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ORIGINAL ARTICLE

Prostate Cancer

Clinical application of free/total PSA ratio in the diagnosis of prostate cancer in men over 50 years of age with total PSA levels of 2.0–25.0 ng ml^{-1} in Western China

Xue-Dan Gao[1], Qiang Miao[1], Jun-Long Zhang[1], Jian-Zhao Zhai[1], Xue-Mei Gui[2], Yi-Han Cai[1], Qian Niu[1], Bei Cai[1]

The goal of this study was to investigate the clinical application of free/total prostate-specific antigen (F/T PSA) ratio, considering the new broad serum total PSA (T-PSA) "gray zone" of 2.0–25.0 ng ml^{-1} in differential diagnosis of prostate cancer (PCa) and benign prostate diseases (BPD) in men over 50 years in Western China. A total of 1655 patients were included, 528 with PCa and 1127 with BPD. Serum T-PSA, free PSA (F-PSA), and F/T PSA ratio were analyzed. Receiver operating characteristic curves were used to assess the efficiency of PSA and F/T PSA ratio. There were 47.4% of cancer patients with T-PSA of 2.0–25.0 ng ml^{-1}. When T-PSA was 2.0–4.0 ng ml^{-1}, 4.0–10.0 ng ml^{-1}, and 10.0–25.0 ng ml^{-1}, the area under the curve (AUC) of F/T PSA ratio was 0.749, 0.769, and 0.761, respectively. The best AUC of F/T PSA ratio was 0.811 when T-PSA was 2.0–25.0 ng ml^{-1}, with a specificity of 0.732, a sensitivity of 0.788, and an optimal cutoff value of 15.5%. The AUC of F/T PSA ratio in different age groups (50–59 years, 60–69 years, 70–79 years, and ≥80 years) was 0.767, 0.806, 0.815, and 0.833, respectively, and the best sensitivity (0.857) and specificity (0.802) were observed in patients over 80 years. The T-PSA trend was in accordance with the Gleason score, tumor node metastasis (TNM) stage, and American Joint Committee on Cancer prognosis group. Therefore, the F/T PSA ratio can facilitate the differential diagnosis of PCa and BPD in the broad T-PSA "gray zone". Serum T-PSA can be a Gleason score and prognostic indicator.

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Keywords: free/total prostate-specific antigen ratio; gray zone prostate-specific antigen; prostate cancer; total prostate-specific antigen

INTRODUCTION

Prostate cancer (PCa) is a common malignancy in men worldwide, and it is characterized by a relatively high mortality rate.[1] Globally, prostate cancer is the second most frequent cancer among men in the global cancer statistics 2018, with a rate of 13.5%, lagging behind lung cancer (a rate of 14.5%).[1] Furthermore, PCa is the fifth leading cause of cancer-related mortality in men (6.7%). It is also the most frequently diagnosed cancer in 105 countries, particularly in the America, Europe, Oceania, and the sub-Saharan Africa region.[1] Moreover, the incidence of PCa in China has been increasing from 4.62/100 000 in 2000 to 21.62/100 000 in 2014 with 11.5% of average annual percent change (95% confidence interval [CI]: 10.3%–12.7%), which can be attributed to a number of factors, including the aging population, economic and lifestyle changes, improved medical care, and growing popularity of health screening programs.[2] Prostate-specific antigen (PSA) is still one of the most common serum tumor markers (sTM) for the early detection of PCa and an important parameter in guiding biopsy decisions. However, since some conditions such as benign prostate diseases (BPD) and digital rectal examination could induce an increase in serum total PSA (T-PSA), serum T-PSA measurements show low specificity in PCa diagnosis, especially in the "diagnostic gray zone", leading to unnecessary biopsies and overdiagnosis. However, the range of the "gray zone" T-PSA is not uniformly agreed upon and therefore remains controversial. The majority of studies consider the range of serum T-PSA of 4.0–10.0 ng ml^{-1} as "gray zone"; however, Catalona *et al.*[3] demonstrated that 22% of patients with serum T-PSA levels between 2.6 ng ml^{-1} and 4.0 ng ml^{-1} were finally diagnosed with PCa on biopsy. Other "gray zone" ranges have also been proposed, such as 2.0–10.0 ng ml^{-1} and a hypothetical higher Chinese T-PSA "gray zone" of 10.1–20.0 ng ml^{-1} related to the lower incidence of PCa.[4,5]

Clinically, the distinction between PCa and BPD in the context of T-PSA "gray zone" is of crucial importance. Several studies have shown that free/total PSA (F/T PSA) ratio could be a valuable marker for the differential diagnosis of PCa, as it is characterized by good accuracy and specificity, especially in the T-PSA "gray zone".[6–8] Free PSA(F-PSA),

