Volume Fraction-Corrected Mitotic Index In Prostate Cancer

DANIELA DIACONESCU, SORIN DIACONESCU, ANTONELLA CHESCA,
SEBASTIAN TOMA
Faculty of Medicine
Transilvania University of Brașov
Nicolaie Balcescu Street, No. 56, 500019, Brașov
ROMANIA
diaconescu_dani@yahoo.com, diaconescu_sorinel@yahoo.com,
anto_chesca@yahoo.com, sebitom2002@unitbv.ro

Abstract: - Mitosis counting remains one of the most valuable prognostic indicators in tumour pathology. The aim of this study was to assess the proliferative activity of primary prostate cancer and lymph node metastases using the volume fraction-corrected mitotic index (M/Vv). Mitotic figures were quantitated as number of mitotic figures/mm² of neoplastic epithelium in a series of 40 prostatic adenocarcinomas, and the results were related to histologic features as well as to prognosis. Gleason score and mitotic indices were not significantly interrelated. High-grade tumours showed higher mitosis counts than intermediate-grade tumours, and metastasis was related to mitotic indices as well. The results show that mitotic indices are useful prognostic parameters in prostatic adenocarcinoma.

Keywords: - prostate cancer, proliferative activity; mitotic index, prostate cancer metastasis

1 Introduction

The comparative features of cells in primary tumours and their metastases have been discussed in numerous papers. The aim of this study was to investigate the proliferative activity in prostate carcinomas and their lymph node metastases metastasis using the volume fraction-corrected mitotic index M/Vv.

The proliferative potential of neoplastic cells can be evaluated in several ways, including determination of the mitotic rate, e.g. by counting the mitotic figures, or determination of the fraction of cells in S-phase. The latter can be estimated by flow citometry or thymidine labelling, or by applying immunohistochemistry using antibodies against S-phase associated proteins.

Counting of mitotic figures is the oldest way of assessing proliferation and has been applied as a diagnostic tool, especially in tumour pathology. The case with which mitoses can be recognized without special equipment – a standard laboratory microscope and a well stained hematoxylin-eosin slide, has led to increasing popularity of this way of counting of mitotic figures [9, 10].

There are several methods for the estimation of the mitotic rate and truly comparable figures are not available. Kuopio and Collan studied the influence of training on performance and suggested that training in mitotic counts provides the means to achieve economical and reproducible estimates of tumour cell proliferation [7].

Collan et al evaluated the value of the standardized mitotic index (volume fraction corrected mitotic index or M/Vv index) giving the results in mitotic figures per square mm of neoplastic epithelium [1].

Volume fraction-corrected mitotic index (M/Vv) is estimated on a microscopic field at high magnification, expressing the number of mitosis per area of tumour epithelium (mm²) [1, 5].

2 Material and method

For the study 40 cases of prostate carcinoma with lymph node metastases were selected, collected during 1.01-31.12.2009 out of the Archive of the Department of Pathology, Clinical County Hospital of Brasov. Samples were fixed in formalin 10%, embedded in paraffin, cut at 5 μm, and stained with hematoxylin and eosin.

All slides were graded using the Gleason three-grade system corresponding to tumours that are well (combined Gleason grades 2 to 4), moderately (combined Gleason grades 5 to 7), and poorly differentiated (combined Gleason grades 8 to 10).
Proliferative activity of tumour cells was estimated using the M/Vv index, expressed as number of mitotic figures/mm² of neoplastic epithelium. The microscope was equipped with a compensating measuring eyepiece x12.4 with a Weibel graticule for stereological measurements.

The Weibel graticule consists of 21 short lines with interruptions the same length as the lines. Basically, the number of intersections falling over the short lines are counted and the number of endpoints falling on the end of the structure are determined [12, 13].

Counting was carried out in each primary tumor and each lymph node metastases, using the objective 40x. Mitotic figures were characterized by an absent nuclear membrane with clear, hairy extensions of nuclear material (condensed chromosomes) that were clumped, in a plane, or in separate chromosomal aggregates. The basic idea was that at least one chromosomal end was seen in mitosis. Two parallel, clearly separate chromosome clumps were counted as one mitotic figure.

