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Clinicopathological and prognostic significance of blood microvessel density in endometrial cancer: a meta-analysis and subgroup analysis

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Abstract

Objective The study aimed to systematically review the association between angiogenesis and clinicopathological characteristics and its prognostic value in patients with endometrial cancer.

Methods Eligible studies were searched in PubMed, Embase, China National Knowledge Infrastructure, and Wanfang database. Studies that assessed blood microvessel density (BMVD) and correlated with clinicopathological features and/or overall survival (OS) were included. Geometric mean values and hazard ratio with 95% confidence interval were pooled to examine the risk or hazard association. Subgroup analyses were conducted based on populations, BMVD criteria, BMVD markers, and type of survival analysis.

Results A total of 29 studies of 2517 patients were included. BMVD was associated with depth of myometrial invasion (MI) [standard mean difference (SMD) 1.24; 95% CI 0.53–1.95; $P = 0.0006$], lymphovascular space invasion (LVSI) (SMD 0.75; 95% CI 0.3–1.21; $P = 0.001$), and lymph node metastasis (LNM) (SMD 0.99; 95% CI 0.46–1.52; $P = 0.0003$). BMVD was also significantly associated with poor OS (HR 2.65; 95% CI 1.86–3.77; $P < 0.00001$). The association remained significant in the subgroups Asian population, BMVD criteria using Weidner method, BMVD marker CD34 for MI, LVSI, and LNM, CD105 for MI, and factor VIII for MI and LNM, respectively. For OS, either Asian or non-Asian population, BMVD criteria using Weidner or non-Weidner method, BMVD marker CD31, or factor VIII antibody and analysis using univariate or multivariate were all significantly associated.

Conclusions BMVD was associated with deeper MI, positive LVSI, positive LNM, and poor OS in patients with endometrial cancer. Therefore, angiogenesis is a useful measure for poor clinicopathological outcomes and prognosis in patients with endometrial cancer.

Keywords Microvessel density · Endometrial cancer · Myometrial invasion · Lymphovascular space invasion · Lymph node metastasis · Overall survival

Introduction

During tumor growth, tumor angiogenesis is a key step, because a tumor needs to recruit new vasculature from existing blood vessels to grow beyond more than 2 mm in diameter. Furthermore, angiogenesis is also critical for tumor invasion, migration, and metastasis [1]. In 1991, Weidner et al. first developed blood microvessel density (BMVD) assessment to qualify tumor angiogenesis through immunohistochemical staining blood microvessel by factor VIII [2]. From then, BMVD count was commonly accepted and applied in assessment of all kinds of tumor angiogenesis, and now the commonly used antibodies include factor VIII, CD31, CD34, and CD105.

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Accumulated evidence indicated that tumor angiogenesis assessed by BMVD is associated with advanced clinicopathological parameters and poor prognostic outcomes in different kinds of cancers. Meta-analysis confirmed that BMVD was associated with poor survival in breast [3], colorectal [4], and bladder cancers [5]. Endometrial cancer represents the most common gynecologic malignancy and the fourth most cause of death among cancer patients with increasing prevalence in United States, Europe, and China [6, 7]. In endometrial cancer, myometrial invasion (MI) represents the first definite evidence of aggressive behavior, and positive lymphovascular space invasion (LVSI) is associated with high risk of lymph node metastasis (LNM) which indicates high recurrence rate and is an independent predictor of survival [8, 9]. Prognosis of endometrial cancer significantly depends upon the above three clinicopathological features, namely depth of MI, positive or negative LVSI, and LNM [10]. Until now, the relationship between BMVD and clinicopathological characteristics and overall survival (OS) remains controversial in endometrial cancer and the effects of study population and methods to define and study BMVD on the relationship are still unknown. Therefore, to confirm the association of BMVD with clinicopathological characteristics including depth of MI, LVSI, and LNM, and with prognostic value including OS in patients with endometrial cancer here, we systematically reviewed and conducted this meta-analysis and subgroup analysis.

Methods

Search strategy

Relevant literature was searched from PubMed, Embase, China National Knowledge Infrastructure, and Wanfang databases from their inception until January 2017. Terms were used as follows: "microvessel density", "MVD", "blood microvessel density", "BMVD", "microvessel count", "endometrial neoplasms", "endometrial carcinoma", "endometrial cancer", "endometrial tumor", "uterine neoplasms", "uterine carcinoma", "uterine cancer", and "uterine tumor".

Inclusion criteria

The included studies met the criteria including that (1) patients were diagnosed as endometrial carcinoma regardless of cancer types; (2) BMVD was assessed after staining with microvessel markers such as factor VIII, CD31, CD34, CD105, and others by immunohistochemistry; (3) clinicopathological factors (MI, LVSI, or LNM) or/and enough information to extract hazard ratio (HR) and standard error (SE) of lnHR for OS; (4) article was published in English or Chinese. Relevant references were further screened by two researchers independently (JZW and YJX). Disagreements were resolved through discussion and consensus.

