Incidental Prostatic Carcinoma

A predictive role of neoangiogenesis and comparison with other prognostic factors

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Incidental prostatic carcinoma (ICP) has good prognosis related to low stage at diagnosis. Few progressive cases demanding aggressive treatment need early identification. Neoangiogenesis proved its predictive role in prostatic carcinoma after radical prostatectomy. To reveal its value in ICP authors investigated specimens after transurethral resection of prostate (TURP). Retrospective study was performed on 68 ICP diagnosed in years 1985–1989. Microvessels highlighted by factor VIII were counted in a x200 microscope field (0.8012 mm²) in most active areas of neovascularisation. Microvessel count was correlated with tumor differentiation degree, Gleason score, disease stage, and patients' survival in at least 9 years after diagnosis. Higher maximal microvessel counts were associated with lower degree of tumor differentiation (p=0.005), Gleason score (p=0.001), and disease stage (0.003). No association with disease progression and patients' survival was found. Mean microvessel counts showed less significant values when correlated with tumor differentiation degree (p=0.003) and Gleason score (p=0.01), and no correlation with other variables. Microvessel density in TURP specimens of ICP retains its prognostic value already demonstrated in carcinoma of peripheral prostatic lobes. Maximal microvessel counts were prognostically more reliable than mean values.

Keywords: angiogenesis, prostate, incidental carcinoma, transurethral resection

Introduction

A denocarcinoma of the prostate is an important cause of morbidity and mortality in elderly men and the incidence is still growing. It represents the second highest cause of cancer-related deaths in American and also Slovenian men. Incidental carcinoma of the prostate (ICP) is diagnosed in prostatic biopsies without previous knowledge of malignant disease. Its good prognosis is related to low stage at diagnosis. It is usually a well-differentiated tumor of limited growth is arising in a periurethral, transition zone of the prostatic gland. Only few cases are progressive and demand aggressive treatment so the lower biological malignancy of these tumors has been suggested. Therapy of advanced disease is difficult and often unsuccessful.

Therefore it is important to uncover those cases of ICP where the disease progression is to be anticipated. Conventional markers of malignant potential include clinical and pathologic stage, histologic grade, DNA ploidy and serum prostate specific antigen (PSA) levels. All neoplasms require angiogenesis for growth and metastatic spread. Clinical and pathomorphologic studies of malignant tumors in different organs have proved the staging and prognostic significance of neoangiogenesis determination in tumor progression and metastatic spread.

The reports on neoangiogenesis in prostatic carcinoma are confirming these results. Most of the research work has been done on biopsy material of clinically manifest prostatic cancer after radical prostatectomy. The aim of our study was to disclose the significance and possible prognostic value of neoangiogenesis in ICP diagnosed in biopsy specimens after transurethral resection of prostate (TURP).

Materials and Methods

Our retrospective study included all patients, in whom the Institute of Pathology, Medical Faculty in Ljubljana, Slovenia, ICP was diagnosed in years 1985–1989. The
biopsy material was obtained by transurethral resection of prostate (TURP) only. The patients with needle biopsy, subtotal or radical prostatectomy were excluded from the study. The prostatic resection was performed to relieve dysuric problems associated with benign prostatic hyperplasia without clinical suspicion of malignant process.

Immediately after operation prostatic tissue was fixed in 10% buffered formalin at pH 7 for 24 hours, processed, and embedded in paraffin. In each paraffin block 2-10 chips were embedded. 4-5 m thick sections were cut from each block and stained with hematoxylin and eosin (H&E).

The cancers were graded using two different methods: with classical histopathologically determined degree of differentiation,24 results were presented as well, moderately and poorly differentiated carcinoma, including the intermediate grades. During statistical evaluation results were translated to numerical values (1-3). According to the method of Gleason,14 the score was recorded (sum of the two most common grades).

