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Research Article

Increased tumor-associated macrophages in the prostate cancer microenvironment predicted patients' survival and responses to androgen deprivation therapies in Indonesian patients cohort

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ABSTRACT

Background: Tumor-associated macrophages (TAMs) and microvessel density (MVD) play an essential role for tumor progression in prostate cancer (PCa). In this study, we evaluated the association between TAMs, the infiltration with tumor angiogenesis and the response to androgen deprivation therapies (ADTs) in PCa to evaluate TAM infiltration as a predictive factor for PCa survival.

Materials and methods: Fifty-four specimens were collected and stained with CD 68 antibody to investigated TAM infiltration in tumor. Von Willebrand factor was stained to evaluate MVD around the cancer foci. We assessed the association between patient's age, preoperative serum prostate-specific antigen, pathologic Gleason sum (GS), TAM infiltration, MVD, and the response to ADT for 5 years after PCa diagnosis.

Results: The median TAM was observed in 28 (6-76)/high power field (x400). Increasing TAM correlated with increasing tumor angiogenesis ($P < 0.001$, $r = 0.61$), and the response to ADT was significantly better in patients with fewer TAMs (<28) than in patients with higher TAMs (>28) ($P = 0.003$). TAM infiltration was significantly higher in those with higher serum prostate-specific antigen, higher GS, and metastasis. Multivariate analysis showed that GS, ADT type, and MVD number were significant prognostic factors for response to ADT in PCa ($P < 0.0001$). An increased infiltration of TAM [hazards ratio (HR) = 4.47; 95% confidence interval (CI): 1.97–10.15], MVD (HR = 2.66; 95% CI: 1.27–5.61), metastatic status (HR = 2.29; 95% CI: 0.14-0.60), and prostate volume (HR = 2.19; 95% CI: 1.27–3.12) significantly correlated with shorter survival in PCa patients by univariate analysis ($P < 0.05$). Multivariate analyses revealed that TAM and metastatic status significantly correlated with poor overall survival.

Conclusions: TAM infiltration is associated with response to ADT and increased tumor angiogenesis in PCa. GS, ADT type, and MVD in PCa specimens are useful predictive factors for poor response to ADT. Increasing TAM and positive metastatic status were prognostic factors for a poorer survival in PCa patients.

Abbreviations: TAM, Tumor associated macrophage; MVD, Microvessel density; PCa, Prostate cancer; ADT, Androgen deprivation therapy; PSA, Prostate-specific antigen; RP, Radical prostatectomy.

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1. Introduction

Prostate cancer (PCa) is the second most common disease and the sixth highest cause of death in men in the world.[1] In Asia, the average incidence of PCa is 7.2 per 100,000 men per year. Indonesian Society of Urologic Oncology in the period of 2006–2010 reported 971 PCa cases in Indonesia with the mean of age 68.3 years, and in them, PCa was found in 563 (57.9%) cases by prostate biopsy.[2] The primary therapy for PCa with metastasis is androgen deprivation therapy (ADT),[3,4] but acquired resistance to the therapies is a serious problem in PCa treatments.[4] Several clinicopathological factors have been reported as prognostic factors for hormonal therapy.[5,6] Other studies have reported on immune response as a prognostic factor in PCa patients.[7,8]

There is strong evidence that tumor-associated macrophages (TAMs) are involved in an inflammatory sequence that promotes tumor development.[9–11] TAMs play an important role in tumor progression and response to ADT.[7,12] However, the clinical significance of TAMs in various cancers is not fully understood. The role of TAMs in PCa progression is multifactorial. TAMs cause tumor invasion and increase tumor angiogenesis, tumor proliferation, tumor metastasis, and immunosuppression.[13] Increased TAM infiltration is related to worse pathological characteristics and poorer patients prognosis in several cancers including breast, colon, bladder, and PCa.[8,12,14,15]

The clinical significance of TAM infiltration for PCa progression and patient survival is gradually becoming clearer. Two studies reported that increased TAM infiltration was associated with poor cancer-specific survival and recurrence-free survival,[7,16] whereas others found that increased TAM infiltration in prostate tumors was predictive of improved disease-free survival.[17]

In PCa, vascular invasion should be also evaluated in addition to tumor assessment because the presence of malignant cells in blood vessels increases the risk of pelvic metastasis.[18] As tumor growth depends on angiogenesis, its inhibition is one of the measures for controlling growth of neoplastic cells.[19] Tumor angiogenesis can be evaluated quantitatively by microvessel density (MVD) technique. Several studies have shown a correlation between MVD and the risk of tumor invasion in PCa and breast cancer. For instance, correlations between MVD, vascular invasion, nuclear pleomorphism, and tumor cell proliferation have been observed.[18] Although the relation between MVD and survival still appears controversial, MVD may be a prognostic and predictive factor for PCa. This study was designed to determine the association between TAMs, response to ADT, and tumor angiogenesis in PCa and to evaluate TAM infiltration as a predictive factor for PCa patients' survival.

