10.1053.paor.2000.0252 available online at http://www.idealibrary.com on IDEAL

ARTICLE

Incidental Prostatic Carcinoma

*A predictive role of neoangiogenesis and comparison with other prognostic factors**

Metka VOLAV• EK, Andrej MA• ERA, Zdenka OV• AK

Institute of Pathology, Medical Faculty, University of Ljubljana, Slovenia

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 mm^2) 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. (Pathology Oncology Research Vol 6, No 3, 191–196, 2000)

Keywords: angiogenesis, prostate, incidental carcinoma, transurethral resection

Introduction

Adenocarcinoma of the prostate is an important cause of morbidity and mortality in elderly men and the incidence is still growing.[18] It represents the second highest cause of cancer-related deaths in American and also Slovenian men.[25]

Incidental carcinoma of the prostate (ICP) is diagnosed in prostatic biopsies without previous knowledge of malignant disease.[24] 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.[15,22] Only few cases are progressive and demand aggressive treatment[21] so the lower biological malignancy of these tumors has been suggested.[16] 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.[9,10] 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.[32,33]

The reports on neoangiogenesis in prostatic carcinoma are confirming these results.[5,6,13,17,34] 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

Recived: Jan 12, 2000; *accepted:* April 15, 2000

Correspondence: Metka VOLAV• EK M.D., M.Sc., Institute of Pathology, Medical Faculty, University of Ljubljana, Korytkova 2, 1000 Ljubljana, Slovenia. Tel:+386-61-1403 042; Fax: +386-61-301 816

*A part of this study has been presented at The 9th "Ljudevit Jurak" International Symposium on Comparative Pathology", June 5-6 1998, Zagreb, Croatia.

 1219-4956/00/030191+06 $ 12.00/0

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,[8,21] 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, or less differentiated carcinoma irrespective of chip number (T1b). American and UICC Staging Systems for prostate cancer have comparable substages for ICP.[8,16] Eventual disease progression was clinically determined using the American staging system.[8] Disease stage was translated into numerical values (1–7) during statistical evaluation.

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 diaminobenzidine 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 malynole. The whole process was performed in a Tech Mate™ 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, 0.8012mm^2 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.**

	Maximal microvessel counts (regression quotation)	*Regression coefficient (r)*	*p*
Differentiation degree	y = 0.41x + 1.40	0.41	0.005
Gleason score	y = 0.39x + 5.11	0.39	0.001
Disease stage – at diagnosis	y = 0.26x + 1.78	0.26	0.03
Disease stage – at progression			0.46*
Survival time			0.08*

* – 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.

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

	Mean microvessel counts (regression quotation)	*Regression coefficient (r)*	*p*
Differentiation degree	y = 0.42x + 1,34	0.42	0.003
Gleason score	y = 0.39x + 5,0	0.39	0.01
Disease stage – at diagnosis			0.15*
Disease stage – at progression			0.57*
Survival time			0.09*

* – not statistically significant

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.

Maximal 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. [21] 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.[22,24] 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.[22] In a study including radical prostatectomies performed after TURP, 98% of the cases showed the residual carcinomatous growth.[15] 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[28] 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,

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.[19] Dividing our patients according to that, 11 patients had carcinomas of low malignancy, 31 of high, and 26 patients of intermediate grades. The disease progression increased proportionally to Gleason scores, confirming the results of other studies.[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 sympoms of prostatic disease. Adolfsson et al[1] found disease progression in 53% in an average time of 50 months. In our patients, disease progression occured 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.[27] When the whole population of stage A cancers was followed, disease progression occured 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.[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 higher than reported for patients in the Veterans Administration Cooperative Urological Research Group with clinical stages I and II prostate cancer,[7] being only 3 to 6% during the follow up, and lower from rates reported elsewhere.[21,23]

In our study the endothelial cells were labeled using F VIII antibody, although different, newer and more specific antibodies are available (CD31, CD34). In a pilot study staining with F VIII antibody gave more consistent results than CD 31. The decision about which antibody to use is supported by report of Schlingemann and coworkers[26] who found different expression of various endothelial and pericytic markers in microvasculature of different tumors and granulation tissue. In 1997 a report published by Fox[11] claimed the reactions with CD 31 and CD 34 to be too sensitive and specific, and that the results of studies performed with the use of those two markers[31] were not comparable to other studies where F VIII has been used.[5,6,17,34]

In the morphometric analysis of the specimens the so called "hot spots" were first identified and the number of microvessels determined. The method described by Weidner[32] is used almost by all authors who studied microvessel density in prostatic cracinoma.[4-6,17,34] Only Barth and coworkers[2] decided to count the microvessels in the whole tumor area, but their results also proved the higher prognostic value of determining the microvessel density in the hot spots.

