Endometrial alterations in endometriosis: a systematic review of putative biomarkers

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BACKGROUND: Endometriosis is usually diagnosed by an invasive procedure such as a laparoscopy. Great interest therefore lies in the potential to identify biomarkers which may be surrogates of disease presence or activity, especially relating to the effects of therapy. We have reviewed the existing literature on endometrial differences in women with endometriosis, and assess their potential use as disease biomarkers.

METHODS: We used QUADAS (Quality Assessment of Diagnostic Accuracy Studies) criteria to conduct a systematic review of published papers over the past 25 years on the subject of endometrial differences in endometriosis. We searched for all studies assessing differences between eutopic endometrium of women with and without endometriosis.

RESULTS: We identified 182 relevant articles that are summarized in the review. These studies assess over 200 potential biomarkers, including hormones and their receptors (n = 29), cytokines (n = 25), factors identified using proteomics (n = 8) and histological analysis (n = 10) of endometrial tissue. Sensitivity and specificity were reported or could be calculated for only 32 articles, and ranged from 0 to 100%. Of the nine highest quality studies, six identified putative biomarkers related to nerve fibre growth or cell cycle control, highlighting these areas as promising candidates for future biomarker research.
CONCLUSIONS: This systematic review identified several reports of endometrial differences which have the potential to be biomarkers of endometriosis. However, larger studies in well-defined populations are clearly required to determine their true usefulness.

Key words: endometrium / endometriosis / biomarker

Introduction

Endometriosis is a common disorder amongst women of reproductive age. It is defined as the presence of endometrial-like tissue outside the uterus, leading to a chronic, inflammatory condition (Kennedy et al., 2005). Current guidelines advise that visual inspection of the pelvis at laparoscopy is the gold standard for diagnosis, but this results in long delays before women acquire a definitive diagnosis (Hadfield et al., 1996). This has led to great interest in identifying peripheral biomarkers of endometriosis. A simple, reliable diagnostic test, in particular for minimal-mild disease, could avoid countless women having to undergo unnecessary surgical procedures and empirical treatment could be applied more specifically. Many studies have focused on identifying biomarkers in blood or urine because of the ease of sampling. However, it is possible to collect endometrium simply and comfortably from conscious patients using devices such as the Pipelle (Cooper-Surgical, Trumbull, CT, USA) or Endosampler (Surgimed-MLB, Newtown, PA, USA) suction curettes, which are commonly used in outpatient settings without the need for anaesthesia.

The most widely accepted theory on the pathogenesis of endometriosis is that of retrograde menstruation (Sampson, 1927). This suggests that shed endometrial fragments may pass back through the fallopian tubes into the peritoneal cavity, initiating endometriotic lesion formation. It is possible that the eutopic endometrium may therefore play a key role in the establishment of disease.

We have conducted a systematic review of the literature on differences between the eutopic endometrium of women with and without endometriosis, published over the past 25 years. Also included are studies investigating differences in uterine or menstrual fluid. Many of these studies were designed simply to investigate the nature of endometriosis, and gain further insights into this complex disease. However, the ease of endometrial biopsy means that endometrial differences in endometriosis could be considered as potential diagnostic tools. The identification of profound differences between women with and without the disease could be used as a simple biomarker for endometriosis. However, even markers which are more modestly altered in endometriosis have the potential to be combined into a clinically useful test. Whilst this may not categorically diagnose women with symptoms of endometriosis, it could possibly identify those women who are more likely to have the disease, and would therefore benefit most from treatment.

Table I Inclusion and exclusion criteria for studies.

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
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<tr>
<td>Markers retrieved from endometrial tissue, menstrual or uterine fluid</td>
<td>Markers retrieved from invasive procedure (e.g. endometriotic tissue or peritoneal fluid) or peripheral markers (e.g. urine/serum/plasma)—these are reviewed separately in a companion paper, May et al., 2010).</td>
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<tr>
<td>Visual and/or histological confirmation of endometriosis, defined as the presence of peritoneal endometriotic lesions, endometriomata and/or rectovaginal endometriotic nodules</td>
<td>Anecdotal reports, editorials, letters to the editor, conference abstracts, duplicate papers and reviews without original data.</td>
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<tr>
<td>Patients and controls clearly shown to have or not have endometriosis, respectively (all participants to have undergone either laparoscopy or laparotomy to confirm presence or absence of disease)</td>
<td>Surgical verification of participants not performed or unclear (including ‘hysterectomy’ unless clearly laparoscopic/laparoscopically assisted or transabdominal)</td>
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<td>Paper included comparison of endometrial tissue from women with and without endometriosis</td>
<td>Papers that exclusively monitored marker levels between women with different stages of endometriosis (no disease-free controls)</td>
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<td>English language publication</td>
<td>Studies involving cell culture</td>
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Table II Modified QUADAS criteria used for assessing studies.

<table>
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<th>Criteria</th>
<th>Yes</th>
<th>No</th>
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<tr>
<td>1. Were patients and controls recruited from women with symptoms consistent with endometriosis?</td>
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<td>2. Were selection criteria clearly described? Did the study describe time frame, consecutive recruitment, inclusion/exclusion criteria?</td>
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<td>3. Was the time period between the diagnosis and biomarker test short enough to avoid a change in disease status?</td>
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<td>4. Were the methods for testing sufficiently explained?</td>
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<td>5. Were the biomarker test results interpreted in a blinded fashion?</td>
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<td>6. Was the diagnosis of endometriosis made without knowledge of the biomarker test results?</td>
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<td>7. Were uninterpretable/intermediate test results reported?</td>
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<td>8. Were withdrawals from the study explained?</td>
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<tr>
<td>9. Were samples collected at a consistent phase of the cycle, or results corrected for cycle phase?</td>
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<tr>
<td>10. Were samples collected from women with a particular stage(s) of disease, or results corrected for stage?</td>
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Articles considering peripheral biomarkers (e.g. from blood or urine) are considered separately in a recently published companion review paper (May et al., 2010).

**Methods**

We conducted a primary computerized search for all publications in PubMed, MEDLINE, EMBASE and CINAHL of publications from January 1984 to August 2010 that related to endometrial differences between women with and without endometriosis. We searched using the following MeSH or key word terms: endometriosis OR endometrio* OR endometriotic cyst OR endometrioma OR endometrium tumour AND endometrium OR endometrial OR menstrua*. Two authors independently identified relevant abstracts, and the full texts were then obtained. Only English language publications were included.

Relevant studies were assessed for eligibility for inclusion by two authors. Inclusion and exclusion criteria for the studies are shown in Table I. Included studies were then assessed using an adapted version of the QUADAS (Quality Assessment of Diagnostic Accuracy Studies) criteria (Whiting et al., 2003; Table II). This adaptation was necessary to fit the general QUADAS criteria to this particular topic, as previously reported (May et al., 2010).