Histologically, tumor substages were determined by counting number of chips involved by carcinoma.8,22 as follows: Stage A1 (T1a N0 M0); three or fewer chips involved with well-differentiated carcinoma (T1a), no regional lymph node metastasis (N0), no distant metastasis (M0). Stage A2 (T1b N0 M0); more than three chips involved with well-differentiated carcinoma (T1a), no regional lymph node metastasis (N0), no distant metastasis (M0). Stage A3 (T1c N0 M0); more than three chips involved with poorly differentiated carcinoma (T1b), no regional lymph node metastasis (N0), no distant metastasis (M0). Stage A4 (T1d N0 M0); more than three chips involved with poorly differentiated carcinoma (T1b), regional lymph node metastasis (N1), no distant metastasis (M0).

The time of patients’ survival was calculated from the date of operation (diagnosis of ICP) to the date of death, and expressed in years. If the patient was alive on December 31, 1998, that date has been used for calculation. Data on patients’ survival and cause of death were obtained from the Registry of Cancer for Slovenia, at the Institute of Oncology, Ljubljana, Slovenia.

For immunohistochemical studies and morphometry all the blocks involved by carcinoma were used. In cases with abundant involvement 6 to 8 blocks were randomly chosen to avoid unnecessary expenses. Sections adjacent to those stained with H&E, the blocks were cut at 4-5 m, deparaffinized and washed in falling concentrations of alcohol, finishing with distilled water. Sections were treated in microwave POLAR PATENT PP-780 using citrate buffer pH 6.0 (S 2031-DAKO, Buffer for Antigen Retrieval) and washed in distilled water. After the sections were digested with Proteinase K (S 2019-DAKO) for 10 minutes, they were covered with primary polyclonal antibody F VIII (von Willebrand factor, rabbit anti-human) at a dilution 1:400. After the sections were washed with buffer, they were incubated with secondary biotinylated antibody against rabbit and mouse immunoglobulins (K 5001-DAKO) for 25 minutes. After the buffer wash, streptavidin complex labelled with horseradish peroxidase was applied for 25 minutes. The slides were developed using H2O2 substrate and diamobenzidine three times for 5 minutes to produce a brown reaction product, and counterstained with Mayer hematoxylin. Sections were dehydrated, cleared in xylene and covered with malmote. The whole process was performed in a Tech Mate24 500/1000 (DAKO Denmark) with the use of Reagents and Buffers Chem Mate.

The areas of invasive tumor containing the highest numbers of capillaries and small venules per area (“hot spots”) were selected by light microscopy at low magnification (x40). After the area of highest neovascularisation was identified, individual microvessel counts were made on a x200 field (x20 objective and x10 ocular, 0.8012mm² per field). In selected areas at least three x200 fields were examined, and in the cases with minimal tumor growth, the microvessels of the whole tumor area were counted. Any brown-staining endothelial cell or endothelial cell cluster, clearly separated from adjacent microvessels, tumor cells, and other identifiable elements of connective tissue, was considered a single, countable microvessel. Red cells were not used to define a lumen neither was a lumen necessary for a structure to be defined as a microvessel.34

Results were expressed in two different ways. Firstly, the highest number of vessels identified within any single x200 field was used. Secondly, the mean value of all the fields in which the determination of microvessel count was made, has been calculated.5 The assessment of microvascularity was made blindly, without previous knowledge of clinical data or other parameters of the disease.

The degree of angiogenesis expressed as maximal or mean microvessel counts was defined as independent variable. The dependent variables were the degrees of tumor differentiation (determined histopathologically or with Gleason score), disease stage, and the time of survival in at least 9 years after the diagnosis of ICP. To determine the association between independent and dependent variables the linear regression method (Statistica for Windows) was used. Statistical significance was considered at p<0.05.

