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Role of angiogenesis in adenomyosis-associated abnormal uterine bleeding and subfertility: a systematic review

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BACKGROUND: Adenomyosis commonly occurs with abnormal uterine bleeding (AUB) and is associated with subfertility and a higher miscarriage rate. Recent evidence showed abnormal vascularization in the endometrium in patients with adenomyosis, suggesting a role of angiogenesis in the pathophysiology of AUB and subfertility in adenomyosis and providing a possible treatment target.

OBJECTIVE AND RATIONALE: We hypothesized that the level of abnormal vascularization and expression of angiogenic markers is increased in the ectopic and eutopic endometrium of adenomyosis patients in comparison with the endometrium of control patients. This was investigated through a search of the literature.

SEARCH METHODS: A systematic search was performed in PubMed and Embase until February 2019. Combinations of terms for angiogenesis and adenomyosis were applied as well as AUB, subfertility or anti-angiogenic therapy. The main search was limited to clinical studies carried out on premenopausal women. Original research articles focusing on markers of angiogenesis in the endometrium of patients with adenomyosis were included. Studies in which no comparison was made to control patients or which were not published in a peer-reviewed journal were excluded. A second search was performed to explore the therapeutic potential of targeting angiogenesis in adenomyosis. This search also included preclinical studies.

OUTCOMES: A total of 20 articles out of 1669 hits met our selection criteria. The mean vascular density (MVD) was studied by quantification of CD31, CD34, von Willebrand Factor (vWF) or factor-VIII-antibody-stained microvessels in seven studies. All these studies reported a significantly increased MVD in ectopic endometrium, and out of the six articles that took it into account, four studies reported a significantly increased MVD in eutopic endometrium compared with control endometrium. Five articles showed a significantly higher vascular endothelial growth factor expression in ectopic endometrium and three articles in eutopic endometrium compared with control endometrium. The vascular and pro-angiogenic markers α-smooth muscle actin, endoglin, S100A13, vimentin, matrix metalloproteinases (MMPs), nuclear factor (NF)-kB, tissue factor (TF), DJ-1, phosphorylated mammalian target of rapamycin, activin A, follistatin and myostatin, CD41, SLIT, roundabout 1 (ROBO1), cyclooxygenase-2, lysophosphatidic acid (LPA) 1,4-5, phospho signal transducer and activator of transcription 3 (pSTAT3), interleukin (IL)-6, IL-22 and transforming growth factor-β1 were increased in ectopic endometrium, and the markers S100A13, MMP-2 and -9, TF, follistatin, myostatin, ROBO1, LPA1 and 4-5, pSTAT3, IL-6 and IL-22 were increased in eutopic endometrium, compared with control endometrium. The anti-angiogenic markers E-cadherin, eukaryotic translation initiation factor 3 subunit and gene associated with retinoic-interferon-induced mortality 19 were decreased in ectopic endometrium and IL-10 in eutopic endometrium, compared with control endometrium. The staining level of vWF and two pro-angiogenic markers (NF-κB nuclear p65 and TF) correlated with AUB in patients with adenomyosis. We found no studies that investigated the possible relationship between markers of angiogenesis and subfertility in adenomyosis patients. Nine articles reported on direct or indirect targeting of angiogenesis in adenomyosis—either by testing hormonal therapy or herbal compounds in clinical studies or by testing angiogenesis inhibitors in preclinical studies. However, there are no clinical studies on the effectiveness of such therapy for adenomyosis-related AUB or subfertility.

WIDER IMPLICATIONS: The results are in agreement with our hypothesis that increased angiogenesis is present in the endometrium of patients with adenomyosis compared with the endometrium of control patients. It is likely that increased angiogenesis leads to fragile and more permeable vessels resulting in adenomyosis-related AUB and possibly subfertility. While this association has not sufficiently been studied yet, our results encourage future studies to investigate the exact role of angiogenesis in the etiology of adenomyosis and related AUB or subfertility in women with adenomyosis in order to design curative or preventive therapeutic strategies.

Key words: adenomyosis / angiogenesis / abnormal uterine bleeding / subfertility / anti-angiogenic therapy / endometriosis

Introduction

Adenomyosis is a common gynecological disorder, predominantly occurring in premenopausal women (Bergeron, 2006; Cohen *et al*., 1995). Adenomyosis may cause abnormal uterine bleeding (AUB) and dysmenorrhea and is associated with a 28% lower clinical pregnancy rate as well as a more than doubled risk of miscarriage in women undergoing IVF with autologous oocytes (Tremellen and Russell, 2011; Martinez-Conejero *et al*., 2011; Vercellini *et al*., 2014; Cohen *et al*., 1995). The main histologic feature of adenomyosis is the presence of endometrial glands and stroma within the myometrium (Bird *et al*., 1972). The ectopic endometrium is generally associated with smooth muscle hyperplasia (Abbott, 2017). Recently, innovations in ultrasound skill and reporting systems have proven to be of essential value in diagnosing adenomyosis without the need for prior hysterectomy (Van den Bosch *et al*., 2018; Stoelinga *et al*., 2018). Therapy options are still scarce and limited to hormonal suppression, hysterectomy, embolization or MRI-guided high-intensity focused ultrasound in experimental settings (de Bruijn *et al*., 2017; de Bruijn *et al*., 2017; de Bruijn *et al*., 2018; Pontis *et al*., 2016; Dueholm, 2017; Vannuccini *et al*., 2018; Fan *et al*., 2012). The pathology of adenomyosis explaining the symptoms of AUB and implantation failure remains inconclusive (Abbott, 2017; Vannuccini *et al*., 2017). Hypotheses include various biological mechanisms such as alterations in endometrial immunological environment, the increased local estrogen production due to P450 overexpression, alterations in apoptosis and/or increased angiogenesis in the endometrium (Tremellen and Russell, 2012; Brosens *et al*., 2004; Vannuccini *et al*., 2017).

Angiogenesis is the process of the outgrowth of new capillary blood vessels from existing blood vessels, which occurs in both physiological and pathological processes (Griffioen and Molema, 2000; Folkman, 1995). It occurs during the proliferative phase of the menstrual cycle when the endometrium is regenerated and is essential for successful embryonic implantation (Burton *et al*., 2009; Moller *et al*., 2001). The notion that the ectopic endometrium is surrounded by increased vascularization suggests that the ectopic endometrium releases factors that signal a quiescent vasculature to initiate capillary sprouting. This mechanism was first described in the cancer arena. Tumor cells mutate to start the production of angiogenic factors, which is the initiating event of the formation of new vasculature. This event is referred to as the angiogenic switch (Hanahan and Folkman, 1996) and has motivated the search for both angiogenic factors and angiogenesis inhibitors (Ramjiawan *et al*., 2017). Estrogen, which is considered a crucial factor in the etiology of adenomyosis, causes mobilization and microvessel

incorporation of endothelial progenitor cells resulting in angiogenesis (Chen *et al*., 2010; Rudzitis-Auth *et al*., 2016). Abnormal angiogenesis may occur due to modulation of the expression of angiogenesis-regulating genes, such as genes that regulate vascular endothelial growth factor (VEGF). Overexpression of VEGF leads to enhanced vascular sprouting (Hillen and Griffioen, 2007), decreased pericyte coverage and augmented vascular permeability and leakage. Such vasculature is expected to bleed more easily (Weis and Cheresh, 2005).

The role of angiogenesis is well established in endometriosis (Rudzitis-Auth *et al*., 2016) and has been recognized as a potential treatment target (Laschke and Menger, 2012). Adenomyosis has a strong relationship with endometriosis and can be found in one-third of patients with endometriosis (Larsen *et al*., 2011). Both endometriosis and adenomyosis are invasive diseases in which the endometrial cells have acquired invasive properties that require angiogenesis to establish at an ectopic site (Kang *et al*., 2010; Yen *et al*., 2017). Accordingly, polymorphisms in the angiogenic factor fibroblast growth factor 1 and 2 gene have been found to be associated with the risk of developing both endometriosis and adenomyosis (Kang *et al*., 2010). In line with endometriosis, where it is known that angiogenesis plays a role in the establishment of ectopic foci outside the uterus, this is also expected to play a role concerning ectopic foci inside the uterus (Groothuis *et al*., 2005). If angiogenesis exists in the eutopic endometrium, it may be additionally relevant if it is associated with AUB or subfertility. In subfertile patients undergoing surgery for deep endometriosis, pregnancy rates were 68% reduced when patients had coexistent adenomyosis (Vercellini *et al*., 2014). Although this suggests that adenomyosis negatively impacts the eutopic endometrium, the finding should be interpreted with caution since it was reported in a systematic review including not more than five observational studies. Prior studies have noted the potential role of angiogenesis in the pathophysiology of adenomyosis (Ota and Tanaka, 2003; Ota, 1992; Benagiano *et al*., 2012). With the knowledge that adenomyosis is found to be a common cause of AUB in women of the perimenopausal age group (Rizvi *et al*., 2013) and is associated with a higher risk of miscarriage and lower pregnancy rates in women treated with IVF, it is suggested that aberrant angiogenesis plays a role in the pathophysiology of adenomyosis-related AUB and subfertility.

However, very little is known about the role of angiogenesis in adenomyosis. To improve our knowledge of angiogenesis in the endometrium of women with adenomyosis, study its possible relation with alterations in vascular characteristics that may cause AUB and/or subfertility and explore the therapeutic potential of targeting angiogenesis, we performed a literature review to summarize the current knowledge of possible markers related to the angiogenesis in the endometrium of women with adenomyosis. The main objective was to test the hypothesis that abnormal vascularization and the level of angiogenic markers are increased in the ectopic and eutopic endometrium of adenomyosis patients in comparison with the endometrium of control patients. In addition, we review the biology of angiogenesis in adenomyosis and discuss possible therapeutic opportunities.

Methods

This systematic review is conducted in accordance to the Prisma guidelines (Liberati *et al*., 2009). The methods, inclusion/exclusion criteria, type of studies, study selection, data collection, risk of bias assessment, type of interventions and type of outcome measures were specified in advance and registered in the PROSPERO International prospective register of systematic reviews (CRD42017069832).

Literature search

Two databases, PubMed and Embase, were consulted until February 2019. Combinations of terms for angiogenesis, angiogenic markers and adenomyosis were applied as well as AUB, infertility or anti-angiogenic therapy. References of the studies and related articles were checked for inclusion if not already included in the primary search (see Supplementary Data for full search strategy).