Table 1
Guideline comparison among NCCN, IUA, and EAU.

Initial therapy	EAU	NCCN[a)]	IUA
Active surveillance, EBRT/ Brachytherapy Radical Prostatectomy Observation	T1-T2a/Low Risk	<10 y and >10 y survival/Low Risk	T1a-T2a/Low Risk
Active surveillance, EBRT/Brachytherapy Radical Prostatectomy with PLND if probable metastasis EBRT or Brachytherapy alone/Observation	cT2c, PSA 10-20	<10 y and >10 y survival/ Intermediate Risk	T2b-T3b
Radical Prostatectomy with PLND if probable metastasis EBRT + Brachytherapy ± ADT	cT2c with PSA >20	<10 y and >10 y survival/unfavorable Intermediate Risk	T2b-T3b, with age <80 y
EBRT + ADT 2-3 y EBRT + Brachytherapy+ ADT 1-2 y Radical Prostatectomy with PLND	cT3-4 with any PSA RP indicate in >10 year life expectancy and locally advanced (cT3a)	High Risk or Very High Risk	T2b-T3b, with age <80 y

NCCN, The National Comprehensive Cancer Network; IUA, Indonesian Urological Association; EAU, European Association of Urology; EBRT, External beam radiation therapy; ADT, androgen deprivation therapy; PLND, pelvic lymph node dissection.
[a)] NCCN Asia 2013: primary ADT may be considered as a possible treatment in all group (low to very high risk).

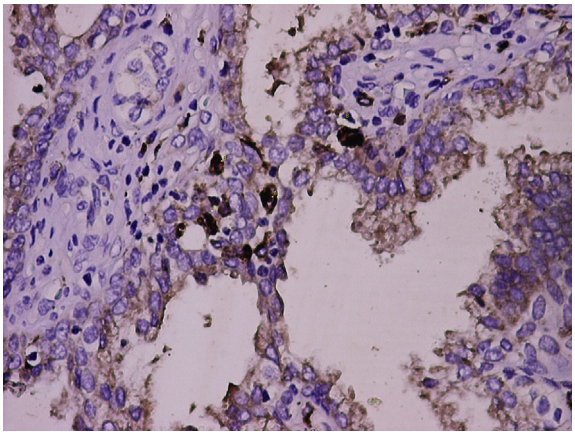

Fig. 1. TAM immunohistochemical specimen using monoclonal antibody to CD68 in PCa tissue. TAM, tumor associated macrophage; PCa, prostate cancer.

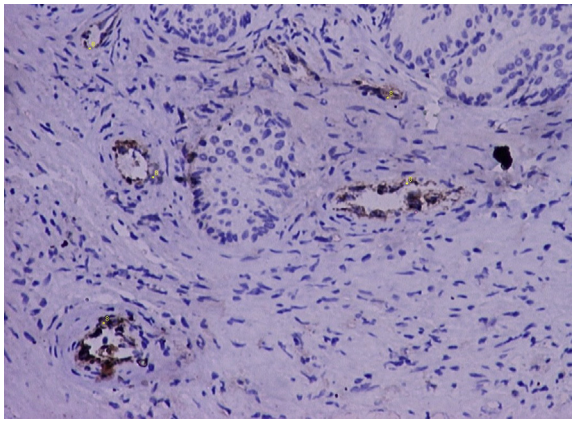

Fig. 2. MVD immunohistochemical specimen using monoclonal antibody to von Willebrand factor (vWF) in PCa tissue. MVD, microvessel density; PCa, prostate cancer.

Table 2
Variable characteristics.

Variable	
Age, Mean ± SD	68.9 ± 9.1
Prostate volume, Median (Min − Max)	45 (21.4 − 393.7)
PSA, Median (Min − Max)	
Before treatment	50.7 (1.89 − 432.6)
After treatment: 3 month	22.3 (1.5 − 221)
6 month	24.7 (0.3 − 243)
TAM, Median (Min − Max)	28 (6 − 76)
MVD, Median (Min − Max)	32.5 (10 − 99)

TAM, tumor-associated macrophage; MVD, microvessel density; PSA, prostate-specific antigen.

Table 3
Bivariate analysis of increasing TAM numbers.

