Prognostic Criteria in Patients with Prostate Cancer: Gleason Score versus Volume-weighted Mean Nuclear Volume

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ABSTRACT

Gleason’s score (GS) has been reported to be the most valuable prognostic factor in cases of prostate cancer. GS is solely dependent on the histological architecture of the prostate cancer, but, it seems doubtful that histological patterns are sufficient for evaluating the degree of malignancy of prostate cancer. We previously reported that the estimation of volume-weighted mean nuclear volume (MNV) might be a more useful prognosticator for prostate cancer than subjective histological grading. However, the previous study was conducted on patients treated in a single hospital, and the number of subjects was too small to draw a definitive conclusion. In this study, we analyzed a larger number of subjects at another institution using a blinded study design. A retrospective prognostic study of 195 patients with prostate cancer diagnosed between January 1966 and December 1988 at Kyoto University Hospital, and treated by conservative therapy, was conducted. Unbiased estimates of MNV were compared with the clinical stage and histological grading according to GS with regard to the prognostic value. Univariate analysis revealed that estimates of MNV, clinical stage, and GS all correlated significantly with disease-specific survival in cases of prostate cancer. Multivariate analysis of all cases also revealed that all of these factors were significant independent prognosticators of disease-specific survival. However, focusing on clinically localized cases (stages A, B, and C), multivariate analysis revealed that the estimation of MNV was the only powerful prognosticator of prostate cancer. This study indicates that the estimation of MNV is prognostically equal or superior to GS in cases of prostate cancer. We emphasized that the estimates of MNV is a more objective method for histological grading to predict the malignant potential of prostate cancer.

INTRODUCTION

Grading of malignancy in prostate cancer is carried out by the subjective, qualitative visual observation of morphological details in ordinary histological sections. Among the many methods of histological grading, many authors have reported GS (1, 2), which is solely dependent on the architecture of the prostate cancer cell, with no consideration to the volume of the nucleus, to be of reliable prognostic value. Some authors, however, have pointed out that the subjective histological grading of GS is characterized by low reproducibility (3, 4). Furthermore, it has been reported that the volume of the cancer nucleus was closely correlated to the degree of malignancy in a variety of human cancers (5–11). These observations suggest that more objective grading systems in consideration of nuclear morphology are required for accurate prediction of the malignant potential of prostate cancer.

Estimates of volume-weighted MNV are the only methods by which unbiased estimates of three-dimensional parameters can be obtained from a single two-dimensional section (12), which was developed by Gunderson and Jensen (13, 14) based on a stereological method, and it was proven by Sørensen et al. (15) and Nielsen et al. (16) to be highly reproducible. Furthermore, estimates of MNV are capable of predicting the recurrence of pTa bladder cancer (5, 6), and it provides an accurate prognosis for other cancers such as cutaneous malignant melanoma (7, 8), papillary carcinomas of the ampulla of Vater (9), and squamous cell carcinoma of the uterine cervix (10). In a preliminary report, we stated that estimates of MNV were a valuable prognostic factor for prostate cancer (17, 18). This is the first study to evaluate the significance of the estimates of MNV in a large number of subjects, and the correlation between the prognosis for prostate cancer and the MNV was compared with GS using a blinded study design and multivariate survival statistics.

MATERIALS AND METHODS

Patients. Two hundred eighty-seven patients were diagnosed with prostate cancer at Kyoto University Hospital during the period of January 1966 to December 1988. Histological slides were obtained before hormone therapy in 228 of the 287 patients. One hundred eighty of these patients underwent needle biopsy, 33 of these patients underwent transurethral resection of the prostate and 15 patients were incidentally diagnosed in the course of treatment for benign prostatic hyperplasia by retropu...
bic subcapsular prostatectomy. In eight of these cases, the specimen was too poor to evaluate volume-weighted MNV. Eight cases who were treated with radiation therapy, 11 cases who underwent radical prostatectomy, and 6 cases with incomplete data were excluded from evaluation. Thus, the prognostic value of GS, clinical stage, and estimates of MNV were analyzed in 195 patients (stage A1, 10 cases; stage A2, 12 cases; stage B1, 10 cases; stage B2, 19 cases; stage C, 53 cases; stage D1, 7 cases; and stage D2, 84 cases) whose mean age at the time of diagnosis was 71.1 years (range, 47–91 years; 40s, 1 case; 50s, 16 cases; 60s, 61 cases; 70s, 82 cases; 80s, 31 cases; and 90s, 4 cases).

