Clinical Implications of Peripheral CD+3CD+69 T-Cell And CD+8CD+28 T-Cell Proportions in Patients Prior to Radical Prostatectomy

Yu Zhang[1], Ziye Zhang[1], Lina Zhang[2], Sheng Zhao[1], Jing Zhao[1], Qing Ye[1], Yingli Gao[1], Chenyi Jiang[1], Di Cui[1], Shujie Xia[1], Bangmin Han[1]*, Yifeng Jing[1]*

Purpose: To investigate the clinical implications of CD+3CD+69 T-cells and CD+8CD+28 T-cells in the peripheral blood of patients prior to radical prostatectomy.

Materials and Methods: A total of 91 prostate cancer (PCa) patients and 50 benign prostatic hyperplasia (BPH) patients were enrolled from January 2016 to December 2017. The proportions of CD+3CD+69 T-cells and CD+8CD+28 T-cells in the peripheral blood of PCa and BPH patients were detected by flow cytometry, and the association of these T-cell populations with pathological Grade Group and pathological TNM classification was evaluated. Data analysis was performed with SAS version 9.4 software.

Results: The proportions of CD+3CD+69 and CD+8CD+28 T-cells in peripheral blood were higher in PCa patients than those in BPH patients. Multivariate analysis identified a higher CD+3CD+69 T-cell proportion as a risk factor for PCa (odds ratio (OR) = 4.783, $P = 0.0013$), but the diagnostic efficacy of the CD+3CD+69 T-cell proportion (area under the curve (AUC)=0.6833, $P = 0.0003$) for PCa was still inferior to that of the tPSA level (AUC=0.7531, $P < 0.0001$). The AUCs for CD+3CD+69 T-cell and CD+8CD+28 T-cell proportions for PCa were 0.6959 ($P = 0.0372$) and 0.6935 ($P = 0.0395$), respectively, among men with tPSA levels of 10.0-4.0 ng/mL. A lower CD+3CD+69 T-cell proportion was associated with higher pathological Grade Group (P=0.0074).

Conclusion: The proportions of CD+3CD+69 T-cells and CD+8CD+28 T-cells in peripheral blood are potential diagnostic indicators for PCa. The preoperative proportion of CD+3CD+69 T-cells in peripheral blood may have prognostic value in terms of the pathological Grade Group in PCa.

Keywords: CD+3CD+69 T-cell; CD+8CD+28 T-cell; immune function; prostate cancer; radical prostatectomy

INTRODUCTION

Prostate cancer (PCa) is the most commonly diagnosed malignancy and the second leading cause of cancer-related mortality in American males, with an estimated 164,690 new cases and 29,430 deaths expected in 2018 [(1)].The occurrence, development, recurrence and metastasis of tumors are processes representative of tumor escape from immune surveillance, which is strongly related to host immune function[(2)]. Detecting subsets of T lymphocytes in peripheral blood may be a beneficial way to understand immune function, and assist in the clinical diagnosis of disease[(2,3)].

CD28 is expressed on approximately 50% of CD8+ T-cells[(4)]. CD28 is known to be the primary T-cell costimulatory molecule that interacts with its natural ligands, CD80 and CD86, located on antigen-presenting cells (APCs). The signal leads to the activation and proliferation of T-cells and cytokine secretion[(5)]. Previous studies have found that CD69+ T lymphocytes downregulate the inflammatory process and could be a negative regulator of the differentiation of T lymphocytes toward the Th17 lineage through TGF-β or Jak3/Stat5 signaling[(6-8)]. Therefore, understanding the mechanisms of CD69+ T-cells that modulate the immune response could enable them to be targeted by cancer immunotherapies.

In the present study, we examined the differences in CD3+CD69+ and CD8+CD28+ T-cell populations in preoperative peripheral blood between PCa and BPH patients to find potential diagnostic targets for PCa and investigated the relationships between CD3+CD69+ and CD8+CD28+ T-cell proportions and clinicopathological characteristics to determine their roles as biomarkers for PCa.