Results

Between 1985-1989, at the Institute of Pathology, Medical Faculty in Ljubljana, the diagnosis of ICP after TURP was made in 68 patients. The patients were 60 to 97 years old (mean 75.1, standard deviation 6.8 years). Well differentiated carcinoma was diagnosed in 16 (23.53%) patients, well to moderately differentiated in 13 (19.12%), moderately differentiated in 16 (23.53%), moderately to poorly
Table 1. Degree of association between maximal microvessel counts per x200 field and dependent variables as determined with linear regression method.

<table>
<thead>
<tr>
<th></th>
<th>Maximal microvessel counts (regression quotation)</th>
<th>Regression coefficient (r)</th>
<th>p</th>
</tr>
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<tbody>
<tr>
<td>Differentiation degree</td>
<td>y = 0.41x + 1.40</td>
<td>0.41</td>
<td>0.005</td>
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<tr>
<td>Gleason score</td>
<td>y = 0.39x + 5.11</td>
<td>0.39</td>
<td>0.001</td>
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<td>Disease stage</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>- at diagnosis</td>
<td>y = 0.26x + 1.78</td>
<td>0.26</td>
<td>0.03</td>
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<tr>
<td>Disease stage</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>- at progression</td>
<td></td>
<td></td>
<td>0.46*</td>
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<tr>
<td>Survival time</td>
<td></td>
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<td>0.08*</td>
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</table>

* - not statistically significant

differentiated in 16 (23.53 %), and poorly differentiated and undifferentiated carcinoma in 7 (10.29%) patients.
The results of scoring the carcinoma according to Gleason are, as follows: Gleason 3 was diagnosed in 1 (1.47%) patient, Gleason 4 in 10 (14.70%), Gleason 5 in 15 (22.06%), Gleason 6 in 11 (16.18%), Gleason 7 in 13 (19.12%), Gleason 8 in 14 (20.59 %), and Gleason 9 in 4 (5.88%) patients. At the time of diagnosis all the patients had stage A disease, defined to be “reserved” for ICP. According to definition 6 (8.82%) patients had A1 and other 62 (91.18%) A2 disease stage. During clinical follow up, available for 48 patients, 28 (58.3%) of them have not shown any signs of prostatic disease. When the disease progression occurred, 6 (12.5%) patients had disease stage B1, 3 (6.2%) B2, 1 (2.1 %) C1, 4 (8.4%) C2, and 6 (12.5%) patients had metastatic disease (stage D). The routine use of serum PSA level determination at the Clinic of Urology, Clinical Centre in Ljubljana, was introduced in 1990. Therefore, the data on patients having surgery between 1985–1989 here available only in 6 patients, and this could not be included in the analysis.

Data on the time of survival was available in 64 patients. Mean survival time was 5.7 years (standard deviation 3.5 years, range 0.1 to 13.6 years). The patient with the shortest survival died of thrombembolisation during hospitalisation shortly after TURP. Only 13 (19.1%) patients died of prostatic carcinoma. Eleven (16.2%) patients died of other malignant diseases (rectosigmoid, gastric cancer, etc.), and 30 (44.1%) patients of other diseases not otherwise specified. At completion of our study (31.12.1998), 9 (13.2%) patients were still alive.

Mean microvessel counts (MVC) identified in any x200 field ranged from 25 to 220 (mean 81.5, standard deviation 44.9). The association of MVC to dependent variables is shown in Table 1. Mean microvessel counts (MEVC) ranged from 19 to 126 (mean 51.9, standard deviation 26.6). The association of MEVC to dependent variables is shown in Table 2.

Discussion

In our study 68 patients with ICP diagnosed after TURP have been included. The patients' mean age of 75.1 years is higher than the 64–72 years as reported for prostatic carcinoma. More than half of our patients had less differentiated carcinoma with Gleason scores from 6 to 10. The results of tumor differentiation degree determination are different from other reports which define ICP as usually a well differentiated tumor originating in the transitional prostatic zone and having relatively benign clinical course. Disconcordance of reported data with our results could be explained, as follows.

