Tertiary Gleason Pattern 5 is An Independent Risk Factor for Prostate Cancer with GS 7: A Retrospective Study from China.

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Research

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Abstract

Background: Tertiary Gleason pattern 5 (TGP5) was found to be prognostic in prostate cancer (PCa) after radical prostatectomy (RP), but related data from China was rare. Our study was aimed at finding out the effect of TGP5 on PCa with Gleason score (GS) 7 and supplementing data from China in this field.

Methods: A total of 229 cases met with inclusion criteria during Jan. 2014 to Dec. 2018 were reviewed. Cases were divided into GS 7 without TGP5 and GS 7 with TGP5. We compared age at diagnosis, preoperative PSA level, prostate volume, PSA density (PSAD), GS variation, clinical T staging, pathological T staging, T staging variation, extra-prostatic extension (EPE), positive surgical margin (PSM) and seminal vesicle invasion (SVI) between the groups. Effects of TGP5 on prognosis of PCa with GS 7 were evaluated using biochemical recurrence (BCR) as the primary end point.

Results: TGP5 was related to higher PSM rate (P=0.001) and BCR rate (P=0.009) but not related to higher preoperative PSA level, larger prostate volume, higher PSAD, GS upgrade, poorer clinical/pathological T staging, T upstaging, EPE and SVI (all P>0.05). The median follow-up time was 24 months (interquartile range 17.5-45.5). TGP5 was an independent risk factor to PCa with GS 7 after RP using Kaplan-Meier log-rank test (P=0.018). Both univariable and multivariable cox-regression analysis pointed out that TGP5 increased the incidence of BCR in PCa with GS 7 (P<0.05). Stratified analyses were also done.

Conclusion: TGP5 is an independent risk factor predicting of BCR after RP in PCa with GS 7 from China. TGP5 is related to higher PSM rate and BCR incidence. It is time to renew the contemporary Grading Group system with the consideration of TGP.

Background

According to the latest data from nation cancer center in China, up to 2015, the incidence of prostate cancer (PCa) had reached sixth place in male malignant tumors (1). So far, according to the latest guidelines from EAU and NCCN on prostate cancer, urologists prefer making treatment strategy by stratifying patients into different groups with different risks. Gleason score (GS) is defined as the sum of primary and secondary patterns of prostate specimens, ranging from 2 to 10 (2, 3). It is a known factor which should be considered when stratifying patients into different risk groups. In 2014 International Society of Urological Pathology (ISUP), experts found different GS had different prognosis. To be noted, GS 3 + 4 has better prognosis than GS 4 + 3 even though they have same GS (4), which indicated that the composition of GS or, in other words, the primary pattern of GS is more related to patients' outcome.

Tertiary Gleason pattern (TGP) was first reported in 2000 (5), but there haven't been consensus on its definition until now. In 2014 ISUP, most of the experts supported the definition that for specimens after radical prostatectomy (RP), TGP, also called minor high-grade pattern, was defined as the third most prevalent differentiation pattern consisting of less than 5% of the cancer in the specimen but worse than primary and secondary patterns (4). Is TGP related to PCa prognosis? Many studies aboard have been conducted since TGP was proposed. They got similar conclusions that patients with the existence of TGP had worse outcome than those without it (5–20). However, data from China, nation with the largest population, is rare and limited. It is important to know the characteristics of TGP in Chinese population. For GS 7 groups, either 3 + 4 or 4 + 3, TGP specifically refers to TGP5.

Our study was aimed at: 1. Finding out the basic characteristic of TGP5 in PCa with GS 7 in our institution; 2. Validating TGP5's prognostic effects on PCa patients with GS 7 in China; 3. Supplementing data from China in this field and doing the first stratifying analysis considering both TGP and GS in Chinese.

Methods

1. Patients selection and data collection

We identified 317 patients who met with following conditions from Peking University First Hospital, which is one of the top institutions in urology field in China: 1) Underwent prostate biopsy in this hospital and pathological diagnosis were PCa; 2) Underwent RP in this hospital and GS in specimens after RP was 7（either 3+4 or 4+3）; 3) Surgery time was from Jan. 2014 to Dec. 2018. Patients who received neo-adjuvant, adjuvant therapy or salvage therapy, which were 61 cases in total, were excluded. In the

remaining 256 patients, 19 patients were lost to follow-up, 8 patients had incomplete or poor reliability of follow-up data. In total, 229 patients were entered analysis finally (Figure 1). Ethical audit was permitted before we conducted this study.

2. Pathological specimens processing

All prostate specimens after RP were processed according to standard pathological procedures. Reports were given by two qualified pathologists separately. Pathological T staging was given according to 2009 American Joint Committee on Cancer TNM staging system. GS was defined as the sum of primary and secondary patterns of prostate specimens. TGP5 was defined as the third prevalent pattern (≤5%) but worse than primary and secondary patterns in RP specimen with GS 7 according to the definition most experts supported on 2014 ISUP. GS upgrade was considered when GS in specimens after RP was poorer than GS in prostate biopsy specimens according to Grading Group system from 2005 ISUP, while TGP5 did not change the GS. Surgical margin was considered positive when tumor cells were in contact with the ink on the RP specimen surface.

3. Follow-up

The primary endpoint of our study was biochemical recurrence (BCR). It was defined as prostate-specific antigen (PSA) >0.2 ng/ml by two consecutive (more than 1 week apart) measurement after RP. Generally, patients would be evaluated with PSA level 3 months after RP. If this PSA values exceeded 0.1 ng/ml, we considered that the surgery did not reach the standard of “radical”. In this case, salvage therapy would be given instead and these people would also be excluded even PSA >0.2 ng/ml. Patients were followed up quarterly within the first year, semiannually in the second year, and annually in the following years mainly with PSA evaluation. Among the primary 317 patients, after telephone follow-up and looking up original medical history, we identified 61 cases who underwent neo-adjuvant/adjuvant/salvage therapy, which were excluded, and 8 patients who had incomplete or poor reliability of follow-up data, which were not included in final analysis. There were 19 patients lost to follow up.