Data extraction and quality assessment

Data were extracted independently by two researchers (JZW and YJX) from each study and disagreements were resolved by a third author (CCW). The extracted information included the first author's name, year of publication, country, antibody for BMVD, cut-off value of BMVD quantitative data, magnification to assess BMVD, BMVD definition, number of field examined, outcome measures (MI, LVSI, LNM, and OS), and duration of follow-up. The quality of the included studies was assessed by Newcastle–Ottawa scale (NOS) criteria categorized by patient selection, study comparability, and outcome for cohort studies [11].

Statistical analysis

Review Manager Version 5.3 software was used for meta-analysis and subgroup analysis. Standard mean difference (SMD) with 95% confidence interval (CI) was calculated to estimate the association between BMVD and clinicopathological features (MI, LVSI, or LNM). HR with 95% CI was pooled to evaluate the effect of BMVD level on OS. If the HR with 95% CI was reported, the data were extracted directly. SE was calculated by the following equation: $\mathrm{SElnHR} = (\mathrm{lnUpperCI} - \mathrm{lnLowerCI})/3.92$ [12]. If Kaplan–Meier survival curve was provided, Engauge Digitizer software was used to obtain HR with SE [13]. A random-effects model was used when there was significant heterogeneity ($P \leq 0.1$, $I^2 > 50\%$) assessed by Cochrane's Q test and I^2 statistics. Sensitivity analysis was also performed to examine the robustness of the combined risk estimates. Subgroup analyses were conducted according to study population, BMVD criteria, and BMVD markers. A P value of less than 0.05 was considered statistically significant.

Results

Search results and study characteristics

Study selection was shown in Fig. 1. One hundred and thirty-nine potentially relevant studies were found from the initial search. After screening of titles and abstracts, 93 articles were excluded, whereas 12 were reviews, 7 were conference/letter/case report, 9 were neither in English nor Chinese, 42 were duplicated publications, and 23 were animal studies. After full text of 46 articles was assessed, 17 articles were further excluded, because proper BMVD or HR was not provided in the details. At last, 29 studies of total

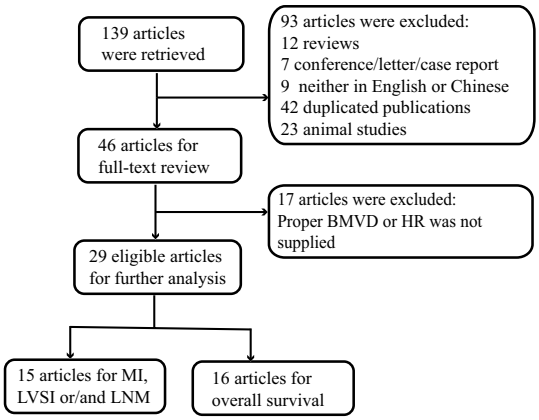


Fig. 1 Flow diagram of included studies. *BMVD* microvessel density, *SD* standard deviation, *HR* hazard ratio, *MI* myometrial invasion, *LVSI* lymphovascular space invasion, *LNM* lymph node metastasis

2517 patients were included, and 15 studies reported clinicopathological characteristics (MI, LVSI, or LNM), while 16 studies reported OS [14–42]. Consensus on study selection was reached by discussion among the authors. Characteristics of all 29 eligible studies were summarized in Table 1. HRs were directly obtained from 13 articles, only 3 studies in which HRs were estimated from Kaplan–Meier survival curves. Quality of all included studies was good (Table 2).

Publication bias

The publication bias in the literature was assessed by funnel plot, which did not indicate an obvious publication bias in all 15 included studies on associations between BMVD expression level and depth of MI, LVSI, or LNM. The shape of the funnel plot was also not significantly asymmetrical for the eligible 16 studies investigating BMVD on OS, which indicated that no obvious publication bias was found (figures not shown).

Association between BMVD and clinicopathological characteristics

BMVD was significantly associated with depth of MI (SMD 1.24; 95% CI 0.53–1.95; $P = 0.0006$), LVSI (SMD 0.75; 95% CI 0.3–1.21; $P = 0.001$), and LNM (SMD 0.99; 95% CI 0.46–1.52; $P = 0.0003$) (Table 3 and Fig. 2). Significant heterogeneity was observed in MI ($\chi^2 = 90.02$, $I^2 = 91\%$; $P < 0.00001$), LVSI ($\chi^2 = 34.34$, $I^2 = 80\%$; $P < 0.0001$), and LNM ($\chi^2 = 81.28$, $I^2 = 85\%$; $P < 0.00001$). Subgroup analyses based on study populations, BMVD criteria, and BMVD markers were shown in Table 3 and corresponding Figs. S1, S2, and S3. The pooled result of Asian populations showed the statistically significant association in MI (SMD 1.24; 95% CI 0.53–1.95; $P = 0.0006$), LVSI (SMD 0.95; 95% CI 0.44–1.46; $P = 0.0002$), and LNM (SMD 1.26; 95% CI 0.65–1.87; $P < 0.0001$), while there was no statistically significant association for non-Asian populations (Table 3 and Fig. S1). The aggregated estimate of BMVD using Weidner method was also significantly associated in MI (SMD 1.54; 95% CI 0.66–2.41; $P = 0.0006$), LVSI (SMD 0.83; 95% CI 0.33–1.32; $P = 0.001$), and LNM (SMD 1.11; 95% CI 0.51–1.72; $P = 0.0003$), while there was no statistically significant association for non-Weidner method (Table 3 and Fig. S2). BMVD detection using CD34 antibody was significantly associated in MI (SMD 1.63; 95% CI 0.63–2.63; $P = 0.001$), LVSI (SMD 1.21; 95% CI 0.43–2.00; $P = 0.002$), and LNM (SMD 1.4; 95% CI 0.52–2.28; $P = 0.002$), while CD105 antibody was significantly associated in MI (SMD 1.03; 95% CI 0.18–1.87; $P = 0.02$), and factor VIII antibody was significantly associated with LNM (SMD 0.69; 95% CI 0.17–1.21; $P = 0.01$) (Table 3 and Fig. S3).