Variable	Total number of patients (%)	TAM count, Mean ± SD	*P*
Age, year			
< 68.9	24 (44.4)	35.5 ± 21.8	0.715[a)]
≥ 68.9	30 (55.6)	32.7 ± 20.2	
PSA, ng/ml			
< 50.7	14 (25.9)	25.0 ± 12.2	0.103[b)]
≥ 50.7	40 (74.1)	37.1 ± 22.3	
Prostate volume, ml			
< 45	27 (50)	29.3 ± 19.8	0.062[b)]
≥ 45	27 (50)	38.6 ± 21.0	
MVD, n			
< 32.5	27 (50)	22.8 ± 13.4	<0.001[b)]
≥ 32.5	27 (50)	45.1 ± 21.0	
Metastasis			
Yes	20 (37)	45.2 ± 22.5	0.005[b)]
No	34 (63)	27.4 ± 16.7	
Gleason score			
< 7	17 (31.5)	24.4 ± 11.6	0.038[b)]
≥ 7	37 (68.5)	38.4 ± 22.6	
ADT			
Surgical	27 (50)	30.8 ± 19.1	0.315[b)]
Medical	27 (50)	37.1 ± 22.2	
Response to ADT			
Good	22 (40.7)	30.8 ± 19.1	0.007[b)]
Poor	32 (59.3)	37.1 ± 22.2	

TAM, tumor-associated macrophage; PSA, prostate-specific antigen; MVD, microvessel density; ADT, androgen deprivation therapy.
[a)] Independent *t*-test.
[b)] Mann-U-Whitney.

2. Materials and methods

2.1. Patients

Tissue samples from 54 patients diagnosed with PCa at Sardjito Hospital, Yogyakarta, Indonesia, from 2009 to 2011 were consecutively selected because we need to follow their outcome at least 5 years. All patients underwent transrectal ultrasound, biopsy to diagnose PCa. Regardless of transrectal ultrasound biopsy results, those patients who had bladder outlet obstruction with lower urinary tract symptoms and refused radical prostatectomy (RP) underwent transurethral resection of the prostate to improve their lower urinary tract symptoms.[20,21] The normal prostate-specific antigen (PSA) value is < 4 ng/dl.[2] Metastatic status was assessed by bone scan, bone survey, and/or contrast computed tomography. All of the patients received ADT. After diagnosis of PCa and staging, these patients were treated by total androgen blockade with gonadotropin-releasing hormone analog and bicalutamide as initial ADT. Patients who refused medical ADT underwent bilateral subcapsular orchidectomy (surgical ADT). After initial therapy, patients were followed with periodical evaluations of digital rectal examination, serum PSA, and imaging findings. Progression of PCa was defined as an elevation of serum PSA levels at three consecutive measurements (PSA failure), the existence of local or metastatic recurrent tumors, or evidence of symptomatic worsening. Patients whose tPSA level did not fall below 0.2 within 6 months after ADT initiation is the poor response, and the time to PSA level <0.2 ng/dl within 6 months is good response.[22] These diagnostic treatments and follow-up methods are based on the national guideline (Table 1). The study was approved by the Universitas Gadjah Mada Institutional Research Committee.

2.2. Histological preparation

Histological specimens from prostate tissues were fixed in 10% neutral buffered formalin and routinely processed for paraffin embedding. Serial 5-μm-thick sections were cut and stained with hematoxylin and reviewed by one pathologist (S.H.) to determine Gleason score based on the Gleason grading system.

2.3. Immunohistochemical examination

For immunohistochemical examination, paraffin blocks were cut by microtome and then incubated for 1 night at 37°C. After incubation, tissues were deparaffinized and rehydrated. Antigen retrieval was performed using citrate buffer at pH 6 heated by microwave for 10 minutes. Blocking serum was given to the tissues for 5 minutes followed by primary antibody (CD 68 for TAM (1:100, Dako, Santa Clara, USA) and Von Willebrand factor (vWF) for MVD (1:25, Dako) with link antibody. Coloring agent was then applied to the tissue using streptavidin, substrate chromogen, and Mayer hematoxylin. Then the blocks were dehydrated, cleared with Xylol, and mounted with Canada Balsam for microscopic examination.

TAMs comprise the macrophages in stromal tissues (peritumoral) assessed by immunohistochemical examination using anti-CD68 antibodies, screening all tumor areas and determining six hot spots (areas most positive for CD68) by weak enlargement (x50), then examined at ×400 magnification to obtain the average value of the 6 areas around the cancer foci.[8]

MVD, an indicator of tumor angiogenesis, was assessed using average microvessel count methods with immunohistochemical examination using vWF antibody to determine endothelial cell vWF expression in six areas straddling the border between tumor and normal tissues, chosen under strong enlargement (x100).[12]

2.4. Statistical methods

Correlations between TAM infiltration as determined by immunohistochemistry and clinicopathological parameters were evaluated using Mann-Whitney U tests. Follow-up periods were measured from the date of the start of therapy for survival. Associations between clinicopathological parameters and hormonal therapy response were assessed using the χ^2 test and Kruskal-Wallis test. Overall survival rates were calculated using the Kaplan–Meier method, and differences in survival curves were estimated with the log-rank test. $P < 0.05$ denoted a statistically significant difference.