MATERIALS AND METHODS

Patients and sample size

Our study was approved and carried out according to the instructions of the Ethics Committee of Shanghai General Hospital. All peripheral blood samples were taken from patients after obtaining written informed consent. From January 2016 to December 2017, 91 patients with PCa underwent laparoscopic radical prostatectomy (LRP) performed by one single experienced surgeon, and they formed the experimental group. Patients in the following situations were excluded: (1) the patients had

[1]Department of Urology, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China
[2]Department of Biostatistics, Shanghai Jiao Tong University School of Medicine, Shanghai, China
*Correspondence: Department of Urology, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, 100 Haining Road, Shanghai, China. Tel: +8613918839913; Fax: +86021-63240825; E-mail: jyf_123@163.com (YFJ); Tel: +8618939757031; Fax: +86-21-63240825; E-mail: hanbm@163.com (BMH)
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Table 1. Clinical characteristics of 91 PCa patients and 50 BPH patients (mean ± sd)

Variables	PCa (n=91)	BPH (n=50)	*P* value
Age (years)	67.62 ± 5.66	68.40 ± 7.71	0.535
tPSA (ng*ml-1)	15.16 ± 13.90	3.50 ± 2.62	0.000**
CD8+CD28+ T-cells (%)	8.50 ± 3.91	6.55 ± 3.77	0.005**
CD3+CD69+ T-cells (%)	1.33 ± 0.12	0.66 ± 0.05	0.000**
Pathologic TNM classification, n (%)		/	/
T2	43(47.3%)		
T3	48(52.7%)		
Grade Group, n (%)		/	/
1	25(27.5%)		
2	21(23.1%)		
3	26(28.6%)		
4	8(8.8%)		
5	11(12.0%)		

Abbreviations: PCa prostate cancer, BPH benign prostate hyperplasia, tPSA total prostate-specific antigen.
**: $P < 0.01$.

accepted any type of neoadjuvant hormonal therapy prior to the operation; (2) the patients had suffered from incurable endocrine diseases; (3) the patients had acute prostatitis, urinary tract infection and previous surgical intervention on the prostate; (4) the patients had systematic tumors or chronic diseases, such as hepatitis; and[5] the patients had other diseases of the immune system[10]. In addition, 50 BPH patients who underwent transurethral thulium laser resection of the prostate were selected to form the control group over the same timeframe; the same exclusion criteria used for patients with PCa was also used for controls; their postoperative pathological results showed BPH.

Tissue samples, histological classification and TNM stage

Paraffin-embedded tissue samples from 91 patients surgically treated for PCa were utilized. Histological classifications were assessed by pathological Grade Group according to the criteria of the International Society of Urological Pathology (ISUP) 2014 grade groups[10]. The pathological tumor node metastasis (TNM) stage was evaluated according to the TNM classification of PCa in European Association of Urology (EAU) 2016 guidelines[10].

Blood sample preparation and flow cytometer

Venous blood was obtained from each patient in the morning, prior to surgery. Blood samples were divided into two separate anticoagulated tubes per subject and were sent to the clinical laboratory for the immediate analysis of T-cell subsets. For immunostaining, the following conjugated antibodies were all obtained from BD Pharmingen, USA. Anti-CD3-PerCP and anti-CD69-FITC mAbs were added to one tube, while anti-CD8-PE and anti-CD28-APC mAbs were added to the other tube. The tubes were then mixed gently and incubated for 30 min. After lysing red blood cells, the tubes were centrifuged at 1000 rpm for 5 min, and the precipitate was obtained. The precipitate was washed with phosphate-buffered saline (PBS), after which the tubes were centrifuged again at 1000 rpm for 5 min, and the precipitate was obtained. The precipitate was resuspended in PBS. The detection of CD3+CD69+ and CD8+CD28+ T-cells was performed by flow cytometry (BD Pharmingen, USA). In addition, the prostate-specific antigen (PSA) level was tested before the operation.

Statistical analysis

An independent t test was used to compare the age, total prostate-specific antigen (tPSA) level and proportions of T-cells in peripheral blood between the two groups. In addition, a Wilcoxon rank-sum test was used to compare the proportions of T-cells in peripheral blood between the two groups when the tPSA threshold was set to 4-10 ng/ml. Data are expressed as the mean and standard deviation (sd). Multivariate logistic regression analyses were performed to evaluate the associations between T-cell proportions and disease outcomes. To determine the optimal cutoff value, Youden's index was calculated using receiver operating characteristic (ROC) curve analysis. Spearman rank correlation analysis was used to explore the association between the T-cell proportions and clinical conditions, including pathological Grade Group, and pTNM classification. All statistical analyses were performed using SAS version 9.4 software.

