PROGNOSTIC SIGNIFICANCE OF VOLUME-WEIGHTED MEAN NUCLEAR VOLUMES IN PROSTATE ADENOCARCINOMAS

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Abstract: *The aim of this study was to investigate the correlation of stereologically estimated volume-weighted mean nuclear volumes (MNV) with Gleason score in prostate adenocarcinomas. To estimate the MNV the point sample intercepts method was used. MNV was significantly associated with tumour differentiation, suggesting that estimates of MNV are prognostically equal or superior to morphological grading of malignancy, such as Gleason score, in prostate cancer.*

Key words: *prostate carcinoma, prognosis, mean-weighted nuclear volume, stereology.*

1. Introduction

Prostate cancer is the most prevalent malignancy in males in the Western world and the second leading cause of male cancer death.

Various factors have been reported to be of value in predicting the prognosis of patients with prostate cancer. Gleason score and prostate-specific antigen (PSA) are the two most powerful prognosticators among them [7]. The value of histologic grading is controversial, being prone to a significant degree of intraobserver and interobserver variation.

There is agreement in the literature that additional objective and reproducible methods of predicting outcome would be desirable. That is why many different parameters have been evaluated for their prognostic significance in adeno-carcinomas, including proliferative activity [18], ploidy [1], apoptosis [19], p53 expression [4], AgNOR count [10], and lymphocytic infiltration of the tumor margins [6]. Investigations on prostatic carcinomas using stereologic methods, however, are rare.

The stereologic point-sampled intercepts method of estimating mean weighted nuclear volume (MNV) allows an unbiased, shape- and orientation-independent, three-dimensional estimation of nuclear size in tissues [11, 12]. The method is simple, fast and objective, with high intraobserver and interobserver reproducibility [8], thus well suited to be used in clinical pathology. This method has already been proven to provide important information concerning prognosis in a variety of malignant tumors [5], [9], [15], [16].

The aim of the present study was to investigate the value of MNV as a prognostic indicator in prostate adenocarcinoma.

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2. Materials and Methods

For this study 30 cases of prostatic carcinoma were selected, collected during 2004-2005 out of the archive of the Department of Pathology, District Hospital of Brasov. The age of the patients ranged from 36 to 87 years (mean: 68). Samples were obtained from routinely processed (formalin-fixed and paraffin-embedded) pathology specimens prepared from transurethral resection of the prostate. As a histopathologic reference for defining tumor regions, 5 μm random sections were cut onto microscope slides and stained with conventional hematoxylin & eosin stain. All slides were graded using the Gleason three-grade system corresponding to tumours that are well (corresponding to combined Gleason grades 2 to 4), moderately (corresponding to combined Gleason grades 5 to 7), and poorly differentiated (corresponding to combined Gleason grades 8 to 10)

Estimation of the MNV was carried out using an original stereologic software, created by a group led by Professor Olinici C.D. from the Department of Pathology, University of Medicine and Pharmacy, and Professor Ing. Vaida M.F. from the Department of Communications, Technical University of Cluj-Napoca. All measurements were performed using an Olympus microscope equipped with an x100 oil-immersion lens at a final magnification of x1000. The quantitative analysis was carried out by one observer. Estimates of three-dimensional MNV distribution were obtained by so-called point sampling of nuclear intercepts, as described by Gundersen and Jensen [Gundersen; Gundersen]. A mean of 30 fields of vision were examined in each case. An average of 100 nuclei was point sampled per case, 50 from each of the main two Gleason's grade. Fields of vision containing extensive necrosis or inflammation were excluded from measurement. A transparent test grid was superimposed on the screen (Figure 1). The nuclear intercepts were measured along the test lines of the grid from nuclear boundary to nuclear boundary. Only nuclear profiles hit by points were sampled. The lengths of nuclear intercepts (l_0) was processed to obtain $\pi.l_0^3/3$, an unbiased estimate of MNV independent of nuclear shape, which because of point sampling emphasizes larger nuclei rather than smaller ones.

A comparison between MNV values in tumor areas with different Gleason grade and score was performed.

Mean ± SD was calculated by Statistica for Windows (StatSoft Inc) package. Comparison between means was performed using the Student's *t*-test; $p < 0.05$ was considered significant. Concerning the reproducibility of the MNV estimation method, consecutive measurements of the same cases showed excellent agreement (0.9-4% variation between two measures).