Pretreatment staging consisted of physical examination, excretory urography, chest X-ray, a bone scan, and computerized tomographic scan of the retroperitoneum when it became available after 1980. Two of the seven stage D1 cases were diagnosed by bilateral pelvic lymphadenectomy. The disease was staged according to the Whitmore-Jewett system (19). All patients except stage A1 prostate cancer patients were treated with hormone therapy and when hormone therapy became refractory, symptomatic treatments were performed in each case. Hormone therapy was undertaken as follows (castration, 22 cases; diethylstilbestrol diphosphate, 84 cases; and castration + diethylstilbestrol diphosphate, 79 cases). Patients were followed on a 1–3-month basis. Routine tests included physical examinations, prostate-specific antigen when it was available after 1989, chest X-rays, skeletal survey, computerized tomographic scan, and bone scintigraphy. At the time of study closure, 78 patients were still alive, 76 patients had died of prostate cancer, and 41 patients had died of unrelated cause. The mean observation period was 54.1 (1–210) months.

**Histological grading.** Specimens were fixed using a routine method and stained with H&E. One pathologist (H. Y.) who was unfamiliar with the clinical outcome evaluated the GS (1, 2).

**MNV.** Slides used for histological grading were used in the stereological calculation of MNV. All the equipment needed is a projector and a microscope. An Olympus BHS microscope (Olympus, Tokyo, Japan) with a projection attachment (Projection Mirror; BICO A/S, Glostrup, Denmark) with a ×100 oil immersion lens (numerical aperture 1.4) was used to obtain the quantitative histopathological variables. The final regularly calibrated magnification was ×1600. Histological sections from prostate cancer were projected onto the used test system. The three-dimensional nucleus are first sampled in proportion to height by the histological slide. These nuclei are then resampled in proportion to sectional area by a point-grid as shown in Fig. 1, and only nuclear profiles hit by points are sampled for the estimation of MNV, i.e., nuclei are sampled proportional to height × sectional area = volume (12). On the point-sampled nuclear profiles, nuclear intercepts were measured from nuclear boundary to nuclear boundary through the sampling point in one direction indicated by the test system (Fig. 1). An unbiased estimate of MNV was obtained by multiplying the mean of the cubed intercept length by \( \pi/3 \). A minimum of four visual fields and a minimum of 50 nuclei are needed to calculate MNV (12–14); thus, 4–12 fields of vision (average, 5.9) obtained at random and 53–143 intercepts (average, 75.6) were examined in each case. A detailed description of the techniques used, including formulas and calculations, has been published previously (5, 12–14). The evaluation of MNV can be performed in 10–15 min per case. All measurements were carried out in Shizuoka City Hospital by one author (K. F.) without knowledge of the clinical outcome.

**Statistics.** Disease-specific survival was estimated by the Kaplan-Meier plots. Differences between groups were tested with the log rank test. Multivariate regression analysis was performed by using the Cox proportional hazards model of SPSS (SPSS, Inc., Chicago, IL) to determine which variables were independently correlated with survival. Comparison between group means was carried out using Student’s \( t \) test. More than two groups were analyzed by one-way ANOVA. The limit of significance for all tests was \( P < 0.05 \).