3. Results

3.1. Patient characteristics

TAMs assessment was performed in 54 patients by immunohistochemical staining of prostate biopsy or transurethral resection of the prostate specimens using monoclonal antibody for CD68

Table 4
Bivariate analysis for response to ADT.

Variable	Response to ADT, n%		$P^{a)}$	OR (95%CI)
	Good	Poor		
Age, years old				
< 68.9	11 (45.8)	13 (54.2)	0.49	0.68 (0.23-2.04)
≥ 68.9	11 (36.7)	19 (63.3)		
PSA, ng/ml				
< 50.7	10 (71.4)	4 (28.6)	0.007	0.17 (0.05-0.66)
≥ 50.7	12 (30)	28 (70)		
Prostate volume, ml				
< 45	17 (63)	10 (37)	0.001	0.13 (0.04-0.47)
≥ 45	5 (18.5)	22 (81.5)		
TAM, n				
< 28	16 (61.5)	10 (38.5)	0.003	0.17 (0.05-0.57)
≥ 28	6 (21.4)	22 (78.6)		
MVD, n				
< 32.5	16 (59.3)	11 (40.7)	0.006	0.19 (0.06-0.65)
≥ 32.5	6 (22.2)	21 (77.8)		
Metastasis				
Yes	3 (15)	17 (85)	0.003	0.14 (0.03-0.57)
No	19 (55.9)	15 (44.1)		
Gleason score				
< 7	14 (82.4)	3 (17.6)	<0.001	0.06 (0.01-0.26)
≥ 7	8 (21.6)	29 (78.4)		
ADT				
Medical	7 (25.9)	20 (74.1)	0.027	0.28 (0.08-0.88)
Surgical	15 (55.6)	12 (44.4)		

ADT, androgen deprivation therapy; PSA: prostate-specific antigen; TAM, tumor-associated macrophage; MVD, microvessel density; OR, odds ratio; CI, confidence interval.
[a)] Chi-square.

(Fig. 1). MVD was evaluated by immunohistochemical staining using the monoclonal antibody for vWF (Fig. 2). Average of age was 68.9 ± 9.1 years. The median of PSA before and after 3 and 6 months of ADT, prostate volume, TAMs, and MVD in all the patients were 50.7 ng/dl, 22.3 ng/dl, 24.7 ng/dl, 45 ml, 28/high power field (HPF), and 32,5/HPF, respectively (Table 2). We found 20 patients with metastatic PCa, and all of them had bone metastatic and nodal and visceral metastatic. Most of the metastatic sites were found in vertebral, femur, and pelvic bone.

3.2. TAMs and hormonal therapy response

TAM infiltration significantly correlated with increasing tumor angiogenesis ($P < 0.001$, r = 0.61). Median TAM infiltration was 28/HPF, and the median value of MVD was 32.5/HPF. TAM infiltration also increased alongside higher PSA and prostate volume but did not quite reach significant difference ($P > 0.05$) (Table 3). Good response to ADT was significantly better in patients with lower prostate volume (<45 ml) and lower Gleason sum (<7) ($P < 0.001$). Fewer TAMs (<28/HPF) was significantly associated with better ADT response compared with those with higher numbers of TAMs (>28/HPF) ($P = 0.003$) (Table 4). Then, TAM infiltration was significantly higher in those with higher PSA, higher Gleason score, and metastasis. Multivariate analysis showed that Gleason score, type of ADT, and MVD number were prognostic factors for response to ADT in PCa ($P < 0.05$) (Table 5). There are no differences among PSA level, MVD, TAM infiltration, age, and prostate volume under the comparison in the ADT type (between medical and surgical ADT) (Table 6).

3.3. TAMs and survival analysis

Increased TAM infiltration [hazards ratio (HR) = 4.47; 95% confidence interval (CI): 1.97−10.15], increased MVD (HR = 2.66; 95% CI: 1.27−5.61), metastatic status (HR = 2.29; 95% CI: 0.14-0.60), and prostate volume ((HR = 2.19; 95% CI: 1.27-3.12) were significantly correlated with a shorter duration of survival in PCa patients in univariate analysis ($P < 0.05$). Multivariate analyses revealed that TAM infiltration and metastatic status were predictors for overall survival rate (Table 7). Increased TAM infiltration, MVD, and PSA value were correlated with metastatic status (Table 8). From the Kaplan-Meier plot, good survival was found for patients with TAM infiltration ≤28/HPF, MVD number ≤32.5/HPF, and no metastatic status (Figs. 3−6).

