RESEARCH ARTICLE

Microvessel Density as a Prognostic Factor in Ovarian Cancer: a Systematic Review and Meta-analysis

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Abstract

Background: The prognostic value of microvessel density (MVD), reflecting angiogenesis, detected in ovarian cancer is currently controversial. Here we performed a meta-analysis of all relevant eligible studies. Materials and Methods: A comprehensive search of online PubMed, Medline, EMBASE and Sciencedirect was performed to identify all related articles. The search strategy was designed as 'microvessel density', 'ovarian cancer', 'ovarian neoplasm', 'CD34' and 'angiogenesis'. Results: The studies were categorized by author/year, number of patients, FIGO stage, histology, cutoff value for microvessel density, types of survival analysis, methods of hazard rations (HR) estimation, HR and its 95% confidence interval (CI). Combined hazard ratios suggested that high MVD was associated with poor overall survival (OS) and progression-free survival (PFS), with HR and 95% CIs of 1.84 (1.33-2.35) and 1.36 (1.06-1.66), respectively. Subgroup analysis showed that high MVD detected by CD34 was relevant for OS [HR=1.67 (1.36-2.35)], but not MVD detected with other antibodies [HR=2.11 (0.90-3.31)]. Another subgroup analysis indicated that high MVD in patients without pre-chemotherapy, but not with pre-chemotherapy, was associated with OS [HR=1.88(1.59-2.18 and HR=1.70 (-0.18-3.59)]. Conclusions: The OS and PFS with high MVD were significant poorer than with low MVD in ovarian cancer patients. However, high MVD detected by CD34 seems to be more associated with survival for patients without pre-chemotherapy.

Keywords: Microvessel density - survival - ovarian cancer - chemotherapy - meta-analysis - CD34

Introduction

Ovarian cancer, as an infamous "silent killer", is one of the most lethal gynecological neoplasms in women. Owing to development of surgery and chemotherapy with empirically optimized combinations of conventional agents, survival rate of ovarian cancer remains approximately 30% (Bast et al., 2009). Independent prognostic factors such as International Federation of Gynecology and Obstetrics (FIGO) stage, residual disease after surgery, histology and lymph node status allow a better understanding of the natural history and process of ovarian cancer and the classification of homogeneous populations with a similar outcome profile. However, these prognostic factors insufficiently predict individual clinical outcome. Therefore, to optimize clinical care, putative molecular marker such as serum CA 125 must be identified.

Angiogenesis has attracted an enormous surge in interest over the past twenty years. Undoubtedly, the hypothesis that targeting angiogenesis could be a promise strategy to overcome cancer has been extraordinary in inspiring scientists to participate in this field (Folkman, 1971). Angiogenesis is implicated in pathogenesis, progression and metastasis of malignancies. Without vessels, tumor can't exceed 1-2mm or metastasize to distant organs. Vessels in an embryo are derived form in situ differentiation of undifferentiated precursor cells to vascular endothelial cells (Risau, 1997). Subsequently, this primeval structure expands by sprouting of capillaries from pre-existing vessels or intussusception, in which interstitial tissue such as tumor cells are integrated into the lumen of pre-existing vessels (Carmeliet, 2000). In addition, tumor cells next to existing vessels have an ability to form a perivascular cuff (Yancopoulos et al., 2000). It is still a controversial question whether these vessels result from tumor cells invading lumen, from 'vasculogenic mimicry' of tumor cells, or from exposing underlying tumor cells due to apoptosis of endothelial cells. Despite of the internal reasons involved the existence of tumor cells in microvessel show momentous significance in metastasis and for the utilization of anti-angiogenic therapy. Recent evidence suggests that formation and maturation of new vessels are inconceivable complex and coordinated processes, requiring cascade reactions which consist of various receptors and ligands (Carmeliet and Jain, 2000). We now recognize several molecules involved in the regulation of 'angiogenic switch' such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor, platelet-derived endothelial cell growth factor and angiopoietin. With the advent of specific antibodies to detect vascular endothelial cells, quantitative observation

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of tumor angiogenesis intensified. Concerning the relationship between angiogenesis and clinical outcome, ovarian cancer is one of the most conspicuous tumors. As a surrogate marker of angiogenesis, microvessel density has been proposed to be a prognostic indicator for human breast cancers in early 1990s (Weidner et al., 1992). Over the past two decades, studies reported that microvessel density is positively correlated to the clinicopathological factors of malignancies in breast cancer (Cao et al., 2013) as well as in prostate cancer (Muhammadnejad et al., 2013). To identify patients at early stage would be of great benefit, allowing for a more opportune surgery and effective chemotherapy to prolong survival and possibly predicting response rate of new drugs.