**RESULTS**

Distributions of GS and clinical stage of all patients are summarized in Table 1. Only 13 cases had well-differentiated cases with a GS of 2–4, and one-half of patients had metastatic lesions (stage D) at the time of diagnosis. Clinical stage was well correlated with disease-specific survival (Fig. 2, \( P < 0.0001 \)). However, age at the time of diagnosis and the type of initial hormone therapy (castration versus diethylstilbestrol diphosphate versus castration + diethylstilbestrol diphosphate) had no prognostic value (data not shown).

**Disease-specific Survival of All Cases: Estimates of MNV versus GS.** MNV of all cases varied from 90.0 \( \mu \text{m}^3 \) to 677.2 \( \mu \text{m}^3 \) with a median of 248 \( \mu \text{m}^3 \). Estimates of MNV grouped for GS are shown in Fig. 3. Among the GS groups, a significant difference was observed with MNV, and MNV was higher in the GS 8–10 group (\( P < 0.0001 \)). Several workers have recently reported that a GS of 7 or higher should indicate a high-grade tumor (20). Even when the patients were divided into two groups (GS = 2–6 versus GS = 7–10), MNV was higher in the GS = 7–10 group (\( P < 0.0001 \)); however, there was a considerable amount of overlap between each group. No relationship was identified between MNV and clinical stage though MNV was higher in the stage D group (\( P = 0.0705 \)). When the arbitrary cutoff is set up at the median MNV, disease-specific survival curves are demonstrated as Fig. 5. The
Table 1 Distribution of GS and clinical stage

<table>
<thead>
<tr>
<th>GS</th>
<th>Clinical stage</th>
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<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>A</td>
<td>4</td>
</tr>
<tr>
<td>B</td>
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<tr>
<td>C</td>
<td>0</td>
</tr>
<tr>
<td>D</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>4</td>
</tr>
</tbody>
</table>

Fig. 2 Kaplan-Meier disease-specific survival functions for 195 patients treated for prostate cancer according to clinical stage.

Fig. 3 Estimates of MNV, obtained in histological sections of 195 patients, grouped for GS. Bars, grouped mean value.

Fig. 4 Estimates of MNV, obtained in histological sections of 195 patients, grouped for clinical stage. Bars, grouped mean value.

Fig. 5 Kaplan-Meier disease-specific survival functions for 195 patients treated for prostate cancer, according to estimates of MNV.

Disease-specific Survival According to MNV and GS in Clinically Localized Prostate Cancer (Stages A, B, and C). One hundred four patients were classified as a group with clinically localized prostate cancer. MNV in this group varied from 103.5 μm³ to 497.6 μm³ with a median of 245 μm³. Both MNV (P = 0.0058) and GS (P = 0.0256) were significantly correlated with disease-specific survival of clinically localized cases (Figs. 7 and 8, respectively). The prognosis for patients with MNV ≥245 μm³ was significantly worse than that for patients with MNV <245 μm³ (P = 0.0058). The estimates of the median disease-specific survival time were 7.80 years (95% confidence interval, 5.16-10.44) for patients with MNV ≥245 μm³ and 13.89 years (95% confidence interval, 10.83-14.05) for patients with MNV <245 μm³.

Disease-specific Survival According to MNV and GS in Stage D2 Prostate Cancer. Eighty-four patients had distant metastasis at the time of diagnosis. MNV of this group varied from 103.5 μm³ to 497.6 μm³ with a median of 245 μm³. Both MNV (P = 0.0058) and GS (P = 0.0256) were significantly correlated with disease-specific survival of clinically localized cases (Figs. 7 and 8, respectively). The prognosis for patients with MNV ≥245 μm³ was significantly worse than that for patients with MNV <245 μm³ (P = 0.0058). The estimates of the median disease-specific survival time were 7.80 years (95% confidence interval, 5.16-10.44) for patients with MNV ≥245 μm³ and 13.89 years (95% confidence interval, 10.83-14.05) for patients with MNV <245 μm³.

prognosis for patients with MNV ≥248 μm³ was significantly worse than that for patients with MNV <248 μm³ (P < 0.0001). The estimates of the median disease-specific survival time were 4.76 years (95% confidence interval, 3.47-6.05) for patients with MNV ≥248 μm³ and 11.27 years (95% confidence interval 6.41-16.13) for patients with MNV <248 μm³.

