Introduction

Prostate cancer (PCa) is the second most common disease and the sixth leading cause of death in males, around 14% (903,500) of the total new cancer cases of the disease and 6% (258,400) of the total deaths caused by cancer in men at the end of 2008. The cause of initiation and progression of PCa is not yet recognized; some studies suggest that genetic factors, race, diets, and environment factors play an important role in the development of the disease.2,3 Tumor-associated macrophages (TAM) played a significant biological role in initiation and progressivity of tumor. However, the clinical significance of TAM in various cancers is not yet determined. This research was designed to determine whether infiltration of TAM is a predictor of a disadvantageously pathological parameter and a poor prognosis in men undergoing radical prostatectomy (RP) for PCa. This research was consistent with previous research, which reported a higher level of TAM in malignant PCa compared to the benign tissue and higher Gleason score. The level of TAM was higher than in prostatic intraepithelial neoplasia (PIN) compared with benign tissue. The higher Gleason score containing a higher number of TAM was also compared with the lower Gleason score. Although the mechanisms of TAM promoting the development and the progression of prostate cancer is not known, animal studies in vivo properties showed that mobilization and infiltration of TAM played a key role in the development and the progression of PCa, and a histopathological study showed that the level of TAM positively correlated with the microvessel density (MVD).4

Association between tumor-associated macrophages and microvessel density on prostate cancer progression

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A B S T R A C T

Background: To evaluate tumor-associated macrophages (TAMs) infiltration and microvessel density as possible prognostic factors related to prostate cancer (PCa) progression.

Methods: Immunostaining of TAMs in prostate biopsy specimens was performed using a monoclonal antibody CD68 and microvessel density (MVD) using von Willebrand factor (vWF) from 25 specimens with high-grade prostatic intraepithelial neoplasia (HGPIN) and 25 specimens with PCa after transurethral resection of the prostate (TURP). Six microscopic (×200) fields were selected for TAM counting and six microscopic (×100) fields were selected for MVD counting around the cancer foci. Association between age, preoperative prostate-specific antigen (PSA), pathologic Gleason sum (GS), TAM, MVD, extracapsular extension, and metastasis were assessed using Pearson/Spearman, Student t test/Mann-Whitney U test and one-way analysis of variance/Kruskal-Wallis test.

Results: The mean of age, PSA, TAMs, and MVD were 69.1 ± 9.9, 67.1 ± 92.4, 26.2 ± 11.9, and 31.4 ± 14.0, respectively, from 50 specimens with PCa and HGPIN. Increasing TAMs number was not correlated with increasing MVD number and there was no significant mean difference statistically (P > 0.05) in TAMs and MVD although the mean of TAMs number was higher in PCa versus HGPIN but significant in PSA level (P < 0.001). In PCa specimens, age, PSA, TAMs, and MVD number were higher in patients with metastatic and extracapsular extension, but not significant statistically (P > 0.005). There was no correlation between TAMs and MVD (P > 0.001).

Conclusions: TAMs and MVD had increased PCa but did not provide independent prognostic value. Increasing numbers of TAMs was not always followed by an increase in MVD. HGPIN is the most likely precursor for PCa.

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Conclusions: TAMs and MVD had increased PCa but did not provide independent prognostic value. Increasing numbers of TAMs was not always followed by an increase in MVD. HGPIN is the most likely precursor for PCa.

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With PCa, in addition to tumor grading, vascular invasion is also evaluated, because the presence of malignant cells in the vessels increases the risk of pelvic metastasis. The theory of tumor dependence growth in angiogenesis is directed by scientists to focus on angiogenesis inhibition as a method to control the growth of neoplastic cell. The number of angiogenesis tumors can be measured quantitatively with MVD techniques. In this technique, endothelial cells have an immune reaction by immunohistochemical means, and then are counted with an optical microscope. Some studies have indicated the correlation between MVD and risk of tumor invasion on prostate and breast cancer. Additionally, correlation between MVD, vascular invasion, nuclear pleomorphism, and proliferation has been observed. Although association between MVD and the level of survival sometimes seems to be controversial, many scientists have suggested MVD as a prognostic and a predictive factor.  