4. Statistical analysis

Categorical variables were presented as numbers and percentages whereas continuous variables as median and interquartile range (IQR). Differences in categorical variables and continuous variables were analyzed with the chi-square test and Kruskal-Wallis test, respectively. BCR-free survival (BFS) was calculated from the date of surgery to BCR or to last follow-up. The Kaplan-Meier analysis and log-rank tests were used to estimate and compare BCR-free survival. Univariable and multivariable analyses were also done with Cox-regression models using hazard ratios (HRs) and 95% confidence indexes (CIs). Statistical significance was considered at P≤0.05 (2-tailed test). IBM SPSS Statistics version 26.0 (SPSS Inc., Chicago, IL, USA) was used for analysis. Graphpad Prism version 8.4.3 (GraphPad Software, LLC, USA) was used for curve illustration.

Results

1. Clinical and pathological characteristics distribution in group without and with TGP5.

Among the 229 patients with GS 7 that were finally included and analyzed, there were 74.7% patients without TGP5 (n = 171) while there were 25.3% patients with TGP5 (n = 58). Clinical and pathological characteristics distributions were shown in Table 1. The median age at diagnosis of patients were 66 (IQR 62–71) and 67 (IQR 61.75-72) years old for GS 7 without TGP5 and GS 7 with TGP5, respectively. The median preoperative PSA level were 8,97 (IQR 6.77–3.39) and 10.755 (IQR 7.48-17.2625) ng/ml in GS 7 without TGP5 and GS 7 with TGP5, respectively. There were no significant differences between groups in age at diagnosis (P = 0.461, P = 0.708 using chi-square test after stratifying ages into < 65 years old and ≥ 65 years old) and preoperative PSA level (P = 0.054, P = 0.176 using chi-square test after stratifying preoperative PSA level into < 10 ng/ml and ≥ 10 ng/ml). Other characteristic distributions were shown in Table 1.

Table 1
Clinical and pathological characteristics of patients between GS = 7 without TGP5 and with TGP5

Variable	Total	without TGP5	with TGP5	P value
Patients, n(%)	229	171(74.67%)	58(25.33%)	-
Age, years old				
Median	66	66	67	0.461
IQR	62–71	62–71	61.75-72	
<65	90(39.3%)	66(38.6%)	24(41.4%)	0.708
≥ 65	139(60.7%)	105(61.4%)	34(58.6%)	
Preoperative PSA level, ng/ml				
Median	9.24	8.97	10.755	0.054
IQR	6.930-14.695	6.77–3.39	7.48-17.2625	
≤ 10	128(55.9%)	100(58.5%)	28(48.3%)	0.176
>10	101(44.1%)	71(41.5%)	30(51.7%)	
Prostate Volume, ml				
Median	37.80	37.00	39.75	0.264
IQR	28.25-50.00	28.00-49.25	29.53–55.48	
<50	171(74.70%)	131(76.60%)	40(69.00%)	0.247
≥ 50	58(25.30%)	40(23.40%)	18(31.00%)	
PSAD, ng/ml/ml				
Median	0.2458	0.2450	0.2507	0.355
IQR	0.1667–0.3857	0.1559–0.3726	0.1805–0.4170	
< 0.15	46(20.10%)	38(22.20%)	8(13.80%)	0.166
≥ 0.15	183(79.90%)	133(77.80%)	50(86.2%)	
GS variation, n(%)				0.093
Non-GS Upgrade(overestimated)	151(65.90%)	118(69.00%)	33(56.90%)	
GS Upgrade(underestimated)	78(34.10%)	53(31.00%)	25(43.10%)	
Clinical T Staging, n(%)				0.260(fisher)
T1c	36(15.70%)	23(13.50%)	13(22.40%)	
T2	183(79.90%)	141(82.50%)	42(72.40%)	
T3a	5(2.20%)	4(2.30%)	1(1.70%)	
T3b	5(2.20%)	3(1.80%)	2(3.40%)	
Pathological T Staging, n(%)				0.224
T2	121(52.8%)	96(56.1%)	25(43.1%)	
T3a	83(36.2%)	58(33.9%)	25(43.1%)	
T3b	25(10.9%)	17(9.9%)	8(13.8%)	
T Staging variation, n(%)				0.120

Variable	Total	without TGP5	with TGP5	P value
Non-upstaging	107(46.70%)	85(49.70%)	22(37.90%)	
Upstaging	122(53.30%)	86(50.30%)	36(62.10%)	
PSM, n(%)				0.001
No	180(78.6%)	143(83.6%)	37(63.8%)	
Yes	49(21.4%)	28(16.4%)	21(36.2%)	
EPE, n(%)				0.345
No	107(46.7%)	83(48.5%)	24(41.4%)	
Yes	122(53.3%)	88(51.5%)	34(58.6%)	
SVI				0.416
No	204(89.1%)	154(90.1%)	50(86.2%)	
Yes	25(10.9%)	17(9.9%)	8(13.8%)	
Operation ways, n(%)				0.728
LPRP	219(95.6%)	164(95.9%)	55(94.8%)	
RARP	10(4.4%)	7(4.1%)	3(5.2%)	
BCR, n(%)				0.009
No	206(90.0%)	159(93.0%)	47(81.0%)	
Yes	23(10.0%)	12(7.0%)	11(19.0%)	

No significant difference between these two groups was found when it came to prostate volume (P = 0.264, P = 0.247 using chi-square test after dividing prostate volume into < 50 ml and ≥ 50 ml), PSAD (P = 0.355, P = 0.166 using chi-square test after dividing PSAD into < 0.15 ng/ml/ml and ≥ 0.15 ng/ml/ml), GS variation (P = 0.093), clinical T staging (P = 0.260), pathological T staging (P = 0.224), T staging variation (P = 0.120), extra-prostatic extension (P = 0.345), seminal vesicles invasion (P = 0.416) and operation ways (P = 0.728). There were statistically difference in positive surgical margin rate (P = 0.001) between GS 7 without TGP5 and GS 7 with TGP5. Table 1 also showed the biochemical recurrence incidence and cases in our study in different groups. There was significant difference in BCR incidence between GS 7 without TGP5 and GS 7 with TGP5 (P = 0.009), which indicated TGP5 might be a risk factor in prognosis of PCa.