Association between BMVD and OS

The pooled HRs of included 16 studies (HR 2.65, 95% CI 1.86–3.77, $P < 0.00001$) indicated that BMVD was significantly associated with poor OS (Table 3 and Fig. 2). Subgroup analysis showed that the combined HRs of Asian populations and non-Asian populations both were significant (HR 2.58, 95% CI 1.28–5.17, $P = 0.008$, and HR 2.8, 95% CI 1.74–4.5, $P < 0.0001$, respectively) (Table 3 and Fig. S1). The combined HRs of MDV assessed by either Weidner or non-Weidner method were also significant (HR 2.25, 95% CI 1.57–3.23, $P < 0.0001$, and HR 5.69, 95% CI 3.04–10.65, $P < 0.00001$, respectively) (Table 3 and Fig. S2). For the BMVD markers used for BMVD staining and quantification, only CD31 and factor VIII, but not CD34 and CD105 showed a significant association (HR 7.79, 95% CI 2.64–23, $P = 0.0002$, and HR 2.24, 95% CI 1.78–2.82, $P < 0.00001$, vs HR 1.9, 95% CI 0.97–3.74, $P = 0.06$, and HR 1.75, 95% CI 0.05–67.06, $P = 0.76$, respectively) (Table 3 and Fig. S3). Based on univariate and multivariate survival analyses, both the pooled HRs were significant (HR 3.95, 95% CI 2.69–5.81, $P < 0.00001$, and HR 2.1, 95% CI 1.42–3.12, $P = 0.0002$, respectively) (Table 3 and Fig. S4). Based on quantile–quantile plot of included cut-off values, Ai et al. [14] as an outlier were further excluded, and the combined HRs with normal distributed cut-off value of BMVD were significant (HR 2.03, 95% CI 1.38–2.97, $P = 0.0003$) (Fig. S5).

Discussion

Angiogenesis is referred to a process that new blood vessels develop from the pre-existing vessels. Angiogenesis can be further divided into physiological and pathological

Table 1 Main characteristics of included studies

Study	Country	Patients	Antibody	Cut-off value	Magnification	Microvessel definition	No. of field	Outcome	Follow-up (months)	Quality[a]
Ai et al. [14]	China	60	CD34	≥ 36.5/mm^2	×200	Weidner	3	HR	8–120	6
Chen et al. [15]	Taiwan	53	CD34	NR	×200	Weidner	3	MI, LVSI, LNM	2–36	7
Chen et al. [16]	China	25	CD105	NR	×400	Weidner	3	MI, LNM	NR	5
Erdem et al. [17]	Turkey	90	CD105	NR	×200	Weidner	Any	LVSI, LNM	60.5	7
Giatromanolaki et al. [18]	Greece	121	CD31	NR	×200	A lumen or a linear vessel shape	3	HR	4–182	7
Guset et al. [19]	Romania	54	CD34	NR	×200	Weidner	5	LNM	NR	7
Huang et al. [20]	China	56	CD105	≥ 43/field	×200	Weidner	3	K–M	2–90	5
Jiang et al. [21]	China	95	CD34	NR	×200	Weidner	5	MI, LVSI, LNM	5–150	7
Kaku et al. [22]	Japan	122	Factor VIII	≥ 76/mm^2	×200	Weidner	Any	LVSI, HR	37–184	6
Kamat et al. [23]	USA	111	CD31	≥ 13.7/field	×160	A lumen with positive signals	3	HR	NR	7
Kirschner et al. [24]	USA	50	Factor VIII	> 10/field	×400	Not reported	NR	K–M	3–148	6
Li et al. [25]	China	51	CD34	> 35.2/field	×200	Weidner	Any	MI, HR	15–96	5
Liang et al. [26]	China	46	CD34	NR	×400	Weidner	5	MI, LNM	NR	5
Merritt et al. [27]	USA	85	CD31	> 13.7/field	×160	A lumen with positive signals	3	HR	NR	5
Niu et al. [28]	China	70	CD34	NR	×200	Weidner	5	MI, LNM	NR	5
Obermair et al. [29]	Austria	93	CD34	> 135/mm^2	×200	Weidner	Any	HR	8–62	6
Ohno et al. [30]	Japan	70	CD31	NR	×400	A lumen with positive signals	5	MI, LNM	1.8–102	8
Ozalp et al. [31]	Turkey	43	Factor VIII	> 109/mm^2	×200	Weidner	Any	HR	NR	6
Ozuysal et al. [32]	Turkey	60	Factor VIII	NR	×200	Weidner	Any	LVSI, LNM	2–84	6
Pansrikaew et al. [33]	Thailand	46	CD31	NR	NR	CD31 positive signals	4	MI, LVSI, LNM	3.6–83.8	7
Salvesen et al. [34]	Norway	142	Factor VIII	> 90/mm^2	×250	Weidner	10	HR	96–180	7
Salvesen et al. [35]	Norway	195	Factor VIII	> 90/mm^2	×250	Weidner	10	HR	48–180	7
Soeda et al. [36]	Japan	84	CD34	NR	×200	Weidner	3	LVSI, LNM	13–130	7
Stefansson et al. [37]	Norway	281	Factor VIII	> 83.2/mm^2	×250	Weidner	10	K–M	48–192	7
Straume et al. [38]	Norway	194	Factor VIII	> 75%	NR	Weidner	NR	HR	48–189	7
Tan et al. [39]	China	48	Factor VIII	≥ 10/field	×400	Weidner	3	HR	3–122	5
Wagatsuma et al. [40]	Japan	93	Factor VIII	≥ 122/mm^2	×100	Weidner	3	K–M	2–61	7
Watanabe et al. [41]	Japan	40	Factor VIII	NR	×250	Weidner	3	LVSI, LNM	NR	5
Zhang et al. [42]	China	39	CD34	NR	×400	Weidner	3	MI, LNM	NR	5