MVD, which increased in PCa tissues caused by proliferation of neovessels and the increase of MVD was associated with the prognosis and development of PCa, and metastasis, the degree of the disease, and the survival rate. Thus, visualization of MVD can improve detection and cancer grading. The Gleason score is based on the microscopic characterization of PCa. This is the most commonly used system for PCa grading and is an important factor in the formulation, therapy schedule, and the prognosis of PCa. Evaluation of a tumor is significant in regarding detection and characterization. The study of MVD in PCa showed the relationship between the increase MVD with the carcinoma and with the higher tumor grade.  

The risk of finding PCa in a subsequent biopsy with HGPIN is 15 times greater than in biopsies without PIN. HGPIN shares many biochemical and genetic changes with cancer. Our objective was to evaluate TAMs infiltration and microvessel density in HGPIN and PCa.

**Materials and methods**

This research was a prospective analytic study with the cross-sectional design to examine the correlation between TAMs and MVD as well as the PCa progression. The patients in whom PCa was diagnosed at Sardjito Hospital from 2009 to 2011 were selected. Patients with prostate preparation after a well-processed treatment (on the evaluation of Hematoxylin and eosin stain (HE)), were examined for prostate specific antigen (PSA), and inspection bone scan/scintigraphy/bone survey was performed about 2 weeks after the treatment of transurethral resection of the prostate (TURP) and the size of prostate was measured through transabdominal/transrectal ultrasonography (TAUS/TRUS) incorporated in the research. In our hospital, RP was rarely done during this period (2 patients). Therefore, The RP specimen was excluded.  

The extracapsular extension, seminal vesicle invasion, and lymph node metastasis were assessed by magnetic resonance imaging (MRI) and/or multi-slice computer tomography (MSCT). The prostate preparation was taken from the result of core biopsy, prostate preparation with the diagnosis besides PIN high-grade and prostate adenocarcinoma, prostate adenocarcinoma preparation consisted of prostate small tumor focus (<5 spacious point), damaged paraffin block lost or impossible to cut off with microtome, preparation that did not meet the requirements for the immunohistochemical examination and the patient who had no any completely medical record data were excluded.

TAMs comprise the macrophages in stroma tissues (peritumoral) assessed through immunohistochemical examination using antibodies of anti-CD68 through screening all tumor areas and determined six hot spot areas (area with the most populous of positive CD68) with a weak enlargement (×50), chosen under a microscope at ×200 magnification, and made the average value of the 6 areas around the cancer foci. MVD was an indicator of angiogenesis assessed using average microvessel count (AMC) methods with considering the expression of vWF at endotelial cell with immunohistochemical examination using antibody of vWF. This method was performed by determining six areas straddling the border between the tumor and normal tissues with strong enlargement (×100), and then the mean of positive expression of vWF positive was made. Association between age, preoperative PSA, pathologic Gleason score (GS), TAM, MVD, extracapsular extension, and metastasis were assessed using Pearson/Spearman, Student t/Mann-Whitney U tests, and the one-way analysis of variance/Kruskal-Wallis test.

**Results**

This study had been carried out using 50 specimens consisting of 25 specimens of prostate cancer and 25 specimens of HGPIN. TAMs assessment was performed with immunohistochemical staining of prostate specimens using monoclonal antibody of CD68 and the evaluation of MVD was through immunohistochemical staining of prostate specimens using the monoclonal antibody of vWF (Fig. 1 and 2). From this research, obtained average age, PSA, TAMs, and MVD in all samples were 69.1 ± 9.5, 67.1 ± 92.4, 2.1 ± 11.9, and 31.4 ± 14.0 respectively. In prostate cancer, there were 11 patients (44%) experiencing metastasis, 7 patients (28%) with extracapsular extension, and 14 patients (56%) with Gleason score ≥8 (Table 1).

