Human Reproduction Update, Vol.16, No.6 pp. 651–674, 2010
Advanced Access publication on May 12, 2010 doi:10.1093/humupd/dmq009

human reproduction update

Peripheral biomarkers of endometriosis: a systematic review

K.E. May [1,†], S.A. Conduit-Hulbert [1,2,†], J. Villar [1], S. Kirtley [1], S.H. Kennedy [1], and C.M. Becker [1,*]

[1]Nuffield Department of Obstetrics & Gynaecology, University of Oxford, Women's Centre, John Radcliffe Hospital, Oxford OX3 9DU, UK
[2]Drexel University College of Medicine, Philadelphia, PA, USA

*Correspondence address. E-mail: christian.becker@obs-gyn.ox.ac.uk

Submitted on December 16, 2008; resubmitted on January 27, 2010; accepted on April 6, 2010

TABLE OF CONTENTS

- Introduction
- Methods
- Results
  - Cytokines
  - Antibodies
  - Cell populations
  - Other immunology
  - Glycoproteins
  - Cell adhesion
  - Growth factors
  - Proteomics
  - Hormones
  - Angiogenesis
  - Apoptosis
  - Other
- Discussion

BACKGROUND: Endometriosis is estimated to affect 1 in 10 women during the reproductive years. There is often delay in making the diagnosis, mainly due to the non-specific nature of the associated symptoms and the need to verify the disease surgically. A biomarker that is simple to measure could help clinicians to diagnose (or at least exclude) endometriosis; it might also allow the effects of treatment to be monitored. If effective, such a marker or panel of markers could prevent unnecessary diagnostic procedures and/or recognize treatment failure at an early stage.

METHODS: We used QUADAS (Quality Assessment of Diagnostic Accuracy Studies) criteria to perform a systematic review of the literature over the last 25 years to assess critically the clinical value of all proposed biomarkers for endometriosis in serum, plasma and urine.

RESULTS: We identified over 100 putative biomarkers in publications that met the selection criteria. We were unable to identify a single biomarker or panel of biomarkers that have unequivocally been shown to be clinically useful.

CONCLUSIONS: Peripheral biomarkers show promise as diagnostic aids, but further research is necessary before they can be recommended in routine clinical care. Panels of markers may allow increased sensitivity and specificity of any diagnostic test.

Key words: endometriosis / infertility / laparoscopy

[†] The first two authors contributed equally to the study.

Introduction

Endometriosis, the presence of endometrial-like tissue outside the uterus, is a disease associated with pelvic pain and infertility. It affects approximately 10% of women of reproductive age (Giudice and Kao, 2004), although its prevalence in women with chronic pelvic pain is much higher, and infertility has been reported in 30–50% of endometriosis patients (Practice committee of the American Society for Reproductive Medicine, 2004). As such, it has significant socio-economic implications for individuals and society as a whole (Simoens *et al.*, 2007).

The symptoms are often non-specific as they may mimic those associated with other chronic pain disorders, such as irritable bowel syndrome and pelvic inflammatory disease. In addition, in the overwhelming majority of cases a surgical procedure is required to make a definitive diagnosis. As a result, women can suffer for 8–12 years before obtaining a diagnosis and receiving appropriate treatment (Hadfield *et al.*, 1996). Therefore, the ability to diagnose patients more easily, using less invasive means (e.g. a biomarker), would be of great value, particularly if the same biomarker could be used to monitor treatment efficacy. A biomarker is defined as 'a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention' (Biomarkers Definitions Working Group, 2001). Hence, if reliable, such a biomarker, or a panel of biomarkers, could be an effective tool to diagnose endometriosis without the need of surgery.

To date, biomarker discovery in this field has utilized hypothesis-free approaches, e.g. differential expression in eutopic endometrium and endometriotic lesions from the same patients, or a more hypothesis-driven approach assessing candidate biomarkers in urine and/or blood samples. The majority of the latter studies have focused on single markers, and many results have been inconsistent and, at times, contradictory. Several narrative reviews have been published, but most authors have failed to use methods that are now recommended for systematic reviews of diagnostic tests (Levine *et al.*, 1994; Stroup *et al.*, 2000).

Our primary aim, therefore, was to conduct a systematic review of the literature from the last 25 years to assess critically the clinical value of all proposed biomarkers for endometriosis in serum, plasma and urine. Although recent research has highlighted endometrial biopsy as a potential diagnostic tool (Al-Jefout *et al.*, 2009; Bokor *et al.*, 2009), the role of endometrial samples, endometrial fluid aspirates and menstrual effluent as biomarkers will be considered separately in a companion review article because of the overwhelmingly large amount of data.

Methods

A primary computerized search was performed in PubMed, MEDLINE, EMBASE, and CINAHL of publications from January 1984 to August 2009. We searched using the following MeSH or keyword terms: *endometriosis* and *urine* or *plasma* or *serum* or *tissue* or *endometrium* or *endometrial* or *blood* or *cell* or *saliva* or *menstrua** or *sputum* and *biological markers* and *diagnosis* or *mass screening*. We then searched in the bibliography of the retrieved articles and reviews and included any additional relevant articles. Only English language publications were included. The potentially relevant studies were retrieved, reviewed and categorized by two authors. Studies were evaluated according to specific criteria (Table I).

Two authors assessed the methodological quality of the studies and extracted relevant data such as sample size, biomarkers evaluated, tissue sampled, visual/histological confirmation of disease state, and whether or not confounding factors were controlled for by matching or adjustment. Where available, we extracted statistical data from the original papers or calculated missing measures using the data provided. The quality of individual studies was judged using a modified version of the QUADAS (Quality Assessment of Diagnostic Accuracy Studies) criteria (Whiting *et al.*, 2003) (Table II).

Results

The primary computerized search produced 11 122 results, of which 10 950 were eliminated after screening their titles and abstracts (Fig. 1). If the abstract did not clearly indicate whether a study met the initial inclusion criteria, the entire article was assessed.

Table I Inclusion and exclusion criteria for studies.

Inclusion criteria	Exclusion criteria
Biomarkers were retrieved from plasma, serum or urine	Biomarkers were retrieved during invasive procedure (e.g. peritoneal tissue or fluid)
Visual and/or histological confirmation of endometriosis, defined as the presence of peritoneal endometriotic lesions, endometriomata and/or rectovaginal endometriotic nodules	Anecdotal reports, editorials, letters to the editor, duplicate papers and reviews without original data
	Papers that exclusively monitored biomarker levels between women with different stages of endometriosis (unless they compared values with 'normal ranges') as they could not demonstrate the diagnostic potential of the test
	Studies assessing the levels of CA-125 published before the comprehensive meta-analysis by Mol and colleagues (Mol *et al.*, 1998)
	Studies that used males in the control group (unless a separate control group of females was identified)
	Studies that required prolonged cell culture (>24 h) in order to demonstrate differences in biomarker expression (impractical)

Table II Modified QUADAS criteria used for assessing studies.

Criteria	Yes	No	Unclear
1. Were patients and controls recruited from women with symptoms consistent with endometriosis?			
2. Were selection criteria clearly described? Did the study describe time frame, consecutive recruitment, inclusion/exclusion criteria?			
3. Was the time period between the diagnosis and biomarker test short enough to avoid a change in disease status?			
4. Were controls surgically verified (not to have endometriosis)?			
5. Were the methods for testing sufficiently explained?			
6. Were the biomarker test results interpreted in a blinded fashion?			
7. Was the diagnosis of endometriosis made without knowledge of the biomarker test results?			
8. Were uninterpretable/intermediate test results reported?			
9. Were withdrawals from the study explained?			
10. Were samples collected at a consistent phase of the cycle, or results corrected for cycle phase?			
11. Were samples collected from women with a particular stage(s) of disease, or results corrected for stage?			

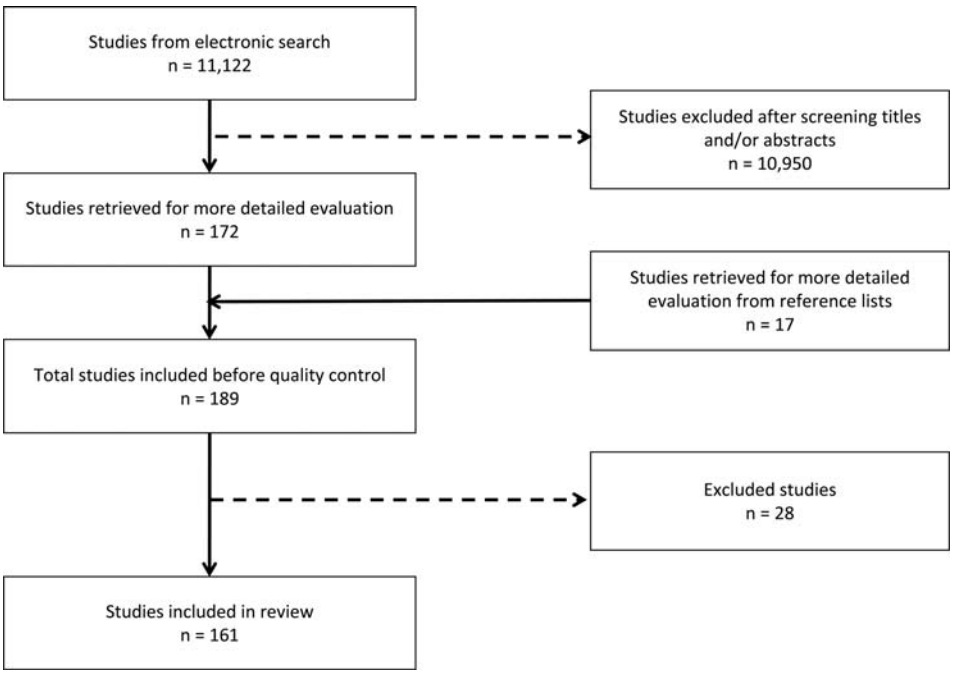

Figure 1 Flow diagram depicting selection of articles for review.

The remaining 172 articles were considered relevant and the full papers were obtained, as well as an additional 17 papers identified from their reference lists. From this pool of 189 papers, 27 studies were excluded because, on more detailed assessment, they did not meet the selection criteria. One further study was excluded as the full text was unavailable, leaving 161 studies that were included in the final review (Fig. 1).

Table III shows the modified QUADAS criteria, biomarkers assessed and number of subjects and controls included in each study. Study sample size ranged from 8 (Panidis *et al.*, 1988) to 775 (Kitawaki *et al.*, 2005). None of the identified studies fulfilled all methodological criteria. The most common flaws were lack of blinding of investigators to disease state, poorly defined patient and control selection criteria, and lack of adjustment for menstrual cycle or stage of disease.

Cytokines

Many authors have sought to identify elevated or decreased levels of a variety of cytokines in women with endometriosis, partly to provide insights into the pathogenesis of disease and partly to assess their use as putative biomarkers. The most studied cytokines have been

Table III Modified QUADAS scoring for studies and main biomarkers assessed.

Paper	Modified QUADAS criteria											Number		Factors assessed
	1	2	3	4	5	6	7	8	9	10	11	Endo	Control	
Abrao et al. (1997)	n	n	u	y	y	u	u	y	y	y	y	35	15	Ca125II, serum amyloid A, anti-cardiolipin antibodies and CRP
Abrao et al. (1999)	n	n	u	y	y	u	u	y	y	y	y	35	15	Ca125, Ca15-3, Ca19-9, CEA, AFP, B2microglobulin
Acien et al. (1989)	n	n	u	u	y	u	u	y	y	y	y	60	16	PRL and TRH
Adamyan et al. (1993)	y	n	u	y	y	u	u	y	y	n	n	54	48	LH, testosterone and estradiol
Agic et al. (2007)	n	n	u	y	y	u	u	y	y	y	n	83	51	CCR1mRNA
Agic et al. (2008)	n	n	y	y	y	u	u	y	y	y	y	102	49	CCR1mRNA, MCP-1 and Ca125
Akoum et al. (1996)	n	n	u	y	y	u	u	y	y	y	y	57	44	MCP-1
Amaral et al. (2006)	y	n	y	y	y	u	u	y	y	y	y	35	17	CA125
Antsiferova et al. (2005)	n	n	y	y	y	u	u	y	y	y	n	15	20	IL-2, -4 and -10
Arumugam (1991)	y	n	u	y	y	u	u	y	y	y	y	43	36	PRL
Badawy et al. (1984)	y	n	u	y	y	u	u	y	y	n	n	37	54	Anti-endometrial antibodies
Badawy et al. (1987)	y	n	u	y	y	u	u	y	y	n	n	23	22	Complement factors and immunoglobulins
Badawy et al. (1990)	y	n	u	y	y	u	u	y	y	y	y	43	46	T and B cells
Bagan et al. (2008)	u	y	y	y	y	u	u	y	y	y	n	9	22	CA125
Barrier and Sharpe-Timms (2002)	n	n	n	y	y	u	u	y	y	n	y	16	16	Soluble ICAM-1, VCAM-1 and E-selectin
Bedaiwy et al. (2002)	y	n	y	y	y	u	u	y	y	y	y	56	27	IL-6, IL-1β, IL-12, IL-13 and TNFα
Bohler et al. (2007)	n	n	u	u	y	u	u	y	y	n	y	58	25	Anti-endometrial antibodies
Borkowski et al. (2008)	n	n	y	y	y	u	u	y	y	y	y	26	17	Vitamin D binding protein
Bourlev et al. (2006a)	n	n	y	y	y	u	u	y	y	y	y	25	14	FGF-2
Bourlev et al. (2006b)	n	n	y	y	y	u	u	y	y	y	y	25	14	VEGF
Chen et al. (1998)	y	y	y	y	y	u	u	y	y	y	y	131	26	CA125
Chishima et al. (2000)	n	n	u	n	y	u	u	y	y	n	n	31	14	B1 cell levels
Cho et al. (2007)	y	n	y	y	y	u	u	y	y	y	y	46	24	VEGF, sFlt1 and TNFα
Cho et al. (2008)	n	n	u	n	y	u	u	y	y	n	y	231	529	Neutrophil:lymphocyte ratio and CA125
Cho et al. (2009)	n	n	y	n	y	u	u	y	y	y	y	79	43	Osteopontin
Confino et al. (1990)	n	n	u	y	y	u	u	y	y	n	n	14	9	Antibody levels
Cunha-Filho et al. (2001)	y	n	u	y	y	u	u	y	y	y	n	27	14	PRL
Daniel et al., (2000)	y	n	y	y	y	u	u	y	y	y	n	5	8	sICAM-1 and sVCAM-1
Darai et al. (2003)	y	n	y	y	y	u	u	y	y	n	n	34	43	IL-6, IL-8 and TNFα
D'Cruz et al. (1996)	n	n	u	y	y	u	u	y	y	n	y	319	200	Carbonic anhydrase antibodies
De Placido et al. (1998)	u	n	y	y	y	u	u	y	y	y	y	15	15	Soluble HLA-1 and sICAM-1
El-Roeiy et al. (1988)	n	n	y	n	y	u	u	y	y	n	n	20	250	Autoantibodies and antibody levels
Fairbanks et al. (2009)	y	n	y	y	y	u	u	y	y	y	y	72	33	IL-12 and IL-18
Fassbender et al. (2009)	y	n	y	y	y	u	u	y	y	y	y	109	51	C3a-des-Arg
Fazleabas et al. (1987)	n	n	u	y	y	u	u	y	y	y	n	16	20	Progesterone
Ferrero et al. (2005)	y	n	y	y	y	u	u	y	y	y	n	72	35	Haptoglobin isoforms
Florio et al. (2007)	y	u	y	y	y	y	u	y	y	n	y	40	40	Urocortin
Florio et al. (2009)	n	n	y	y	y	u	u	y	y	n	y	63	79	Follistatin
Fu and Lang (2002)	n	n	y	n	y	u	u	y	y	y	y	32	30	Soluble E cadherin
Gagne et al. (2003a)	n	n	y	y	y	u	u	y	y	y	y	131	146	VEGF
Gagne et al. (2003b)	n	n	y	y	y	u	u	y	y	y	n	175	131	Leucocyte subsets
Gajbhiye et al. (2008)	n	n	u	y	y	u	u	y	y	y	y	40	30	Anti-endometrial antibodies
Galleri et al. (2009)	y	n	y	y	y	u	u	y	y	y	y	77	70	CXCL10