Only clearly identified mitotic figures within tumour cells were counted, and care was taken to exclude mitoses in reactive stromal and endothelial cells as well as apoptotic bodies.

The number of mitotic figures was estimated in 30 consecutive microscopic fields.

The M/Vv index was calculated using the following equation:

\[ M/Vv = \frac{\sum MI}{\sum a \times A_A} \]  

where \( MI \) is the mitotic count in a microscopic field (number of mitosis/field), \( \sum MI \) is the mitotic count in all studied fields, \( a \) represents the area of the microscopic field, \( A_A \) the area fraction of neoplastic epithelium in a microscopic field, and \( \sum a \times A_A \) is the sum of area fractions multiplied by the area of the microscopic field.

\( A_A \) can be easily calculated according to the following equation:

\[ A_A / A = P_A / P \]  

where \( P \) is the total number of points counted (30 fields of 42 points = 1260 points), and \( P_A \) the number of points corresponding to neoplastic tissue (number of points superimposed on tumour cells) (fig. 1 and fig. 2) [12].

With the microscope that has been used in this study, the area of the microscopic field included in the Weibel graticle \( (A) \) had the value of 0.050625 mm².

Let us suppose that in the 30 fields which were scanned in a tumour there were 47 mitoses (M/Vv = 47) and that 975 points corresponded to neoplastic tissue.

In the 30 examined microscopic field of the primary tumour 47 mitotic figures were counted (\( \sum IM = 47 \)), and the number of points superimposed on the neoplastic tissue was 975; in this case the M/Vv index was calculated as follows:

\[ A_A / A = P_A / P = 0.75 / 1260 = 0.77 \]

\[ M/Vv = 47 / 0.050625 \times 30 \times 0.77 = 47 / 1.17 = 40.2 \]

i.e. in this case there are 40.2 mitosis/mm² of neoplastic tissue.

In a similar way, at 77 mitosis/30 microscopic fields, and 668 points superimposed on the
neoplastic tissue, in the lymph node metastases the value of M/V<sub>v</sub> was 88.75 mitosis/mm<sup>2</sup> neoplastic tissues.

The same method was applied to all studied tumours.

For statistical analysis the Statistica for Windows (StatSoft Inc.) system was used. Correlation between quantitative parameters was analysed with the Student t test. Data were considered statistically significant at p<0.05.

3 Results

3.1. General data

The mean age at diagnosis of prostate cancer among patients was 68±16.16 years.

None of the analyzed tumours were well differentiated (Gleason score 2 to 4). Most tumours (32 cases – 80%) had medium differentiation (Gleason score 5 to 7), and 8 cases (20%) were poorly differentiated (Gleason score 8 to 10).

The poorly differentiated carcinomas had a higher value of M/V<sub>v</sub> (76.45±23.93) than those with intermediate differentiation (61.42±21.73), but there were no significant differences between the two groups.

The mean values of M/V<sub>v</sub> were 64.42±22.70 in primary tumours and 75.66±25.06 in metastases (Table 1).

| Mean values of M/V<sub>v</sub> in primary tumours and metastases |
|-----------------|-----------------|-----------------|
| Type of tumour  | No. of cases    | Mean M/V<sub>v</sub> (mitosis/mm<sup>2</sup>) | Limits            |
| Primary tumours | 40              | 64.42±22.70     | Minim 33.53±27.61 |
|                 |                 |                 | Maxim 132.59±81.87 |
| Lymph node metastases | 40 | 75.66±25.06 | Minim 28.79±36.6 | Maxim 142.25±86.39 |

All analyzed tumours showed lymph node metastases, their number varying between 1 and 12.

3.2. Relation between M/V<sub>v</sub> and some histologic parameters of the primary tumours

The relation between M/V<sub>v</sub> values and grade of histologic differentiation (Gleason score) were analyzed. Tumours with medium malignancy (score 8-10) showed higher M/V<sub>v</sub> values (M/V<sub>v</sub> = 76.45±23.93) than those well differentiated (score 5-7; M/V<sub>v</sub> = 61.42±21.73), but these mean values were not statistically different.