**Univariate analysis**

The increase of TAMs was not related to the increase of MVD with Spearman test (P = 0.103) and there was no mean difference between in the number of TAMs and MVD, between PCa and HGPIN, though TAMs mean was higher on PCa than HGPIN (27.6 ± 14.2 vs. 24.7 ± 9.3). Nevertheless, there were differences in mean of PSA between PCa and HGPIN with P = 0.001 (117.5 ± 106.8 versus 27.1 –16.6 ±) (Table 2).

In the cancer prostate group, based on the incident of metastasis (yes vs. no), age (70.9 ± 11.8 vs 68.1 ± 8.4), PSA (143.5 ± 135.9 vs. 97.0 ± 76.1), TAMs (32.2 ± 16.3 vs. 24.1 ± 11.7) and MVD (38.3 ± 20.5 vs. 25.4 ± 9.7), overall data had mean score higher on metastatic patient, but statistically not significant (P > 0.05) (Table 3).

In the PCa group, based on the extracapsular extension (yes vs. no), age (72.3 ± 10.3 vs. 68.2 ± 9.8), PSA (148.2 ± 131.9 vs. 105.5 ± 96.9) and TAMs (32.3 ± 22.2 vs. 25.8 ± 9.9), all data had mean score higher on patients with extracapsular extension but statistically not significant (P > 0.05). Mean of MVD (27.7 ± 15.2 vs. 32.4 ± 17) was higher in patients who did not experience the extracapsular extension, but statistically not significant (P > 0.05). (Table 4).

For mean of age, TAMs and MVD was not higher than in the Gleason score, except for PSA which had higher mean for patients with higher Gleason score (Table 5). On Spearman correlation testing, no correlation was found between TAMs and MVD on prostate cancer with metastasis (P = 0.157) and the extracapsular extension (P = 1.492).

**Discussion**

The increase of TAM infiltration had been associated with the pathological characteristics and poor prognosis in various cancers, including breast, colorectal, and bladder cancer. However, in other research, an infiltration of TAM was associated with prognosis or did not have the prognostic value in colorectal cancer and breast cancer. Similarly, the clinical significance of TAM in the...
development of prostate cancer and survival has not been clear. Two previous studies indicated that the increase of TAM infiltration was associated with survival-specific cancer that is associated with a worse survival, whereas the other study found that the increase of TAM infiltration in prostate tumor was a predictor of patient survival rates. These studies had been restricted by the small sample size and the lack of treatment modality uniformity, so that it was difficult to draw a conclusion about the significance of TAM in

**Fig. 1.** Coloration of immunohistochemical TAMs to specimens using monoclonal antibody of CD68 >200. (A) High-grade PIN, (B) Prostate Cancer. TAM, tumor-associated macrophages; PIN, prostatic intraepithelial neoplasia.

**Fig. 2.** Coloration of immunohistochemical MVD to specimens using monoclonal antibody von Willebrand factor (vWF) >100. (A) High-grade PIN, (B) Prostate Cancer. MVD, microvessel density; PIN, prostatic intraepithelial neoplasia.
prostate cancer; histopathological study demonstrated that the
level of TAM was positively correlated with MVD.4

The clinical significance of TAMs and MVD in prostate cancer is
not yet determined and is still controversial. This research was
designed to determine whether infiltration of TAMs and the in-
crease of MVD were predictors of disadvantageous pathological
parameters and poor prognosis in men who are undergoing TURP
for PCa. Studies have been performed using 50 specimens, con-
stituting of 25 specimens of PCa and 25 specimens of HGPIN. From
this research, mean of age, PSA, TAMs, and MVD in all samples was
69.1 ± 9.9, 67.1 ± 22.4, 26.2 ± 11.9, and 31.4 ± 14.0, respectively. In
PCa, 11 patients (44%) experienced metastasis, seven patients (28%)
with extracapsular extension, and 14 patients (56%) with Gleason
score < 6. Nonomura et al4 examined TAMs infiltration in 71 pa-
tients with prostate cancer and obtained the median of age 74, TAM
22, and PSA 50.1 ng/mL with 21 patients (29.6%) with ≥ 8 Gleason
score and 11 patients (15.5%) with extracapsular extension.

