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# Nuclear Grading Versus Gleason Grading in Small Samples Containing Prostate Cancer: A Tissue Microarray Study

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**Abstract** In this study we addressed the question whether nuclear grading in very small samples of prostate cancer would provide additional prognostic information as compared to Gleason grading. Therefore, a tissue microarray (TMA) was constructed comprising a total number of 3,261 prostate cancers. Blinded for all clinical and pathological data, the TMA spots (diameter 0.6 mm) containing cancer were graded with two systems: First, for nuclear features according to a modified Fuhrman grading system, and second, by using a simplified Gleason system. The results were compared with tumour stage, tumour grade and follow-up data. Although nuclear grading could easily be performed on the TMA spots, no correlation was found with tumour stage, grade or PSA recurrence after prostatectomy. However, Gleason grading, even when performed on the small TMA spots, provided significant prognostic information. Correlation with Gleason scores determined in the complete prostatectomy specimens showed moderate agreement in low-grade (score≤6) or intermediate (score= 7) tumours, but poor agreement with high-grade (score≥8) tumours. In conclusion, the Fuhrman grading of prostate cancer does not appear to be of any prognostic importance so the Gleason grading remains the system of choice, even in tumour specimens smaller than 1 mm.

**Keywords** Prostate cancer · Gleason grading · Fuhrman nuclear grading · Prognosis · TMA

**Abbreviations**

| | |
|---|---|
| PSA | prostate-specific antigen |
| TMA | tissue microarray |
| H&E | hematoxylin and eosin |

## Introduction

Prostate cancer is the most common of all malignant tumours of men beyond the age of 70. This cancer shows great variation in biological behaviour and also in the histological grade of differentiation. Thus, in order to take up a prognostic evaluation, the Gleason grading is the mostly used grading system. The Gleason grading is only based on histological growth patterns of the tumour glands and totally disregards cytological features [1–3]. Consistently, it has been confirmed as a predictor for final pathological stage (TNM classification) and multiple prognosis factors [4–9]. Additionally, the Gleason grade has been shown to correlate with tumour volume, extraprostatic extension and lymph node metastasis [10–13]. However, in very small specimens, such as in prostate needle biopsies with minute amounts of cancer, pathologists are often reluctant in assigning a Gleason score, because the growth pattern can hardly be determined.

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Fatefully, undergrading of prostate cancer in needle biopsies occurs more frequently than overgrading (33–45% vs. 4–32%) depending on tumour volume included and grade of tumour differentiation [9]. Therefore, among uropathologists the reliability, accuracy and predictability of the Gleason grading within needle biopsies is an ongoing and unresolved debate.

Within the last decades also nuclear features of prostate adenocarcinoma have been incorporated in several grading systems. In such systems nuclei are graded based on their size, shape, chromatin distribution, mitoses and nucleoli in order to optimize adjuvant therapy by a more accurate prediction of the likelihood for disease recurrence [14–18].

However, there are only few studies that investigated the predictive potential of nuclear features in very small samples of prostate cancer. We chose the tissue microarray (TMA) format to address this question, because it provides an excellent means to assess a very large cohort of homogeneously treated patients and guarantees maximal experimental standardization concerning cutting, fixation and H&E staining of the specimens. Furthermore, the evaluation of a TMA can be performed in a blinded fashion with regard to stage, tumour size and clinical data including post-operative follow-up. With respect to the growing importance of nuclear features for prognosis of prostate cancer, we aimed on the comparison of the Gleason grading with an established nuclear grading system. Therefore, we chose the nuclear grading by Fuhrman et al. (1982) [19], originally developed for renal cell carcinoma and applied a modified version. To our knowledge, this is the first study which investigated a nuclear grading of prostate cancer in a TMA format.

## Material and Methods

### Patients and Tissues

An anonymous list of all radical prostatectomies performed between 1994 and 2002 at the Department of Urology, University Medical Center Hamburg Eppendorf, was constructed (Table 1). The list included pre-operative PSA values, clinical and pathological TNM-classification, Gleason grades of prostatectomy specimens, tumour location and tumor volumes, margin status of the prostatectomy specimens and post-operative follow-up of PSA values whenever available. All patients had been scheduled for an annual follow-up visit at the institutional outpatient clinic. Information about external post-treatment prostate-specific antigen (PSA) testing (usually done every 6 months) had been added to the institutional database. Follow-up data were available for 2,385 patients, ranging from 1 to 144 months (mean, 34 months). PSA recurrence was defined as persisting or rising postoperative PSA values >0.1 ng/ml in two separate measurements.