Continued

Table III *Continued*

Paper	Modified QUADAS criteria											Number		Factors assessed
	1	2	3	4	5	6	7	8	9	10	11	Endo	Control	
Garcia-Velasco *et al.* (2002)	n	n	u	y	y	u	u	y	y	y	y	57	14	sFas L
Garza *et al.* (1991)	n	n	y	u	y	u	u	y	y	n	n	12	6	Anti-endometrial antibodies
Garzetti *et al.* (1993)	y	n	u	y	y	u	u	y	y	n	y	33	11	T cells, NK cells and cytotoxicity
Garzetti *et al.* (1998)	n	n	u	y	y	u	u	y	y	y	y	20	10	PMN levels
Gazvani *et al.* (1998)	n	n	u	y	y	u	u	y	y	y	y	25	22	IL-8
Gmyrek *et al.* (2005)	n	n	y	u	y	u	u	y	y	y	y	15	36	MCP-1
Gmyrek *et al.* (2008)	n	n	u	y	y	u	u	y	y	y	y	36	12	T cells and monocytes
Górski *et al.* (2007)	n	n	y	y	y	u	u	y	y	n	y	24	12	$CD8^+$ T cells
Gungor *et al.* (2009)	y	n	y	y	y	u	u	y	y	y	y	33	79	leptin
Gurgan *et al.* (1999)	u	n	y	y	y	y	u	y	y	y	y	29	15	IGF 1 and 2, IGFBP3
Harada *et al.* (2002)	y	n	u	y	y	u	u	y	y	n	y	101	22	CA125 and CA19-9
Hassa *et al.* (2009)	n	n	u	y	y	y	u	y	y	y	y	60	37	IL-2, -4, -10 and IFNγ, also lymphocytes
Hatayama *et al.* (1996)	y	n	u	y	y	u	u	y	y	y	n	20	20	Anti-endometrial antibodies
Ho *et al.* (1995)	y	n	y	y	y	u	u	y	y	y	y	17	16	Peripheral lymphocyte numbers
Hsu *et al.*, (1997)	y	n	y	y	y	u	u	y	y	y	y	36	7	IL-4, -10, -2 and IFNγ
Huang *et al.* (2004)	n	n	y	y	y	u	u	y	y	y	y	40	18	MMP-2
Illera *et al.* (2001)	n	n	y	y	y	u	u	y	y	y	n	32	20	LH
Inagaki *et al.* (2003)	y	n	y	y	y	u	u	y	y	n	y	42	39	Anti-laminin-1 antibodies
Iwabe *et al.* (2003)	y	n	y	y	y	u	u	y	y	y	n	22	13	IL-6
Iwasaki *et al.* (1993)	y	n	y	y	y	u	u	y	y	y	n	19	26	T cell subsets and NK cells
Izumiya *et al.* (2003)	n	n	u	y	y	u	u	y	y	y	y	45	48	Monocyte levels and markers
Jee *et al.* (2008)	y	n	y	y	y	u	u	y	y	n	y	44	51	IL-6 and CD163, CA125
Jing *et al.* (2008)	n	n	y	n	y	y	u	y	y	n	y	59	61	Proteomics—mass spectrometry
Joshi *et al.* (1986)	y	n	u	y	y	u	u	y	y	y	y	36	19	Serum PEP
Kabut *et al.* (2007)	y	n	u	y	y	u	u	y	y	n	y	82	30	Complement components
Kafali *et al.* (2004)	y	n	u	y	y	u	u	y	y	y	n	16	12	CA125
Kalu *et al.* (2007)	y	n	y	y	y	u	u	y	y	y	n	26	31	IL-8, IL-6, IL-1β, PDGF, RANTES, TNFα, sFas
Khan *et al.* (2006)	n	n	u	y	y	u	u	y	y	y	y	37	21	HGF
Kiechle *et al.* (1994)	n	n	u	u	y	u	u	y	y	n	y	23	17	Carbonic anhydrase antibodies
Kikuchi *et al.* (1993)	n	n	u	n	y	u	u	y	y	n	n	10	59	NK cells and T cells pre/post op
Kilpatrick *et al.* (1991)	u	n	u	u	n	u	u	y	y	n	n	32	25	Anti-cardiolipin antibodies
Kitawaki *et al* (2005)	n	y	u	y	y	u	u	y	y	n	y	433	342	CA125
Kondera-Anasz *et al.* (2005)	y	n	u	y	y	u	u	y	y	y	y	49	20	IL-1α, IL-1sRII and IL-1Ra antagonist
Kurdoglu *et al.* (2009)	n	n	y	y	y	u	u	y	y	n	y	101	26	CA125 and CA19-9
Lambrinoudaki *et al.* (2009)	u	n	y	y	y	u	u	y	y	n	y	45	21	HSP70, HSP70b′, TRX and IMA
Lima *et al.* (2006)	n	n	y	y	y	u	u	y	y	n	y	28	21	PRL and cortisol
Linghu *et al.* (2004)	y	n	u	u	y	u	u	y	y	n	n	20	30	Fas and sFasL
Liu *et al.* (2007)	n	n	u	n	y	y	u	y	y	n	n	52	46	Proteomics—mass spectrometry
Maeda *et al.* (2002a)	n	n	u	y	y	u	u	y	y	n	y	28	26	NK subsets and ICAM-1
Maeda *et al.* (2002b)	n	n	u	y	y	u	u	y	y	n	y	42	40	KIR2DL1 expression
Maeda *et al.* (2004)	n	n	y	y	y	u	u	y	y	y	y	98	104	NK subsets
Maiorana *et al.* (2007)	n	n	u	y	y	u	u	y	y	y	y	69	17	CA125
Markham *et al.* (1997a)	n	n	y	y	y	u	u	y	y	n	y	23	9	TNFα and RANTES

Continued

Table III *Continued*

Paper	Modified QUADAS criteria											Number		Factors assessed
	1	2	3	4	5	6	7	8	9	10	11	Endo	Control	
Markham *et al.* (1997b)	n	n	y	y	y	u	u	y	u	n	n	145	80	TNFα
Martinez *et al.* (2007)	n	n	y	y	y	u	u	y	y	y	y	47	38	IL-6, Ca125
Matalliotakis *et al.* (1996)	n	n	u	n	y	u	u	y	y	y	n	10	10	PRL and TSH
Matalliotakis *et al.* (1997)	n	n	u	n	y	u	u	y	y	n	n	10	10	TNFα, sCD8 and sCD4
Matalliotakis *et al.* (1998)	y	n	y	y	y	n	u	y	n	n	n	15	0	CA19-9 with treatment
Matalliotakis *et al.* (2000a)	u	n	u	y	y	u	u	y	y	n	n	20	10	Leptin
Matalliotakis *et al.* (2000b)	y	n	y	y	y	y	u	y	y	y	y	20	10	CD23
Matalliotakis *et al.* (2001a)	y	n	u	y	y	u	u	y	y	n	n	38	30	Soluble HLA I and II
Matalliotakis *et al.* (2001b)	y	n	u	y	y	y	u	y	y	n	n	38	30	Soluble ICAM-1
Matalliotakis *et al.* (2003)	u	n	u	y	y	u	u	y	y	n	n	38	30	VEGF and EGFR, soluble HLA I and II
Matalliotakis *et al.* (2004)	y	n	y	y	y	u	u	y	y	y	y	50	50	CA125 with treatment
Matarese *et al.* (2000)	n	n	y	y	y	u	u	y	y	y	y	13	15	Leptin
Mathur *et al.* (1990)	n	n	y	y	y	u	u	y	y	y	n	21	10	Antibody levels
Mathur *et al.* (1998)	n	n	u	n	y	u	u	y	y	y	n	123	105	Anti-transferrin and α2-HS glycoprotein antibodies
Mathur *et al.* (1999)	u	n	y	y	y	u	u	y	y	n	n	40	60	Serum transferrin and α2-HS glycoprotein
Matsuzaki *et al.* (2006)	n	n	y	y	y	u	u	y	y	y	n	59	40	Progesterone
Meek *et al.* (1988)	n	n	u	y	y	y	u	y	y	y	n	20	20	IgG, IgA, IgM, C3, C4 and total complement
Medl *et al.* (1997)	y	y	y	y	y	y	u	y	y	n	y	71	112	TATI
Molo *et al.* (1994)	y	n	y	y	y	u	u	y	y	y	y	19	16	CA125, CA72
Morin *et al.* (2005)	n	n	y	y	y	u	u	y	y	y	y	55	38	Macrophage migration inhibitory factor
Muscatello *et al.* (1992)	y	n	y	y	y	u	u	y	y	y	y	81	38	CA125, CA15-3, TAG-72
Odukoya *et al.* (1995a)	n	n	y	y	y	u	u	y	y	n	y	55	43	Anti-endometrial antibodies
Odukoya *et al.* (1995b)	n	n	y	y	y	u	u	y	y	y	y	21	18	CD23
Odukoya *et al.* (1996)	n	n	y	y	y	u	u	y	y	y	y	57	40	Anti-endometrial Abs and CD23
Ohata *et al.* (2008)	y	n	u	y	y	u	u	y	y	y	y	70	21	IL-8
Oosterlynck *et al.* (1991)	y	n	u	y	y	u	y	y	y	n	y	24	10	NK cells and subsets
Oosterlynck *et al.* (1994)	y	n	y	y	y	u	u	y	y	n	n	35	24	PBMC subsets
Othman *et al.* (2008)	y	n	y	y	y	u	u	y	y	y	y	68	70	IL-6, MCP-1, IFNγ, VEGF, TNFα and GM-CSF
Panidis *et al.* (1988)	y	n	y	y	y	u	u	y	y	n	n	8	0	CA125, CA19-9 and CA15-3
Panidis *et al.* (1992)	u	n	u	u	y	u	u	y	y	y	n	10	10	PRL (in response to TRH)
Pellicer *et al.* (1998)	y	n	y	y	y	u	u	y	y	y	y	12	11	IL-6, VEGF and IL-1β
Philippoussis *et al.* (2004)	y	n	y	y	y	u	u	y	y	y	y	36	36	EGF, AFP, IGFBP-3 and cErbB2
Pillai *et al.* (1996)	y	n	y	y	y	u	u	y	y	n	n	46	46	anti-transferrin and α2-HS glycoprotein antibodies
Pizzo *et al.* (2002)	y	n	y	y	y	u	u	y	y	y	y	26	5	TGFβ, IL-8 and MCP-1
Potlog-Nahari *et al.* (2004)	y	n	y	y	y	u	u	y	y	y	n	40	22	Urine VEGF
Pupo-Nogueira *et al.* (2007)	y	n	y	y	y	u	u	y	y	y	n	32	14	VEGF
Radwanska *et al.* (1987)	y	n	y	y	y	u	u	y	y	n	n	32	23	PRL
Rajkumar *et al.* (1992)	n	n	u	u	y	u	u	y	y	n	n	7	6	Anti-endometrial antibodies
Randall *et al.* (2007)	y	n	u	y	y	y	u	y	y	n	n	278	249	Anti-endometrial antibodies
Rosa e Silva *et al.* (2007)	y	n	y	y	y	u	u	y	y	y	y	148	53	CA125
Seeber *et al.* (2008)	n	n	u	y	y	u	u	y	y	n	n	63	78	IL-6, TNFα, MIF, MCP-1, IFNγ, leptin and CA125

Continued

Table III *Continued*

Paper	Modified QUADAS criteria											Number		Factors assessed
	1	2	3	4	5	6	7	8	9	10	11	Endo	Control	
Seeber *et al.* (2009)	n	n	u	y	y	u	u	y	y	n	n	63	78	Mass spec
Sha *et al.* (2009)	n	n	u	y	y	u	u	y	y	y	n	35	20	Gremlin-1
Shanti *et al.* (1999)	n	n	y	y	y	u	u	y	y	n	n	36	16	Autoantibodies to oxidative stress markers
Sharpe-Timms *et al.* (1998b)	n	n	u	y	y	u	u	y	y	y	n	8	8	TIMP-1 in serum
Somigliana *et al.* (2002)	n	y	y	y	y	u	u	y	y	y	y	71	49	ICAM-1
Somigliana *et al.* (2004)	n	y	y	y	y	u	u	y	y	n	y	45	35	CA125, CA19-9 and IL-6
Steff *et al.* (2004a)	n	n	y	y	y	u	u	y	y	y	y	176	198	ICAM-1
Steff *et al.* (2004b)	n	n	y	y	y	u	u	y	y	y	y	77	71	TNFR-1, angiogenin and IGF-I
Suzumori *et al.* (1999)	n	n	u	y	y	u	u	y	y	n	n	27	21	Leucocyte protease inhibitor
Szyllo *et al.* (2003)	n	n	u	y	y	u	u	y	n	n	n	60	30	T/B/NK cells and cytokines
Szymanowski (2007)	u	n	y	y	y	u	u	y	y	y	y	52	73	Progesterone
Takemura *et al.* (2005)	n	n	y	y	y	u	u	y	y	y	y	48	30	Adiponectin
Telimaa *et al.* (1989)	n	n	y	n	y	u	u	y	y	y	y	77	7	Serum PP14
Vercellini *et al.* (1992)	y	n	y	y	y	u	u	y	y	y	y	45	30	β-endorphin levels in PBMCs
Vercellini *et al.* (1993)	y	n	y	y	y	u	u	y	y	n	n	46	48	TNFα
Verit *et al.* (2008)	y	y	y	y	y	u	u	y	y	y	y	47	40	Serum paroxonase -1
Vigano *et al.* (2002)	y	y	y	y	y	u	u	y	y	n	n	42	25	Leptin
Wang *et al.* (2007)	n	n	y	n	y	u	u	y	y	n	n	16	16	Proteomics—mass spectrometry
Wang *et al.* (2008)	n	n	u	n	y	y	u	y	y	n	n	36	30	Proteomics—mass spectrometry
Wild and Shivers (1985)	y	n	u	y	y	u	u	y	y	n	n	34	38	Anti-endometrial antibodies
Wild *et al.* (1991a)	y	n	u	y	y	y	u	y	y	n	y	82	23	Anti-endometrial antibodies
Wild *et al.* (1991b)	y	n	u	y	y	y	u	y	y	n	n	77	52	Anti-endometrial antibodies and Ca125
Wolfler *et al.* (2009)	y	n	y	y	y	u	u	y	y	n	y	51	39	Proteomics—mass spectrometry
Wu *et al.* (1998)	y	n	y	y	y	u	u	y	y	n	n	36	35	ICAM-1 and IFNγ
Wu *et al.* (2003)	y	n	u	u	y	u	u	y	y	y	n	22	26	Leptin levels
Xavier *et al.* (2005)	n	n	u	y	y	u	u	y	y	y	n	25	13	CA125, CA19-9
Xavier *et al.* (2006)	n	n	u	y	y	u	u	y	y	y	n	25	13	VEGF, TNFα
Zachariah *et al.* (2009)	n	n	u	n	y	u	u	y	y	n	y	19	15	Circulating cell free DNA
Zeller *et al.* (1987)	y	n	u	y	y	u	u	y	y	y	y	19	6	reactive oxygen species production
Zhang *et al.* (2005)	n	n	y	y	y	u	u	y	y	y	y	22	22	IL-16
Zhang *et al.* (2006a)	n	n	y	y	y	u	u	y	y	n	n	56	68	KIR and HLA
Zhang *et al.*, (2006b)	u	n	u	y	y	u	u	y	y	y	n	6	6	Proteomics—mass spectrometry
Zhang *et al.* (2009)	n	n	u	n	y	y	u	y	y	n	n	48	32	Proteomics—mass spectrometry
Zong *et al.* (2003)	n	n	y	y	y	u	u	y	y	y	y	72	54	HGF

y, yes; n, no; u, unclear; Endo, number of endometriosis patients included; Control, number of control patients included.

interleukin 6 (IL-6) and tumour necrosis factor-alpha (TNFα), but the results from these (and other studies) have sometimes been conflicting.

Interleukin 6

IL-6 is a pro-inflammatory cytokine involved in the activation of T cells; it also promotes the differentiation of B cells (Kishimoto *et al.*, 1995). Six studies have indicated a link between raised serum levels of IL-6 and endometriosis (Pellicer *et al.*, 1998; Bedaiwy *et al.*, 2002; Darai *et al.*, 2003; Iwabe *et al.*, 2003; Martinez *et al.*, 2007; Othman *et al.*, 2008), but other studies have shown no link (Somigliana *et al.*, 2004; Kalu *et al.*, 2007; Jee *et al.*, 2008; Seeber *et al.*, 2008).

The accuracy of the test for diagnostic purposes varied in the six positive studies. Martinez *et al.* (2007) found elevated levels of serum IL-6, but only in women with Stages I–II disease yielding a sensitivity of 75% and specificity of 83.3% for disease of this severity, using a threshold of 25.75 pg/ml. A separate study used a much lower threshold point of 1.3 pg/ml: it yielded a sensitivity of 81%, with a specificity of only 51% to diagnose all women regardless of stage (Othman *et al.*, 2008).

Various differences between these studies may account for their very different findings. For example, some studies compare only women with ovarian cysts (endometriomas versus other benign cysts) (Jee et al., 2008). Others use different control groups: healthy, fertile controls or women with infertility other than that attributed to endometriosis (Kalu et al., 2007; Martinez et al., 2007). Furthermore, results may have been affected because of different assay sensitivities (Iwabe et al., 2003; Kalu et al., 2007). Finally, the stage of disease may considerably alter the cytokine levels: one study that showed no change in IL-6 levels between endometriosis patients and controls excluded women with Stage I disease from the analysis (Seeber et al., 2008). This resulted in the majority of women in their study being at Stages III–IV, which may therefore have affected their overall results.

Interleukin 8

IL-8 is a monocyte/macrophage-derived chemokine, capable of attracting and activating neutrophils (Baggiolini and Clark-Lewis, 1992). One paper found no difference in serum IL-8 levels in women with endometriosis compared with controls, and another reached the same conclusion for women with endometriomas (Gazvani et al., 1998; Darai et al., 2003). Kalu et al. (2007) reported a non-significant trend towards increased serum IL-8 levels in women with endometriosis . In another study, the serum levels were below the detection threshold of the test used (Othman et al., 2008).

Two studies have shown elevated IL-8 levels. The first demonstrated significantly higher levels in women with endometriosis versus controls (Pizzo et al., 2002). Interestingly, levels were higher in Stages I–II than in Stage III disease. Similarly, the second paper found increased IL-8 levels in women with endometriomas (Ohata et al., 2008).

Tumour necrosis factor-alpha

TNFα, a cytokine with pro-inflammatory and pro-angiogenic roles (Yan et al., 2006), appears in a variety of studies as a putative biomarker. Seven studies demonstrated an increase in TNFα levels in women with endometriosis (Markham and I., 1997a,b; Matalliotakis et al., 1997; Pizzo et al., 2002; Darai et al., 2003; Xavier et al., 2006; Cho et al., 2007), but four studies showed no such difference (Vercellini et al., 1993; Kalu et al., 2007; Othman et al., 2008; Seeber et al., 2008).

Two papers from the same group have shown elevated levels of serum TNFα in women with endometriosis compared with controls (Markham and I., 1997a,b). The first study also assessed TNFα levels in women with adhesions or a past history of endometriosis. These were not found to be significantly different to women with current endometriosis, raising concerns over the specificity of TNFα as a diagnostic test. The second (smaller) study showed that the elevated level was only significant in the group with an AFS (American Fertility Society) score of 11–20.

Matalliotakis et al. (1997) showed that serum TNFα levels were elevated in women with endometriosis compared with healthy controls and that levels were reduced by treatment with danazol for 6 months. Three months after discontinuation of treatment, TNFα levels were no longer significantly lower than pretreatment levels. A more recent study found a similar serum TNFα increase in endometriosis, but no increase in urinary TNFα levels (Cho et al., 2007). Analysis of subgroups showed no difference in serum levels between controls and women with Stages I–II disease, but showed a significant increase in women with Stages III–IV endometriosis. One further paper demonstrated an apparent association of increasing TNFα levels and worsening stage of disease (Pizzo et al., 2002). Infertility could be a confounding factor when assessing TNFα levels, as women with endometriosis had equivalent levels to those with idiopathic infertility, both of which were higher than in controls (Bedaiwy et al., 2002).