We performed follow-up patient monitoring for more than 5 years. From the Kaplan-Meier survival plot of PSA level and survival rate in PCa patients (Fig. 3), patients with PSA level ≤50.7 ng/dl had longer survival than patients with PSA level ≥50.7 ng/dl, but statistically not significant.

4. Discussion

ADT is now an accepted therapy worldwide for advanced PCa.[3,4] Urologists often choose ADT instead of RP or radiotherapy for localized PCa considering the patient's life expectancy or high risk of complications,[23,24] especially for elderly patients, and importantly, need to think of treatment modality, and the spread is varied among countries or regions.

In our study, TAM infiltration was significantly correlated with increasing tumor angiogenesis, Gleason score, metastasis, and response to ADT. Previous studies also found that TAM infiltration was a predictive factor for PSA failure or PCa progression after ADT.[7] Good response to ADT was demonstrated in the group of patients with TAMs <28/HPF in our study, and this is comparable with Nonomura's study (<22/HPF). However, it is controversial in the role of TAMs. Other studies have shown an association between TAM infiltration and disease-free survival after RP.[8] Disease-free survival significantly become worse with high level TAMs compared with low-level TAMs.[17] In contrast, increased TAMs infiltration is not a predictive factor for biochemical recurrence after prostatectomy.[15]

TAMs play a critical biological role in tumor initiation and progression, but the clinical significance of TAMs in various cancers is still undefined.[25,26] To date, it cannot be explained yet why the

Table 5
Multivariate analysis predicting response to ADT.

Variable	HR	95% Confidence interval		*P*
		Lower	Upper	
MVD, numbers (≥32.5)	9.775	1.271	75.179	0.028
Gleason score (≥7)	25.249	2.741	232.543	0.005
ADT (medical)	17.113	1.494	195.987	0.021

MVD, microvessel density; ADT, androgen deprivation therapy.

Table 6
Variable characteristic in medical and surgical ADT.

Variable	Type of ADT, Mean ± SD		*P*
	Medical	Surgical	
Age, years old	66.7 ± 10.3	71.2 ± 7.2	0.068
PSA, ng/ml	87.6 (1.89-432.6)	50 (4.01-216.75)	0.184
Prostate volume, ml	45.3 (21.43-393.7)	43.75 (23.7-87.40)	0.355
TAM, n	30 (8-76)	28 (6-74)	0.315
MVD, n	32 (10-99)	33 (13-86)	0.959

TAM, tumor-associated macrophage; PSA, prostate-specific antigen; MVD, microvessel density; ADT, androgen deprivation therapy.

response to ADT is better in patients with low levels of TAM infiltration. It may be that TAM recruitment and infiltration contributes to PCa progression.[12,15,27,28]

TAMs are one of key orchestrators of the smoldering inflammation present in the tumor microenvironment. In the majority of experimental and clinical studies, TAM levels have been associated with cancer progression. TAMs produce a host of growth factors for epithelial and endothelial cells as well as inflammatory cytokines and chemokines, contributing to tumor survival, angiogenesis, proliferation, and invasion.[13] In addition, immunosuppressive mediators released by local inflammatory or tumor cells extinguish host-mediated antitumor responses and facilitate tumor progression.[10] This helps explain biomolecularly why TAM infiltration is a predictor of ADT success in PCa patients.

The MVD level is a quantitative measurement of angiogenesis. The increase of MVD in PCa is similar to TAMs[18] and show similar results to this study. Anti-CD 163 and anti-CD 206 could be used to count TAMs and reports TAMs could also predict patient prognosis in PCa but only in nonconfined or localized tumors.[29,30] Previous investigations showed a weak correlation between MVD, pathological parameters, and metastasis.[19] Muhammadnejad et al demonstrated the significant relationship between MVD and vascular invasion for predictive value in PCa.[18] Haese et al reported that MVD immunohistochemical analysis using CD31 showed a significant relationship with pathological stage and higher Gleason scores. Wiedner et al also reported a relationship between MVD and invasive PCa and metastasis by the study of factor VII-related immunohistochemical antigens (F8-RA).[12]

Table 8
Bivariate analysis for metastatic status.

variable	Metastatic status		P
	Yes	No	
TAM	41 (8–76)	23 (6–74)	0.005[#]
MVD	44 (10–99)	27.5 (13–86)	0.012[*]
PSA	125 (19.34–432.6)	43.44 (1.89–228)	0.003[*]
Prostate volume	47.5 (25.65–393.7)	43.5 (21.43–289)	0.361
ADT			
Medical	11 (20.4)	9 (16.7)	0.573
Surgical	16 (29.6)	18 (33.3)	

TAM, tumor-associated macrophage; PSA, prostate-specific antigen; MVD, microvessel density; ADT, androgen deprivation therapy; PCa, Prostate cancer.
[#] Independent T Test.
[*] Mann-U-Whitney.

