and pregnancy of unknown location

Andrew Horne
Outline

• Clinical and economic need
• Existing candidates and methodological approaches
• Challenges and future directions
Clinical problem

• 1-2% of pregnancies are ectopic
• Rupture – potentially life-threatening intra-abdominal haemorrhage and impaired future fertility
• 9-13% maternal deaths in Western world (10-30% in developing countries)
• Leading cause of morbidity in pregnancy worldwide

Jurkovic & Wilkinson BMJ 2011
Pain and bleeding
+ve urinary pregnancy test
Pain and bleeding
+ve urinary pregnancy test

TV USS
Pain and bleeding +ve urinary pregnancy test → TV USS → Viable intrauterine pregnancy
Pain and bleeding +ve urinary pregnancy test → TV USS

- Viable intrauterine pregnancy
- Non-viable intrauterine pregnancy (miscarriage)
Pain and bleeding
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- Ectopic pregnancy
Pain and bleeding +ve urinary pregnancy test → TV USS →

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- Pregnancy of unknown location (PUL)
- Ectopic pregnancy
Definitions

Definite Ectopic Pregnancy
Extraterine gestational sac with yolk sac and/or embryo (with or without cardiac activity)

Probable Ectopic Pregnancy
Inhomogeneous adnexal mass or extraterine sac-like structure

Pregnancy of unknown location
No signs of intrauterine or extraterine gestation on transvagal sonography

Probable Intrauterine Pregnancy
Intrauterine echogenic sac-like structure

Definite Intrauterine Pregnancy
Intrauterine gestational sac with yolk sac and/or embryo (with or without cardiac activity)

Barnhart et al. Fertil Steril 2011
Pain and bleeding +ve urinary pregnancy test → TV USS

Viable intrauterine pregnancy

Non-viable intrauterine pregnancy (miscarriage)

Pregnancy of unknown location (PUL)

Ectopic pregnancy
Pain and bleeding +ve urinary pregnancy test → TV USS

- Viable intrauterine pregnancy
- Non-viable intrauterine pregnancy (miscarriage)
- Pregnancy of unknown location (PUL) → Ectopic pregnancy
Healthcare costs of diagnosing and excluding ectopic pregnancy

- Retrospective cost-description analysis
- Health-care costs incurred by patients presenting to a large Scottish teaching hospital with pain and bleeding in early pregnancy
- Cost minimization analysis for the costs of current ectopic pregnancy investigations versus those of a theoretical single diagnostic serum biomarker
- This included sensitivity analyses where the biomarker was priced at increasing values and assumed to have less than 100% diagnostic sensitivity and specificity

Wedderburn et al. Hum Rep 2010
The total yearly cost for diagnosing and excluding ectopic pregnancy was 21 million Euros in UK.

Using a theoretical diagnostic serum biomarker we calculated that we could save health services up to 14 million Euros (lowest saving 3.5 million Euros after subanalysis) every year in UK.

*Wedderburn et al. Hum Rep 2010*
Financial costs to patients of diagnosing and excluding ectopic pregnancy

- Cost-description analysis
- Patients presenting with abdominal pain and/or bleeding and a positive pregnancy test
- Patients were provided with questionnaires to be completed at home and designed to record and quantify costs that they incurred until a final diagnosis of their condition was made

*Unger et al. JFPRHC 2012*
Financial costs to patients of diagnosing and excluding ectopic pregnancy

- 203 patients were recruited, of whom 52 returned their questionnaires (a 26% response rate)