Two papers identified raised serum TNFα levels in women with endometriomas. The first study found significantly higher TNFα concentrations in women with endometriomas than in those with benign cysts, but no difference compared with levels in women with malignant cysts (Darai et al., 2003). The second paper reported higher levels in women with endometriomas compared with healthy controls (Xavier et al., 2006).

Vercellini et al. (1993) found no difference in serum TNFα levels between women with and without endometriosis, although the levels were below the detection limits of their assay in >80% of subjects. However, other groups have also reported no significant change in TNFα levels (Kalu et al., 2007; Seeber et al., 2008), even after stratifying women into two groups based on stage of disease (Othman et al., 2008).

Finally, one study measured levels of soluble TNF receptor in women with endometriosis and found a significant elevation in these levels during the follicular phase of the cycle (Steff et al., 2004b).

Monocyte chemotactic protein 1

It is known that macrophages play a role in endometriosis, and numbers of peritoneal macrophages are often increased in women with the disease compared with controls (Olive et al., 1985). One study found significant monocyte chemotactic protein 1 (MCP-1) increases but only during the luteal phase (Akoum et al., 1996): a threshold of 100 pg/ml gave a sensitivity of only 65% with 61% specificity. Disease stage may also affect the diagnostic value of this cytokine, although one study has shown higher levels in early disease stages (Pizzo et al., 2002) and another demonstrated higher values in more severe stages (Gmyrek et al., 2005).

Othman et al. (2008) studied MCP-1 as part of a panel of potential biomarkers. Although MCP-1 levels were higher in women with disease, IL-6 was a better discriminator; the use of MCP-1 and IL-6 together did not improve sensitivity or specificity. Two further studies did find MCP-1 to be of use in a panel (Agic et al., 2008; Seeber et al., 2008). The first used MCP-1 with cancer antigen 125 (CA125) and cognate chemokine receptor 1 (CCR1) mRNA levels (Agic et al., 2008). The second generated a sensitive and specific biomarker panel using MCP-1 in combination with leptin and CA125 (Seeber et al., 2008).

Interferon gamma

Despite the pro-inflammatory nature of interferon gamma (IFNγ), most studies have failed to find a correlation between peripheral blood levels and endometriosis. Two studies failed to demonstrate any change in serum IFNγ levels in women with endometriosis, using ELISA (Wu et al., 1998; Hassa et al., 2009). One paper identified increased IFNγ levels in affected women versus controls, but noted

that IL-6 was a better discriminator (Othman *et al*., 2008); another study attempted to assess IFNγ but levels were below the detection threshold for the assay used (Seeber *et al*., 2008).

A recent study looked at levels of CXCL10, also known as IFNγ-inducible protein-10, which is involved in TH1-type immune responses (Galleri *et al*., 2009). Reduced serum levels were found in women with endometriosis compared with healthy controls.

Other cytokines

One study found elevated levels of IL-1α and IL-1 receptor antagonist, and decreased levels of IL-1 soluble receptor type II, in the serum of women with endometriosis (Kondera-Anasz *et al*., 2005). These differences showed an association with stage of disease.

Levels of IL-1β in infertile women with endometriosis have been found to be equivalent to those in women with tubal factor infertility (Pellicer *et al*., 1998). This finding is supported by more recent studies (Bedaiwy *et al*., 2002; Kalu *et al*., 2007).

No difference in serum IL-2, -4 or -10 levels has been found (Hassa *et al*., 2009). One further paper found IL-2 levels to be undetectable with their assay (Othman *et al*., 2008).

In some studies, IL-12 and -18 have been found to be elevated in peritoneal fluid of women with endometriosis (Arici *et al*., 2003; Gallinelli *et al*., 2004). However, no change in serum IL-18 levels has been identified (Fairbanks *et al*., 2009). Overall, IL-12 levels were no different in women with endometriosis, although levels were higher in women with Stages III–IV than Stages I–II disease (Fairbanks *et al*., 2009). Another study failed to find a correlation between IL-12 levels and endometriosis (Bedaiwy *et al*., 2002).

Serum IL-13 levels have not been found to alter with endometriosis (Bedaiwy *et al*., 2002). IL-15 levels were assessed in one study, but levels were below the detection threshold for the assay used (Othman *et al*., 2008).

IL-16 is known to be involved in regulation of the TH1/2 balance (Center *et al*., 1997), but no association has been found between serum levels and endometriosis (Zhang *et al*., 2005). Increased levels of serum TGFβ have been reported in women with endometriosis (Pizzo *et al*., 2002). Furthermore, the concentration appeared to correlate with stage of disease, such that the highest levels were found in women with more severe disease.

Levels of RANTES (regulated on activation normal T cell expressed and secreted) have been measured without finding any significant differences (Markham and I., 1997b). This result was supported by a separate study (Kalu *et al*., 2007).

Serum levels of macrophage migration inhibitory factor (MIF) have also been studied (Morin *et al*., 2005; Seeber *et al*., 2008). Levels were significantly higher in women with endometriosis, especially in women with more advanced disease stages, in one report (Morin *et al*., 2005). However, the second paper was unable to identify a difference in this cytokine between women with and without the disease (Seeber *et al*., 2008).

Intracellular cytokines

Some studies have looked at intracellular levels of cytokines in various cell populations to try to identify differences between women with and without endometriosis. One study looked at intracellular staining of a variety of cytokines (TNFα, IFNγ, IL-2, -4, -6, -10 and -12) and found increased expression of TNFα and IL-6 in peripheral T cells of women with endometriosis. (Szyllo *et al*., 2003). A similar study looked at a panel of cytokines (TNFα, IFNγ, IL-8, IL-6, IL-10 and MCP-1) and assessed their intracellular expression after stimulating different cell types (Gmyrek *et al*., 2008). The authors found that cytokine levels were similar in women with and without disease. The only statistically significant differences were in women with advanced disease who showed a reduction in IFNγ levels in $CD3^{+}CD8^{-}$ cells, and an increase in MCP-1 levels in $CD14^{+}$ cells.

Other studies have used RT–PCR and western blotting to identify intracellular mRNA and protein concentrations. Two studies showed increased IL-4 mRNA and protein in peripheral blood mononuclear cells of women with endometriosis, but no change in IL-2 (Hsu *et al*., 1997; Antsiferova *et al*., 2005). However, the first of these studies showed an increase in IL-10 mRNA and protein, whereas the second identified unchanged IL-10 levels in women with endometriosis. Finally, one study has found equivalent IFNγ mRNA levels in peripheral mononuclear cells of women with and without disease (Hsu *et al*., 1997).

Two studies reported mRNA levels of CCR1 (a RANTES receptor) in peripheral blood leucocytes of women with endometriosis (Agic *et al*., 2007; Agic *et al*., 2008). The first used CCR1 mRNA levels alone as a diagnostic test and found good sensitivity and specificity (90% and 74%, respectively) (Agic *et al*., 2007). The second combined CCR1 mRNA levels with MCP-1 and CA125 measurements to try to increase the test's diagnostic accuracy (Agic *et al*., 2008). Sensitivity and specificity were improved to 92% and 82%, respectively, by using this biomarker panel.

Antibodies

A great deal of interest has focused on circulating antibodies that may be a marker of endometriosis or involved in disease pathogenesis, especially because the interaction of the immune system with the endometrium is thought to have a major influence on how, and if, the disease develops.

Total immunoglobulin

Two studies have assessed total immunoglobulin levels in women with and without endometriosis, but found them unaffected by disease (El-Roeiy *et al*., 1988; Confino *et al*., 1990). However, El-Roeiy and colleagues did identify a positive correlation between increasing disease stage and IgG and IgM levels; they also noted a significant reduction in all immunoglobulin subtypes studied (IgG, IgM and IgA) after treatment with danazol for 6 months. A separate study found reduced IgA levels throughout the cycle, and reduced IgG in the follicular phase, but no significant change in IgM levels (Meek *et al*., 1988).

Anti-endometrial antibodies

Studies aimed at identifying circulating antibodies directed against endometrial antigens commenced in the early 1980s with the work of Methur (Methur *et al*., 1982). Later, Wild and Shivers (1985) analysed peripheral blood from women with infertility and found antibodies to be more common in women with, than in those without, endometriosis. Similar results were found by later studies (Badawy *et al*., 1990; Garza *et al*., 1991; Wild *et al*., 1991b; Hatayama *et al*., 1996; Meek *et al*., 1988; Bohler *et al*., 2007). One study reported a sensitivity of 86% and specificity of 76% for the diagnosis of endometriosis in women with infertility or other gynaecological pathology

(Wild et al., 1991a). When compared with CA125, the diagnostic accuracy of endometrial antibody testing appeared favourable with a sensitivity of 83% and specificity of 79% versus 27 and 83%, respectively, for CA 125 (Wild et al., 1991b). Another study showed the presence of anti-endometrial antibodies in the serum of women with and without endometriosis, but demonstrated reactivity to different antigens in each group (Rajkumar et al., 1992).

It appears that IgG shows the strongest correlation with disease (Mathur et al., 1990; Odukoya et al., 1995a). One study identified anti-endometrial IgG antibodies in 56% of endometriosis patients, but only 5% of healthy control women (Odukoya et al., 1996). Another study demonstrated the presence of anti-endometrial IgG antibodies in 33% of women with endometriosis and anti-endometrial IgM antibodies in 27% (Gajbhiye et al., 2008).

The potential value of anti-endometrial antibodies as a diagnostic test has been revisited recently in a prospective multi-centre study (Randall et al., 2007). The authors tested for anti-endometrial antibodies using indirect immunofluorescence in a large group of women who consulted a medical practitioner for infertility, chronic pelvic pain or dysmenorrhoea: the sensitivity and specificity were both 87%.

Autoantibodies may also be affected by treatment: levels of nine types of autoantibody were reduced by 6 months treatment with danazol (El-Roeiy et al., 1988).

Specific antibodies

Other researchers have tried to determine the exact nature of autoantibodies in endometriosis. One early study looked for antibodies against progestagen-associated endometrial protein (PEP) and endometrial glycoproteins, but was unable to identify these autoantibodies in either patients or controls (Joshi et al., 1986).

Some studies have looked at antibodies directed against carbonic anhydrase. The first of these demonstrated the presence of antibodies against human carbonic anhydrase in women with endometriosis (Kiechle et al., 1994). A further study showed significantly higher levels of anti-carbonic anhydrase I antibodies in women with all disease stages compared with controls (D'Cruz et al., 1996). Antibodies against carbonic anhydrase II were also detected, but these were only significantly elevated in women with Stage II disease. However, the absolute numbers of women with strongly positive sera were relatively low in the endometriosis group. This study also measured levels of other common autoantibodies, including antinuclear, anti-DNA and anti-RNA protein antibodies (extractable nuclear antigens and anti-Ro). Several women with positive anti-carbonic anhydrase antibodies had significant titres of anti-Ro or ENA, but no analysis was done to indicate whether these may also be relevant tests for women with endometriosis.

Pillai et al. (1996) used western blotting and sequencing to identify transferrin, collagen, albumin, IgG and α2-Heremans Schmidt glycoprotein (α2-HS glycoprotein) as potential autoantigens in endometriosis. Antibodies to collagen, albumin and IgG were infrequent, but 89% of endometriosis patients had antibodies against transferrin (versus 28% of controls); 86% of patients had antibodies to α2-HS glycoprotein (versus 38% of controls). These figures correspond to sensitivities of 89% and 86% and specificities of 72% and 63%, respectively, for transferrin and α2-HS glycoprotein antibodies. The same group followed this up with a larger study using ELISA detection of antibodies, and again found significantly elevated antibody levels against these two antigens (Mathur et al., 1998). The sensitivity and specificity for both tests was 95% or greater.

Another group has assessed autoantibodies directed against markers of oxidative stress (Shanti et al., 1999). Their study identified significantly increased levels of three autoantibodies against lipid peroxide modified rabbit serum albumin, copper oxidized low-density lipoprotein and malondialdehyde-modified low-density lipoprotein in women with endometriosis.

Anti-laminin-1 antibodies have previously been found to be associated with recurrent miscarriage (Inagaki et al., 2001). Levels were increased in infertile women compared with fertile controls and were found to be significantly associated with endometriosis (Inagaki et al., 2003).

Anti-cardiolipin antibodies have also been measured (Kilpatrick et al., 1991; Abrao et al., 1997). One study showed no significant difference in levels between women with and without endometriosis, regardless of stage or fertility status (Kilpatrick et al., 1991). However, a second study found IgM anti-cardiolipin at significantly increased titres in women with endometriosis, but there was no difference in IgG levels (Abrao et al., 1997).

In summary, some autoantibodies appear promising candidates as biomarkers for endometriosis. However, further work is required to elucidate which antigens are triggers for some of these autoantibodies, to refine laboratory testing for the disease.

Cell populations

One of the mechanisms that is probably involved in the development of endometriotic lesions is the interaction between sloughed endometrial tissue and the immune system. Consequently, various populations of immune cells have been studied to gain insights into the disease pathogenesis, and test their utility as biomarkers.

T cells

T lymphocytes are critical players in cell-mediated, adaptive immune responses, and their levels and markers have been subject to scrutiny in women with endometriosis. One of the earliest studies demonstrated increased numbers of both T and B lymphocytes, as well as an increase in the CD4:CD8 ratio in women with endometriosis (Badawy et al., 1987). A later study failed to verify this change in lymphocyte numbers, but did show alterations in specific subsets of T cells, with increased numbers of suppressor ($CD8^+$, $CD11b^+$) and activated ($CD3^+$, $HLA\text{-}DR^+$) T cells and reduced numbers of cytotoxic T cells ($CD8^+$, $CD11b^-$) (Iwasaki et al., 1993). These studies used very different methods to assess cell numbers (rosette formation with sheep erythrocytes versus flow cytometry), which probably accounts for their different findings. Other studies using flow cytometry techniques have also failed to identify differences in T cell numbers (using anti-CD3 antibodies) or major subsets ($CD4^+$ or $CD8^+$) (Garzetti et al., 1993; Oosterlynck et al., 1994; Ho et al., 1995; Maeda et al., 2002b; Zhang et al., 2006a; Gmyrek et al., 2008; Hassa et al., 2009). One of these studies in fact found a decrease in the activated T cell subset in women with the disease ($CD25^+CD3^+$ cells) (Ho et al., 1995).

One study has assessed changes in lymphocyte subsets following surgery (Kikuchi et al., 1993). There was a significant increase in

peripheral blood levels of suppressor ($CD8^+CD11^+$), suppressor inducer ($CD4^+4B4^-$) and helper T cells ($CD4^+2H4^+$) 1 month after a wide range of operations (ovarian cystectomy to total abdominal hysterectomy), but lymphocyte subsets did not change in a control group having surgery for leiomyomas.

$CD4^+CD25^+$ T cells were first identified in 1995 as a cell population capable of reducing the occurrence of autoimmunity (Sakaguchi *et al*., 1995). No differences in the proportion of these cells have been found in women with endometriosis (Górski *et al*., 2007). However, this study did show a reduction in the percentage of $CD8^+$ cells and a subsequent increase in the $CD4^+$:$CD8^+$ ratio. These findings were confirmed in a separate study (Szyllo *et al*., 2003).

Finally, Gagne *et al*. (2003b) have cautioned against using data that have not been adjusted for important confounders, such as age, use of oral contraceptives and history of acute infection. Their study identified a significant decrease in both total T cell levels and a population of activated T cells ($CD3^+$HLA-DR^+), but these changes were not seen after adjusting for confounders.

B cells

The possible role of autoantibodies in endometriosis suggests that B cell populations may be important in the development of disease. However, several studies have failed to identify a difference in B cell levels when comparing healthy women to those with endometriosis (Iwasaki *et al*., 1993; Oosterlynck *et al*., 1994; Ho *et al*., 1995; Maeda *et al*., 2002a; Zhang *et al*., 2006a). One study in fact found a small but significant reduction in peripheral B cell numbers in women with the disease (Szyllo *et al*., 2003).

It is possible that subsets of B cells may be altered in women with endometriosis. One study has looked at levels of B-1 cells, a group of B cells involved in the production of autoantibodies, which express CD5 (Chishima *et al*., 2000). There was no overall difference in B-1 cell levels; however, when the women with endometriosis were subdivided into those with and without antinuclear autoantibodies (ANA), they found significantly increased B-1 cell levels in women who were ANA positive. Another study identified decreased numbers of $CD20^+$, $CD20^+$HLA-DR^+ (activated B cells) and $CD20^+CD44^{high+}$ cells in women with endometriosis, even after adjusting for important confounders (Gagne *et al*., 2003b). However, this finding was not supported by a more recent study, which found equivalent numbers of total B cells, B-1 cells and $CD20^+$HLA-DR^+ B cells in women with and without endometriosis (Antsiferova *et al*., 2005).

Natural killer cells

Natural killer (NK) cells have been a focus of intense research in endometriosis. For example, studies have looked at cytotoxicity by measuring the lytic effects on target cells, but whether there is any correlation with disease stage remains controversial (Oosterlynck *et al*., 1991; Garzetti *et al*., 1993; Ho *et al*., 1995).

Several studies have failed to identify different levels of NK cells in peripheral blood (Garzetti *et al*., 1993; Iwasaki *et al*., 1993; Oosterlynck *et al*., 1994; Ho *et al*., 1995; Gagne *et al*., 2003b; Zhang *et al*., 2006a; Hassa *et al*., 2009). However, we identified two studies that have reported a difference in NK cell numbers. The first showed a reduction only in the $CD57^+CD16^+$ subset of moderately differentiated NK cells (Kikuchi *et al*., 1993). There was also a significant increase in this subset of NK cells 1 month after surgery for endometriosis. The second study identified a small reduction in the percentage of NK cells in peripheral blood of women with endometriosis (Szyllo *et al*., 2003).

Expression of a killer inhibitory receptor (KIR), known as KIR2DL1, on NK cells has been found to be increased in women with endometriosis, perhaps accounting for their decreased activity against target cells (Maeda *et al*., 2002a). No difference in the expression of KIR2DL2 was identified. The same group confirmed this finding in further studies, when they also found that the elevated levels persisted for at least 1 month after laparoscopic surgery or after 12 weeks of GnRH analogue treatment (Maeda *et al*., 2002b; Maeda *et al*., 2004). Similar findings were reported later confirming increased expression of CD158a (a form of KIR) on peripheral blood NK cells (Zhang *et al*., 2006a).