Firstly, ICP has been diagnosed after TURP, and that does not exclude a possibility of a undiagnosed less differentiated carcinoma of peripheral prostatic lobes invading transitional zone where it has been resected by TURP. Invasion from the periphery of prostate has been reported to occur with increasing volume of the tumor. In a study including radical prostatectomies performed after TURP, 98% of the cases showed the residual carcinomatous growth. The residual tumors could be found in transitional or peripheral lobes, the peripheral ones being significantly less differentiated. Secondly, the routine use of PSA determination and the use of transrectal ultrasound (TRUS) had not yet been introduced at Clinic of Urology, Clinical Centre in Ljubljana in years 1985–1989. Therefore, these patients could probably have been diagnosed clinically nowadays, and the diagnosis of ICP not made. Detection of PSA levels and the use of TRUS as methods of early prostatic cancer detection have proven effective with a significantly falling incidence of ICP at our institution in the last ten years.

The prognostic value of scoring the carcinoma according to Gleason has been discussed extensively. Nowadays,

Table 2. Degree of association between mean microvessel counts per x200 field and dependent variables as determined with linear regression method.

<table>
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<td>Disease stage</td>
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<td>- at diagnosis</td>
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<td>0.15*</td>
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<tr>
<td>Disease stage</td>
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<td>- at progression</td>
<td></td>
<td></td>
<td>0.57*</td>
</tr>
<tr>
<td>Survival time</td>
<td></td>
<td></td>
<td>0.09*</td>
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</tbody>
</table>

* - not statistically significant
carcinomas with Gleason score 4 or less are supposed to be of low, with Gleason score 7 to 10 of high, and other values of intermediate malignancy.\(^\text{19}\) Dividing our patients according to that, 11 patients had carcinomas of low malignancy, 31 of high, and 26 of intermediate grades. The disease progression increased proportionally to Gleason scores, confirming the results of other studies.\(^\text{3}\)

The disease stage in most of our 68 patients has been A2, and only in 6 patients the prostatic tissue has been minimally involved with carcinomatous growth (disease stage A1). For pathological determination of the lowest disease stage, the criteria between American System and TNM classification for prostatic cancer have international consensus, are comparable and determined according to similar criteria. So disease stage A1 corresponds to T1a, and A2 to T1b. The same is true for clinical disease staging.

In our study complete data on patients' follow up including clinical disease stage at eventual disease progression was available in 48 patients. During the follow up of minimum 9 years or till the patients' death, most of them (58.3 %) have not shown any signs or symptoms of prostatic disease. A dolfsson et al found disease progression in 53% in an average time of 50 months. In our patients, disease progression occurred in 41.7%. In most cases the disease has been locally progressive and only 12.5% of patients developed metastatic disease.

Disease progression in ICP has been reported to occur in 23 to 35% of patients in stage A2, and 10% in stage A1 disease.\(^\text{21}\) When the whole population of stage A cancers was followed, disease progression occurred in 29%. Local progression has been detected in 10% and metastatic disease in only 9%. In our patients disease progression has been found in a higher percentage. This finding can be explained with a relatively high rate of less differentiated tumors in our patients.

The minimal follow up time in our study was 9 years. The mean survival time in 63 patients with data available was 5.7 years (0.1 to 13.6 years). After 5 years 52% of the patients were still alive. The survival rate is lower than the 81% or 87% reported elsewhere.\(^\text{20,21}\) This is probably the consequence of a higher number of less differentiated tumors and the higher mean age in our patients.

Data about the cause of death were available in the same 64 patients as above. Only 13 (19.1%) patients died of prostatic carcinoma. The cancer specific mortality rate is 12.5% of patients developed metastatic disease. When the whole population of stage A cancers was followed, disease progression occurred in 41.7%. In our patients disease progression has been found in a higher percentage. This finding can be explained with a relatively high rate of less differentiated tumors in our patients.