2. Clinical and pathological characteristics distribution in groups 3 + 4 without/with TGP5 and 4 + 3 without/with TGP5.

Gleason Score was acknowledged as an independent risk factor in prostate cancer. Patients with GS 4 + 3 have poorer prognosis than patients with GS 3 + 4.(4) We then stratified our patients into four groups, details and distribution were as followed: 113 (4.3%), 31 (13.5%), 58 (25.3%), and 27 (11.8%) with GS 3 + 4 without TGP5, GS 3 + 4 with TGP5, GS 4 + 3 without TGP5, and GS 4 + 3 with TGP5, respectively. New clinical and pathological characteristics distributions were shown in Table 2. The median ages of the patients were 66 (IQR 62-70.5), 71 (IQR 61–73), 66 (IQR 60.75-71), and 65 (IQR 62–71) years old for GS 3 + 4 without TGP5, GS 3 + 4 with TGP5, GS 4 + 3 without TGP5 and GS + 4 + 3 with TGP5. respectively. There was no difference among the groups (P = 0.461, P = 0.944 using chi-square test after stratifying ages into < 65 years old and ≥ 65 years old). The median preoperative PSA level was 9.24 (IQR 6.955–13.800), 9.74 (IQR 6.800–18.200), 8.69 (IQR 6.583–13.053), and 12.17 (IQR 8.310–16.950) ng/ml for GS 3 + 4 without TGP5, GS 3 + 4 with TGP5, GS 4 + 3 without TGP5, and GS 4 + 3 with TGP5, respectively. There was no difference among the groups (P = 0.129, P = 0.521 using chi-square test after stratifying preoperative PSA level into < 10 ng/ml and ≥ 10 ng/ml), too.

Table 2
Clinical and pathological characteristics of patients among 4 groups.

Variable	Total	Gleason Score				P value
		3 + 4	3 + 4 + TGP5	4 + 3	4 + 3 + TGP5	
Patients, n(%)	229	113(49.3%)	31(13.5%)	58(25.3%)	27(11.8%)	-
Age, years old						
Median	66	66	71	66	65	0.461
IQR	62–71	62-70.5	61–73	60.75-71	62–71	
<65	90(39.3%)	43(38.1%)a	12(38.7%)a	23(39.7%)a	12(44.4%)a	0.944
≥ 65	139(60.7%)	70(61.9%)a	19(61.3%)a	35(60.3%)a	15(55.6%)a	
Preoperative PSA level, ng/ml						
Median	9.24	9.24	9.74	8.69	12.17	0.129
IQR	6.930-14.695	6.955–13.800	6.800–18.200	6.5825–13.0525	8.310–16.950	
≤ 10	128(55.9%)	65(57.5%)a	16(51.6%)a	35(60.3%)a	12(44.4%)a	0.521
>10	101(44.1%)	48(42.5%)a	15(48.4%)a	23(39.7%)a	15(55.6%)a	
Prostate Volume, ml						
Median	37.80	38.19	44.00	36.00	38.50	0.545
IQR	28.25-50.00	27.15–51.50	29.70–56.00	28.85–43.93	29.00-52.40	
<50	171(74.70%)	82(72.60%)a	20(64.50%)a	49(84.50%)a	20(74.10%)a	0.178
≥ 50	58(25.30%)	31(27.40%)a	11(35.50%)a	9(15.50%)a	7(25.90%)a	
PSAD, ng/ml/ml						
Median	0.2458	0.2423	0.2257	0.2596	0.2898	0.487
IQR	0.1667–0.3857	0.1503–0.3654	0.1718–0.4108	0.1816–0.3959	0.2105–0.4358	
< 0.15	46(20.10%)	28(24.80%)a	5(16.10%)a	10(17.20%)a	3(11.10%)a	0.321
≥ 0.15	183(79.90%)	85(75.20%)a	26(83.90%)a	48(82.80%)a	24(88.90%)a	
GS Variation, n(%)						0.002
Non-GS Upgrade(overestimated)	151(65.90%)	88(77.90%)a	17(54.80%)a,b	30(51.70%)b	16(59.30%)a,b	
GS Upgrade(underestimated)	78(34.10%)	25(22.10%)a	14(45.20%)a,b	28(48.30%)b	11(40.70%)a,b	
Clinical T Staging, n(%)						0.315(fisher)
T1c	36(15.70%)	17(15.00%)a	8(25.80%)a	6(10.30%)a	5(18.50%)a	
T2	183(79.90%)	92(81.40%)a	21(67.70%)a	49(84.50%)a	21(77.80%)a	
T3a	5(2.20%)	3(2.70%)a	0(0.00%)a	1(1.70%)a	1(3.70%)a	
T3b	5(2.20%)	1(0.90%)a	2(6.50%)a	2(3.40%)a	0(0.00%)a	

Variable	Total	Gleason Score				P value
Pathological T Staging, n(%)						<0.001
T2	121(52.8%)	76(67.3%)a	17(54.8%)a,b	20(34.5%)b	8(29.6%)b	
T3a	83(36.2%)	29(25.7%)a	9(29.0%)a,b	29(50.0%)b	16(59.3%)b	
T3b	25(10.9%)	8(7.1%)a	5(16.1%)a,b	9(15.5%)b	3(11.1%)b	
T staging variation, n(%)						0.001
Non-upstaging	107(46.70%)	67(59.30%)a	14(45.20%)a,b	18(31.00%)b	8(29.60%)b	
Upstaging	122(53.30%)	46(40.70%)a	17(54.80%)a,b	40(69.00%)b	19(70.40%)b	
PSM, n(%)						0.002
No	180(78.6%)	95(84.1%)a	23(74.2%)a,b	48(82.8%)a	14(51.9%)b	
Yes	49(21.4%)	18(15.9%)a	8(25.8%)a,b	10(17.2%)a	13(48.1%)b	
EPE, n(%)						0.001
No	107(46.7%)	66(58.4%)a	16(51.6%)a,b	17(29.3%)b	8(29.6%)b	
Yes	122(53.3%)	47(41.6%)a	15(48.4%)a,b	41(70.7%)b	19(70.4%)b	
SVI, n(%)						0.231
No	204(89.1%)	105(92.9%)a	26(83.9%)a	49(84.5%)a	24(88.9%)a	
Yes	25(10.9%)	8(7.1%)a	5(16.1%)a	9(15.5%)a	3(11.1%)a	
Operation ways, n(%)						0.860
LPRP	219(95.6%)	109(96.5%)a	29(93.5%)a	55(94.8%)a	26(96.3%)a	
RARP	10(4.4%)	4(3.5%)a	2(6.5%)a	3(5.2%)a	1(3.7%)a	
BCR, n(%)						0.006
BCR-free	206(90.0%)	108(95.6%)a	27(87.1%)a,b	51(87.9%)a,b	20(74.1%)b	
BCR	23(10.0%)	5(4.4%)a	4(12.9%)a,b	7(12.1%)a,b	7(25.9%)b	