Macrophages/monocytes

No changes in peripheral $CD14^+$ macrophage numbers have been identified in women with endometriosis (Oosterlynck *et al*., 1994). Similarly, Zhang *et al*. (2006a) reported similar macrophage numbers in women with and without disease and found no differences in the expression of HLA-ABC, -DR or a variety of other co-stimulatory molecules (CD40, CD54, CD58, CD80, CD86). A more recent study also found no difference in the expression levels of various markers on the macrophage surface (CD14, ICAM-1 and HLA-DR) as measured by relative fluorescence intensity (Izumiya *et al*., 2003). One study did find a significant increase in the $CD14^+CD44^{high+}$ leucocyte subset (Gagne *et al*., 2003b). CD44 is an adhesion molecule involved in cell homing to sites of inflammation (Jalkanen *et al*., 1986), so this may reflect the pro-inflammatory nature of endometriosis.

Functional studies on monocytes have also been conducted. One study used chemiluminescence to assess the generation of reactive oxygen species by macrophages (Zeller *et al*., 1987). The authors showed no difference in resting monocyte activity, but saw increased activity in response to various stimuli (e.g. serum-opsonized zymosan), in monocytes from women with endometriosis.

Polymorphonuclear neutrophils

One study showed no change in absolute numbers of polymorphonuclear neutrophils (PMN), but did reveal a small but significant reduction in chemotactic index (i.e. PMN from women with endometriosis showed reduced chemotaxis in response to a stimulus than those from control women) (Garzetti *et al*., 1998). More recently, the ratio of neutrophils:lymphocytes (NLR) has been suggested as a diagnostic test (Cho *et al*., 2008). This study identified an increase in total white blood cell levels, and a particular increase in neutrophil levels in endometriosis. The NLR gave a sensitivity and specificity of 60%. Furthermore, combining NLR and CA125 levels gave improved sensitivity over either test alone, but with slightly reduced specificity compared with CA125 alone. This recent study demonstrates the continued interest and effort placed into identifying possible cell-type alterations in endometriosis, which itself reflects the widespread view that immunological alterations lie at the heart of this disease.

Other immunology

One early study identified significant increases in levels of C3c and C4, but no difference in Factor B or properdin (components of the alternative complement pathway) in women with endometriosis (Badawy

et al., 1984). Elevated levels of C3c and C4 were also found in a more recent study, along with an increase in SC5b-9—the membrane attack complex (Kabut et al., 2007). One study has reported lower levels of C3 and C4 in women with endometriosis, during the follicular phase (Meek et al., 1988). A further study looked at levels of C3a, a proteolytic fragment of the complement pathway that induces inflammatory reactions, but found levels to be similar in infertile women with and without endometriosis (Fassbender et al., 2009).

Significant reductions in peripheral mononuclear cell β-endorphin levels have been noted in women with endometriosis, particularly in the luteal phase (Vercellini et al., 1992). However, the finding only applied to symptomatic women with endometriosis when compared with symptomatic controls; healthy controls and asymptomatic women with endometriosis had equivalent levels.

Levels of soluble CD4 have been found to be increased in endometriosis, but levels of soluble CD8 appear to be unchanged by the disease (Matalliotakis et al., 1997).

Three studies have looked at levels of CD23, an IgE receptor that also exists in a soluble form (Gordon, 1991). All have shown raised levels of soluble CD23 in peripheral blood of women with endometriosis (Odukoya et al., 1995b; Odukoya et al., 1996; Matalliotakis et al., 2000b). Two of these studies also revealed a reduction in CD23 levels during treatment (Odukoya et al., 1995b; Matalliotakis et al., 2000b).

Levels of CD163, a macrophage scavenger receptor that also exists in a soluble form, were similar in women with endometriosis and healthy controls (Jee et al., 2008).

Three studies have investigated soluble HLA in endometriosis. The first of these showed that, overall, there was no association between endometriosis and serum soluble HLA-1 concentrations, although women with Stages I–II disease had significantly higher levels than those with Stages III–IV (De Placido et al., 1998). The second study found that levels of soluble HLA class I and II were significantly lower in women with endometriosis than control women (Matalliotakis et al., 2001b). One explanation for the contradictory findings is that different controls were used in these studies: women with various gynaecological complaints, including infertility, Müllerian malformations and those attending for tubal ligation were used in the first and infertile women only in the second. However, a more recent study has also demonstrated reduced HLA class I and II levels in serum of women with endometriosis, using slightly larger numbers of women, suggesting that there may be a true difference (Matalliotakis et al., 2003) (38 women with endometriosis, 30 controls with pelvic pain but no disease).

One group has measured levels of secretory leucocyte protease inhibitor in the serum of women with and without endometriosis, but there was no significant difference in levels (Suzumori et al., 1999).

Glycoproteins

Many authors have assessed levels of a variety of serum glycoproteins as potential diagnostic tools. The majority of these studies have looked at glycoproteins which have been assessed in the past as 'tumour markers' because of their association with malignant disease.

Cancer antigen 125

The most consistently studied glycoprotein in endometriosis has been CA125. A very comprehensive meta-analysis was published over 10 years ago, which found that CA125 may be of more benefit in diagnosing Stages III–IV, than Stages I–II, disease (Mol et al., 1998). Hence, studies published before 1998 were not included in our review.

Studies published since continue to demonstrate a correlation between raised CA125 levels and endometriosis (Abrao et al., 1999; Somigliana et al., 2004; Agic et al., 2008; Seeber et al., 2008), and some imply a correlation with stage of disease (Chen et al., 1998; Amaral et al., 2006; Martinez et al., 2007; Rosa e Silva et al., 2007). One study has indicated that CA125 may be more accurate at diagnosing women with later stages of disease, in concordance with the review by Mol (Maiorana et al., 2007).

One interesting study looked at fluctuations in CA125 levels across the menstrual cycle, and whether alterations in this flux could be used as a possible diagnostic tool (Kafali et al., 2004). Infertile women without endometriosis tended to have slight elevations in serum CA125 levels during menstruation; however, the magnitude of this increase was much greater in women with endometriosis. A sensitivity of 93% and specificity of 92% for the diagnosis of endometriosis was achieved, using a threshold of an 83% increase in CA125 during menses. The use of cycle fluctuations in CA125 has not yet been assessed in women without infertility.

A difficulty that has been noted in the past with CA125 is that levels tend to be higher in women with endometriomas: the accuracy CA125 certainly seems significantly better for women with, compared with those without, endometriomas (sensitivity 79% versus 44% with 30 IU/ml threshold) (Kitawaki et al., 2005). CA125 may also be helpful in the detection of unusual presentations of endometriosis: for example, measuring CA125 levels in women presenting with recurrent pneumothorax may identify thoracic endometriosis (Bagan et al., 2008).

The type of assay used to detect CA125 may also affect its clinical performance. One method known as CA125II (O'Brien et al., 1991), which uses a combination of two monoclonal antibodies that bind at different sites, could not distinguish women with Stages I–II disease from those without, although it did identify women with Stages III–IV endometriosis (Abrao et al., 1997).

Some studies have assessed the value of CA125 measurements during treatment. Chen et al. (1998) found that CA125 levels fell significantly after 3 months treatment with danazol. Ten women in this study underwent a 'second-look' laparoscopy while on treatment with danazol; however, despite having normal CA125 measurements, all of the women still had laparoscopic evidence of disease (albeit at a lesser stage than pre-treatment). A further study looked at the effect of leuprolide acetate and danazol on CA125 levels (Matalliotakis et al., 2004). Reduced levels were seen during treatment with both drugs. Three months after treatment, levels tended to rise again, but were still significantly reduced compared with pre-treatment levels in the danazol group.

CA19-9

In the first study to assess CA19-9, Panidis et al. (1988) reported that baseline levels of CA19-9 were elevated above the usual normal range (<37 IU/ml) in five of eight women with endometriosis, although no control group was studied. Levels were found to drop significantly during treatment with danazol. A similar study was conducted in 1998, which again demonstrated elevated baseline CA19-9 levels and a significant decrease during treatment with danazol (Matalliotakis

et al., 1998). Levels rose 3 months after treatment was completed, but remained significantly lower than baseline due perhaps, the authors postulated, to disease recurrence. Other studies have failed to find an association between raised CA19-9 levels and endometriosis (Abrao et al., 1999; Somigliana et al., 2004).

Several studies have attempted to compare CA19-9 and CA125 measurements. Harada et al. (2002) found that CA19-9 had similar specificity but reduced sensitivity compared with CA125. The authors did note that CA125 levels tend to be increased in women with adenomyosis, whereas CA19-9 levels were often normal in these women. In a more recent study, CA125 and CA19-9 had similar sensitivities and specificities (86–89% and 61–52%, respectively) (Kurdoglu et al., 2009).

Xavier et al. (2005) have highlighted the importance of choosing the correct threshold for a putative biomarker. These authors calculated cut-off values using ROC curves (a comparison of sensitivity against false-positive rate) to identify the level at which most women were correctly diagnosed: 22.6 IU/ml for CA125 and 5.4 IU/ml for CA19-9—much lower levels than those often reported in the literature. At these thresholds, the sensitivity and specificity of both CA125 and CA19-9 were greatly improved.

CA15-3

We identified three papers that considered CA15-3 levels in endometriosis. The first reported elevated levels, compared with the 'normal' range, in six of eight women with endometriosis (Panidis et al., 1988). Levels fell during treatment with danazol and were significantly reduced from baseline 3 months after treatment ended. The lack of control group means that sensitivity/specificity could not be calculated.

Two further papers found no association between CA15-3 and endometriosis (Muscatello et al., 1992; Abrao et al., 1999). Hence, it seems unlikely that CA15-3 has potential as a diagnostic test.

CA-72

CA-72 (also known as TAG72) has been studied in two papers, neither of which found any association with endometriosis (Muscatello et al., 1992; Molo et al., 1994).

Other glycoproteins

As autoantibodies to transferrin and α2-HS glycoprotein have been identified in women with endometriosis (see Antibodies section), one group investigated serum levels of these glycoproteins and found significantly reduced levels of serum transferrin and increased levels of α2-HS glycoprotein in women with endometriosis (Mathur et al., 1999).

Serum levels of alpha-fetoprotein (AFP) have been measured in endometriosis, but not shown to be different in women with and without disease (Abrao et al., 1999; Philippoussis et al., 2004). Carcinoembryonic antigen (CEA) and beta-2 microglobulin levels were also measured in one of these studies, but the findings were similarly unhelpful (Abrao et al., 1999).

Haptoglobin has been identified as a secretory product of endometriotic lesions (Sharpe-Timms et al., 1998a) leading to the study of haptoglobin-β (Hpβ) chain isoforms (Ferrero et al., 2005). Serum HpβE levels were significantly higher in women with endometriosis than controls, during the follicular phase of the cycle, although levels of the other isoforms were unchanged.

Follistatin is a glycoprotein involved in the inhibition of activin (de Winter et al., 1996). One study has shown significant increases in serum follistatin levels in women with endometriosis and endometriomas compared with controls (Florio et al., 2009). Follistatin had superior specificity and sensitivity than CA125 levels.

Gremlin-1 is a secreted glycoprotein that has previously been found to be up-regulated in the endometrial stromal cells of women with endometriosis (Sha et al., 2007). One study has found increased Gremlin-1 levels in the serum of women with endometriosis, but the difference was only significant during the proliferative phase of the cycle (Sha et al., 2009).

Cell adhesion

A variety of factors involved in cell adhesion have been studied in endometriosis. These may have important roles in cell–cell interaction or other accessory roles.

Intracellular adhesion molecule-1

The first studies to assess this protein found conflicting results. One showed a significant increase in soluble intracellular adhesion molecule-1 (ICAM-1) levels in plasma of women with endometriosis (Wu et al., 1998), whereas the other showed no change (De Placido et al., 1998). However, these differences may have been stage dependent: the first study mainly recruited women with Stages I–II disease, whereas the second mainly looked at women with Stages III–IV. Increased levels of ICAM-1 with Stages I–II disease have also been reported more recently (Matalliotakis et al., 2001b). In this study, levels tended to rise further during treatment (danazol or leuprolide acetate) and were still significantly higher than baseline 3 months after treatment stopped. Only one study reported an increase in serum ICAM-1 levels in women with Stages III–IV disease (Daniel et al., 2000). Another paper showed a significant increase in levels in women with deep pelvic endometriosis only (Somigliana et al., 2002).

Conversely, a reduction in serum ICAM-1 levels in women with Stages III–IV endometriosis has also been shown (Barrier and Sharpe-Timms, 2002). Hence, it is possible that levels increase during the early stages of the disease, but decrease at more advanced stages. However, the importance of confounders has been highlighted (Steff et al., 2004a). These authors identified a reduction in ICAM-1 levels in women with Stages III–IV disease, but this was not apparent after adjusting for the surgical indication or for infertility.

Other

Soluble E-cadherin levels in endometriosis have been found to be elevated in women with endometriosis, but no association with stage of disease was noted (Fu and Lang, 2002).

One study has measured levels of osteopontin, a glycoprotein involved in interactions between integrins, known to be expressed in the endometrium (Cho et al., 2009). Plasma levels were elevated in women with endometriosis throughout the cycle, although they did not correlate with disease stage. The authors calculated a sensitivity of 93% and specificity of 72% for the test.

Raised serum levels of soluble VCAM-1 were identified in one study (Barrier and Sharpe-Timms, 2002), consistent with another study that

showed a trend which did not reach statistical significance (Daniel et al., 2000). P-selectin and E-selectin levels were unchanged in women with endometriosis in these two studies (Daniel et al., 2000; Barrier and Sharpe-Timms, 2002).

Growth factors

One study has showed a significant increase in insulin-like growth factor-I (IGF-I) levels in Stages III–IV, but not Stages I–II, disease (Gurgan et al., 1999), whereas another showed no significant difference from control patients (Steff et al., 2004b). Levels of IGF-II have not been found to be altered in the disease (Gurgan et al., 1999).

IGFBP3 is a protein that regulates the transport of IGF and influences the growth of endometrial cells (Koutsilieris et al., 1995). However, two studies have demonstrated no significant difference in serum IGFBP3 levels between women with and without endometriosis (Gurgan et al., 1999; Philippoussis et al., 2004).

Levels of granulocyte macrophage colony-stimulating factor (GM-CSF), a growth factor that stimulates stem cells to produce granulocytes and monocytes, were unchanged in women with endometriosis compared with controls (Othman et al., 2008).

Proteomics

In the area of biomarker discovery, proteomic techniques are proving particularly powerful tools to identify protein 'fingerprints' in blood or tissues that may be markers of disease. From patterns of expression, individual peptides or proteins that are present or absent (or up- or down-regulated) in various disease states can be identified and assessed as possible biomarkers. Alternatively, the actual protein/peptide pattern itself can be used as a distinctive marker of disease presence.

The search for differential serum protein expression in endometriosis commenced more than 20 years ago (Joshi et al., 1986): however, gel electrophoresis could not distinguish serum from women with and without disease in this study. More recently, Zhang et al. (2006b) have identified 13 differentially expressed proteins in sera of women with endometriosis, using two-dimensional gel electrophoresis. Some of these proteins were characterized by searching a computerized database using molecular weight and isoelectric points, but some remain elusive.

A variety of groups are now attempting to identify specific peptide and protein patterns to diagnose endometriosis, mainly using mass spectrometry. Different serum protein peaks have been identified using this technique (Liu et al., 2007). Twenty protein peaks were found to be different between women with and without the disease; three of these were used to generate a diagnostic model with 88% sensitivity and 86% specificity.

Another group used a similar technique and surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS) to identify 288 differently expressed peaks (Wang et al., 2007). A further study by the same group showed that a distinctive pattern of five protein peaks gave a sensitivity of 92% and a specificity of 90.0% (Wang et al., 2008).

A third group, using the same technique, reported a sensitivity of 87% and specificity of 97% for their diagnostic algorithm, which was based on just two protein peaks (Jing et al., 2008). This study also found that one of these two peaks was significantly altered 1 month after surgery, suggesting that it may be a possible marker of disease stage or activity.

Three further studies were published last year, all evaluating mass spectrometry of serum proteins in endometriosis, but these have generally shown less diagnostic accuracy then the initial studies. One identified 24 differently expressed protein peaks, and generated a diagnostic model with 92% sensitivity, but only 75% specificity (Zhang et al., 2009). A further study looked only at women with symptoms consistent with endometriosis (e.g. pelvic pain, infertility) and used two different analytic methods to distinguish endometriosis from non-endometriosis subjects (Wolfler et al., 2009). However, these two methods only gave a sensitivity of 78–81% and specificity of 59–50%. The final study we identified used only proteins of small molecular mass, and identified six discriminatory peaks (Seeber et al., 2009). This model correctly diagnosed 66% of the women with endometriosis and 45% of those without the disease.

Proteomic technologies are providing innovative ways to identify biomarkers of disease and may be of use in identifying diseases by their protein fingerprints. However, the time and cost associated with these technologies currently prohibit their widespread use.

Hormones

Prolactin

An association between galactorrhoea and endometriosis was first identified over 30 years ago (Hirchowitz et al., 1978), which led to further investigation of the role of prolactin (PRL) in endometriosis. Although one study indicated a higher basal prolactin level in women with endometriosis (Acien et al., 1989), others have failed to confirm this finding (Arumugam, 1991; Panidis et al., 1992; Matalliotakis et al., 1996). Timing of sampling is particularly important for prolactin, as levels have a diurnal pattern. One study showed that the 8 am decline in prolactin levels (seen in healthy women) failed to occur in women with endometriosis (Radwanska et al., 1987).

Two more recent studies have revisited this issue. The first assessed prolactin levels in fertile controls, as well as fertile and infertile women with Stages I–II endometriosis (Cunha-Filho et al., 2001). Significantly higher prolactin levels were found in women with endometriosis, compared with controls: 30% of the women with endometriosis-associated infertility had hyperprolactinaemia (>20 ng/ml), but none of the controls or fertile women with endometriosis. The second study showed significantly higher prolactin levels in infertile women with all stages of endometriosis compared with fertile controls (Lima et al., 2006).

Three studies have assessed the response of serum prolactin levels to a challenge with thyrotropin releasing hormone (TRH), and particularly the effect of treatment on this (Acien et al., 1989; Panidis et al., 1992; Matalliotakis et al., 1996). The first of these studies found that the prolactin response, following a challenge with TRH and luteinising hormone (LH) releasing hormone, was greater in women with endometriosis than controls (Acien et al., 1989). Responses were significantly reduced following 6 months treatment with danazol. A similar study was carried out that showed a significantly reduced prolactin response during treatment with danazol (Panidis et al., 1992). The final study confirmed these findings but also showed that, conversely, the levels of thyroid stimulating hormone (TSH) in response to a TRH

challenge were increased in women on danazol treatment compared with pretreatment (Matalliotakis *et al*., 1996).

Pituitary hormones

One study found LH levels to be significantly increased in women with endometriomas compared with healthy controls, but equivalent to levels in women with other ovarian cysts (Adamyan *et al*., 1993). Significant elevations in LH levels in women with endometriosis have also been reported, throughout the cycle (Illera *et al*., 2001). Conversely, a second study showed no association between LH levels and endometriosis, when measured in the early follicular phase (Cunha-Filho *et al*., 2001). This study also measured TSH and follicle stimulating hormone, but found no association with endometriosis.

Steroids

Six studies were identified that measured serum steroid hormone levels. No significant change in progesterone levels has been identified in women with endometriosis (Fazleabas *et al*., 1987; Adamyan *et al*., 1993; Matsuzaki *et al*., 2006; Szymanowski, 2007). One study has also found no change in estradiol levels, but elevated testosterone levels in women with an endometrioma (Adamyan *et al*., 1993). However, a more recent study did indicate that infertile women with endometriosis had lower early follicular estradiol levels than fertile controls (Cunha-Filho *et al*., 2001). The final study looked at cortisol levels and found them to be increased in women with Stages III–IV disease, but not at earlier stages (Lima *et al*., 2006).

Other

The most common other hormone to be assessed has been leptin. The first study found serum leptin levels to be significantly higher in patients with endometriosis than in controls (Matarese *et al*., 2000). A similar finding was reported in a more recent paper, which used leptin levels as part of a biomarker panel to diagnose endometriosis (Seeber *et al*., 2008). However, three other studies have concluded that serum leptin is unchanged in endometriosis (Vigano *et al*., 2002; Wu *et al*., 2003; Gungor *et al*., 2009). One further study found baseline serum leptin levels to be no different in women with endometriosis, but did demonstrate an increase in levels during treatment with danazol or leuprolide acetate, suggesting that this may be a means of monitoring treatment (Matalliotakis *et al*., 2000a).

Finally, one study has measured levels of serum adiponectin and found them to be significantly lower in women with endometriosis than in controls (Takemura *et al*., 2005).

Angiogenesis

Several studies have sought to identify a link between endometriosis and pro-angiogenic factors in serum or urine—principally vascular endothelial growth factor (VEGF)—which may have use as biomarkers. However, no significant difference in VEGF levels was seen when comparing women with endometriosis-associated infertility to those with tubal factor infertility (Pellicer *et al*., 1998). Similarly, no difference in serum VEGF levels was found by four further studies (Gagne *et al*., 2003a; Bourlev *et al*., 2006b; Pupo-Nogueira *et al*., 2007; Othman *et al*., 2008).

One study has demonstrated elevated VEGF levels during the secretory phase in women with endometriomas (Xavier *et al*., 2006). A second study found a similar increase in VEGF levels in women with all types of endometriosis (Matalliotakis *et al*., 2003). There were no obvious methodological differences between this study and the others, therefore the reason for this discrepancy is unclear.

Increased levels of serum angiogenin (a polypeptide that stimulates angiogenesis) have been identified in women with endometriosis; however, this difference was only seen during the follicular phase of the cycle (Steff *et al*., 2004b).

Some studies have also analysed pro-angiogenic factors in urine as possible biomarkers. No association between urinary VEGF and endometriosis has been found (Potlog-Nahari *et al*., 2004). Raised urinary soluble Flt-1 levels (a VEGF receptor) have been noted in endometriosis patients, although there was no overall difference in serum levels (Cho *et al*., 2007). Interestingly, significantly higher urine and serum sFlt-1 levels were identified in women with earlier stage disease.

One study has found levels of fibroblast growth factor-2 (FGF-2) to be increased throughout the cycle in women with endometriosis (Bourlev *et al*., 2006a).

No significant difference in levels of soluble epidermal growth factor (EGF) receptor has been found between women with and without endometriosis (Matalliotakis *et al*., 2003). Serum levels of EGF itself have also been assessed and were not found to correlate with the disease (Philippoussis *et al*., 2004).

One paper measured levels of platelet-derived growth factor (PDGF) in women with endometriosis, but found similar levels to those in infertile controls (Kalu *et al*., 2007).

Two studies have looked at levels of hepatocyte growth factor (HGF), a protein with important roles as a mitogen and chemoattractant for endothelial cells (Bussolino *et al*., 1992). Serum HGF levels were initially found to be elevated in women with endometriosis (Zong *et al*., 2003). Levels did not change throughout the cycle, but did correlate with disease stage (levels in Stages I–II were lower than in Stages III–IV disease). However, this finding was not confirmed by a smaller, second study, which showed no correlation with incidence or stage of disease (Khan *et al*., 2006).

Apoptosis

Cells expressing Fas undergo apoptosis on interaction with other cells expressing Fas ligand (for review see Nagata and Goldstein, 1995). However, it also exists in a soluble form, due to cleavage from the cell surface by matrix metalloproteinases (Kayagaki *et al*., 1995; Powell *et al*., 1999). Women with Stages I–II disease had equivalent levels of soluble Fas ligand (sFasL) in serum compared with controls, but levels were significantly increased in women with Stages III–IV disease (Garcia-Velasco *et al*., 2002). A second study also identified significantly higher levels of sFasL in women with endometriosis, compared with both fertile and infertile controls (Linghu *et al*., 2004). This study also considered Fas levels, which did not differ between the subject groups. Equivalent serum Fas levels were also found in another study (Kalu *et al*., 2007).

Other

Three studies have measured levels of C-reactive protein (CRP), an acute phase protein used widely to monitor inflammatory and infectious processes. The first of these showed that CRP appeared to be increased in women with endometriosis, but more markedly in

those with more advanced disease (Abrao et al., 1997). However, a more recent study was unable to identify an increase in CRP level with the disease (Xavier et al., 2006). CRP results were below the sensitivity threshold of the test used in the final study (Matarese et al., 2000).

Urocortin is a peptide belonging to the corticotrophin releasing hormone family, known to be expressed in the endometrium (Florio et al., 2002). Serum levels of urocortin were found to be significantly higher in women with endometriomas than in women with other benign ovarian cysts, giving a sensitivity of 88% and specificity of 90% (Florio et al., 2007).

One study has assessed antioxidant and cholesterol levels (Verit et al., 2008). Women with endometriosis showed significant reductions in serum paroxonase-1 (PON-1) and high-density lipoprotein levels, as well as increased levels of total cholesterol, triglycerides, low-density lipoprotein and lipid peroxidises. PON-1 yielded a sensitivity of 98% and specificity of 83%. Other markers of oxidative stress were measured in a recent study, which found elevated levels of heat shock protein 70B′ (HSP70b′), a stress-induced protein involved in cell protection, in endometriosis, but no change in HSP70, ischaemia modified albumin (IMA) or thioredoxin (TRX) (Lambrinoudaki et al., 2009).

Levels of circulating free DNA appear to be elevated in women with endometriosis, with a sensitivity of 70% and specificity of 87% (Zachariah et al., 2009).

One study has measured serum levels of vitamin D binding protein, but did not demonstrate significant differences compared with controls (Borkowski et al., 2008).

In one study, no differences in the levels of PEP could be identified between women with and without endometriosis (Joshi et al., 1986). Levels of endometrial protein PP14 (related to PEP) have also been assessed, and were found to be increased in all women with endometriosis, but particularly so in more advanced stages (Telimaa et al., 1989). Treatment (either surgical, danazol or medroxyprogesteronee acetate) reduced PP14 levels.

Tumour-associated trypsin inhibitor (TATI) is a polypeptide known to be associated with gynaecological neoplasms (Huhtala et al., 1983). One study has shown that levels are elevated in endometriosis, although only significantly so in women with Stage II disease or greater (Medl et al., 1997). The test gave poor sensitivity (34%) but reasonable specificity (85%). Levels of TATI were also elevated in women taking a GnRH analogue after surgery, although they fell when treatment was discontinued.

Levels of the proto-oncogene c-erbB-2 were not found to differ between women with and without the disease (Philippoussis et al., 2004).

Levels of serum amyloid A (an acute phase protein) were increased in women with Stages III–IV disease during menses, but not in those with earlier stages (Abrao et al., 1997).

A reduction in serum tissue inhibitor of metalloproteinase-1 (TIMP-1) levels has been shown in women with endometriosis (Sharpe-Timms et al., 1998b). This appeared to be the case throughout the proliferative and secretory phases. Furthermore, levels of TIMP-1 were shown to rise significantly after 6 months GnRH analogue treatment.

Finally, elevated levels of serum matrix metalloproteinase-2 (MMP-2) have been found in women with endometriosis, throughout the cycle (Huang et al., 2004) (Fig. 2).

Discussion

Establishing a correct diagnosis of endometriosis is often problematic, because the presenting symptoms can be non-specific and associated with a number of different conditions (Giudice and Kao, 2004). Imaging methods such as transvaginal ultrasound and magnetic resonance imaging may help to identify ovarian endometriomas or a recto-vaginal endometriotic nodule, but they have no value in diagnosing peritoneal endometriosis (Moore et al., 2002; Kennedy et al., 2005). Consequently, it is recommended that pelvic endometriosis should be diagnosed surgically (Kennedy et al., 2005).

An accurate blood or urine test could avoid the need for an invasive procedure (Brosens et al., 2003a, b), or at the very least could enable symptomatic women to be screened. It has previously been suggested that a biomarker may be of most clinical use in specific subgroups of women with endometriosis (D'Hooghe et al., 2006). For example, women with symptoms consistent with Stages I–II disease may benefit from laparoscopic treatment if endometriosis is present. However, for those women with the same symptoms but no endometriosis, the risks of laparoscopy may outweigh the benefits. As such, this may be the group of women in whom a biomarker test might be most useful.

Another role for a biomarker would be to identify early signs of therapeutic efficacy, other than symptom relief itself, which is so difficult to measure. Such a molecule would be vitally important for novel drug design and early clinical studies. In addition, as recurrence rates of up to 50% after 5 years have been reported (Guo, 2009), it would be desirable if a biomarker or a panel of biomarkers could predict the likelihood of disease recurrence. This could lead to different therapeutic approaches depending on the outcome of the test.

We therefore chose to conduct a systematic review of the literature to determine which biomarkers have been proposed over the last 25 years as potential diagnostic tests. The search identified over 100 possible biomarkers that have been investigated; however, none of these have been clearly shown to be of clinical use. Some have undoubtedly shown promise as diagnostic tools, but further research needs to be conducted to establish their true value in clinical practice.

We find the lack of high-quality studies investigating large numbers of well-phenotyped patients surprising, given the obvious clinical need. Symptoms suggestive of the disease are common in the general population and it is currently difficult to establish a firm diagnosis. All these factors contribute to the well-recognized delay in diagnosis of 8–12 years (Hadfield et al., 1996) and further exacerbate the effects on quality of life and work productivity.

Although the diagnostic value of many of these biomarkers in endometriosis remains unclear, some appear to be more reliable in other conditions. For example, IGF has been used as a urine marker to diagnose urothelial carcinoma of the bladder (Watson et al., 2008). MIF has been used in urine to screen for acute pyelonephritis in children with urinary tract infections (Otukesh et al., 2009). Anti-cardiolipin antibodies and VEGF have both been implicated in systemic lupus erythematosus (Tanaseanu et al., 2007). CA125 has been used as both a diagnostic and surrogate marker in epithelial ovarian cancer, and CA 19-9 is highly sensitive in detecting mucinous ovarian cancer, a tumour type that does not express high levels of CA125 (Gadducci et al., 2004). Urocortin is a valuable biomarker in cardiac failure, with elevated levels correlating with the degree of cardiac dysfunction (Wright et al., 2009). These examples should convince

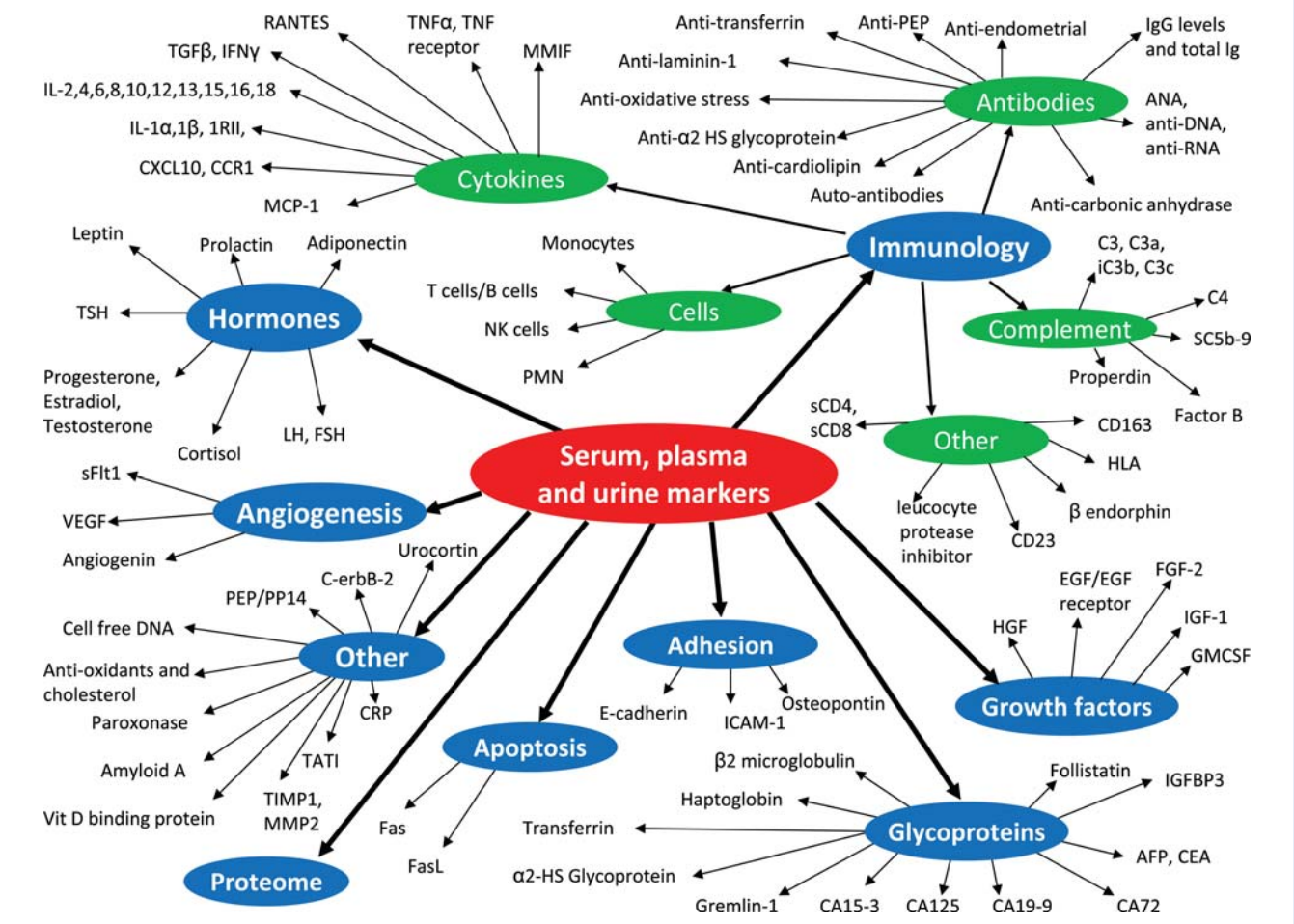

Figure 2 Spider diagram depicting putative biomarkers.

gynaecologists, patients and industry that the search for a biomarker in endometriosis is worth pursuing.

Problems with the studies identified in this review include the wide range of assay methods, recruitment strategies and study designs employed; failing to correct for the phase of the menstrual cycle, and the use of different types of patients as controls. These factors have undoubtedly contributed to some of the conflicting results we report above. For example, three of the nine papers investigating IL-6 failed to adjust for the phase of the menstrual cycle, despite evidence that levels are known to change throughout the cycle (Angstwurm *et al.*, 1997).

The numbers of patients recruited vary widely, and studies clearly differ in their scope—some studies seek to demonstrate a proof of concept (that a difference in a biomarker may be seen) and others aim to prove the clinical usefulness of a particular marker/panel of markers. Direct comparison of studies would therefore be unjust. In addition, few of the studies reported power calculations to guide recruitment figures, and this should be considered when planning high-quality biomarker research in the future.

Selection of an appropriate control group is often a challenge. Screening tests for disease can be employed in one of two ways—either applied to the entire population (e.g. cervical screening programme) or offered to women at increased risk of having disease, due to their clinical presentation. As the benefits of treating women with asymptomatic endometriosis are unclear, it is likely that any biomarker would be used only to investigate women with symptoms suggestive of endometriosis. Consequently, to show true promise as an aid to diagnosis, a prospective biomarker needs to distinguish women with endometriosis from unaffected women with a similar presentation (e.g. dysmenorrhoea, pelvic pain or subfertility). This has not been the case in all of the studies included in this review. For example, one study of MCP-1 levels found increased levels in infertile women with endometriosis compared with women with leiomyomas or fertile controls, but no significant difference compared with women with unexplained infertility (Gmyrek *et al.*, 2005). This may be partly due to the small numbers in the infertile control group, but the possibility that infertility itself may affect biomarker levels cannot be ignored. Similarly, studies investigating women with endometriomas may need to consider using women with other benign ovarian cysts as a control group, rather than those with no disease.