Venous extension of carcinomas was present in 7 (17.5%) cases; in these cases the M/V<sub>v</sub> mean value was 59.18±19.55 mitoses/mm<sup>2</sup> tumour epithelium. In tumours without venous spread the M/V<sub>v</sub> mean value was 65.35±23.43 mitoses/mm<sup>2</sup>. The difference between the two values was not statistically significant (p<0.51).

Perineural invasion was observed in a half of the cases, with a M/V<sub>v</sub> mean value of 65.05±25.58, not statistically different from those without perineural invasion (M/V<sub>v</sub> = 63.78±20.08; p<0.86).

All tumours analyzed in this study showed pelvic lymph node metastases. Cases were separated in two groups: with local, pelvic metastases (N), and with distance metastases (M) respectively. Most of the tumours (24-60%) had distance metastases and a mean value of M/V<sub>v</sub> = 60.43±19.95. Tumours with local metastases had a mean value of M/V<sub>v</sub> = 70.40±25.81 (p<0.18).

3.3. Comparison between mitotic activity in primary tumors and metastases

The mean value of M/V<sub>v</sub> in the studied group was 64.42±22.70 in primary tumours, and 75.66±25.06 in lymph node metastases. The statistical analysis (one-tailed paired t-test) showed significant differences between the two groups. In 75% of the cases M/V<sub>v</sub> values in lymph nodes were higher than in primary tumours. In metastases, M/V<sub>v</sub> was significant higher than in primary tumours (p=0.0017): 75.66±25.06, versus 64.42±22.70 mitosis/mm<sup>2</sup>.

Statistical analysis of M/V<sub>v</sub> values using linear regression method revealed a strong correlation of the M/V<sub>v</sub> in the two series, especially for values between 40 and 80 mitosis/mm<sup>2</sup> (fig. 3).

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Linear Regression (95% confidence bands)
y = 0.6756x + 32.137; R^2 = 0.3746; p = 0.000086
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Fig. 3. Linear regression analysis of the M/V<sub>v</sub> values in primary and metastatic prostate carcinoma.
4 Discussions

Proliferation markers, especially the mitotic index, have proved to be important prognostic factors in some malignant tumours. Therefore, mitosis counting is a major tool for the cytologic and histologic grading systems in mammary, pulmonary, ovarian, and other cancer types [2, 8, 13].

Concerning prostate adenocarcinomas there are no concluding data in the literature about the influence of mitotic index on prognosis. The results concerning the proliferative activity are rather conflicting.

Some studies, analysing the mitotic and the apoptotic index in comparison with different prognostic factors, showed that mitotic indeces are useful prognostic parameters in prostate carcinoma, but only in addition to the conventional histologic grading system [3], [11]. Howe et al. [6] showed a decrease of the mitotic index after radiation exposure, while other authors [4] describe a decrease of the apoptotic index, without significant changes of mitosis after experimental castration.

The investigations made in this study have shown that there are significant differences in the volume fraction-corrected mitotic index in primary tumours and metastases. Probably these differences could be explained by different conditions of vascularization and nutrition in the lymph node, and on the other hand by clonal selection, which could lead to the growth of clones with particular kinetic properties in the lymph node metastases. A decrease of the growth rate in the metastases could be the result of local inhibitory mechanisms, such as cell mediated inhibitory mechanisms.

Finally, the differences between primary and metastatic tumours may be explained by the heterogeneity of the tumours.

5 Conclusions

The proliferative activity, estimated by analysis of M/V, was significantly higher in lymph node metastasis than in primary tumours. There was no important correlation between mitotic activity and histologic differentiation (Gleason score) in primary tumours. Nevertheless, M/V can be considered an useful until, quick, and efficient factor in evaluation of malignant tumor proliferative activity, and can be an useful prognostic parameters in prostatic adenocarcinoma.

References: