

## Peer Review File

**Article Information:** Available at <http://dx.doi.org/10.21037/tau-20-448>.

### Reviewer's Comment 1:

1) The first major concern of this study is regarding the value of the data of this study to represent the degree of inflammation in the entire prostate specimens, which would greatly differ among the different regions of the prostate (e.g., TZ vs. peripheral zone). However, it is not known how many TURP-resected tissue cores are examined and how the authors dealt with the location-to-location differences of inflammation to determine the pattern or severity of inflammation. The authors probably need to show some types of mapping of inflammatory conditions by using multiple resected core tissues from different regions of the prostate.

### Reply 1:

We appreciate the reviewer's attention to the tissue we used. In this research, we aimed to study the inflammation patterns of BPH tissues. BPH is mainly developed in the transitional zone of the prostate. In transurethral prostatic resection (TURP), it is the prostate tissues at the transitional zone that are removed and sent for pathological examination. Of note, for every BPH patient, all the TURP tissues are sent for pathological examination. Therefore, the TURP samples are adequate to analyze the inflammatory condition of BPH tissues. Meanwhile, the published literatures use the similar method to analyze the inflammatory condition of BPH tissues [*M2 macrophage-mediated interleukin-4 signaling induces myofibroblast phenotype during the progression of benign prostatic hyperplasia. Cell Death Dis. 2018;9(7):755*].

However, the above sampling method fails to exactly localize the inflammation condition on the transitional zone of the prostate. We have added to the insufficiency of above sampling method to the section of *limitation* in the revised manuscript. Thank you for your advice.

### Changes in the text:

We have modified our text as advised (see Page 11, line 10-15).

### Reviewer's Comment 2:

2) The next concern is regarding the classification of inflammation patterns (glandular, periglandular and stromal). Because these different patterns co-exist in the same prostate, the correlation of each pattern with clinical parameters may not be meaningful. The authors rather need to determine the most dominant pattern in each patient by examining the location, severity and also "extent" of each inflammation pattern in the whole prostate and, then, investigate the relationship.

### Reply 2:

We appreciate the reviewer's attention to the definition of the most dominant inflammation pattern of

BPH patients. For every BPH patient, all the TURP tissues, the hyperplastic prostatic tissues at the transitional zone, were sent for pathological examination to evaluate the inflammation degree and whether the malignancy existed. During the pathological examination, hematoxylin-eosin (H&E) staining was performed. All the H&E sections of one BPH patient were blindly reviewed of by two pathologists. For each case, the prostatic inflammatory infiltrates in each histological structure (glandular, periglandular and stromal) were recorded by the extent of inflammation (focal, multifocal or diffuse) and the grade of inflammation (mild, moderate and severe). And the most dominant inflammation pattern of one patient was defined as the histological structure with the most extent and grade of inflammation. In addition, we chose the paraffin-embedded tissue with the most dominant inflammation pattern for immunohistochemistry. Thank you very much for pointing it out. We would add the definition of the most dominant inflammation pattern in the revised manuscript.

**Changes in the text:**

We have modified our text as advised (see Page 4, line 9-26).

**Reviewer's Comment 3:**

*3) Another concern is regarding the blinding of histological analyses as the classification method is qualitative, rather than quantitative. How many pathologists were involved for histological evaluation or were they blinded when looking at the specimens?*

**Reply 3:**

We appreciate the reviewer's attention to the evaluation of pathological sections in the manuscript. The two pathologists evaluated the sections back to back without each other's knowledge. When the results of the two pathologists were inconsistent, the third pathologist was invited for the additional evaluation. We have revised our text. Thank you for your advice.

**Changes in the text:**

We have modified our text as advised (see Page 4, line 19-23).

**Reviewer's Comment 4:**

*4) Methods: Page 4 line 11-15, supplemental Table. 1; The histological criteria that you referred from Ref. 12 include the parameter of "extent", which should be assessed.*

**Reply 4:**

Thanks for pointing it out. Actually, we assessed the *extent* of inflammation of prostate in the present study. The histopathological criteria that we referred was originated from the chronic prostatitis. Different from that of chronic prostatitis, the extent of inflammation in BPH is mainly multifocal infiltration and scarcely focal infiltration or diffuse infiltration. We would add the point in our revised manuscript.