No statistical difference was found among these four group when comparing prostate volume (P = 0.545), PSAD (P = 0.487), clinical T staging (P = 0.315), seminal vesicle invasion (P = 0.231) and operation way (P = 0.860) in our study, too. However, there were overall statistical differences among 4 groups when comparing GS variation (P = 0.002), pathological T staging (P < 0.001), T staging variation (P = 0.001), positive surgical margin (P = 0.002) and extra-prostatic extension (P = 0.001). Multiple comparison only found significant differences (P < 0.05) existing in: (1) GS variation between GS 3 + 4 without TGP5 and GS 4 + 3 without TGP5; (2) pathological T staging distribution, T staging variation and EPE rate between GS 3 + 4 without TGP5 and GS 4 + 3 without/with TGP5; (3) PSM rate between GS 4 + 3 with TGP5 and GS 3 + 4/4 + 3 without TGP5, all P < 0.05. Similar results were found when comparing biochemical recurrence incidence. Biochemical recurrence incidence was higher in GS 4 + 3 with TGP5 than GS 3 + 4 without TGP5 (P < 0.05) while no statistical difference was found in other multiple comparisons (P > 0.05).

3. Association of TGP5 with biochemical recurrence after radical prostatectomy.

The median follow-up time was 24 months (IQR 17.5–45.5)for all patients. 10% of patients experienced biochemical recurrence (n = 23). The Kaplan-Meier BCR-free curves for different groups after RP were illustrated in Fig. 2. GS 7 with TGP5 had poorer BCR-free survival than GS 7 without TGP5 (log-rank test, P = 0.018). We also illustrated Kaplan-Meier BCR-free curves for 4 groups, which were GS 3 + 4 without TGP5, GS 3 + 4 with TGP5, GS 4 + 3 without TGP5 and GS 4 + 3 with TGP5, see Fig. 3. There was overall difference among 4 groups (p = 0.0038). But in multiple comparison, statistical difference was only found between GS 3 + 4 without TGP5 and GS 4 + 3 with TGP5 (P < 0.001). There was no statistical difference when comparing BFS between GS 3 + 4 with

TGP5 and GS 4 + 3 without TGP5 (P > 0.05). In GS 3 + 4/GS 4 + 3 respectively, the presence of TGP5 wasn't related to poorer BFS (P > 0.05).

In univariable cox-regression analysis, pathological T staging, T upstaging, positive surgical margin, Gleason score and TGP5 were found as independent risk factors to BCR after RP. Compared to T2 stage, T3a and T3b had 5.065-fold and 10.116-fold risk to experience BCR with P = 0.005 and P < 0.001, respectively. Patients with T upstaging had 3.226-fold risk to experience BCR than non-upstaging. As a well-acknowledged risk factors in prostate cancer, GS 4 + 3 had 2.896-fold risk to BCR than GS 3 + 4 (P = 0.013). Patients with PSM had 2.362-fold risk to BCR than negative surgical margin (P = 0.042). Patients with TGP5 had about 2.640-fold risk to BCR after RP than patients without TGP5 (P = 0.023). Elder age at diagnosis (≥ 65 years old), higher preoperative PSA level (>10 ng/ml), higher prostate volume (≥ 50 ml), higher PSAD (≥ 0.15 ng/ml/ml), GS upgrade and higher clinical T staging did not increase the risk to BCR compared to age 3.

Table 3
Univariable and multivariable cox regression model predicting BCR of 229 PCa patients with GS = 7 included

Variable	Category	Univariable			Multivariable (LR)				
		HR	LCI	UCI	P value	HR	LCI	UCI	P value
Ages		0.754	0.331	1.718	0.502				
Preoperative PSA level, ng/ml	≤ 10 vs. >10	1.436	0.612	3.371	0.405				
Prostate volume, ml	< 50 vs. ≥50	0.671	0.227	1.983	0.470				
PSA density, ng/ml/ml	< 0.15 vs. ≥0.15	0.944	0.319	2.792	0.917				
GS Variation	Non-GS Upgrade vs. GS Upgrade	0.970	0.407	2.314	0.945				
Clinical T staging	T1c	1.000	-	-	0.864				
	T2	0.764	0.255	2.288	0.630				
	T3a	1.655	0.184	14.863	0.653				
	T3b	0.954	0.106	8.619	0.967				
Pathological T staging	T2	1.000	-	-	0.001	1.00			0.003
	T3a	5.065	1.627	15.770	0.005	4.254	1.341	13.499	0.014
	T3b	10.116	2.935	34.870	< 0.001	8.989	2.573	31.396	0.001
T staging variation	Non-upstaging vs. Upstaging	3.226	1.263	8.238	0.014				
PSM	No vs. Yes	2.362	1.031	5.411	0.042				
Operation ways	LSRP vs. RARP	2.987	0.881	10.123	0.079				
GS	3 + 4 vs. 4 + 3	2.896	1.251	6.700	0.013	2.491	1.052	5.902	0.038
TGP5	No vs. Yes	2.640	1.143	6.097	0.023	2.36	1.019	5.464	0.045

Discussion

As one of the major male malignant tumors in urology, prostate cancer has gradually surpassed other tumors in incidence in recent years (1). Meanwhile, diversified treatments have also appeared in the management of PCa. Assigning the most suitable treatment for patients depends on the risk stratification of prostate cancer itself. As described in the latest EAU and NCCN

guidelines, prostate cancer is now divided into low, intermediate and high-risk groups, or even more with intermediate risk group being subdivided into unfavorable intermediate-risk group and unfavorable intermediate-risk group. The basis of this classification mainly derived from the acknowledged risk factors related to the prognosis of prostate cancer, for example, preoperative PSA level, Gleason score composition (primary and secondary pattern), clinical and pathological staging (4, 21–23). GS plays an important role in risk classification so as in treatment strategy making. To those patients with non-regional and non-distant metastases prostate cancer, if GS ≤ 6 (low-risk), active surveillance or surgical treatment are both available while if GS ≥ 8 (high-risk localized), multimodal treatments are preferred, for example, radical prostatectomy or radical radiotherapy as initial treatment, androgen deprivation therapy (or radiotherapy) as adjuvant therapy. If GS = 7, either 3 + 4 or 4 + 3, radical prostatectomy or radiotherapy is recommended. However, one should receive adjuvant therapy after RP depends on whether he has lymph node metastases or adverse features, which now include positive surgical margin, seminal vesicle invasion and extracapsular extension (see NCCN). Identifying the risk factors for PCa patients with GS 7 is extremely important because it helps identify the certain group of patients who need adjuvant therapy and can benefit from it maximumly in prolonging survival times, meanwhile helps avoid over treatment and unnecessary side effects for those who don't need adjuvant therapy.

GS was first proposed by Gleason and defined as the sum of primary and secondary patterns of prostate specimens (2, 3), which indicated that prognosis of prostate cancer was mainly decided by its primary and secondary Gleason patterns. Primary Gleason pattern was found to play an even more important role in predicting malignancy degree of PCa with more investigation being conducted. However, researchers were wondering what role tertiary but higher Gleason pattern played in PCa since GS was proposed until Pan C.C., et al. first proposed TGP was also a risk factor to PCa (5).

The main purpose of this study was to decide whether TGP5 was an independent risk factor to PCa with GS 7 in Chinese population and whether clinician should take it into account as adverse feature when assigning further treatment for PCa patients with GS 7 after RP.

There have been many studies published concerning TGP's effects on prostate cancer. Pan, C. C., et al. first brought tertiary Gleason pattern to the public. They analyzed 114 radical prostatectomies with tertiary components (≥5%), which were compared with a prostatectomy database comprised of 2,276 cases without a tertiary component. In their study, GS 7 with TGP5 showed significantly worse pathologic stages than GS 7 without TGP5 and were not different statistically from typical GS 8 (4 + 4) tumors. GS 7 with TGP5 revealed significantly higher progression rates than GS 7 without TGP5 (5). Adam, M., et al. compared 2,396 patients with (22.4%) and 8,260 without (77.5%) a tertiary Gleason pattern for adverse histopathological features (extra-prostatic extension, seminal vesicle invasion, positive surgical margins and lymph node invasion) and analyzed the effect of a tertiary Gleason pattern on biochemical recurrence. They found TGP was statistically significantly associated with all evaluated histopathological parameters and it was an independent predictor of biochemical recurrence (HR 1.43, $p < 0.001$). On subanalysis, TGP independently predicted biochemical recurrence in patients with GS 3 + 4 and 4 + 3 after RP, respectively (6). Borhan, W. et al. analyzed a total of 4060 specimens with a GS 7 with and without TGP5. Cases were subdivided into 3 + 4, 3 + 4 with TGP5, 4 + 3, and 4 + 3 with TGP5. They compared prostate-specific antigen, clinical stage, pathologic stage, and surgical margin status between the groups. The impact of TGP5 on biochemical recurrence was also assessed. They found TGP5 was related to higher PSA level, pathologic stage, positive surgical margin and worse prognostic outcome (8). Although many studies got the same results that TGP5 related to one or more clinicopathological characteristics and PCa outcome (4, 21–23), some studies failed. It remains uncertain that what variables can predict TGP5 and whether 3 + 4 + TGP5 has same prognostic outcome as 4 + 3 without TGP5.

In this study, we retrospectively analyzed 229 patients who met with inclusion criteria and had complete and reliable data from Jan. 2014 to Dec. 2018 in Peking University First Hospital. Clinical and pathological characteristics distribution between different groups with and without TGP5 was being analyzed. Differing from some researches abroad, our study found that TGP5 wasn't related to elder age at diagnosis (≥ 65 years old) and higher preoperative PSA level (≥ 10 ng/ml). Besides, no statistical difference was found between GS 7 without TGP5 and GS 7 with TGP5 when comparing prostate volume, PSAD, GS variation, clinical T staging, pathological T staging, T staging variation, extra-prostatic extension, seminal vesicle invasion and operation ways.

Recently, a study by Jiakun Li et al. from China was published on OncoTargets and Therapy. In their study, they included 350 patients in total from 2009 to 2017, among which ~ 10% (n = 34) had TGP5. They also found that TGP5 wasn't related to elder age

at diagnosis, higher preoperative PSA levels and pathological staging (24). In our study, ~ 25.3% (n = 58) patients had TGP5, which was higher than Jiakun Li's study and also higher than some studies from other countries. Cases were all within 6 years, strictly followed the recommended criteria proposed during ISUP 2014 on TGP5, which meant our study were of more time-effectiveness and reliability. So, even we were surprised by the finding that TGP5 was not related to those variables, we could not fully rule out the possibility that TGP5 was indeed not associated with those variables mentioned above. We believed race and genetics difference might account for and explain it. But given that our sample size was still small, more investigation needs to be done in the future.

To be noted, the PSM rate in the entire cohort was 21.4% and we also found the presence of TGP5 was related to higher PSM with statistical significance (P = 0.002). In GS = 7 with TGP5, PSM rate was 36.2% while in GS = 7 without TGP5, PSM rate was only 16.4%. In Sauter G, et al.'s study, the PSM rates were 16.09%, 13.18% and 48.54% in GS = 7, GS = 7 without and with TGP5, respectively (25). In Li, J. et al.'s study, the PSM rates were 20.17%, 19.81% and 23.53% in GS = 7, GS = 7 without and with TGP5, respectively (24). Some other research also had similar findings that the presence of TGP5 was related to higher PSM rate while there weren't many explanation or discussion about it (8, 16). Compared to these studies with larger population, our comparison results were consistent with them. However, PSM rate was higher in our cohort. Especially for group GS = 4 + 3 with TGP5, PSM rate was even higher as 48.1%. There were many factors including pathological T staging, operation ways, experience of surgeons etc. having impact on PSM rates, it was hard to attribute higher PSM to TGP5 alone. In Zhang Z. et al.'s study, they included 113 PCa patients with GS 7 after laparoscopic RP in Peking University First Hospital from 2016 to 2017 and the PSM rate of PCa patients with GS 7 was 30.97%. The number of positive cores, positive percentage of needle biopsy, and pathological stage were correlated with PSM rate ($P < 0.05$) in their study (26). In our view, TGP5 was related to poorer pathological characteristics, like higher T staging, according to other studies mentioned above (although it was not shown in our study), so PSM occurred more easily when TGP5 was present. But in our cohort, small sample size might be the source of deviation. Further investigation with larger population should be done to validate this.