The type of endometriosis may also impact on biomarker levels, i.e. women with peritoneal disease may have different markers to those with rectovaginal endometriosis or endometriomas. This does not necessarily mean that a biomarker is not of use, but interpretation of the result may have to take other clinical findings into account.

For example, no difference in IL-8 levels was found between women with endometriosis and those with other benign gynaecological disease (Gazvani *et al*., 1998). However, a more recent study did find significantly elevated serum IL-8 levels in women with an endometrioma compared with women with other ovarian cysts (Ohata *et al*., 2008).

The one biomarker that has been used in clinical practice over the last 20 years is CA125. However, in a meta-analysis published in 1998, Mol *et al*. (1998) showed convincingly that the biomarker's performance in diagnosing endometriosis was low, even though it showed some promise in detecting more severe disease. Since their meta-analysis was published, we identified 15 further studies reporting a correlation between endometriosis and CA125. More recent studies tend to assess the use of CA125 in monitoring treatment (Chen *et al*., 1998; Matalliotakis *et al*., 2004). Although these studies suggest CA125 levels fall during treatment, they have not shown a correlation with disease response. Owing to the ethical constraints on performing second-look laparoscopies in these studies (and the impracticalities in a modern clinical setting), it may be useful to include some form of health-related questionnaire such as the Endometriosis Health Profile-30 (Jones *et al*., 2001), to try to gain more information about disease activity.

In our analysis, we took into consideration the strong possibility of reporting bias. There is statistical evidence to suggest that the data available are heavily skewed as a result of investigators' failure to present negative data for publication and the disinclination of journals to publish negative data (Dwan *et al*., 2008). It is common knowledge that negative data are often either not submitted to scientific journals or not accepted by the reviewers or editors. However, if a study is well designed then negative data can be extremely informative and should, in our opinion, be published. In fact, as patients consent to collect and use their samples to help increase knowledge of the disease and/or find new diagnostic tools, it is ethically highly questionable when negative data are not published.

It is worth noting that surgery often plays a vital role in the treatment of endometriosis. Furthermore, it may also be of importance in the management of other conditions which present in a similar manner (e.g. tubal infertility). The use of a biomarker may well be tempered in these circumstances, but it may still help to reduce the need for diagnostic surgery in some women, enable monitoring of the disease progression by non-surgical methods, and potentially allow for better pre-operative assessment of women with endometriosis.

Finally, the majority of the studies included in our review focused on assessing the diagnostic performance of single biomarkers. Realistically, however, a reliable diagnostic tool for endometriosis is likely to consist of a panel of biomarkers, not a single molecule, as has been the case for example with screening for Down's syndrome (Pihl *et al*., 2008). Ultimately, with more studies investigating the use of technologies such as genomics, proteomics and metabolomics, it can be expected that a panel of molecules or a typical profile of gene or protein expression will in the future help to distinguish between patients with and without disease. In combination with imaging techniques, such a panel of biomarkers may indicate which women need a laparoscopy and eliminate countless unnecessary operations. Future, larger well-designed studies together with increased knowledge of the pathogenesis of endometriosis are essential to improve overall health-related quality of life for patients suffering from this debilitating disease.

Funding

Supported by an MRC New Investigator Award (G0601458 to C.B.) and the Oxford Partnership Comprehensive Biomedical Research Centre with funding from the Department of Health's NIHR Biomedical Research Centres scheme. K.M. was funded by a MRC Doctoral Training Account (DTA).

References

Abrao MS, Podgaec S, Filho BM, Ramos LO, Pinotti JA, de Oliveira RM. The use of biochemical markers in the diagnosis of pelvic endometriosis. *Hum Reprod* 1997;**12**:2523–2527.

Abrao MS, Podgaec S, Pinotti JA, de Oliveira RM. Tumor markers in endometriosis. *Int J Gynaecol Obstet* 1999;**66**:19–22.

Acien P, Lloret M, Graells M. Prolactin and its response to the luteinizing hormone-releasing hormone thyrotropin-releasing hormone test in patients with endometriosis before, during, and after treatment with danazol. *Fertil Steril* 1989;**51**:774–780.

Adamyan LV, Fanchenko ND, Alexeyeva ML, Andreyeva YN, Novikov YA, Jahan I. Hormonal and immunologic methods in the diagnosis and treatment of patients with benign ovarian tumors and endometriotic cysts. *Int J Fertil Menopausal Stud* 1993;**38**:92–98.

Agic A, Xu H, Rehbein M, Wolfler M, Ebert A, Hornung D. Cognate chemokine receptor 1 messenger ribonucleic acid expression in peripheral blood as a diagnostic test for endometriosis. *Fertil Steril* 2007;**87**:982–984.

Agic A, Djalali S, Wolfler MM, Halis G, Diedrich K, Hornung D. Combination of CCR1 mRNA, MCP1, and CA125 measurements in peripheral blood as a diagnostic test for endometriosis. *Reprod Sci* 2008;**15**:906–911.

Akoum A, Lemay A, McColl SR, Paradis I, Maheux R. Increased monocyte chemotactic protein-1 level and activity in the peripheral blood of women with endometriosis. Le Groupe d'Investigation en Gynecologie. *Am J Obstet Gynecol* 1996;**175**:1620–1625.

Al-Jefout M, Dezarnaulds G, Cooper M, Tokushige N, Luscombe G, Markham R, Fraser I. Diagnosis of endometriosis by detection of nerve fibres in an endometrial biopsy: a double blind study. *Hum Reprod* 2009;**24**:3019–3024.

Amaral VF, Ferriani RA, Sa MF, Nogueira AA, Rosa e Silva JC, Rosa e Silva AC, Moura MD. Positive correlation between serum and peritoneal fluid CA-125 levels in women with pelvic endometriosis. *Sao Paulo Med J* 2006;**124**:223–227.

Angstwurm M, Gartner R, Ziegler-Heitbrock H. Cyclic plasma IL-6 levels during normal menstrual cycle. *Cytokine* 1997;**9**:370–374.

Antsiferova YS, Sotnikova NY, Posiseeva LV, Shor AL. Changes in the T-helper cytokine profile and in lymphocyte activation at the systemic and local levels in women with endometriosis. *Fertil Steril* 2005; **84**:1705–1711.

Arici A, Matalliotakis I, Goumenou A, Koumantakis G, Vassiliadis S, Mahutte N. Altered expression of interleukin-18 in the peritoneal fluid of women with endometriosis. *Fertil Sterility* 2003;**80**:889–894.

Arumugam K. Serum prolactin levels in infertile patients with endometriosis. *Malays J Pathol* 1991;**13**:43–45.

ASRM. Endometriosis and infertility. *Fertil Steril* 2004;**81**:1441–1446.

Badawy SZ, Cuenca V, Stitzel A, Jacobs RD, Tomar RH. Autoimmune phenomena in infertile patients with endometriosis. *Obstet Gynecol* 1984;**63**:271–275.

Badawy SZ, Cuenca V, Stitzel A, Tice D. Immune rosettes of T and B lymphocytes in infertile women with endometriosis. *J Reprod Med* 1987;**32**:194–197.

Badawy SZ, Cuenca V, Freliech H, Stefanu C. Endometrial antibodies in serum and peritoneal fluid of infertile patients with and without endometriosis. *Fertil Steril* 1990;**53**:930–932.

Bagan P, Berna P, Assouad J, Hupertan V, Le Pimpec Barthes F, Riquet M. Value of cancer antigen 125 for diagnosis of pleural endometriosis in females with recurrent pneumothorax. *Eur Respir J* 2008;**31**:140–142.

Baggiolini M, Clark-Lewis I. Interleukin-8, a chemotactic and inflammatory cytokine. *FEBS Lett* 1992;**307**:97–101.

Barrier BF, Sharpe-Timms KL. Expression of soluble adhesion molecules in sera of women with stage III and IV endometriosis. *J Soc Gynecol Investig* 2002;**9**:98–101.

Bedaiwy MA, Falcone T, Sharma RK, Goldberg JM, Attaran M, Nelson DR, Agarwal A. Prediction of endometriosis with serum and peritoneal fluid markers: A prospective controlled trial. *Hum Reprod* 2002;**17**:426–431.

Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin Pharmacol Ther* 2001;**69**:89–95.

Bohler HC, Gercel-Taylor C, Lessey BA, Taylor DD. Endometriosis markers: immunologic alterations as diagnostic indicators for endometriosis. *Reprod Sci* 2007;**14**:595–604.

Bokor A, Kyama C, Vercruysse L, Fassbender A, Gevaert O, Vodolazkaia A, Moor BD, Fulop V, D'Hooghe T. Density of small diamter sensory nerve fibres in endometrium: a semi-invasive diagnostic test for minimal to mild endometriosis. *Hum Reprod* 2009;**24**:3025–3032.

Borkowski J, Gmyrek GB, Madej JP, Nowacki W, Goluda M, Gabrys M, Stefaniak T, Chelmonska-Soyta A. Serum and peritoneal evaluation of vitamin D-binding protein in women with endometriosis. *Postepy Hig Med Dosw (Online)* 2008;**62**:103–109.

Bourlev V, Larsson A, Olovsson M. Elevated levels of fibroblast growth factor-2 in serum from women with endometriosis. *Am J Obstet Gynecol* 2006a;**194**:755–759.

Bourlev V, Volkov N, Pavlovitch S, Lets N, Larsson A, Olovsson M. The relationship between microvessel density, proliferative activity and expression of vascular endothelial growth factor-A and its receptors in eutopic endometrium and endometriotic lesions. *Reproduction* 2006b;**132**:501–509.

Brosens I, Puttemans P, Campro R, Gordts S, Brosens J. Non-invasive methods of diagnosis of endometriosis. *Curr Opin Obstet Gynecol* 2003a;**15**:519–522.

Brosens J, Timmerman D, Starzinski-Powitz A, Brosens I. Noninvasive diagnosis of endometriosis: the role of imaging and markers. *Obstet Gynecol Clin North Am* 2003b;**30**:95–114. viii-ix.

Bussolino F, Renzo MD, Ziche M, Bocchietto E, Olivero M, Naldini L, Gaudino G, Tamagnone L, Coffer A, Comoglio P. Hepatocyte growth factor is a potent angiogenic factor which stimulates endothelial cell motility and growth. *J Cell Biol* 1992;**119**:629–641.

Center D, Kornfield H, Cruikshank W. Interleukin-16. *Int J Biochem Cell Biol* 1997;**29**:1231–1234.

Chan A, Altman D. Identifying outcome reporting bias in randomised trials on PubMed: review of publications and survey of authors. *BMJ* 2005; **330**:753.

Chen F, Soong Y, Lee N, Kai S. The use of serum CA-125 as a marker for endometriosis in patients with dysmenorrhea for monitoring therapy and for recurrence of endometriosis. *Acta Obstet Gynecol Scand* 1998; **77**:665–670.

Chishima F, Hayakawa S, Hirata Y, Nagai N, Kanaeda T, Tsubata K, Satoh K. Peritoneal and peripheral B-1-cell populations in patients with endometriosis. *J Obstet Gynaecol Res* 2000;**26**:141–149.

Cho S, Oh Y, Nam A, Kim H, Park J, Kim J, Park K, Cho D, Lee B. Evaluation of serum and urinary angiogenic factors in patients with endometriosis. *Am J Reprod Immunol* 2007;**58**:497–504.

Cho S, Cho H, Nam A, Kim HY, Choi YS, Park KH, Cho DJ, Lee BS. Neutrophil-to-lymphocyte ratio as an adjunct to CA-125 for the diagnosis of endometriosis. *Fertil Steril* 2008;**90**:2073–2079.

Cho S, Ahn YS, Choi YS, Seo SK, Nam A, Kim HY, Kim JH, Park KH, Cho DJ, Lee BS. Endometrial osteopontin mRNA expression and plasma osteopontin levels are increased in patients with endometriosis. *Am J Reprod Immunol* 2009;**61**:286–293.

Confino E, Harlow L, Gleicher N. Peritoneal fluid and serum autoantibody levels in patients with endometriosis. *Fertil Steril* 1990; **53**:242–245.

Cunha-Filho JS, Gross JL, Lemos NA, Brandelli A, Castillos M, Passos EP. Hyperprolactinemia and luteal insufficiency in infertile patients with mild and minimal endometriosis. *Horm Metab Res* 2001;**33**:216–220.

D'Cruz OJ, Wild RA, Haas GGJ, Reichlin M. Antibodies to carbonic anhydrase in endometriosis: prevalence, specificity, and relationship to clinical and laboratory parameters. *Fertil Steril* 1996;**66**:547–556.

D'Hooghe T, Mihalyi A, Simsa P, Kyama C, Peeraer K, De Loecker P, Meeuwis L, Segal L, Meuleman C. Why we need a noninvasive diagnostic test for minimal to mild endometriosis with a high sensitivity. *Gynecol Obstet Invest* 2006;**62**:136–138.

Daniel Y, Geva E, Amit A, Eshed-Englender T, Baram A, Fait G, Lessing J. Do soluble cell adhesion molecules play a role in endometriosis? *Am J Reprod Immunol* 2000;**43**:160–166.

Darai E, Detchev R, Hugol D, Quang NT. Serum and cyst fluid levels of interleukin (IL) -6, IL-8 and tumour necrosis factor-alpha in women with endometriomas and benign and malignant cystic ovarian tumours. *Hum Reprod* 2003;**18**:1681–1685.

De Placido G, Alviggi C, Di Palma G, Carravetta C, Matarese G, Landino G, Racioppi L. Serum concentrations of soluble human leukocyte class I antigens and of the soluble intercellular adhesion molecule-1 in endometriosis: relationship with stage and non-pigmented peritoneal lesions. *Hum Reprod* 1998;**13**:3206–3210.

de Winter JP, ten Dijke P, de Vries CJM, van Achterberg TAE, Sugino H, de Waele P, Huylebroeck D, Verschueren K, van den Eijnden-van Raaij AJM. Follistatins neutralize activin bioactivity by inhibition of activin binding to its type II receptors. *Mol Cell Endocrinol* 1996; **116**:105–114.

Dwan K, Altman DG, Arnaiz JA, Bloom J, Chan AW, Cronin E, Decullier E, Easterbrook PJ, Von Elm E, Gamble D *et al.* Systematic review of the empirical evidence of study publication bias and outcome reporting bias. *PLoS One* 2008;**3**:e3081.

El-Roeiy A, Dmowski W, Gleicher N, Radwanska E, Harlow L, Binor Z, tummon I, Rawlins R. Danazol but not gonadotropin-releasing hormone agonists suppresses autoantibodies in endometriosis. *Fertil Steril* 1988;**50**:864–871.

Fairbanks F, Abrao MS, Podgaec S, Dias JAJ, de Oliveira RM, Rizzo LV. Interleukin-12 but not interleukin-18 is associated with severe endometriosis. *Fertil Steril* 2009;**91**:320–324.

Fassbender A, D'Hooghe T, Mihalyi A, Kyama C, Simsa P, Lessey BA. Plasma C3a-des-Arg levels in women with and without endometriosis. *Am J Reprod Immunol* 2009;**62**:187–195.

Fazleabas AT, Khan-Dawood FS, Dawood MY. Protein, progesteronee, and protease inhibitors in uterine and peritoneal fluids of women with endometriosis. *Fertil Steril* 1987;**47**:218–224.

Ferrero S, Gillott DJ, Remorgida V, Anserini P, Price K, Ragni N, Grudzinskas JG. Haptoglobin beta chain isoforms in the plasma and peritoneal fluid of women with endometriosis. *Fertil Steril* 2005; **83**:1536–1543.

Florio P, Arcuri F, Ciarmela P, Runci Y, Romagnoli R, Cintorino M, Blasio AD, Petraglia F. Identification of urocortin mRNA and peptide in the human endometrium. *J Endocrinol* 2002;**173**:R9–R14.

Florio P, Reis FM, Torres PB, Calonaci F, Toti P, Bocchi C, Linton EA, Petralgia F. Plasma urocortin levels in the diagnosis of ovarian endometriosis. *Obstet Gynecol* 2007;**110**:594–600.

Florio P, Reis FM, Torres PB, Calonaci F, Abrao MS, Nascimento LL, Franchini M, Cianferoni L, Petraglia F. High serum follistatin levels in women with ovarian endometriosis. *Hum Reprod* 2009;**24**:2600–2606.

Fu C, Lang J. Serum soluble E-cadherin level in patients with endometriosis. *Chin Med Sci J* 2002;**17**:121–123.

Gadducci A, Cosio S, Carpi A, Nicolini A, Genazzani A. Serum tumor markers in the management of ovarian, endometrial and cervical cancer. *Biomed Pharmacother* 2004;**58**:24–38.

Gagne D, Page M, Robitaille G, Hugo P, Gosselin D. Levels of vascular endothelial growth factor (VEGF) in serum of patients with endometriosis. *Hum Reprod* 2003a;**18**:1674–1680.

Gagne D, Rivard M, Page M, Shazand K, Hugh P, Gosselin D. Blood leukocyte subsets are modulated in patients with endometriosis. *Fertil Steril* 2003b;**80**:43–53.

Gajbhiye R, Suryawanshi A, Khan S, Meherji P, Warty N, Raut V, Chehna N, Khole V. Multiple endometrial antigens are targeted in autoimmune endometriosis. *Reprod Biomed Online* 2008;**16**:817–824.

Galleri L, Luisi S, Rotondi M, Romagnani P, Cobellis L, Serio M, Petraglia F Low serum and peritoneal fluid concentration of interferon-gamma-induced protein-10 (CXCL10) in women with endometriosis. *Fertil Steril* 2009;**91**:331–334.

Gallinelli A, Chiossi G, Giannella L, Marsella T, Genazzani A, Volpe A. Different concentrations of interleukins in the peritoneal fluid of women with endometriosis: relationships with lymphocyte subsets. *Gynecol Endocrinol* 2004;**18**:144–151.

Garcia-Velasco JA, Mulayim N, Kayisli UA, Arici A. Elevated soluble Fas ligand levels may suggest a role for apoptosis in women with endometriosis. *Fertil Steril* 2002;**78**:855–859.

Garza D, Mathur S, Dowd MM, Smith LF, Williamson HO. Antigenic differences between the endometrium of women with and without endometriosis. *J Reprod Med* 1991;**36**:177–182.