<table>
<thead>
<tr>
<th>Resource use</th>
<th>Units (women)</th>
<th>Total costs (£ sterling) [total (mean, SE, median)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct health care costs</td>
<td></td>
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<tr>
<td>Medication</td>
<td>21</td>
<td>49.85 (2.33, 0.58, 1.6)</td>
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<tr>
<td>Direct non-health care costs</td>
<td></td>
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<tr>
<td>GP visits</td>
<td>11</td>
<td>56.20 (5.11, 1.74, 3)</td>
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<tr>
<td>PSC visits</td>
<td>45</td>
<td>727.50 (16.17, 3.66, 10)</td>
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<tr>
<td>Hospital admission</td>
<td>3</td>
<td>41.50 (13.83, 1.97, 15)</td>
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<tr>
<td>Sanitary towels and toiletry products</td>
<td>25</td>
<td>130.10 (5.20, 0.95, 4)</td>
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<tr>
<td>Home help</td>
<td>3</td>
<td>275.00 (91.67, 14.24, 80)</td>
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<tr>
<td>Holiday cancellations</td>
<td>14</td>
<td>3105.00 (388.13, 186.96, 147.50)</td>
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<tr>
<td>Indirect costs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Income loss</td>
<td></td>
<td>2641.38 (377.34, 277.85, 95)</td>
</tr>
<tr>
<td>Total</td>
<td>52 combined</td>
<td>7026.53 (135.13, 51.60, 20.70)</td>
</tr>
</tbody>
</table>

GP, general practitioner; PSC, Pregnancy Support Centre; SE, standard error.

- The mean cost to patients of diagnosing or excluding ectopic pregnancy was ~160 Euros

Unger et al. JFPRHC 2012
There is an unmet clinical and economic need for a serum biomarker for diagnosing ectopic pregnancy.
Updated from Cartwright J et al. Reproduction 2009;138:9-22
Fallonian Tube
Creatine Kinase (CK)
Myoglobin
Smooth Muscle Heavy Chain Myosin

Embryo/Trophoblast
β-human chorionic gonadotrophin (β-hCG)
Pregnancy associated Plasma Protein (PAPP-A)
Pregnancy Specific β-1-glycoprotein
Human placental lactogen (HPL)
Activin A
A Disintegrin and Metalloproteinase 12 (ADAM-12)
Nucleic acid markers

Angiogenesis
Vascular Endothelial Growth Factor (VEGF)
Placenta-like growth factor (PIGF)

 Corpus luteum
Progestrone
Oestradiol (E2)
Relaxin
Renin
Inhibin A

Inflammation
Cancer Antigen 125 (MUC16)
Interleukin-2 receptor
Interleukin-6
Interleukin-8
Tumour Necrosis Factor α (TNFα)
Fibronectin (FNI)

Uterus
Leukaemia inhibitory factor (LIF)
Glycoprotein (placental protein-14)
Activin B
MUC14
Adrenomedullin

Updated from Cartwright J et al. Reproduction 2009;138:9-22
Updated from Cartwright J et al. Reproduction 2009;138:9-22
Progesterone

- Readily available clinical assay
- Extensively studied as a single marker
- Meta-analysis of 26 studies suggested that single serum P< 3.2-6 ng/ml has good prediction for non-viable pregnancies
- Unable to differentiate EPs from non-viable IUPs

- Used in combination with hCG to aid in identifying those at risk of EP in some EPAUs
- Phase IV of development

Updated from Cartwright J et al. Reproduction 2009;138:9-22
Tubal implantation is manifest by an abnormal angiogenic response and that the trophoblast outside its intrauterine environment determines the altered angiogenic secretome.
Placenta-like growth factor (PIGF)

Horne et al. J Clin Endocrinol Metabol 2011
Placenta-like growth factor (PIGF)

- Screening test based on PIGF and soluble fms-like tyrosine kinase (sflt-1) levels suggested that a PIGF level $> 15.73$ pg/ml had good prediction for non-viable pregnancies
- Unable to differentiate EPs from non-viable IUPs
- Potential to aid in identifying those at risk of EP
- Used in combination with other biomarkers
- Phase III of development

Daponte et al. J Clin Endocrinol Metabol 2011
Updated from Cartwright J et al. Reproduction 2009;138:9-22
Creatine Kinase

- A number of markers of muscle damage have been explored for prediction of EP
- Conflicting data suggesting that creatine kinase is increased in EP compared with IUP
- More likely to detect rupture rather than simply extrauterine location

- **Phase III of development**
- **Use for diagnostic triage in a stable PUL is likely limited**

