Measurement of Serum Levels of Macrophage Inhibitory Cytokine 1 Combined with Prostate-Specific Antigen Improves Prostate Cancer Diagnosis

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Abstract **Purpose:** Current serum testing for the detection of prostate cancer (PCa) lacks specificity. On diagnosis, the optimal therapeutic pathway is not clear and tools for adequate risk assessment of localized PCa progression are not available. This leads to a significant number of men having unnecessary diagnostic biopsies and surgery. A search for novel tumor markers identified macrophage inhibitory cytokine 1 (MIC-1) as a potentially useful marker. Follow-up studies revealed MIC-1 overexpression in local and metastatic PCa whereas peritumoral interstitial staining for MIC-1 identified lower-grade tumors destined for recurrence. Consequently, we sought to assess serum MIC-1 measurement as a diagnostic tool.

Experimental Design: Using immunoassay determination of serum MIC-1 concentration in 1,000 men, 538 of whom had PCa, we defined the relationship of MIC-1 to disease variables. A diagnostic algorithm (*MIC-PSA* score) based on serum levels of MIC-1, total serum prostate-specific antigen, and percentage of free prostate-specific antigen was developed.

Results: Serum MIC-1 was found to be an independent predictor of the presence of PCa and tumors with a Gleason sum $\geq$7. We validated the *MIC-PSA* score in a separate population and showed an improved specificity for diagnostic blood testing for PCa over percentage of free prostate-specific antigen, potentially reducing unnecessary biopsies by 27%.

Conclusions: Serum MIC-1 is an independent marker of the presence of PCa and tumors with a Gleason sum of $\geq$7. The use of serum MIC-1 significantly increases diagnostic specificity and may be a future tool in the management of PCa.

Prostate cancer (PCa) is the most common male noncutaneous malignancy in the Western world, with 232,090 estimated new cases and 30,350 estimated deaths in the United States annually (1). However, cross-sectional postmortem studies estimate that PCa will spread in only 25% of men with histologically defined disease (2). Reliable tools for diagnosis and prediction of cancer progression are desirable but have been elusive to date.

Currently, measurement of total serum prostate-specific antigen is the most widely used tool for early detection, staging, and monitoring of PCa (3). Although total serum prostate-specific antigen is almost organ specific, it is not cancer specific, with elevated serum levels found in benign prostatic diseases. Approaches have been developed to improve the specificity of total serum prostate-specific antigen for the detection of PCa (4). The most successful of these, measurement of alternative molecular forms of prostate-specific antigen, expressed as the percentage of free prostate-specific antigen, improves the diagnostic specificity of prostate-specific antigen testing (5, 6) and can decrease the number of negative prostatic biopsies by 20% to 25% (7, 8).

In an attempt to improve the specificity of PCa diagnostics, microarray technology has been used to identify a number of mRNA species modulated in PCa. Notable among these is macrophage inhibitory cytokine 1 (MIC-1; ref. 9), a divergent member of the transforming growth factor-β superfamily. MIC-1 was originally identified by some members of our group on the basis of increased mRNA expression associated with macrophage activation (10, 11). It has subsequently been

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reported under a wide variety of other names (12–14) including growth and differentiation factor-15 (15) and prostate-derived factor (16). MIC-1 is synthesized as a 60-kDa dimer which is cleaved by furinlike proconvertases from its propeptide to release a 25-kDa mature protein (17, 18). However, in tumors and tumor cell lines, MIC-1 is frequently secreted from cells in an unprocessed, propeptide-containing form. This remains localized in tissues due to strong matrix binding mediated by its propeptide (18). Only processed mature MIC-1 diffuses into the circulation.

The major biological role of MIC-1 is still uncertain but among its suggested functions (10, 16, 19–21) are growth inhibition (22), induction of apoptosis (23, 24), cell detachment (24), and enhanced tumor invasiveness (25) in epithelial and other tumor cell lines. Like transforming growth factor-β, MIC-1 is expressed in normal prostate and has been implicated in p53-dependent and p53-independent cellular functions (22, 23). A number of *in vivo* and *in vitro* studies have shown that MIC-1 production and secretion are related to p53 pathway activation (26). Additional data linking MIC-1 to cancer in general, and PCa specifically, indicate that MIC-1 is likely to be involved in all stages of PCa development and progression.

