Pathol. Oncol. Res. (2012) 18:615–621
DOI 10.1007/s12253-011-9484-6

RESEARCH

Improving the Reproducibility of the Gleason Scores in Small Foci of Prostate Cancer - Suggestion of Diagnostic Criteria for Glandular Fusion

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Received: 26 September 2011 / Accepted: 23 November 2011 / Published online: 17 December 2011

Abstract High upgrading rates of Gleason score 6 to 7 carcinomas between biopsy and radical prostatectomy specimens may be produced by change of fused glands of pattern 3 to pattern 4. Therefore, inter-observer reproducibility of fused and non-fused glands in biopsy specimens was analysed. Images of H&E stained slides of glands of carcinomas with Gleason score 6 and 7 (3+4) with and without glandular fusions with different lens magnification were analysed by 4 specialized genitourinary pathologists and 3 non-specialized pathologists. The definition of glandular fusion was a complete lack of any stromal fibres between a minimum of two glands and only one line of nuclei within the area of fusion. Overall agreement and inter-observer reproducibility of fused versus non-fused glands of non- and uro-pathologically specialized pathologists were lower in lens magnification of 50× in contrast to 200×. The inter-observer reproducibility of fused glands by specialized observer was higher than that of non-specialized pathologists. The results support the importance of strict but practicable criteria for the diagnosis of fused tumor glands in order to decrease the interobserver variability of Gleason scores, particularly in non-specialised pathologists.

Keywords Prostate carcinoma · Glandular fusion · Inter-observer reproducibility

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Introduction

The modified Gleason grading system of prostate cancer that was introduced by the International Society of Urological Pathology (ISUP) and accepted by the World Health Organisation (WHO) has been in use since 2004 for both diagnostic and scientific purposes.

As opposed to the old Gleason grading system, poorly formed, cribriform and very small complexes of fused glands are currently scored as Gleason pattern 4, instead of Gleason pattern 3 [1].

This change has led to a shift towards diagnosing Gleason score 7 (3+4=7a) more frequently at the expense of Gleason score 6 [2–5]. Thus, an increasing number of patients, now diagnosed with a Gleason score 7, qualify for lymphadenectomy.

In histopathological diagnostics this effect hinges on glandular pattern 4, which is characterized by poorly formed, cribriform and fused glands.

Cribriform structures or poorly formed glands are readily detectable whereas recognition of fused glands can be difficult and thus influences the frequency distributions of Gleason scores 6 and 7 (especially 3+4=7a) [2–6]. Following the original introduction of his grading system, Gleason himself mentioned fused glands of the hypernephroid type as pattern 4 in 1974 [7–12]. This finding of large areas of fused clear cell glands can be

found in the illustrations of virtually all text books and corresponding uro-pathological reports.

A problem frequently encountered with core needle biopsy technique is that very small foci of carcinoma are frequently captured and diagnosed. Those foci often only consist of two to four glands. This microfocal aspect has so far barely been examined on conventional core needle biopsy specimens (as opposed to tissue microarray (TMA)).

Gleason has not enclosed a definition of gland fusion in his work [7, 8, 12]. Definitions of glandular fusion have later been postulated by Bostwick (1994) and Mostofi (2002). Both agree that fused glands are defined by a lack of intervening stroma. The first defined glands as fused, if no connective tissue stroma is detectable between multiple (>=2) glands [13, 14], whereas the latter demanded tightly packed glands without intermediate stroma as the defining criterion [15].

The WHO classification of uro-genital tumors (2004) describes the Gleason pattern 4 as follows: "The glands appear fused, cribriform or they may be poorly defined. Fused glands are composed of a group of glands that are no longer completely separated by stroma. The edge of a group of fused glands is scalloped and there are occasionally thin strands of connective tissue within this group" [16]. Glands that are cut tangentially or that are shaped like a V, Y, or 8 should not be counted as true gland fusion.

According to Bonkhoff's definition no gland fusion is present if a virtual line can be drawn around each individual gland and therefore qualifies as Gleason pattern 3 [17]. The assessment of gland fusion is especially difficult in microfocal carcinomas and it is common practice to ask for a second opinion in these cases. This is particularly prominent in the consultancy practice during histological reevaluation of prostate carcinomas.

Accurately differentiating Gleason pattern 3 (lacking gland fusion) from Gleason pattern 4 (with gland fusion) and thereby differentiating Gleason score 3+3=6 from Gleason score 3+4=7a is also of great importance in the ongoing debate on insignificant prostate cancer [6, 18, 19].