**Results**

The primary computerized search yielded 8037 results; of these, 7676 (95.6%) were excluded after screening their titles and abstracts (Fig. 1). Full texts of the remaining 361 studies were obtained. After reviewing the full text of all papers, 178 (49.3%) were excluded for not meeting the inclusion criteria (see Supplementary data, Table S1). The full text of one paper was unavailable at the time of writing (Szymanowski et al., 2008). The remaining 182 papers are included in the review.

The quality criteria results of each paper are shown in Supplementary data, Table S2 (Supplementary data, Table S3 shows additional information for this table). No paper scored less than 2 of 10 in the quality assessment. The majority of studies scored 5 of 10 (n = 58). The distribution of quality scores is shown in Fig. 2. The nine studies with the highest QUADAS scores (8 of 10 or greater) are shown in Table III. No study met all quality criteria. The most common flaws were a lack of blinding of the investigators to disease state and poorly described subject selection. Subject numbers varied from 6 (Kyama et al., 2006a) to 368 (Gagne et al., 2003). Where available, data on the stages of endometriosis assessed in the studies is also included. Comparatively few studies were found to include only women with stages I–II endometriosis, or to analyse changes for this subgroup of women separately. Only 11 papers included a calculation of sensitivity and specificity, or receiver operating characteristic curve analysis for a diagnostic test of endometriosis. However, a further 21 papers contained sufficient raw data to allow a calculation to be made.

Not every study could be included in the text of the review because the search identified such a large number. However, Supplementary data, Tables S2 and S3 contain results from all studies, including those that identified significant differences in endometriosis, as well as those where marker levels were found to be unchanged.

**Cytokines**

Many authors have sought to identify differences in endometrial cytokines of women with endometriosis. One study has suggested that endometrial IL-1β levels may be increased in women with...
endometriosis during the secretory phase (Kyama et al., 2008). The
decoy receptor IL-1R type II has been comprehensively investigated.
In six studies, levels of IL-1R type II have consistently been reduced
in the endometrial epithelium of women with endometriosis
(Akoum et al., 2001; Kharfi and Akoum, 2001; Kharfi et al., 2002;
Bellehumeur et al., 2005; Akoum et al., 2007; Lawson et al., 2008).

One study has identified a reduction in IL-6 (mRNA and protein)
during the secretory phase (Ponce et al., 2009). A second study
found equivalent secretory phase endometrial IL-6 levels in women
with and without endometriosis (Kyama et al., 2006b).

Endometriosis patients appear to have increased endometrial
expression of IL-8 (Kyama et al., 2006b; Ulukus et al., 2009).
However, no difference in IL-8 levels in endometrial endothelial cells
was seen between women with and without endometriosis (Luk
et al., 2005). Levels of IL-8 receptors (CXCR1 and 2) were increased
in the endometrial epithelium of affected women (Ulukus et al., 2005).
Proliferative phase CXCR1 levels were increased; CXCR2 levels were
increased throughout the cycle.

IL-13 and IL-15 were also raised during the proliferative phase of
the cycle in one study, at both the mRNA and protein level
(Chegini et al., 2003). Levels of IL-18 were found to be reduced in
women with endometriosis, compared with controls (Luo et al.,
2006).

One study found elevated menstrual phase tumour necrosis factor
(TNF-α) mRNA levels in women with endometriosis compared with
controls (Kyama et al., 2006b). Conversely, TNF-α receptor type II
levels were reduced (Kharfi et al., 2003).

Several groups have examined endometrial monocyte chemotactic
protein 1 (MCP-1). Two studies showed increased glandular MCP-1
expression in women with endometriosis (immunohistochemistry
(Jolicoeur et al., 1998; Kharfi and Akoum, 2001). Ulukus et al.
(2009) confirmed this finding, but found a significant difference only
during the proliferative phase. However, mRNA levels for MCP-1
were not found to differ in endometriosis (Kyama et al., 2008), and
no difference in endometrial endothelial MCP-1 expression was identi-
fied by immunohistochemistry (Luk et al., 2010).

Macrophage-stimulating protein was also up-regulated in the gland-
ular epithelium of women with endometriosis, during the late
secretory phase (Matsuzaki et al., 2005a). One study has revealed
an increase in levels of macrophage migration inhibitory factor in the
endometrium of women with endometriosis (Akoum et al., 2006).
Another study found raised mRNA levels of the chemokine Regu-
ulated upon Activation, Normal T-cell Expressed and Secreted (RANTES) in the endometrium of women with endometriosis during the secretory phase (Kyama et al., 2008).

Therefore, studies assessing cytokines highlight some fundamental principles in endometriosis research—in particular, the critical impor-
tance of analysing data based on the cycle phase when samples were obtained. This can affect results considerably and could determine whether statistical significance is obtained.

**Immunology**

The failure to recognize shed endometrial tissue in the peritoneal cavity as abnormal may be due to increased immunological tolerance. Researchers have therefore tried to identify immunological variation within endometrial tissue, on a background of a similar approach using serum markers.

Several studies have measured HLA expression. An increase in the expression of HLA-DR antigens on endometrial glandular cell surfaces has been identified in women with endometriosis (Liu et al., 2002). However, equivalent staining intensity of HLA-DR in the stroma and epithelium was found in an earlier study (Mettler et al., 1997). One group has demonstrated increased expression levels of HLA class I in the glands and stroma of women with endometriosis (strongest sig-
ificance with stromal cells during the secretory phase; Vernet-Tomas et al., 2006a).

Levels of endometrial IgG were initially shown to be increased in the endometrium of women with endometriosis (Kreiner et al., 1986). These authors assessed the accuracy of endometrial IgG as a diagno-
tic test: its sensitivity was 88.8%, but specificity was only 62.5% (levels were also increased in women with chronic pelvic inflamma-
tory disease). One further study suggested increased endometrial IgG in women with endometriosis but no statistical analysis was presented (Mathur et al., 1990). However, a third study identified no glandular IgG staining in patients or controls, and equal stromal staining in both groups (Nomiyama et al., 1997).

Haematopoietic cell populations that are important in immune defence have been investigated. Several early studies failed to detect changes in T cells, CD4⁺ or CD8⁺ lymphocytes, B cells, macrophages, Langerhans cells, NK cells, HLA-DR⁺ cells or granulated lymphocytes (CD56⁺CD38⁺) (Klentzeris et al., 1995; Mettler et al., 1996, 1997; Nomiyama et al., 1997).