A study of >2,000 patients in Sweden examined four of the single nucleotide polymorphisms known to occur in and around the MIC-1 sequence and identified the most common and well-characterized histidine 6-to-aspartate (H6D) polymorphism as being associated with sporadic and familial cases of PCa (27). The H6D is due to a C-to-G point mutation, which alters a histidine to an aspartic acid residue in the mature domain of MIC-1. Carriage of the DD or HD alleles was protective for both familial and sporadic PCa whereas the HH genotype accounted for 19.2% and 7.2% of familial and sporadic cases, respectively (27). In established PCa, MIC-1 mRNA expression is higher in Gleason sum ≥7 tumors compared with lower-grade lesions (28).

Development of an immunoassay for MIC-1 has made it possible to assess serum MIC-1 concentrations for the diagnosis and management of disease (29). Whereas MIC-1 is detectable in the serum of all patients, significant deviation from normal levels have been defined in pregnancy, with relatively decreased serum levels predicting miscarriage (30). Additionally, increasing serum MIC-1 levels within the reference range are associated with increased risk of atherosclerotic events (31). In the area of malignancy, serum MIC-1 estimation may be useful in the diagnosis of pancreatic cancer (32) and in predicting the course of colorectal carcinoma (33). Examination of a cohort of premalignant and malignant colonic lesions has shown that serum MIC-1 levels rose progressively from normal to adenoma to cancer in proportion to the stage and extent of the disease. It is an independent predictor of colon cancer relapse-free and overall survival. In the same study, allelic H6D variation of MIC-1 was an independent predictor of the presence of metastasis at presentation (33). These findings suggest a role for MIC-1 in the regulation of epithelial growth as well as malignant epithelial tumor development and progression.

Because of data linking MIC-1 to PCa at the mRNA and protein level, as well as to other epithelial malignancies, we reasoned that increased MIC-1 might be a discriminating factor in the serum of patients with PCa. We have previously shown that serum MIC-1 levels are grossly elevated in patients with metastatic PCa (34). However, in this retrospective study of 1,000 patients presenting for treatment at a specialist clinic, we have focused on early, localized disease and examined the relationship between PCa variables and serum MIC-1 levels. We show that compared with any marker alone, a combination of serum MIC-1, total serum prostate-specific antigen, and percentage of free prostate-specific antigen improves diagnostic specificity by more than 27% over percentage of free prostate-specific antigen alone and has the potential to significantly decrease the number of unnecessary biopsies.

Patients and Methods

Patient samples

Cohort. A total of 1,000 patients were enrolled in this study between 1997 and 2002, who were referred to the Department of Urology or the affiliated outpatient department at the University Hospital Charité, Berlin. Of these patients, 538 had PCa [age = 63.03 ± 6.18 years (mean ± SD); total serum prostate-specific antigen = 10.41 ± 10.16 ng/L; percentage of free prostate-specific antigen = 6.86 ± 7.37%]; 380 patients had benign prostatic hyperplasia (BPH; age = 66.95 ± 7.75 years; total serum prostate-specific antigen = 5.59 ± 6.99 ng/L; percentage of free prostate-specific antigen = 19.57 ± 10.87%); and 82 patients had no prostatic disease (age = 56.40 ± 12.58 years; total serum prostate-specific antigen = 1.844 ± 1.9 ng/L; percentage of free prostate-specific antigen = 17.66 ± 11.87%) as determined by histologic examination of the prostate. A summary of total cohort characteristics is shown in Table 1. Of the 538 PCa patients, 312 underwent radical prostatectomy. The remaining 226 PCa patients received radiation therapy and/or antiandrogenic medication or no treatment (watchful waiting). Cancer stage was assigned according to the revised tumor-node-metastasis system from 1997 and the histologic grade was classified as grade 1, 2, or 3. The pathologic stages and grades of the 312 patients receiving operative therapy were pT_1, $n = 7$; pT_2, $n = 172$; pT_3, $n = 126$; pT_4, $n = 7$; G1, $n = 7$; G2, $n = 173$; G3, $n = 132$. Gleason sum scores were available from 245 of the 312 patients who had a radical prostatectomy (Gleason sum 2-6, $n = 86$; Gleason sum 7, $n = 92$; Gleason sum 8-10, $n = 67$).