The characteristics of microvasculature in prostatic cancer compared to benign and premalignant lesions have already been described in detail.\(^\text{29}\) The vascular density rises significantly from benign to premalignant and finally malignant changes, where the highest number of microvessels is found in the centre of the tumor followed by the peripheral part. The increase of the microvessel density between benign peritumoral tissue and central part of the tumor is twofold.\(^\text{6,29}\) This findings are in keeping with Folkman's observations\(^\text{8}\) which suggests that angiogenesis one of the most important steps in a process of
cancerogenesis. Tumors larger than one mm\(^3\) induce neoangiogenesis required for additional tumor growth.\(^6\)\(^,\)\(^9\) 
In the process of invasive growth the tumors switch from prevascular into the vascular phenotype. This has been 
firmed in the case of cervical dysplasia\(^3\) and an autopsy 
study of latent prostatic carcinoma.\(^3\)\(^,\)\(^1\)

Studies of neoangiogenesis in breast carcinoma have 
shown the association between microvessel density 
expressed as M VC in a x200 field and disease progression 
and especially metastatic spread.\(^2\) \(^3\) Neoangiogenesis has 
been called an independant and highly significant prog 
nosticator of the patients’ survival.\(^3\)

First reports about neoangiogenesis in prostatic cancer 
made the comparison between localized and metastatic 
tumors. An association between neoangiogenesis and 
pathologic disease stage\(^3\)\(^,\)\(^1\)\(^4\) and response to treatment has 
been found.\(^1\)\(^5\) The prognostic value of neoangiogenesis has 
also been confirmed in clinically localised prostatic cancer 
treated with irradiation only.\(^1\)\(^7\) To our knowledge, this has 
been the only study of neoangiogenesis performed on 
biopsy specimens after TURP.

The M VC in our 68 patients with ICP showed statisti 
cally significant association with the degree of tumor 
differentiation determined histopathologically or with Glea 
son score, and disease stage in primary tumors. Similar 
results have been reported from the studies made on rad 
ical prostatectomy specimens, where the vascularisation has 
been compared to degree of tumor differentiation, local 
disease extension, and the presence of metastatic disease.\(^2\) 
Determination of M VC has shown higher statistical signifi 
cance of the results compared to M EVC or microvessel 
density/tumor area. The results of our study confirmed 
their observation. M EVC showed significant association 
only with the degree of tumor differentiation determined 
by both ways, and not with other parameters of the disease. 
Correlation of prostatic cancer in different disease stages 
has shown the high significance of microvessel number 
and pathological disease stage. Among other prognostic 
factors similar prognostic value has been proved only in 
the case of Gleason score and tumor area\(^6\) but not tumor 
volume.\(^1\)\(^5\) In prostatic cancer with Gleason score 5 to 7 and 
yet undetermined prognostic validity the prognostic value 
of neoangiogenesis has been high and even higher with 
Gleason score combined to serum PSA values.\(^4\)\(^,\)\(^6\)\(^,\)\(^9\) The PSA 
levels have due to low number of patients not been used to 
compare to other studies which confirmed the microvessel 
density as a better prognosticator of pathological disease 
stage than PSA values or Gleason score.\(^6\)

In our study, the M EVC compared to M VC has shown 
lower association of variables compared. Higher M EVC 
was associated only with less differentiated tumors (p=0.003) of 
higher Gleason score (p=0.01) and not with other disease 
parameters. Similar results have been obtained elsewhere 
when both ways of results expression have been compared.\(^2\)

In conclusion, our results showed that determination of 
neoangiogenesis in ICP biopsies after TURP is an important 
method showing association with different disease parame 
ters. The results obtained from such specimens can compare 
well with results of the studies performed on prostatic can 
cer of peripheral prostatic lobes after radical prostatect 
omies and have similar prognostic value. The determina 
tion of M VC compared to M EVC in highly vascularized 
tumor areas is associated with higher number of disease 
parameters and has a better predictive value.

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