Research Article

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Microvessel density as a prognostic indicator of prostate cancer: A systematic review and meta-analysis

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Abstract: The aim of this study is to evaluate prognostic and therapeutic implications of microvessel density (MVD) in the recurrence-free survival (RFS), disease-specific survival (DSS), and overall survival (OS) in prostate cancer (PCa). As of April 2019, EMBASE, PubMed, Cochrane Library, Science Direct/Elsevier, MEDLINE, and CNKI are used for systematic literature retrieval to investigate the correlation between MVD and PCa. Meta-analysis was performed using Review Manager and Stata software. Combined hazard ratio (HR) was identified with 95% confidence intervals (95% CI) in a random or fixed effects model. Thirteen studies were identified in this article. Of which, 8 studies analyzed for the recurrence-free survival (2,399 patients) demonstrated that MVD significantly elevated in the poor recurrence-free survival (HR 2.57, 95% CI 2.21–2.97). Other 2 eligible studies (330 patients) with 3 data sets for the MVD-OS analysis and the pooled HR (HR 1.70, 95% CI 1.27–2.28) suggested a weak risk of overall death rate in patients with high-MVD levels. The last 3 studies for disease-specific survival (220 patients) suggested that the association with high MVD and disease-specific survival may not have statistically significance (HR 1.32, 95% CI 0.49–3.56). This study suggests that high intratumoral MVD appears a significant progenitor for poor recurrence-free survival of PCa.

Keywords: microvessel density, angiogenesis, PCa, prognosis, meta-analysis

1 Introduction

Prostate cancer (PCa) is the most common malignancy and the second leading cause of cancer death among men in the United States and Western countries. In Europe, PCa is the most common solid neoplasm, with an incidence rate of 214 cases per 1,000 men, outnumbering lung cancer and colorectal cancer [1,2]. The incidence of PCa has been dramatically increasing in China over the last decade. The biological heterogeneity of PCa is highly variable. Some patients with PCa have normal longevity similar to the general population, whereas the survival time of other patients is only few months due to developing metastatic disease [3–5]. Several prognostic indicators, such as histological grade as Gleason's grading system, the prostate-specific antigen (PSA), the volume of the tumor, vascular invasion, extension of the tumor through the prostate capsule, and invasion to the seminal vesicle have been investigated in PCa [6]. But few of these prognostic factors can help in forecasting the clinical outcome.

Angiogenesis is the process of formation of new blood vessels from the endothelium of the existing vasculature and is vital for tumor development. As a new tumor extends to the size of 1–2 mm, its growth requires the induction of new blood vessels, which may consequently lead to the development of metastases [7,8]. In addition, angiogenesis is implicated in pathogenesis and progression of malignancies. Vascular endothelial growth factor (VEGF), platelet-derived endothelial cell growth factor (PDGF), and basic fibroblast growth factor (bFGF), produced by tumors and the intratumor stromal cells, are potent inducers of the angiogenic switch. However, angiogenesis is necessary but not sufficient to the development of metastases. The angiogenic activity is

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heterogeneous within different tumor types. Concerning the relationship between angiogenesis and clinical outcomes, PCa has been one of the well-investigated tumors. Microvessel density (MVD), a surrogate marker of tumor angiogenesis, has been proposed to be a prognostor for human breast cancers in the early 1990s [9,10]. Over the past two decades, hundreds of studies revealed the prognostic role of microvessel density in lung, breast, bladder, and hepatic cancer [11–14]. The identification of such patients at the early stage of their disease would be attractive, allowing for a more appropriate and effective treatment (by adjuvant chemotherapy or in the near future by specific antiangiogenic drugs) of patients at higher risk and possibly predicting the activity of the latter treatment drugs.

MVD can be evaluated by using antipan-endothelial antibodies, such as CD31 and Von Willebrand Factor (vWF). Weidner et al. first proposed the method of MVD measurement in breast cancer in 1990s [9,10]. Most of the studies support positive correlations between MVD and overall survival, recurrence-free survival, disease-free survival, or disease-specific survival. Furthermore, increased MVD in PCa has been demonstrated when compared with that in benign prostate tissue according to radical prostatectomy specimens [15]. Many observational researches have confirmed that MVD is inversely related to survival in PCa, while others studied the opposite results. We examined the evidence explicitly by conducting a systematic review and a meta-analysis to evaluate the prognostic value of the elevated MVD among patients suffering from PCa.