Table 1. Total, training, and validation cohort composition

Cohort	MIC-1	tPSA	Age (y)	%fPSA	Non-PCa	PCa	GS < 7	GS = 7
Total	837 ± 674	7.9 ± 9.1	64.0 ± 8.0	14.7 ± 10.0	462	538	86	159
Training	862 ± 802	7.5 ± 7.0	64.2 ± 8.0	14.5 ± 9.5	239	261	40	75
Validation	812 ± 514	8.2 ± 10.9	63.7 ± 8.1	14.9 ± 10.6	223	277	46	84

NOTE: Training and validation data sets are similar. There was no significant differences between the cohorts using Mann-Whitney *U* test for nonparametric serum marker comparison or χ^2 analysis for Gleason sum and PCa/non-PCa case comparisons.
Abbreviations: tPSA, total serum prostate-specific antigen; %fPSA, percentage of free prostate-specific antigen; GS, Gleason sum.

Table 2. Logistic regressions in the whole cohort for PCa and Gleason sum

Variable	*P*	Coefficient size of effect
Univariate logistic regression for the presence of PCa		
MIC-1	<0.0001	−5.456
tPSA	<0.0001	9.615
%fPSA	<0.0001	−12.140
Age	<0.0001	−3.960
Multivariate logistic regression for the presence of PCa*		
MIC-1	<0.0001	−3.943
tPSA	<0.0001	7.396
%fPSA	<0.0001	−9.992
Model	<0.0001	
Age	0.6796	−0.413
Univariate logistic regression for the presence of Gleason sum ≥7 tumors		
MIC-1	0.0054	2.315
tPSA	0.0094	2.409
Age	0.0129	2.450
%fPSA	0.0810	−1.723
Multivariate logistic regression for the presence of Gleason sum ≥7 tumors†		
MIC-1	0.0164	2.021
tPSA	0.0059	2.530
Age	0.0077	2.617
%fPSA	0.0298	−2.125
Model	<0.0001	

NOTE: Serum MIC-1 is an independent marker of the presence of PCa and high-grade tumors.
*Model used multivariate logistic regression for the determination of the presence of PCa.
†Model used multivariate logistic regression for the determination of the presence of Gleason sum >7 in the presence of PCa.

The 226 patients who were not treated with radical prostatectomy were clinically staged; T_1, n = 24; T_2, n = 100; T_3, n = 102. Pathologic features on biopsy were; G1, n = 15; G2, n = 141; G3, n = 70. Three patients showed distant metastasis before therapy and 18 patients had previously received antiandrogen therapy. There was no significant difference in serum variables when compared with nontreated patients.

Prostate-specific antigen assay

All blood samples were drawn before any prostate manipulation or at least 3 to 4 weeks after an earlier manipulation and centrifuged within 2 to 3 hours after sampling. The samples were analyzed immediately or stored as previously described (34, 35).

MIC-1 serum concentration determination

We analyzed baseline blood samples from all subjects for serum concentrations of MIC-1 using a sensitive immunoassay as previously described (29, 36). Data defining sensitivity and specificity of the MIC-1 sandwich ELISA have been published (29, 36). All samples were assayed in duplicate and the coefficient of variation between samples was <12%.

Statistical analysis

Continuous data were analyzed using ANOVA and logistic and linear regression analyses. Nonparametric data were log normalized where parametric analysis was used. Forward stepwise regression modeling was used to determine the optimal diagnostic algorithm on a training data set constructed using random number generation (Excel, 2004, Microsoft, Redmond, WA) with 500 patients assigned to the training data set and the remaining 500 patients to the validation data set. Using the principles of a functional link neural network (37), compound variables were established by multiplication or division of pairs of variables as outlined below. These variables and single variables were compared with outcome data using forward stepwise logistic regression in the training data set only. Those variables significantly associated with disease presence in the resultant multivariate logistic regression were used to generate diagnostic algorithms. The numerical score for the algorithm was calculated using a modified form of the method of Le Gall et al. (38). Briefly, β-coefficients derived from the model logistic regression were multiplied by the corresponding variable and tallied for all cases. The algorithm was then validated using the randomly generated validation data set only. Diagnostic algorithms and traditional diagnostic variables were compared by receiver operator curve analysis and McNare's test. Areas under the curve were compared using Rockit 0.9.1B (Charles E. Metz, Department of Radiology, University of Chicago, Chicago, IL). All other data were analyzed using the StatView v 5.0 statistical software (SPPS, Inc., Cary, NC). $P < 0.05$ was considered significant.

