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Clinical utility of serum macrophage migration inhibitory factor in men with prostate cancer as a novel biomarker of detection and disease progression

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Abstract. Macrophage migration inhibitory factor (MIF) has been shown to play an important role in the growth and metastasis of prostate cancer. The objective of this study was to determine whether the serum level of MIF could be used as a diagnostic biomarker for prostate cancer as well as a predictor of disease progression. A total of 369 men who underwent systematic prostate biopsy from January 2000 to April 2004 and 30 healthy controls were included in this study. The serum MIF level was measured using an enzyme linked immunosorbent assay. Associations between the serum MIF level and several clinicopathological factors were analyzed. Among 359 patients, 137 were diagnosed as having prostate cancer. The mean values of serum MIF in the control, benign and cancer-groups were 2.1, 3.5 and 10.8 ng/ml, respectively. The MIF levels of patients with prostate cancer were significantly higher than those of the other two groups. A comparison of MIF and PSA values in all patients showed a positive correlation (correlation coefficient r^2=0.56, p<0.0001). The MIF value in patients with prostate cancer was significantly associated with clinical T stage, Gleason score and percentage of positive biopsy core (PPBC). MIF levels in patients with metastasis were significantly elevated compared with those in patients without metastasis. Among patients undergoing radical prostatectomy, the level of MIF in those with pathologically confirmed extraprostatic disease was significantly higher than that in patients with organ-confined disease. Moreover, multivariate analysis showed that MIF values, PSA values and PPBC were independent predictors for extraprostatic disease. These findings suggest that an increased serum MIF level is closely associated with the progression of prostate cancer, and thus the serum MIF level could be useful as a novel biomarker for the detection of prostate cancer as well as a predictor of disease progression.

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Introduction

Prostate cancer is the most frequently diagnosed malignancy in men in Western industrialized countries and the second leading cause of cancer-related death (1). Prostate-specific antigen (PSA) has been proved to be the most valuable tool for early detection, staging and monitoring of prostate cancer (2,3). Nevertheless, the rate of false-positive findings in prostate biopsies is approximately 65% using the conventional cut-off value of 4 ng/ml to discriminate between cancer and benign prostatic diseases (4). This is attributed to the fact that PSA is as an organ-specific, not a tumor-specific, marker and can be elevated in patients with non-malignant prostatic diseases as well. Moreover, despite recent advances in imaging modalities and the introduction of serum PSA, 20-50% of patients who underwent surgery based on a diagnosis of clinically organ-confined disease showed extraprostatic extension at the final pathological examination of prostatectomy specimens (5). Therefore, there is a pressing need to develop a novel tumor marker which is useful in both the diagnosis and prediction of disease extension in order to further improve the prognosis of patients with prostate cancer.

It is widely accepted that cytokines play an important role in the responses of individuals with malignant tumors (6). Several cytokines and growth factors have been shown to be involved in the progression of various kinds of cancer (7). Among them, macrophage migration inhibitory factor (MIF) has been reported to be associated with the growth and progression of a number of malignant tumors (8-11), including prostate cancer (12,13). MIF was first identified as a T cell-derived lymphokine that inhibits the random migration of macrophages out of capillary tubes *in vitro* (14,15). MIF functions as a pluripotent cytokine involved in a broad spectrum of pathophysiological events in association with inflammation and immune responses (16). Recent reports have demonstrated that MIF plays an essential role in the proliferation and differentiation of tumor cells (17). Moreover, several studies reported that MIF gene expression is highly

upregulated in prostate cancer cells during the progression to androgen-independence (13), MIF expression is associated with the metastatic stage of prostate cancer (18,19), and a significant positive correction was observed with a higher Gleason score (20).

