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A Comparison of Prostatic Acinar Adenocarcinoma Gleason 3+4 versus 4+3 in Different Laboratory Practice Settings

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Abstract

Objective: To evaluate Gleason 7 tumors from radical prostatectomy specimens that were stratified by 3+4 and 4+3 status, focusing on the clinical and morphologic features.

Methods and results: In the first series of 20 Gleason 7 tumors processed by total embedding, cases with 4+3 status were associated with a higher proliferation index. In a second series of 106 Gleason 7 tumors processed by partial embedding, 4+3 status was associated with higher preoperative serum prostate-specific antigen levels and a higher frequency of extraprostatic extension and seminal vesicle invasion.

Conclusion: For those laboratories that employ partial samplings as the adopted method for processing radical prostatectomy specimens, inclusion of the entire prostatic periphery and extraprostatic tissue is advised to avoid understaging caused by missing extraprostatic extension.

Keywords

Prostatic neoplasms, Pathology, Prostate-specific antigen, Neoplasm grading

Introduction

The Gleason grading system is the method used to grade acinar adenocarcinoma of the prostate [1]. Although a Gleason score of 7 is one of the most frequent scores reported in radical prostatectomy specimens, tumors with Gleason score 7 show heterogeneous behavior on follow up. Approximately 14 years ago, a body of literature began to accrue that explored the importance of distinguishing a final Gleason score of 7 based on Gleason 3 pattern predominance (3+4) or Gleason 4 pattern predominance (4+3). Several studies have shown varying associations with respect to morphologic parameters and prognosis when comparing Gleason 3+4 and 4+3 status. Some of these differences might be attributed to specific methods of radical prostatectomy specimen handling and evaluation. The present study compared the clinical and morphologic features of Gleason 7 tumors from radical prostatectomy specimens stratified according to 3+4 or 4+3 status. Specimens were obtained from two different series using distinct protocols: one employed total embedding and an original point-count method to assess tumor burden [2], and a second series used partial embedding and an adapted point-count method [3].

Material and Methods

Patients and protocols

The study included two series of consecutive radical prostatectomy specimens evaluated in Salvador, Brazil. The first series comprised 48 specimens collected from January 2000 to December 2003: 34 from Hospital Universitário Professor Edgard Santos (HUPES), 12 from CLINAZZA Pathology Laboratory and two from Centro Estadual de Oncologia (CICAN). All of these prostatectomy specimens were processed by complete embedding. The cone method was used to assess each specimen's apical and basal margins, while the original point-count method described by Billis and colleagues was employed to evaluate tumor extent and the percentage of prostate involvement by carcinoma [2].

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Table 1: Clinical and morphologic features of prostate carcinoma with prognostic value in cases of prostatic acinar adenocarcinoma Gleason 7 stratified by 3+4 or 4+3 sum. Specimens were processed by complete embedding and evaluated by the original point-count method (n=48).

	Gleason 7 (3+4) n/N (%)	Gleason 7 (4+3) n/N (%)	p value
Preoperative serum PSA			
≤ 4,00ng/ml	0	0	0.06
> 4,00 and ≤ 9,99ng/ml	5/7 (71)	2/7 (29)	
> 9,99ng/ml	2/7 (29)	5/7 (71)	
Percentage of gland involvement by carcinoma			
≤ 10%	2/11 (18)	0	0.03
> 10% and ≤ 20%	5/11 (46)	2/9 (22)	
> 20% and ≤ 30%	2/11 (18)	3/9 (33)	
> 30% and ≤ 40%	2/11 (18)	3/9 (33)	
> 60% and ≤ 80%	0	1/9 (11)	
Positive circumferential surgical margin	4/11 (36)	4/9 (44)	1.00
Positive basal surgical margin	0	4/9 (44)	0.03
Positive apical surgical margin	3/11 (27)	0	0.22
Extraprostatic extension	2/11 (18)	5/9 (56)	0.15
Seminal vesicle invasion	1/11 (9)	4/9 (44)	0.12
Ki-67 immunostaining (% of cancer cells)			
Negative	2/11 (18)	0	0.04
> 0% and ≤ 3,0%	9/11(82)	7/9 (78)	
> 3,0%		2/9 (22)	

The second series comprised 224 radical prostatectomy specimens from the IMAGEPAT Pathology Laboratory (Salvador, Brazil), obtained from May 2010 to May 2013 (n=195), and 29 specimens from HUPES (2013). All specimens were processed by partial embedding with the cone method to assess the apical and basal margins and an evaluation of the tumor extent (and the percentage of prostate involvement by carcinoma) was performed using a recently described modified point-count method [3]. This project was approved by the Research Ethics Committee of Centro de Pesquisas Gonçalo Moniz (CPqGM/FIOCRUZ) in Salvador, Brazil.