**Table 1** Clinical and histopathological data of 3,261* prostate cancer patients undergoing radical prostatectomy at a single institution

| Characteristics | No. of patients |
|---|---|
| Age (years) | |
| <50 | 83 (3%) |
| 50–60 | 998 (33%) |
| 60–70 | 1,807 (59%) |
| >70 | 175 (6%) |
| Preoperative PSA (ng/ml) | |
| <4 | 513 (17%) |
| 4–10 | 1,673 (55%) |
| 10–20 | 641 (21%) |
| >20 | 225 (7%) |
| pT classification | |
| pT2 | 2,080 (67%) |
| pT3a | 609 (20%) |
| pT3b | 372 (12%) |
| pT4 | 42 (1%) |
| Pathologic lymph node status | |
| pN0 | 1,544 (50%) |
| pN1 | 96 (3%) |
| pNx | 1,457 (47%) |
| Gleason score (prostatectomy) | |
| ≤3+3 | 1,426 (46%) |
| 3+4 | 1,311 (42%) |
| 4+3 | 313 (10%) |
| ≥4+4 | 55 (2%) |
| Surgical margin status | |
| negative | 2,475 (80%) |
| positive | 627 (20%) |
| Tumor location | |
| transitional zone | 402 (19%) |
| peripheral zone | 1,704 (81%) |

*Numbers do not always add up to 3,261 in the different categories because of cases with missing data

At the Department of Pathology, the Hematoxylin and Eosin (H&E) stained histological sections of 3,261 prostatectomy specimens were reviewed and the index tumours, as defined by the largest tumour focus and/or the focus with the worst Gleason grade, were marked on the slides. The corresponding paraffin blocks were retrieved from the archive. With the help of the marked histological sections, a 0.6 mm thick tissue core was punched out from the index tumours of each case, and then transferred into a tissue microarray (TMA) format as described previously [20]. From the resulting seven TMA paraffin blocks, serial histological sections were taken. The first set of sections was routinely H&E stained. A further set of sections was then used for immunohistochemistry against high molecular weight cytokeratins (34βE12), which labels basal cells in normal prostate glands, and

against PSA. For internal controls, each TMA block also contained different kinds of normal and tumour tissues. This TMA had been successfully used for previous studies on prognostic factors [21–23].

H&E sections were taken from all TMA blocks and analysed for both, a novel nuclear grade for prostate cancer modified after Fuhrman et al. (1982) [19] and the Gleason grade. All gradings were executed by one pathologist (D. W.), blinded for all pathological and clinical data.

## Nuclear Grading for Prostate Cancer Modified After Fuhrman

Based on the well established nuclear grading used for renal cell carcinoma by Fuhrman et al. (1982) [19], we defined the following criteria:

Nuclear grade 1: Tumour cell nuclei were small (approximately 10 μm) and composed of dense chromatin with inconspicuous or absent nucleoli (Fig. 1a).

Nuclear grade 2: Tumour cells had larger nuclei (approximately 15 μm) which exhibited irregularities in chromatin structure and nucleoli seen under high power (400×) (Fig. 1b).

Nuclear grade 3: Tumour cells contain even larger nuclei (approximately 20 μm) with an obviously irregular distribution of chromatin and prominent large nucleoli even at low power (100×) (Fig. 1c).

A Fuhrman nuclear grade 4 obviously does not exist in prostate cancer. Each tumour was graded by the most malignant or highest grade exhibited even if only focal.

## Gleason Grading of TMA Spots

Gleason score was determined by using the instructions originally described and developed by Donald F. Gleason [1–3]. Following international recommendations of grading prostate cancer in needle biopsies [9, 24, 25], Gleason grades 1 and 2 were not given. Furthermore, it was not differentiated between Gleason grades 3+4 and 4+3 because of the very small diameters of the TMA spots (0.6 mm). Thus, for final evaluation these gradings were assembled in one group of Gleason score=7. For the same reason, patterns with Gleason score≥8 were assembled in one group as well.

## Statistics

Statistical calculations were performed with Graph Pad Prism 2.01. Contingency tables were calculated with the $X^2$