Our study showed that response to ADT was influenced by hormonal therapy type, Gleason sum, and MVD number. Our results help predict the prognosis of PCa patients with better response to ADT. Although there have been many clinicopathological studies, only few reported the relation between PCa prognosis and host immune response.[7,8,12] The progression of PCa depends on the aggressiveness of the cancer cells and also on body immune responses through macrophage cell infiltration. Our study found that the efficacy of ADT on PCa may be also influenced by immune response.

Our multivariate analyses revealed that the volume infiltration of TAMs and metastatic status were predictors for overall survival. These results were consistent with the study by Lissbrant et al, describing that increased volume infiltration of TAMs was associated with poorer cancer-specific survival,[16] However, in multivariate analysis, metastasis was the most important prognostic factor for cancer-specific survival. Hu et al reported Gleason score, PSA level, and number of TAMs were predictors for overall survival rate.[29] These results indicate that TAMs may play important role on PCa metastasis, so may be potential biomarkers of poor prognosis in late-stage PCa patients, and it was supported by the study of Nonomura et al finding significantly better recurrence-free survival in patients with fewer TAMs (<22/HPF) than those with higher numbers of TAMs (≥22/HPF) ($P < 0.001$).[7]

Recent studies demonstrated that TAMs play a critical biological role in PCa initiation and progression compared with benign status.[12,18,26,31,32] Gollapudi et al demonstrated that TAM levels were higher in prostatic intraepithelial neoplasia compared with levels in benign tissue, while patients with higher Gleason scores had higher TAM infiltration than those with lower Gleason scores.[15] Further studies from Indonesian cohort needs to be done for comparison and definitive conclusions.

Table 7
Univariate and multivariate overall survival rate analysis in PCa patients.

	Variable	Univariate analysis			Multivariate analysis		
		HR	95%CI	*P*	HR	95%CI	*P*
Age, year	≤68.9 vs. > 68.9	1.11	0.54–2.27	0.769	N/A		
PSA, ng/ml	≤50.7 vs. > 50.7	2.46	0.94–6.43	0.066	N/A		
Prostate volume, ml	≤45 vs. > 45	2.99	1.41–6.31	0.004	N/A		
TAM	≤28 vs. > 28	4.47	1.97–10.15	<0.001	3.51	1.49–8.26	0.004
MVD	≤32.5 vs. 32.5	2.66	1.27–5.61	0.010	N/A		
Metastatic status	No vs. Yes	2.29	0.14–0.60	0.001	0.41	0.19–0.89	0.023
Gleason sum	≤7 vs. > 7	2.23	0.95–5.2	0.065	N/A		

PCa, prostate cancer; TAM, tumor-associated macrophage; PSA, prostate-specific antigen; MVD, microvessel density; CI, confidence interval; HR, hazard ratio; ADT, Androgen deprivation therapy; DRE, Digital rectal examination.