In this study, we illustrate the criteria for gland fusion with pictures of small prostate cancer specimens and compare the reproducibility of gland fusion (yes vs. no) as assessed by specialized genito-urinary (GU) pathologists and non-specialized general pathologists. Furthermore, we reevaluated cases that were submitted for second opinion and discuss the value and validity of Gleason score 3+3=6 and 3+4=7a in those cases of microfocal cancer.

Methods

The specimens used were prostate punch biopsies from daily practice, containing predominantly microfocal adenocarcinomas (n=268). Sections of 3 μm thickness were cut and stained with haematoxylin-eosin. An additional test with a staining after van Gieson demonstrates no better results. Using both 50× and 100× magnification, the Gleason grading system was then applied according to the ISUP 2004 criteria [1]. Gleason pattern 3 was assigned to carcinoma glands without any signs of fusion.

Glands of Gleason pattern 4 were additionally analyzed in 200× magnification regarding their extent of glandular fusion.

Criteria of fusion were defined as follows: no stromal connective tissue strands or bridges and only a single line of nuclei or remnants thereof and hence no traceable virtual line between at least two glands, at least two distinct and diverse gland lumina.

Confluence of lumina alone is not a fusion criterion, as this may be mimicked by a three-dimensional branching of glands in tangential sections. Branching glands, i.e. gland configurations in shapes of Vs,Ys or 8 s were excluded.

The gold standard was defined by an internationally recognized high volume uro-pathologist specialized on prostate cancer who took part in the modification of the Gleason grading system by the International Society of Urological Pathology (ISUP) and who has been collaborating with the Mostofi group since 1980–2002 [1, 15].

Essentially the carcinomas had Gleason scores 3+3=6 and 3+4=7a with a predominant Gleason pattern 3 and small areas of pattern 4 without cribriform parts [3, 6].

Digital pictures (Zeiss) were taken of the carcinomas in all magnifications mentioned above. A recent study has shown good intra- and inter-observer variability comparing digital imaging and standard light microscopy in the evaluation of prostate cancer in biopsy specimens [20].

Areas chosen for photography showed no cribriform or poorly formed glands, so that only round glandular structures were available for analysis.

Seven pathologists participated in this study. Among these, four are specialized uro-pathologists (two in-house and two from other institutions) and three are pathologists without any particular uro-pathological background.

268 anonymous pictures of prostate cancer glands were then analyzed regarding gland fusion (yes vs. no) by all participating pathologists.

The results were then compared, establishing values for overall agreement, inter- and intra-observer reproducibility for all participants and for the specialized versus non-specialized group respectively with a free marginal Kappa Test according to Randolph (2005) and Brennan and Prediger (1981). Kappa values greater than 0.7 were regarded as adequate consensus [21, 22].

Statistical validity was backed with a Chi-square test, p-values smaller than 0.05 were regarded as significant.

An additional set of 252 prostate cancer biopsy specimens of a total of 1,800 cases with Gleason scores 6 and 7 that was pre-graded by external pathologists and had been submitted for reference pathology (2008–2009) were re-evaluated.

Results

Glandular Fusion

Along with unambiguous cases with fused glands (pattern 4) and non-fused glands (pattern3), there were also borderline cases that did not show definite intermediate stromal components between the glands on 50× magnification. Certain fusion or non-fusion was only detectable on higher magnification (100× or more frequently 200×) in those cases (Figs. 1 and 2).

Furthermore there were also tangentially cut glands that did not allow certain classification (Table 3), as well as expanded glands in shapes of Vs, Ys, and 8 s (pseudo-fusion). Glands lacking fusion showed an unequivocal separation by delineating stroma (Fig. 1). Cribriform or poorly formed glands were not detected in the pictures.

The individual results of all seven pathologists for all 268 pictures are the following.

The overall agreement in all participants was 0.71 for 50×, 0.76 for 100× and 0.81 for 200× magnification respectively. The inter-observer reproducibility (free marginal Kappa value) was 0.48 / 0.51 / 0.61 (50×, 100× and 200× magnification). In the specialized group, overall agreement was 0.88/ 0.91 / 0.94 (50×,100× and 200× magnification) and inter-observer reproducibility was 0.77/ 0.82 / 0.87 (50×,100× and 200× magnification). Comparison of the non-specialized group to the specialized group, overall agreement of 0.67 / 0.68 / 0.74 (50×,100× and 200× magnification) and inter-observer reproducibility of 0.34 / 0.35 / 0.48 (50×,100× and 200× magnification) were found (Table 1).