GS (2-4 versus 5-7 versus 8-10) was also significantly correlated with the prognosis (P = 0.0002; Fig. 6).
from 90.0 μm³ to 677.2 μm³ with a median of 250 μm³. Similar to the clinically localized prostate cancer, both MNV (P = 0.0199) and GS (P = 0.0283) were significantly correlated with disease-specific survival (Figs. 9 and 10, respectively). Prognosis for patients with MNV ≥250 μm³ was significantly worse than that for patients with MNV <250 μm³. The estimates of the median disease-specific survival time were 3.22 years (95% confident interval, 1.20–5.25) for patients with MNV ≥250 μm³ and 5.83 years (95% confident interval, 1.96–9.69) for patients with MNV <250 μm³.

**Multivariate Analysis.** The results of multivariate analysis, simultaneously quantifying the relationship between disease-specific survival time and a set of prognostic factors, are shown in Table 2. Estimates of MNV, GS, and clinical stage all proved to be significant independent prognosticators in disease-specific survival. A 2.39-fold increased risk of disease-specific death for the patients with MNV ≥248 μm³ was found when compared with that for patients with MNV <248 μm³.

Focusing on clinically localized cases (stages A, B, and C), multivariate analysis revealed that estimates of MNV was the only powerful prognosticator of prostate cancer (Table 2). MNV has much more significant prognostic value when it is used as a continuous variables (P = 0.0015) than when it is used with cutoff points. Conversely, no prognostic value of GS was evident, even when the patients were divided into two groups (GS = 2–6 versus GS = 7–10).

**DISCUSSION**

GS has been considered to be the most valuable criteria for predicting the prognosis for prostate cancer (1, 2). GS determines the degree of malignancy based on the histological archi-

Fig. 6 Kaplan-Meier disease-specific survival functions for 195 patients treated for prostate cancer according to GS.

Fig. 7 Kaplan-Meier disease-specific survival functions for 104 patients treated for clinically localized prostate cancer according to estimates of MNV.

Fig. 8 Kaplan-Meier disease-specific survival functions for 104 patients treated for clinically localized prostate cancer according to GS.

Fig. 9 Kaplan-Meier disease-specific survival functions for 84 patients treated for stage D2 prostate cancer according to estimates of MNV.

Fig. 10 Kaplan-Meier disease-specific survival functions for 84 patients treated for stage D2 prostate cancer according to GS.
The volume of the cancer nucleus has been reported to be closely correlated to the degree of malignancy in many other cancers (5-11). Although the histological grading of the WHO classification (21), Böcking et al. (22), and Gaeta et al. (23) take into consideration the volume of the cancer nucleus, none is as widely used as GS, meaning that there has been no method of histological grading to take the place of GS in predicting a prognosis for prostate cancer.

Since Weibel (24) discussed the application of stereology to cancer research, the impact of MNV as a prognostic factor has been demonstrated in several entities (5-12). In spite of the fact that prostate cancer is very common in the United States and Europe, few authors have reported the relationship between the prognosis of prostate cancer and MNV. Analyzing cases at Shizuoka City Hospital, we previously reported that MNV was a more useful prognosticator for prostate cancer than subjective histological grading (17, 18). By analyzing a larger number of subjects, using a Cox multivariate model, the present study confirmed that, in addition to the cell architecture on which GS is dependent, the volume of the nucleus is also an important prognosticator in predicting the prognosis of prostate cancer.