2 Materials and methods

2.1 Search strategy

We followed the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidance and the Cochrane handbook for systematic reviews of interventions. Two independent reviewers searched PubMed, EMBASE, Science Direct/Elsevier, MEDLINE, CNKI, and the Cochrane Library from inception to April 2019 with no language or study type restrictions. The search terms combined text words and MeSH terms. For example, the search terms for finasteride were “microvessel density,” “microvessel density of tissue,” “microvessel density in tumor,” or “MVD,” while the search terms for prostatic cancer were “prostatic cancer,” “prostatic tumors,” “prostate

malignancies,” “prostate carcinoma,” “prostate malignant diseases,” “prostatic neoplasms,” “cancer of prostate,” “neoplasms prostate,” “neoplasms prostatic,” “prostate neoplasms,” or “PCa.” In addition, the reference list of the original research articles and reviews were also manually searched to implement our study.

2.2 Selection criteria

The eligible studies must meet all the following inclusion criteria: Clinical studies exploring the association between MVD expression and one or more of the following survival outcomes: overall survival, disease-free survival, disease-specific survival, or recurrence-free survival. Studies were excluded based on any of the following criteria: (1) a review, a case report, an editorial, or a comment; (2) for duplicate data, we used the most recent or largest population data; (3) study with the total number of cases less than 20; and (4) not enough information to calculate the log hazard ratio (log HR) and its standard error (SE). Two independent reviewers performed an initial assessment by identifying the eligibility of abstracts from the identified studies according to the included and excluded criteria. The final selection decision was made by the full text reading if the eligibility was unclear from the sole abstract. Available data related to this study were collected from the literature, which consist of the number of aberrant expression in each group.

2.3 Data extraction

We tried carefully to avoid duplication of data by examining for each publication the names of all authors and the different medical centers involved. When studies were published by the same medical center, journal with higher influence factor or the larger sample size or the longer follow-up time was selected. However, we included both studies including different patients in two studies of the same research center. Similarly, if a study consisted of multiple sets of data, such as different detection markers (CD34, CD31, and vWF), then we regarded all data sets as separate ones. When additional data were needed for the statistical analysis, the authors of those studies were contacted for further information by E-mail if possible. Two authors (Feng and Wang) reviewed the retrieved articles independently for data extraction. Any

disagreement was resolved through discussion to reach a common consensus. For the condition of persistent discrepancy, the final decision was made by one expert (zhao) from this department. Multivariate Cox hazard regression analysis was first chosen in our analysis, and when not available, the Kaplan–Meier curves of the survival outcomes with log-rank p values or Cox hazard regression analysis were extracted. Additional data were carefully extracted from all eligible publications as follows: first author's name, publication year, country, number of patients involved, patient's age, treatment of the patients, marker of MVD, detection method, follow-up period, types of survival analysis, and other relevant clinical characteristics.

2.4 Statistical methods

The log HR and SE were required for aggregation of survival results. We calculated the parameters on the basis of the available data with the methods developed by Parmar *et al.*, Williamson *et al.*, and Tierney *et al.* [16–18]. The statistics derived from Kaplan–Meier survival curves with log-rank p values were calculated by using the software designed by Matthew and Tierney (Medical Research Council Clinical Trials Unit, London, UK). Forrest plots were used to estimate the predictive role of the elevated MVD in PCa patients. RevMan 5.3 (Cochrane collaboration, Oxford, UK) was used to perform the meta-analysis. A pooled result was considered statistically significant as $P < 0.05$ for the statistical test comparing survival distributions between the groups with high and low MVD. For every single study, we marked the results as “positive” when a high MVD predicted poorer survival and as “negative” when a high MVD did not predict a poor survival or even once predicted a better survival. For the quantitative aggregation of OS, RFS, and DSS, we measured MVD on survival by combining HR and its 95% CI. A combined HR > 1 frequently indicated a poorer prognosis in the high MVD cohort [19]. Both the subgroup meta-analysis and sensitivity analysis were used in this study. The heterogeneity between studies was evaluated by I^2 . As $I^2 > 50\%$ indicated heterogeneity, a random-effect model should be applied to the secondary analysis, otherwise a fixed-effect model was used. Publication bias that was evaluated using the Begg's funnel plot were performed by STATA 11.0 (STATA Corporation, College Station, TX), and $p > 0.05$ was considered as no potential publication bias [20].