Previous research reported a higher level of TAM in malignant
PCa compared with benign tissues and the higher Gleason score.
The lever of TAM is higher in PIN than in benign tissues. The higher
Gleason score containing a higher number of TAM was also compared
with the lower Gleason score.4 Nonomura et al4 reported that the
median of PSA is 50.1 ng/mL and median of TAM is 22. Survival,
based on recurrence rate, was better on patients with < 22 total
TAMs than with ≥ 22 total TAMs, and TAMs infiltration had
significant relationships with PSA levels, and the Gleason score and
stage T in prostate cancer.4 Lisfranc et al5 found that the increase
of TAMs could be a nasty predictive factor in prostate cancer pa-
tients after undergoing TURP.5 Gannon et al13 investigated some
immune cells in the prostate after RP with or without neoadjuvant-
reducing androgen therapy, and found that the increase of TAMs
was a predictor of biochemical recurrence (PSA) on univariate test
but not a predictor on multivariate analysis.13

### Table 1

<table>
<thead>
<tr>
<th>Variables</th>
<th>Age (mean ± SD, median)</th>
<th>PSA (mean ± SD, median)</th>
<th>TAMs (mean ± SD, median)</th>
<th>MVD (mean ± SD, median)</th>
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<tbody>
<tr>
<td></td>
<td>69.1 ± 9.9 (71)</td>
<td>67.1 ± 9.2 (44)</td>
<td>26.2 ± 11.9 (24)</td>
<td>31.4 ± 14.0 (27)</td>
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<td>Metastasis (N, %)</td>
<td>Yes</td>
<td>11 (44)</td>
<td>14 (56)</td>
<td>7 (28)</td>
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<tr>
<td></td>
<td>No</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gleason score (N, %) ≤ 6</td>
<td>4 (16)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>7</td>
<td></td>
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<tr>
<td></td>
<td>≥ 8</td>
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</table>

MVD, microvessel density; PSA, prostate-specific antigen; SD, standard devi-
ation; TAMs, tumor-associated macrophages.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Yes</th>
<th>No</th>
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<tbody>
<tr>
<td>Age (mean ± SD, median)</td>
<td>72.0 ± 11.8 (75)</td>
<td>68.1 ± 8.4 (70.5)</td>
</tr>
<tr>
<td>PSA (mean ± SD, median)</td>
<td>141.5 ± 135.9 (100)</td>
<td>97.0 ± 76.1 (68.8)</td>
</tr>
<tr>
<td>TAMs (mean ± SD, median)</td>
<td>32.2 ± 16.3 (29)</td>
<td>24.1 ± 11.7 (24.5)</td>
</tr>
<tr>
<td>MVD (mean ± SD, median)</td>
<td>18.3 ± 20.5 (33)</td>
<td>25.4 ± 9.3 (22.5)</td>
</tr>
</tbody>
</table>

a) Independent t-test.
b) Mann-Whitney U test.

In this research, the increase in the total of TAMs was not related
to the increase of MVD (P = 0.103), no mean difference was found
between the total of TAMs and MVD between PCa and HGPIN
though mean of TAMs in PCa was higher than in HGPIN, and in the
metastasis and in extracapsular extension PCa group. This research
was consistent with the study of Gollapudi et al,4 which reported
that the mean of TAMs in PCa is higher than in PIN and benign
prostate enlargement. But, TAMs infiltration was not predictive
of biochemical recurrence (PSA level) after RP.4 The variations noted
in TAM levels in these studies compared to our own can partly be
explained by different quantification methods and amount of tissue
used to determine TAM levels. There is presently no standardized
method for quantification of TAM levels, thus making it difficult to
compare studies.