Garzetti GG, Ciavattini A, Provinciali M, Fabris N, Cignitti M, Romanini C. Natural killer cell activity in endometriosis: correlation between serum estradiol levels and cytotoxicity. *Obstet Gynecol* 1993;**81**:665–668.

Garzetti GG, Ciavattini A, Provinciali M, Amati M, Muzzioli M, Governa M. Decrease in peripheral blood polymorphonuclear leukocyte chemotactic index in endometriosis: role of prostaglandin E2 release. *Obstet Gynecol* 1998;**91**:25–29.

Gazvani M, Christmas S, Quenby S, Kirwan K, Johnson P, Kingsland C. Peritoneal fluid concentrations of interleukin-8 in women with endometriosis: relationship to stage of disease. *Hum Reprod* 1998; **13**:1957–1961.

Giudice L, Kao L. Endometriosis. *Lancet* 2004;**364**:1789–1799.

Gmyrek GB, Sozański R, Jerzak M, Chrobak A, Wickiewicz D, Skupnik A, Sieradzka U, Fortuna W, Gabrys M, Chelmońska-Soyta A. Evaluation of monocyte chemotactic protein-1 levels in peripheral blood of infertile women with endometriosis. *Eur J Obstet Gynecol Reprod Biol* 2005; **122**:199–205.

Gmyrek GB, Sieradzka U, Goluda M, Gabryś M, Sozański R, Jerzak M, Zbyryt I, Chrobak A, Chelmónska-Soyta A. Flow cytometric evaluation of intracellular cytokine synthesis in peripheral mononuclear cells of women with endometriosis. *Immunol Invest* 2008;**37**:43–61.

Gordon J. CD23: novel disease marker with a split personality. *Clin Exp Immunol* 1991;**86**:356–359.

Górski J, Szyllo K, Banasik M, Lewkowicz P, Tchórzewski H. CD4+, CD8+ and CD4+CD25+ T lymphocytes in peripheral blood and peritoneal fluid of women with endometriosis - Preliminary report. *Arch Med Sci* 2007;**3**:37–42.

Gungor T, Kanat-Pektas M, Karayalcin R, Mollamahmutoglu L. Peritoneal fluid and serum leptin concentrations in women with primary infertility. *Arch Gynecol Obstet* 2009;**279**:361–364.

Guo SW. Recurrence of endometriosis and its control. *Hum Reprod Update* 2009;**15**:441–461.

Gurgan T, Bukulmez O, Yarali H, Tanir M, Akyildiz S. Serum and peritoneal fluid levels of IGF I and II and insulin-like growth binding protein-3 in endometriosis. *J Reprod Med* 1999;**44**:450–454.

Hadfield R, Mardon H, Barlow D, Kennedy S. Delay in the diagnosis of endometriosis: a survey of women from the USA and the UK. *Hum Reprod* 1996;**11**:878–880.

Harada T, Kubota T, Aso T. Usefulness of CA19-9 versus CA125 for the diagnosis of endometriosis. *Fertil Steril* 2002;**78**:733–739.

Hassa H, Tanir HM, Tekin B, Kirilmaz SD, Sahin Mutlu F. Cytokine and immune cell levels in peritoneal fluid and peripheral blood of women with early- and late-staged endometriosis. *Arch Gynecol Obstet* 2009; **279**:891–895.

Hatayama H, Imai K, Kanzaki H, Higuchi T, Fujimoto M, Mori T. Detection of antiendometrial antibodies in patients with endometriosis by cell ELISA. *Am J Reprod Immunol* 1996;**35**:118–122.

Hirchowitz J, Soler N, Wortsman J. The galactorrhea-endometriosis syndrome. *Lancet* 1978;**311**:896–898.

Ho HN, Chao KH, Chen HF, Wu MY, Yang YS, Lee TY. Peritoneal natural killer cytotoxicity and CD25+ CD3+ lymphocyte subpopulation are decreased in women with stage III-IV endometriosis. *Hum Reprod* 1995;**10**:2671–2675.

Hsu C, Yang C, Wu M, Huang K. Enhanced interleukin-4 expression in patients with endometriosis. *Fertil Steril* 1997;**67**:1059–1164.

Huang H, Hong H, Tan Y, Sheng J. Matrix metalloproteinase 2 is associated with changes in steroids hormones in the sera and peritoneal fluid of patients with endometriosis. *Fertil Steril* 2004;**81**:1235–1239.

Huhtala M, Kahanpaa K, Seppala M, Halila H, Stenman U. Excretion of a tumor-associated trypsin inhibitor (TATI) in urine of patients with gynecological malignancy. *Int J Cancer* 1983;**31**:711–714.

Illera JC, Silvan G, Illera MJ, Munro CJ, Lessey BA, Illera M. Measurement of serum and peritoneal fluid LH concentrations as a diagnostic tool for human endometriosis. *Reproduction* 2001;**121**:761–769.

Inagaki J, Matsuura E, Nomizu M, Sugiura-Ogasawara M, Katano K, Kaihara K, Kobayashi K, Yasuda T, Aoki K. IgG antilaminin-1 autoantibody and recurrent miscarriages. *Am J Reprod Immunol* 2001; **45**:232–238.

Inagaki J, Sugiura-Ogasawara M, Nomizu M, Nakatsuka M, Ikuta K, Suzuki N, Kaihara K, Kobayashi K, Yasuda T, Shoenfeld Y *et al.* An association of IgG anti-laminin-1 autoantibodies with endometriosis in infertile patients. *Hum Reprod* 2003;**18**:544–549.

Iwabe T, Harada T, Sakamoto Y, Iba Y, Horie S, Mitsunari M, Terakawa N. Gonadotropin-releasing hormone agonist treatment reduced serum interleukin-6 concentrations in patients with ovarian endometriomas. *Fertil Steril* 2003;**80**:300–304.

Iwasaki K, Makino T, Maruyama T, Matsubayashi H, Nozawa S, Yokokura T. Leukocyte subpopulations and natural killer activity in endometriosis. *Int J Fertil Menopausal Stud* 1993;**38**: 229–234.

Izumiya C, Maeda N, Kusume T, Masumoto T, Yamashita C, Yamamoto Y, Oguri H, Fukaya T. Coordinated but depressed expression of human leukocyte antigen-DR, intercellular adhesion molecule-1, and CD14 on peritoneal macrophages in women with pelvic endometriosis. *Fertil Steril* 2003;**80**(Suppl. 2):768–775.

Jalkanen S, Reichert R, Gallatin W, Bargatze R, Weissman I, Butcher E. Homing receptors and the control of lymphocyte migration. *Immunol Rev* 1986;**91**:39–60.

Jee BC, Suh CS, Kim SH, Moon SY. Serum soluble CD163 and interleukin-6 levels in women with ovarian endometriomas. *Gynecol Obstet Invest* 2008;**66**:47–52.

Jing J, Qiao Y, Suginami H, Taniguchi F, Shi H, Wang X. Two novel serum biomarkers for endometriosis screened by surface-enhanced laser desorption/ionization time-of-flight mass spectrometry and their change after laparoscopic removal of endometriosis. *Fertil Steril* 2008; **92**:1221–1227.

Jones G, Kennedy S, Barnard A, Wong J, Jenkinson C. Development of an endometriosis quality-of-life instrument: The Endometriosis Health Profile-30. *Obstet Gynecol* 2001;**98**:2–64.

Joshi SG, Zamah NM, Raikar RS, Buttram VC Jr, Henriques ES, Gordon M. Serum and peritoneal fluid proteins in women with and without endometriosis. *Fertil Steril* 1986;**46**:1077–1082.

Kabut J, Kondera-Anasz Z, Sikora J, Mielczarek-Palacz A. Levels of complement components iC3b, C3c, C4, and SC5b-9 in peritoneal fluid and serum of infertile women with endometriosis. *Fertil Steril* 2007;**88**:1298–1303.

Kafali H, Artuc H, Demir N. Use of CA125 fluctuation during the menstrual cycle as a tool in the clinical diagnosis of endometriosis; a preliminary report. *Eur J Obstet Gynecol Reprod Biol* 2004;**116**:85–88.

Kalu E, Sumar N, Giannopoulos T, Patel P, Croucher C, Sherriff E, Bansal A. Cytokine profiles in serum and peritoneal fluid from infertile women with and without endometriosis. *J Obstet Gynaecol Res* 2007; **33**:490–495.

Kayagaki N, Kawasaki A, Ebata T, Ohmoto H, Ikeda S, Inoue S, Yoshino K, Okumura K, Yagita H. Metalloproteinase-mediated release of human Fas ligand. *J Exp Med* 1995;**182**:1777–1783.

Kennedy S, Bergqvist A, Chapron C, D'Hooghe T, Dunselman G, Greb R, Hummelshoj L, Prentice A, Saridogan E. ESHRE guideline for the diagnosis and treatment of endometriosis. *Hum Reprod* 2005; **20**:2698–2704.

Khan KN, Masuzaki H, Fujishita A, Kitajima M, Hiraki K, Miura S, Sekine I, Ishimaru T. Peritoneal fluid and serum levels of hepatocyte growth factor may predict the activity of endometriosis. *Acta Obstet Gynecol Scand* 2006;**85**:458–466.

Kiechle FL, Quattrociocchi-Longe TM, Brinton DA. Carbonic anhydrase antibody in sera from patients with endometriosis. *Am J Clin Pathol* 1994;**101**:611–615.

Kikuchi Y, Ishikawa N, Hirata J, Imaizumi E, Sasa H, Nagata I. Changes of peripheral blood lymphocyte subsets before and after operation of patients with endometriosis. *Acta Obstet Gynecol Scand* 1993;**72**:157–161.

Kilpatrick D, Haining R, Smith S. Are cardiolipin levels elevated in endometriosis? *Fertil Steril* 1991;**55**:436–437.

Kishimoto T, Akira S, Narazaki M, Taga T. Interleukin-6 family of cytokines and gp130. *Blood* 1995;**86**:1243–1254.

Kitawaki J, Ishihara H, Koshiba H, Kiyomizu M, Teramoto M, Kitaoka Y, Honjo H. Usefulness and limits of CA-125 in diagnosis of endometriosis without associated ovarian endometriomas. *Hum Reprod* 2005;**20**:1999–2003.

Kondera-Anasz Z, Sikora J, Mielczarek-Palacz A, Jonca M. Concentrations of interleukin (IL)-1alpha, IL-1 soluble receptor type II (IL-1 sRII) and IL-1 receptor antagonist (IL-1 Ra) in the peritoneal fluid and serum of infertile women with endometriosis. *Eur J Obstet Gynecol Reprod Biol* 2005;**123**:198–203.

Koutsilieris M, Akoum A, Lazure C, Frenette G, Lemay A. N-terminal truncated forms of insulin-like growth factor binding protein-3 in the peritoneal fluid of women without laparoscopic evidence of endometriosis. *Fertil Steril* 1995;**63**:314–321.

Kurdoglu Z, Gursoy R, Kurdoglu M, Erdem M, Erdem O, Erdem A. Comparison of the clinical value of CA 19-9 versus CA 125 for the diagnosis of endometriosis. *Fertil Steril* 2009;**92**:1761–1763.

Lambrinoudaki IV, Augoulea A, Christodoulakos GE, Economou EV, Kaparos G, Kontoravdis A, Papadias C, Creatsas G. Measurable serum markers of oxidative stress response in women with endometriosis. *Fertil Steril* 2009;**91**:46–50.

Levine M, Walter S, Lee H, Haines T, Holbrook A, Moyer V. Users' guides to the medical literature. IV. How to use an article about harm. Evidence-based medicine working group. *JAMA* 1994; **271**:1615–1619.

Lima AP, Moura MD, Rosa e Silva AA. Prolactin and cortisol levels in women with endometriosis. *Braz J Med Biol Res* 2006;**39**: 1121–1127.

Linghu H, Xu X, Luo J, Zhuang L. Changes of soluble fas and soluble fas ligand in serum and peritoneal fluid of infertile patients with endometriosis. *Chin Med Sci J* 2004;**19**:56–59.

Liu H, Lang J, Zhou Q, Shan D, Li Q. Detection of endometriosis with the use of plasma protein profiling by surface-enhanced laser desorption/ ionization time-of-flight mass spectrometry. *Fertil Steril* 2007; **87**:988–990.

Maeda N, Izumiya C, Oguri H, Kusume T, Yamamoto Y, Fukaya T. Aberrant expression of intercellular adhesion molecule-1 and killer inhibitory receptors induces immune tolerance in women with pelvic endometriosis. *Fertil Steril* 2002a;**77**:679–683.

Maeda N, Izumiya C, Yamamoto Y, Oguri H, Kusume T, Fukaya T. Increased killer inhibitory receptor KIR2DL1 expression among natural killer cells in women with pelvic endometriosis. *Fertil Steril* 2002b; **77**:297–302.

Maeda N, Izumiya C, Kusum T, Masumoto T, Yamashita C, Yamamoto Y, Oguri H, Fukaya T. Killer inhibitory receptor CD158a overexpression among natural killer cells in women with endometriosis is undiminished by laparoscopic surgery and gonadotropin releasing hormone agonist treatment. *Am J Reprod Immunol* 2004;**51**:364–372.

Maiorana A, Cicerone C, Niceta M, Alio L. Evaluation of serum CA 125 levels in patients with pelvic pain related to endometriosis. *Int J Biol Markers* 2007;**22**:200–202.

Markham R, Fraser IS, Song JY, Jansen RPS. The measurement of tumour necrosis factor alpha in patients with endometriosis. *Aust J Med Sci* 1997a;**18**:56–59.

Markham R, Fraser IS, Song JY, Young L, Chullapram T. Blood and peritoneal fluid concentrations of TNFalpha and RANTES in patients with and without endometriosis. *Aust J Med Sci* 1997b;**18**:116–118.

Martinez S, Garrido N, Coperias JL, Pardo F, Desco J, Garcia-Velasco JA, Simon C, Pellicer A. Serum interleukin-6 levels are elevated in women with minimal-mild endometriosis. *Hum Reprod* 2007;**22**:836–842.

Matalliotakis I, Panidis D, Vlassis G, Vavilis D, Neonaki M, Koumantakis E. PRL, TSH and their response to the TRH test in patients with endometriosis before, during, and after treatment with danazol. *Gynecol Obstet Invest* 1996;**42**:183–186.

Matalliotakis I, Neonaki M, Zolindaki A, Hassan E, Georgoulias V, Koumantakis E. Changes in immunologic variables (TNF-a, sCD8 and sCD4) during danazol treatment in patients with endometriosis. *Int J Fertil Womens Med* 1997;**42**:211–214.

Matalliotakis I, Panidis D, Vlassis G, Neonaki M, Goumenou A, Koumantakis E. Unexpected increase of the CA 19-9 tumour marker in patients with endometriosis. *Eur J Gynaecol Oncol* 1998;**19**:498–500.

Matalliotakis I, Koumantaki Y, Neonaki M, Goumenou A, Koumantakis G, Kyriakou D, Koumantakis E. Increase in serum leptin concentrations among women with endometriosis during danazol and leuprolide depot treatments. *Am J Obstet Gynecol* 2000a;**183**:58–62.

Matalliotakis IM, Neonaki MA, Koumantaki YG, Goumenou AG, Kyriakou DS, Koumantakis EE. A randomized comparison of danazol and leuprolide acetate suppression of serum-soluble CD23 levels in endometriosis. *Obstet Gynecol* 2000b;**95**:810–813.

Matalliotakis IM, Vassiliadis S, Goumenou AG, Athanassakis I, Koumantakis GE, Neonaki MA, Koumantakis EE. Soluble ICAM-1 levels in the serum of endometriotic patients appear to be independent of medical treatment. *J Reprod Immunol* 2001b;**51**:9–19.

Matalliotakis IM, Goumenou AG, Koumantakis GE, Athanassakis I, Dionyssopoulou E, Neonaki MA, Vassiliadis S. Expression of serum human leukocyte antigen and growth factor levels in a Greek family with familial endometriosis. *J Soc Gynecol Investig* 2003;**10**:118–121.

Matalliotakis IM, Arici A, Goumenou AG, Katassos T, Karkavitsas N, Koumantakis EE. Comparison of the effects of leuprorelin acetate and danazol treatments on serum CA-125 levels in women with endometriosis. *Int J Fertil Womens Med* 2004;**49**:75–78.

Matarese G, Alviggi C, Sanna V, Howard JK, Lord GM, Carravetta C, Fontana S, Lechler RI, Bloom SR, De Placido G. Increased leptin levels in serum and peritoneal fluid of patients with pelvic endometriosis. *J Clin Endocrinol Metab* 2000;**85**:2483–2487.

Mathur S, Garza DE, Smith LF. Endometrial autoantigens eliciting immunoglobulin (Ig)G, IgA, and IgM responses in endometriosis. *Fertil Steril* 1990;**54**:56–63.

Mathur SP, Holt VL, Lee JH, Jiang H, Rust PF. Levels of antibodies to transferrin and alpha 2-HS glycoprotein in women with and without endometriosis. *Am J Reprod Immunol* 1998;**40**:69–73.

Mathur SP, Lee JH, Jiang H, Arnaud P, Rust PF. Levels of transferrin and alpha 2-HS glycoprotein in women with and without endometriosis. *Autoimmunity* 1999;**29**:121–127.

Matsuzaki S, Canis M, Darcha C, Dechelotte P, Pouly J, Mage G. Expression of WT1 is down-regulated in eutopic endometrium obtained during the midsecretory phase from patients with endometriosis. *Fertil Steril* 2006;**86**:554–558.

Medl M, Ogris E, Peters-Engl C, Mierau M, Buxbaum P, Leodolter S. Serum levels of the tumour-associated trypsin inhibitor in patients with endometriosis. *Br J Obstet Gynaecol* 1997;**104**:78–81.

Meek S, Hodge D, Musich J. Autoimmunity in infertile patients with endometriosis. *Am J Obstet Gynecol* 1988;**158**:1365–1373.

Methur S, Peress M, Williamson H, Youmans C, Maney S, Garvin A, Rust P, Fudenberg H. Autoimmunity to endometrium and ovary in endometriosis. *Clin Exp Immunol* 1982;**50**:259–266.