Most of the studies support positive correlations between microvessel density and overall survival (OS), progression free survival (PFS) or disease free survival (DFS). Some of the discrepancies may be elucidated by the influence of methodology such as antibody (e.g. CD34, CD105, CD31, VIII, von Willebrand factor or PECAM-1). Regardless of these intrinsic reasons, hotspot method is confirmed to be an independent prognostic variable (Zhang et al., 2006). Recommended methods for MVD count include Chalkley counting (Fox et al., 1994) and hot spots areas on a x400 or x200 field (e.g. 20 objective and 10 ocular, 0.785 mm2 per field).

Biomarkers such as MMP, E-cad and epididymis protein-4 have been confirmed to be associated with the prognosis of ovarian cancer (Lin et al., 2012; Peng et al., 2012; Li et al., 2013). Many observational researches have confirmed that MVD is inversely related to survival in ovarian cancer, while others yield opposite results. We examined the evidence explicitly, by conducting systematic literature review and a meta-analysis to discuss clinical relevance of MVD and survival of ovarian cancer patients.

Materials and Methods

Search strategy

A comprehensive search of online PubMed, Medline, EMBASE and Sciencedirect was done to identify all related articles focused on MVD and ovarian cancer. Published time was limited between 1990 and May 1st, 2012. Search strategy was designed as 'microvessel density', 'ovarian cancer', 'ovarian neoplasm', 'CD34' and 'angiogenesis'. Furthermore, references from eligible articles as well as reviews and editorials were reviewed manually to implement our search. We tried to avoid duplication of data by examining authors and medical center. When studies were published by the same medical center, journal with higher influence factor or larger sample size was chosen.

Selection criteria

We established included criteria as follow: *(i)* MVD count was performed by hotspots or Charkley count. *(ii)* The endpoints of investigation should include OS or PFS. *(iii)* Sufficient data for determining an estimate of Log-Hazard ratio (HR) and its 95%CI. *(iv)* All observed patients must be diagnosed as ovarian cancer by pathology

and enroll more than 30 patients. *(v)* Study population was divided into high MVD (or positive) and low MVD group (or negative) for survival analysis. *(vi)* Only articles written in English were included. Studies should be excluded: *(i)* the same author or the same medical center with duplicate data, the article with higher influence factor was chosen. *(ii)* Follow-up was less than 3 year. *(iii)* Animal studies focused on subjects such as rabbit, BALB/c mouse or sheep.

Two authors (Lei He and Qiao Wang) independently evaluated titles and abstracts of all studies (n=423) to decide whether full-text should be screened. Disagreement was resolved by consensus between two authors. For the condition of persistent disagreement, our professor made the final decision. We examined 73 full-text and pick up information with included and excluded criteria.

We did not set a predefined minimal duration of median follow-up. We failed to weigh each study by a quality score because no such score has been generally accepted in prognostic meta-analysis (Altman, 2001).

Data extraction and analysis

For every single study, we marked the results as 'positive' when higher MVD predicted poorer survival. In the sake for quantitative aggregation of OS, DFS and PFS, we measured MVD on survival by combining HR and its 95%CI which was first published by Peto. We recorded the following information from eligible studies: first author/ year of publication, number of patients, FIGO stage, histology, cutoff value of MVD, types of survival analyses, HR and 95% CI if available. According to the patients with or without pre-chemotherapy, we classified patients as 'baseline' and 'chemotherapy' subgroup. When more than one antibody was used to detect MVD, we recorded the HR and its 95% as independent data sets. HR and its 95% CI was either directly collected from original article or calculated by survival (Parmar et al., 1998).

Heterogeneity between studies was evaluated by Chi-square test and expressed by I2 index. As $I^2 > 35\%$ indicated heterogeneity, random effect (I-V heterogeneity) was used. Potential causes of heterogeneity were assessed by meta-regression analyses. We considered a worse survival when observed HR>1 for high MVD populations (Barraclough et al., 2011). This impact of high MVD on OS, DFS, and PFS was considered with statistical significance if the combined HR and its 95%CI didn't overlap 1.

Publication bias was evaluated by Begg's Test, Egger's Test and Contour-enhanced funnel plot (presented by STATA 12.0). Publication bias was considered when $p<0.05$. Furthermore, contour-enhanced funnel plot is helpful to indicate regions of statistical significance, to interpret funnel plot and to identify whether the cause of asymmetry is due to factors such as variable study quality.