Eligibility criteria

The first search (Search I: angiogenic-related outcome in adenomyosis patients) was limited to laboratory studies carried out on the endometrium of premenopausal women. Original research articles, such as cohort or case control studies, and prospective clinical trials, which investigated the presence of angiogenesis in the endometrium of adenomyotic patients and were published as full papers in peer-reviewed journals, were included. The research needed to focus on markers that indicate the presence of angiogenesis or are known to (among other functions) induce angiogenesis. Papers had to be written in English. Case reports, studies on cells cultured *in vitro* not taken from women with adenomyosis or studies that did not compare the endometrium of adenomyosis with the endometrium of control patients without adenomyosis or uterine fibroids were excluded. A second search (Search II: anti-angiogenic therapy for adenomyosis) was carried out to evaluate the current status of research into therapeutic options targeting angiogenesis in adenomyosis. For this search, all original research articles, including preclinical studies that evaluated angiogenesis inhibitors in experimental settings, were included.

Data extraction

Data from the included studies were extracted according to a predefined standardized format. The following information was extracted from each of the included articles: first author, year of publication, study design, number of patients included, patient and control characteristics, use of hormones, phase in menstrual cycle, study method, angiogenic-related outcome, type of cells and method of evaluation. All the angiogenic-related markers studied in the articles are summarized by category: vascular outcomes (including mean vascular density: MVD), pro-angiogenic markers and anti-angiogenic markers. We present findings on the differences in tissue expression of the angiogenic-related markers by endometrial type: ectopic (the displaced endometrium to a location within the myometrium, also noted as endometrium with abnormal location (Goteri *et al*., 2009), adenomyosis (Schindl *et al*., 2001), adenomyotic lesions (Huang *et al*., 2014), adenomyotic nodules (Carrarelli *et al*., 2015), adenomyotic tissue (Li *et al*., 2013) or adenomyotic foci (Liu *et al*., 2016) in the articles) or eutopic (the endometrium lining the uterine cavity in adenomyotic patients also noted as endometrium with normal location (Goteri *et al*., 2009)). Also, we report on the type of cells (endothelial, epithelial or stromal cells) where the angiogenic markers were found and on the tissue expression of angiogenic-related markers during the menstrual phase.

Quality assessment of the included studies

The quality assessment of the included studies was performed by two independent reviewers (MH and CW). The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) checklist was used to assess reporting quality of the included studies (Vandenbroucke *et al*., 2014), and the Newcastle Ottawa Scale was used to assess methodological quality of all included studies (Stang, 2010). Although the use of quality assessment scales is less common in the field of preclinical medicine, we considered the use of the Gold Standard Publication Checklist (GSPC) to Improve the Quality of Animal Studies, a valid method to enable quality evaluation of the preclinical studies (Hooijmans *et al*., 2010). The scales will be used as a guide to critically evaluate the quality of the studies, but will not be used to rate articles (da Costa *et al*., 2011; Sanderson *et al*., 2007).

Results

Study characteristics

The search using the abovementioned databases resulted in 1669 hits. After removal of duplicates and screening of both the titles and abstracts, 73 articles were retrieved for full-text screening. A total of 20 articles met our selection criteria and were included in our review (Fig. 1a).

The second search yielded 120 articles, out of which 17 full-text articles were assessed for eligibility and nine were included in this review (Fig. 1b).

The study characteristics of the included articles are shown in Tables I and II. The factors that were identified in the search are not all solely angiogenic factors. Some are usually categorized in the group of inflammatory cytokines, with angiogenesis regulatory functions (Table III). Since the factors were included in this study for their (anti-)angiogenic capacity, they will be referred to as (anti-)angiogenic factors for the remainder of this paper. All articles used immunohistochemical staining to study endometrial samples from premenopausal women. Some articles also used other techniques, such as western blotting, real-time PCR and ELISA. The terms ectopic and eutopic were used by most studies to describe the type of endometrium studied.

Assessment of risk of bias

The methodological quality was assessed using the STROBE checklist for the quality of reporting (Table IV), the Newcastle Ottawa Scale for possible sources of bias (Table V) and the GSPC for the preclinical studies (Table VI) to assess the reporting as well as the methodological quality of the included studies. Only 12 studies reported specifications of the location and inclusion dates of the conducted study. The lack of information poses risk of selection bias of study participants. Only a limited number of studies controlled for possible confounders, such as comorbidity, menstrual phase, age or parity (Liu *et al*., 2016; Yang *et al*., 2017; Cai *et al*., 2018; Liu *et al*., 2018). The preclinical animal studies on anti-angiogenic therapy often lacked clear reasoning why a specific animal model was chosen, a clear description of how the disease was defined and full disclosure on the type of anesthesia/euthanasia, description of general well-being and relevant physiological parameters of the animals. The discussion of the findings and clinical relevance, on the other hand, was accurate in all included studies.

Microvascular density and vascular characteristics

In nine articles, vascular characteristics or the MVD in endometrium of patients with adenomyosis was studied by quantification of microvessels through either CD31, CD34, von Willebrand Factor (vWF) or factor VIII (FVIII) antibody staining (Liu *et al*., 2016; Wang *et al*., 2016; Nie *et al*., 2011; Tokyol *et al*., 2009; Huang *et al*., 2014; Li *et al*., 2006; Schindl *et al*., 2001; Nie and Liu, 2016; Ota *et al*., 1998). Staining of these markers exclusively occurs in endothelial cells of blood vessels (Table III). In all articles that assessed the ectopic endometrium, the MVD was significantly increased compared with the control endometrium. Six studies found a significantly increased MVD in the eutopic endometrium from adenomyosis patients compared with the control endometrium (Liu *et al*., 2016; Huang *et al*., 2014; Wang *et al*., 2016; Nie *et al*., 2011; Ota *et al*., 1998; Nie and Liu, 2016), while two studies reported no significant difference (Schindl *et al*., 2001; Tokyol *et al*., 2009), and one did not study the difference between eutopic endometrium and control endometrium (Liu *et al*., 2016).

One article assessed the characteristics of capillaries in the endometrium through staining vWF (Ota *et al*., 1998). Compared with the control endometrium, the eutopic endometrium displayed an increase in mean and total surface area of capillaries, irrespective of the menstrual cycle phase. The ectopic endometrium was not investigated in this study. The highest increase was reported for the total surface area of capillaries; this was 11.6 times higher in the eutopic endometrium in adenomyosis patients than in the endometrium of controls during the proliferative phase. One group studied endoglin (Eng), a transmembrane homodimer glycoprotein that is known as an angiogenic marker because it is overexpressed in activated endothelial cells (Hayrabedyan *et al*., 2005). Eng staining was not observed in the endothelial cells of microvessels of the control endometrium, while it was expressed in all microvessels of the eutopic and ectopic endometrium, with stronger expression in eutopic than in ectopic endometrium.

Pro-angiogenic and vascular markers

A total of 22 markers were studied for their angiogenic characteristics in the 20 articles retrieved by our search (Table III). Five articles compared VEGF expression in the endometrium of adenomyosis patients with controls (Li *et al*., 2006; Wang *et al*., 2016; Huang *et al*., 2014; Liu *et al*., 2016; Li *et al*., 2013). All studies reported a significantly higher VEGF expression in the ectopic endometrium compared with control endometrium. In three articles, the eutopic endometrium was compared with the control endometrium (Wang *et al*., 2016; Huang *et al*., 2014; Li *et al*., 2006), where VEGF expression was reported to be increased in the eutopic endometrium compared with control endometrium. Immunoreactivity of cyclo-oxygenase 2 (COX-2) was studied in three studies (Tokyol *et al*., 2009; Ota *et al*., 2001; Li *et al*., 2013). One study reported significantly increased protein expression levels of COX-2 in adenomyotic tissue compared with control tissue (Li *et al*., 2013). Both other studies reported no significant difference in COX-2 expression between the groups.

α-Smooth muscle actin (SMA), S100A13, vimentin, matrix metalloproteinases (MMPs) 2 and 9, nuclear factor (NF)-kB (subunits p50/p65), tissue factor (TF), DJ-1, phosphorylated mammalian