Results

Serum MIC-1 levels are higher in benign disease compared with PCa. Overall, all three variables, MIC-1, total serum prostate-specific antigen, and percentage of free prostate-specific antigen, were independent predictors of the presence of PCa in univariate and multivariate logistic regression (all $P < 0.0001$; Table 2). The 380 patients with BPH had lower total serum prostate-specific antigen (5.6 ± 10.2 versus 10.4 ± 7.0) and higher percentage of free prostate-specific antigen (M = 19.6 ± 10.9 versus 10.8 ± 7.0) than those with PCa (all $P < 0.0001$, ANOVA; Fig. 1A and B). Patients with BPH (MIC-1 = 983 ± 850 pg/mL) had significantly higher serum MIC-1 levels compared with the normal group of 82 patients (859 ± 619 pg/mL) who in turn had significantly higher serum MIC-1 levels than the PCa group of 538 [731 ± 500 pg/mL; P = 0.048 and 0.0323, respectively

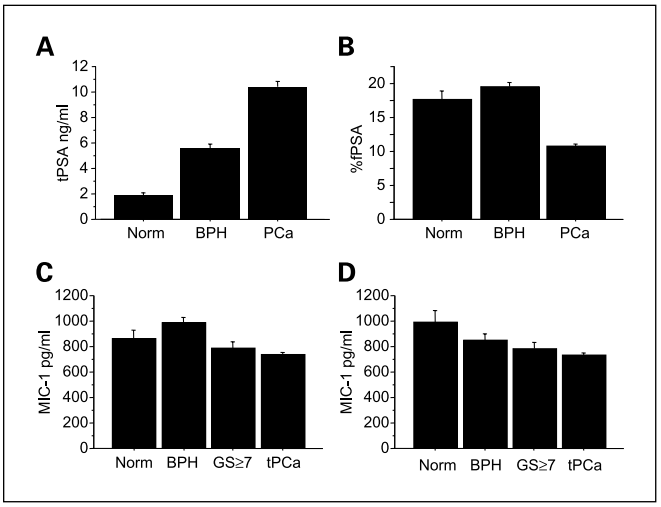

Fig. 1. Serum MIC-1 and other established markers are different in BPH and normals compared with PCa. *A,* PCa patients have the highest total serum prostate-specific antigen (*tPSA*) followed by BPH then normal ($P < 0.0001$, ANOVA). *B,* patients with PCa have the lowest percentage of free prostate-specific antigen (*%fPSA*) followed by normal, with BPH having the highest level ($P < 0.0001$, ANOVA). *C,* serum MIC-1 level is lowest in PCa and highest in BPH ($P < 0.0001$, ANOVA) with Gleason sum ≥7 tumors intermediate between normal and PCa. *D,* in the age-corrected cohort, MIC-1 is highest in normals and decreases in disease progression from BPH to PCa ($P < 0.0001$, ANOVA). Gleason sum ≥7 tumors had intermediate levels of serum MIC-1 between BPH and PCa. Bars, SE.

(ANOVA)]. Subjects with BPH also had significantly higher levels of serum MIC-1 compared with those who had PCa ($P < 0.0001$, ANOVA; Fig. 1C). However, the three groups were significantly mismatched for age with the healthy controls being significantly younger than the patients with BPH and PCa whereas the patients with BPH were significantly older than both normal and PCa patients (all $P < 0.0001$, unpaired t test). A subgroup of 855 subjects was generated by sequential exclusion of older to younger patients in the BPH group ($n = 264$) and exclusion of youngest to oldest in the healthy controls ($n = 56$) until all groups were age matched with the PCa patients ($n = 538$; mean age of the three groups, 63 years; $P > 0.9$, unpaired t test). The MIC-1 serum levels were significantly different between the three groups [normal/BPH, $P = 0.034$; BPH/PCa, $P = 0.0004$; normal/PCa, $P < 0.0001$ (ANOVA)]. However, in this case, the normal patients had the highest MIC-1 serum level (993 ± 690 pg/mL), BPH patients had intermediate level (860 ± 850 pg/mL), and PCa patients had the lowest MIC-1 serum level (731 ± 500 pg/mL; Fig. 1D), indicating that age was a confounding factor. Indeed, simple regression revealed that age was significantly correlated with MIC-1 in the full cohort ($P = 0.0009$; $r = 0.2588$, linear regression). This association was significant in all subgroups (BPH, normal, and PCa: $P = 0.0003$, $P < 0.0001$, and $P < 0.0001$, linear regression). Whereas age was negatively associated with the presence of PCa in the full cohort ($P < 0.0001$, univariate logistic regression), it was not significant in multivariate logistic regression ($P = 0.6796$; Table 2).