However, more recent studies have shown some differences. Gagne et al. (2003) identified reduced numbers of CD3⁺, CD3⁺CD16⁻ and CD3⁺CD56⁻ cells, but increased numbers of CD16⁺, CD16b⁺, CD3⁺HLA-DR⁺, CD3⁺CD45RA⁻ and CD56⁺CD16⁺ cells in the endometrium. The authors used these data in combination with clinical history (duration of menses) and serum CA125 to generate a predictive model. This showed 61% sen-
sitivity and 95% specificity to diagnose endometriosis; these values were better than those obtained for CA125 alone.

T cell subsets have been analysed by Berbic et al. (2010), who found a significant increase in Foxp3⁺ regulatory T cells during the secretory phase of the cycle.

Recently, using immunohistochemistry and antibodies to CD68, macrophage increases were seen during the proliferative phase (Berbic et al., 2009). Conversely, one paper has identified a significant decrease in the number of endometrial macrophages found during the early proliferative phase in women with endometriosis (Braun et al., 2002).

The issue of immune cell population levels in endometriosis remains controversial, and thus far no consistent differences between women with and without disease have been identified. It is possible that functional studies, assessing the behaviour of cells in vivo will help us under-
stand the disease process better, although whether they can be used as biomarkers is uncertain.

**Steroids and hormones**

It is well known that endometriosis is a hormone responsive disease, and that disease progression is inhibited by an anti-estrogenic environ-
ment. Since Bulun’s initial report (Noble et al., 1996), there has been a great deal of interest in the possibility of endometrial aromatase expression identifying women with endometriosis. The original findings have been confirmed by several other groups (Hudelist et al., 2007; Bukulmez et al., 2008; Kyama et al., 2008; Hatok et al., 2010). However, three papers report no detectable aromatase expression in the endometrium of patients or controls (Velasco et al., 2006; Colette et al., 2009; Delvoux et al., 2009), and one further paper found no difference in expression between infertile women with and without endometriosis (Morsch et al., 2009).

The influence of other gynaecological pathology on aromatase expression remains unclear. Some authors have found expression to be unchanged by fibroids (Bukulmez et al., 2008). However, one group reported good sensitivity and specificity for aromatase expression (91 and 100%, respectively), but only for distinguishing ‘disease-free’ women from those with ‘disease’, including adenomyo-
sis, endometriosis and fibroids (Kitawaki et al., 1999). The same issue was highlighted by a more recent paper, which found a relatively poor specificity of aromatase expression (59%; Dheenadayalu et al., 2002). Woller et al. (2005) were able to improve the test’s diagnostic accu-
racy using a model incorporating aromatase expression and symptoms (the presence or absence of moderate to severe dysmenorrhoea).

Clearly, aromatase is not the only protein involved in steroid hormone regulation. Several papers have considered levels of hydroxyoysteroid dehydrogenase (HSD) enzymes, involved in steroid hormone pathways. For example, 17βHSD type 1 is involved in estradiol synthesis, whilst types 2 and 4 convert estradiol into less active forms (Luu-The, 2001). 17βHSD type 2 expression was unchanged in the endometrium of affected women in one study (Carneiro et al., 2007), although another identified significantly increased levels in secretory phase epithelial cells (Matsuzaki et al., 2006a). Finally, one group found overall levels of oxidising (17βestradiol → estrone) and reducing (estrone → 17βestradiol) 17βHSD enzymes to be equivalent in women with and without the disease (Delvoux et al., 2009).

Whilst no overall difference in estrogen receptors (ERs) has been shown in endometriosis, both ER forms have been investigated separ-
ately. One group found no change in ERβ levels in women with endo-
metriosis (Rey et al., 1998). However, one study discovered that glandular expression of ERβ was increased compared with controls (Hudelist et al., 2005a), whilst a second found reduced ERβ in the endothelial, stromal and perivascular compartments (Hapangama et al., 2008).
These studies indicate possible dysregulation of steroid biosynthesis in the endometrium of women with endometriosis. A combination of tests, perhaps including symptoms and/or clinical findings could potentially increase the sensitivity and specificity of a biological marker for determining disease status.

Growth factors

The TGFβ family has been studied in endometriosis. One paper reported lower mid to late secretory phase TGFβ1 mRNA levels in women with endometriosis (Johnson et al., 2005). This result was not confirmed by a second study, which used menstrual and secretory samples but found equivalent results from women with and without endometriosis (Kyama et al., 2006b). A further paper looked at the TGFβ superfamily member activin, important for endometrial growth and stromal decidualization. This study showed higher activin A mRNA levels in endometriosis patients throughout the cycle (Torres et al., 2009). Cripto expression, an antagonist of activin, was reduced during the proliferative phase only (Torres et al., 2009).

Insulin-like growth (IGF) factors are known to play an important role in diverse tissues by stimulating growth and differentiation. IGF-binding proteins (IGF-BPs) are involved in regulating transport of IGFs, but also have direct effects on cell growth (Cohen et al., 1993). Increased expression of IGF-BP3 has been found in the endometrial glands of women with endometriosis, compared with controls (Akoum et al., 1999).

Hepatocyte growth factor (HGF) and its receptor, c-Met, have both been found to be expressed more highly in endometrium of women with endometriosis (Khan et al., 2003). HGF was expressed throughout the endometrium; c-Met was found to be more highly expressed in the epithelium only.

Finally, increased levels of annexin-1 have been found in women with endometriosis (Li et al., 2008), and endometrial mRNA levels of midkine and pleiotrophin appear to be increased during the secretory phase (RT–PCR; Chung et al., 2002a).

Cell adhesion and extracellular matrix

It is possible that the endometrium of women with disease has altered expression of a variety of cell adhesion molecules, perhaps affecting how sloughed cells adhere. The majority of studies on this subject have assessed the expression of integrins—important proteins involved in cell–cell interactions.

The β3 integrin subunit has been described as defectively expressed in women with endometriosis (Lessey et al., 1994). Using endometrial samples from women after Day 19 (the window of implantation), reduced β3 expression was found in women with endometriosis compared with healthy controls or women with other causes of infertility. The same group identified decreased levels of α1β1 integrin during the window of implantation in patients with endometriosis (Khorram and Lessey, 2002). However, another group demonstrated equivalent levels in women with infertility and endometriosis, as in fertile and infertile controls (Ordi et al., 2003). A third group actually suggested that levels of α1β1 and αv integrins are increased in women with endometriosis during menses (Kyama et al., 2008), highlighting the importance of knowing the cycle phase to interpret results.