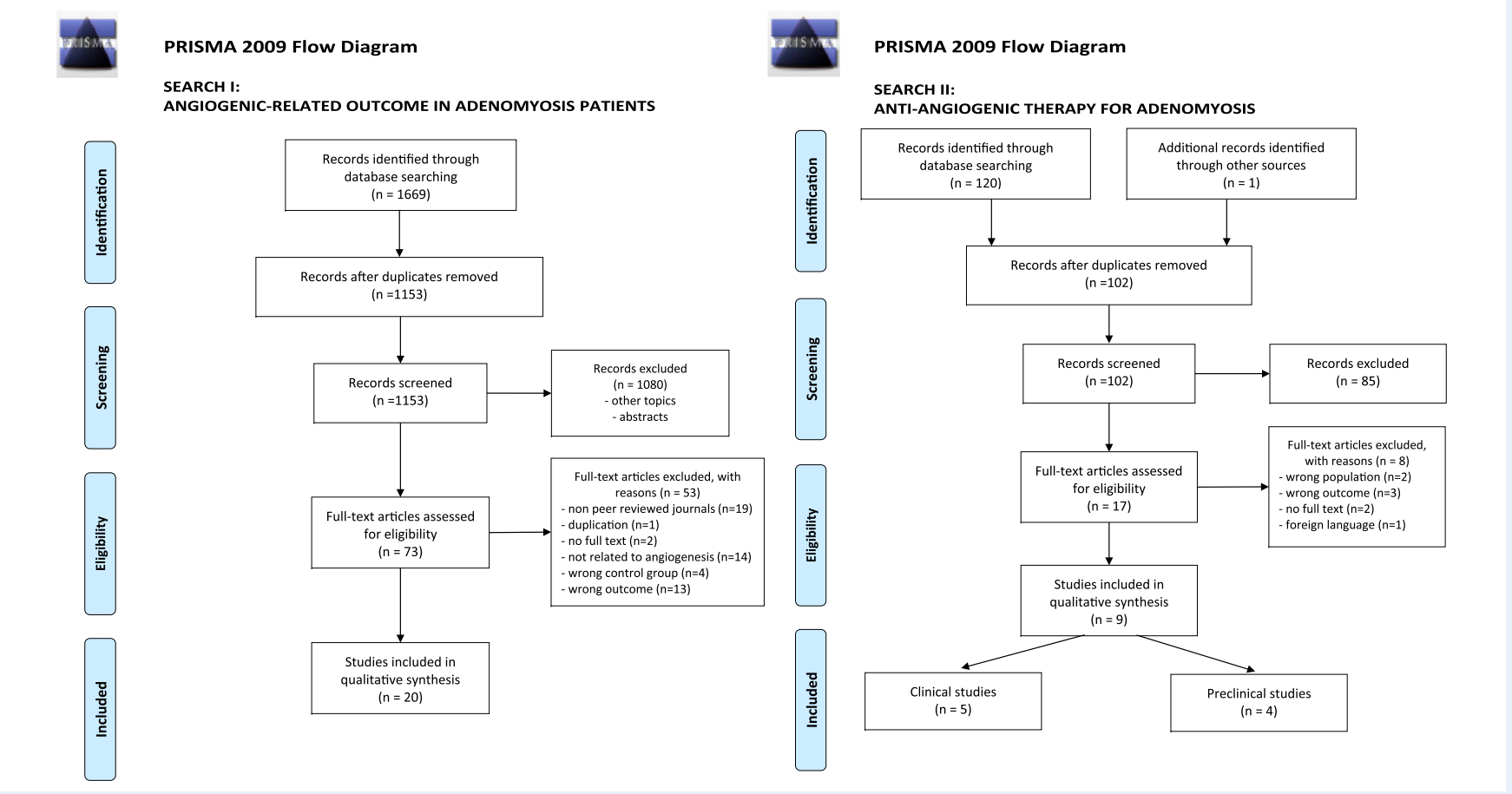

Figure 1 Prisma flow diagrams for the two searches carried out for the systematic review. (**a**) PRISMA 2009 Flow Diagram Search I: angiogenic-related outcome in adenomyosis patients. The search was limited to laboratory studies carried out on the endometrium of premenopausal women. (**b**) PRISMA 2009 Flow Diagram Search II: anti-angiogenic therapy for adenomyosis. The search was for all original research articles, including preclinical studies that evaluated angiogenesis inhibitors in experimental settings.

Table I Study characteristics of the included articles on angiogenic-related outcome.

Study, year	Study type	*N* participants	Patient group	Control group	Matching	Menstrual cycle phase	Time of no hormone use	Methods	Angiogenic parameters	Outcome		Association clinical symptom
										Eutopic	Ectopic	
Cai *et al.*, 2018	Case–control	80	40[1]	40	NA	Proliferative and secretory	6 months	IHC	eIF3e	-	↓	-
									TGF-β	-	↑	
									E-cadherin	-	↓	
									Vimentin	-	↑	
									Snail	-	↑	
									PCNA	-	↑	
Yang *et al.*, 2017	Case–control	60	10	10	Age	Proliferative	6 months	IHC and ELISA	LPA1[a]	↑↑	↑↑	-
									LPA2[a]	↓	↓↓	
									LPA3[a]	↓	↓↓	
									LPA4[a]	↑↑	↑↑	
									LPA5[a]	↑↑	↑↑	
									LPA6[a]	=	=	
Nie *et al.*, 2016	Case–control	68	50[2]	18	NR	Proliferative and secretory	6 months	IHC	vWF	↑	↑	Menorrhagia
									CD68	↑	↑	
Liu *et al.*, 2016	Cross-sectional case control	81	34[1]	20[6]	Age, parity, menstrual phase	Proliferative and secretory	6 months	IHC, IF and western blot	TGF-β1[a]	-	↑↑↑	-
									CD41[a]	-	↑↑↑	
									Vimentin[a]	-	↑↑↑	
									E-cadherin[a]	-	↓↓↓	
									VEGF[a]	-	↑↑↑	
									CD31[a]	-	↑↑↑	
									α-SMA[a]	-	↑↑↑	
Zhihong *et al.*, 2016	Case–control	42	18	24	NA	Secretory	1 year[1]	IHC, quantitative RT-PCR	IL-6[a]	↑	-	-
									IL-10[a]	↓		
Wang *et al.*, 2016	Case–control	40	30[2]	10	NR	Proliferative and secretory	3 months	IHC, *in situ* TUNEL, western blot	GRIM-19[b]	↓/↓↓	↓↓/↓↓	-
									VEGF[b]	↑↑/↑↑	↑↑/↑↑	
									CD34[b]	↑↑/↑	↑/↑↑	
Guo *et al.*, 2015	Prospective case–control	47	30	17	NR	Proliferative and secretory	3 months	IHC and western blot	DJ-1[e]	↑/↑	↑/↑	-
									p-mTOR[e]	↑/↑	↑/↑	

(Continued)

Table I *(Continued)*

Study, year	Study type	*N* participants	Patient group	Control group	Matching	Menstrual cycle phase	Time of no hormone use	Methods	Angiogenic parameters	Outcome		Association clinical symptom
										Eutopic	Ectopic	
Carrarelli *et al.*, 2015	Prospective case–control	20	8[3]	12	NR	Proliferative	1 month	IHC, RT-PCR	Activin A[a]	=	↑	-
									ActRIIA[a]	↑	↑↑↑	
									ActRIIB[a]	↑↑	↑↑	
									Follistatin[a]	↑↑↑	↑	
									Myostatin[a]	↑↑↑	↑	
Huang *et al.*, 2014	Prospective case–control	130	100	30	NR	Proliferative, secretory	3 months	IHC, ELISA, western blot	VEGF[c]	↑↑↑/↑↑↑	↑↑↑/↑↑↑	-
									CD31[c]	↑↑↑/↑↑↑	↑↑↑/↑↑↑	
Wang *et al.*, 2014	Case–control	20	10	10	NA	NR	NR	IHC	IL-22, IL-22R1, IL-10R2	↑	↑	-
Li *et al.*, 2013	Case–control	31	23	8	None	NR[b]	3 months	Electrophoretic shift assay, gene, protein expression	NF-kB (p50, p65)	-	↑↑↑	Dysmenorrhea
									COX-2	-	↑↑	
									VEGF	-	↑↑	
									TF	-	↑↑	
Nie *et al.*, 2011	Case–control	68	50[2]	18	NR	Proliferative and secretory	6 months	IHC	SLIT[b]	=	↑	Dysmenorrhea (SLIT)
									ROBO1[b]	↑	↑	
									CD34[b]	↑	↑	
Liu *et al.*, 2011	Case–control	68	50[2]	18	NR	Proliferative and secretory	6 months	IHC	TF[b]	↑↑↑	↑↑	AUB dysmenorrhea
Nie *et al.*, 2009	Retrospective case–control	68	50	18	None	Proliferative and secretory	6 months	IHC	PR-B[a]	↓↓↓	↓	AUB dysmenorrhea
									NF-kB (p50, p52, p65)[a]	↑	↑	
Tokyol *et al.*, 2009	Retrospective case–control	45	25	20	NR	Proliferative and secretory	NR	IHC	MMP-2	=/=	↑/↑	-
									COX-2	=/=	=/=	
									CD34	=/=	↑↑/↑↑	
Li *et al.*, 2006	Prospective case–control	88	68	20	NR	Proliferative and secretory	3 months	IHC	MMP-2	↑/↑	↑/↑	-
									MMP-9	↑/↑	↑/↑	
									F-VIII Rag[b]	=	↑/↑	
									VEGF[b]	↑/↑	↑/↑	
Hayrabedyan *et al.*, 2005	Case–control	50	20	5	NR	Secretory	NR	IHC	Endoglin[c]	↑	↑↑	-
									S100A13[c]	↑	-	

(Continued)

Table I *(Continued)*

Study, year	Study type	N participants	Patient group	Control group	Matching	Menstrual cycle phase	Time of no hormone use	Methods	Angiogenic parameters	Outcome		Association clinical symptom
										Eutopic	Ectopic	
Schindl *et al.*, 2001	Retrospective case–control	70	53	17	NR	Proliferative and secretory	4 months	IHC	CD34[b]	=	↑↑↑/↑↑↑	-
Ota *et al.*, 2001	Case LISTNUM – control	83	33[4]	50	NR	Proliferative and secretory	None	IHC	COX-2[b]	=/=	=/=	-
Ota *et al.*, 1998	Case–control	71	42[5]	29	NR	Proliferative and secretory	None	IHC	vWF[b]	↑/↑	-	-

Superscripts: [1]focal and diffuse, [2]all diffuse, [3]all focal adenomyosis, [4]group includes nine patients with ovarian cyst, [5]includes nine patients with leiomyoma less than one-third of uteri corpus uteri, [6]includes patients with carcinoma *in situ*; [a]no comparison performed between the phases, [b]no (significant) differences observed between the phases, [c]no *P*-value known for difference between the phases

Symbols: - not included in the study, = no difference observed compared with control endometrium; ↑ increase, ↓ decrease; ↑ or ↓ significant difference compared with control endometrium with $P < 0.05$; ↑↑ or ↓↓: significant difference compared with control endometrium with $P \leq 0.01$; ↑↑↑ or ↓↓↓ significant difference compared with control endometrium with $P \leq 0.001$

/: difference between the menstrual phases indicating: proliferative phase / secretory phase

NR, not reported; IHC, immunohistochemistry; IF, immunohistofluorescence; VEGF, vascular endothelial growth factor; MMP, matrix metalloproteinase; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; *p*-mTOR, phosphorylated mammalian target of rapamycin; COX-2, cyclooxygenase-2; LPA, Lysophosphatidic acid; pSTAT3, phospho signal transducer and activator of transcription 3; IL-, interleukin-; α-SMA, alpha smooth muscle actin; TGF-β, transforming growth factor beta; GRIM-19, gene associated with retinoic-interferon-induced mortality 19; eIF3e, eukaryotic translation initiation factor 3 subunit; PCNA, proliferating cell nuclear antigen; vWF, von Willebrand factor; TF, tissue factor; ROBO1, roundabout 1; AUB, abnormal uterine bleeding

target of rapamycin (*p*-mTOR), activin A, folli- and myostatin, platelet count through CD41, SLIT, roundabout 1 (ROBO1), lysophosphatidic acid (LPA) 1,4-5, phospho signal transducer and activator of transcription 3 (pSTAT3), interleukin (IL)-6, IL-22 and transforming growth factor (TGF)-β1 are markers that were identified in this search as being involved in angiogenesis and vascular establishment. They are studied in one or two articles per factor (Table III). The immunohistochemical staining and protein expression levels of all markers were significantly increased in the ectopic endometrium compared with the endometrium of control patients. For the markers S100A13, MMP-2 and MMP-9, TF, follistatin, myostatin, ROBO1, LPA1 and 4-5, pSTAT3, IL-6 and IL-22, an increase in expression was also observed in eutopic compared with control endometrium.