The finding of lower MIC-1 serum levels in patients with BPH and even lower values in those with PCa was surprising. Investigation of a separate cohort of >200 patients from Finland confirmed that serum MIC-1 levels are not elevated and tend to be depressed in local PCa.[9] This contrasts with clear evidence of increased mRNA and protein expression in PCa and suggests that MIC-1 is being retained in the prostate. As will be discussed later, this may be due to binding of MIC-1 to local extracellular matrix in the prostate (18). In the case of BPH, areas of prostate intraepithelial neoplasia may lead to increased extracellular matrix–bound MIC-1. Development of frank PCa may well be associated with a further increase in the proportion of secreted MIC-1 being matrix bound and resulting in decreased serum concentrations of MIC-1.

MIC-1 is an independent predictor of Gleason sum. Gleason scores were available for 246 patients. The remainder of the samples had been graded by the WHO grading system. In these 246 patients, increasing MIC-1, total serum prostate-specific antigen, and age were significantly associated with the presence of Gleason sum ≥7 tumors ($P = 0.0054$, 0.0094, and 0.0129, univariate logistic regression; Table 2). Decreasing percentage of free prostate-specific antigen failed to be significantly associated with the presence of Gleason sum ≥7 tumors ($P = 0.0810$, univariate logistic regression). When all three markers and subject age were included in a multivariate logistic regression increasing MIC-1, total serum prostate-specific antigen and age remained independent predictors of the presence of Gleason sum ≥7 tumors. Additionally, decreasing percentage of free prostate-specific antigen became an independent predictor of the presence of high-grade Gleason sum tumors. There was no relationship of serum MIC-1 with surgical tumor staging ($P > 0.5$, Kruskal-Wallis test), suggesting that the association of increasing serum MIC-1 with higher-grade tumors is independent of tumor burden in nonmetastatic disease. The initial depression of serum MIC-1 levels in early PCa and likely progressive elevation with increasing grade and/or dissemination of disease may indicate a role for serum MIC-1 estimation for disease monitoring in biopsy proven PCa.

MIC-1 is weakly correlated with percentage of free prostate-specific antigen but not with total serum prostate-specific antigen. Using linear regression analysis for the total cohort, MIC-1 serum levels were weakly, but significantly, correlated with the percentage of free prostate-specific antigen ($P = 0.0063$; $r = 0.21$). As MIC-1 was correlated with age, we investigated the relationship of total serum prostate-specific antigen and percentage of free prostate-specific antigen with age. The age of a subject was significantly and positively correlated with percentage of free prostate-specific antigen ($P < 0.0001$; $r = 0.20$). This relationship was significant for all three subgroups of the cohort (normal, BPH, and PCa: $P < 0.0001$). However, there was no correlation between total serum prostate-specific antigen and age ($P > 0.35$; $r = 0.041$). Additionally, serum MIC-1 was not correlated with total serum prostate-specific antigen or free PSA ($P > 0.4$; $r = 0.034$). These data in combination with previous results, indicating regulation of MIC-1 release at the cellular and extracellular matrix level (18), suggest that PCa-related alteration in MIC-1 levels reflects aspects of tumor behavior not captured by total serum prostate-specific antigen or percentage of free prostate-specific antigen measurements. Because of the superior relationship of percentage of free prostate-specific antigen to PCa presence, we have chosen to use this as an independent rather than a compound variable. Because of the lack of a relationship of MIC-1 to total serum prostate-specific antigen and the weak relationship to percentage of free prostate-specific antigen, both validated markers of PCa, in addition to the association

[9] K.S. Selander, D.A. Brown, G. Blanco-Sequeiros, et al. unpublished data.

of age with these markers, it seemed possible that a combination of these variables may improve diagnostic sensitivity and/or specificity.