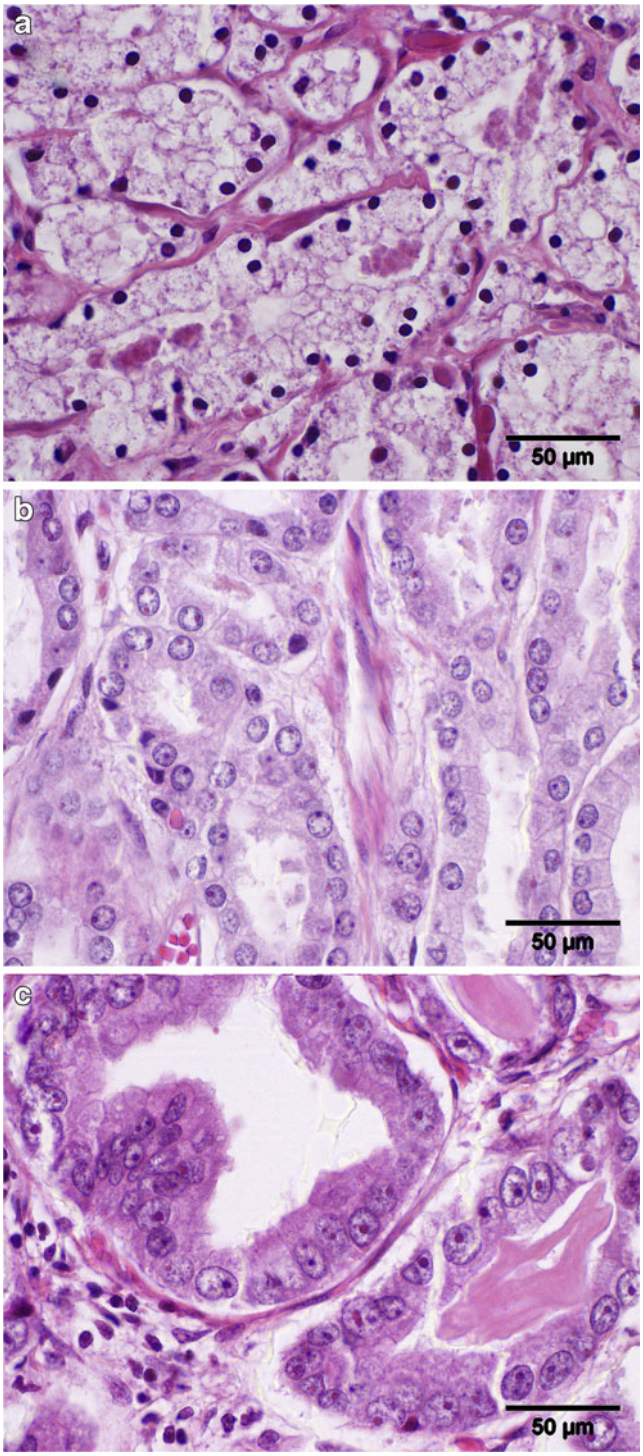


**Fig. 1** Nuclear grades 1–3 were defined modified after Fuhrman et al. (1982): **a**) Fuhrman nuclear grade 1: Tumour cell nuclei were small (approximately 10 μm) and composed of dense chromatin with inconspicuous or absent nucleoli **b**) Fuhrman nuclear grade 2: Tumour cells had larger nuclei (approximately 15 μm) which exhibited irregularities in chromatin structure and nucleoli seen under high power (400×) **c**) Fuhrman nuclear grade 3: Tumour cells contain even larger nuclei (approximately 20 μm) with an obviously irregular distribution of chromatin and prominent large nucleoli even at low power. Magnification of all pictures presented: 400×

test. Survival curves were calculated by the Kaplan-Meier method and compared with the Logrank test.

## Results

### Data Obtained from TMA Grading

Definite prostate cancer was present in 2,556 of 3,261 arrayed tissue samples. Noninformative cases were caused by missing spots on the tissue microarray or absence of definite invasive cancer tissue. The latter contained normal prostatic tissue, high-grade prostatic intraepithelial neoplasia or stromal tissue only (Table 2).

Blinded for all clinical and pathological data, 2,510 of the 2,556 TMA spots were graded after Fuhrman and subdivided into the three different nuclear grades described above. Fuhrman nuclear grade 1 was given only in 4% ($n$=88) of the cases, whereas Fuhrman nuclear grade 2 was present in 44% ($n$=1,116) and Fuhrman nuclear grade 3 in 52% ($n$=1,306). In contrast to the Fuhrman grading for renal cell carcinoma, a Fuhrman nuclear grade 4 obviously does not exist in prostate cancer.

Within the same cohort of TMA spots containing prostate cancer and again blinded for all clinical and pathological data, 1,912 of the 2,556 tissue samples were graded by using a simplified Gleason system as described above. Gleason score≤6 was given in nearly half of the cases (54%, $n$=1,028). Gleason grades 3+4 and 4+3 were assembled in one group of Gleason score=7 and appeared in 29% ($n$=545). Only 18% ($n$=18) showed a histomorphological pattern corresponding to a Gleason score ≥8.