Different expression patterns may be specific to a certain endometrial cell type. Szymanowski et al. (2003) found increased expression of αvβ3 integrin in epithelial cells of women with endometriosis, but reduced expression of the β1 subunit in stromal cells. Similarly, αvβ3 levels in vascular endothelial cells were increased in endometrial samples from endometriosis patients (Hii and Rogers, 1998). A more recent paper found equivalent stromal and epithelial levels of αvβ3 and αvβ6 integrins in endometriosis patients and controls (Puy et al., 2002). However, levels of these integrins in endometrial blood vessels were increased in women with endometriosis. The precise cellular location of integrins may also differ in women with endometriosis (Vernet-Tomas et al., 2006b). The αv integrin subunit was mainly present on the basal cell surface in glandular cells of normal women. In contrast, this subunit was also found on other cell surfaces in women with endometriosis (termed ‘depolarized’ expression).

E-cadherin has been assessed in two papers. One group found equivalent expression in women with and without endometriosis (van der Linden et al., 1994). The second paper suggested that epithelial E-cadherin levels were higher in the mid, but lower in the late secretory phase in women with endometriosis (Matsuzaki et al., 2010a).

Extracellular matrix molecules (ECM) have also been assessed. Microarray analysis suggested down-regulation of osteopontin (a glycoprotein that binds αvβ3 integrin) during the secretory phase (Burney et al., 2007). Vimentin was initially found to be unchanged in endometriosis (Kyama et al., 2008), although more recent microarray data suggest a reduction in vimentin levels in affected women (Stephens et al., 2010).

ICAM-1 (CD54) was found to have reduced density of expression on endometrial cells of women with endometriosis (Prefumo et al., 2002). Protein levels of β-catenin have recently been reported as elevated in women with endometriosis during the mid-secretory phase of the cycle (Matsuzaki et al., 2010a).

One study looked at focal adhesion kinase (FAK)—a cell receptor that interacts with integrin subunits and transmits signals from the ECM to the cytoskeleton (Mu et al., 2008). Secretory phase endometrium from women with endometriosis had increased mRNA levels of FAK, and increased protein expression.

It seems likely that differences in cell adhesion molecules/pathways may play a role in disease pathogenesis. However, none of these proteins has yet been demonstrated to be useful as a biomarker. Clarification of the cycle phase at which changes occur or verification of changes by several methods may help translate some of these findings into clinical practice.

Tissue remodelling

Establishment of endometriotic lesions requires endometrial cells to adhere and invade through the peritoneal mesothelium. As such, it is plausible that these cells express proteins to break down and repair ECM. The major group of proteins studied is the matrix metalloproteinase (MMP) family.

The first study showed no difference in MMP-2 expression between women with endometriosis and healthy controls (Wenzl and Heinzl, 1998). However, Chung et al. (2002b) reported significantly higher expression of MMP-2 mRNA in affected women throughout the cycle. Similarly, another study has reported increased MMP-2 protein levels in endometriosis using immunohistochemistry (Uzan et al., 2002).
Increased mRNA levels of MMP-2 were reported in two more recent studies (Di Carlo et al., 2009; Sotnikova et al., 2010). Levels of MT1-MMP (membranous type 1 MMP, responsible for activation of MMP-2) were also elevated in endometriosis patients (Chung et al., 2002b).

Hudelist et al. (2005a, b) found a reduction in stromal MMP-1 but no difference in epithelial expression in women with endometriosis. Reduced MMP-1 expression in endometriosis patients was also shown using RT–PCR, but not confirmed by immunohistochemistry, by another group (Di Carlo et al., 2009). However, other authors reported unchanged MMP-1 expression (Kyama et al., 2006b). This study also assessed MMP-3 expression, and found mRNA levels to be increased in women with endometriosis. Raised protein levels of MMP-3 were similarly reported in four separate studies using immunohistochemistry, RT–PCR and ELISA (Gilabert-Estelles et al., 2003; Uzan et al., 2004; Ramon et al., 2005; Gilabert-Estelles et al., 2007), although one study has shown levels of MMP-3 to be unchanged (Meola et al., 2009a).

A more recent study identified an increase in MMP-7 levels in epithelial cells (Matsuzaki et al., 2010b). MMP-9 has also been investigated, with conflicting results. One study showed similar expression levels in women with and without disease (Chung et al., 2001). However, Collette et al. (2006) showed that MMP-9 levels were increased in endometriosis. This was confirmed in three more recent studies (Bellehumeur et al., 2005; Pan et al., 2008; Di Carlo et al., 2009).

Levels of tissue inhibitors of metalloproteinases (TIMPs) have been assessed. However, no difference in expression of TIMP-1 was identified using ELISA or RT–PCR (Gilabert-Estelles et al., 2003; Ramon et al., 2005; Collette et al., 2006). Immunohistochemistry has also failed to show a difference in TIMP-1 levels (Uzan et al., 2004). One paper has shown an increase in secretory phase TIMP-1 protein, but not mRNA levels (Gilabert-Estelles et al., 2007). Two papers identified reduced levels of TIMP-2 (all cycle phases) and TIMP-3 (secretory phase) in endometrium of women with endometriosis (Chung et al., 2001, 2002b). However, TIMP-2 levels were also found to be unaltered (Uzan et al., 2004), or even increased in endometriosis (Sotnikova et al., 2010).

Three studies have demonstrated an increase in endometrial urokinase in endometriosis (Gilabert-Estelles et al., 2003; Ramon et al., 2005; Gilabert-Estelles et al., 2007). One of these studies only identified a significant increase during the secretory phase (Gilabert-Estelles et al., 2007).

It therefore appears that there are some significant differences in factors involved in tissue remodelling in the endometrium of women with endometriosis. Whether these differences may translate into clinical practice as useful and relevant biomarkers is currently undetermined.

Angiogenesis

Vascular endothelial growth factor (VEGF) is the most commonly studied proangiogenic factor in the endometrium. VEGF induces blood vessel development, vasodilation and promotes vessel hyperpermeability; it therefore plays an important role in physiological endometrial remodelling (Jabbour et al., 2006).

Donnez et al. (1998) were the first to assess VEGF expression in eutopic endometrium from women with and without endometriosis. Immunostaining of samples taken throughout the menstrual cycle demonstrated elevated glandular VEGF levels in women with the disease, but only during the late secretory phase. No significant difference was found in stroma. Other studies have demonstrated significant increases in VEGF during the secretory phase. Elevated VEGF levels in women with endometriosis were shown by RT–PCR, and northern blots demonstrated that this was more marked during the secretory phase (Tan et al., 2002). Di Carlo et al. (2009) used secretory phase samples to show increased VEGF expression in women with endometriosis (immunohistochemistry, RT–PCR). Glandular epithelium was also reported to have elevated secretory VEGF-A levels in women with endometriosis (Bourlev et al., 2006). This study also found reduced levels of VEGF receptor-1 and -2 in women with endometriosis—throughout the cycle in stromal cells, and in the secretory phase in glandular epithelium. The same results were found in another similar study (Burlev et al., 2005). The latter study also found increased secretory phase expression of VEGFR-2 in endometrial microvessels (Burlev et al., 2005).