3 Results

3.1 Characteristics of eligible studies

In total, 374 references were identified after comprehensive searching. However, after screening of these titles and abstracts, 343 studies were excluded because they were review articles, laboratory studies, duplicated data, or irrelevant to this current study. The remaining 31 potentially relevant full-text publications were reviewed for detail. Eighteen articles were further excluded because of the insufficient survival data available for further calculation (Figure 1). In addition, 13 studies were included in our meta-analysis, all of which were published ranging from 1997 to 2010 [21–33]. Three of the eligible studies detected MVD using different markers (CD31, CD34, and factor VIII) [21,26,30]. These data groups for further statistical analysis were all extracted as separate data sets. A total of 2,949 patients were included in our meta-analysis, with a mean number of 226 patients per study. The main characteristics of the 13 included studies are presented in Table 1. The biochemical recurrence was defined as persisting or increasing post-operative PSA in serum, which also named PSA failure or PSA recurrence. Finally, we considered the 13 eligible articles, including 16 data sets, for the analysis of survival outcomes: 8 articles contained 10 data sets and used recurrence-free survival as the prognostic endpoint index, 2 articles included 3 data sets and estimated overall survival, and 3 articles included disease-specific survival.

3.2 Correlation between MVD and survival outcome

HRs for recurrence-free survival were available in 8 studies (2,409 patients) with 10 data sets. In the pooled analysis, HR for all the studies showed a high risk of shorter RFS in PCa patients with higher MVD expression (HR 2.57, 95% CI 2.21–2.97, $I^2 = 37\%$; Figure 2). There are 330 patients in the 2 eligible studies with 3 included data sets for the MVD-OS analysis, and the estimated pooled HR (HR 1.70, 95% CI 1.27–2.28, $I^2 = 0\%$) suggested a significant although weak risk of overall death rate in patients with high-MVD levels (Figure 3). Since the heterogeneity test performed among the two studies could be accepted ($I^2 = 37\%$ and $I^2 = 0\%$, separately), the fixed-effect model was chosen to calculate the summary HR. However, the pooled HR for DSS

Figure 1: Flow diagram of the study selection process.

was 1.32 (HR 1.32, 95% CI 0.49–3.56, $I^2 = 77\%$), suggesting that the association with high MVD and DSS may not have a statistical significance. The random-effect model was applied (Figure 4).

was 0.788, indicating no significant publication bias (Figure 5).

4 Discussion

3.3 Subgroup analysis

We performed the subgroup analysis considering endothelial markers used in OS, RFS, and DSS groups. CD34 was used in 6 studies to analyze the survival of PCa patients, the pooled HR (95% CI) was 3.42 (2.32, 5.02) for RFS and 1.38 (0.78, 2.43) for overall survival [21,24,26,29–31]. While the HR (95% CI) for CD31 in three studies was 2.30 (1.95, 2.72) [23,26,33]. In all the remaining studies with the endothelial marker factor VIII, the pooled HR (95% CI) for overall survival was 1.83 (1.3, 2.58), for RFS was 4.08 (2.49, 6.70), and for disease-specific survival was 1.32 (0.49, 3.56; Table 2) [21,22,25,27,28,30,32].

3.4 Publication bias

Begg's funnel plot and test were used to assess the possible publication bias of our meta-analysis. Because of insufficient articles, it is meaningless to perform the Begg's funnel plot and test for overall survival and disease-specific survival. P value of Begg's test for recurrence-free survival

The previous study found that many factors affect the prognosis of prostate cancer, such as tumor size, pathological type, tumor stage, and Gleason score [1,34,35]. Stevan et al. proved that the mean age of a patient was significantly higher in patients with high MVD index. In addition, the patients with high MVD index had significantly greater Gleason score in comparison to those with low MVD index in prostate tumor tissue [36]. Previous studies have found that urinary PCA3, urinary miRNA, and prostate tissue novel localization of the low-affinity NGF receptor (p75) have a potential diagnostic and prognostic value for prostate cancer [37–40]. In this study, we demonstrated that an elevated MVD, as a marker of angiogenesis, does indeed predict high recurrence rate in prostate cancer. The pooled results of the subgroup analysis were consistent also when stratifying by different endothelial markers. As a rule of thumb, a risk ratio (RR) >2 was considered as a useful prognostic value in clinical practice. The combined HR and 95% CI for recurrence-free survival met this standard. Patients with high intratumor MVD showed a slight higher risk of overall decreased survival rates. But we failed to evaluate the influence of MVD on disease-specific survival in patients with prostate cancer.