Biochemical recurrence was the primary endpoint in our study, which is now considered as a sign of prostate cancer relapse. To identify the predictors of BCR is crucial since it will help to select patients needing multimodal therapy while spare the others. In our study, for prostate cancer with GS 7, the incidence of BCR in the group with TGP5 was significantly higher than that in the group without TGP5, indicating TGP5 was a risk factor to PCa. Kaplan-Maier survival analysis and log-rank test validated it with biochemical recurrence-free survival (BFS) curve showing that the presence of TGP5 was negatively correlated with BFS. Univariable cox-regression analysis got the same result that TGP5 was a risk factor to prostate cancer with GS = 7, HR 2.640 (95%CI 1.143–6.097). In univariable cox-regression analysis, GS composition and pathological T staging were also risk factors to PCa prognosis. Given that the composition of GS and pathological T staging might interfere with TGP5 in survival analysis, multivariable cox-regression analysis was also conducted, showing TGP5 was an independent risk factor to BCR, with HR 2.360 (95%CI 1.019–5.464).

This study had many limitations. Firstly, like other retrospective studies, recall bias was the biggest source of bias in this study. Secondly, sample size was small compared to previous studies, with only 229 cases finally entered into our analysis and only n = 23(10%) patients experiencing BCR. However, our cohort was of more time-effectiveness and reliability since the pathological reports of specimen accorded with the latest criteria from 2014 ISUP and variables affecting the accuracy of survival analysis were considered as exclusion criteria. Thirdly, there were some variables not recorded such as pelvic lymph nodes dissection (PLND) during operation. In this cohort, there were only 67 cases (29.26%) experiencing PLND during RP, and all were negative findings. According to the existing studies comparing oncological and non-oncological outcomes between no PLND and PLND, the benefits and harms of PLND during RP still remain controversial (27). Urologists in this hospital also have different opinions on whether performing PLND during RP or not. We finally decided to discard this variable to ensure the accuracy of the survival analysis. But we believed that lymph nodes dissection during RP was theoretically related to the survival of prostate cancer and might affect some results of this study.

We conducted a stratified analysis considering and eliminating the influence of GS composition on prostate cancer. The cohort was divided into 4 groups as 3 + 4 without TGP5, 3 + 4 with TGP5, 4 + 3 without TGP5 and 4 + 3 + TGP5. Results showed that overall differences among 4 groups were found in terms of GS variation, pathological T staging, prostate extracapsular invasion, positive surgical margins, and biochemical recurrence rate. But these differences mainly existed between group 3 + 4 without

TGP5 and group 4 + 3 with TGP5. In 3 + 4/4 + 3 group alone in our study, the existence of TGP5 was not related to poorer clinicopathological features than 3 + 4/4 + 3 without TGP5. K-M survival analysis among 4 groups also showed statistical difference only between group 3 + 4 without TGP5 and group 4 + 3 with TGP5. BFS in group 3 + 4 with TGP5 were not statistically different from group 4 + 3 without TGP5. Whether it indicated that 3 + 4 with TGP5 had same risk as 4 + 3 without TGP5 was worthy of more investigations. Although the stratifying analyses did not find many positive findings or consistent with previous studies, due to the small sample size, this study was still the first stratifying analyses grouped by GS and TGP5 in China, which was the novelty of this study.

While exploring the prognostic effect of TGP on prostate cancer, researchers are also working on how to integrate tertiary Gleason pattern (5) into new grading standard in prostate cancer (with GS 7). Sauter, G., et al. proposed the integrated quantitative Gleason score (IQ-Gleason) to evaluate the risk of prostate cancer. It ranges from 0-117.5 and is calculated as follows: percentage of unfavorable Gleason pattern (Gleason 4 + Gleason 5) + 10 score points if any Gleason 5 pattern was seen + another 7.5 score points in case of Gleason 5 quantities of > 20%. For example, the IQ-Gleason of a Gleason 3 + 4 = 7 cancer with 40% Gleason 4 is 40, the IQ-Gleason of a Gleason 3 + 4 = 7/tertiary grade (TG) 5 cancer with 40% Gleason 4 and 5% Gleason 5 is 40 + 5 + 10 = 55. The IQ-Gleason of a (Gleason 4 + 5 = 9) cancer with 60% Gleason 4 and 40% Gleason 5 is 60 + 40 + 10 + 7.5 = 117.5. This model was published on European Urology and got some initial and promising results (25). Its quantification idea originated from Gleason Score but got more. Other models were also created by different researchers but lack of large population-based validation for now. These models remind that urological pathologists should keep an idea of quantitation when processing and reporting specimens after RP. We are looking forward to establish a new grading system for PCa concerning TGP to assign best treatment for patients.

Conclusion

Based on data from the top medical institution in urology field in China, despite the limitations mentioned above, we still achieved some persuasive conclusions: (1) TGP5 is associated with poorer pathological characteristics like positive surgical margin in prostate cancer specimens but not related to elder age at diagnosis, preoperative PSA level, prostate volume, PSAD, GS variation, clinical T staging, pathological T staging, T upstaging and seminal vesicle invasion in our cohort ; (2) TGP5 is an independent risk predictor of prostate cancer with GS 7, which should be considered when assigning further treatment for these patients after RP in a clinical scenario;(3) Race and genetics difference might exist in TGP5 between China and other countries. More studies with larger population, more possible variables and stratified analysis are needed to conducted in China. It is time to renew the contemporary Grading Group system, with the consideration of TGP and being quantitative.