In addition, one study found that the amount of MIF secreted by cultured prostate cancer cells was greater than that of prostate epithelial cells and, thus, increased serum levels of MIF in patients with prostate cancer were expected (12). Although some clinical investigations have been carried out to evaluate the clinical usefulness of measuring serum MIF, these studies provided conflicting data on the association of prostate cancer with serum MIF levels (21,22). Furthermore, there is no report concerning the usefulness of serum MIF in predicting the disease progression of prostate cancer. Therefore, in the present study, we retrospectively analyzed the usefulness of the serum MIF level as a novel tumor marker in the diagnosis of prostate cancer and also as a predictor of disease progression.

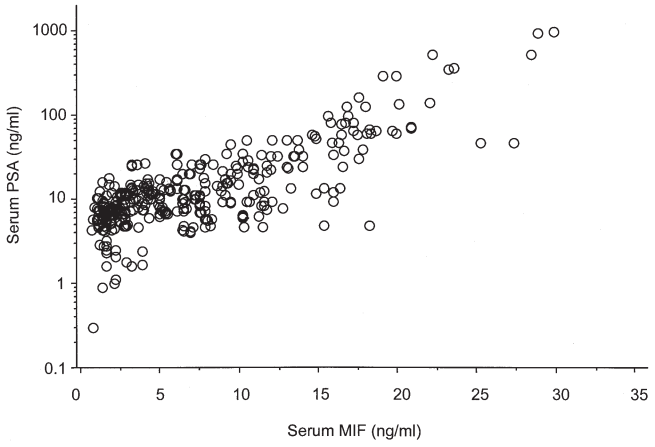

Figure 1. Correlation between serum PSA and serum MIF values in patients who underwent prostate needle biopsy. The serum MIF value is significantly correlated with the serum PSA value (n=359, r^2=0.594, p<0.0001; Spearman correlation coefficient).

Materials and methods

Between January 2000 and April 2004, 359 patients (median age, 68 years; range, 48-86 years) underwent a systemic sextant transrectal ultrasound (TRUS)-guided biopsy of the prostate. Indications for prostate biopsies were a serum PSA level >4.1 ng/ml and/or a suspicious digital rectal examination (DRE) irrespective of TRUS findings. Presentation of urinary tract infection was the exclusion criteria of the prostate biopsy. Blood samples were collected before DRE and TRUS of the prostate from patients who had not received any treatment for prostate cancer. After the blood had been allowed to clot for 60 min at room temperature, serum was separated by centrifugation at 2000 g for 15 min at 4˚C, and stored at -80˚C for 1 h until assessed. Sera were also collected from 30 healthy male volunteers (median age, 67 years; range, 56-79 years) who had no evidence of malignant disease on clinical examination including DRE, TRUS and the measurement of serum PSA value.

Of the 137 prostate cancer patients who were pathologically diagnosed based on the findings of the prostate biopsy, 79 received hormonal therapy, while the remaining 58 underwent radical retropubic prostatectomy (RRP) and pelvic lymphadenectomy. The resected prostatectomy specimens were fixed and whole-mount step-sections were cut transversely at 5-mm intervals from the apex of the prostate to the tips of the seminal vesicles. Each section was examined for cancer location, capsular penetration and seminal vesicle invasion. Among the 58 surgically resected patients, 38 had pathologically confirmed organ-confined disease (pT1N0M0 or pT2N0M0) and 20 had non-organ-confined disease (pT3≤ and/or pN^+). The pathological stages were determined according to the UICC (TNM) tumor stage classfication system (23).

The concentration of MIF was determined using a quantitative sandwich ELISA kit for human MIF (Sapporo I.D.L., Sapporo, Japan). A monoclonal antibody specific for MIF was pre-coated onto a microplate. Samples were pipetted into the wells with peroxidase-conjugated anti-human MIF monoclonal antibody and then allowed to incubate for 2 h at room temperature. After a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells and color developed in proportion to the amount of MIF bound in the present step. The color development was then stopped and the intensity of the color was measured spectrophotometrically using a microculture plate reader (Bio-Rad, CA). All analyses and calibrations were performed in duplicate. Each microtiter plate included a recombinant human MIF standard for calibration. The blank value was subtracted from the duplicate readings for each standard and sample. A standard curve was created using StatView software packages (SAS Institute Inc., NC) by plotting the logarithm of the mean absorbance of each sample versus the sample concentration.