All of the microvessels were counted in a 200x field . The results have been expressed as MVC and MEVC. The the two methods have been used because of different reports which present their results in different ways. We wanted to determine which is better. The determination of MVC,[17,32-34] or MEVC[12] gives us absolute numbers and the calculation into microvessel density (number of microvessels/ mm^2) is simple if the area of x200 magnification in a light microscope is known. Namely, the microvessel density expressed as described is also present as the result of some studies,[5,6,31] but in some of them the use of the term is not strict and consequently the results are not comparable to the others. Some of the authors use the term microvessel density and present absolute numbers. We decided to use a more oldfashionate but a more precise presentation of results.

The counting of microvessels has been done without the use of computerised systems in common with most authors.[2,17,31-34] Computerised optical systems are claimed to give more reproducible and reliable results,[5,19] but this is available at our institution. Nevertheless, the use of counting under the control of the naked eye is simple, not so time-comsuming, much cheaper, and can be used in routine work as well.[11]

The characteristics of microvasculature in prostatic cancer compared to benign and premalignant lesions have already been described in detail.[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.[6,29] This findings are in keeping with Folkman's observations[9] 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 confirmed in the case of cervical dysplasia[30] and an autopsy study of latent prostatic carcinoma.[13]

Studies of neoangiogenesis in breast carcinoma have shown the association between microvessel density expressed as MVC in a x200 field and disease progression and especially metastatic spread.[32] Neoangiogenesis has been called an independant and highly significant prognosticator of the patients' survival.[33]

First reports about neoangiogenesis in prostatic cancer made the comparison between localized and metastatic tumors. An association between neoangiogenesis and pathologic disease stage[5,34] and response to treatment has been found.[11] The prognostic value of neoangiogenesis has also been confirmed in clinically localised prostatic cancer treated with irradiation only.[17] To our knowledge, this has been the only study of neoangiogenesis performed on biopsy specimens after TURP.

The MVC in our 68 patients with ICP showed statistically significant association with the degree of tumor differentiation determined histopathologically or with Gleason score, and disease stage in primary tumors. Similar results have been reported from the studies made on radical prostatectomy specimens, where the vascularisation has been compared to degree of tumor differenciation, local disease extension, and the presence of metastatic disease.[2] Determination of MVC has shown higher statistical significance of the results compared to MEVC or microvessel density/tumor area. The results of our study confirmed their observation. MEVC 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.[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] 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 MEVC compared to MVC has shown lower association of variables compared. Higher MEVC 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 parameters. The results obtained from such specimens can compare well with results of the studies performed on prostatic cancer of peripheral prostatic lobes after radical prostatectomies and have similar prognostic value. The determination of MVC compared to MEVC in highly vascularized tumor areas is associated with higher number of disease parameters and has a better predictive value.

References

1. [2] *Adolfsson J, Rönström L, Carstensen J, et al:* The natural course of low grade, non-metastatic prostatic carcinoma. Br J Urol 65:611-614, 1990.
2. [2] *Barth PJ, Weingärtner K, Köhler HH, et al:* Assessment of the vascularisation in prostatic carcinoma: A morphometric investigation. Hum Pathol 27:1306-1310, 1996.
3. [2] *Bostwick DG, Qian J:* Current and proposed biologic markers in prostate cancer. J Cell Biochem 19(Suppl): 197-201, 1994.
4. [2] *Bostwick DG, Wheeler TM, Blute M, et al:* Optimized microvessel density analysis improves prediction of cancer stage from prostate needle biopsies. Urology 48:47-57, 1996.
5. [2] *Brawer MK, Deering RE, Brown M, et al:* Predictors of pathologic stage in prostatic carcinoma. The role of neovascularity. Cancer 73:678-687, 1994.
6. [2] *Brawer MK:* Quantitative microvessel density. A staging and prognostic marker for human prostatic carcinoma. Cancer 78:345-349, 1996.
7. [2] *Byar DP, Corle DK, and Veterans Administration Cooperative Urological Research Group:* VACURG randomized trial of radical prostatectomy for stages I and II prostate cancer. Urology 17(Suppl): 7-11, 1981.
8. [2] *Eble JN, Epstein JI:* Stage A carcinoma of the prostate In: Contemporary issues in surgical pathology. Pathology of the prostate. (Ed: Bostwick DG), Churchill Livingstone, 1990, pp. 61-82.
9. [2] *Folkman J, Watson K, Ingber D, et al:* Induction of angiogenesis during the transition from hyperplasia to neoplasia. Nature 339:58-61, 1989.
10. [2] *Folkman J:* Tumor angiogenesis. In: The molecular basis of cancer. (Eds: Mendelsohn J, Howley PM, Israel MA, Liotta LA), WB Saunders Company, 1995, pp. 206-32.
11. [2] *Fox SB:* Tumor angiogenesis and prognosis. Histopathology 30:294-301, 1997.
12. [2] *Fregene TA, Khanuja PS, Noto AC, et al:* Tumor-associated angiogenesis in prostate cancer. Anticancer Res 13:2377-2382, 1993.
13. [2] *Furusato M, Wakui S, Sasaki H, et al:* Tumour angiogenesis in latent prostatic carcinoma. Br J Cancer 70:1244-1246, 1994.
14. [2] *Gleason DF:* Histologic grading of prostatic carcinoma. In: Contemporary issues in surgical pathology. Pathology of the prostate. (Ed: Bostwick DG), Churchill Livingstone, 1990, pp. 83-93.
15. [2] *Greene DR, Egawa S, Neerhut G, et al:* The distribution of residual cancer in radical prostatectomy specimens in stage A prostate cancer. J Urol 145:324-329, 1991.
16. [2] *Grignon DJ, Sakr WA:* Zonal origin of prostatic adenocarcinoma: Are there biologic differences between transition zone and peripheral zone adenocarcinomas of the prostate gland? J Cell Biochem 19(Suppl): 267-269, 1994.