Abbreviations

PCa, prostate cancer; GS, Gleason score; ISUP, International Society of Urological Pathology; TGP, tertiary gleason pattern; RP, radical prostatectomy; BCR, biochemical recurrence; PSA, prostate specific antigen; IQR, interquartile range; BFS, BCR-free survival; HR, hazard ratios; CIs, confidence indexes; PSAD, prostate specific antigen density; PSM, positive surgical margin; EPE, extra-prostatic extension; SVI, seminal vesicle invasion; LSRP, laparoscopic radical prostatectomy; RARP, robot-assistant laparoscopic radical prostatectomy.

Declarations

1. *Ethics approval and consent to participate*

This project had gained the approval of the Institutional Ethics Committee of Peking University First Hospital.

2. *Consent for publication*

Not applicable.

3. *Availability of data and materials*

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

4. *Competing interests*

The authors declare that they have no competing interests

5. *Funding*

Not applicable.

6. *Authors' contributions*

DC, YF and KG conceptualized and designed this study. DC, ZW acquired the data and did the follow-up. DC, YF and LC analyzed and interpreted the patient data. DC did original draft writing. DC, LC and KG did the draft review and revision. All authors read and approved the final manuscript.

7. *Acknowledgements*

Not applicable.

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Figures

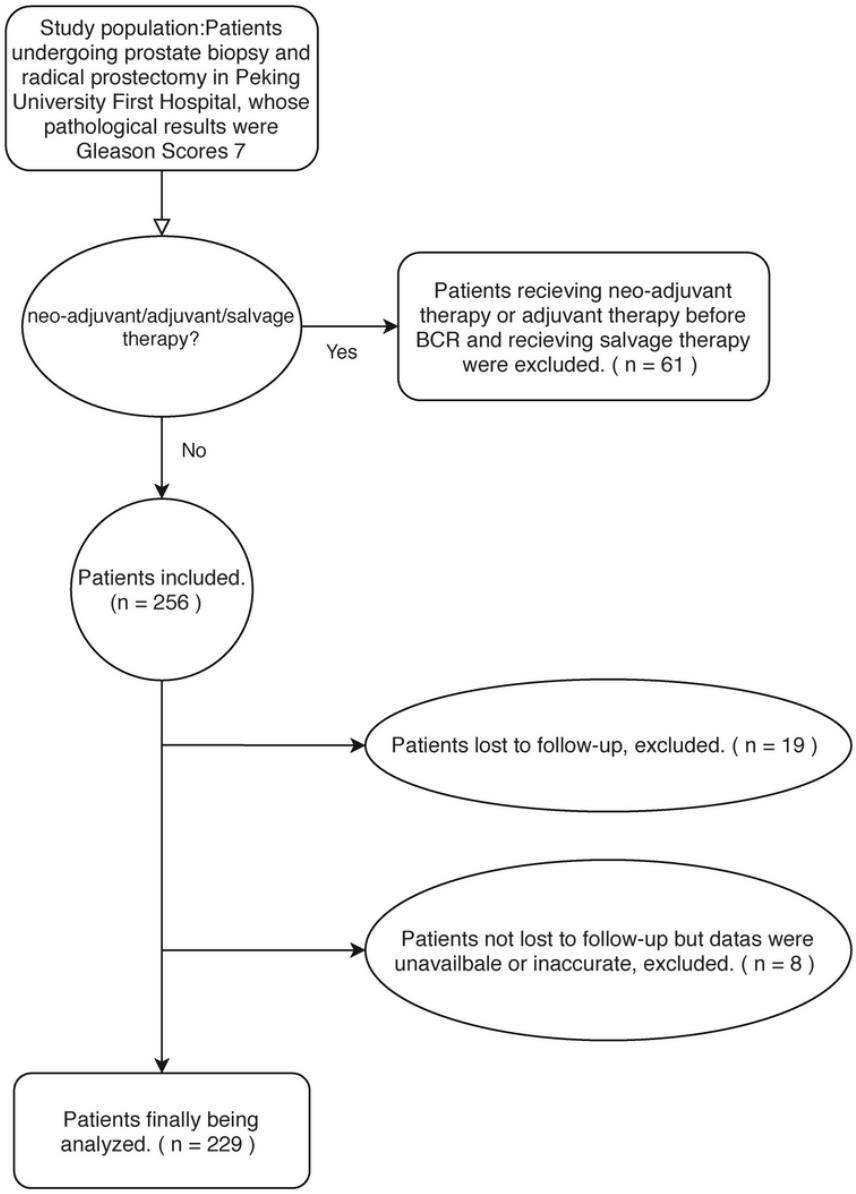

Figure 1

Flow chart of patients who met the study's inclusion/exclusion criteria.