Four studies have indicated that increased VEGF expression may persist throughout the cycle (Khan et al., 2003; Takehara et al., 2004; Gilabert-Estelles et al., 2007; Cosin et al., 2009). Increased epithelial immunostaining was found in women with endometriosis in both proliferative and secretory phases (Khan et al., 2003). Increased VEGF-A mRNA was found in endometriosis patients throughout the cycle by another group (Takehara et al., 2004). Similarly, two more studies have demonstrated increased VEGF mRNA and protein levels in endometrial samples of women with endometriosis during both phases (Gilabert-Estelles et al., 2007; Cosin et al., 2009). Conversely, a recent study detected increased VEGF mRNA expression in women with endometriosis during the proliferative phase, but unchanged VEGFR-2 expression (Novella-Maestre et al., 2010). This study also assessed levels of dopamine receptor type-2, thought to be involved in VEGF signalling regulation, and found mRNA levels to be decreased in women with endometriosis. One study was unable to detect a significant difference in VEGF mRNA levels using samples from both menstrual and secretory phases (Kyama et al., 2006b). Finally, VEGF-C levels were measured by one group, and found to be significantly reduced in women with endometriosis (Takehara et al., 2004).

Angiopoietin-1 and -2 are cytokines, which regulate angiogenesis through binding to the Tie-2 (tunica interna cell kinase-2) receptor (Thomas and Augustin, 2009). Increased endometrial Ang-1 mRNA and protein have been found in women with endometriosis, throughout the cycle (Hur et al., 2006). Furthermore, mRNA levels of Ang-2 and Tie-2 were significantly increased in the secretory phase endometrium of women with the disease (Hur et al., 2006). Di Carlo et al. (2009) found that secretory phase samples had increased Ang-1 and 2, both by immunostaining and RT–PCR.

Several papers have assessed microvessel density (MVD) in the endometrium. An early study found no correlation between MVD or vascular surface area and presence of disease (Liu et al., 2003); another found MVD in the endometrial stroma was increased in endometriosis (Khan et al., 2003). Bourlev et al. (2006) found normal endometrium to have a consistent MVD throughout the cycle, whilst women with endometriosis tended to show cyclical variation with
significantly increased MVD in the secretory phase. This finding was replicated in another paper (Burlev et al., 2005).

By immunohistochemistry, the number of endoglin positive vessels during the secretory phase was higher in women with endometriosis than in unaffected controls (Kim et al., 2001). Platelet derived growth factor-A was decreased in the secretory endometrium of women with advanced endometriosis (Lee et al., 2007).

One study reported reduced thrombospondin-1 levels in the endometrium of women with endometriosis (Tan et al., 2002), although a more recent study found no significant change from control endometrium (Gilabert-Estelles et al., 2007).

Finally, two papers have considered the expression of prokineticin-1, an angiogenic factor expressed in normal endometrium. One paper found a reduction in prokineticin-1 levels in the endometrial glands of women with endometriosis (Tiberi et al., 2010). The second paper was unable to determine any difference in prokineticin-1 level in endometriosis (Lee et al., 2010).

Apoptosis and cell cycle control

The concept that retrograde menstruation is the key step in initiating disease has led researchers to investigate factors contributing to endometrial cell survival in the peritoneal cavity.

The first relevant studies used single cell suspensions of endometrial cells (Dmowski et al., 1998; Gebel et al., 1998). The authors found reduced susceptibility to apoptosis in endometrial cells from women with endometriosis. Studies have also used the TUNEL assay to demonstrate reduced numbers of apoptotic cells in the endometrium of women with endometriosis (Meresman et al., 2000; Dmowski et al., 2001; Braun et al., 2002; Meresman et al., 2002; Szymanowski, 2007). One study showed altered apoptosis in different cycle phases—women with endometriosis showed increased apoptosis during the early secretory phase, but reduced apoptosis during the late secretory phase (Johnson et al., 2005).

Three studies have shown endometrial levels of Bcl-2 to be unchanged in endometriosis (Burlev et al., 2006; Braun et al., 2007; Hassa et al., 2009). However, three studies identified increased Bcl-2 expression using immunohistochemistry (Meresman et al., 2000, 2002; Park et al., 2009).

Penna et al. (2008) reported decreased calpain 5, an apoptosis regulator, in the endometrium of women with endometriosis, as well as reduced activated caspase 3 levels (western blotting). Reduced levels of caspase 1 have also been noted, and the same study found reduced p53 levels in samples taken throughout the cycle (Braun et al., 2007). However, one group has suggested that p53 levels may be increased in women with endometriosis during the proliferative phase (Zubor et al., 2009). This study also showed a significant increase in pro-apoptotic Bcl-xS mRNA levels in women with endometriosis (Zubor et al., 2009).

A significant increase in MCL-1 and reduction in Bak expression has also been shown in secretory phase glandular epithelium of women with endometriosis (Burlev et al., 2006). Whilst no overall difference was found in various other inhibitors and promoters of apoptosis, an alteration in the ratio of these factors was seen in women with endometriosis. The authors demonstrated increased ratios of MCL-1:Bax, Bcl-2:Bax, Bcl-2:Bak and Bcl-xL:Bax, indicating an enhanced anti-apoptotic environment (Burlev et al., 2006). Whilst an increased ratio of Bcl-xL to Bcl-xS was seen by one group (Braun et al., 2007), another group found no significant difference (Zubor et al., 2009).

Five papers have suggested that Bax levels are unchanged by the disease (Meresman et al., 2000, 2002; Burlev et al., 2006; Hassa et al., 2009; Zubor et al., 2009), whilst one reported that Bax levels may be reduced in the late secretory phase, but increased in the early secretory phase in women with endometriosis (Johnson et al., 2005).

Equivalent levels of Ki67, a proliferation marker, have been found in women with and without the disease in three studies (Jurgensen et al., 1996; Mettler et al., 1997; Bourlev et al., 2006). However, three other studies found increased Ki67 immunohistochemical staining in affected women (Burlev et al., 2005; Johnson et al., 2005; Park et al., 2009).

Telomerase activity has been studied and was initially found not to differ overall in women with endometriosis (Kim et al., 2007). The same study found increased human telomerase reverse transcriptase mRNA in endometrium from women with endometriosis (Kim et al., 2007). More recent studies have suggested that telomerase activity may be increased in women with endometriosis during the secretory phase (Hapangama et al., 2008, 2009). Furthermore, mean telomere length was increased in women with endometriosis at this cycle phase (Hapangama et al., 2008).