In PCa, in addition to the tumor grading, the vascular invasion is
evaluated, because the existence of malignant cells in the vessels
increases the risk of pelvic bone metastasis. The theory of depend-
ence tumor growth in angiogenesis is directed by scientists to
focus on angiogenesis inhabitation as a method to control
neoplastic cell growth. The amount of tumor angiogenesis can be
measured quantitatively and MVD techniques.5 In PCa, the invasion
of vascular toward the development of cancer depends on the
extension extracapsular, the inclusion vesika vesicle, tumor size,
tumor metastasis to the lymph node, positive surgery limits, and
the grade of pathological examination.5 The increase of MVD in
PCa is similar as TAMs which is debatable.5,6 Previous in-
vestigations showed a weak correlation between MVD, the patho-
logical parameters, and PCa exodus.5 Muhammadnejad et al
reported that there was a relationship between MVD and vascular
invasion, and provide the predictive value in prostate cancer.7
Haese et al12 reported that MVD with immunohistochemical CD31
showed a significant relationship between pathological stage and
the higher Gleason score. Wiedner et al11 also reported the rela-
tionship between MVD and invasive PCa and metastasis incident
with factor VII-related immunohistochemical antigens (FB-RA).

In this research, there was no MVD difference between prostate
cancer with HGPIN and in the PCa group with metastasis or
extracapsular extension. This was not in accordance with the the-
ory and research. However, this result was same as several studies
reporting that MVD is not associated with some parameters of PCa

### Table 2

Univariate analysis between prostate cancer versus high-grade prostate intra-
epithelial neoplasia.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Prostate cancer</th>
<th>High-grade PIN</th>
<th>P</th>
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<tbody>
<tr>
<td>Age (mean ± SD, median)</td>
<td>69.4 ± 9.9 (71)</td>
<td>68.8 ± 6.8 (68)</td>
<td>0.817</td>
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<tr>
<td>PSA (mean ± SD, median)</td>
<td>117.5 ± 106.8 (87.6)</td>
<td>16.6 ± 27.1 (9.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TAMs (mean ± SD, median)</td>
<td>27.6 ± 14.2 (26)</td>
<td>24.7 ± 9.3 (24)</td>
<td>0.393</td>
</tr>
<tr>
<td>MVD (mean ± SD, median)</td>
<td>31.1 ± 16.4 (26)</td>
<td>31.7 ± 11.6 (28)</td>
<td>0.392</td>
</tr>
</tbody>
</table>

a) Independent t-test.
b) Mann-Whitney U test.

MVD, microvessel density; PIN, prostatic intraepithelial neoplasia; PSA, prostate-
specific antigen; SD, standard deviation; TAMs, tumor-associated macrophages.

### Table 3

Univariate analysis on prostate cancer based on the incident of metastasis.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean ± SD, median)</td>
<td>72.0 ± 11.8 (75)</td>
<td>68.1 ± 8.4 (70.5)</td>
</tr>
<tr>
<td>PSA (mean ± SD, median)</td>
<td>141.5 ± 135.9 (100)</td>
<td>97.0 ± 76.1 (68.8)</td>
</tr>
<tr>
<td>TAMs (mean ± SD, median)</td>
<td>32.2 ± 16.3 (29)</td>
<td>24.1 ± 11.7 (24.5)</td>
</tr>
<tr>
<td>MVD (mean ± SD, median)</td>
<td>18.3 ± 20.5 (33)</td>
<td>25.4 ± 9.3 (22.5)</td>
</tr>
</tbody>
</table>

a) Independent t-test.
b) Mann-Whitney U test.

MVD, microvessel density; PSA, prostate-specific antigen; SD, standard deviation; TAMs, tumor-associated macrophages.

### Table 4

Univariate analysis on prostate cancer based on the extracapsular extension.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean ± SD, median)</td>
<td>72.3 ± 10.3 (77)</td>
<td>68.2 ± 9.8 (71)</td>
</tr>
<tr>
<td>PSA (mean ± SD, median)</td>
<td>148.2 ± 131.9 (120)</td>
<td>105.5 ± 96.9 (64.5)</td>
</tr>
<tr>
<td>TAMs (mean ± SD, median)</td>
<td>32.3 ± 22.2 (34)</td>
<td>25.8 ± 9.3 (25)</td>
</tr>
<tr>
<td>MVD (mean ± SD, median)</td>
<td>27.7 ± 15.2 (22)</td>
<td>32.4 ± 17.2 (27.5)</td>
</tr>
</tbody>
</table>

a) Independent t-test.
b) Mann-Whitney U test.