[1]Department of Laboratory Medicine/Research Center of Clinical Laboratory Medicine, West China Hospital of Sichuan University, Chengdu 610041, China; [2]Department of Laboratory Medicine, Nan'an Chinese Medicine Hospital, Chongqing 400060, China.
Correspondence: Dr. Q Niu (niuqian991@126.com) or Dr. B Cai (evacaieyou@126.com)
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a form of unbound PSA, and combined PSA constitute the T-PSA. The lower the F/T PSA ratio, the higher the probability of being diagnosed with PCa. Recently, the T-PSA "gray zone" range was proposed to be broader. Moreover, it remains unclear whether F/T PSA ratio could play a critical role in differentiating PCa outside of the classical T-PSA "gray zone" of 4.0–10.0 ng ml^{-1}. Hence, the current study aimed at exploring the clinical significance of serum F/T PSA ratio in the context of a broad serum T-PSA "gray zone" of 2.0–25.0 ng ml^{-1} in differential diagnosis of PCa and BPD in men in Western China and defining the cutoff value of the F/T PSA ratio.

PATIENTS AND METHODS

Patients

This study retrospectively included 1655 patients with prostate diseases registered between January 2011 and September 2016 at the West China Hospital of Sichuan University (Chengdu, China). Five hundred and twenty-eight of these patients were diagnosed with PCa and 1127 patients with BPD (including benign prostatic hypertrophy and prostatitis), according to the prostate biopsy results or clinical data. The inclusion criteria were as follows: (a) patients diagnosed with PCa on the basis of the transrectal ultrasound (TRUS)-guided systematic prostatic biopsy results or clinical diagnosis; (b) patients with BPD diagnosed through noninvasive ultrasound or biopsy results, and via assessments of clinical characteristics and PSA levels with/without biopsy; (c) patients with prostate diseases as primary diagnoses; and (d) patients whose data were obtained from an initial diagnosis, and not subjected to any treatment. Patients with kidney diseases or other male genitourinary system diseases were excluded. Patients with PCa were categorized according to the tumor node metastasis (TNM) classification of malignant tumors, Gleason score, and the American Joint Committee on Cancer (AJCC) prognostic group criteria.[9] In this study, the prognostic groups were simplified into four groups, as shown in **Table 1**. The clinical information is also detailed in **Table 1**. The study protocol was reviewed and approved by the Ethics Committee of West China Hospital of Sichuan University (Approval No. 2020.823) with the need for informed consent waived off, and the study was conducted according to the principles expressed in the Declaration of Helsinki.

Serum T-PSA and F-PSA detection

Blood samples were collected prior to prostate biopsies or surgeries in patients without digital rectal examination, catheterization, cystoscopy of the urethra, or prostate injury. The serum was separated within 3 h after blood drawing. Serum T-PSA and F-PSA were simultaneously detected on a Roche Elecsys MODULAR E170 analyzer using corresponding reagent kits (Roche Diagnostics GmbH, Mannheim, Germany), according to the manufacturer's instructions. All samples were detected as routine clinical samples, and Lyphochek Tumor Marker Plus Control, Level 1 and Level 3 (Bio-Rad Laboratories, Hercules, CA, USA) were used to conduct an internal quality control.

Statistical analyses

All data were analyzed using the IBM SPSS 20.0 software (IBM Corp., Armonk, NY, USA). Normal and nonnormal data are presented as mean ± standard deviation and median with interquartile range, respectively. Group comparisons were performed using the Student's *t*-test or Mann–Whitney U test. Receiver operating characteristic (ROC) curves were used to evaluate the diagnostic performance of serum T-PSA, F-PSA, and F/T PSA ratio. The cutoff value was defined using the Youden index. Spearman's correlation was used to detect associations. Statistical significance was set at $P < 0.05$.

RESULTS

Basic clinical characteristics of patients with prostate diseases

Regardless of the PCa or BPD diagnosis, the patients were mainly 60–80 years old, and this age group constituted 80.1% and 81.3% of the PCa and BPD patients, respectively. In patients with PCa, 57.8% had serum T-PSA <25.0 ng ml^{-1}, and 47.4% had serum T-PSA ≥2.0 ng ml^{-1} and <25.0 ng ml^{-1}. Among patients with BPD, 73.0% had serum T-PSA ≥2.0 ng ml^{-1} and <25.0 ng ml^{-1}.

PCa patients with TNM stages I/II and III accounted for 74.0% of the cohort. Patients with Gleason grades 2 and 3 categorized by AJCC were the most common (25.9% and 29.4%, respectively) among the PCa patients who underwent prostate biopsy. According to the AJCC prognostic guidelines, patients with PCa were divided into four groups. More than 70% of patients were in group III or IV, which accounted for 53.0% and 27.0% of the cohort, respectively (**Table 1**).