Immunohistochemistry using proliferating cell nuclear antigen (PCNA) has been used to assess cell proliferation in endometrium from women with endometriosis (Wingfield et al., 1995). This study showed increased numbers of proliferating cells in the endothelium, stroma, and glandular and luminal epithelium of women with the disease. A second study has assessed markers of cell replication in the endometrium (Hapangama et al., 2009). The authors found increased nucleolin and PCNA in secretory phase biopsies of women with endometriosis. Conversely, levels of γH2AX (indicative of DNA damage) were reduced during the secretory phase. Increased PCNA staining was also seen in a third study (Khan et al., 2003), but was not confirmed by a fourth (Braun et al., 2007).

Levels of Pak-1 (p21 activated kinase-1), an essential protein for cell survival that plays a role in many signalling pathways, were increased in women with endometriosis, mainly in the mid-secretory phase (Kim et al., 2009). Similarly, levels of phosphorylated ERK1/2 (crucial for up-regulation of cyclin D1 and passing out of G1 phase) were increased in stroma and epithelium during the early secretory and early proliferative phases, respectively (Murk et al., 2008). A second study found similar increases, but during the early secretory phase in both cell types (Velarde et al., 2009).

Levels of c-myc (involved in promoting cell growth and proliferation) were increased in endometriosis during the proliferative phase (Johnson et al., 2005). Levels of c-fos were also increased throughout the cycle (Pan et al., 2008). However, another study did not replicate these findings (Morsch et al., 2009).

Survivin is a protein capable of regulating apoptosis and cell proliferation. Levels of this protein were initially shown to be equivalent in women with and without endometriosis (Fujino et al., 2006). However, a second study showed an increase in the proliferative endometrium of women with endometriosis (Zhang et al., 2009a). Expression of p27Kip1 (involved in cyclin regulation, preventing progression from G1 to S phase) was found to be down-regulated in the endometrium of women with endometriosis (Schor et al., 2009).
Various tumour suppressor genes were assessed by Laudanski et al. (2009). Whilst many of the genes studied were unchanged, 4EPB1 and AKT1 were both up-regulated in the endometrium of women with endometriosis. RT–PCR indicated reduced late secretory phase NfkB1A levels in women with endometriosis, although similar reductions in protein levels were not seen with western blotting (Ponce et al., 2009). Reduced CHUK levels were seen by western blotting in the late secretory phase, but mRNA levels were unchanged (Ponce et al., 2009).

Alterations in cell proliferation and cell cycle control may therefore be critical features of the endometrium in endometriosis. These features, perhaps in combination with other factors, could be of potential benefit as biomarkers in the future.

Reactive oxygen and nitrogen species

One group has suggested increased endothelial xanthine oxidase expression in women with endometriosis (Ota et al., 2001a). However, the data are difficult to interpret as two control groups (healthy women and women with adenomyosis) were used. Statistical analysis revealed a difference between the three groups, but it is not clear whether levels are altered in endometriosis when compared specifically to the healthy controls. Ota et al. (2002) also found increased expression of catalase in women with endometriosis, although no statistical analysis was shown.

One paper reported increased levels of eNOS in women with endometriosis (Khorram and Lessey, 2002). Whilst the data regarding reactive oxygen and nitrogen species is relatively sparse compared with other areas, there may be scope for further studies in this field.

Genetic studies

A study by Matsuzaki et al. (2005b) failed to identify any consistent change in endometrial gene expression in women with and without disease, throughout the cycle. Using laser capture microdissection (LCM), some differential gene expression was identified specifically in epithelium or stroma, but not in whole tissue; the authors point out that LCM and microarray technologies are expensive and time consuming. As such, these may not be valid techniques for a diagnostic test, but may be helpful in identifying biomarkers that could be measured by an alternative method. The same group followed up this paper by confirming their microarray data with immunohistochemistry, showing down-regulation of WT-1 (a Wilm’s tumour susceptibility protein) in the mid-secretory stroma of women with endometriosis (Matsuzaki et al., 2006b).

Wu et al. (2006a) also analysed eutopic endometrium from women with and without endometriosis. Cluster analysis was unable to correctly distinguish disease-free from diseased endometrium in this paper, although small numbers of subjects were included (five patients and four controls).

Burney et al. (2007) found that differences in gene expression may be cycle dependent—this study showed up-regulation of genes involved in mitosis and proliferation during the secretory phase. The same study also identified abnormal secretory phase expression of genes regulated by progesterone, supporting the notion of progesterone resistance as a feature of endometriosis. However, a separate study found only eight significantly up-regulated genes and one down-regulated gene in late secretory phase samples from women with endometriosis (Sherwin et al., 2008).

Chand et al. (2007) used cDNA arrays on pooled endometrial epithelial samples, obtained with LCM. They identified 22 up-regulated and 11 down-regulated chemokine genes. They went on to study CCL16 and CCL21 in depth, which were consistently up-regulated in endometriosis (immunohistochemistry and RT–PCR).

A reduction in HOXA10 (a homeobox gene) expression has been shown in women with either endometriosis or infertility, when compared with fertile controls (Matsuzaki et al., 2009). A target of HOXA10 has also been studied. EMX2 is negatively regulated by HOXA10; it is expressed at significantly higher levels in women with endometriosis during the mid and late secretory phases (Daitary and Taylor, 2004) (N.B. control women included in this study were confirmed to be free of endometriosis by laparoscopy during the preceding 5 years, but were not necessarily verified as disease-free at the time of sample collection).

One paper suggests that abnormal expression of HOXA10 is due to hypermethylation in women with endometriosis (Wu et al., 2005). The same group studied DNA methyltransferases (DNMT) with conflicting results (Wu et al., 2007). Semi-quantitative PCR showed raised levels of DNMT1 in the endometrium of women with endometriosis, but unchanged DNMT3A or B. However, real-time RT–PCR showed DNMT3A levels to be increased; DNMT1 and 3B levels were equivalent. Further studies will be needed to reach firmer conclusions regarding these proteins.

Finally, the emerging field of microRNA is being explored by some groups. One study found secretory endometrium from women with endometriosis to have six differentially regulated microRNAs (Burney et al., 2009). This may open up new avenues of research for a better understanding of gene regulation in endometriosis.

Proteomics

Proteomic analysis is becoming increasingly important in a variety of fields as evidence emerges that protein production and regulation play critical roles in disease processes.

Recent studies have used new proteomic technologies to identify putative biomarkers in endometrial tissue. The first of these, although very small (three subjects, three controls) showed significantly different expression of certain peptides and proteins in women with endometriosis, using protein arrays and mass spectrometry (Kyama et al., 2006a). Zhang et al. (2006) also identified different protein expression using 2D electrophoresis followed by mass spectrometry. A slightly larger study identified 223 differentially expressed protein peaks between women with and without the disease using SELDI-TOF mass spectrometry (Wang et al., 2010a). The same group assessed the mitochondrial proteome in women with endometriosis (Ding et al., 2010). A predictive model was generated using three protein peaks, which distinguished women with endometriosis with 87.5% sensitivity and 86.2% specificity.