Serum marker combinations increase specificity of PCa detection. To derive and validate a diagnostic algorithm, training and validation data sets were constructed using random number generation, with 500 patients assigned to the training data set and the remaining 500 patients to the validation data set. As there was a clear relationship between all three variables, MIC-1, total serum prostate-specific antigen, and percentage of free prostate-specific antigen, with PCa, it seemed likely that there are interrelationships between these markers that are currently not understood. Additionally, there were significant relationships between MIC-1, percentage of free prostate-specific antigen, and age. One approach to develop predictive algorithms wherein all the interactions among a number of variables are not clear is the use of the principles of functional link neural networks (37). Employing these principles to develop a disease-predictive model based on all three serum variables, we looked at all possible combinations of two of the three markers combined by multiplication or division in the training data set. Additional variables were generated by the multiplicative or divisive combinations of MIC-1 with age and percentage of free prostate-specific antigen with age. Using forward stepwise logistic regression, we determined the best combination of single and compound variables for discriminating between noncancer (healthy subjects and BPH patients) and PCa (Table 3). Each of the model variables is an independent predictor of the presence of PCa in univariate and multivariate logistic regression in the training data sets (Table 3). The model was significantly better than any of total serum prostate-specific antigen, percentage of free prostate-specific antigen, and MIC-1 as single markers, with percentage of free prostate-specific antigen being the best of the single markers or all three markers included as single variables in an algorithm constructed in an equivalent manner (area under the curve = 0.8425 versus 0.7712; $P < 0.0001$ (two tailed), receiver operator curve correlated area test). Using the β coefficient obtained in the training data set, we scored each subject of the validation set according to our model and multiplied by a scaling factor of 100 and rounded to the nearest integer. This model gave a score for individual subjects that ranged between −4,192 and 3,176. We named this algorithm the *MIC-PSA* score.

Table 3. Logistic regressions for *MIC-PSA* score in training data set

Variable	*P*	Coefficient size of effect
Univariate logistic regression for the *MIC-PSA* score (training)*		
MIC-1/tPSA	<0.0001	−7.394
tPSA/%fPSA	<0.0001	8.090
MIC-1 × %fPSA	<0.0001	−6.888
MIC-1	0.0001	−3.255
Multivariate logistic regression for the *MIC-PSA* score (training)*		
MIC-1/tPSA	<0.0001	−3.838
tPSA/%fPSA	<0.0001	3.830
MIC-1 × %fPSA	0.0007	−3.141
MIC-1	0.0154	2.444
Model	<0.0001	

*Models used univariate and multivariate logistic regression for the determination of the presence of PCa in the training data set only.

Receiver operator curve analysis in the validation data set revealed that the *MIC-PSA* score (generated from β coefficients derived in the training data set) did significantly better than any other single marker with the best single marker being percentage of free prostate-specific antigen (area under the curve = 0.8360 versus 0.7990; $P = 0.0244$ (two tailed), receiver operator curve correlated area test; Fig. 2A). A box plot comparing the range of serum markers levels compared with the *MIC-PSA* score is also shown (Fig. 2B). We compared the ability of the *MIC-PSA* score with percentage of free prostate-specific antigen to correctly identify cases of PCa and non-PCa in the validation data set using cutoffs set at sensitivities of 90% and 95% (Table 4) defined in the training data set. When these cutoff values were applied to the validation data set, the *MIC-PSA* score detected similar numbers of tumors and correctly identified significantly more nontumor cases at both levels of sensitivity [$P < 0.0001$ for both 90% and 95% levels (McNemar's test); Table 4]. At the 90% sensitivity cutoff, the *MIC-PSA* score detected an extra tumor case and extra 29 benign cases. This represented a saving of 29 of the 107 unnecessary biopsies that would be indicated by percentage of free prostate-specific antigen testing, a potential 27% reduction in negative biopsies. Additionally, when looking at the 95% sensitivity cutoff, the *MIC-PSA* score had further increased specificity compared with percentage of free prostate-specific antigen, reducing unnecessary biopsies by 55 (34%) with little or no significant attenuation in sensitivity ($P = 0.0809$, McNemar's test). Further analysis of the specificity of testing of the *MIC-PSA* score compared with percentage of free prostate-specific antigen in the noncancer group revealed that the *MIC-PSA* score correctly classified more benign cases at the 90% sensitivity level in BPH patients (108 versus 98) but this failed to reach significance ($P = 0.2120$, McNemar's test). Using these same cutoffs in normal patients, the *MIC-PSA* score was more specific ($P < 0.0001$, McNemar's test). At the 95% specificity level, the *MIC-PSA* score gave significantly more specific results in both normal and BPH patients ($P < 0.0001$, McNemar's test). These results suggested superior performance of the *MIC-PSA* score over percentage of free prostate-specific antigen levels in normal subjects.

Discussion

In this retrospective study, we have shown that the relationship of serum MIC-1 level to prostatic tumors seems to be largely independent of other serum markers. However, combining serum MIC-1 with other established markers of PCa significantly improves the specificity of diagnostic testing. Additionally, serum MIC-1 is an independent predictor of the presence of higher Gleason sum prostatic tumors. The increased specificity afforded by the use of serum MIC-1 measurement improves the detection of PCa and could potentially lead to a significant decrease in unnecessary prostate biopsies.