Results

Eligible studies

Four hundred twenty-two records were identified by primary search strategy. However, after screening of titles and abstracts, 324 original articles and 26 reviews

were excluded because either of non-English, duplicate data, animal and cell studies or irrelevant to MVD and prognosis. Seventy-three full-texts were reviewed for detail. Fifty-one were further excluded for insufficient survival data or duplication. In two references (Gadducci et al., 2003; Raspollini et al., 2005), patients were overlapped; we picked up the larger sample size. Finally, 22 studies (Hollingsworth et al., 1995; Gasparini et al., 1996; Heimburg et al., 1999; Obermair et al., 1999; Shen et al., 2000; Nakayama et al., 2001; Hata et al., 2002; Ogawa et al., 2002; Raspollini et al., 2004; Chan et al., 2005; Goodheart et al., 2005; Gadducci et al., 2006; Ino et al., 2006; Taskiran et al., 2006; Palmer et al., 2007; Suhonen et al., 2007; Labiche et al., 2009; Rubatt et al., 2009; Han et al., 2010; Ferrero et al., 2011; Liu et al., 2012; Qin et al., 2012) fulfilled in this meta-analysis. The included 22 studies encompassed 1,918 ovarian cancer patients. 'Baseline' (n=1369) and 'chemotherapy' (n=549) patients were enrolled in 17 and 5 studies, respectively. 'CD 34' (n=1129) and 'other antibody' (n=789) were enrolled in 9 and 13 studies. The main characteristics were presented in Table 1.

Analysis of MVD on survival

HRs for OS were available in 20 studies accounting for 1770 patients. The estimated HR for all studies indicated a significantly risk of death in patients with higher MVD
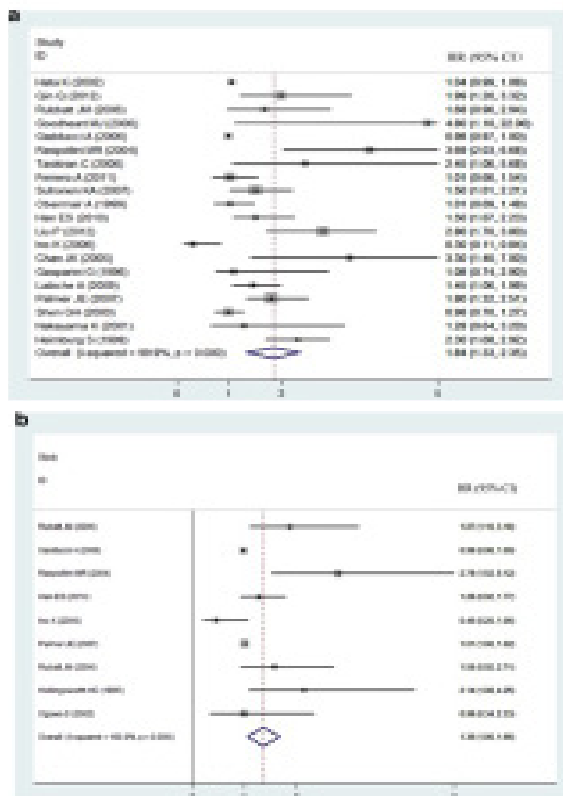

Figure 2. (a) Contour-enhanced Funnel Plot of 20 Eligible Studies Assessing the Influence of high MVD in OS of Ovarian Cancer Patients. (b) Contour-enhanced funnel plot of 8 eligible studies assessing the influence of high MVD in PFS of ovarian cancer patients