It is noteworthy that MNV was the only meaningful independent prognosticator for patients with clinically localized prostate cancer, whereas clinical stage and GS had no prognostic value in multivariate analysis (Table 2). Fortunately, through the simultaneous use of transrectal ultrasound-guided biopsy and prostate-specific antigen, which are other important prognostic factors for prostate cancer (25, 26), we have an effective means for the earlier diagnosis of prostate cancer, often at a small size and early clinical stage (27-29). However, a number of concerns still remain, such as the possibility that insignificant tumors might also be identified (30). At the present time, there is no consensus with regard to an acceptable and effective therapy for clinically localized prostate cancer. For example, most urologists in the United States believe that the anatomical radical prostatectomy is safe and is the most effective curative therapy against prostate cancer (31, 32); conversely, urologists from Scandinavian countries contend that radical prostatectomy should be considered experimental because in their well-selected series of patients treated with watchful waiting death rates at 10 years are low (33). In addition, there are no objective criteria to decide the optimal treatment for given patients. Although new parameters will be provided by molecular genetic approaches (34-36), the present study suggests the possibility that MNV determined on conventional histopathological specimens might be a useful criteria.

Several problems remain to be resolved before the practical application of the estimates of MNV. One problem is the possible bias originating from the artifact of sampling methods. To test this possibility, the same analysis was conducted on the 158 cases whose specimens were obtained by transrectal needle biopsy. The results of all 158 cases reveal that clinical stage (P = 0.0001), GS (P = 0.0056) and MNV (P = 0.0015) were significantly correlated with the prognosis. In the multivariate analysis of clinically localized cases (n = 79), MNV was determined to be the single parameter correlating with the prognosis (P = 0.0095). Another problem is concerning the cutoff point of MNV to determine the biological characteristics of prostate cancer. To elucidate these issues, further investigation on a larger number of cases at multiple institutions should be required.

The test would have to be performed at other institutions as the next step in the evaluation of MNV, which would indicate reproducibility for this organ site.

The present study suggests that estimates of MNV are prognostically equal or superior to morphological grading of malignancy, such as GS, in prostate cancer. In particular, MNV proved to be the only meaningful prognosticator for the patients with clinically localized prostate cancer. We emphasized that the estimates of MNV might be a useful method of objective histological grading for prostate cancer.

Table 2 Results of Cox multivariate analysis with regard to disease-specific survival of the 195 patients with prostate cancer

<table>
<thead>
<tr>
<th>Variables</th>
<th>B</th>
<th>P</th>
<th>Risk ratio</th>
<th>95% Confidence interval</th>
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<tbody>
<tr>
<td>All stage (n = 195)</td>
<td></td>
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<tr>
<td>GS (2-10)</td>
<td>0.6932</td>
<td>0.0021</td>
<td>2.0001</td>
<td>1.2849-3.1132</td>
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<tr>
<td>Clinical stage (A-D)</td>
<td>0.6073</td>
<td>0.0001</td>
<td>1.8355</td>
<td>1.3531-2.4899</td>
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<td>MNV (classified with median)</td>
<td>0.8698</td>
<td>0.0004</td>
<td>2.3865</td>
<td>1.4732-3.8600</td>
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<td>Clinically localized disease (n = 104)</td>
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<tr>
<td>GS (2-10)</td>
<td>0.5540</td>
<td>0.1156</td>
<td>1.7401</td>
<td>0.8728-3.4692</td>
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<td>Clinical stage (A-C)</td>
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<td>0.2599</td>
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<tr>
<td>MNV (classified with median)</td>
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<td>0.0423</td>
<td>2.3159</td>
<td>1.0297-5.2088</td>
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<td>Stage D2 disease (n = 84)</td>
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<tr>
<td>GS (2-10)</td>
<td>0.8235</td>
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<td>2.2786</td>
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<td>MNV (classified with median)</td>
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<td>2.2249</td>
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</table>

ACKNOWLEDGMENTS

We thank Dr. Sørensen, Stereological Research Laboratory, and University Institute of Pathology, Aarhus Kommunehospital, University of Aarhus, Denmark, for technical assistance.

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*Clin Cancer Res* 1997;3:613-618.

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