Table 1: Main characteristics of the 13 included studies with 16 data sets

First author [ref.]	Year	Country	Treatment	No.	Median (y) follow-up	Blinded reading	Reader	Antibody	MVD assessment	Survival analysis	HR estimation
Erbersdobler et al. [33]	2010	Germany	RP	1,521	3	Yes	2	CD31	IHC	PSA RFS	Kaplan–Meier survival curves
Concato et al. [32]	2007	United States	?	228	11	Yes	1	Factor VIII	IHC	OS	Multivariate analysis
EKici et al. [31]	2004	United States	RP	66	8.3	Yes	3	CD34	IHC	PSA RFS	Univariate analysis
Offersen et al. [30]	2002	Denmark	TURP	51	2.4	Yes	1	CD34	IHC	OS	Kaplan–Meier survival curves
Offersen et al. [30]	2002	Denmark	TURP	51	2.4	Yes	1	Factor VIII	IHC	OS	Kaplan–Meier survival curves
Bono et al. [29]	2002	Italy	RP	75	?	Yes	2	CD34	IHC	RFS	Kaplan–Meier survival curves
Halvorsen et al. [27]	2000	Norway	RP	104	4.6	?	?	Factor VIII	IHC	PSA RFS	Multivariate analysis
Krupski et al. [28]	2000	United States	RP	42	10	?	?	Factor VIII	IHC	DSS	Kaplan–Meier survival curves
de la Taille et al. [26]	2000	United States	RP	102	3.6	Yes	1	CD31	IHC	PSA RFS	Kaplan–Meier survival curves
de la Taille et al. [26]	2000	United States	RP	102	3.6	Yes	1	CD34	IHC	PSA RFS	Kaplan–Meier survival curves
Bettencourt et al. [24]	1998	United States	RP	149	5	Yes	2	CD34	IHC	PSA RFS	Kaplan–Meier survival curves
Borre et al. [25]	1998	Denmark	TURP	125	3.5	Yes	1	Factor VIII	IHC	DSS	Kaplan–Meier survival curves
Lissbrant et al. [22]	1997	Sweden	TURP	53	?	Yes	1	Factor VIII	IHC	DSS	Kaplan–Meier survival curves
Silberman et al. [23]	1997	United States	RP	87	?	Yes	1	CD31	IHC	PSA RFS	Multivariate analysis
Arakawa et al. [21]	1997	United States	RP	100	7	Yes	1	CD34	IHC	PSA RFS	Kaplan–Meier survival curves
Arakawa et al. [21]	1997	United States	RP	93	7	Yes	1	Factor VIII	IHC	PSA RFS	Kaplan–Meier survival curves

Figure 2: Meta-analysis (forest plot) of 8 eligible studies with 10 data sets assessing (microvessel density) MVD in DFS of Prostate cancer patients. The pooled HR (its 95% CI) for DFS is 2.57 (2.21, 2.97). Subgroup analysis considering endothelial markers for CD34, HR = 3.42 (2.32, 5.02); for CD31, HR = 2.07 (1.45, 2.70); for factor-VIII, HR = 4.08 (2.49, 6.70). Each study is shown by the first author/year and the HR with 95% CI.

Figure 3: Meta-analysis (forest plot) of 2 eligible studies with 3 data sets assessing (microvessel density) MVD in OS of prostate cancer patients. The pooled HR (its 95% CI) for OS is 1.70 (1.27, 2.28). Subgroup analysis considering endothelial markers for factor-VIII, HR = 1.83 (1.30, 2.58); for CD34, HR = 1.38 (0.78, 2.43). Each study is shown by the first author/year and the HR with 95% CI.

Obviously, using different endothelial marker for immunohistochemical staining may modify the conclusions. With the work by de la Taille *et al.*, both CD31 and CD34 were used to evaluate MVD, but only CD34 was an independent predictor of PSA failure [26]. Therefore, de la Taille *et al.* thought some of the confusion about MVD value as a prognostic indicator might be due to the antibodies used. Of the 3 studies using FVIII in the MVD-DSS group indicated poor prognostic influence. Factor VIII was one of the first markers used for staining MVD, but not all endothelial cells express FVIII. According to the subgroup analysis based on different endothelial markers in the MVD- RFS group, studies using factor