### Fuhrman Grading of Prostate Cancer in a TMA does not Reveal any Correlation to pT Tumour Stage or Disease Recurrence

Based on follow-up data from 2,385 patients, the data obtained from TMA Fuhrman grading were analysed with regard to PSA recurrence and pT tumour stage.

However, neither the correlation of Fuhrman grading to PSA recurrence free survival nor to pT tumour stage reveals any prognostic information. Independent of the Fuhrman nuclear grading score all the survival curves are nearly at the same range and do not show differences of any significance ($p$=0.5968; Fig. 2a). Comparison of the Fuhrman nuclear grades with pT tumour stages does not reveal any significant correlation either ($p$=0.1796; Table 3).

### High Prognostic Relevance Is Presented by Gleason Grading Even in the Small Tumour Samples of the TMA Examined

As well as in the analyses of the TMA Fuhrman grades, the TMA Gleason scores were correlated to PSA recurrence and pT tumour stage extracted from the follow-up data of 2,385 patients.

In contrast to the TMA Fuhrman grading, in the TMA Gleason grading all associations expected were found at high levels of statistical significance. The severity of the Gleason score determined in the TMA is significantly

**Table 2** Data obtained from TMA grading. Blinded for all clinical and pathological data, the TMA spots (diameter 0.6 mm) containing prostate cancer were graded with both, a nuclear grading system modified after Fuhrman et al. (1982) and a simplified Gleason grading system. Correlation with Gleason scores determined on the complete prostatectomy specimens shows a moderate agreement in low grade (Gleason score ≤6) or intermediate (Gleason score = 7) tumours, but a poor agreement with high grade (Gleason score ≥8) tumours

| Characteristics | No. of patients |
|---|---|
| Cohort considered | |
| TMA spots, H&E stained | 3,261 |
| TMA spots with cancer | 2,556 |
| TMA Fuhrman grading | |
| TMA spots, Fuhrman graded | 2,510 |
| Fuhrman nuclear grade 1 | 88 (4%) |
| Fuhrman nuclear grade 2 | 1,116 (44%) |
| Fuhrman nuclear grade 3 | 1,306 (52%) |
| TMA Gleason grading | |
| TMA spots, Gleason graded | 1,912 |
| Gleason ≤6 | 1,028 (54%) |
| Gleason = 7 | 545 (29%) |
| Gleason ≥8 | 339 (18%) |
| Concordance of the Gleason scores determined in TMA and prostatectomy | |
| Gleason ≤6 | 561 (55%) |
| Gleason = 7 | 318 (59%) |
| Gleason ≥8 | 23 (7%) |

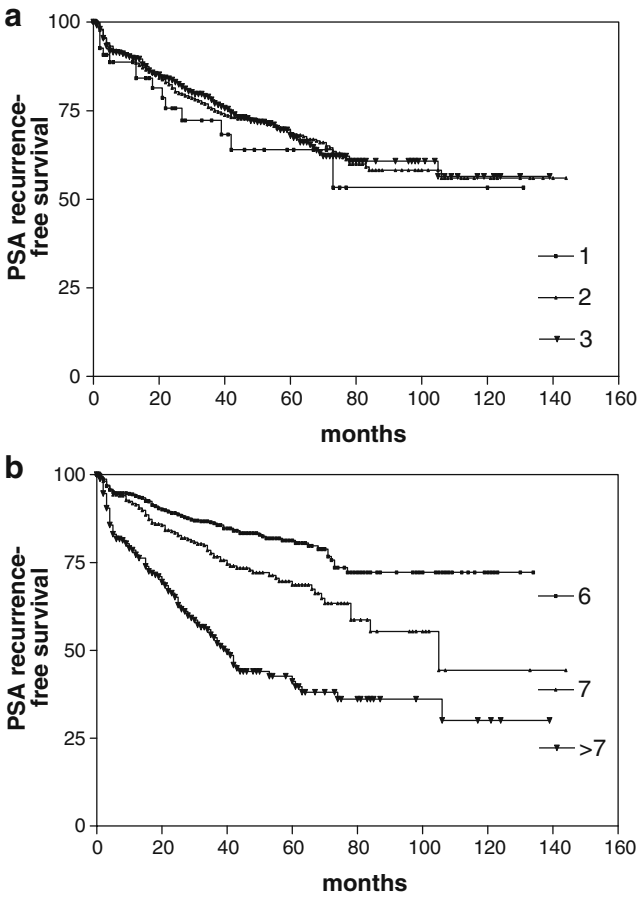


**Fig. 2** Correlation of PSA recurrence with Fuhrman grade (**a**) and Gleason score (**b**) determined in a TMA. Fuhrman grading does not show any correlation ($p$=0.5968) whereas the Gleason grading is significantly related to PSA recurrence ($p$<0.0001)