Table 1: Characteristics of the study population

Characteristic	*PCa (n=528)*	*BPD (n=1127)*	*P*
Age (year)[a]	69.3±8.1	70.2±7.8	0.050
<50[c]	5 (0.9)	6 (0.5)	0.231[d]
50–59[c]	52 (9.8)	81 (7.2)	
60–69[c]	198 (37.5)	415 (36.8)	
70–79[c]	225 (42.6)	502 (44.5)	
≥80[c]	48 (9.1)	123 (10.9)	
T-PSA (ng ml^{-1})[b]	20.2 (7.7–58.2)	4.5 (2.2–9.3)	<0.001
<2.0[c]	55 (10.4)	242 (21.5)	<0.001[d]
≥2.0 and <4.0[c]	21 (4.0)	280 (24.8)	
≥4.0 and <10.0[c]	87 (16.5)	347 (30.8)	
≥10.0 and <25.0[c]	142 (26.9)	196 (17.4)	
≥25.0 and <50.0[c]	77 (14.6)	46 (4.1)	
≥50.0 and <100.0[c]	54 (10.2)	15 (1.3)	
≥100.0[c]	92 (17.4)	1 (0.1)	
TNM stage (*n*=100)			
I or II group (localized limited)[c]	32 (32.0)	-	
III group (localized advanced)[c]	42 (42.0)	-	
IV group 1 (regional metastasis)[c]	10 (10.0)	-	
IV group 2 (distant metastasis)[c]	16 (16.0)	-	
AJCC grade group (*n*=394)			
Grade 1 (GS≤6)[c]	24 (6.1)	-	
Grade 2 (GS=3+4)[c]	102 (25.9)	-	
Grade 3 (GS=4+3)[c]	116 (29.4)	-	
Grade 4 (GS=8)[c]	60 (15.2)	-	
Grade 5 (GS≥9)[c]	92 (23.4)	-	
AJCC prognosis stage group[e] (*n*=100)			
I stage[c]	3 (3.0)	-	
II stage[c]	17 (17.0)	-	
III stage[c]	53 (53.0)	-	
IV stage[c]	27 (27.0)	-	

[a]Data were described with mean±standard deviation; [b]data were described with median and interquartile range; [c]data were described with number (*n*) and percentage (%); [d]*P* value of composition analysis of age and serum T-PSA; [e]AJCC prognosis group is classified by combining TNM, T-PSA level, and AJCC grade group. I stage: T1–2N0M0 (except cT2b-c), AJCC grade 1, and T-PSA <10 ng ml^{-1}; II stage: T1–2N0M0, AJCC grade 2–4, and T-PSA <20 ng ml^{-1}, or T1–2N0M0, AJCC grade 1, and T-PSA >10 ng ml^{-1} and <20 ng ml^{-1}, or cT2b–cN0M0, AJCC grade 1, and T-PSA <20 ng ml^{-1}; III stage: T3–4, AJCC grade 5, and T-PSA ≥20 ng ml^{-1}; IV stage: T1–4N1/M1. PCa: prostate cancer; BPD: benign prostate disease; T-PSA: total prostate-specific antigen; AJCC: American Joint Committee on Cancer; GS: Gleason score; -: not applicable

Serum T-PSA, F-PSA, and F/T PSA ratio in patients with prostate diseases

Serum T-PSA and F-PSA levels in PCa patients were both higher than those in BPD patients, while the F/T PSA ratio was higher in BPD patients (**Figure 1a**). **Figure 1b** shows that the ratios of F/T PSA in the PCa group were significantly lower than those in the BPD group with T-PSA <25.0 ng ml^{-1} (all $P < 0.05$). However, no difference in F/T PSA ratios between PCa and BPD patients was found when T-PSA ≥25.0 ng ml^{-1}. Furthermore, both in PCa and BPD patients, the F/T PSA ratio gradually decreased with increasing T-PSA levels.

Clinical efficiency of F/T PSA ratio for differential diagnosis of PCa in the 2.0–25.0 ng ml^{-1} T-PSA range

ROC curves were applied to evaluate the clinical significance of T-PSA, F-PSA, and F/T PSA ratio in all patients and in patients with different T-PSA levels (<2.0 ng ml^{-1}, ≥2.0 ng ml^{-1} and <4.0 ng ml^{-1}, ≥4.0 ng ml^{-1} and <10.0 ng ml^{-1}, ≥10.0 ng ml^{-1} and <25.0 ng ml^{-1}, ≥25.0 ng ml^{-1} and <50.0 ng ml^{-1}, and ≥50.0 ng ml^{-1} and <100.0 ng ml^{-1}). F/T PSA ratio had a better diagnostic performance (AUC = 0.791, $P < 0.001$) in facilitating the diagnosis of PCa in all of the patients, compared with T-PSA and F-PSA (**Figure 1c**). The AUCs of F/T PSA ratios in patients with T-PSA <2.0 ng ml^{-1} and ≥25.0 ng ml^{-1} were all lower than