Weidner, after the area of highest neovascularization (hot spot) was found, any brown-staining endothelial cell or endothelial-cell cluster that was clearly separate from adjacent microvessels, tumor cells, and other connective tissue elements was considered a single, countable microvessel, and vessel lumens, although usually present, were not necessary for a structure to be defined as a microvessel, and red cells were not used to define a vessel lumen

HR hazard ratio, *MI* myometrial invasion, *LVSI* lymphovascular space invasion, *LNM* lymph node metastasis, *K–M* Kaplan–Meier curve, *NR* not reported

[a]The details of quality assessment were shown in Table 2

Table 2 Quality assessment of included studies using the Newcastle–Ottawa scale

Studies	Score for selection				Score for comparability		Score for outcome			Total score
	REC	SNEC	AE	DO	SC	AF	AO	FU	AFU	
Ai et al. [14]	1	1	1	1	0	0	0	1	1	6
Chen et al. [15]	1	1	1	1	0	0	1	1	1	7
Chen et al. [16]	1	1	1	1	0	0	0	1	0	5
Erdem et al. [17]	1	1	1	1	1	0	1	1	0	7
Giatromanolaki et al. [18]	1	1	1	1	0	0	1	1	1	7
Guset et al. [19]	1	1	1	1	1	0	1	1	0	7
Huang et al. [20]	1	1	1	1	0	0	0	1	0	5
Jiang et al. [21]	1	1	1	1	1	0	1	1	0	7
Kaku et al. [22]	1	1	1	1	0	0	1	1	0	6
Kamat et al. [23]	1	1	1	1	0	1	1	0	1	7
Kirschner et al. [24]	1	1	1	1	0	0	1	1	0	6
Li et al. [25]	1	1	1	1	0	0	0	1	0	5
Liang et al. [26]	1	1	1	1	0	0	1	0	0	5
Merritt et al. [27]	1	1	1	1	0	0	1	0	0	5
Niu et al. [28]	1	1	1	1	1	0	0	0	0	5
Obermair et al. [29]	1	1	1	1	0	0	1	1	0	6
Ohno et al. [30]	1	1	1	1	1	0	1	1	1	8
Ozalp et al. [31]	1	1	1	1	0	0	1	0	1	6
Ozuysal et al. [32]	1	1	1	1	0	0	1	1	0	6
Pansrikaew et al. [33]	1	1	1	1	1	0	0	1	1	7
Salvesen et al. [34]	1	1	1	1	1	0	1	1	0	7
Salvesen et al. [35]	1	1	1	1	1	0	1	1	0	7
Soeda et al. [36]	1	1	1	1	1	0	1	1	0	7
Stefansson et al. [37]	1	1	1	1	0	0	1	1	1	7
Straume et al. [38]	1	1	1	1	0	0	1	1	1	7
Tan et al. [39]	1	1	1	1	0	0	0	1	0	5
Wagatsuma et al. [40]	1	1	1	1	1	0	1	1	0	7
Watanabe et al. [41]	1	1	1	1	0	0	1	0	0	5
Zhang et al. [42]	1	1	1	1	1	0	0	0	0	5