Mol B, Bayram N, Lijmer J, Wiegerinck M, Bongers M, Veen Fvd, Bossuyt P. The performance of CA-125 measurement in the detection of endometriosis: a meta-analysis. *Fertil Steril* 1998;**70**:1101–1108.

Molo MW, Kelly M, Radwanska E, Binor Z. Preoperative serum CA-125 and CA-72 in predicting endometriosis in infertility patients. *J Reprod Med* 1994;**39**:964–966.

Moore J, Copley S, Morris J, Lindsell D, Golding S, Kennedy S. A systematic review of the accuracy of ultrasound in the diagnosis of endometriosis. *Ultrasound Obstet Gynecol* 2002;**20**:630–634.

Morin M, Bellehumeur C, Therriault MJ, Metz C, Maheux R, Akoum A. Elevated levels of macrophage migration inhibitory factor in the peripheral blood of women with endometriosis. *Fertil Steril* 2005; **83**:865–872.

Muscatello R, Cucinelli F, Fulghesu A, Lanzone A, Caruso A, Mancuso S. Multiple serum marker assay in the diagnosis of endometriosis. *Gynecol Endocrinol* 1992;**6**:265–269.

Nagata S, Goldstein P. The Fas death factor. *Science* 1995; **267**:1449–1456.

O'Brien T, Raymond L, Bannon G, Ford D, Hardardottir H, Miller F, Quirk J. New monoclonal antibodies identify the glycoprotein carrying the CA 125 epitope. *Obstet Gynecol* 1991;**165**:1857–1864.

Odukoya OA, Bansal A, Wilson AP, Weetman AP, Cooke ID. Serum-soluble CD23 in patients with endometriosis and the effect of treatment with danazol and leuprolide acetate depot injection. *Hum Reprod* 1995b;**10**:942–946.

Odukoya OA, Wheatcroft N, Weetman AP, Cooke ID. The prevalence of endometrial immunoglobulin G antibodies in patients with endometriosis. *Hum Reprod* 1995a;**10**:1214–1219.

Odukoya OA, Bansal A, Cooke I, Odukoya O, Bansal A, Cooke I. Serum endometrial IgG antibodies and soluble CD23 concentrations in patients with endometriosis. *Acta Obstet Gynecol Scand* 1996;**75**:927–931.

Ohata Y, Harada T, Miyakoda H, Taniguchi F, Iwabe T, Terakawa N. Serum interleukin-8 levels are elevated in patients with ovarian endometrioma. *Fertil Steril* 2008;**90**:994–999.

Olive D, Weinberg J, Haney A. Peritoneal macrophages and infertility: the association between cell number and pelvic pathology. *Fertil Steril* 1985; **44**:772–777.

Oosterlynck D, Cornillie F, Waer M, Vandeputte M, Koninckx P. Women with endometriosis show a defect in natural killer activity resulting in a decreased cytotoxicity to autologous endometrium. *Fertil Steril* 1991; **56**:45–51.

Oosterlynck DJ, Meuleman C, Lacquet FA, Waer M, Koninckx PR. Flow cytometry analysis of lymphocyte subpopulations in peritoneal fluid of women with endometriosis. *Am J Reprod Immunol* 1994;**31**:25–31.

Othman EE-D, Hornung D, Salem HT, Khalifa EA, El-Metwally TH, Al-Hendy A. Serum cytokines as biomarkers for nonsurgical prediction of endometriosis. *Eur J Obstet Gynecol Reprod Biol* 2008; **137**:240–246.

Otukesh H, Fereshtehnejad S, Hoseini R, Hekmat S, Chalian H, Chalian M, Bedayat A, Yazdi RS, Sabaghi S, Mahdavi S. Urine macrophage migration inhibitory factor (MIF) in children with urinary tract infection: a possible predictor of acute pyelonephritis. *Pediatr Nephrol* 2009;**24**:105–111.

Panidis D, Vlassis G, Matalliotakis J, Skiadopoulos S, Kalogeropoulos A. Serum levels of the oncofetal antigens CA-125, CA 19-9 and CA 15-3 in patients with endometriosis. *J Endocrinol Invest* 1988; **11**:801–804.

Panidis D, Vavilis D, Rousso D, Panidou E, Kalogeropoulos A. Provocative tests of prolactin before, during and after long-term danazol treatment in patients with endometriosis. *Gynecol Endocrinol* 1992;**6**:19–24.

Pellicer A, Albert C, Mercader A, Bonilla-Musoles F, Remohl J, Simon C. The follicular and endocrine environment in women with endometriosis: local and systemic cytokine production. *Fertil Steril* 1998;**70**:425–431.

Philippoussis F, Gagne D, Hugo P, Gosselin D. Concentrations of alpha-fetoprotein, insulin-like growth factor binding protein-3, c-erbB-2, and epidermal growth factor in serum of patients with endometriosis. *J Soc Gynecol Investig* 2004;**11**:175–181.

Pihl K, Sorensen T, Norgaard-Pedersen B, Larsen S, Nguyen T, Krebs L, Larsen T, Christiansen M. First-trimester combined screening for Down syndrome: prediction of low birth weight, small for gestational age and pre-term delivery in a cohort of non-selected women. *Prenat Diagn* 2008;**28**:247–253.

Pillai S, Zhou GX, Arnaud P, Jiang H, Butler WJ, Zhang H. Antibodies to endometrial transferrin and alpha 2-Heremans Schmidt (HS) glycoprotein in patients with endometriosis. *Am J Reprod Immunol* 1996;**35**:483–494.

Pizzo A, Salmeri FM, Ardita FV, Sofo V, Tripepi M, Marsico S. Behaviour of cytokine levels in serum and peritoneal fluid of women with endometriosis. *Gynecol Obstet Invest* 2002;**54**:82–87.

Potlog-Nahari C, Stratton P, Winkel C, Widra E, Sinaii N, Connors S, Nieman LK. Urine vascular endothelial growth factor-A is not a useful marker for endometriosis. *Fertil Steril* 2004;**81**:1507–1512.

Powell W, Fingleton B, Wilson C, Boothby M, Matrisian L. The metalloproteinase matrilysin proteolytically generates active soluble Fas ligand and potentiates epithelial cell apoptosis. *Curr Biol* 1999; **9**:1441–1447.

Pupo-Nogueira A, de Oliveira RM, Petta CA, Podgaec S, Dias JA Jr, Abrao MS. Vascular endothelial growth factor concentrations in the serum and peritoneal fluid of women with endometriosis. *Int J Gynaecol Obstet* 2007;**99**:33–37.

Radwanska E, Henig I, Dmowski W. Nocturnal prolactin levels in infertile women with endometriosis. *J Reprod Med* 1987;**32**:605–608.

Rajkumar K, Malliah V, Simpson CW. Identifying the presence of antibodies against endometrial antigens: a preliminary study. *J Reprod Med* 1992;**37**:552–556.

Randall GW, Gantt PA, Poe-Zeigler RL, Bergmann CA, Noel ME, Strawbridge WR, Richardson-Cox B, Hereford JR, Reiff RH. Serum antiendometrial antibodies and diagnosis of endometriosis. *Am J Reprod Immunol* 2007;**58**:374–382.

Rosa e Silva AC, Rosa e Silva JC, Ferriani RA. Serum CA-125 in the diagnosis of endometriosis. *Int J Gynaecol Obstet* 2007;**96**:206–207.

Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor α-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune disease. *J Immunol* 1995;**155**:1151–1164.

Seeber B, Sammel MD, Fan X, Gerton GL, Shaunik A, Chittams J, Barnhart KT. Panel of markers can accurately predict endometriosis in a subset of patients. *Fertil Steril* 2008;**89**:1073–1081.

Seeber B, Sammel MD, Fan X, Gerton GL, Shaunik A, Chittams J, Barnhart KT. Proteomic analysis of serum yields six candidate proteins that are differentially regulated in a subset of women with endometriosis. *Fertil Steril* 2009; doi:10.1016/j.fertnstert.2008.12.121.

Sha G, Wu D, Zhang L, Chen X, Lei M, Sun H, Lin S, Lang J. Differentially expressed genes in human endometrial endothelial cells derived from eutopic endometrium of patients with endometriosis compared with those from patients without endometriosis. *Hum Reprod* 2007; **22**:3159–3169.

Sha G, Zhang Y, Zhang C, Wan Y, Zhao Z, Li C, Lang J. Elevated levels of gremlin-1 in eutopic endometrium and peripheral serum in patients with endometriosis. *Fertil Steril* 2009;**91**:350–358.

Shanti A, Santanam N, Morales A, Parthasarathy S, Murphy A. Autoantibodies to markers of oxidative stress are elevated in women with endometriosis. *Fertil Steril* 1999;**71**:1115–1118.

Sharpe-Timms K, Piva M, Ricke E, Surewicz K, Zhang Y, Zimmer R. Endometriotic lesions synthesize and secrete a haptoglobin-like protein. *Biol Reprod* 1998a;**58**:988–994.

Sharpe-Timms KL, Keisler LW, McIntush EW, Keisler DH. Tissue inhibitor of metalloproteinase-1 concentrations are attenuated in peritoneal fluid and sera of women with endometriosis and restored in sera by gonadotropin-releasing hormone agonist therapy. *Fertil Steril* 1998b; **69**:1128–1134.

Simoens S, Hummelshoj L, D'Hooghe T. Endometriosis: cost estimates and methodological perspective. *Hum Reprod Update* 2007; **13**:395–404.

Somigliana E, Vigano P, Candiani M, Felicetta I, Di Blasio AM, Vignali M. Use of serum-soluble intercellular adhesion molecule-1 as a new marker of endometriosis. *Fertil Steril* 2002;**77**:1028–1031.

Somigliana E, Vigano P, Tirelli AS, Felicetta I, Torresani E, Vignali M, Di Blasio AM. Use of the concomitant serum dosage of CA 125, CA 19-9 and interleukin-6 to detect the presence of endometriosis. Results from a series of reproductive age women undergoing laparoscopic surgery for benign gynaecological conditions. *Hum Reprod* 2004;**19**:1871–1876.

Steff AM, Gagne D, Page M, Hugo P, Gosselin D. Concentration of soluble intercellular adhesion molecule-1 in serum samples from patients with endometriosis collected during the luteal phase of the menstrual cycle. *Hum Reprod* 2004a;**19**:172–178.

Steff AM, Gagne D, Page M, Rioux A, Hugo P, Gosselin D. Serum concentrations of insulin-like growth factor-1, soluble tumor necrosis factor receptor-1 and angiogenin in endometriosis patients. *Am J Reprod Immunol* 2004b;**51**:166–173.

Stroup D, Berlin J, Morton S, Olkin I, Williamson G, Rennie D, Moher D, Becker B, Sipe T, Thacker S. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis of observational studies in epidemiology (MOOSE) group. *JAMA* 2000;**283**:2008–2012.

Suzumori N, Sato M, Yoneda T, Ozaki Y, Takagi H, Suzumori K. Expression of secretory leukocyte protease inhibitor in women with endometriosis. *Fertil Steril* 1999;**72**:857–867.

Szyllo K, Tchorzewski H, Banasik M, Glowacka E, Lewkowicz P, Kamer-Bartosinska A. The involvement of T lymphocytes in the pathogenesis of endometriotic tissues overgrowth in women with endometriosis. *Mediators Inflamm* 2003;**12**:131–138.

Szymanowski K. Apoptosis pattern in human endometrium in women with pelvic endometriosis. *Eur J Obstet Gynecol Reprod Biol* 2007; **132**:107–110.

Takemura Y, Osuga Y, Harada M, Hirata T, Koga K, Morimoto C, Hirota Y, Yoshino O, Yano T, Taketani Y. Serum adiponectin concentrations are decreased in women with endometriosis. *Hum Reprod* 2005;**20**:3510–3513.

Tanaseanu C, Tudor S, Tamsulea I, Marta D, Manea G, Moldoveanu E. Vascular endothelial growth factor, lipoprotein-associated phospholipase A2, sP-selectin and antiphospholipid antibodies, biological markers with prognostic value in pulmonary hypertension associated with chronic obstructive pulmonary disease and systemic lupus erythematosus. *Eur J Med Res* 2007;**12**:145–151.

Telimaa S, Kauppila A, Ronnberg L, Suikkari A, Seppala M. Elevated serum levels of endometrial secretory protein PP14 in patients with advanced endometriosis. *Am J Obstet Gynecol* 1989;**161**:866–871.

Vercellini P, Sacerdote P, Panerai AE, Manfredi B, Bocciolone L, Crosignani G. Mononuclear cell beta-endorphin concentration in women with and without endometriosis. *Obstet Gynecol* 1992; **79**:743–746.

Vercellini P, De Benedetti F, Rossi E, Colombo A, Trespidi L, Crosignani PG. Tumor necrosis factor in plasma and peritoneal fluid of women with and without endometriosis. *Gynecol Obstet Invest* 1993;**36**:39–41.

Verit FF, Erel O, Celik H. Serum paraoxonase-1 activity in women with endometriosis and its relationship with the stage of the disease. *Hum Reprod* 2008;**23**:100–104.

Vigano P, Somigliana E, Matrone R, Dubini A, Barron C, Vignali M, di Blasio AM. Serum leptin concentrations in endometriosis. *J Clin Endocrinol Metab* 2002;**87**:1085–1087.

Wang L, Zheng W, Yu JK, Jiang WZ, Mu L, Zhang SZ. Artificial neural networks combined with surface-enhanced laser desorption/ionization mass spectra distinguish endometriosis from healthy population. *Fertil Steril* 2007;**88**:1700–1702.

Wang L, Zheng W, Mu L, Zhang SZ. Identifying biomarkers of endometriosis using serum protein fingerprinting and artificial neural networks. *Int J Gynaecol Obstet* 2008;**101**:253–258.

Watson J, Burling K, Fitzpatrick P, Kay E, Kelly J, Fitzpatrick J, Dervan P, McCann A. Urinary insulin-like growth factor 2 identifies the presence of urothelial carcinoma of the bladder. *BJU Int* 2009;**103**:694–697.

Whiting P, Rutjes AW, Reitsma JB, Bossuyt PM, Kleijnen J. The development of QUADAS: a tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews. *BMC Med Res Methodol* 2003;**3**:25.

Wild RA, Shivers CA. Antiendometrial antibodies in patients with endometriosis. *Am J Reprod Immunol Microbiol* 1985;**8**:84–86.

Wild RA, Hirisave V, Podczaski ES, Coulam C, Shivers CA, Satyaswaroop PG. Autoantibodies associated with endometriosis: can their detection predict presence of the disease? *Obstet Gynecol* 1991a; **77**:927–931.

Wild RA, Hirisave V, Bianco A, Podczaski ES, Demers LM. Endometrial antibodies versus CA-125 for the detection of endometriosis. *Fertil Steril* 1991b;**55**:90–94.

Wolfler MM, Schwamborn K, Otten D, Hornung D, Liu H, Rath W. Mass spectrometry and serum pattern profiling for analyzing the individual risk for endometriosis: promising insights? *Fertil Steril* 2009; **91**:2331–2337.

Wright S, Doughty R, Frampton C, Gamble G, Yandle T, Richards A. Plasma urocortin 1 in human heart failure. *Circ Heart Fail* 2009; **2**:465–471.

Wu MH, Yang BC, Hsu CC, Lee YC, Huang KE. The expression of soluble intercellular adhesion molecule-1 in endometriosis. *Fertil Steril* 1998; **70**:1139–1142.

Wu MH, Tsai SJ, Pan HA, Hsiao KY, Chang FM. Three-dimensional power Doppler imaging of ovarian stromal blood flow in women with endometriosis undergoing in vitro fertilization. *Ultrasound Obstet Gynecol* 2003;**21**:480–485.

Xavier P, Beires J, Belo L, Rebelo I, Martinez-de-Oliveira J, Lunet N, Barros H. Are we employing the most effective CA 125 and CA 19-9 cut-off values to detect endometriosis? *Eur J Obstet Gynecol Reprod Biol* 2005;**123**:254–255.

Xavier P, Belo L, Beires J, Rebelo I, Martinez-de-Oliveira J, Lunet N, Barros H. Serum levels of VEGF and TNF-a and their association with C-reactive protein in patients with endometriosis. *Arch Gynecol Obstet* 2006;**273**:227–231.

Yan L, Anderson G, DeWitte M, Nakada M. Therapeutic potential of cytokine and chemokine antagonists in cancer therapy. *Eur J Cancer* 2006;**42**:793–802.

Zachariah R, Schmid S, Radpour R, Buerki N, Fan AX-C, Hahn S, Holzgreve W, Zhong XY. Circulating cell-free DNA as a potential biomarker for minimal and mild endometriosis. *Reprod BioMed Online* 2009;**18**:407–411.

Zeller JM, Henig I, Radwanska E, Dmowski WP. Enhancement of human monocyte and peritoneal macrophage chemiluminescence activities in women with endometriosis. *Am J Reprod Immunol Microbiol* 1987; **13**:78–82.

Zhang X, Lin J, Deng L, Chen Z, Chen L. Peritoneal fluid and serum concentration of interleukin-16 in women with endometriosis. *Acta Obstet Gynecol Scand* 2005;**84**:297–298.

Zhang C, Maeda N, Izumiya C, Yamamoto Y, Kusume T, Oguri H, Yamashita C, Nishimori Y, Hayashi K, Luo J *et al.* Killer immunoglobulin-like receptor and human leukocyte antigen expression as immunodiagnostic parameters for pelvic endometriosis. *Am J Reprod Immunol* 2006a;**55**:106–114.

Zhang H, Niu Y, Feng J, Guo H, Ye X, Cui H. Use of proteomic analysis of endometriosis to identify different protein expression in patients with endometriosis versus normal controls. *Fertil Steril* 2006b;**86**:274–282.

Zhang H, Feng J, Chang XH, Li ZX, Wu XY, Cui H. Effect of surface-enhanced laser desorption/ionization time-of-flight mass spectrometry on identifying biomarkers of endometriosis. *Chin Med J (Engl)* 2009;**122**:373–376.

Zong L, Li Y, Ha X. Determination of HGF concentration in serum and peritoneal fluid in women with endometriosis. *Di Yi Jun Yi Da Xue Xue Bao* 2003;**23**:757–760.