Anti-angiogenic markers

Four articles that reported on markers with an anti-angiogenic capacity were identified (Tables I and III). Two articles reported decreased immunostaining levels of E-cadherin in the ectopic endometrium compared with control endometrium (Cai *et al.*, 2018; Liu *et al.*, 2016). IL-10 was investigated in endometrial samples obtained during the implantation window after ovarian stimulation and was lower in the eutopic endometrium of adenomyosis patients than in endometrium samples of control patients (Zhihong *et al.*, 2016). The immunohistochemical staining of gene associated with retinoic-interferon-induced mortality (GRIM)-19 was less positive in the ectopic endometrium compared with the eutopic endometrium, which in turn was less positive than the control endometrium (ectopic<eutopic<control) (Wang *et al.*, 2016). Eukaryotic translation initiation factor 3 subunit (eIF3e) immunoreactivity was significantly decreased in the ectopic endometrium compared with the control endometrium. eIF3e staining levels correlated positively with those of E-cadherin and negatively with those of TGF-β1 and vimentin (Cai *et al.*, 2018).

Interferon (IFN)-α and tumor necrosis factor (TNF)-α are other known (pro- and anti-, respectively) angiogenic markers that have been reported to play an important role in several angiogenesis-related pathologies (von Marschall *et al.*, 2003; Jin *et al.*, 2019). We found no studies that investigated the expression of these markers in the endometrium of adenomyosis patients versus control patients. Sotnikova *et al.* (2002) studied cytokine expression in supernatants of mononuclear cells from the eutopic and ectopic endometrium from adenomyosis patients in comparison with control patients and found increased levels of both IFN-α and TNF-α in supernatants derived from the eutopic and ectopic endometrium compared with the control endometrium. However, no association with angiogenesis was considered. Li *et al.* (2013) studied the effect of TNF-α on the expression of NF-κB DNA-binding activity and protein expression of NF-κB-mediated genes COX-2, VEGF and TF in stromal cells of the ectopic endometrium of adenomyosis patients and the endometrium of control patients. The NF-κB-binding activity, assessed by electrophoretic mobility shift assay, and its protein (p50 and p65) expression levels were significantly higher in the ectopic endometrium than in the control endometrium ($P < 0.01$ and $P < 0.001$ respectively). Also, the protein levels of COX-2 ($P < 0.0017$), VEGF ($P < 0.0006$) and TF ($P < 0.0013$) were significantly higher in the ectopic endometrium than in controls.

Table II Potential anti-angiogenic strategies for treatment of adenomyosis.

Substance group	Mechanism	Anti-angiogenic agent	Studies	Effect on endo- or myometrium	Effect on clinical symptoms
GnRH agonist	Reduction of estrogen and progesterone concentrations	Leuprolide acetate	(Khan *et al.*, 2010)	Decreased staining of vWF (MVD) and angiogenesis in endo- and myometrium. No difference in MVD in adenomyotic lesions	-
Progestogens	Reduction of estrogen concentration and progesterone receptors	Levonorgestrel (LNg-IUS)	(Laoag-Fernandez *et al.*, 2003)	Decrease in VEGF expression in endometrial epithelium and stroma	No correlation between number of bleeding days and expression levels of VEGF
Growth factor inhibitors	Alleviating expression of IL-6, IL-8, TGF-β, EGF, VEGF, MMP-2	Berberine	(Liu *et al.*, 2018) (Liu *et al.*, 2017)	Inhibit proliferation and viability of eutopic and ectopic endometrial stromal cells	-
	NF-κB inhibitor	Andrographolide (*Andrographis paniculata*)	(Li *et al.*, 2013)	Decrease in mRNA and protein levels of COX-2, VEGF and TF	-
Preclinical studies	**Mechanism**	**Anti-angiogenic agent**	**Studies**	**Model**	**Effect on endo- or myometrium**	**Effect on clinical symptoms**
Anti-platelet therapy	Thromboxane A2 (TXA2) synthesis inhibitor or platelet depletion	Ozagrel; rat anti-mouse GPIbα polyclonal IgG antibody	(Zhu *et al.*, 2016)	ICR mice with neonatal dosing of tamoxifen	Both ozagrel and platelet depletion reduced the depth of myometrial invasion, platelet aggregation and staining level of COX-2 and NF-κB p65	Diminished hyperalgesia (improvement in hot plate latency)
Growth factor inhibitors	Anti-VEGF antibody	Bevacizumab	(Huang *et al.*, 2014)	Xenotransplantation of human adenomyosis in NODSCID mice	Reduce blood vessel formation	-
	Annexin A2 inhibition (ANXA2)	ANXA2 knockout via siRNA	(Zhou *et al.*, 2012)	Nude mouse model	Inhibition of endometrial tissue growth	Diminished hyperalgesia (improvement in hot plate latency)
Fumagillin analogues	Reduction mean surface area blood vessels	TNP-470	(Zhou *et al.*, 2003)	Virgin female SHN mice implanted with pituitary gland	Inhibition of endothelial cell migration and proliferation	-

MVD, mean vascular density; LNG-IUS, levonorgestrel-releasing intrauterine system; EGF, epidermal growth factor; NODSCID, non-obese diabetic severe combined immunodeficient,

Table III Outcome of vascular characteristics and angiogenic markers.

	Location in uterus						Studies
	Eutopic endometrium			Ectopic endometrium			
	Endothelium	(Glandular) epithelium	Stroma	Endothelium	(Glandular) epithelium	Stroma	
MVD and vascular characteristics							
MVD by CD31	↑			↑↑↑			(Huang *et al*,. 2014, Liu *et al*., 2016)
MVD by CD34	↑/=			↑			(Schindl *et al*., 2001; Tokyol *et al*., 2009; Nie *et al*., 2011; Wang *et al*., 2016)
MVD by FVIII-Rag	=			↑↑↑			(Li *et al*., 2006)
vWF	↑						(Ota *et al*., 1998; Nie and Liu, 2016)
Endoglin	↑			↑			(Hayrabedyan *et al*., 2005)
Pro-angiogenic and vascular markers							
VEGF	↑	↑	↑	↑	↑	↑	(Li *et al*., 2006; Li *et al*., 2013; Huang *et al*. 2014; Liu *et al*., 2016; Wang *et al*., 2016; Liu *et al*., 2016; Wang *et al*., 2016; Huang *et al*., 2014; Li *et al*., 2006; Li *et al*., 2013)
S100A13	↑	↑	↑	↑	↑	↑	(Hayrabedyan *et al*., 2005)
Vimentin			=		↑↑	↑	(Liu *et al*., 2016; Cai *et al*., 2018)
MMP-2, -9		↑	↑		↑	↑	(Li *et al*., 2006; Tokyol *et al*., 2009)
NF-kB (p50, p65)						↑	(Nie *et al*., 2009; Li *et al*., 2013)
Tissue factor		↑	↑		↑	↑	(Liu *et al*. 2011; Li *et al*., 2013; Liu *et al*., 2011; Li *et al*., 2013)
DJ-1					↑↑↑	↑	(Guo *et al*., 2015)
p-mTOR					↑↑↑	↑	(Guo *et al*., 2015)
Activin A					↑*		(Carrarelli *et al*., 2015)
Follistatin		↑	↑		↑	↑	(Carrarelli *et al*., 2015)
Myostatin		↑	↑		↑	↑	(Carrarelli *et al*., 2015)
Platelet count by CD41						↑	(Liu *et al*., 2016; Cai *et al*., 2018)
SLIT					↑		(Nie *et al*., 2011)
ROBO1	↑	↑		↑	↑		(Nie *et al*., 2011)
COX-2	=	=	=	=	=	↑	(Ota *et al*., 2001; Tokyol *et al*., 2009; Li *et al*., 2013)

(Continued)

Table III ***(Continued)***

	Location in uterus						
	Eutopic endometrium			Ectopic endometrium			Studies
	Endothelium	(Glandular) epithelium	Stroma	Endothelium	(Glandular) epithelium	Stroma	
LPA1, 4-5		↑	↑		↑↑↑	↑↑↑	(Yang *et al.*, 2017)
pSTAT3		↑	↑		↑↑↑	↑↑↑	(Wang *et al.*, 2016)
*IL-6***		↑	↑				(Zhihong *et al.*, 2016)
α-SMA					↑	↑	(Liu *et al.*, 2016)
*IL-22***		↑	↑		↑	↑	(Wang *et al.*, 2014)
*TGF-β1***				↑	↑	↑	(Liu *et al.*, 2016; Cai *et al.*, 2018; Liu *et al.*, 2016; Cai *et al.*, 2018)
Anti-angiogenic markers							
GRIM-19		↓					(Wang *et al.*, 2016)
*IL-10***		↓	↓		↓↓↓		(Zhihong *et al.*, 2016)
E-cadherin					↓		(Liu *et al.* 2016; Cai *et al.* 2018)
eIF3e					↓		(Cai *et al.*, 2018)

= No difference; ↑/↓ increase/decrease compared with control endometrium; ↑/ ↓ significant increase/decrease compared with control endometrium; ↑↑/↓↓ significant increase/decrease compared with eutopic endometrium; ↑↑↑/↓↓↓ significant increase/decrease compared with control and eutopic endometrium. *Type of cells not reported, **Inflammatory cytokines with angiogenesis regulatory functions.

Type of cells expressing (anti-)angiogenic markers

All markers for MVD and vascular characteristics were specific for endothelial cells in order to quantify and characterize blood vessels in the studied samples. Immunoreactivity of S100A13 (Hayrabedyan *et al.*, 2005), MMP-2 and ROBO1, a receptor for SLIT, was also observed on the surface and within vascular endothelial cells (Nie *et al.*, 2011; Tokyol *et al.*, 2009). Glandular epithelial cells (Wang *et al.*, 2016; Li *et al.*, 2006; Goteri *et al.*, 2009; Liu *et al.*, 2016; Lai *et al.*, 2016; Orazov *et al.*, 2016), stromal cells (Li *et al.*, 2006; Goteri *et al.*, 2009; Li *et al.*, 2013; Orazov *et al.*, 2016) and endothelial cells (Goteri *et al.*, 2009; Orazov *et al.*, 2016) in the endometrium were stained positive for VEGF in women with adenomyosis whereas the staining in the control endometrium was either very low or not reported (Wang *et al.*, 2016; Li *et al.*, 2006; Goteri *et al.*, 2009; Liu *et al.*, 2016; Li *et al.*, 2013). The expression of COX-2 was observed not only in the endothelial, glandular (Ota *et al.*, 2001; Tokyol *et al.*, 2009) and stromal (Li *et al.*, 2013) cells but also in plasma cells (Ota *et al.*, 2001). S100A13 (Hayrabedyan *et al.*, 2005) staining was present in glandular epithelial cells, stromal cells and vascular smooth muscle cells as well as in the perivascular space. For the markers activin A and its receptors ActRIIA and ActRIIB, the type of cell was not studied.