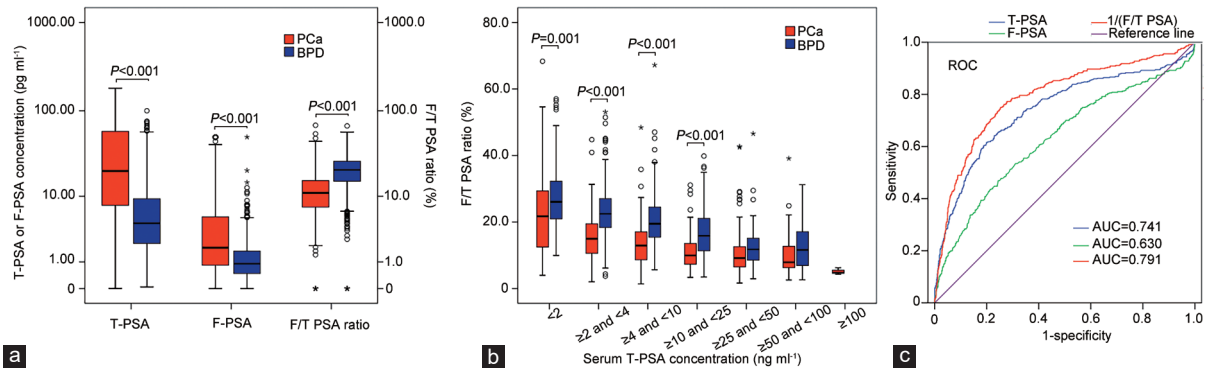

Figure 1: Serum T-PSA, F-PSA, and F/T PSA ratio analysis in patients with prostate diseases. (**a**) Serum T-PSA, F-PSA, and F/T PSA ratio in patients with PCa and BPD. (**b**) The F/T PSA ratio in groups with different T-PSA ranges. (**c**) ROC analysis of T-PSA, F-PSA, and F/T PSA ratio in differentiating PCa from BPD in the whole population. T-PSA: total prostate-specific antigen; F-PSA: free prostate-specific antigen; PCa: prostate cancer; BPD: benign prostate disease; ROC: receiver operating characteristic; AUC: area under the curve; F/T PSA: free/total prostate-specific antigen.