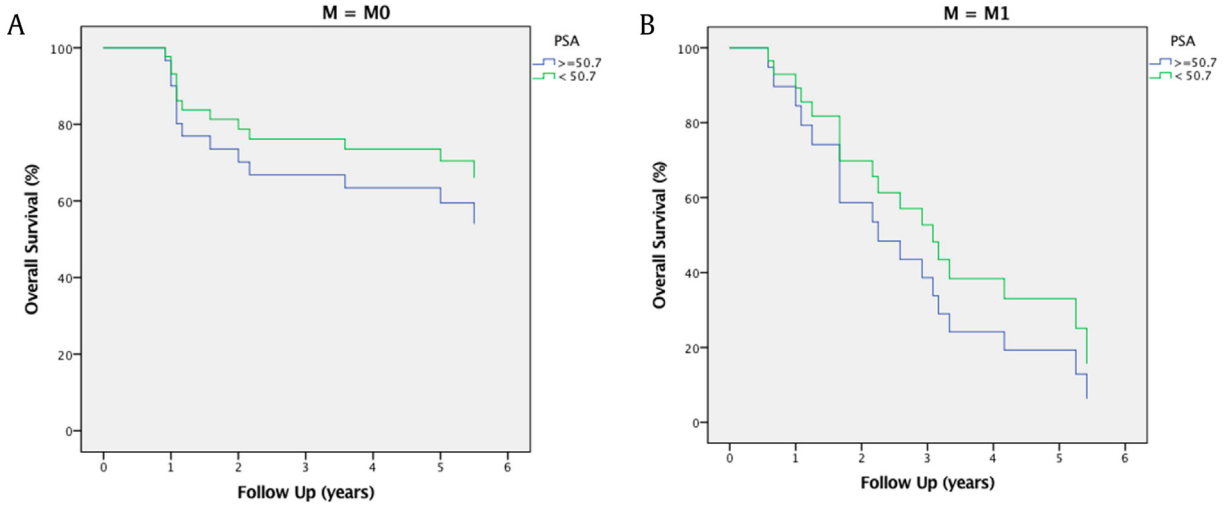

Fig. 3. (A) Kaplan-Meier survival plot of PSA level alone (HR = 2.95; 95% CI 1.73–4.16; $P = 0.027$) (B) Kaplan-Meier survival plot of PSA >16.8 withpositive metastatic status (HR = 5.2; 95% CI 25.36–45.77). PSA cut-off value 16.8 ng/ml. All patients with positive metastatic status have PSA value > 16.8 ng/dl. PSA, prostate-specific antigen; HR, hazards ratio; CI, confidence interval.

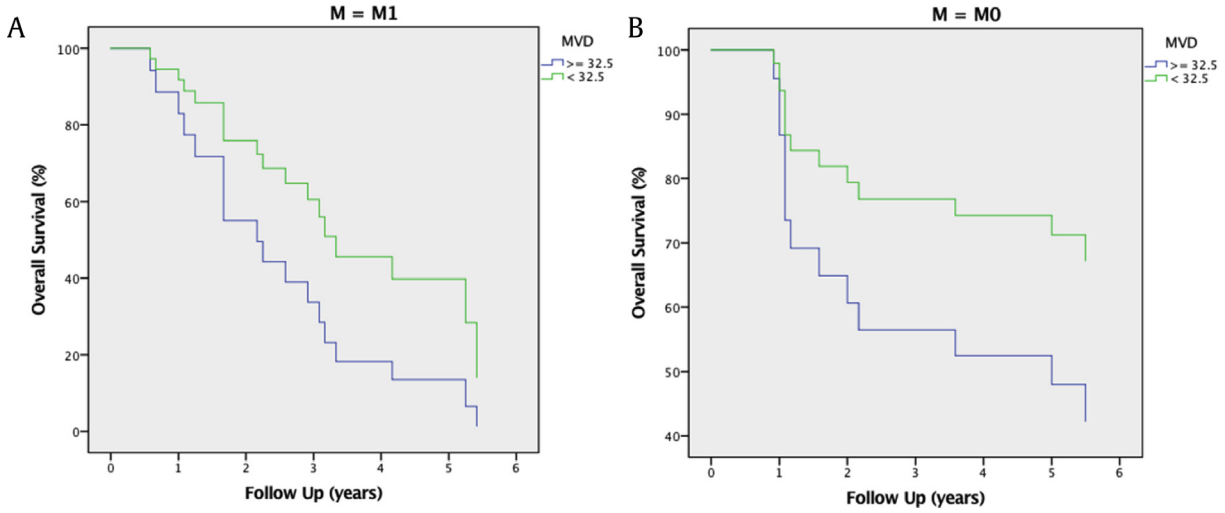

Fig. 4. (A) Kaplan-Meier survival plot of TAMs infiltration alone (HR = 2.19; 95% CI 0.61–3.78; $P = 0.002$); (B) Kaplan-Meier survival plot of TAM infiltration >28 with positive metastatic status (HR = 3.57; 95% CI 18.66–32.67; $P = 0.034$). TAM, tumor associated macrophage; HR, hazards ratio; CI, confidence interval.