RESULTS

The patients' clinicopathological and demographic characteristics are summarized in **Table 1**. The 91 PCa patients and 50 BPH patients involved in this study showed no significant difference in age ($P = 0.535$).

Table 2. Multivariate logistic regression analysis of proportion of CD3+CD69+ T-cells in peripheral blood in predicting PCa

Variable	OR	95% CI of OR		*P* value
CD3+CD69+ T-cells (%)	4.783	1.840	12.432	0.0013**

Abbreviations: OR odds ratio, CI confidence interval.
**: $P < 0.01$.

Table 3. Comparison of proportions of CD3+CD69+ and CD8+CD28+ T-cells in peripheral blood between PCa and BPH patients with tPSA levels ranging from 4.0-10.0 ng/ml (mean ± sd)

Variables	PCa (n=31)	BPH (n=14)	*P* value
CD8+CD28+ T-cells (%)	9.57±0.78	7.13±0.71	0.0389*
CD3+CD69+ T-cells (%)	1.50±0.22	0.76±0.11	0.0362*

Abbreviations: PCa prostate cancer, BPH benign prostate hyperplasia, tPSA total prostate-specific antigen.
*: $P < 0.05$.

However, the preoperative tPSA level was higher in the PCa group than in the BPH group ($P < 0.001$).
The comparison of the proportions of CD3+CD69+ and CD8+CD28+ T-cells in the peripheral blood of PCa and BPH patients is shown in **Table 1**. The proportions of CD8+CD28+ and CD3+CD69+ T-cells were higher in the PCa group than in the BPH group.
Multivariate logistic regression analysis was performed to evaluate the associations between the proportions of CD8+CD28+ or CD3+CD69+ T-cells and disease outcomes. **Table 2** shows that a high proportion of CD3+CD69+ T-cells in peripheral blood was associated with a higher risk of PCa when adjusted for age and tPSA.
ROC curve analysis was performed to determine the optimal cutoff value of the CD3+CD69+ T-cell population for PCa (area under the curve (AUC)=0.6833, $P = 0.0003$, **Figure 1**). Because a CD3+CD69+ value of 0.9 showed the maximal Youden's index on this curve, the cutoff value of CD3+CD69+ for PCa was set at 0.9. The AUC for CD8+CD28+ was 0.6645 ($P = 0.0013$), and the AUC for tPSA was 0.7531 ($P < 0.0001$). There was no significant difference in the AUC between the two biomarkers, but the AUC for tPSA was the highest. When we set the threshold of the tPSA level (4-10 ng/ml), there were 31 patients in the PCa group and 14 patients in the BPH group. A nonparametric test was performed to determine the differences in the two T-cell proportions between the two groups. Table 3 shows that the proportions of CD8+CD28+ and CD3+CD69+ T-cells in the PCa group were higher than those in the BPH group.
The ROC curves of CD3+CD69+ and CD8+CD28+ for PCa were analyzed to determine the optimal cutoff values (AUC=0.6959 for CD3+CD69+; AUC=0.6935 for CD8+CD28+, Figure 2). The cutoff values of CD3+CD69+ and CD8+CD28+ for PCa were set at 1.6 and 8.2, respectively. In addition, Figure 2 shows that the AUC for the ratio of free to total PSA (f/tPSA) was 0.9055 (P<0.0001). These data suggest that a higher proportion of CD3+CD69+ or CD8+CD28+ T-cells is a predictor of PCa in men with tPSA levels of 4.0-10.0 ng/ml.
We compared the distribution of clinicopathological characteristics between the two groups, along with the two T-cell proportions. **Table 4** shows that the proportion of CD3+CD69+ T-cells in peripheral blood was weakly negatively correlated with the pathological Grade Group in PCa patients. Figure 3 shows that the AUC for CD3+CD69+ T-cells for the pathological Grade Group (GS≥3) was 0.6429 ($P = 0.0189$). Youden's index was still 0.9. In addition, the proportion of CD8+CD28+ T-cells in circulating blood was not significantly associated with any of the analyzed clinicopathological parameters.