Immunohistochemistry

Paraffin-embedded sections were prepared at 4-μm thickness. Paraffin was removed by xylene, followed by rehydration with graded ethanol. Heat-induced antigen retrieval was carried out for all sections in 0.01 M citrate buffer, pH=6.00, at 95°C for 30 min. After washing in PBS with 0.1% Tween-20, subsequent steps were performed with the LSAB (DAKO) kit. The sections were incubated with the primary antibodies overnight at 4°C in a humidified chamber. Primary antibodies (mouse IgG, clone MIB-1; DAKO, Carpinteria, USA) were diluted in antibody diluent Af (2% BSA in PBS; pH 7.4; DAKO). After washing in PBS, the sections were incubated in 10% skim milk for 20 min to block non-specific binding. A blockade of endogenous peroxidase was achieved with 3% H_2O_2 for 10 min at room temperature. Reactions were developed with 3,3-diamino-benzidine tetrahydrochloride (DAB) (DAKO). Sections were counterstained with Harris hematoxylin for 2 min, then dehydrated and mounted using Permount.

Statistical analyses

Statistical analyses were performed using the GraphPad Prism 4.03 software package (GraphPad,La Jolla, CA, USA). Categorical data were compared by Chi-square test or Fisher´s exact test, and the numerical data were compared using the Student´s t-test or the Mann-Whitney test. The Chi-square test for trend was used to compare stratified groups with different serum Prostate-specific Antigen (PSA) levels and the percentage of gland involvement by carcinoma.

Results

In the first series, 20 of 48 (42%) radical prostatectomy specimens exhibited tumors with Gleason score 7. In this series, we observed a trend (p=0.06) with respect to higher serum preoperative PSA levels in patients with 4+3 status. Five of seven (71%) patients with 3+4 status had serum PSA levels between 4.00 and 9.99 ng/ml, whereas 5/7 (71%) patients with 4+3 status had serum PSA levels > 9.99 ng/ml. Significant differences were observed between the groups regarding the percentage of gland involvement by carcinoma (PGIC) and the percentage of cells stained for a cellular marker of proliferation (Ki-67 antigen immunostaining). Seven of nine patients with 4+3 status (77%) had PGIC > 20%, whereas 7/11 patients with 3+4 status (64%) had PGIC ≤ 20% (p=0.03). Two of nine patients with 4+3 status (22%) had > 3.0% cells stained positively for Ki67, whereas 11/11 patients with 3+4 status (100%) had ≤ 3.0% cells stained for Ki67 (p=0.04). There were no differences observed between the groups regarding circumferential margins, basal margin, apical margin, extraprostatic extension or seminal vesicle invasion. No tertiary Gleason 5 component was observed in this series. Data from the first series are detailed in Table 1. Among these 48 prostatectomy specimens, the percentage of Ki67-positive cells was positively correlated with PGIC and Gleason grade.

We extended the comparison of 3+4 and 4+3 status in a larger series of radical prostatectomies processed by a partial sampling method and using an adapted modified point-count method to estimate PGIC and tumor volume. In this series, 106 of 224 (47%) radical prostatectomy specimens contained tumors with Gleason score 7. Patients with 4+3 status had higher preoperative serum PSA levels. Despite a lack of differences in PGIC and tumor volume, tumors with 4+3 status were more likely to exhibit extraprostatic extension and seminal vesicle invasion. The rate of tertiary Gleason 5 component detection was similar in each group. Data from the second series are detailed in Table 2.

Discussion

In 2000, in a series of 567 radical prostatectomies for localized acinar Gleason score 7 adenocarcinoma, Partin and colleagues reported that Gleason 4 pattern predominance was associated with lower recurrence-free survival intervals. The tumor 4+3 status was an independent predictor of serum PSA progression [4]. That same year, Sakr and colleagues reported a series of 534 patients with Gleason 7 tumors. Patients with 4+3 status were more likely to be older African-Americans and exhibited higher preoperative serum PSA levels, a higher stage and larger tumors. In a multivariate analysis among patients with organ-confined disease, primary Gleason 4 status was the only independent predictor of PSA recurrence [5]. In 2001, in a series of 237 Gleason score 7 tumors, Lau and colleagues reported that 4+3 status was associated with seminal vesicle involvement, a higher stage, extraprostatic extension and higher preoperative serum PSA levels, but not with cancer-specific survival. Under multivariate analysis, preoperative PSA, seminal vesicle involvement and DNA

Table 2: Clinical and morphologic features of prostate carcinoma with prognostic value in cases of prostatic acinar adenocarcinoma Gleason 7 stratified by 3+4 or 4+3 sum. Specimens were processed by partial embedding and evaluated by the adapted point-count method (n=106).