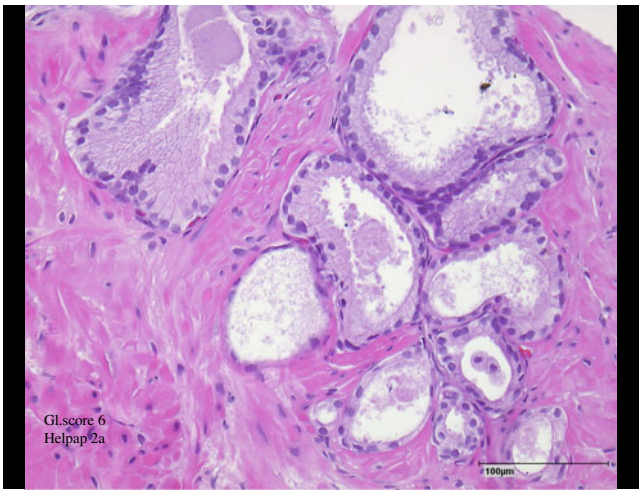

Fig. 1 Prostate carcinoma, Gleason pattern 3 with very thin connective tissue fibres between the glands. No glandular fusion, Gleason score 6. H&E 10×

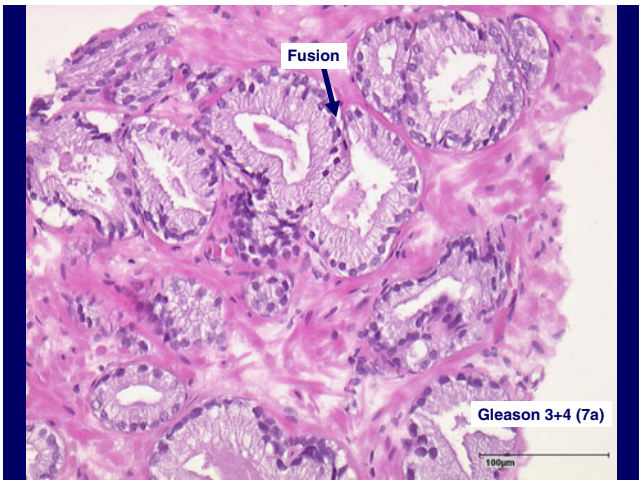

Fig. 2 Prostate carcinoma, Gleason score 3+4=7a with distinct glandular fusion. H&E 10×

A second assessment round in the non-specialized group after 6 months yielded higher kappa values for inter-observer reproducibility and also adequate inter-observer agreement between the two groups (Table 1).

Intra-observer reproducibility of all participants (specialized uro-pathologists and non-specialized pathologists) was excellent for all three magnifications used, yielding kappa values of 0.909 / 0.935 / 0.926 (50×,100× and 200× magnification) and free marginal kappa values of 0.819 / 0.870 / 0.852 (50×,100× and 200× magnification) (Table 2).

The difference of the kappa values between the specialized and the non-specialized group was highly significant ($p<0.0001$). The difference between the 50× and 100× magnification failed significance ($p=0.071$), whereas the difference between 100× and 200× magnification was significant ($p=0.015$). The percent distribution of the glandular findings "uncertain, no fusion versus fusion" is demonstrated in Table 3. The differences of the values between the specialized and non-specialized group was significant ($p<0.00117$). There were no significant differences between both observer groups ($p=0.352$) after reevaluation by general pathologists.

Gleason Patterns and Gleason Score

We re-evaluated the presence of glandular fusion in 252 consultancy cases.

Mostly, these cases harboured only minute carcinoma infiltrates consisting of 3 to 4 glands and that had been sent in either as malignant or suspicious for malignancy. Following consult pathology, the suspect cases were nearly exclusively designated as pattern 3/Gleason score 6 carcinomas.