MVD, microvessel density; PSA, prostate-specific antigen; SD, standard deviation; TAMs, tumor-associated macrophages.
Luczynska et al reported that the increase of MVD was not related to the tumor stage, the Gleason score, tumor size, and invasion of the tumor to seminal vesicles. The absence of MVD correlation on PCA parameters in this research could be caused by several factors involving the small size of the sample, the different method of MVD calculation, and the kind of immunohistochemical staining used. Some theories described that MVD was not an indicator to assess an angiogenic process in tumor and is a reflection of the metabolic process of a cancer. This description was also in contrast with a previous theory, which stated that MVD did not represent angiogenesis activities, but only described the distance among tumor capillaries. Oxygen and other nutrition were a limit or how far the distance of vascularization in a tumor was.

Currently, no research is associated with the increase of TAMs and MVD. Nevertheless, in this research, findings suggest that there are associations between the increase of TAMs, MVD in PCA, and HGPIN. These findings were not in accordance with the theory described by several studies in the literature about the importance of TAM in PCA and histopathological studies show that the level of TAM positively correlated with MVD. TAM was obtained and kept in the neoplastic tissue by various chemokines and cytokines such as CCL2 and macrophage colony-stimulating factor-CSF. Initially TAM was suspected to have tumoricidal activities, the recent evidence suggests that there were associations between the increase of TAMs, MVD in PCA as well as in metastasis and extracapsular extension of PCA. These findings were not in accordance with the theory described by several studies in the literature about the importance of TAM in PCA and histopathological studies show that the level of TAM positively correlated with MVD. TAM was obtained and kept in the neoplastic tissue by various chemokines and cytokines such as CCL2 and macrophage colony-stimulating factor-CSF. Initially TAM was suspected to have tumoricidal activities, the recent evidence showed that TAM might be involved in the development of cancer because they released cytokine, growth factor, and extracellular matrix protein (e.g., interleukin-6, vascular endothelial growth factor, matrix metalloproteinases) that promote proliferation, angiogenesis, and metastasis tumor.

HGPIN is the most likely precursor for prostate cancer (CaP). The incidence of HGPIN averages about 9% (range 4–16%) in prostate biopsies, representing 115,000 new cases of HGPIN each year in the United States. It is found predominantly (about 85%) in the peripheral zone of the prostate, much as is CaP. The presence of PIN and HGPIN can be found in about one third of men with HGPIN on repeat biopsy have prostate cancer, but others have found variable results. PINs associated with increased MVD may have the potential of progressing to CaP. Therefore, we compare the differences of increasing TAMs and MVD in both HGPIN as precancer lesion and CaP not included BPH.

This study had several limitations. First, the results may have been influenced by the heterogeneity of patients, immunohistochemical staining techniques, and prostate specimen (only CaP and HGPIN specimen). Second, in our center RP procedure was rare; therefore we could not include the RP specimen. Third, this study had fewer samples compared with the others. Owing to small numbers of patients in our data sets, we were unable to assess for other clinically relevant endpoints. 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.

### Conclusion

TAMs and MVD were increased in PCA but could not be used as an independent predictor. The increase of TAMs infiltration was not always followed by the increase of micro-vascularization and in the development of PCA. HGPIN is characterized by increased crowding of prostatic cells that have enlarged nuclei and nucleoli, and intact to discontinuous basal cell layer and shares many biochemical and genetic changes with cancer. We emphasize that our conclusion is tentative and ought to be confirmed in a study of a larger sample size before making a clinical decision. This is the first report to show that a group of molecular markers, when used in concert, can distinguish PINs that are precursors to CaP from those that are not.

### Conflicts of interest

None declared.

### Acknowledgments

Our thanks go to Dr. Sri Hidayah Nurlela Syafii, Dr. Iriani Widodo, SpPa (K) and Dr. Sagiri Mangunsudirdjo, SpPa (K) from Department of Anatomical Pathology, Medical Faculty, Gajah Mada University, Yogyakarta who drew the illustrations and gave some advise for this study.

### References