VIII or CD34 as the endothelial marker show a stronger poor prognostic value. However, CD34, a highly glycosylated transmembrane protein, is expressed on immature hematopoietic cells and on luminal endothelial cells. It has been suggested that CD34 and CD31 showed a better sensitivity than FVIII for identifying microvessel [41]. CD31 is known to be more specific for endothelial cells. When compared to CD34, it is clear that CD34 has a much broader reactivity. During cellular differentiation, CD31, also named platelet endothelial cell adhesion molecule-1, is expressed by vascular endothelial cells, platelets, monocytes, neutrophils, and naive T lymphocytes [42]. CD34 has the same characteristics as CD31, and then CD34 and

Figure 4: Meta-analysis (forest plot) of 3 eligible studies assessing (microvessel density) MVD in DSS of prostate cancer patients. The pooled HR (its 95% CI) for DSS is 1.32 (0.49, 3.56). Each study is shown by the first author/year and the HR with 95% CI.

CD31-MVD counts are approximately 30% higher than FVIII. Standardization with the optimal marker involving multicenter studies is needed before the use of MVD as a surrogate marker of angiogenesis for predictive purposes.

The chalkley or hot spot is considered as a useful procedure for daily clinical use to calculate MVD. Most studies used the similar technique, but many variations to the MVD assessment exist. The sizes of the area for

counting in studies are different. Also the cutoff value for MVD varied among studies. Many authors used the median value of MVD as a cutoff, some studies used 40 microvessels/field 200 0.74 mm², and some divided the values of MVD by quartile. As these variations, it makes difficult to determine a standard cutoff value in clinical practice. Furthermore, the methodology for extrapolating HR might be a potential bias in HR estimates. Some studies did not report HR and 95% CI directly, so we need to

Table 2: Meta-analysis of MVD expression in prostate cancer: hazard ratios and confidence intervals to predict the survival

	Survival outcome	Data sets (n)	Model	HR (95% CI)	log-rank p	Heterogeneity (p, I ²)	Patients (n)	Conclusion
Total	OS	3	Fixed	1.70 (1.27, 2.28)	0.0004	0.66, 0%	330	Positive
	RFS	10	Fixed	2.57 (2.21, 2.97)	<0.00001	0.11, 37%	2,399	Positive
	DSS	3	Random	1.32 (0.49, 3.56)	0.58	0.01, 77%	220	Negative
CD34	OS	1	—	1.38 (0.78, 2.43)	0.27	—	51	Negative
	RFS	5	Fixed	3.42 (2.32, 5.02)	<0.00001	0.2, 33%	492	Positive
CD31	RFS	3	Fixed	2.30 (1.95, 2.72)	<0.00001	0.56, 0%	1,710	Positive
Factor VIII	OS	2	Fixed	1.83 (1.30, 2.58)	0.0005	0.72, 0%	279	Positive
	RFS	2	Fixed	4.08 (2.49, 6.70)	<0.00001	0.68, 0%	197	Positive
	DSS	3	Random	1.32 (0.49, 3.56)	0.58	0.01, 77%	220	Negative

Figure 5: Funnel plot analysis on the detection of the publication bias for the MVD-DFS group. *P* value of Begg's test for RFS was 0.788.

calculate the data according to survival curves, assuming that censored observations were well distributed.

There are some limitations in our study, which need to be taken into consideration when interpreting the results of this meta-analysis. First, all eligible articles were observational studies, more prone to bias than prospective randomized controlled studies. Second, our conclusion was based on the data of the published studies. We could not weigh each study by a quality score because no such score has been generally accepted in the prognostic meta-analysis. Third, not all studies reported the multivariate analysis of HR and its 95% CI, while the data sets extracted from the survival curves and the *p* values are by the univariate analysis; then, we have no choice but to combine the univariate analysis and the multivariate analysis together to ensure data integrity. What's more, the different cutoff values for MVD may modify the conclusions. Finally, it is unavoidable that all the eligible articles have differed in patient's baseline status such as age, tumor size, chemotherapy strategy of preoperation, the duration of follow-up, even insufficient data, and so on.

In conclusion, our meta-analysis found a significant inverse relationship between MVD and the recurrence-free survival of men with prostate cancer. Patients with elevated intratumor MVD showed a higher in weak risk of overall decreased survival rates. But the association with high MVD and disease-specific survival may not have statistically significance. According to this analysis, the following recommendations should be made: (1) a large number of consecutive patients should better come from a single cohort, and the baseline status of the study population must be clearly described and stratified by a standard; (2) we recommend using antibodies

directed against CD31 and vWF for immune staining, and MVD counting also should be followed up systematically; and (3) a full description of survival events should be given to allow future calculations.

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