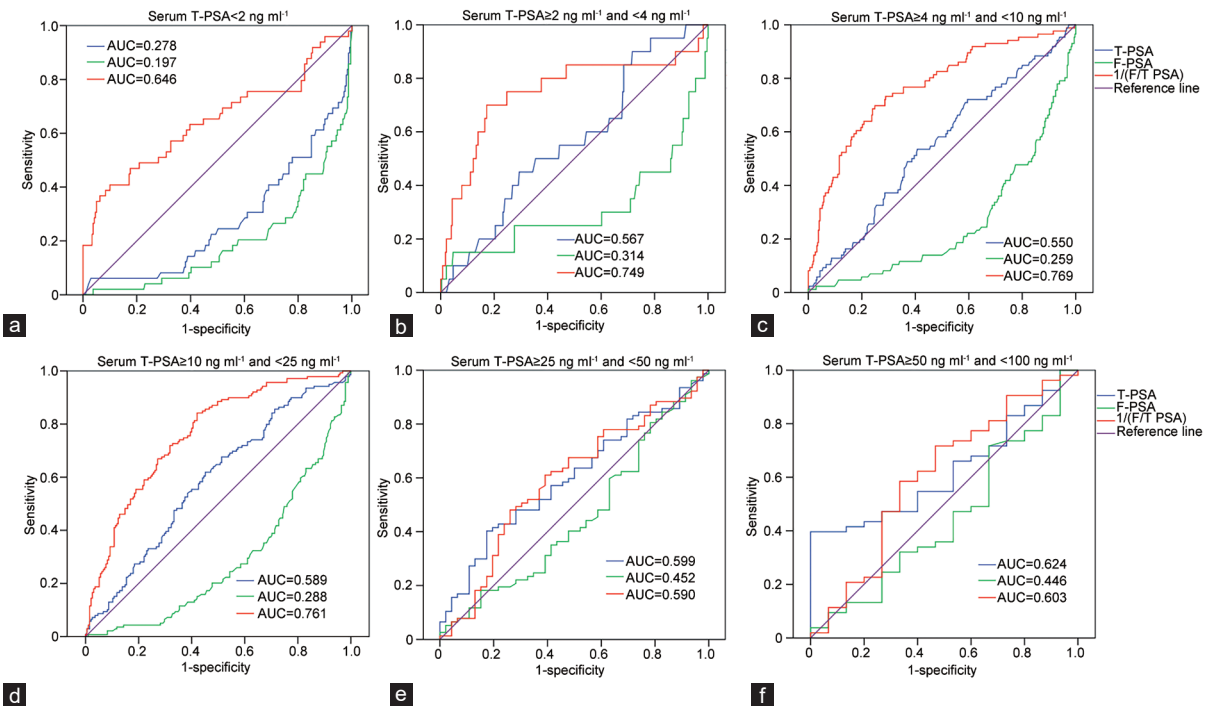

Figure 2: ROC analysis of T-PSA, F-PSA, and F/T PSA ratio in different T-PSA ranges. ROC analysis of T-PSA, F-PSA, and F/T PSA ratio in serum T-PSA (**a**) <2.0 ng ml^{-1}, (**b**) ≥2.0 ng ml^{-1} and <4.0 ng ml^{-1}, (**c**) ≥4.0 ng ml^{-1} and <10.0 ng ml^{-1}, (**d**) ≥10.0 ng ml^{-1} and <25.0 ng ml^{-1}, (**e**) ≥25.0 ng ml^{-1} and <50.0 ng ml^{-1}, and (**f**) ≥50.0 ng ml^{-1} and <100.0 ng ml^{-1}, respectively. T-PSA: total prostate-specific antigen; AUC: area under the curve; F-PSA: free prostate-specific antigen; ROC: receiver operating characteristic; F/T PSA: free/total prostate-specific antigen.

0.700 (**Figure 2a** and **2e–2f**). However, the AUC of F/T PSA ratio was strikingly larger than those of T-PSA and F-PSA in the T-PSA ≥2.0 ng ml^{-1} and <4.0 ng ml^{-1} (AUC = 0.749), ≥4.0 ng ml^{-1} and <10.0 ng ml^{-1} (AUC = 0.769), ≥10.0 ng ml^{-1} and <25.0 ng ml^{-1} (AUC = 0.761), as shown in **Figure 2b–2d**. And the AUC of F/T PSA ratio can get to 0.811 in patients with T-PSA ≥2.0 ng ml^{-1} and <25.0 ng ml^{-1}. Meanwhile, the optimal cutoff, sensitivity, specificity, and positive/negative predictive value (PPV/NPV) of F/T PSA ratio when T-PSA ≥2.0 ng ml^{-1} and <25.0 ng ml^{-1} were calculated and are shown in **Table 2**.

Age-dependent changes of serum T-PSA in PCa and BPD patients

In all age groups, the T-PSA level of PCa was significantly higher than that of BPD, and the F/T PSA ratio in PCa patients was strikingly lower than that in BPD patients. In patients with BPD or PCa, the T-PSA level and F/T PSA ratio both gradually increased with age, except for those aged <50 years (**Supplementary Figure 1**).

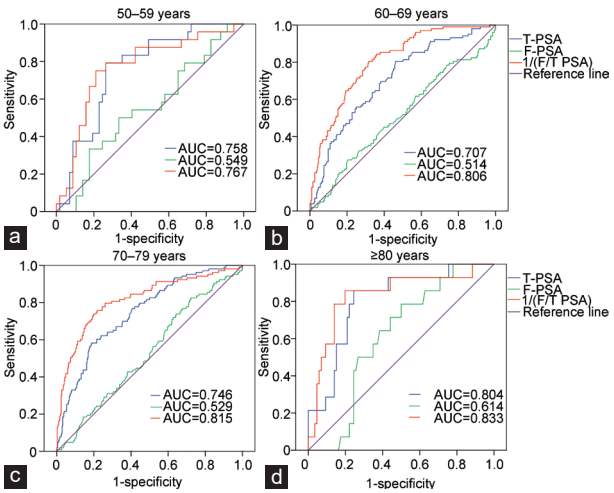

Figure 3: ROC analysis of T-PSA, F-PSA, and F/T PSA ratio for serum T-PSA ≥2.0 ng ml^{-1} and <25.0 ng ml^{-1} in different age groups. ROC analysis of T-PSA, F-PSA, and F/T PSA ratio for (**a**) 50–59 years, (**b**) 60–69 years, (**c**) 70–79 years, and (**d**) age ≥80 years, respectively. ROC: receiver operating characteristic; T-PSA: total prostate-specific antigen; F-PSA: free prostate-specific antigen; F/T PSA: free/total prostate-specific antigen; AUC: area under the curve.

Diagnostic efficiency of the F/T PSA ratio in the 2.0–25.0 ng ml^{-1} T-PSA range in different age groups

All patients were stratified into five age groups (<50 years, 50–59 years, 60–69 years, 70–79 years, and ≥80 years), and ROC curve analyses were performed for each group in which serum T-PSA was in the 2.0–25.0 ng ml^{-1} range (there were only 5 cases in the <50 years' age group, so the ROC curve analysis was not performed). The AUC of F/T PSA ratio in the other four age groups was 0.767, 0.806, 0.815, and 0.833, respectively. The optimal cutoff of the F/T PSA ratio in the 50–59 years group was the lowest among the four age groups, but the optimal cutoff of the F/T PSA ratio in patients over 60 years of age was approximately 15.5% (**Figure 3** and **Table 2**).

The sensitivity and specificity of the F/T PSA ratio at different cutoff levels in various age groups were calculated and are shown in **Table 2**. The sensitivity of F/T PSA ratio in the four age groups was all above 0.750, and the specificity of F/T PSA ratio ranged between 0.684 and 0.802. Obviously, the over 80 years' age group was characterized by the best sensitivity (0.857) and specificity (0.802). In the four age groups, the NPV of the F/T PSA ratio was over 88%.