DISCUSSION

PCa is one of the most common cancers in men, and the global burden of this disease is rising[11]. Early diagnosis is vital for the treatment of PCa. The current gold standard, prostate biopsy, is an invasive testing method for the diagnosis of PCa, but PCa does not present with obvious clinical manifestations, therefore, the decision to perform a prostate biopsy depends on serum PSA, digital rectal examination (DRE) and multiparametric magnetic resonance imaging results[12]. However, there are drawbacks of PSA as an early detection biomarker of PCa. The gray area of PSA (4.0-10.0 ng/ml) leads to a high rate of negative biopsies and overtreatment. DRE is a subjective procedure that can lead to false-positive results and unnecessary biopsies. As a consequence, there is still an urgent need for novel biomarkers that could further improve diagnostic capability[13,14].
Jamali et al. found that the combined contribution of SPOP, DAXX, RARRES1, and LAMP2 could be a putative regulatory element acting as a prognostic signature and therapeutic target in PCa. Guo J et al. demonstrated that quantitative analysis of ultrasound real-time tissue diffusion elastography is helpful in the diagnosis of benign and malignant prostate lesions and provides a relatively accurate evaluation method in clinical practice, with broad application prospects. Taheri et al. assessed the associations between two genomic variants (rs1800795 and rs2069845) of the IL-6 gene and risk of PCa. Saffari et al. showed that miR-let7b and/or mir-548 can be considered as potential targets in prostate

Table 4. Clinicopathological characteristics of the PCa patient cohort in relation to CD3+CD69+ T-cells (Spearman correlation analysis)

Variables	r_s	*P* value
Pathological TNM classification (T2, T3a, ≥ T3b)	-0.17455	0.098
Pathological Grade Group (2016 WHO new classification, 1-5)	-0.22729	0.0303*

Abbreviations: PCa prostate cancer, BPH benign prostate hyperplasia, rs Spearman rank correlation coefficient, TNM tumor, node and metastasis.
*: $P < 0.05$; **: $P < 0.01$.

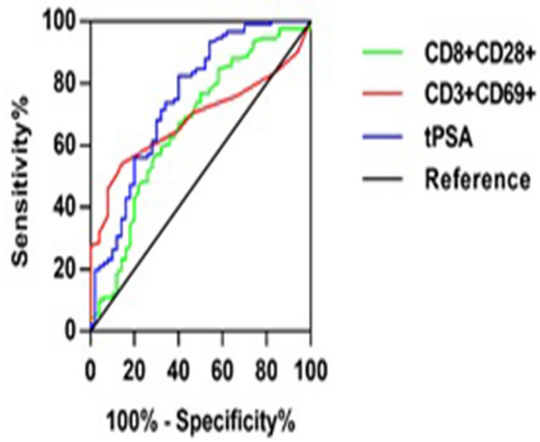

Figure 1. Receiver operating characteristic (ROC) curve of CD3+CD69+ T-cells, CD8+CD28+ T-cells and tPSA for PCa diagnosis

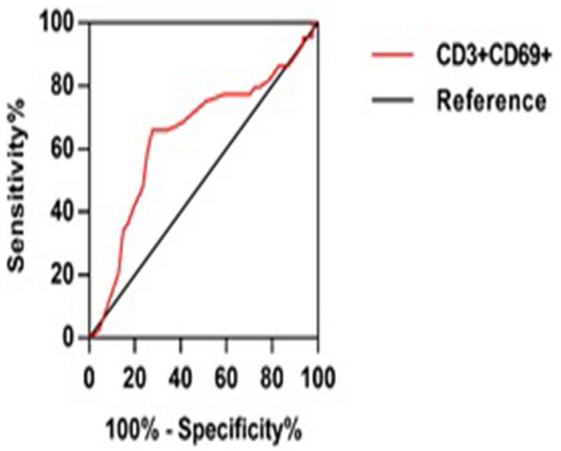

Figure 3. Receiver operating characteristic (ROC) curve of CD3+CD69+ T-cells for pathological Grade Group

cancer therapy.