17. *Hall MC, Troncoso P, Pollack A, et al:* Significance of tumor angiogenesis in clinically localized prostate carcinoma treated with external beam radiotherapy. Urology 44:869-875, 1994.
18. *Isaacs JT:* Prostatic cancer: An age-old problem. In: The underlying molecular, cellular, and immunological factors in cancer and aging. (Eds: Yang SS, Warner HR), Plenum Press, 1993, pp. 167-84.
19. *Johnstone PA, Riffenburgh R, Saunders EL, et al*: Grading inaccuracies in diagnostic biopsies revealing prostatic adenocarcinoma: implications for definitive radiation therapy. Int J Radiat Oncol 32:479-482, 1995.
20. *Jones EC, Young RH*: The differential diagnosis of prostatic carcinoma. Its distinction from premalignant and pseudocarcinomatous lesions of the prostate gland. Am J Clin Pathol 101:48-64, 1994.
21. *Lerner SP, Seale-Hawkins C, Carlton Jr CE, et al*: The risk of dying of prostate cancer in patients with clinically localised diasease. J Urol 146:1040-1045, 1991.
22. *Mc Neal JE, Redwine EA, Freiha FS, et al*: Zonal distribution of prostatic adenocarcinoma. Correlation with histologic pattern and direction of spread. Am J Surg Pathol 12:897-906, 1988.
23. *Mohler JL, Partin AW, Epstein JI, et al:* Prediction of prognosis in untreated stage A2 prostatic carcinoma. Cancer 69:511-519, 1992.
24. *Mostofi FK, Davis Jr CJ, Sesterhenn IA*: Pathology of carcinoma of the prostate. Cancer 70:235-253, 1992.
25. *Pompe-Kirn V*: Epidemiolo•ke zna•ilnosti raka prostate v Sloveniji. In: Rak prostate. 12. onkolo•ki vikend. Zbornik predavanj. (Ed: Marolt F), La•ko, 1997, pp 25-33.
26. *Schlingemann RO, Rietveld FJR, Kwaspen F, et al*: Differential expression of markers for endothelial cells, pericytes, and basal lamina in the microvasculature of tumors and granulation tissue. Am J Pathol 138:1335-1347, 1991.
27. *Schröder FH*: The natural history of incidental prostatic carcinoma. In: Incidental carcinoma of the prostate. (Eds: Altwein JE, Faul P, Schneider W), Springer-Verlag, 1991, pp 56-62.
28. *Sedmak B*: Diagnosti•ni postopki pri raku prostate. In: Rak prostate. 12. onkolo•ki vikend. Zbornik predavanj. (Ed: Marolt F), La•ko, 1997, pp 54-61.
29. *Siegal JA, Yu E, Brawer MK*: Topography of neovascularity in human prostate carcinoma. Cancer 75:2545-2551, 1995.
30. *Smith-Mc Cune KK, Weidner N*: Demonstration and characterisation of the angiogenic properties of cervical dysplasia. Cancer Res 54:800-804, 1994.
31. *Vartanian RK, Weidner N*: Endothelial cell proliferation in prostatic carcinoma and prostatic hyperplasia: Correlation with Gleason's score, microvessel density, and epithelial cell proliferation. Lab Invest 73:844-850, 1995.
32. *Weidner N, Semple JP, Welch WR, et al*: Tumor angiogenesis and metastasis-correlation in invasive breast carcinoma. N Engl J Med 324:1-8, 1991.
33. *Weidner N, Folkman J, Pozza F, et al*: Tumor angiogenesis: A new significant and independent prognostic indicator in early-stage breast carcinoma. J Natl Cancer Inst 84:1875-1887, 1992.
34. *Weidner N, Carroll PR, Flax J, et al*: Tumor angiogenesis correlates with metastasis in invasive prostate carcinoma, Am J Pathol 143:401-409, 1993.