REC representativeness of the exposed cohort, *SNEC* selection of the non-exposed cohort, *AE* ascertainment of exposure, *DO* demonstration that outcome of interest was not present at start of study, *SC* study controls for the most important factor (i.e., age), *AF* study controls for any additional factor (treatments for cancer), *AO* assessment of outcome, *FU* follow-up long enough for outcomes to occur (maximum follow-up period was over 36 months), *AFU* adequacy of follow-up of cohorts (over 90%)

angiogenesis. Physiological angiogenesis takes place mainly during embryonic development, normal menstruation, and wound healing, while pathological angiogenesis is seen in inflammatory, immunological, malignant, and ischemic disorders [43]. In 1971, Folkman first presented atypical angiogenesis in tumor [44]. Now, it is clear that solid tumors without blood vessels do not grow when tumor size is larger than 2 mm in diameter, because oxygen and nutrients can be transported less than 2 mm by simple diffusion, so angiogenesis is necessary in the tumor mass for further growth [1]. Furthermore, the tumor vasculature is unevenly distributed and chaotic. Their unusual permeability, potential for rapid growth and remodeling, and abnormalities of basement membrane are responsible for mediating hematogenous spread of tumor cells [45]. Therefore, it was through that tumor angiogenesis not only promotes the growth of the primary tumor but also contributes to the invasion, migration, and distant metastases of tumor cells. The degree of tumor angiogenesis, usually measured by BMVD, is commonly determined by factor VIII, CD31, CD34, and CD105 antibodies to stain the endothelial cells of the blood microvessel. Weidner criteria for BMVD assessment were first presented in 1991 [2]. When the area of highest neovascularization (hot spot) in the field was found, any positively stained endothelial cell or endothelial-cell cluster that was clearly separate from adjacent microvessels, tumor

Table 3 Meta-analysis and subgroup analysis of BMVD with myometrial invasion, lymphovascular space invasion, lymph node metastasis, and overall survival in endometrial cancer patients

Group	Subgroup	Study number	Pooled result			Heterogeneity		
			SMD/HR	95% CI	*P* value	χ^2	*P* value	I^2
Myometrial invasion								
All		9	1.24	(0.53, 1.95)	0.0006	90.02	< 0.00001	91%
Populations[a]	Asian	9	1.24	(0.53, 1.95)	0.0006	90.02	< 0.00001	91%
	Non-Asian	0	NA	NA	NA	NA	NA	NA
BMVD criteria[b]	Weidner	7	1.54	(0.66, 2.41)	0.0006	74.06	< 0.00001	92%
	Non-Weidner	2	0.32	(− 0.08, 0.72)	0.12	0.43	0.51	0%
BMVD markers[c]	CD31	2	0.32	(− 0.08, 0.72)	0.12	0.43	0.51	0%
	CD34	6	1.63	(0.63, 2.63)	0.001	73.75	< 0.00001	93%
	CD105	1	1.03	(0.18, 1.87)	0.02	NA	NA	NA
	Factor VIII	0	NA	NA	NA	NA	NA	NA
Lymphovascular space invasion								
All		8	0.75	(0.30, 1.21)	0.001	34.34	< 0.0001	80%
Populations[a]	Asian	6	0.95	(0.44, 1.46)	0.0002	24.71	0.0002	80%
	Non-Asian	2	0.14	(− 0.32, 0.59)	0.56	0.07	0.78	0%
BMVD criteria[b]	Weidner	7	0.83	(0.33, 1.32)	0.001	30.72	< 0.0001	80%
	Non-Weidner	1	0.25	(− 0.33, 0.83)	0.40	NA	NA	NA
BMVD markers[c]	CD31	1	0.25	(− 0.33, 0.83)	0.40	NA	NA	NA
	CD34	3	1.21	(0.43, 2.00)	0.002	12.08	0.002	83%
	CD105	1	0.19	(− 0.40, 0.78)	0.53	NA	NA	NA
	Factor VIII	3	0.63	(0.01, 1.26)	0.05	5.98	0.05	67%
Lymph node metastasis								
All		13	0.99	(0.46, 1.52)	0.0003	81.28	< 0.00001	85%
Populations[a]	Asian	10	1.26	(0.65, 1.87)	< 0.0001	59.21	< 0.00001	85%
	Non-Asian	3	0.17	(− 0.24, 0.57)	0.42	3.13	0.21	36%
BMVD criteria[b]	Weidner	11	1.11	(0.51, 1.72)	0.0003	75.29	< 0.00001	87%
	Non-Weidner	2	0.36	(− 0.17, 0.89)	0.18	0.51	0.48	0%
BMVD markers[c]	CD31	2	0.36	(− 0.17, 0.89)	0.18	0.51	0.48	0%
	CD34	7	1.40	(0.52, 2.28)	0.002	58.94	< 0.00001	90%
	CD105	2	0.54	(− 0.49, 1.57)	0.30	3.85	0.05	74%
	Factor VIII	2	0.69	(0.17, 1.21)	0.01	0.22	0.64	0%
Overall survival								
All		16	2.65	(1.86, 3.77)	< 0.00001	123.72	< 0.00001	88%
Populations[a]	Asian	6	2.58	(1.28, 5.17)	0.008	59.84	< 0.00001	92%
	Non-Asian	10	2.80	(1.74, 4.50)	< 0.0001	46.96	< 0.00001	81%
BMVD criteria[b]	Weidner	12	2.25	(1.57, 3.23)	< 0.0001	102.88	< 0.00001	89%
	Non-Weidner	4	5.69	(3.04, 10.65)	< 0.00001	4.48	0.21	33%
BMVD markers[c]	CD31	3	7.79	(2.64, 23.00)	0.0002	4.22	0.12	53%
	CD34	3	1.90	(0.97, 3.74)	0.06	84.51	< 0.00001	98%
	CD105	1	1.75	(0.05, 67.06)	0.76	NA	NA	NA
	Factor VIII	9	2.24	(1.78, 2.82)	< 0.00001	6.74	0.57	0%
Survival analysis[d]	Univariate	7	3.95	(2.69, 5.81)	< 0.00001	6.79	0.34	12%
	Multivariate	9	2.10	(1.42, 3.12)	0.0002	93.96	< 0.00001	91%