Association of serum T-PSA, F-PSA concentration, or F/T PSA ratio with Gleason score, TNM stage, and AJCC prognosis category

We found that with increasing Gleason score or TNM stage and AJCC prognosis group, the serum T-PSA and F-PSA levels were increasing, while the F/T PSA ratio did not change in a regular

Table 2: Diagnostic efficiency of F/T PSA ratio in 2.0–25.0 ng ml^{-1} T-PSA and in different age groups

Parameter	*Cutoff value (%)*	*Sensitivity*	*Specificity*	*PPV (%)*	*NPV (%)*
T-PSA (ng ml^{-1})					
≥2.0 and <4.0	16.4	0.700	0.828	22.6	97.5
≥4.0 and <10.0	15.4	0.698	0.751	41.1	90.9
≥10.0 and <25.0	14.6	0.842	0.579	58.8	83.7
≥2.0 and <25.0	15.5	0.788	0.732	46.7	92.0
Age (year)					
50–59	10.6	0.750	0.789	60.0	88.2
60–69	15.5	0.804	0.684	46.1	91.2
70–79	15.5	0.757	0.782	49.1	92.1
≥80	15.8	0.857	0.802	41.4	97.2

F/T PSA: free/total prostate-specific antigen; T-PSA: total prostate-specific antigen; PPV: positive predictive value; NPV: negative predictive value

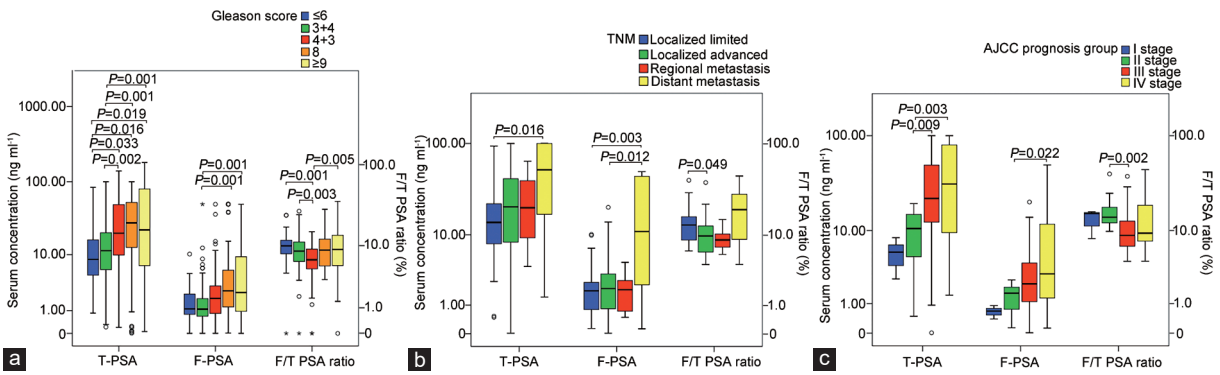

Figure 4: Analysis of serum T-PSA, F-PSA concentration, and F/T PSA ratio in patients with different (**a**) Gleason scores and (**b**) TNM stages, and (**c**) in AJCC prognosis groups. T-PSA: total prostate-specific antigen; F-PSA: free prostate-specific antigen; F/T PSA: free/total prostate-specific antigen; AJCC: American Joint Committee on Cancer; TNM: tumor node metastasis.

manner (**Figure 4**). Further correlation analysis also showed that serum T-PSA and F-PSA levels were both associated with Gleason score, TNM stage, and AJCC prognosis category, with Spearman correlation coefficients of 0.243, 0.273, and 0.363 for T-PSA and 0.230, 0.272, and 0.334 for F-PSA, respectively (all $P < 0.05$; **Supplementary Table 1**).

DISCUSSION

Serum T-PSA is widely used as an assisting parameter in identification of benign versus malignant prostate diseases and as a reference indicator for biopsy recommendation. When T-PSA level is in the "gray zone" range, it has a low negative and positive predictive value due to its poor specificity, which may result in unnecessary biopsies and wrong diagnoses.[10] However, the application of the F/T PSA ratio in the "gray zone" of T-PSA has been shown to be helpful in increasing the diagnostic efficiency. Here, we found that serum T-PSA may have a wider "gray zone" range (≥2.0 ng ml^{-1} and <25.0 ng ml^{-1}). Hence, defining the new Chinese T-PSA "gray zone" and cutoff values of the F/T PSA ratio in the T-PSA "gray zone" is of paramount importance for accurate differentiation of PCa from BPD.