Figure 1. (a) The Association between High MVD and Overall Survival of Ovarian Cancer Stratified by HR Estimation. Meta-analysis of 20 eligible studies evaluating high MVD in overall survival. HR and its 95% CI for OS : 1.84 (1.33-2.35). **(b)** The association between high MVD and progression-free survival (PFS) of ovarian cancer. Meta-analysis of 8 eligible studies evaluating high MVD in PFS. HR and its 95%CI for PFS : 1.36 (1.06-1.66)
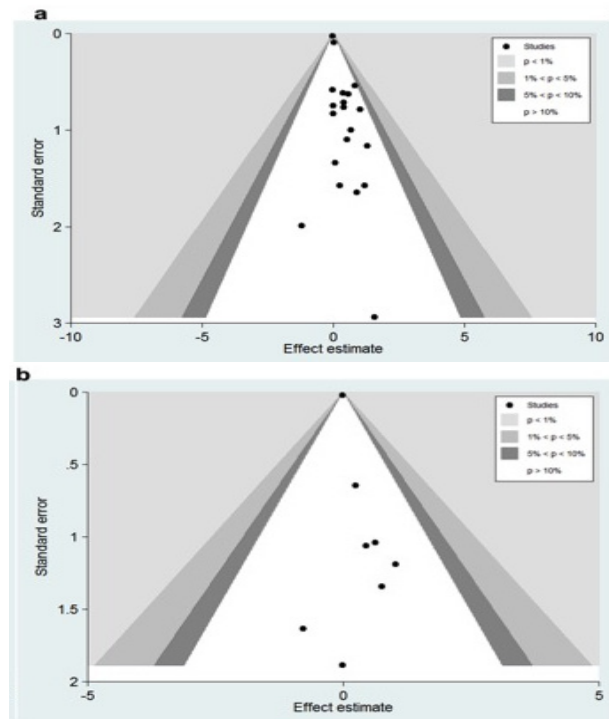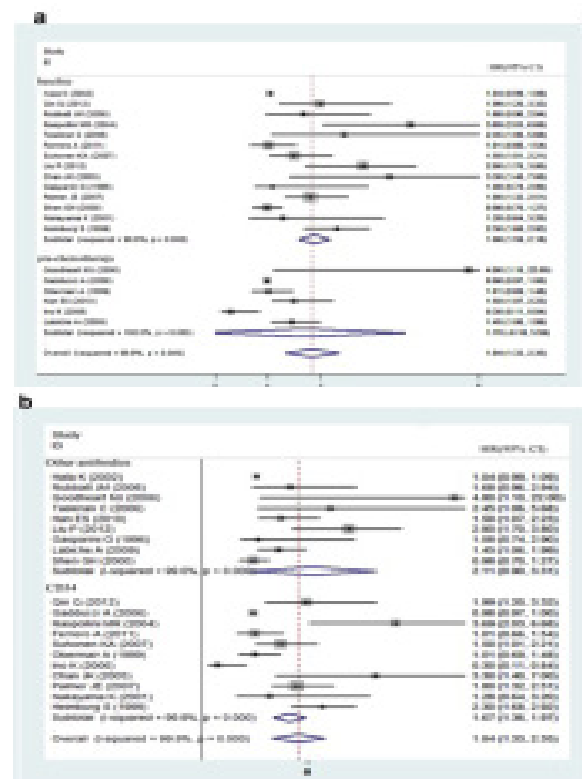

Figure 3. (a) Subgroup Analysis for Detecting Antibodies, HR=1.32 (0.82-1.82), for CD 34, HR and 95% CI=1.67 (1.36-2.35); for other Antibodies, HR and 95% CI=2.11 (0.90-3.31). **(b)** Subgroup analysis for pre-chemotherapy, HR=1.70 (-0.18-3.59), for baseline, HR and 95% CI=1.88 (1.59-2.18)