Table 1 Inter-observer reproducibility of fused glands of prostate carcinoma of Gleason score 6 and 7a

Lens magnification a	overall agreement	fixed marginal kappa	free marginal kappa
50×			
Uropathologist	X 0.884	X 0.727	X 0.771
Uropath. / non Uropath.	X 0.668	X 0.350	X 0.336
Reevaluation Uropath/ non Uropath.	X 0.802	X 0.548	X 0.604
100×			
Uropathologist	X 0.910	X 0.792	X 0.820
Uropath./ non Uropath.	X 0.677	X 0.197	X 0.351
Reevaluation Uropath/ non Uropath.	X 0.793	X 0.470	X 0.593
200×			
Uropathologist	X 0.937	X 0.843	X 0.874
Uropath/non Uropath.	X 0.741	X 0.276	X 0.483
Reevaluation Uropath/ non Uropath.	X 0.865	X 0.677	X 0.743

However, also carcinomas with higher Gleason scores were commonly observed as minute foci. The data regarding the relationship of primary and consult pathology is given in Table 4. All cases with Gleason scores 3 und 4 were upgraded to scores 5 and 6. Gleason scores 5 were upgraded to GS 6 in over 70% of cases. Glandular fusion played no role in this respect, decisive were size and shape of the glands for upgrading (Fig.1). Gleason score 6 showed a higher degree of variation: identical grades were assigned in 62.5% of cases (p=< 0.001), 2.8% of cases were downgraded to GS 5, 33.3% cases were upgraded to GS 3+4=7 (=GS7a) and 1.2% were upgraded to GS 4+3=7 (=GS7b) and GS 8. In all cases of the latter, fused areas could be demonstrated that had escaped the attention of the primary pathologist. At low power these small and often tightly packed acini resemble the classical Gleason 3 pattern. A closer look at higher magnification allows to discriminate the lack of stromal bridges, which define fusion and that had apparently been missed at first evaluation. For cases with GS7a, the rate of concordance between external primary pathologists and expert uro-pathologists was 90.6%. The pattern 4 contained fusion, but no cribriform structures. In approximately 10% of cases, the Gleason pattern 4 (fusion) predominated (>50%), leading to a GS of 4+3=7b. In higher grade carcinomas (GS = >7b), the percent variations of concordance rates (7b, 8, 9=100, 87.6, 100%) can be attributed to the distribution patterns of fused and cribriform glands or solid tumor areas.

Table 2 Intra-observer reproducibility of fused glands in prostate carcinomas of Gleason score 6 and 7

All observer Lens magnification	overall agreement kappa	fixed marginal kappa	free marginal kappa
50×	X 0.909	X 0.935	X 0.819
100×	X 0.935	X 0.818	X 0.870
200×	X 0.926	X 0.776	X 0.852

Discussion

This study demonstrates a significantly higher rate of inter-observer reproducibility concerning glandular fusion among specialized genitourinary pathologists in comparison to general pathologists. Additionally, after a hermeneutic evaluation process, we achieved excellent kappa values also for intra-observer reproducibility with the definition of glandular fusion used in this study.

The differences in inter-observer reproducibility were very small, even in hands of non-expert genitourinary pathologists and nearly reached concordance rates typical of experts. This can be attributed to strict adherence to the criteria of glandular fusion described here and continuous training. However, these criteria may not be successfully applicable in every instance. Tangential sectioning, the difficulties to demonstrate collagenous fibers between glands as well as diverse configurations of glandular lumina can constitute relevant diagnostic problems. It also became apparent, that low power magnification yielded significantly worse results than higher (200x) magnifications. This observation has already been noted by Allsbrook and colleagues who also reported these difficulties of low power

Table 3 Distribution (%) of glandular fusion (uncertain finding, no fusion and fusion) in prostate carcinoma with Gleason score 6 and 7a after histological analysis by specialized uropathologists and not specialized general pathologists

Observer	diagnosis uncertain	no fusion	fusion
Uropathologist	7.0	19.2	73.8
General pathologist (p < 0.00117)	33.0	10.7	56.3
General pathologist Reevaluation (p=0.352)	18.9	14.4	66.9

Table 4 Gleason scores of 252 external (a) diagnosed and (b) re-evaluated prostate carcinomas. (2008/2009)

Cases after consultation (b)										
Gleason score	3	4	5	6	7a	7b	8	9	n	%
External cases (a)										
3	0	0	3	2	0	0	0	0	5	2.0
4	0	0	0	3	0	0	0	0	3	1.2
5	0	0	4 (18.2%)	16 (72.7%)	2 (9.1%)*	0	0	0	22	8.7
6	0	0	4 (2.8%)	90 (62.5%)	48 (33.3%)	1	1	0	144 p=<0.001	57.1
7a (3+4)	0	0	0	0	39	4 (90.6%)	0 (9.4%)	0	43	17.4
7b (4+3)	0	0	0	0	0	14 (100%)	0	0	14	5.6
8	0	0	0	0	0	5 (31.3%)	9 (56.3%)	2	16	6.3
9	0	0	0	0	0	0	0	5	5	2.0
n	0	0	11	111	89	24	10	7	252	
%	0	0	6.0	41.9	33.3	11.1	5.1	2.6		

magnification, although the use of these is explicitly recommended or even demanded for correct Gleason grading [23, 24]. Several studies on Gleason grading have been conducted by researchers in the United Kingdom (UK) or Italy that also involved expert GU pathologists [25, 26]. These confirmed, that specialized pathologists had more accurate results [2, 23, 24, 27, 28].