	Gleason 7 (3+4) mean ± SD or n/N (%)	Gleason 7 (4+3) mean ± SD or n/N (%)	p value
Age (years)	64.3 ± 6.7	62.9 ± 10.6	0.38
Preoperative serum PSA	11.0 ± 3.3	11.8 ± 6.05	0.01
≤ 4,00ng/ml	01/39 (03)	01/16 (06)	0.04
> 4,00 and ≤ 9,99ng/ml	28/39 (72)	05/16 (32)	
> 9,99ng/ml	10/39 (26)	10/16 (62)	
Percentage of gland involvement by carcinoma	16 ± 11.4	19 ± 11.7	0.29
≤ 10%	27/76 (23)	07/30 (35)	0.14
> 10% and ≤ 20%	22/76 (29)	09/30 (30)	
> 20% and ≤ 30%	20/76 (26)	07/30 (23)	
> 30% and ≤ 40%	03/76 (04)	06/30 (20)	
> 60% and ≤ 80%	07/76 (05)	01/30 (03)	
Tumor volume (ml)	12.0 ± 3.48	12.0 ± 3.47	0.6
Positive circumferential surgical margin	18/76 (23)	09/30 (30)	0.3
Positive basal surgical margin	04/76 (05)	03/30 (10)	0.3
Positive apical surgical margin	02/76 (03)	02/30 (07)	0.3
Extraprostatic extension	15/76 (20)	11/30 (38)	0.04
Seminal vesicle invasion	03/76 (04)	05/30 (17)	0.02
Tertiary Gleason 5	07/76 (09)	02/30 (07)	0.8

ploidy – but not the primary Gleason pattern – were associated with progression-free survival [6]. In 2001, Herman and colleagues reported a series of 823 patients with Gleason 7 tumors treated by radical prostatectomy in which primary pattern 4 was associated with lower recurrence-free survival. However, multivariate analysis, including variables such as preoperative PSA, tumor volume, margin status, seminal vesicle involvement, extraprostatic extension and nodal metastasis, did not show that Gleason 4 pattern predominance had the predictive power to be independently associated with disease progression [7].

More recently, some authors have called attention to the importance of tertiary pattern 5 in radical prostatectomy specimens. In a series of 228 patients with Gleason score 7, 4+3 status was associated with a higher stage, serum PSA recurrence and the presence of tertiary Gleason 5 patterns. Using multivariate analysis, tertiary pattern 5 (but not primary Gleason pattern) was an independent predictor of PSA recurrence [8]. In another series of 509 patients with Gleason score 7, tertiary pattern 5 was independently associated with a higher stage and PSA recurrence. The impact of tertiary pattern 5 on PSA recurrence was observed in both 4+3 and 3+4 subsets [9]. Interestingly, in the present study, the frequency of tertiary Gleason 5 did not differ between 3+4 and 4+3 status.

The long-term prognostic value of stratifying Gleason 7 tumors was evaluated in 1,256 men in a report from the Mayo Clinic. After ten years, patients with 4+3 and 3+4 status differed in terms of PSA recurrence-free survival (38% vs 48%), systemic recurrence (15% vs 8%) and cancer-specific survival (93% vs 97%). Primary Gleason patterns among patients with Gleason score 7 remained an independent predictor of all these endpoints when controlling for preoperative PSA, seminal vesicle involvement, margin status, DNA ploidy and TNM staging [10]. In a more recent series of 756 men with prostate cancer, cancer-specific survival after ten years was 98%, 92%, 77% and 70% for patients with tumors of Gleason score ≤ 6, 3+4, 4+3 and ≥ 8, respectively. Another study found that, in patients with Gleason score 7, the primary Gleason pattern was an independent predictor of cancer-specific survival [11].

In a series of 530 patients who underwent brachytherapy for Gleason 7 tumors, the primary Gleason pattern was not found to be predictive of PSA recurrence or survival [12]. It is worth noting, however, that primary Gleason patterns assessed in needle biopsy specimens exhibit a well-recognized level of discrepancy when compared to the (usually upgraded) Gleason score obtained from radical prostatectomy specimens. Thus, at least in part, sampling error may contribute to the lack of an effect observed in patients treated by brachytherapy.

Discrepancies reported in the literature, in which factors emerge as independent predictors of outcome, are dependent on which particular variables were included in a given study. Additionally, certain differences between studies might be attributed to different methods of radical prostatectomy specimen handling and examination. In this study, we demonstrated that Gleason grade 7 tumors with 3+4 and 4+3 status exhibited important differences, even when evaluated by different protocols.

We highlight the observation from the first series that Gleason 7 tumors with 4+3 status were associated with higher proliferative indices. Importantly, Ki67 expression has been reported as the most consistent protein/immunohistochemical marker associated with prognosis in prostate cancer [13,14]. From the second series, which was powered by a larger sample, 4+3 status was associated with extraprostatic extension and seminal vesicle invasion.

This study has several limitations. It was not possible to evaluate the expression of Ki67 antigen in the second (larger) series of radical prostatectomy specimens. As most included specimens were obtained from different services in different cities, it was not possible to obtain patient follow-up data.

Conclusion

In conclusion, the present study found that Gleason 7 tumors with 4+3 status have higher proliferative indices. They also appear to be more infiltrative or locally aggressive than 3+4 tumors, which may be related to a higher frequency of extraprostatic extension and seminal vesicle invasion, despite no differences in the percentage of gland involvement by carcinoma or tumor volume. For those laboratories that employ partial samplings as the adopted method for processing radical prostatectomy specimens, the inclusion of the entire prostatic periphery and extraprostatic tissue is advised to avoid understaging due to missing evidence of extraprostatic extension.

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