Immunoreactivity of α-SMA, TGF-β1 and vimentin (only ectopic) (Liu *et al.*, 2016; Cai *et al.*, 2018), MMP 2–9 (Li *et al.*, 2006; Tokyol *et al.*, 2009), TF (Liu *et al.*, 2011; Li *et al.*, 2013), DJ-1/*p*-mTOR (Guo *et al.*, 2015), follistatin, myostatin (Carrarelli *et al.*, 2015), LPA1,4–5 (Yang *et al.*, 2017), pSTAT3 (Wang *et al.*, 2016), IL-6 and IL-10 (only eutopic) (Zhihong *et al.*, 2016) and IL-22 (Wang *et al.*, 2014) was reported in both glandular epithelial cells and stromal cells. Tissue expression of E-cadherin, eIF3e (Liu *et al.*, 2016; Cai *et al.*, 2018), SLIT (Nie *et al.*, 2011) and GRIM-19 (Wang *et al.*, 2016) was only reported in epithelial cells, while NF-κB (p50, p65) (Li *et al.*, 2013; Nie *et al.*, 2009) and CD41 (Liu *et al.*, 2016) expression was only reported in the stroma. ROBO1 (Nie *et al.*, 2011) and MMP-2 were reported in both endothelial and epithelial cells.

Tissue expression of angiogenic markers during the menstrual cycle

Eighteen studies mentioned the phase of the menstrual cycle in which the samples were collected (Table I). Two of the six articles on MVD in which the difference between the menstrual phases was taken into account reported an increased MVD in the secretory phase compared with the early proliferative phase (Huang *et al.*, 2014; Tokyol *et al.*, 2009), whereas the other studies found no difference in MVD between the secretory and proliferative phases (Nie *et al.*, 2011; Li *et al.*, 2006; Schindl *et al.*, 2001; Wang *et al.*, 2016; Nie and Liu, 2016). All Eng samples were taken in the secretory phase (Hayrabedyan *et al.*, 2005). The levels of staining for vWF were increased in all adenomyotic samples, with no difference between the menstrual phases (Ota *et al.*, 1998). One study reported that the staining of VEGF had a greater density in the secretory phase compared with the proliferative phase (Huang *et al.*, 2014), while two studies found no difference between the phases (Li *et al.*, 2006; Wang *et al.*, 2016) and two others did not compare menstrual phases (Li *et al.*, 2013; Liu *et al.*, 2016).

Table IV STROBE: Strengthening the Reporting of Observational Studies in Epidemiology.

	Cai *et al.*, 2018	Liu, 2018	Liu, 2017	Yang *et al.*, 2017	Liu, 2016	Zhihong, 2016	Wang, 2016	Guo, 2015	Carrarelli, 2015	Huang, 2014	Wang, 2014	Li *et al.*, 2013	Nie, 2011	Liu, 2011	Goteri, 2009	Khan, 2010	Nie, 2009	Tokyol, 2009	Li, 2006	Hayrabedyan, 2005	Laoag-Fernandez, 2003	Schindl, 2001	Ota, 2001	Ota, 1998
Item 5: setting, locations, dates	1	0	1	0	1	1	0	1	0	0	0	0	1	1	1	1	1	1	0	0	0	0	0	1
Item 6: eligibility criteria, selection of participants	1	0	0	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1	0	0	1	1	1
Item 6b: matching criteria	0	NA	NA	1	1	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	NA
Item 7: define variables	1	0	0	1	1	1	1	1	1	1	0	1	1	1	1	0	0	1	1	0	0	1	1	1
Item 8: data sources and methods of assessment	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1
Item 9: sources of bias	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Item 10: study size	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Item 12: statistical methods	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
Item 13: participant results	NA	NA	NA	1	NA	1	NA	NA	NA	NA	NA	1	NA	NA	NA	NA	1	NA	NA	NA	1	NA	NA	NA
Item 14: descriptive results	1	0	0	1	1	1	1	0	0	0	0	1	0	1	0	0	1	0	0	0	0	0	0	1
Item 16: unadjusted estimates results	NA	1	1	NA	0	NA	0	0	0	0	0	1	0	0	0	NA	1	NA	NA	0	1	NA	NA	NA

Assessment of risk of bias according to the STROBE checklist for observational studies: each item is classified as adequate (1), inadequate (0) or not applicable 'NA' in the evaluation of a paper

Table V **Newcastle Ottawa Scale for case control studies.**

	Cai *et al.*, 2018	Liu, 2018	Liu, 2017	Yang *et al.*, 2017	Nie, 2016	Liu, 2016	Zhihong, 2016	Wang, 2016	Guo, 2015	Carrrelli, 2015	Huang, 2014	Wang, 2014	Li *et al.*, 2013	Nie, 2011	Liu X. 2011	Khan. 2010	Nie, 2009	Tokyol, 2009	Li, 2006	Hayrabedyan, 2005	Laoag-Fernandez, 2003	Schindl, 2001	Ota, 2001	Ota, 1998
Selection:																								
1. Is the case definition adequate?	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0
2. Representativeness of the cases	1	1	1	1	1	1	1	0	1	1	0	0	0	1	1	1	1	0	1	0	0	0	0	0
3. Selection of controls	1	1	1	1	0	1	1	0	0	1	0	0	1	0	0	1	0	1	1	0	NA	0	0	0
4. Definition of controls	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	1	1	0	NA	0	1	1
Comparability:																								
1a. Study controls for important factor	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1b. Study controls for additional factor	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Exposure																								
Ascertainment of exposure	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
Same method of ascertainment	0	1	1	1	1	0	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1

Assessment of risk of bias according to the Newcastle Ottawa Scale for case control studies: each item is classified as adequate (1), inadequate (0) or not applicable 'NA' in the evaluation of a paper

Table VI Gold standard publication checklist.

	Zhu, 2016	Huang, 2014	Zhou, 2012	Zhou, 2003
Introduction				
Background information (3p)	3	2	2	2
Research question or hypothesis (2p)	1	0	1	1
Clinical relevance (2p)	1	0	1	2
Methods				
Experimental design (1p)	1	1	1	1
Experimental groups and controls (19p)	14	6	13	13
Regulations and ethics (2p)	2	1	1	1
The intervention (12p)	12	4	7	11
Outcome (3p)	2	1	2	2
Results (7p)	6	4	3	3
Discussion (3p)	3	3	3	3
Total score out of 54	45	22	34	39

Assessment of risk of bias: each topic contains a list of items which ought to be addressed in every research article on animal experiments (Hooijmans et al., 2010). The total of items per topic (1 point per item) is stated behind each topic

In the secretory phase, there was an increased expression reported of the pro-angiogenic parameters COX-2, MMP-2 and MMP-9 (only for eutopic endometrium) (Tokyol et al., 2009; Li et al., 2006). However, Ota et al. (2001) reported no difference in COX-2 expression between the phases. No difference in expression between the phases was reported for SLIT and ROBO1 (Nie et al., 2011), TF (Liu et al., 2011) and GRIM-19 or pSTAT3 (Wang et al., 2016). DJ-1 and *p*-mTOR were the only parameters with a higher expression reported in the proliferative phase than in the secretory phase (Guo et al., 2015). Differences between the two menstrual phases were not studied for α-SMA, TGF-β1, platelet aggregation (CD41), vimentin, E-cadherin, eIF3e (Liu et al., 2016; Cai et al., 2018), NF-κB (Nie et al., 2009; Li et al., 2013) and IL-22 (Wang et al., 2014). The samples for LPA (Yang et al., 2017), activin A, follistatin and myostatin (Carrarelli et al., 2015) were all collected in the proliferative phase, while the samples for IL-6 and IL-10 (Zhihong et al., 2016) and Eng were only taken in the secretory phase (Hayrabedyan et al., 2005).

Angiogenesis in relation to AUB and subfertility

Most studies included in this review only described indirect effects of the MVD or (anti-)angiogenic markers on adenomyosis-related symptoms of AUB or subfertility (Fig. 2). One study found that the MVD in both eutopic and ectopic endometrium was significantly higher in women who reported heavy menses than those who reported light/moderate menses (Nie and Liu, 2016). Two studies assessed the relation between an angiogenic marker and the presence of AUB. One study (Nie et al., 2009) reported NF-κB nuclear p65 immunoreactivity to be associated with heavier menstrual bleeding and increased p65 in the ectopic endometrium to be significantly associated with the severity of dysmenorrhea in adenomyosis patients. Another study reported an increased level of TF staining in patients that reported symptoms of heavy menstrual bleeding compared with patients with light/moderate menstrual bleeding (Liu et al., 2011).

Anti-angiogenic therapy for adenomyosis

The search on anti-angiogenic therapy in adenomyosis identified nine articles reporting on targeting angiogenesis in (pre)clinical studies (Table II). GnRH agonists (GnRHa) may act indirectly, through estrogen or estrogen's effect on the expression of VEGF, on angiogenesis in the endometrium (Khan et al., 2010). As GnRHa induces a hypo-estrogenic state, they lower VEGF and COX-2, which in turn may result in a downregulation of the abnormal endometrial vascularity (Khan et al., 2010). The vWF-positive MVD in biopsy specimens obtained after reduction surgery and hysterectomy for adenomyosis in women with GnRHa therapy for 3–6 months was significantly decreased compared with the non-treated group (Khan et al., 2010). The levonorgestrel-releasing intra-uterine system (LNg-IUS) is a common treatment modality for menorrhagia, and the first line of treatment for adenomyosis (Vannuccini et al., 2018). One study demonstrated that after 3 months of LNg-IUS use, the level of expression of VEGF was decreased in the eutopic endometrium of patients with adenomyosis, while the effect on the ectopic endometrium was not studied (Laoag-Fernandez et al., 2003). On the contrary, the level of VEGF expression was not correlated to the number of bleeding days. A number of herbal compounds have been studied for their anti-angiogenic potential. Andrographolide has been reported to disrupt NF-κB activation (Li et al., 2013). Treating adenomyosis patients with andrographolide resulted in reduced expression levels of COX-2, VEGF and TF (Li et al., 2013; Zheng et al., 2018). Another study investigated the growth inhibitory effect of berberine and