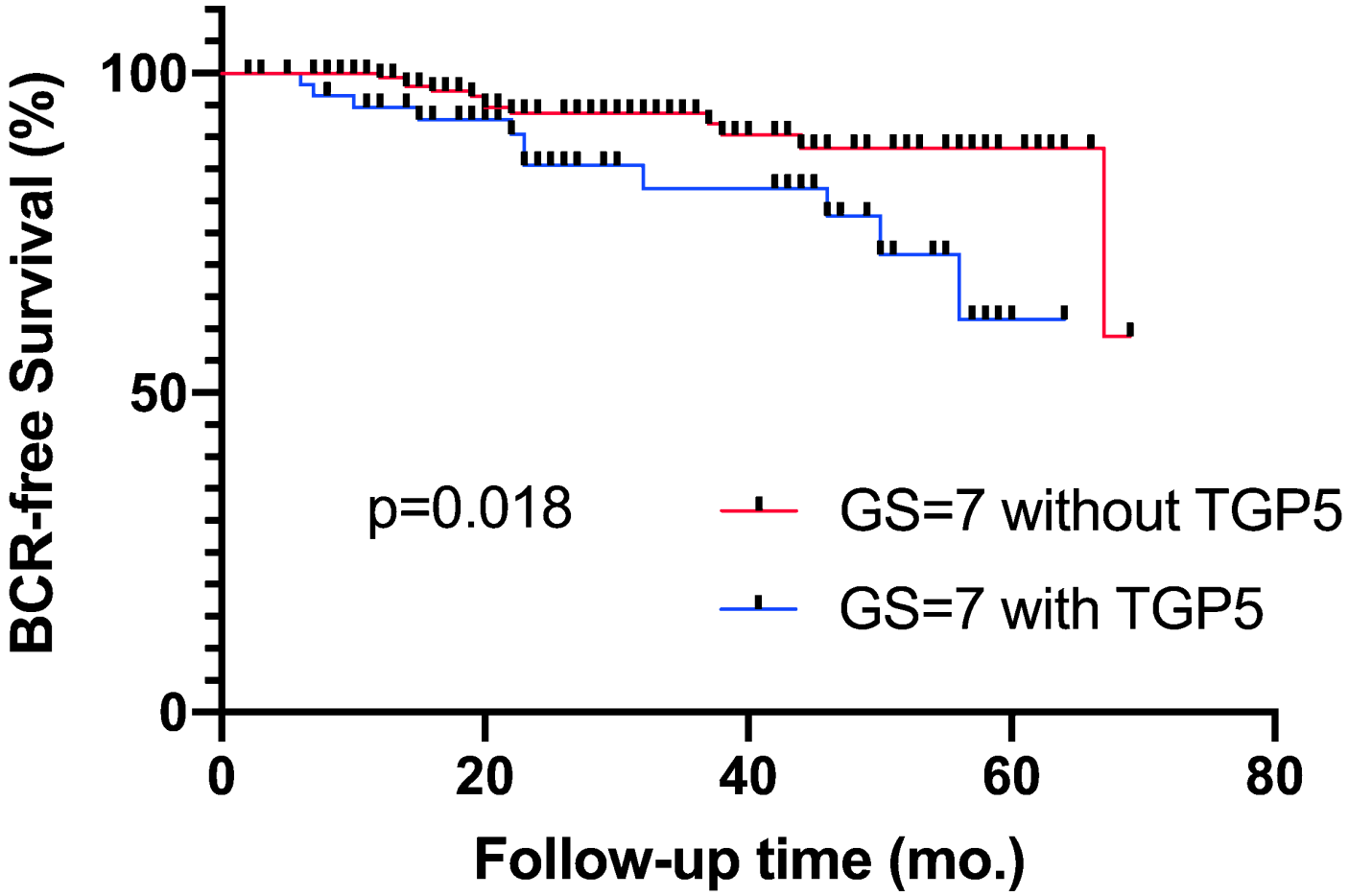

Figure 2

Kaplan-Meier curves showing biochemical recurrence-free survival in patients between two groups.

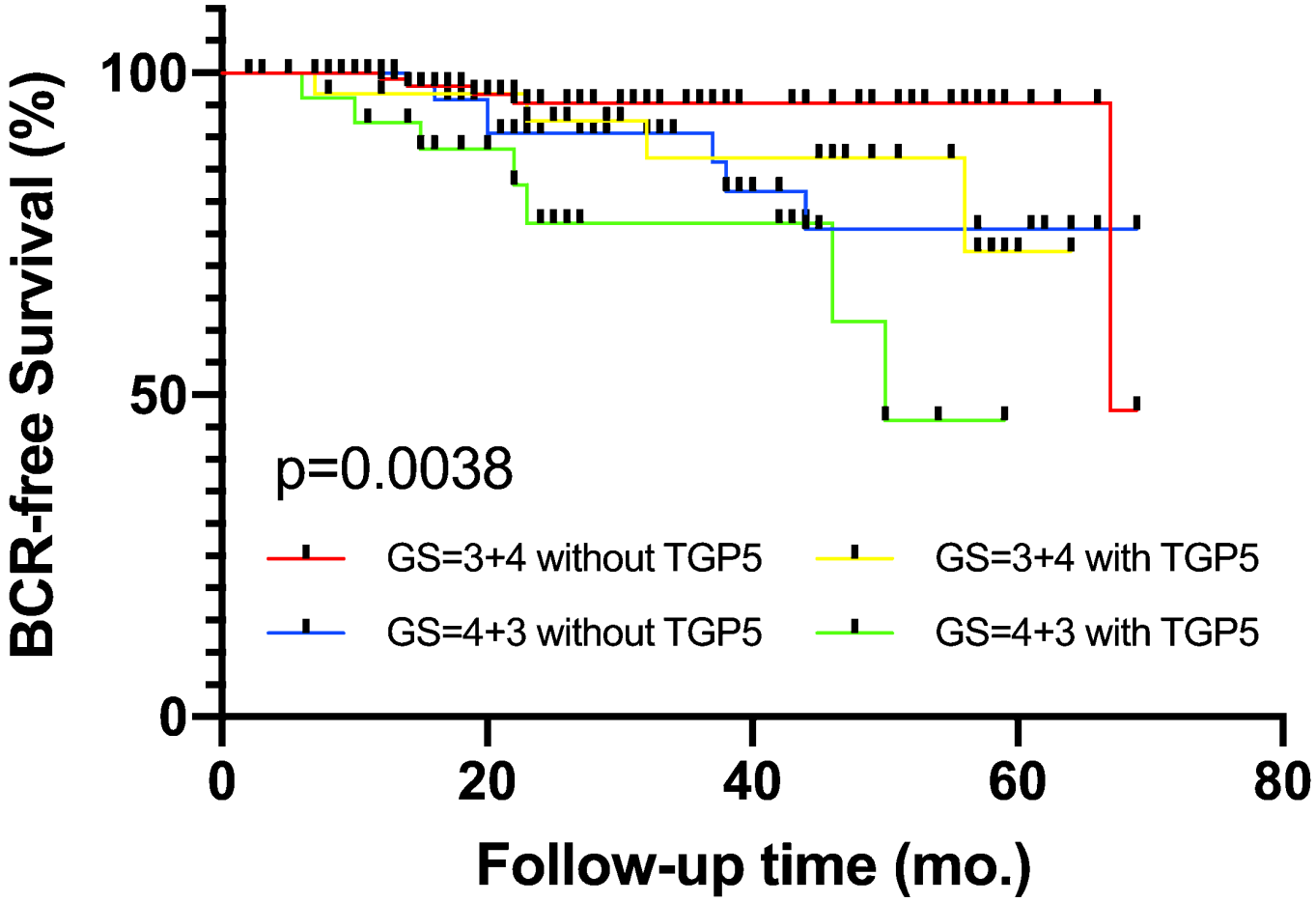

Figure 3

Kaplan-Meier curves showing biochemical recurrence-free survival in patients among four groups.