NA not applicable

[a]The detail was shown in Fig. S1

[b]The detail was shown in Fig. S2

[c]The detail was shown in Fig. S3

[d]The detail was shown in Fig. S4

cells, and other connective tissue was considered as a single, countable microvessel. The vessel lumens, although usually present, were not necessary for a structure to be defined as a microvessel, and red cells were not used to define a vessel lumen [2]. Since then, it was found that Weidner criteria are more accurate than tumor size or grade to predict the prognosis of cancer patients [46] and have been commonly applied in BMVD assessment of various kinds of tumors such as breast [3], colorectal [4], bladder [5], and ovarian [47] cancers.

The value of measuring BMVD in cancer, including endometrial cancer, is still in debate. Many previous studies have reported that BMVD was associated with clinicopathological characteristics such as depth of MI, LVSI, and LNM, and could serve as a prognostic marker and in endometrial cancer [14, 16, 18, 28, 29, 36], but others had different conclusions [20, 33]. Therefore, we undertook this present meta-analysis to collect all relevant data from publications to investigate the overall relationship between BMVD and clinicopathological characteristics and OS in endometrial cancer. In addition, the effect of study populations and BMVD detection methods were often neglected, so we also conducted a subgroup analysis to study the roles of population and BMVD detection method on the effect of pooled results.

Our results indicated that endometrial cancer with higher BMVD was associated with deeper MI and positive LVSI or LNM, and also showed inverse relationship between BMVD and OS, suggesting poor clinical outcome and prognosis. The association remained significant in Asian populations, but not in non-Asian populations. Studies of Asian populations were included from China, Japan, Thailand, and Taiwan, and studies of non-Asian populations from USA, Austria, Greece, and Norway, etc. (Table 1). There were not many included studies from non-Asian population available for analysis, none for MI, only 2 for LVSI [17, 32] and 3 for LNM [17, 19, 32]. The association between BMVD and clinical outcomes remained significant when BMVD defined by Weidner criteria, not non-Weidner criteria, and BMVD markers by CD34, not by other antibodies. For studies used non-Weidner method, various non-validated methods were employed to define BMVD. For non-Weidner method, necessity of a lumen or linear vessel shape with positive signals, simplification of positive signals without other excluded requirements or non-definition of hotspot area may under-estimate or over-estimate microvessel counts. In this meta-analysis, only few studies using non-Weidner criteria were included to analyze the association for MI [30, 33], LVSI [33] and LNM [30, 33]. On the other hand, the choice of BMVD marker may also influence the results. Factor VIII, also termed von Willebrand's factor, is the first marker used for BMVD measurement, which stains mainly mature vessels but also reacts with lymphatic endothelium [48]. CD31 can stain immature blood vessels, but it also stains fibroblasts and some plasma cells and has a high chance of staining failure because of rarely strong reactivity to endothelial cells [3]. Compared to mature blood vessels, immature vessels are irregularly shaped, lack the normal vascular network organization and abnormal basement membranes and pericytes, and have an increased permeability. However, to differentiate mature blood vessel from immature blood vessel, it is better to counterstain with smooth muscle actin [49]. Like CD31, CD34 can stain immature blood vessels and also can stain fibroblasts and some plasma cells, but it usually does not have the risk of staining failure because of strong reactivity with endothelial cells [3]. This may be why BMVD detected by CD34 is more significantly associated with MI, LVSI, and LNM. CD105 is a novel independent prognostic marker in detecting malignant tumors angiogenesis, but whether it is better than factor VIII, CD31 and CD34 still does not reach an agreement until now [50]. In contract, either Asian or non-Asian population, BMVD criteria using Weidner or non-Weidner and analysis using univariate or multivariate were all statistically significant, but only BMVD markers CD31 and factor VIII were significantly associated with OS. Indeed, only three articles [14, 25, 29] studying CD34 were pooled for OS in this meta-analysis, and a significant heterogeneity was observed ($\chi^2 = 84.51$, $I^2 = 98\%$; $P < 0.00001$). According to Fig. 5S, quantile–quantile plot of included cut-off values indicated that the value (36.5, [14]) was an outlier, and the pooled HRs of the remaining two studies [25, 29] showed statistical significance of overall effect without a significant heterogeneity after it was excluded. Only one study [20] for CD105 was included, so more relevant original studies should be performed.