Very recently, a tissue microarray (TMA) based survey allowed to analyse the reproducibility of Gleason grading as well as combinations of Gleason grades with nuclear grading according to Fuhrman [29]. The intra-observer consistency for specialized and non-specialized pathologists demonstrated good to excellent kappa values of 0.65 and 0.73 respectively. The inter-observer values changed between excellent, good and moderate. The evaluated criteria were the glandular patterns of Gleason or their modifications [20, 30]. This becomes also apparent in the analysis of re-evaluated consult cases with Gleason scores 6 and 7a. The unequivocal separation of fused versus non-fused glands is particularly challenging in microfocal carcinomas which can necessitate higher magnifications and longer microscopy times. In these, it is obviously not so rare to overlook subtle signs of glandular fusion on low power in a routine diagnostic situation, resulting in an under-grading of this focus. This underscores that a high degree of training is necessary for proper Gleason grading, which implies not only to recognize the most appropriate pattern but also to estimate glandular fusion correctly. This study with its accurate analysis of the stromal boundary of single carcinoma glands supports the observation of a Gleason score shift from 6 to 7 introduced by the ISUP2005 modification [1, 31]. This upgrading has been reported by several studies already [3–5, 32]. Mostly, these data resulted from routine reports of biopsies. The Gleason scores 7a and 7b outnumbered GS 6.

In combined analyses of biopsies with subsequent radical prostatectomy specimens rates of concordance of over 80% have been reported [33, 34]. The pT categories of these cases (GS 6/7a) were mostly pT2c and pT3. Only rarely, the category pT2a was found in cases with microfocal GS6 cases [33]. In the daily routine diagnostic workup of prostate biopsies with a microfocal carcinoma that consists at time of only two to three atypical glands, a second opinion may be obtained before definitive therapy is planned [35]. Most cases suspicious for cancer harbour GS 5 or GS6 carcinomas, but rarely also GS7a cases. This explains the different percentages of Gleason scores 6, 7a and 7b in routine diagnosis and consult cases. In our own files of biopsies sent in for a second opinion GS6 carcinomas clearly predominate. In nearly 40% of cases an upgrading to GS 7a due to the demonstration of glandular fusion was favoured. Cribriform glandular patterns played no role in this group. The discrimination of GS6 and GS7a may have immediate therapeutic implications [36]. This is of particular importance, if brachytherapy or active surveillance is an otherwise (serum PSA <10 ng/ml, less than two positive biopsy cores) feasible therapeutic option [1, 33]. Active surveillance and brachytherapy may be applied to cases with a small unilateral (pT2a) carcinoma often seen with GS6. In cases with higher Gleason scores (>7a) more advanced pT categories of pT2b/c or even, in a third of cases, a pT3a stage can be assumed, which excludes active surveillance [37]. Similar difficulties as described in our paper about microfocal Gleason 6 and 7a carcinomas are recently published by Egevad and coworkers with a novel method to improve the reproducibility of Gleason grading by inactive digital slides with heat maps. Similar to our own findings fused glands are the main characteristic of microfocal Gleason pattern 4 and score 7a carcinomas [38].

Take Home Message

In addition to the recognition of cribriform and poorly formed glands, the demonstration of fused glands on prostate biopsies is important for accurate determination of the Gleason score, particularly the discrimination of GS6 from GS7a, which is highly relevant for therapy planning. This requires a careful analysis and continuous training to achieve a satisfactory inter- and intra-observer reproducibility with a kappa value = >0.7. This is the kappa value that is achieved by expert GU pathologists, applying the definition suggested in this study: glandular fusion can be diagnosed,

– if no stromal fibres can be discerned in a minimum of two glands,
– and if no double-layered row of nuclei is seen in the putative fusion area.

Non-specialized general pathologists ought to make use of higher magnifications (200×) to ensure that minute areas of glandular fusion, that define a Gleason 4 pattern, are not overlooked.

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