### Activin B

**Horne et al.** *J Clin Endocrinol Metab* 2008

#### Table

<table>
<thead>
<tr>
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<th>P all</th>
<th>P &lt; 0.05</th>
<th>P &lt; 0.02</th>
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<td>3122</td>
<td>1240</td>
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<td>FC &gt; 2.0</td>
<td>953</td>
<td>671</td>
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<tr>
<td>FC &gt; 3.0</td>
<td>285</td>
<td>251</td>
<td>130</td>
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<tr>
<td>Expected by chance</td>
<td>744</td>
<td>297</td>
<td></td>
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</table>
Activin B

Horne et al.  J Clin Endocrinol Metab 2008
Activin B

- First use of unbiased discovery approach
- Can aid in identifying those at risk of EP
- Phase III of development

Horne et al.  J Clin Endocrinol Metab 2008
Updated from Cartwright J et al. Reproduction 2009;138:9-22
A Disintegrin and Metalloproteinase-12 (ADAM-12)

Beer et al. J Proteome Res 2011
A Disintegrin and Metalloproteinase-12 (ADAM-12)

- Via a proteomics evaluation, a statistically significant decrease in ADAM-12 was found in the sera of patients with EP
- Case-control study demonstrated that a level of <4849 ng/ml was 97% sensitive and 37% specific for distinguishing EP from viable IUP. Rausch et al. Fertil Steril 2011
- Subsequent analyses in our UK cohort of 120 women showed that it did not discriminate EP. Horne et al. PLoS One 2012

- First use of a proteomics approach
- Use in isolation is likely limited
- Phase III of development
Updated from Cartwright J et al. Reproduction 2009;138:9-22
Fibronectin (FN1)

Unidentified protein extracted from gel

Split into fragment peptides

Determine mass using mass spectrometry

Determine amino acid sequence by comparison with sequence database

Protein identified

Fibronectin (FN1)

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*Brown et al. PLoS One 2013*
Fibronectin (FN1)

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- Viable intrauterine pregnancy
- Non-viable intrauterine pregnancy (miscarriage)
- Viable intrauterine pregnancy
- Pregnancy of unknown location (PUL)
- Probable ectopic pregnancy
- Spontaneously resolving PUL
- Treated PUL
- Definite ectopic pregnancy
Fibronectin (FN1)

- Can aid in identifying those at risk of EP
- Phase III of development

*Brown et al. PLoS One 2013*
Updated from Cartwright J et al. Reproduction 2009;138:9-22
Multiple marker tests

• A case-control study of 200 patients with EP or VIUP demonstrated that a four-marker test (P, VEGF, Inhibin A and Activin A) could predict EP with 90-98% sensitivity and 100% specificity in women with hCG <1500IU/ml Rausch et al. Obstet Gynecol 2011

• Sequential application of hCGt and CA-125 in a case-control study of 441 patients has 100% sensitivity and 87% specificity for EP Butler et al. Clin Chem 2013

• Multivariate analysis of 8 biomarkers including risk factors for EP did not progress beyond a 2 step diagnostic algorithm……. Horne et al, unpublished
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  Horne et al, unpublished
Challenges and future directions

- **GESTATION**: Gestational age independent markers e.g. pregnancy associated circulating microRNAs Zhao et al. Clin Chem 2012

- **OTHER PATHOLOGICAL PROCESSES**: Markers that do not change with other pathological processes e.g. PET

- **CLINICAL POPULATIONS**: Validation in prospective cohorts representative of dEPs, pEPs, vIUPs, nvIUPs, tPULs and srPULs

- ‘**HIGH AND LOW RISK**’: Markers that aid in the discrimination of pregnancies that require surgical or medical management versus those that can be managed expectantly Kirk et al. Hum Rep Update 2013
Acknowledgements

Laboratory support
Jeremy Brown
Jean Wade
Helen Dewart
Ann Doust
Ronnie Grant

MRC-CRH
Hilary Critchley
Colin Duncan

University of Melbourne
Stephen Tong
Monika Skubisz

Imperial College London
Tom Bourne

NHS Lothian
Pregnancy support staff