related to the PSA recurrence after prostatectomy ($p$< 0.0001; Fig. 2b). Additionally, the correlation between the TMA Gleason score and the pT tumour stage reveals differences of high significance ($p$<0.0001; Table 3). The number of patients with prostate cancer restricted to organ (pT2 stage) decreases with increasing Gleason score (78% with Gleason≤6 versus 40% with Gleason≥8), whereas patients with a tumour stage ≥ pT3 (tumour is penetrating the organ capsule) tend to have a higher Gleason score (22% with Gleason≤6 versus 60% with Gleason≥8).

### Concordance of the Gleason Scores Determined in TMA and Prostatectomy

The correlation of the Gleason scores determined in the complete prostatectomy specimens with the TMA Gleason scores showed different agreements in grade severity, respectively (Table 2). Low and intermediate grade cancers of Gleason score≤6 as well as Gleason score=7 reveal a concordance of more than 50%. However, only 7% of high grade cancers on the TMA represented by Gleason score≥ 8 agree with those in the prostatectomy.

## Discussion

In this study we compared two different grading systems for their usefulness in very small tissue spots containing prostate cancer. The grading of malignant tumours is one of the most important tools for the pathologist to give prognostic information to the treating doctors on the ward. The grading system developed by Donald F. Gleason [1–3] represents the mostly used grading system for prostate cancer due to its reliability and reproducibility. However, in very small specimens, such as in prostate needle biopsies with minute amounts of cancer, the Gleason score is often difficult to determine. To many pathologists, the size of the tissue sample appears too small to evaluate the histological growth pattern, which is crucial for correct Gleason grading.

Another approach to obtain predictive information even from very small samples of prostate cancer is seen in considering nuclear features. In our study, we chose a TMA format because it provided the assessment of a very large cohort of homogeneously treated patients, it guaranteed the maximal experimental standardisation and, moreover, it allowed working in a blinded fashion concerning all clinical and pathological data.

However, the TMA specimens investigated only ensures a restricted representativeness due to the very small spot size of only 0.6 mm in diameter. Therefore, overgrading of Gleason scores in a TMA appeared to be very likely because the tissue cores for constructing the TMA were punched out from the index tumours, which often had to be those with the worst Gleason grade. This could explain the poor concordance of

**Table 3** Correlation between Grading system[a] and tumour stage. TMA Fuhrman grading and pT tumour stage does not show any significance ($p$=0.1796), whereas the correlation between TMA Gleason grading and pT tumour stage is shown to be highly significant ($p$<0.0001)

| Grading system | pT2 | pT3 or pT4 |
|---|---|---|
| TMA Fuhrman grading | | |
| Nuclear grade 1 | 45 (54%) | 38 (46%) |
| Nuclear grade 2 | 689 (64%) | 383 (36%) |
| Nuclear grade 3 | 787 (64%) | 442 (36%) |
| TMA Gleason grading | | |
| Gleason ≤6 | 756 (78%) | 218 (22%) |
| Gleason = 7 | 323 (62%) | 194 (38%) |
| Gleason ≥8 | 128 (40%) | 189 (60%) |

[a] Numbers do not always add up to 2510 (TMA Fuhrman grading) and 1912 (TMA Gleason grading) because of cases with missing data

7% between the Gleason scores of TMA and prostatectomy in high grade cancers with Gleason scores≥8.

Furthermore, this study shows that the Fuhrman nuclear grading applied on prostate cancer does not reveal any correlation to pT tumour stage or disease recurrence. However, several groups demonstrated that nuclear morphometry partially supported by computer technology appears to be objective and reproducible [26–29]. Interestingly, recent reports indicate a patient-specific quantitative nuclear grade (QNG) to be even more accurate in predicting PSA recurrence compared to routine pathology information alone [17, 18]. However, as demonstrated by Zhou et al. (2001) [16], those cytological criteria depend on artefacts due to fixation and staining. Moreover, those methods do not seem to be readily applicable in the daily routine. Thus, the prognostic significance of nuclear grading systems in prostate cancer still remains controversial and, accordingly, the College of American Pathologists only recommends the Gleason system for histological grading of prostate cancer [8].

Concluding, in this study we presented that Gleason grading provides significant prognostic information even if performed on TMA cancer spots of only 0.6 mm in diameter. The Fuhrman grading of prostate cancer obviously does not appear to be of any prognostic importance, hence, the Gleason grading remains the system of choice for prostate cancer, even in tumour specimens smaller than 1 mm.

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