Table 1. Main Characteristic of 22 Included Studies

Author/year	N	FIGO stage (N)	Follow-up (Median)	Cutoff of MVD	Outcome	HR and 95% CI	Antibody	stage	Results
Hata K (2002)	49	I-II(28) III-IV(21)	57 months	Mean number	OS	1.04(0.99-1.09)	VIII	baseline	Positive
Qin Q (2012)	123	III-IV	?	75.8 vessels/ field 200	OS	1.993(1.195-3.323)	CD34	baseline	positive
Rubatt JM (2009)	50	III	158 months	Median value	OS; PFS	CD105[OS:1.125(0.654-1.935);PFS:1.873(1.102-3.184)];CD31[OS:1.678 (0.957-2.943);PFS: 1.578 (0.918-2.711)]	CD31 CD105	baseline	OS: negative PFS: positive
Goodheart MJ (2006)	77	I	74 months	12 vessels/ field	OS	4.8 (1.1-22)	CD31	pre-chemo therapy	positive
Gadducci A (2006)	101	III(90) IV(11)	65 months	40 microvessels/ field 200x 0.74 mm²	OS; PFS	PFS:0.988(0.978-0.999) OS: 0.985(0.973-0.998)	CD34	pre-chemo-therapy	positive
Raspollini MR (2004)	83	III	44.8 months	70 microvessels/ field	OS; PFS	OS: 3.69(2.03-6.68) PFS: 2.79(1.52-5.12)	CD34	basaline	positive
Taskiran C (2006)	58	I-II(26) III-IV(74)	34 months	12 vessels/field 200	OS	2.45(1.06-5.67)	CD105	baseline	positive
Ferrero A (2011)	113	II(7) III-IV(106)	60 months	median	OS	1.01 (0.66-1.54)	CD34	baseline	negative
Suhonen KA (2007)	175	I-II(58) III-IV(117)	23 months	Chalkley count: 8%	OS	1.50(1.01-2.21)	CD34	baseline	positive
Obermair A (1999)	63	I-II(25) III-IV(38)	6.75 years	10 vessels/200 field 0.25mm²	OS	1.01 (0.69-1.48)	CD34	pre-chemo-therapy	negative
Han ES (2010)	61	?	43 month	Not clear	PFS; OS	OS:1.50(1.073-2.23) PFS:1.276(0.918-1.774)	CD31	pre-chemo-therapy	OS: positive PFS: negative
Liu P (2012)	166	I-II(14) III-IV(152)	42.8 months	25% tumor cells	OS	4.1 (1.0-15.2)	CD105	baseline	positive
Ino K (2006)	67	I-II(39) III-IV(28)	60 months	70 vessels/ field 400	OS; PFS	OS:0.30 (0.11 -0.84) PFS: 0.46 0.20 -1.06	CD34	baseline	negative
Chan JK/ (2005)	44	III-IV	>60 months	11 vessels/ field 400	OS	3.3(1.4-7.0)	CD34	baseline	positive
Hollingsworth HC (1995)	43	III-IV	60m months	15 vessels/ field 400	PFS	2.14(1.08, 4.25)	CD34	pre-chemo-therapy	positive
Gasparini G (1996)	60	III-IV	46 months	48 microvessels/ field 200 0.74mm²	OS	1.08(p=0.21) 0.74-2.9	CD31	baseline	positive
Labiche A (2009)	204	III-IV	46 months	?	OS	1.45(1.06 to 1.99)	FVIII	pre-chemo-therapy	positive
Palmer JE (2007)	132	I-II(40) III-IV(92)	?	Median	OS; PFS	OS:1.038(1.027-1.050) PFS:1.010 (1.005- 1.016)	CD34	baseline	positive
Shen GH (2000)	64	I-II(37) III-IV(27)	31 months	40 microvessels/field OS 200 0.74mm²	OS:0.98 (0.10-1.86)	FVIII		baseline	negative
Nakayama K (2001)	42	I-II(16) III-IV(26)	?	Median value	OS	1.28(0.64-3.20)	CD34	baseline	negative
Ogawa S (2002)	105	I-II(8) III-IV(49)	>60 months	70 microvessels/ field 200 0.75mm²	PFS	0.99(0.34-2.33)	CD34	baseline	positive
Heimburg S (1999)	38	I-II(7) III-IV(31)	?	40 microvessels/ field 200	OS	2.3(1.68-2.92)	CD34	baseline	positive

(HR=1.84, 95%CI: 1.33-2.35, random-effects, Figure.1a). Since the heterogeneity among studies was significant (I^2=99.9%, p=0.000), random effect was selected. Twenty studies yielded a Begg's and Egger's test which p=0.697 and p=0.233 respectively. Furthermore, confunnel plot (contour-enhanced funnel plot) was undertaken which also indicates absence of publication bias (Figure 2a).

HRs for PFS were available in 9 studies accounting for 747 patients. In one study (Rubatt et al., 2009), more than one HR was extracted because multi-antibodies were used to detect MVD and HRs were reported accordingly. The estimated HR for all studies indicated a significantly risk of disease progression in patients with higher MVD

(HR=1.36, 95%CI: 1.06-1.66, random-effects, Figure 1b). Since the heterogeneity among studies was significant (I2=100%, p=0.000), random effect was selected. Nine studies yielded a Begg's and Egger's test which p=0.10 and p=0.12 respectively. Furthermore, confunnel plot (contour-enhanced funnel plot) was undertaken which also indicates absence of publication bias (Figure 2b).

Influence of sampling time point and antibody
    The prognostic value of MVD for OS was significant in the 'baseline' subgroup (HR=1.88, 95%CI: 1.59-2.18), while it was not significant in 'chemotherapy' subgroup (HR=1.70, 95%CI: -0.18-3.59, Fig. 3a) which the 95%CI

did not cross the threshold of 1.0. The prognostic value of MVD for OS was significant in the 'CD 34' subgroup (HR=1.67, 95%CI: 1.36-1.97), while it was not significant in 'other antibodies' subgroup (HR=2.11, 95%CI: -0.90-3.31, Figure 3b) which the 95%CI did not cross the threshold of 1.0.