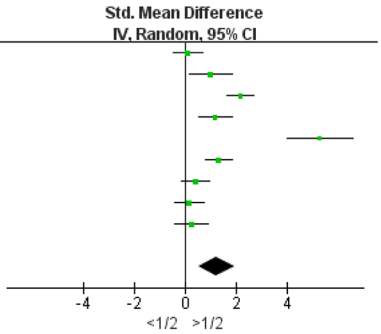
However, potential limitations still might exist in this study. (1) The level of evidence from meta-analysis is always lower than that of randomized and case-controlled trials. In addition, the data included in this meta-analysis were from the published articles not the original data of individual patient. (2) Weidner method do not divide intra and peritumoral microvascular density, so link between peritumoral microvascular density and myometrial invasion could not be studied. (3) The statistics of three included studies were calculated from Kaplan–Meier survival curve instead of the original data from the studies. (4) Many studies did not conduct the multivariate survival analysis. Although our subgroup analysis of HR showed both significant in univariate and multivariate analyses, the sample size is still small. (5) A significant heterogeneity was found in aggregated results, which may come from different methodology, antibodies for BMVD, cut-off value, and duration of follow-up in included studies, so a conservative evaluation was conducted with a random-effect model in this meta-analysis.

In conclusion, this meta-analysis found a statistically significant positive association between angiogenesis, evaluated

Myometrial invasion

Study or Subgroup	>1/2 Mean	SD	Total	<1/2 Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
Chen 2001	74.6	36.4	16	69.4	44	37	11.5%	0.12 [-0.46, 0.71]
Chen 2006	31.24	4.87	14	25.61	5.82	11	10.6%	1.03 [0.18, 1.87]
Jiang 2013	55.4	14.31	35	28.33	10.83	60	11.7%	2.20 [1.67, 2.72]
Li 2010	44.01	9.9	15	31.69	10.21	36	11.3%	1.20 [0.55, 1.85]
Liang 2006	30.46	1.88	15	23.3	0.97	31	8.8%	5.30 [4.01, 6.58]
Niu 2008	53.58	11.18	40	38.4	11.05	30	11.7%	1.35 [0.82, 1.88]
Ohno 2007	26.6	16.9	17	17.9	20	53	11.6%	0.45 [-0.11, 1.00]
Pansrikaew 2010	35.39	12.78	23	33.17	11.91	23	11.5%	0.18 [-0.40, 0.76]
Zhang 2010	48.7	13.38	16	45.04	13.69	23	11.3%	0.26 [-0.38, 0.91]
Total (95% CI)			**191**			**304**	**100.0%**	**1.24 [0.53, 1.95]**

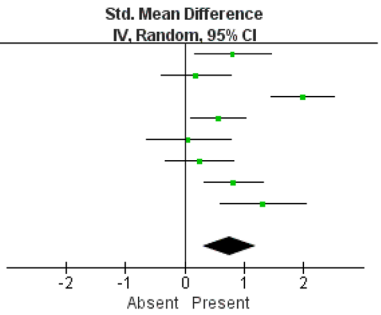
Heterogeneity: Tau² = 1.05; Chi² = 90.02, df = 8 (P < 0.00001); I² = 91%
Test for overall effect: Z = 3.43 (P = 0.0006)



Lymphovascular space invasion

Study or Subgroup	Present Mean	SD	Total	Absent Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
Chen 2001	94.4	33.2	13	62.4	40.7	40	12.0%	0.81 [0.16, 1.45]
Erdem 2006	36.1	12.8	13	32.3	20.9	77	12.5%	0.19 [-0.40, 0.78]
Jiang 2013	54.44	14.58	29	28.88	11.95	66	13.1%	1.98 [1.46, 2.50]
Kaku 1997	73	23.48	29	59.5	23.88	56	13.7%	0.56 [0.11, 1.02]
Ozuysal 2003	26.8	13.9	9	26	13	51	11.4%	0.06 [-0.65, 0.77]
Pansrikaew 2010	35.83	12.97	23	32.74	11.6	23	12.6%	0.25 [-0.33, 0.83]
Soeda 2008	22.3	15	24	10.5	13.8	52	13.3%	0.82 [0.32, 1.33]
Watanabe 2003	22.54	5.93	13	15.48	4.91	27	11.3%	1.32 [0.59, 2.05]
Total (95% CI)			**153**			**392**	**100.0%**	**0.75 [0.30, 1.21]**

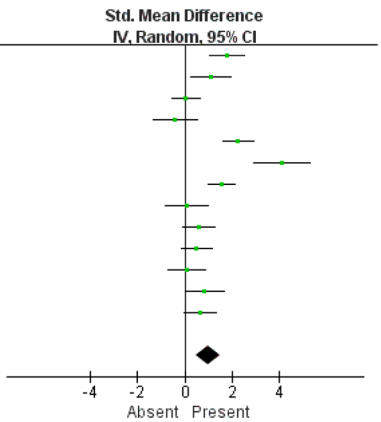
Heterogeneity: Tau² = 0.34; Chi² = 34.34, df = 7 (P < 0.0001); I² = 80%
Test for overall effect: Z = 3.26 (P = 0.001)