Four other studies used 2D gel electrophoresis to identify proteins that were up- or down-regulated in endometriosis (Fowler et al., 2007; ten Have et al., 2007; Chehna-Patel et al., 2010; Stephens et al., 2010). The first, using pooled endometrial samples, demonstrated significant changes in protein expression between women with and without disease (Fowler et al., 2007). The second study...
found 119 differentially regulated proteins in the endometrium of women with endometriosis; most were cell structure proteins or involved in the immune system (ten Have et al., 2007). One study identified 20 differentially expressed proteins in endometriosis using 2DE (Stephens et al., 2010). Some results were confirmed by immunohistochemistry or western blotting, but the results were not all consistent. For example, peroxiredoxin 6 was up-regulated by 2DE, but down-regulated using western blotting. This study also suggested a reduction in ribonuclease/angiogenin inhibitor 1, vimentin, transgelin 2 and coronin 1A in women with endometriosis. Finally, one proteomic study (mainly comparing ectopic and eutopic endometrium) showed increased expression of haptoglobin in the endometrium of women with endometriosis (Chehna-Patel et al., 2010).

It is worth noting that all these studies have used small numbers of patients, but may identify proteins that will be worth studying in more detail. In the future, metabolomic and metabonomic studies may shed more light on potential differences in endometrial cell metabolism.

**Histology**

Fedele et al. (1990) reported altered endometrial ultrastructure in women with endometriosis-associated infertility, using light, scanning and transmission electron microscopy. The endometrial surface showed more heterogeneity with irregular glands, and the numbers of mitotic epithelial and stromal cells were reduced.

It has long been suggested that discrepancies exist in a variety of gynaecological conditions between a woman’s menstrual dates and the histological dating of an endometrial biopsy. One study has shown increased secretory insufficiency (defined as a lag of more than 2 days) in women with endometriosis (Cunha-Filho et al., 2001). This was a trend in fertile women with stage I–II disease, but reached significance in women with infertility and stage I–II disease. However, no differences in histological dating were found by two other studies, even accounting for disease stage (Moeloek and Moeyn, 1993; Ordi et al., 2003).

Over recent years there has been increasing interest in nerve fibre density in the endometrium. The first study (Tokushige et al., 2006) yielded promising results—small fibres in the functional layer were identified in all women with endometriosis, and in no controls. The same group followed up this paper with a preliminary trial using nerve fibre staining (with PGP9.5, a pan-neuronal marker) as a diagnostic tool (Al-Jefout et al., 2007). They recruited 20 women with endometriosis and 17 controls, and reported 100% sensitivity and 100% specificity for their test. More recently, the same group has conducted a larger, double-blind study, which showed a sensitivity of 98% and specificity of 83% (Al-Jefout et al., 2009). Interestingly, four out of the six cases with false positive biopsies either had a past history of endometriosis or a very convincing history (severe dysmenorrhea, dyspareunia and infertility) but no surgical evidence of disease, raising the possibility that disease was missed at surgery. Clearly, surgical diagnosis is the current gold standard, but it would be prudent to consider the possibility of that changing in the future.

One group has assessed endometrial nerve fibres (Bokor et al., 2009) and developed a predictive model based on expression of PGP9.5, vasoactive intestinal polypeptide and substance P, which gave 95% sensitivity and 100% specificity. Levels of neuroendocrine cells have also been identified as altered in the eutopic endometrium of endometriosis (Wang et al., 2010b). The density of cells stained for synaptophysin and neuron-specific enolase was increased in women with endometriosis.

However, a recent study has implied that nerve fibres may simply be a marker of pain (Zhang et al., 2009b). These authors identified nerve fibres only in women with painful conditions (endometriosis, adenomyosis or fibroids), but not in women who did not complain of pain. This may limit the diagnostic application of such a test, and indicates that further research must focus on assessing symptoms as well as clinical findings.

**Other**

Several papers have assessed expression of cyclo-oxygenase 2 (COX-2), an important enzyme in prostaglandin production. The first, using immunohistochemistry, showed stronger epithelial COX-2 expression during the mid- to late proliferative phase in women with endometriosis (Ota et al., 2001b). Interestingly, a second study did not demonstrate significant changes in epithelial immunostaining, but showed increased secretory phase stromal expression in women with disease (Matsuzaki et al., 2004). One paper did not find a statistically significant increase in COX-2 in endometriosis (Bukulmez et al., 2008) although Cho et al. (2010) reported a significant increase in proliferative phase COX-2 mRNA levels.

One group has identified a significant reduction in endometrial iron deposits in women with endometriosis (Van Langendonckt et al., 2002a). Levels of 1α hydroxylase (part of the vitamin D pathway) were higher in women with endometriosis than controls, but not significantly different from women with endometrial or ovarian cancer (Agic et al., 2007).

Finally, one paper demonstrated altered lectin binding—staining with dolichos biflorus agglutinin was significantly reduced in the endometrium of women with endometriosis (Miller et al., 2010).

**Menstrual effluent and uterine fluid**

Two studies have measured CA125 levels in menstrual fluid and found marked increases in women with endometriosis (Takahashi et al., 1988, 1990). The authors suggest that menstrual CA125 levels could provide a diagnostic test with 66.7% specificity and 92.3% sensitivity.

A separate study has identified reduced progesterone levels in uterine fluid from women with endometriosis (Fazleabas et al., 1987). These authors also assessed levels of total protease inhibition, and found them to be greater in endometriosis, but only during the early secretory phase.

A recent study has attempted to use proteomic analysis of endometrial fluid to diagnose endometriosis (Ametzazurra et al., 2009). 2D gel electrophoresis revealed 31 proteins that were differently expressed in women with endometriosis, which may hold insights into the nature of the disease, as well as showing promise as putative biomarkers.

Finally, one study has demonstrated an increased level of endotoxin in menstrual blood, as well as higher contamination of menstrual blood with Escherichia coli in women with endometriosis (Khan et al., 2010).
Discussion

In this review, we set out to conduct a comprehensive analysis of the literature relating to alterations in endometrial tissue, menstrual and uterine fluid in endometriosis. The majority of the studies assessed eutopic endometrium from women with and without endometriosis. Many were designed primarily to gain a better understanding of the disease process rather than to identify biomarkers, but they have been revisited because endometrial sampling has been proposed as a diagnostic tool. Whilst no marker has conclusively been shown to diagnose endometriosis, we found several high-quality studies that identified endometrial nerve fibres and molecules involved in cell cycle control, collagen deposition and angiogenesis as being promising candidates for future biomarker research.