The Spearman correlation coefficient was calculated to evaluate the association between serum PSA and MIF. Values from patients with and without prostate cancer were compared using Student's t-test. The distribution of each value by several clinicopathological factors was evaluated using one-way analysis of variance (ANOVA). Relations between predictors for extracapsular disease were first estimated using univariate logistic regression analysis. Then, multivariate logistic regression analysis was used to estimate the independent prognostic factors of extracapsular disease. In the last part of this study, the positive and negative predictive values for extracapsular disease were analyzed and probability values were calculated using Fisher's exact test. A difference with p<0.05 was considered significant.

Results

The levels of serum PSA and MIF were compared using the Pearson correlation coefficient which identifies a positive and significant correlation between each value (correlation coefficient r^2=0.56, p<0.0001) (Fig 1.). In order to evaluate the usefulness in distinguishing patients with prostate cancer from healthy controls or patients with benign prostatic disease, we first calculated the mean value of serum PSA and MIF in healthy controls, patients with BPH and patients with prostate cancer, respectively. As shown in Table I, there were

Correlation between prostate-specific antigen, macrophage migration-inhibitory factor and prostate pathology.

Histological findings	Sample size (n)	PSA (ng/ml)[a]	MIF (ng/ml)[a]
Control	30	2.9±1.3	2.1±1.8
BPH	222	7.8±4.7[b]	3.5±2.1
CaP	137	14.2±63.4[c]	10.8±6.96[d]

[a]Mean ± SD; [b]Differs from control (p<0.01) using Student's t-test; [c]Differs from BPH (p<0.0001) using Student's t-test; [d]Differs from control or BPH (p<0.0001) using Student's t-test; PSA, prostate specific-antigen; MIF, macrophage migration-inhibitory factor; BPH, benign prostatic hyperplasia; Cap, prostate cancer.

no significant differences in MIF levels between healthy controls and patients with BPH. In contrast, the level of PSA in patients with BPH was significantly greater than that in healthy controls (p<0.01) and the levels of MIF and PSA were significantly greater than those in patients with BPH (p<0.0001, p<0.0001, respectively).

We then compared the serum levels of PSA and MIF across the clinical T classification, the presence of metastasis, the biopsy Gleason score and the percentage of positive biopsy cores (PPBC) using one-way ANOVA. The MIF value in patients with prostate cancer was significantly associated with clinical T stage (p<0.0001), metastasis (p<0.0001), Gleason score (p<0.0001), and PPBC (p<0.0001), while the PSA value correlated significantly with the clinical T stage (p<0.0001) and metastasis (p<0.0001) but not Gleason score or PPBC (Table II).

The characteristics of the patients who underwent RRP and pelvic lymphadenectomy are summarized in Table III. The levels of both PSA and MIF were significantly higher in patients with pathologically confirmed extraprostatic disease than in those with organ-confined disease (p=0.015 and p=0.033, respectively; Student's t-test). In this series, the cut-off values of PSA and MIF for predicting extracapsular disease were determined by calculating the modification of hazard ratio by serum PSA and MIF levels (data not shown). The cut-off values of PSA and MIF were determined as 10 ng/ml and 9 ng/ml, respectively. We then examined the multivariate analysis in those patients to evaluate whether the serum level of PSA or MIF, the Gleason score, or PPBC could be independent predictors of extracapsular disease. Among these variables, the serum levels of PSA and MIF, and PPBC were found to be independent predictors of extracapsular disease [hazard ratio (HR)=7.22, p=0.019; HR=3.86, p=0.046; and HR=4.81, p=0.039, respectively] (Table IV). Using the cut-offs for PSA and MIF as well as a value of >7 for Gleason score and >33% for PPBC, we analyzed their positive and negative predictive value for extracapsular disease. Significant results were found for PSA and MIF (p=0.001, p=0.012,

Table II. Correlation between prostate-specific antigen, macrophage migration-inhibitory factor and characteristics of prostate cancer.