We found that the F/T PSA ratio had the most satisfying diagnostic efficacy for PCa, with an AUC value over 0.700 when the T-PSA level was in the range of 2.0–25.0 ng ml^{-1}, while no superiority of F/T PSA ratio was found in PCa prognosis when the serum T-PSA <2.0 ng ml^{-1} and ≥25.0 ng ml^{-1}. Therefore, the F/T PSA ratio can be considered a better auxiliary diagnostic parameter for PCa than T-PSA in the "gray zone" of T-PSA. Interestingly, we observed that when T-PSA was lower than 2.0 ng ml^{-1}, approximately 10% of patients were diagnosed with PCa. Does this mean that there is a distinct PCa type which does not abnormally secrete PSA? There is no clear answer yet. Nevertheless, there is an infrequent type of neuroendocrine prostate cancer (NEPC) which accounts for about 5%–10% of PCa cases, characterized by variable T-PSA levels, which can even be in a normal range. NEPC includes a small-cell variant, which represents an aggressive and hormone-resistant malignancy.[11,12] Here, we hypothesize that different types of PCa may be characterized by different T-PSA levels.

Furthermore, an 18-year prospective randomized screening trial by Frånlund *et al.*[13] revealed that an initial T-PSA below 3.0 ng ml^{-1} was strongly associated with the risk of PCa diagnosis and related death. Some men aged 50–66 years with serum T-PSA of 1.0–3.0 ng ml^{-1} could therefore benefit from an earlier diagnosis by T-PSA screening or a costly prebiopsy magnetic resonance imaging (MRI), but F/T PSA ratio has a low predictive value if initial serum T-PSA level is within the 1.0–3.0 ng ml^{-1} range. Here, we showed that for serum T-PSA <2.0 ng ml^{-1}, the F/T PSA ratio had no diagnostic significance, and for serum T-PSA in 2.0–4.0 ng ml^{-1}, 4.0–10.0 ng ml^{-1}, 10.0–25.0 ng ml^{-1}, and 2.0–25.0 ng ml^{-1}, the F/T PSA ratio had a good diagnostic significance with an AUC of 0.749, 0.769, 0.761, and 0.811 and a cutoff value of 16.4%, 15.4%, 14.6%, and 15.5%, respectively, and this observation is consistent with other studies.[7,14,15]

A linear relationship between serum T-PSA concentration and age has been reported.[16] With increasing age, the T-PSA concentration tends to increase gradually, which was also observed in our study. However, there is still no consensus on the application of the reference range of T-PSA concentration divided by age in clinical practice. Surprisingly, the AUC value of F/T PSA ratio increased with age in our study (0.767, 0.806, 0.815, and 0.833 in the four age groups, respectively), which indicated that the diagnostic efficiency of F/T PSA ratio could become increasingly high for older men. In patients aged over 80 years, the sensitivity and specificity of F/T PSA ratio to differentiate PCa both exceeded 0.800 in our study. Paradoxically, guidelines from the European Association of Urology,[17] American Urological Association,[18] and US Preventive Services Task Force[19] all recommend PSA-based screening for men aged between 55 years and 69 years, which is associated with the prevalence of a lower stage disease and a reduction in cancer-specific mortality, but not recommend routine PSA screening for men aged over 70 years or with less than 10–15 years of life expectancy, due to unexpected overdiagnoses and overtreatment.[20] Therefore, the clinical application of T-PSA and F/T PSA ratio screening to identify PCa is thought to be associated with the age of patients and other factors that could influence their life expectancy.

The current study found that the cutoff value of F/T PSA ratio in the T-PSA gray zone of 2.0–25.0 ng ml^{-1} was 10.58, 15.50, 15.50, and 15.80 at age groups of 50–59 years, 60–69 years, 70–79 years, and ≥80 years, respectively. Similar results were found in a study in the Turkish population.[21] However, studies on changes of the F/T PSA ratio associated with aging are rare. Here, we found that the optimal cutoff of the F/T PSA ratio in the T-PSA gray zone of 2.0–25.0 ng ml^{-1} and in the 50–59 years' age group was strikingly lower than that in other age groups. However, establishing an age-dependent cutoff value of F/T PSA ratio, especially for patients <60 years, should be confirmed by large sample studies or multicenter studies. A cutoff F/T PSA ratio of over 15.5% can facilitate the diagnosis of PCa in men over 60 years in Western China, with T-PSA in the "gray zone" of 2.0–25.0 ng ml^{-1}.