PCa patients always present with immunological dysfunction. In the present study, we assessed the immune function variation by measuring CD3+CD69+ and CD8+CD28+ T-cell subsets in the peripheral blood of PCa patients prior to any form of treatment, including hormonal therapy, surgery, chemotherapy, and radiotherapy. We found that the proportions of CD3+CD69+ and CD8+CD28+ T-cells were higher in the circulating blood of PCa patients than BPH patients. Previous research has shown that the mean proportion of CD8+CD28+ T-cells is significantly lower in patients with B-cell chronic lymphocytic leukemia than in healthy controls[4]. In 2011, Katarzyna Starska et al. found that the expression of CD69+ antigen on CD3+CD4+ T-cells was higher for pT3 and pT4 tumors than for pT2 squamous cell laryngeal carcinomas[15]. Our results suggest that the proportions of CD3+CD69+ and CD8+CD28+ T-cells in peripheral blood could be associated with the occurrence of PCa.

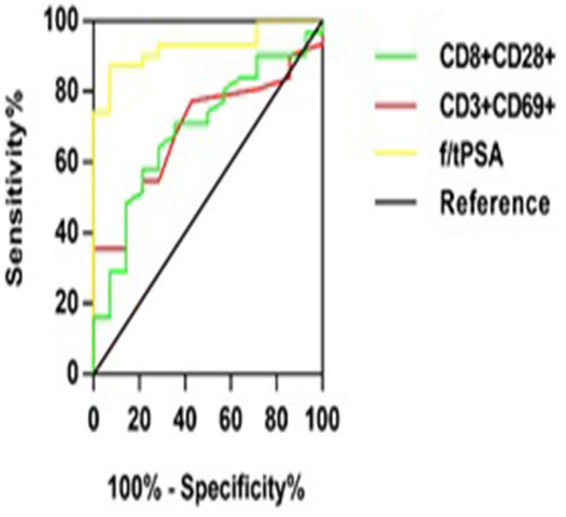

Figure 2. Receiver operating characteristic (ROC) curve of CD3+CD69+ T-cells, CD8+CD28+ T-cells and f/tPSA for PCa diagnosis in men with PSA level 4.0 ng/ml-10.0 ng/ml

In addition, we identified CD3+CD69+ T-cells as an independent risk factor for PCa. Our data suggest that CD3+CD69+ and CD8+CD28+ T-cell proportions are effective for the diagnosis of PCa, especially in patients with tPSA levels of 4.0-10.0 ng/ml. Thus, these data indicate that the proportions of CD3+CD69+ and CD8+CD28+ T-cells in the peripheral blood of patients may be potential diagnostic biomarkers for PCa and that high proportions of CD3+CD69+ and CD8+CD28+ T-cells in the peripheral blood of patients could reflect an increased risk of PCa.

The prognosis of PCa can be evaluated by the Gleason grading system based on its microscopic appearance because the histological differentiation of PCa is closely related to the prognosis, treatment and patient outcome [16]. Our results show a correlation between the proportion of CD3+CD69+ T-cells in peripheral blood and the pathological Grade Group. PCa patients with a lower CD3+CD69+ T-cell proportion in peripheral blood had a higher pathological Grade Group, indicating earlier recurrence [17]. Nonetheless, fundamental research and further studies with more cases are needed.

Although there are still some limitations in our study, on the one hand, T-cells in PCa should be analyzed carefully, as blood cell proportions may be affected by inflammation. On the other hand, it is likely that some participants in the present study were taking drugs, such as steroids or nonsteroidal anti-inflammatory drugs, which could have affected the circulating T-cell populations. To the best of our knowledge, this study is the first to investigate the value of subtypes of circulating T-cells in the diagnosis of PCa. The fact that complete blood count tests are performed during routine workups makes T-cell proportions in peripheral blood accessible, inexpensive clinical parameters[18].

CONCLUSIONS

We conclude that the proportion of CD3+CD69+ T-cells in circulating blood may be an effective predictor of PCa diagnosis combined with the PSA level, especially in those with tPSA levels ranging from 4.0

ng/ml to 10.0 ng/ml, and it also may be a useful prognostic tool in prostate cancer; however, further study is required.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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