Using the adapted QUADAS criteria we were able to assess the quality of all studies included in the review. Whilst no papers achieved the top score, nine studies were notable for achieving high scores of 8 or 9 out of 10. The most common flaw in these studies was a lack of detailed description of selection criteria—a criticism that may be related to pressure on word counts for publication, rather than a true problem. The studies are notable for their consistent recognition of the importance of accounting for the phase of the menstrual cycle, and the stage of disease. The majority of these studies also report on the phase of the menstrual cycle—critical for the robust interpretation of data. Despite their obvious merits, only three of these nine studies report on the sensitivity and specificity of the investigated markers for the diagnosis of endometriosis. This is clearly of critical importance when analysing the potential of a biomarker. Nonetheless, these studies can still be regarded as valuable pilot data to justify further work in certain areas of biomarker research. For example, the study by Kyama et al. (2006a) uses endometrial tissue from only six women. This number of subjects is clearly insufficient to generate meaningful data regarding sensitivity and specificity, but still highlights the future potential for proteomics to identify promising biomarkers.

Two studies (from independent investigators) assessed the presence of nerve fibres in the endometrium (Ali Jefout et al., 2009; Bokor et al., 2009). These studies were also 2 of only 10 studies which identified a test with greater than 80% sensitivity and specificity for the diagnosis of endometriosis. Nerve fibres show great promise as a putative biomarker of disease, as they appear to fall into two distinct categories (present or absent) which correlates with the presence of disease. This therefore has the potential to be a unique biomarker, although further work is needed to determine whether nerve fibres may also be present in other gynaecological pathology.

Four of the highest quality papers assessed factors related to cell survival and cell cycle control (Dmowski et al., 2001; Braun et al., 2002; Schor et al., 2009; Zubor et al., 2009). Whilst none of these papers assess sensitivity or specificity of the markers identified, it is promising that they all identify similar biological mechanisms as being altered in endometriosis. However, notably, one paper finds an increase in pro-apoptotic factors in endometriosis (Zubor et al., 2009), whilst two papers found a reduction in apoptosis in endometriosis (Dmowski et al., 2001; Braun et al., 2002). Further analysis of the apoptotic pathway in endometriosis may lead to a better understanding of these apparently contradictory results, and potentially identify reasons for the discrepancy.

Three other well conducted studies assess integrin β3 levels, endoglin expression and the endometrial proteome in endometriosis (Lessey et al., 1994; Kim et al., 2001; Kyama et al., 2006a). The first of these studies analysed a remarkably large number of samples, but only from infertile women. It may therefore be useful to assess integrin β3 levels in women with symptoms of pain, rather than infertility, and identify whether the reduced level is still present in those women with endometriosis. The study by Kim et al. shows significantly increased endoglin expression in endometrial vessels of women with endometriosis, but compares samples to control women with carcinoma in situ of the cervix, rather than symptoms that could be attributable to endometriosis. However, the role of angiogenic factors may still be a promising area for future research, providing altered levels can be identified in the target population for a biomarker test.

Endometriosis presents with a broad spectrum of symptoms. Laparoscopy is the current gold standard for a definitive diagnosis of endometriosis, preferably with histological confirmation (Kennedy et al., 2003). However, this procedure carries associated surgical risk, is time consuming for the patient, and involves significant cost to healthcare systems. A simpler, quicker, cheaper diagnostic test with fewer associated risks would clearly be preferable. Endometrial biopsy has become a useful outpatient investigation to manage abnormal menstrual bleeding for women who would previously have undergone curettage under general anaesthesia (Seamark, 1998). This hastens the journey from referral to diagnosis, and ultimately treatment. The success of endometrial biopsy in this context should encourage gynaecologists that this is a viable and promising tool for the future management of endometriosis.

Although endometrial biopsy is minimally invasive, it may be regarded as unpleasant or uncomfortable. Most studies assessing patient tolerance of endometrial biopsy have shown that the procedure is associated with discomfort, but that the majority of women would be willing to undergo it again (Lau et al., 1999; De Iaco et al., 2000). Adequacy of sampling is another potential concern. It is important to note that many of the samples used in the above studies would have been collected at the time of surgery. Even studies that specifically assessed the use of biopsies as a diagnostic tool tended to acquire samples at the time of laparoscopy (Bokor et al., 2009). This could potentially result in higher quality samples—the use of Pipelle sampling on an outpatient basis has previously been shown to produce adequate tissue for a histological diagnosis (Gordon and Westgate, 1999). However, many potential diagnostic tests involving molecular techniques require only small amounts of tissue. The adequacy of sampling would therefore need to be assessed with respect to the individual biomarker test employed.

It could ultimately be more worthwhile to develop a blood or urinary biomarker test as only minimal or no training of staff would be required. However, if endometrial biopsy can be shown to be well tolerated in this patient population and provide adequate tissue for whichever technique employed, then it shows considerable promise as a minimally invasive diagnostic test for endometriosis. The risks associated with the procedure are tiny, particularly when compared with the potential risks of diagnostic laparoscopy. If a suitable biomarker is found, endometrial biopsy could potentially provide a simple, rapid and cost-effective means of diagnosing and monitoring endometriosis.
Over the last decade there has been a wealth of suggestive evidence showing intrinsic differences between the eutopic endometrium of women with endometriosis and ‘normal’ controls. However, extrapolating these data to find putative biomarkers is fraught with difficulties. The majority of the research reviewed in this article has been generated by studies seeking understanding of the mechanisms that drive endometriosis. Although this may highlight areas of potential importance it is vital that future research focuses on conducting trials specifically designed to identify consistent and clear biomarkers of disease presence and/or activity. These studies will need to be adequately powered, multicentre collaborations in order to recruit sufficient women and demonstrate the ability to standardize results across different populations and locations. Furthermore, reliable data will only be obtained if the patient populations are clearly defined, with women assigned to appropriate subgroups. As such it will be essential that all clinical and surgical data are meticulously documented. Concurrent medication, surgical treatments, cycle phase and stage of disease are all variables with the potential to affect the result of any biomarker test markedly. Ignoring these important confounders only leads to confusion, and mistrust of results. Taking a standardised and systematic approach in future studies should generate robust data, and will allow better comparison of research carried out by different groups.

Supplementary data
Supplementary data are available at http://humupd.oxfordjournals.org/.

Authors’ roles
K.E.M. identified the articles, acquired and analysed the data and drafted the manuscript. J.V. conceived and designed the study and revised the manuscript. S.K. developed the search strategy for identification of articles and revised the manuscript. S.H.K. conceived and designed the study, identified the articles, acquired the data and revised the manuscript. All authors approved the final version of the manuscript.

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