Discussion

The present meta-analysis showed that the prognostic significance of high MVD vary substantially between studies. We confirmed that alternations of MVD were predictive of mortality and progression in ovarian cancer. Owing to limited eligible studies, our studies failed to evaluate the influence of MVD on recurrence. Further, results were also shown in subgroup analyses for patients with or without pre-chemotherapy, as well as different antibodies. CD34-MVD has an effect on predicting prognosis while other antibodies-MVD fails to indicate stronger relationship with prognosis. Moreover, subgroup analysis of patients with pre-chemotherapy does not predict clinical outcome. This is the first meta-analysis of published studies to evaluate the association between MVD count and prognosis in ovarian cancer. However, all eligible studies in our meta-analysis were observational studies, more prone to bias than randomized controlled trails (Grimes and Schulz, 2002). Hence, these conclusions should be interpreted with caution.

Our study was carried out using published results; there are some bias of our meta-analysis which are the same as the bias reported in the meta-analysis of relations between epididymis protein-4 and prognosis in ovarian cancer (Lin et al., 2012) . We did not look for unpublished trails because our study required data available in full-published articles. And the cut off values for MVD+ were different, some studies used median value, some studies used 40 microvessel/field 200 0.74mm^2 or other values. These distinctions are responsible for the difficulty in determining a standard cut off in clinical practice. Another variability of bias is related to the language selection which positive results are more favorable in English, whereas negative results tend to be reported in native language (Egger et al., 1997). Moreover, methodology for extrapolating HR might be a potential bias in HR estimates. For some studies without HR and 95%CI directly, we need to obtain data from survival curves, assuming that censored observations were well distributed. Finally, it is inevitable that all the included articles have difference in patient's baseline status such as age, tumor size, lymph node status, chemotherapy strategy of pre-chemotherapy, antibodies for MVD detecting and duration of follow-up, even the counting method of MVD.

Despite its excellence as a prognostic factor in untreated ovarian cancer, MVD has not been shown to be an indicator to guide antiangiogenic treatment or monitor response of chemotherapy (Hlatky et al., 2002). Our analysis also showed that MVD failed to predict survival after initial chemotherapy. It is widely hypothesized that tumors with high MVD are superior candidates of antioangiogenic therapies, while tumors with low MVD are regarded as a poor candidate (Wesseling et al., 1998).

However, evidence revealed that bladder tumors with low MVD were found to be more effective by low-dose angiostatin than those tumors with high MVD (Beecken et al., 2001). Thus, MVD is not a useful tool for stratifying patients for clinical trials.

Undoubtedly, our study indicated that the choice of antibody for MVD detecting was crucial for conclusion. There are 13 studies in our meta-analysis using CD34 as an endothelial marker, 9 studies using other markers such as factor VIII (FVIII), CD31, and CD105. Of the 3 studies using FVIII, results indicated poor prognostic influence. Factor VIII is one of the first markers used for staining MVD, but not all endothelial cells express FVIII. On the other hand, FVIII is also expressed in lymphatic endothelium, and platelets leading to cross-reactivity with non-endothelial cells. CD34 is a highly glycosylated transmembrane protein which is expressed on immature hematopoietic cells as well as on luminal endothelial cells. It was reported that CD34 display a better sensitivity and specificity than FVIII for endothelial cells induced by tumor angiogenesis (Tanigawa et al., 1996). CD32 is also a transmembrane glycosylated protein which is expressed both in mature and immature vascular endothelium. During cellular differentiation, CD31 is also expressed which results in cross-react with neutrophils, lymphatic B cells and platelets. Consequently, CD34 and CD31-MVD counts are approximately 30% higher than FVIII (Uzzan et al., 2004). The chemical structure of CD 31 and CD 34 are resembled, but CD34 is more stable than CD31 which cross-react with fibroblasts and plasma (Leek, 2001). Although optimal marker for MVD has not been established, we recommend using CD34 for MVD in future studies.

To conclude, a systematic review suggested a poor overall survival and progression-free survival of high MVD in patients with ovarian cancer. Data are insufficient to determine its role in disease-free survival. To achieve clinical utility of MVD in ovarian cancer, more high-quality interventional researches are acquired. Formal process for patients baseline status, MVD detecting and MVD count should be followed systematically before introducing into clinical practice.

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100.0

6.3

75.0

56.3

50.0

31.3

25.0

0

Newly diagnosed without treatment