Variables	PSA (ng/ml)[a]	p-value	MIF (ng/ml)[a]	p-value
Clinical T classification (n)				
T1c (64)	8.6 (2.8-65.2)		6.8 (1.6-17.5)	
T2a (22)	14.3 (5.8-133.5)		8.9 (1.6-20.1)	
T2b (20)	15.9 (2.8-285.3)		9.2 (1.7-19.1)	
T3a (21)	41.5 (7.5-529.3)	p<0.0001[b]	12.5 (2.9-23.2)	p<0.0001[b]
T3b (6)	45.7 (14.6-139.0)		20.3 (3.2-25.2)	
T4 (4)	548.6 (125.3-971.9)		23.4 (16.8-29.9)	
Metastasis (n)				
+ (18)	82.5 (31.2-971.9)		19.4 (12.4-29.9)	
- (119)	10.9 (2.8-91.2)	p<0.0001[c]	8.7 (1.6-21.3)	p<0.0001[c]
Gleason score (n)				
2-6 (44)	9.9 (2.8-78.1)		7.1 (1.6-16.4)	
7 (63)	14.9 (4.1-971.9)	p=0.17[b]	9.5 (1.7-29.9)	p<0.0001[b]
8-10 (30)	22.6 (4.9-529.3)		15.4 (5.0-23.2)	
Percentage of positive biopsy cores (n)				
PPBC<33 (47)	10.1 (2.8-350.6)		4.2 (1.6-23.2)	
33≤PPBC<50 (36)	16.7 (6.3-70.8)	p=0.28[b]	9.5 (1.6-20.1)	p<0.0001[b]
PPBC≥50 (54)	20.6 (4.6-971.9)		12.9 (1.7-29.9)	

[a]Median (range); [b]Analysis of variance (ANOVA); [c]Student's t-test; PSA, prostate-specific antigen; MIF, macrophage migration-inhibitory factor; PPBC, percentage of positive biopsy cores.

Table III. Summary of characteristics of patients according to pathological stage.

Variables	Organ-confined (n=38)	Extraprostatic (n=20)
Age (year)[a]	64.2±6.1	65.4±5.9
Clinical stage (n)		
T1N0M0	21	10
T2aN0M0	9	4
T2bN0M0	8	6
PSA (ng/ml)[a]	11.7±8.9	20.5±11.8[b]
MIF (ng/ml)[a]	8.4±4.4	14.9±7.1[b]
Gleason score (n)		
2-6	16	4
7	17	11
8-10	5	5
Percentage of positive biopsy cores (%)		
<33	23	4
≥33	15	16

[a]Mean ± SD; [b]Differs from organ-confined group ($p<0.05$) by Student's t-test; PSA, prostate-specific antigen; MIF, macrophage migration-inhibitory factor.

Table IV. Multivariate analysis for prediction to extracapsular disease extension at RRP.[a]

Variables	95% Cl	HR	p-value
PSA (<10 vs. ≥10)	1.34-35.9	7.22	0.019
MIF (<9 vs. ≥9)	0.82-13.2	3.86	0.046
Gleason score (<7 vs. ≥7)	0.25-2.36	1.69	0.32
PPBC (<33 vs. ≥33)	1.03-36.1	4.81	0.039

[a]Logistic regression analysis; Cl, confidence interval; HR, hazard ratio; PSA, prostate-specific antigen; MIF, macrophage migration-inhibitory factor; PPBC, percentage of positive biopsy cores.

respectively); a higher sensitivity but a lower specificity in predicting extracapsular disease at RRP was found for serum PSA when compared with serum MIF. The PPBC showed a significant predictive value ($p=0.009$); however, no significant predictive value was found using biopsy Gleason score ($p=0.541$) (Table V).