The cohort of patients that we examined had a higher rate of PCa than would be expected in a primary screening population because patients were referred to a specialist urology outpatient clinic or referred with known PCa for surgery. This may raise

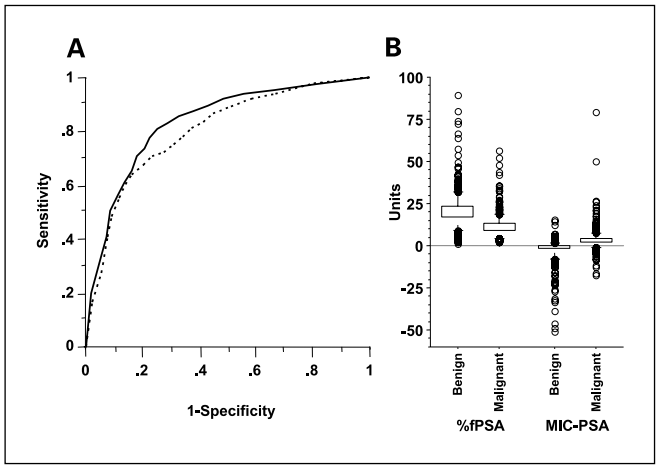

Fig. 2. The MIC-PSA score is significantly better than percentage of free prostate-specific antigen for the diagnosis of benign and malignant prostatic disease. *A,* the area under the curve for *MIC-PSA* score (area under the curve = 0.8360) was significantly greater than the best single marker, percentage of free prostate-specific antigen (*broken line*; area under the curve = 0.8013; $P = 0.0369$ [two tailed], receiver operator curve correlated area test). *B,* box plot comparing the percentage of free prostate-specific antigen serum levels for benign and malignant disease with *MIC-PSA* scores in the same categories. The values for the *MIC-PSA* score have been scaled by a factor of 40 and the point representing −4,191 was removed for ease of representation.

concerns that the application of our screening algorithm to a lower-risk population may give increased numbers of false positives. However, further comparisons of the *MIC-PSA* score within the noncancer (BPH patients and normal subjects) revealed superior performance in the normal population at clinically appropriate testing sensitivities compared with BPH. This suggests that in an unselected population with relatively more normal patients, the use of *MIC-PSA* score may lead to enhanced specificity. Consequently, as the main advantage of the *MIC-PSA* score is increased specificity, it is possible that our model represents a significant improvement in diagnostic specificity with maintained sensitivity.

The value of our method of algorithm generation would seem to be that relationships between variables that may not be immediately obvious are revealed. Whereas the source of serum MIC-1 is not exclusively prostatic, as is the case with prostate-specific antigen, combinations of these independently predictive markers of PCa compensated for potential confounding factors, such as age, and may also correct for production of serum markers outside the prostate. In deriving the *MIC-PSA* score, the addition of age itself or the inclusion of compound variables generated from products and quotients of serum markers and age did not improve diagnostic capability. Additionally, whereas older age was significantly associated with benign disease by univariate logistic regression, as was the case with MIC-1 and other serum variables, it was not an independent predictor of PCa presence in multivariate logistic regression.

Serum MIC-1 was an independent marker of the presence of higher-grade (Gleason sum ≥7) tumors. In these tumors, serum levels of MIC-1 tend to be raised independently of pathologic tumor stage and lymph node spread, suggesting that these findings are not solely due to tumor burden. In contrast to colonic neoplastic disease in which serum MIC-1 levels increase with progression from benign to malignant neoplasia, there is depression of serum levels with early localized prostatic neoplasia despite increased local tumor expression of MIC-1. Higher-grade disease leads to increased serum MIC-1 levels compared with initially depressed levels. With metastatic disease development, there are further, often massive, increases in serum MIC-1 levels. This difference between PCa and colonic carcinoma is likely to be due to either differential production of processed MIC-1 or altered extracellular matrix characteristics.