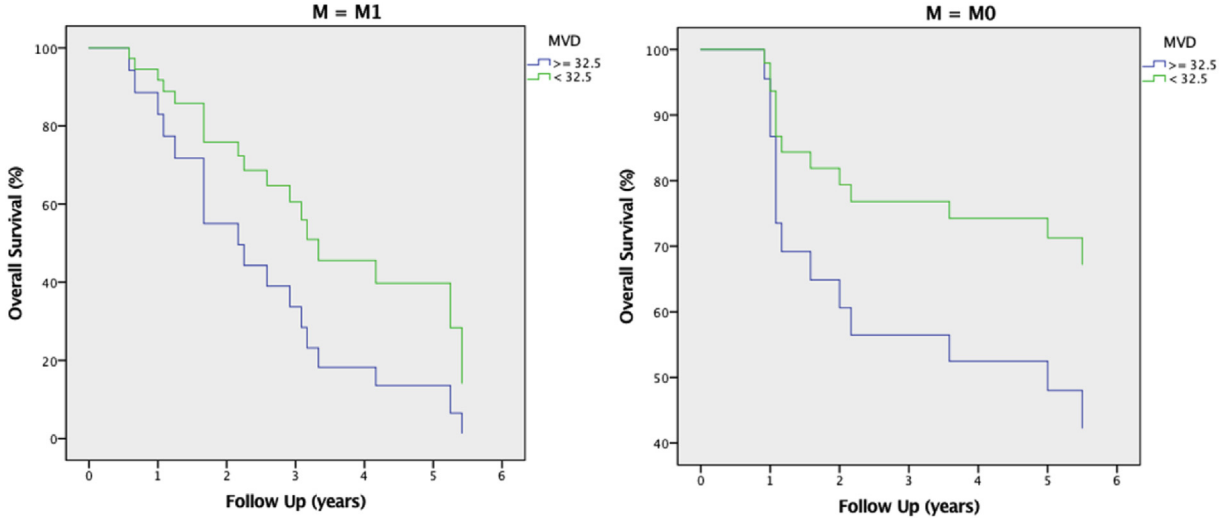

Fig. 5. (A) Kaplan-Meier survival plot of MVD alone (HR = 2.24; 95% CI 0.25–4.22; $P = 0.039$); (B) Kaplan-Meier survival plot of MVD >35 with positive metastatic status (HR = 4.47; 95% CI 18.6–36.2; p = 0.186). HR, hazards ratio; CI, confidence interval; MVD, microvessel density.

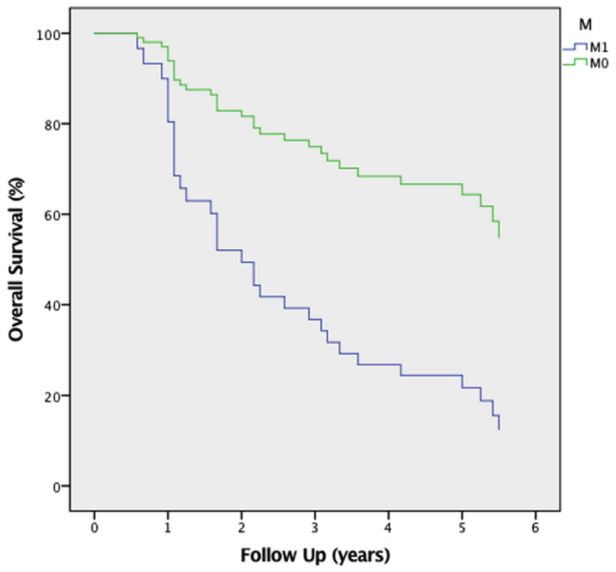

Fig. 6. Kaplan-Meier survival plot of metastatic status and survival rate in PCa patients (HR = 2; 95% CI 1.24–2.76; $P < 0.001$). HR, hazards ratio; CI, confidence interval; PCa, prostate cancer.

4.1. Study limitation

This study had several limitations. First, the results may have been influenced by the heterogeneity of patients, immunohistochemical staining techniques, and prostate specimen. Second, in our center RP procedure was rare; therefore, we could not include the RP specimen. Third, owing to small numbers of patients in our data sets, we were unable to assess for other clinically relevant endpoints. Our data showed somewhat higher PSA in 6 months than 3 months after ADT initiation. They include nonresponders to ADT, so the average of PSA values was higher. This could be more investigated focusing on ADT. Future studies evaluating subsets of TAMs and MVD with different biological functions may further elucidate the potential role of TAMs and MVD in PCa development and progression especially for predicting hormonal therapy response and survival.

5. Conclusions

Increased TAM infiltration correlates with poorer response to ADT, and increasing tumor angiogenesis in PCa. Gleason score, type of ADT, and MVD number in PCa specimens are useful predictive factors for poor response to ADT. Increasing of TAM infiltration and positive metastatic status were prognostic factors for a poor survival rate in PCa patients. Further studies with more number of patients and longer follow-up are necessary for definitive conclusion.

Declaration

Ethics approval and consent to participate

The Ethical Committee of Faculty of Medicine, Universitas Gadjah Mada/Dr. Sardjito Hospital gave approval for this study (KE/FK/545/EC). Written informed consent was obtained from all parents for participating this study.

Consent to Publish

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in the submission. The raw data can be requested to the corresponding author.

Conflicts of interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Authors' contribution

PY, EH, MR, AZ collected the patient data and PY, IS, DN, AZH analyzed and interpreted data. DH, PY performed the histological and immunohistochemical examination of the prostate. PY, AI, SY, YY, KS, KK, MF were major contributors in writing the manuscript. All authors' read and agreed for the final manuscript.

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