Since the 1990s, the Gleason score, PSA level, and TNM grade have been considered as the most important indicators of therapeutic regimens, according to the National Comprehensive Cancer Network (NCCN) guidelines. The Gleason score has also been included in the 2016 edition of the WHO Urological and Male Reproductive System Tumor Classification.[22] The AJCC established a prognostic staging system of PCa based on the PSA level, Gleason score, and TNM grade.[9] Hence, we conducted a relationship analysis between serum PSA levels and PCa prognosis. We found that patients with AJCC prognosis stages III and IV (poor prognosis) accounted for 80% of all patients. However, this observation was inconsistent with the fact that the proportion of PCa patients with T-PSA >25.0 ng ml^{-1} was lower than half (42.4%), which suggested that nearly half of the patients had a delayed diagnosis related to T-PSA "gray zones" or even lower T-PSA levels. Consequently, in the T-PSA range of 2.0–25.0 ng ml^{-1}, the cutoff value of F/T PSA ratio as an auxiliary diagnostic indicator can increase the diagnostic accuracy of PCa patients. Our results clearly demonstrated that both T-PSA and F-PSA trends were in accordance with the Gleason score, TNM stage, and AJCC prognosis. The T-PSA level increased with Gleason score, TNM stage, and AJCC prognosis categories (**Figure 4**). Moreover, the Spearman correlation coefficient between the T-PSA and different disease condition classification groups was higher than that between the F-PSA and different classification groups (**Supplementary Table 1**). Therefore, serum T-PSA, rather than F/T PSA ratio, was more suitable for predicting the Gleason score, TNM stage, and AJCC prognosis.

The current study is not free from certain limitations. First, this retrospective study relied on data collected from a single center, with a small sample size. Second, a patient selection bias might have occurred. Therefore, further prospective studies on larger patient samples are necessary to verify the findings presented hereby.

CONCLUSIONS

The F/T PSA ratio can be used as an accurate and specific PCa diagnostic parameter in a new broad T-PSA "gray zone" of 2.0–25.0 ng ml^{-1}. In men aged 50 years or more in Western China, the F/T PSA ratio has a specificity of 0.732, a sensitivity of 0.788, and an optimal cutoff value

of 15.5%. In PCa patients, serum T-PSA levels are more suitable in auxiliary assessing malignancy, tumor stage, and related prognosis.

AUTHOR CONTRIBUTIONS

BC and QN contributed to the conception of the study and revised the manuscript. XDG performed data acquisition, data analyses, and wrote the manuscript. QM and JLZ assisted with the analysis and provided constructive input during discussions. JZZ, XMG, and YHC contributed to data acquisition, data interpretation, and manuscript preparation. All authors read and approved the final version.

COMPETING INTERESTS

All authors declare no competing interests.

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Supplementary Information is linked to the online version of the paper on the *Asian Journal of Andrology* website.

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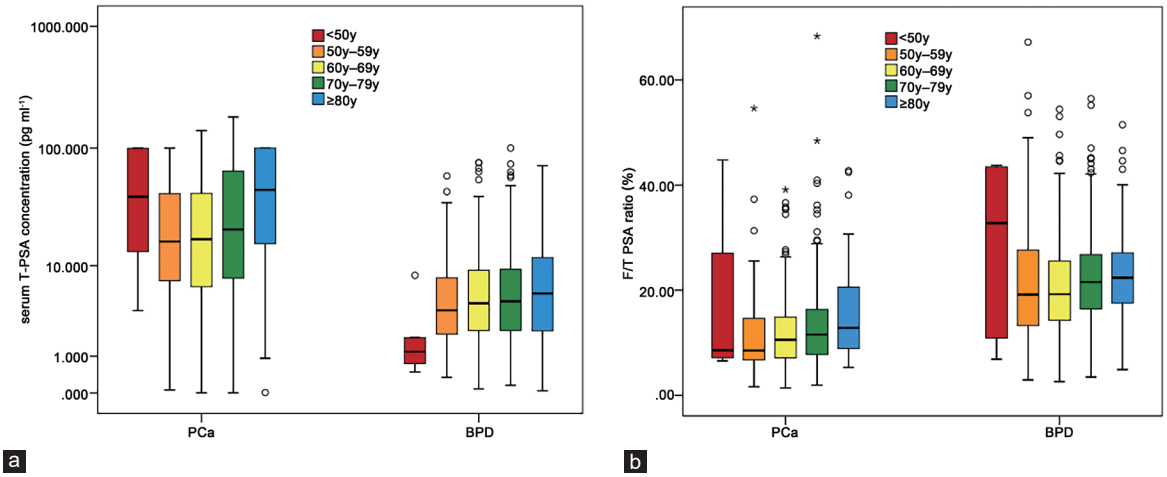

Supplementary Figure 1: Age-associated changes in serum T-PSA levels (**a**) and F/T PSA ratio (**b**) in PCa and BPD patients. PCa: prostate cancer; BPD: benign prostate disease; T-PSA: total prostate-specific antigen; F/T PSA: free/total prostate-specific antigen.

Supplementary Table 1: Rank correlation of serum T-PSA and F-PSA concentration with Gleason score, TNM stage, and AJCC prognosis group

	Gleason score	*TNM stage*	*AJCC prognosis group*
T-PSA			
Spearman correlation	0.243	0.273	0.363
P	<0.001	0.006	<0.001
F-PSA			
Spearman correlation	0.230	0.272	0.334
P	<0.001	0.006	0.001

T-PSA: total prostate-specific antigen; AJCC: American Joint Committee on Cancer; F-PSA: free prostate-specific antigen; TNM: tumor node metastasis