Discussion

MIF was originally described as a T cell-derived lymphokine (14,15), and this protein has been reevaluated as a pluripotent cytokine involved in a broad spectrum of functions within and beyond the immune system (16). Recently, MIF has been shown to be involved in not only tumor growth and angiogenesis, but also tumor invasion and metastasis in a number of experimental tumor models, and the expression level of MIF is closely associated with disease progression in various kinds of human malignancy (8-11,17-20,24). Following these findings, several attempts have been made to evaluate the clinical utility of the serum MIF concentration as a biomarker of cancer (21,22,25). However, it remains unclear whether the serum MIF level can provide information to detect prostate cancer.

In the present study, the serum levels of MIF were found to be significantly higher in patients with prostate cancer than patients with benign prostatic disease or healthy controls, while the difference in levels of MIF between patients with benign prostatic disease and healthy controls was insignificant. Moreover, the serum MIF level paralleled the progression of prostate cancer, suggesting that the serum MIF concentration may be useful for differentiating patients with prostate cancer from those with benign prostatic disease, and could be used for predicting prostate cancer progression. In multivariate analysis, our results consistently showed that pretreatment PSA and PPBC are independent predictors of disease extension at RPP, supported by a previous report (26). Using the same data set, we showed for the first time that the serum MIF level is also a significant and independent predictor of extracapsular disease. Furthermore, the positive and negative predictive value of serum MIF level for extracapsular disease extension was comparable to that of serum PSA. Since it remains difficult to accurately identify patients with non-organ confined disease, even when several contemporary diagnostic methods are combined (4,27), these findings suggest that the measurement of serum MIF in combination with other conventional para-

Table V. Predictive value of preoperative PSA, MIF, biopsy Gleason score and percentage of positive biopsy cores for extracapsular disease extension at RRP.

Variables	Sensitivity (95% Cl)	Specificity (95% Cl)	PPV (95% Cl)	NPV (95% Cl)	p-value[a]
PSA (<10 vs ≥10)	0.82 (0.62-0.92)	0.58 (0.41-0.72)	0.48 (0.35-0.64)	0.89 (0.74-0.94)	0.001
MIF (<9 vs. ≥9)	0.55 (0.39-0.74)	0.61 (0.47-0.75)	0.52 (0.31-0.70)	0.78 (0.65-0.89)	0.012
Gleason score (<7 vs. ≥7)	0.31 (0.09-0.45)	0.68 (0.52-0.69)	0.28 (0.11-0.56)	0.65 (0.53-0.79)	0.541
PPBC (<33 vs. ≥33)	0.79 (0.63-0.91)	0.49 (0.38-0.65)	0.44 (0.29-0.56)	0.84 (0.71-0.90)	0.009

[a]Fisher's exact test; Cl, confidence interval; PSA, prostate-specific antigen; MIF, macrophage migration-inhibitory factor; PPBC, percentage of positive biopsy cores.

ly contribute to differentiating between patients with
lfined and extracapsular diseases.

As described above, there has been conflicting data regarding the serum MIF concentration in the detection and staging of prostate cancer (21,22). Many factors may be proposed to explain why a similar diagnostic significance of the serum MIF value in prostate cancer could not be demonstrated. For example, the investigators employed different conditions for the preparation and storage of serum samples, as well as different assay procedures during ELISA for the measurement of MIF. Moreover, since MIF is one of the essential regulators of the inflammatory and immune response, the existence of inflammation before prostate biopsy may influence the value of serum MIF. Although patients with systematic or symptomatic inflammatory disease were excluded from the present as well as previous studies, the influence of localized and non-symptomatic inflammatory disease, such as chronic prostatitis, on the serum level of MIF was not completely excluded, resulting in a possible migration of data which may affect the results presented. Therefore, further studies involving a larger number of samples should be performed under identical conditions and with identical assay systems to draw definitive conclusions regarding the clinical utility of the serum MIF level.

In conclusion, these findings presented in the current study suggest that an increased serum MIF level is closely associated with progression of prostate cancer and, thus, the serum MIF level could be useful as a novel biomarker for the detection of prostate cancer as well as a predictor of disease progression. However, further studies aimed at validation of the clinical utility of MIF are warranted.

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