Lymph node metastasis

Study or Subgroup	Present Mean	SD	Total	Absent Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
Chen 2001	116.5	30.8	12	56.7	33.3	41	7.8%	1.80 [1.06, 2.53]
Chen 2006	31.86	5.14	12	25.9	5.25	13	7.4%	1.11 [0.26, 1.96]
Erdem 2006	33	14	12	31.9	20	67	8.2%	0.06 [-0.56, 0.67]
Guset 2010	57.88	4.76	5	62.36	11.35	49	7.2%	-0.40 [-1.33, 0.52]
Jiang 2013	66.33	17.39	13	34	13.67	82	8.0%	2.26 [1.59, 2.93]
Liang 2006	27.87	1.42	8	22.78	1.18	38	6.4%	4.10 [2.94, 5.26]
Niu 2008	57.89	12.84	27	40.28	9.92	43	8.4%	1.57 [1.01, 2.12]
Ohno 2007	21.8	24.8	5	19.9	20	65	7.2%	0.09 [-0.82, 1.00]
Ozuysal 2003	32.1	6.68	11	24.7	13.3	49	8.0%	0.59 [-0.07, 1.25]
Pansrikaew 2010	38.69	11.54	13	32.55	12.27	33	8.1%	0.50 [-0.15, 1.15]
Soeda 2008	14.91	15.7	7	14.14	8.7	69	7.7%	0.08 [-0.70, 0.86]
Watanabe 2003	22	5.29	7	16.88	6.04	33	7.5%	0.85 [0.01, 1.69]
Zhang 2010	50.37	11.28	15	43.62	9.56	24	8.0%	0.65 [-0.02, 1.31]
Total (95% CI)			**147**			**606**	**100.0%**	**0.99 [0.46, 1.52]**

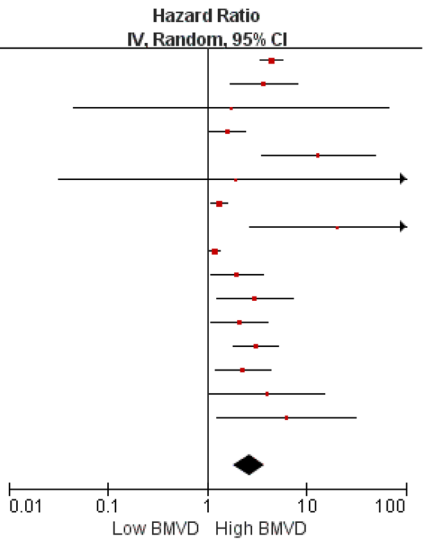
Heterogeneity: Tau² = 0.81; Chi² = 81.28, df = 12 (P < 0.00001); I² = 85%
Test for overall effect: Z = 3.63 (P = 0.0003)



Overall survival

Study or Subgroup	log[Hazard Ratio]	SE	Weight	Hazard Ratio IV, Random, 95% CI
Ai 2009	1.4929	0.131	9.9%	4.45 [3.44, 5.75]
Giatromanolaki 2001	1.3083	0.3941	6.9%	3.70 [1.71, 8.01]
Huang 2008	0.56	1.86	0.9%	1.75 [0.05, 67.06]
Kaku 1997	0.4752	0.2158	9.1%	1.61 [1.05, 2.46]
Kamat 2007	2.5764	0.6753	4.2%	13.15 [3.50, 49.40]
Kirschner 1996	0.68	2.1	0.7%	1.97 [0.03, 121.02]
Li 2010	0.2776	0.0965	10.1%	1.32 [1.09, 1.59]
Merritt 2010	3.0121	1.0415	2.3%	20.33 [2.64, 156.55]
Obermair 1999	0.1823	0.059	10.3%	1.20 [1.07, 1.35]
Ozalp 2003	0.6821	0.3064	8.0%	1.98 [1.08, 3.61]
Salvesen 1999	1.0953	0.4491	6.3%	2.99 [1.24, 7.21]
Salvesen 2000	0.7419	0.3419	7.6%	2.10 [1.07, 4.10]
Stefansson 2006	1.13	0.27	8.4%	3.10 [1.82, 5.26]
Straume 2002	0.8329	0.3276	7.7%	2.30 [1.21, 4.37]
Tan 2003	1.39	0.68	4.2%	4.01 [1.06, 15.22]
Wagatsuma 1998	1.8423	0.8225	3.3%	6.31 [1.26, 31.64]
Total (95% CI)			**100.0%**	**2.65 [1.86, 3.77]**

Heterogeneity: Tau² = 0.31; Chi² = 123.72, df = 15 (P < 0.00001); I² = 88%
Test for overall effect: Z = 5.43 (P < 0.00001)



◂**Fig. 2** Meta-analysis of BMVD association with myometrial invasion, lymphovascular space invasion, lymph node metastasis, and overall survival in patients with endometrial cancer

by BMVD, and three clinicopathological characteristics, MI, LVSI, and LNM, and an inverse relationship between BMVD and OS in endometrial cancer. Therefore, it provided strong evidence that angiogenesis is a useful measure to associate with poor clinical outcomes and prognosis in patients with endometrial cancer.

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Author contributions JZW: project development, data collection, data analysis, and manuscript writing. YJX: data collection and data analysis. GCWM: data collection and data analysis. XYC: data analysis. JK: project development. CCW: project development, data analysis, and manuscript editing.

Compliance with ethical standards

Conflict of interest All authors declare that there is no conflict of interest. All authors have had full control of all primary data and agree to allow the Journal to review their data if requested.

Ethical approval This article does not contain any studies with human participants performed by any of the authors.

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