3. Results

According to the Gleason three-grade system, 8 cases (26,67%) were well-differentiated, 15 cases (50%) were moderately-differentiated, and 7 cases (23,33%) were poorly-differentiated adenocarcinomas.
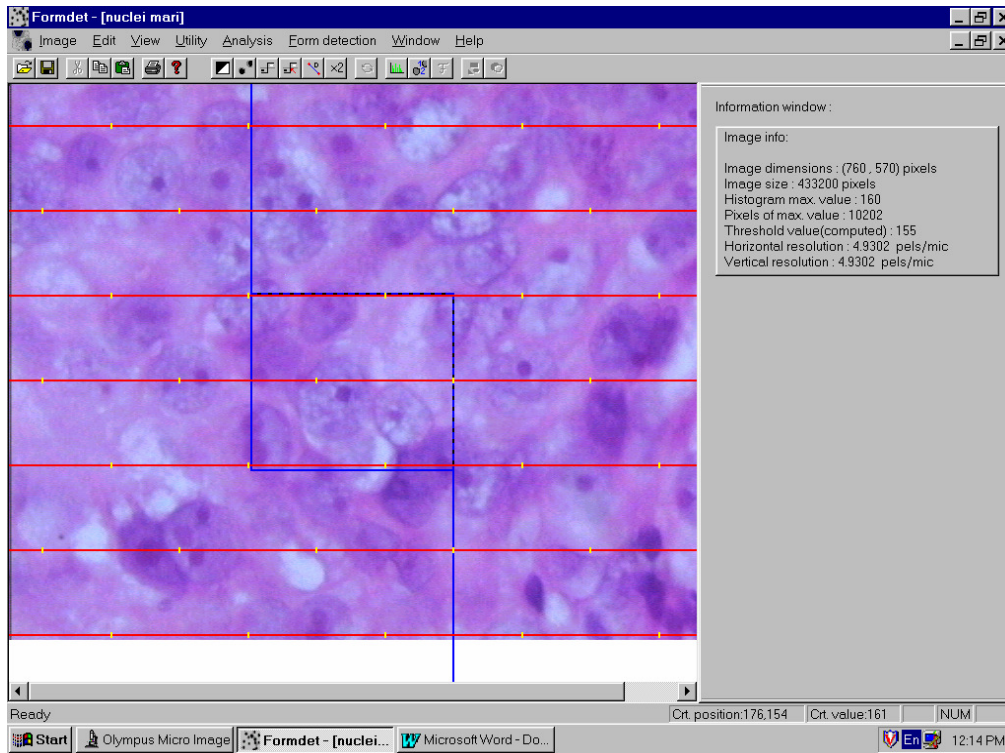
Fig.1. *Micrograph from a Gleason grade 3 prostate adenocarcinoma (MNV=257,09 μm³) with the test system superimposed*

MNV of tumor cells increased highly significant in parallels with Gleason grade from 117.44 (SD, 44.87 μm³) in Gleason grade 1 to 302.56 (SD, 105.56 μm³) in Gleason grade 5 (p = 0,00002) (Table 1).

MNV (μm³) in fields with different Gleason grades   Table 1

Gl. grade	Mean ± SD	Limits
1	117.4±44.8	74.7–194.2
2	182.5±34.6	124.1–243.8
3	216.1±80.5	138–424.2
4	270.5±111.5	155.6–541.6
5	302.5±105.6	203.2–492.4

MNV of tumor cells in well-differentiated adenocarcinoma (Gleason score 2-4) was 138.54 μm³ (SD, 39.92 μm³), ranging from 76.5 to 188.43 μm³, in moderately-differentiated (Gleason score 5-7) – 217.83 μm³ (SD, 83.47 μm³), ranging from 101.11 to 482.91 μm³, and in poorly-differentiated adenocarcinoma (Gleason score 8-10) – 288.8 μm³ (SD, 84.45 μm³), ranging from 201.9 to 408.57 μm³.

Correlation between MNV values and combined Gleason grades was statistically significant for the entire group (p = 0.002), between well- and moderately-differentiated carcinoma (p = 0.01), whereas no significance could be found between moderately- and poorly-differentiated adenocarcinomas. Nuclear volume increased twice from well- to poorly-differentiated prostate tumors.

Unbiased stereologic estimation of MNV has been proven to be an excellent prognostic parameter in several types of cancer [5], [9], [15], [16].

In spite of the fact that prostate cancer is very common in the United States and in Europe, few authors have reported a relationship between the prognosis of prostate cancer and MNV.

This study provides a correlation between MNV and patient survival. There was a significant correlation between MNV and established independent prognostic parameters, like histologic grading according to the Gleason system. Therefore, estimation of MNV could be of use as a prognostic parameter in prostate adenocarcinoma.

The results of this study are in agreement with the results of other authors [2, 3], [7, 8, 9], [13, 14]. However, all of this study was performed on cases in a single institution, and its has remained unclear whether MNV calculations obtained at one institution apply to cases at another institution. To solve this problem, some authors [8] made a prognostic index based on data from one hospital, and tested whether these data could be used to predict the prognosis of patients at another hospital. They concluded that estimates of MNV can be evaluated at multiple institutions with the use of prognostic index.

The comparative study of Teba *et al* [17] could not demonstrate any prognostic superiority of MNV over other nuclear morhometric parameters, including mean nuclear area, coefficients of variation for nuclear area and form factor in patients with prostate cancer.

5. Conclusions

Stereologic estimation of MNV is simple and needs no expensive, special technical equipment. In the present study a significant association could be found between MNV and histologic grading according to the Gleason system.

Although this study was performed on a limited number of cases, our results suggests that estimates of MNV are prognostically equal or superior to morphological grading of malignancy, such as Gleason score, in prostate cancer.

Further study with a larger patient population is needed to confirm the findings. However, we emphasize that the estimates of MNV is a more objective method for histological grading to predict the malignant potential of prostate cancer.

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