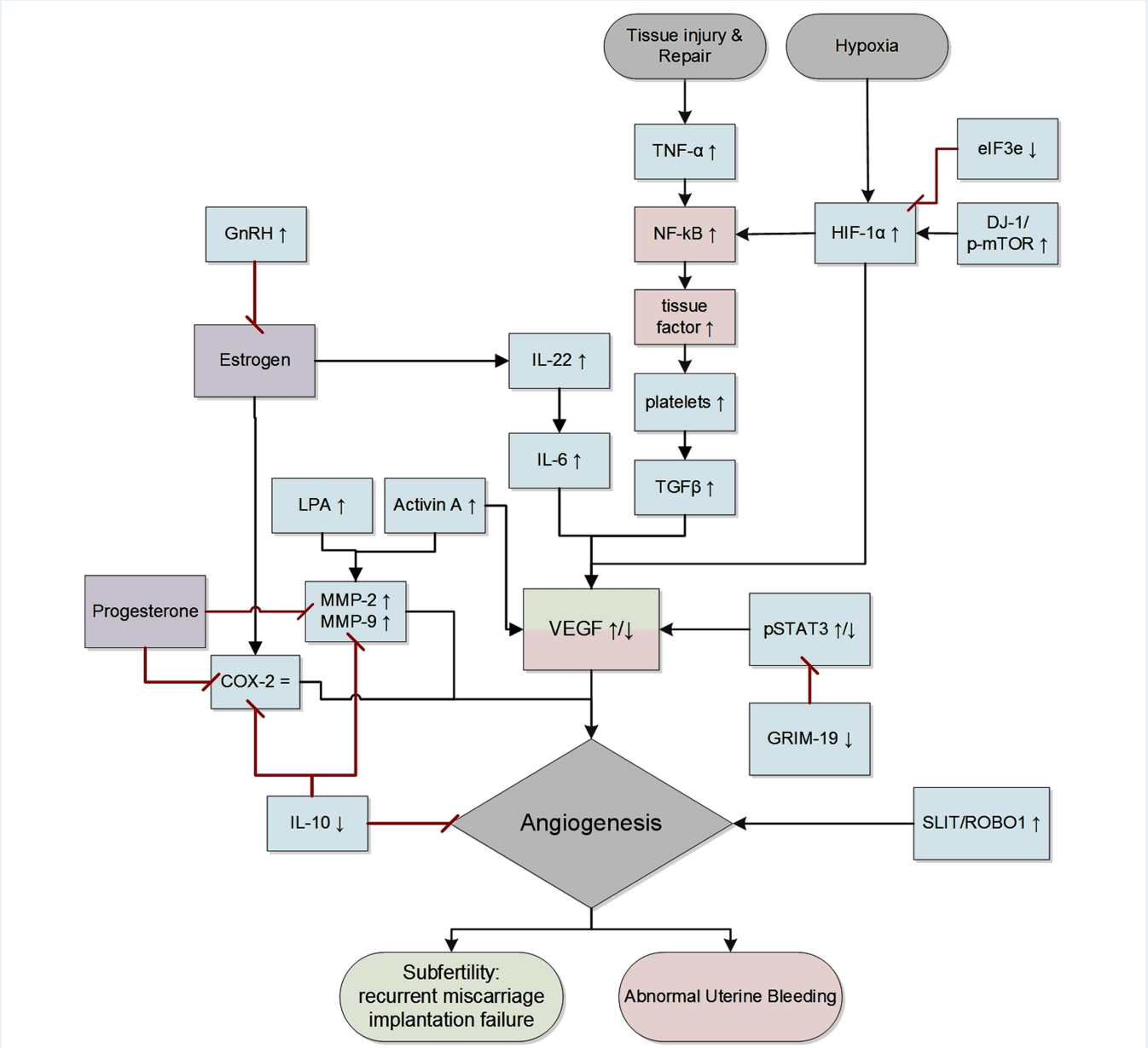

Figure 2 Overview of interactions between the (anti-)angiogenic markers reported in this review. Red: association with abnormal uterine bleeding; green: association with subfertility. ↑ increase, = no change, ↓ decrease. TNF-α, tumor necrosis factor alpha; NF-κB nuclear factor kappa-light-chain-enhancer of activated B cells; HIF-1α, hypoxia-inducible factor 1 alpha; eIF3e, eukaryotic translation initiation factor 3 subunit; DJ-1; *p*-mTOR, phosphorylated mammalian target of rapamycin; TGF-β, transforming growth factor beta; VEGF, vascular endothelial growth factor; IL-, interleukin-; LPA, lysophosphatidic acid; MMP, matrix metalloproteinase; COX-2, cyclooxygenase-2; pSTAT3, phospho signal transducer and activator of transcription 3; GRIM-19, gene associated with retinoic-interferon-induced mortality 19; SLIT; ROBO1, roundabout 1. Note: adjusted figure based on information described in the literature. However, these interactions do not necessarily represent the actual or complete biological pathways. Diagram made with Microsoft Visio 2010.

reported a decrease in the expression levels of IL-6, IL-8, TGF-β, EGF, VEGF and MMP-2, thus inhibiting the growth of ectopic endometrial stromal cells (Liu *et al*., 2017; Liu *et al*., 2018). This effect was also partly exerted through the inhibition of nuclear NF-κB/p65 translocation.

Four studies were identified that targeted angiogenesis in a preclinical study (Table II). In ovariectomized mice xenografted with human adenomyosis lesions and treated with bevacizumab (anti-VEGF monoclonal antibody), the MVD decreased and the expression of VEGF was reduced (Huang *et al*., 2014). Another study targeted angiogenesis in mice that developed adenomyosis after pituitary gland implantation using TNP-470, a potent inhibitor of angiogenesis known from arthritis research (Zhou *et al*., 2003). The mean surface area of blood vessels in the endometrium of the TNP-470-treated group reduced to 60.5% of that in the control group, and the TNP-470-treated group did not develop signs of uterine adenomyosis as opposed to 80% in the

control group. Zhou et al. (2012) identified the angiogenic potential of overexpressed annexin A2 (ANXA2), an estrogen-responsive protein, through hypoxia inducible factor (HIF)-1α/VEGF-A activation, in the ectopic endometrium compared with the eutopic endometrium of adenomyosis patients. Expression of ANXA2 was associated with the epithelial-to-mesenchymal transition (EMT), as well as to the severity of dysmenorrhea in adenomyosis patients. In an *in vivo* nude mouse model, they demonstrated that ANXA2 knockdown reduced angiogenesis, suggesting its potential as therapeutic target.

Zhou et al., 2016 investigated the effect of high- and low-dose anti-platelet therapy using a thromboxane A2 synthesis inhibitor (ozagrel) or platelet depletion therapy using the rat anti-mouse GPIbα polyclonal IgG antibody in an adenomyosis mouse model. Platelet activation is related to angiogenesis since its co-occurrence with TGF-β1 release leads to EMT and fibroblast-to-myofibroblast transdifferentiation. Both ozagrel and platelet depletion therapy reduced depth of myometrial invasion, platelet aggregation and staining level of COX-2 and NF-κB p65. There were no adverse effects observed after ozagrel treatment. However, in the high-dose platelet depletion group, one mouse died and two appeared lethargic, while there were no apparent side effects in the low-dose depletion therapy group. Both treatment strategies resulted in significantly reduced platelet counts, depth of myometrial infiltration and staining level of COX-2 and NF-κB p65.

Discussion

Main findings

This study gives an overview of the angiogenesis-related markers in the endometrium of women with adenomyosis. Out of all outcomes of the included articles, MVD and VEGF were the most often studied parameters. The majority of articles that included these two markers reported an increase in levels in both ectopic and eutopic endometrium in adenomyosis patients compared with control patients. Increased VEGF leads to more angiogenesis, which in turn results in an increased MVD. The markers studied in the included 20 articles either directly or indirectly influence the expression of VEGF, MVD or capillary characteristics, suggesting their impact on angiogenesis in both ectopic and eutopic located endometrium (Table III and Fig. 2). This is in agreement with our hypothesis that adenomyosis patients have an increased MVD and display more angiogenic markers in the eutopic and ectopic endometrium than healthy individuals. Direct correlation with AUB or fertility outcomes has not been studied extensively, and so far only the staining levels of vWF, NF-κB nuclear p65 and TF had a direct correlation with AUB.

The staining of pro- and anti-angiogenic markers was reported in all types of endometrium cells: these include endothelial cells, epithelial cells and stromal cells (Table III). The endometrium consists of endothelial cells lining the blood vessels, glandular columnar epithelial cells lining the cavity of the uterus and the glands and stromal cells that make up the layer of connective tissue underlying the layer of epithelial cells. The expression of angiogenic markers in all cell types and in both eutopic and ectopic endometrium suggests that angiogenesis stimulates the invasiveness of eutopic endometrial tissue and the maintenance of the ectopic endometrium (Zhou et al., 2012). The formation of new and fragile blood vessels in the eutopic endometrium might lead to AUB and impair embryo receptivity.

There was no strong evidence for a difference in expression of angiogenic markers between the proliferative or secretory phases of the menstrual cycle. Continuous regeneration and degeneration of the endometrium during the menstrual cycle under the influence of estradiol (E_2) has been associated with VEGF expression in endometrial epithelial cells (Moller et al., 2001), causing a higher expression of VEGF in the secretory phase than in the early (Day 6–10) proliferative phase (Chen et al., 2010; Huang et al., 2014). This is surprising, since we expected an increased level of angiogenesis in the proliferative phase after the menses to rebuild the endometrial layer under the influence of increased E_2 levels. The inconsistency with our findings may be explained by different expression of VEGF within the early, mid and late proliferative phases (Sugino et al., 2002), which were not taken into account in the included studies.

Interpretation of the findings

The potential role of angiogenesis in the pathophysiology of adenomyosis and its related symptoms is complex. It is a process that is suggested to be triggered by tissue injury and repair (TIAR) causing reactive EMT in response to hypoxic and hormonal stimuli (Leyendecker et al., 2009; Ibrahim et al., 2017). An interpretation of the findings of our review and their potential interaction is depicted in Fig. 2.

The underlying pathogenesis relies on the theory that endometrial cells invade the myometrium at sites in the junctional zone that are weakened, either from genetic predisposition or from uterine auto-traumatization and induced hypoxia (Benagiano et al., 2012). In this theory, hyper and non-synchronized peristalses of the uterus following physiological processes such as menstruation or sperm transport cause chronic damage to the junctional zone, where the endometrium lies adjacent to the myometrium (Leyendecker, 2015). When the endometrium invades these disruptions in the junctional zone, ectopic foci of endometrium establish and cause local inflammation and hypoxia. Exposure to ovarian estrogens, but also local estrogen production due to local estrogen sulfatase and aromatase activity in the adenomyotic tissue, may play an additional role (Bulun, 2009; Yamamoto et al., 1993). These events are directly related to the increased angiogenesis in these tissues since they lead to the production of VEGF, thus causing angiogenesis (Goteri et al., 2009). Hypoxia is suggested to be a driving factor for physiological angiogenesis to induce endometrial repair during menstruation (Maybin et al., 2018). A vicious cycle is established when estrogen increases uterine peristalsis again and more auto-traumatization follows (Vannuccini et al., 2017).

Hereby, this TIAR theory provides insight into the role of the immune system and inflammation in adenomyosis. The reciprocal relationship between angiogenesis and the immune system has long been recognized (Griffioen and Molema, 2000). On the one hand, it is known that angiogenesis can be regulated by immune cells (Dirkx et al., 2006; Sica et al., 2008; Dings et al., 2011) and cytokines produced by immune cells (Castermans et al., 2008; Yao et al., 1999). On the other hand, immunity is heavily regulated by angiogenesis (Griffioen et al., 1996; Griffioen et al., 1996; Dirkx et al., 2003; Dirkx et al., 2006). This makes the separation between angiogenesis and immunity difficult, also reflected by the cytokines in Table III, and the correlation with increased macrophage number in adenomyotic tissue (Nie and Liu, 2016). Most leukocytes produce a series of angiogenic factors, including VEGF, TGF-β and ILs (Carmeliet, 2003;

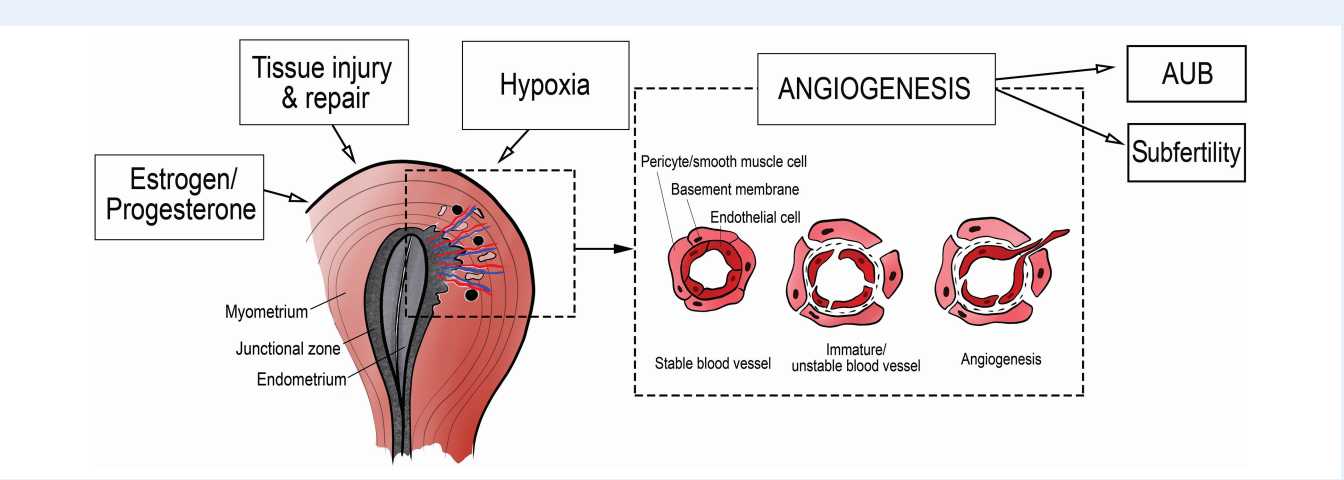

Figure 3 Hypothesis on angiogenesis and abnormal uterine bleeding. AUB, abnormal uterine bleeding.

Ramjiawan *et al*., 2017), and neutrophils and natural killer cells were recognized as initiators of abnormal angiogenesis in endometriosis (Li *et al*., 2001).

VEGF and angiogenesis appear to play a key role in the development of adenomyosis, which is represented by the fact that polymorphisms in the VEGF gene seem to be related to the risk of developing adenomyosis (Kang *et al*., 2009). VEGF is a specific and important angiogenic parameter given its stimulating effect on the proliferation of endothelial cells (Hillen and Griffioen, 2007). The increased VEGF activity in the eutopic and ectopic endometrium in adenomyosis compared with control endometrium suggests increased vascular sprouting and number of fragile blood vessels that bleed more easily (Fig. 3) (Hillen and Griffioen, 2007; Griffioen and Molema, 2000). Moreover, increased VEGF has been reported to be associated with increased neurogenesis, possibly also explaining symptoms of dysmenorrhea (Orazov *et al*., 2016). During hypoxia, the expression of HIF-1α may lead to upregulation of the VEGF gene through activation of the NF-κB pathway (Groenman *et al*., 2007; Semenza *et al*., 1991; Tong *et al*., 2006; van Uden *et al*., 2011; Chen *et al*., 2015b; de Rooij *et al*., 2004). This can take place via DJ-1 (Vasseur *et al*., 2009; Slomovitz and Coleman, 2012), a multifunctional protein overexpressed in a variety of endometrial tumors and various stages of endometriosis that is associated with cell proliferation, migration and invasion (Rai and Shivaji, 2011). Enhanced staining of DJ-1 and HIF-1α in adenomyosis patients implies increased VEGF, which is known to be associated with more permeable and leaky vessels (Zhang and Salamonsen, 2002). Also, platelet aggregation was increased in adenomyotic lesions (Liu *et al*., 2016; Cai *et al*., 2018). Platelets are considered an important player in angiogenesis, as they contain over 80% of the total circulating VEGF (Peterson *et al*., 2010).

Estrogen and progesterone are known angiogenic factors in the uterus, implicating the hormonal role for angiogenesis in adenomyosis patients (Hyder *et al*., 2000). Estrogen is known to induce EMT—the transition of endometrial epithelial cells (associated with a decrease in E-cadherin) to mesenchymal cells, accompanied by an increase in vimentin (Chen *et al*., 2010; Ribatti, 2017). This process is linked to angiogenesis, and the same factors that are involved in EMT may drive endothelial cells toward a pro-angiogenic state in adenomyosis (Chen *et al*., 2010; Khan *et al*., 2015; An *et al*., 2017). The presence of EMT was evident by the increased expression of TGF-beta and vimentin and decreased E-cadherin staining in the epithelial cells of the ectopic endometrium in adenomyosis patients (Liu *et al*. 2016; Chen *et al*., 2010; Cai *et al*. 2018). TGF-beta derived from platelets is known to activate transcription markers involved in EMT (Zhang *et al*., 2016), and its presence was previously reported in adenomyotic mice (Ribatti, 2017; Shen *et al*., 2016). eIF3e, a eukaryotic initiation factor that seems to have an anti-angiogenic effect [silencing of eIF3e has previously been associated with increased angiogenesis through HIF-1α activation (Chen *et al*. 2010)] was studied for its supposed role in EMT in adenomyosis. Indeed, decreased expression of eIF3e was related to increased TGF-beta and vimentin levels in the ectopic endometrium (Cai *et al*. 2018). Staining of vimentin appeared in the cytoplasm of stromal cells in control and adenomyosis groups. It was stained positive in the cytoplasm of epithelial cells in the ectopic adenomyotic endometrium but negative in the control endometrium (Liu *et al*., 2016; Cai *et al*., 2018). Thus, vimentin may serve as a marker for adenomyotic tissue. α-SMA is a molecular marker for pericytes (used to determine the maturity of vessels) and predominates within vascular smooth muscle cells (Morikawa *et al*., 2002). Decreased levels of α-SMA expression were reported in women with menorrhagia compared with controls (Abberton *et al*., 1999), suggesting an increase in immature leaky vessels. Our expectation was contradicted in this review since increased α-SMA expression was reported in adenomyosis patients. However, the increase in TGF-β1 and α-SMA was reported only in the endometrial epithelium and stromal cells (Liu *et al*., 2016) and only provides evidence of fibrogenesis, which might have evolved secondary to angiogenesis in the myometrium (Liu *et al*., 2016).

Estrogen is also a potent stimulus for COX-2, which in turn leads to increased levels of prostaglandins in the uterus (Bulun *et al*., 2000; Chen *et al*., 2010). Symptoms of AUB and dysmenorrhea are associated with increased prostaglandin levels (Maia *et al*., 2005), while COX-2 was associated with angiogenic markers *in vitro* (Leung *et al*., 2003). However, this could not be confirmed *in vivo*, since COX-2 expression was not different between the ectopic and control endometrium (Tokyol *et al*., 2009).

The other angiogenic markers identified in this review, such as IL-6, IL-22, LPA (1-6), SLIT/ROBO, activin A, follistatin, myostatin and pSTAT3, stimulate endometrial stromal cells to produce VEGF (Carrarelli et al., 2015; Rocha et al., 2012; Wang et al., 2014; Wang et al., 2016) and induce autocrine activity, attraction, migration and proliferation of vascular endothelial cells (Wu et al., 2005; Kozian et al., 1997; Dickinson and Duncan, 2010; Wang et al., 2003; Wang et al., 2008; Yang et al., 2017). However, previous studies reported no positive correlation of SLIT/ROBO with MVD (Wang et al., 2003), and when LPA was added to a culture of endometrial stromal cells, there were no differences in the release of VEGF between cases and controls (Yang et al., 2017). The presence of activin A was previously linked to the development of AUB and dysmenorrhea since it increased VEGF in endometriosis patients (Rocha et al., 2012). Activin A has been linked to physiological processes, such as embryo implantation, and pathologies including endometrical carcinoma, where it acts by activating MMP-2 and -9 (Jones et al., 2006). MMPs are known for their great impact on tissue degradation during menstruation (Salamonsen and Woolley, 1996), and a link with increased angiogenesis in tumors has been reported (Bergers et al., 2000; Fang et al., 2000; Tang et al., 2005). MMP-2 and -9 were increased in adenomyosis patients, and the positive correlation with VEGF indicates increased angiogenesis (Li et al., 2006). STAT3 is reported to upregulate VEGF expression and stimulate growth of tumors through angiogenesis (Niu et al., 2002). By inhibiting STAT3, GRIM-19 can downregulate the VEGF expression. IL-10 is a known anti-angiogenic and anti-inflammatory factor that is produced by uterine natural killer cells and macrophages. IL-10 inhibits angiogenesis through downregulation of COX-2 and MMP-2 and -9 (Moore et al., 2001; Zhihong et al., 2016). A significantly lower expression of IL-10 reported in the adenomyosis endometrium compared with the control endometrium indicates an upregulation of angiogenesis.

Pathology of angiogenesis in adenomyosis in relation to AUB

Increased angiogenesis resulting in an increased amount of leaky fragile vessels may be the cause of AUB in women with adenomyosis (Leyendecker et al., 2009) (Fig. 3). The correlation between AUB and the MVD through staining of vWF, and increased levels of TF and NF-κB was the only evidence found to directly support this theory. TF is a glycoprotein in the cell membrane that is involved in the clotting cascade and platelet activation (Liu et al. 2011; Krikun et al., 2008). Increased levels of TF with the co-occurrence of enlarged and widened blood vessels in bleeding sites of the endometrium were linked to angiogenesis in the endometrium (Krikun et al., 2009; Krikun et al., 2008; Liu et al., 2011). The expression of TF was positively correlated with AUB and dysmenorrhea (Liu et al., 2011).

The NF-κB heterodimer belongs to a family of nuclear transcription markers and is known to be involved in the degradation of basement membrane and the remodeling of the extracellular matrix, which is one of the earliest processes in angiogenesis. Also, it has been suggested to play a pivotal role in endometriosis (Guo 2007). However, the exact role of NF-κB in angiogenesis seems to differ among cell types and is inconclusive (Tabruyn and Griffioen 2008; Tabruyn et al. 2009). Thus, suggesting that NF-κB's role in angiogenesis explains its association with dysmenorrhea and the severity of AUB in adenomyosis patients is disputable (Nie et al., 2010; Li et al., 2013). TNF-α, a known pro-angiogenic factor, can activate NF-κB, which translocates and binds to the nucleus of its target genes (Gilmore, 2006) and leads to higher protein levels of COX-2, VEGF and TF. This binding activity was associated with the severity of dysmenorrhea in adenomyosis patients (Li et al., 2013).

Pathology of angiogenesis in adenomyosis in relation to subfertility

VEGF and angiogenesis are crucial for embryonic implantation, decidual vascularization and the early stages of development (Burton et al., 2009). Any disruption in this process might lead to fertility problems. Unfortunately, a direct relationship between angiogenic markers and subfertility has not been studied in adenomyosis patients and could therefore not be established in this review. According to our hypothesis, there is indirect evidence to support the relationship between the increase in angiogenic markers in the endometrium of adenomyosis patients summarized in this review and the observed lower probability of clinical pregnancy and a 2-fold risk of miscarriage in this population undergoing IVF (Campo et al., 2012; Vercellini et al., 2014). One study reported increased levels of the angiogenic factor IL-6 and decreased IL-10 during the window of implantation in adenomyosis patients versus control patients after ovarian stimulation with GnRHa, indicating that increased angiogenesis might be involved in the observed compromised endometrium receptivity (Zhihong et al., 2016). No pregnancy outcomes were available to support this hypothesis.

Other studies have explored the relation between VEGF and abortions or miscarriages. Compared with control patients, there was an increased VEGF-A expression in women with spontaneous miscarriages. In women with recurrent miscarriages, decreased VEGF-A and VEGF-C with increased levels of VEGF-R1 and -R2, TGF-β1 and α-SMA staining and reduced levels of IL-10 were reported (Lash et al., 2012; Plevyak et al., 2002). Another study reported a decreased GRIM-19, resulting in an increased STAT3 expression in villous samples of patients with miscarriages compared with controls (Chen et al., 2015). They reported a lower VEGF expression in women with miscarriages in the same study (Chen et al., 2015). Suppression of adenomyosis by long-term downregulation with GnRHa, which might indirectly reduce VEGF expression, resulted in improvement in IVF outcomes with no difference in clinical pregnancy rates and early pregnancy loss in comparison to controls (Mijatovic et al., 2010; Costello et al., 2011). These results suggest that abnormal levels of VEGF (subtypes) may lead to a disbalance in the angiogenesis process that may play a role in a higher miscarriage rate. According to Lash et al. (2012), women with recurrent miscarriage have more matured vessels in the endometrium compared with controls, which contradicted our hypothesis of less matured vessels being the cause of infertility. This contradiction might be explained by the following: for an ongoing pregnancy, a certain balance of vessel maturity should be achieved. Possibly both overly matured vessels and less matured vessels can lead to miscarriages. In case of more matured vessels, less production of blood vessels or less adaptation of blood flow to the implanted fetus might be the cause of a higher risk for miscarriage. However, to the best of our knowledge there is no literature to further explore this theory.

Strengths and limitations

This is the first review of available literature describing possible angiogenic-related markers in the adenomyotic endometrium (both

ectopic and eutopic) and relating the pathophysiology of angiogenesis to clinical symptoms of adenomyosis, such as AUB and fertility problems. Strengths of this review include the systematic approach in accordance with the Prisma guidelines using two databases (PubMed and Embase), its registration in the PROSPERO International prospective register of systematic reviews and the use of quality checklists to assess the included articles for risk of bias. Another strength of the review is the stratified presentation of the results concerning the outcomes of vascular characteristics (MVD), (anti-) angiogenic markers, type of cells and menstrual phase. However, not all articles reported the menstrual phase, age or parity of the participants or adjusted the results accordingly. Neither were other potential confounders, such as medication use or comorbidity, most importantly endometriosis, taken into account in most articles. The lack of information on these factors seriously limits the interpretability of the observed differences in the angiogenic profile of the endometrium and prevents further insight into the possible correlation of these individual factors with angiogenesis in the endometrium. Despite the limitation of the unknown effect of concomitant endometriosis, the presence of angiogenesis in the eutopic endometrium in adenomyosis seems most relevant for the association with AUB or subfertility (Vercellini *et al*., 2014).

Other limitations of the articles include the relatively small sample size and the heterogeneity of the study population and of the angiogenic markers. Most of the studied markers were only studied and reported in a few articles, hampering the ability to draw solid conclusions on all individual factors in relation to angiogenesis in adenomyosis. Some possible relevant angiogenic markers, such as IFN-alpha and TNF-alpha, were not studied in the endometrium of adenomyosis patients. Additionally, some articles included other endometrial abnormalities in their control group, and these abnormalities may share pathological pathways with adenomyosis (Tokyol *et al*. 2009). Also, many articles lacked a pre-reported definition of adenomyosis or a description of the location of the biopsies taken. Differences in depth of the endometrial invasion might lead to different angiogenic results. The additional search on potential anti-angiogenic therapy for adenomyosis further strengthens this review and stresses the clinical relevance of the topic. However, the heterogeneity of the anti-angiogenic agents and (animal) models used severely limits the interpretability of these results. Moreover, the animal studies lacked a complete description of the applied methods and the results. For example, incomplete reporting of physiological parameters of the animals limits the understanding of the side-effect profile of the applied therapy.

Conclusion

The increased expression of angiogenic markers and decrease in anti-angiogenic markers, as well as an increase in MVD and capillary characteristics in the ectopic and eutopic endometrium in comparison with the endometrium in control patients without adenomyosis, support our hypothesis that increased angiogenesis plays a role in the pathogenesis of adenomyosis.

Future perspectives

The implicated role of these markers in AUB and subfertility outcomes, such as lowered pregnancy rates and increased miscarriage rates during IVF, is only based on indirect evidence. Direct evidence requires future studies focusing on the relation between angiogenesis and these clinical outcomes in adenomyosis patients. Confirmation of this hypothesized relation might give future tools for the development of targeted selective therapies. When a positive correlation between ongoing angiogenesis and the occurrence of adenomyosis can be confirmed, it is tempting to speculate on the use of angiostatic drugs for treatment of adenomyosis. Similar to endometriosis, where the benefit of angiostatic therapy has been validated (Laschke and Menger, 2012; Zheng *et al*., 2018), it is suggested that adenomyosis can be efficiently treated with inhibitors of angiogenesis as well. To date, studies of anti-angiogenic treatment regimens for adenomyosis are still lacking. Available literature on the treatment of adenomyosis report on hormonal therapies, some of which (in)directly influence angiogenesis, on preclinical studies or on the effects of herbal compounds which appear to inhibit vascular growth factors (Table II) (Streuli *et al*., 2014; Benagiano *et al*., 2009). Since endometriosis has been recognized as an angiogenic-dependent disease for a long time, anti-angiogenic compounds have been studied in endometriosis to a greater extent and a number of *in vitro* and *in vivo* studies show promising results (Nap *et al*., 2005; Nap *et al*., 2004; Korbel *et al*., 2018; Hu *et al*., 2017). However, there are no clinical studies on the effectiveness of such therapies for adenomyosis-related AUB or subfertility. Treatment that is targeted at attenuating angiogenesis has been studied most extensively in the field of cancer research (Wentink *et al*., 2015; Hurwitz *et al*., 2004; Dings *et al*., 2006; Wu *et al*., 2019). Angiostatic approaches in the field of cancer, however, have only limited effects on patient survival. This is due to the fact that cancer cells have the capacity to become resistant to angiogenesis inhibitors, e.g. through drifting towards dependency to alternative growth factors (van Beijnum *et al*., 2015; Bani *et al*., 2017; Huijbers *et al*., 2016). Inhibition of angiogenesis may be extremely attractive for non-malignant diseases in which cells are not genetically unstable, as was observed in ophthalmology applications. Treatment of age-related macula degeneration, for example, appears to have resulted in a renaissance of therapeutic intervention (Rosenfeld *et al*., 2006). Therefore, such treatment also seems attractive for reversing or inhibiting angiogenesis in adenomyosis. Nonetheless, the clinical applicability of these compounds in the population of adenomyosis patients is much more restricted than in the patients with cancer, whose compounds were originally developed for: in the latter population, a life-threatening disease needs to be treated, and for that reason, the safety requirements differ completely from the requirements for a drug that treats a benign disease in a population that wants to preserve fertility and accepts fewer side effects (Laschke and Menger, 2012). Since angiogenesis in the reproductive tract is one of the only physiologically occurring angiogenesis mechanisms in the human body, this function would need to be preserved.

Local application of a therapeutic agent would ideally reduce angiogenesis and adenomyosis-related AUB and fertility difficulties without disrupting physiological angiogenesis in the reproductive organs.

Supplementary data

Supplementary data are available at *Human Reproduction Update* online.

Authors' roles

M.H.: conceptualization, data analysis and writing. C.W.: conceptualization and data analysis. F.G.: supervision, review and editing. V.M.:

review and editing. W.H.: review and editing. J.H. and A.W.G: supervision, review and editing. All authors also contributed to the critical revision of the intellectual content and approved the final version of the paper.

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Conflict of interest

The authors have no conflicts of interest to declare.

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