We believe that only processed MIC-1 contributes to high-grade tumor–associated increase in serum MIC-1 levels. We have never been able to detect unprocessed MIC-1 free in circulation.[9] This is in keeping with tumor xenograft studies (18), which indicate that only unprocessed MIC-1 is detectable in the tumor matrix whereas only processed MIC-1 diffuses into the circulation and is the sole contributor to serum MIC-1. This, combined with our previous findings of decreased stromal MIC-1 staining in higher-grade tumors (18), would seem to indicate that MIC-1 serum levels reflect tumor-extracellular matrix interface interaction. Results of previous studies of MIC-1 staining in human PCa indicate that lower Gleason sum cancers produce proportionally more stromal associated full-length, unprocessed MIC-1. Presumably, this proportionally increased production of unprocessed MIC-1 over processed MIC-1 by lower-grade early tumors leads to a relative decrease in serum MIC-1 concentration as proportionally more MIC-1 is bound to extracellular matrix. Massive elevation of serum MIC-1 in metastatic PCa (34) may indicate that there is significantly less MIC-1 bound to the extracellular matrix as a result of further increases in the production of mature MIC-1 or changes

Table 4. Diagnostic specificity and detection of high-grade tumors by MIC-1

Variable	PCa	Noncancer	Cutoff*	Sensitivity validation data set (%)	Specificity validation data set (%)	Difference sensitivity, P †	Difference specificity, P †
Cohort data totals	277	223	NR				
Sens-95 *MIC-PSA* score	256	115	≥76	92	53	0.0809	<0.0001
Sens-95-%fPSA	265	60	≤23.51	96	27		
Sens-90 *MIC-PSA* score	241	145	≥29	87	66	0.8551	<0.0001
Sens-90-%fPSA	240	116	≤17.72	87	52		

NOTE: The *MIC-PSA* score is significantly more specific when used for high-sensitivity testing.
*The data shown indicate the number of correctly diagnosed cases, in the validation data set, for each category using cutoffs derived from the training data set.
†The significance of the difference between %fPSA and the *MIC-PSA* score was evaluated with McNemar's test and expressed as the p value of this test.

in the extracellular matrix at the sites of metastasis. This finding may suggest a role for MIC-1 in the regulation of PCa dissemination.

Locally immobilized MIC-1 may represent an available pool of bioactive cytokine, possibly inhibiting the dissemination process. From previous studies (18) we know that higher Gleason sum tumors have relatively less associated stromal MIC-1 staining. Decreased locally available MIC-1 may lead to compromised local disease control and subsequent dissemination. In support of this notion, decreased stromal staining for MIC-1 is the single best predictor for the progression of Gleason sum ≤6 tumors to metastatic disease, irrespective of the treatment given (18). This finding, in combination with the serum MIC-1 data in this article, may indicate that tumors not picked up with algorithms, including MIC-1 serum level, are less likely to progress to disseminated disease. Additionally, evidence of changes in the serum MIC-1 levels among normal, BPH, and PCa suggests a role for serial serum MIC-1 determination for the monitoring of benign prostatic disease as well as in localized malignant disease to predict tumor progression over time.

In this study, normal patients did not have significantly different serum MIC-1 levels compared with previously studied age-matched normal blood donors (ref. 33; data not shown). However, it would be useful to study a larger cohort of normal men to refine age-specific reference ranges for serum MIC-1. This may allow future use of serum MIC-1 estimation to indicate the likelihood of higher-grade PCa presence, missed due to biopsy sampling error, or to determine a higher chance of disease progression in the context of biopsy-proven disease. It is also possible that serial measurements, showing increasing serum MIC-1 levels over time, may be much more informative for early PCa than a single measure. Additionally, when used for disease monitoring, increasing serum MIC-1 levels may indicate a need for intervention. For the diagnostic use of the *MIC-PSA* score, a carefully defined age-related reference range is less critical as the use of this algorithm relies on the relationship between MIC-1 and prostate-specific antigen. For example, where serum MIC-1 levels approach noncancer levels in higher-grade PCa (Fig. 1C and D), there will also be significantly higher total serum prostate-specific antigen and lower percentage of free prostate-specific antigen levels (Table 2), leading to a higher *MIC-PSA* score and differentiation from noncancer patients.

We have shown that MIC-1 serum determination combined with other markers of PCa may significantly increase the diagnostic specificity for PCa detection. Additionally, our results indicate that it may be worthwhile to investigate the use of MIC-1 serum levels combined with MIC-1 staining of PCa biopsies for the assignment of patients to "watchful waiting" and subsequent disease monitoring. Clearly, there are problems with the analysis of patient cohorts retrospectively and prospective studies are needed for confirmation and extension of our findings. Nevertheless, serum MIC-1, in combination with other serum markers, would seem to hold promise as a diagnostic and monitoring tool in benign and malignant prostatic disease.

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