**Changes in the text:**

We have revised our text as advised (see Page 4, line 14-23).

**Reviewer's Comment 5:**

*5) The authors need to describe the timing and methods of prostate size measurement; transrectal ultrasound, computed tomography or magnetic resonance imaging?*

**Reply 5:**

We appreciate the reviewer's attention to the timing and methods of prostate size measurement. Patient's prostate size was measured by transrectal ultrasound one day before the TURP surgery. We have added related contents in the revised manuscript as advised. Thanks for pointing it out.

**Changes in the text:**

We have revised our manuscript as advised (see Page 4, line 7-8).

**Reviewer's Comment 6:**

*The authors need to describe the timing of PSA testing.*

**Reply 6:**

The PSA test was performed for two times: about one month before surgery at outpatient department and one day before surgery at inpatient ward. We excluded patients with interfering factors for PSA test, such as indwelling catheter, acute urinary retention and urinary tract infection. We have added related contents in the revised manuscript as advised. Thanks for pointing it out.

**Changes in the text:**

We have added related contents in the revised manuscript as advised (see Page 4, line 5-7).

**Reviewer's Comment 7:**

*Additional evaluation of subtypes of macrophages (M1, M2) or T-lymphocytes would be helpful.*

**Reply 7:**

We appreciate the reviewer's attention to the additional evaluation of immune cells by subtypes. In the past, the function of various inflammatory cells in subtypes has been studied in BPH. For instance, M2 macrophages can induce myofibroblast phenotype in BPH [*M2 macrophage-mediated interleukin-4 signalling induces myofibroblast phenotype during the progression of benign prostatic hyperplasia. Cell Death Dis. 2018;9(7):755*]. The ratio of CD4 to CD8 T cells influences the development of BPH [*Intraepithelial and stromal lymphocytes in the normal human prostate. The Prostate. 2003;55(3):187-*

193].

In the present study, we aimed to depict inflammation patterns of the general T lymphocyte, B lymphocyte and macrophage in the BPH tissues in combination with histological structures. Therefore, we did not evaluate every subtype of the above three inflammatory cells. We will conduct a follow-up study on the relationship between the subtypes of inflammatory cells and clinical indicators. We added relevant contents in *Discussion*. Thanks for pointing it out.

**Changes in the text:**

We added relevant contents in *Discussion* (see Page 11, line 15-20).

**Reviewer's Comment 8:**

*This study used the absence or presence of inflammatory cell types; however, the infiltration of T-lymphocytes, B-lymphocytes and macrophages does not seem to be all or none. In addition, it is not clear what the statistical results here are indicating in Table 2.*

**Reply 8:**

In our study, all samples were infiltrated with inflammatory cells. We use the degree of inflammation to determine the general degree of inflammation. However, for specific inflammatory cells, due to the limited sample size, we used dichotomy method to describe the degree of infiltration. We defined the proportion of T-lymphocytes, B-lymphocytes and macrophages less than 5% in all cells per high magnification as no infiltration. We added relevant contents in the revised manuscript. Thanks for pointing it out.

**Changes in the text:**

We added relevant contents in the revised manuscript (see Page 5, line 12-14).

**Reviewer's Comment 9:**

*Table 3: There are no detail descriptions or representative histological photomicrographs of epithelial or stromal hyperplasia of BPH specimens.*

**Reply 9:**

Thanks for pointing it out. In the epithelial hyperplasia-dominant BPH patients' tissue, tall columnar prostatic epithelial cells are often seen, with prominent hyperplasia protruding into the glandular cavity. There is a marked increase in glands with glandular (adenomatous) nodules or mixed nodules around the glands. In the stromal hyperplasia-dominant BPH patients' tissue, stromal thickening/nodules, adenomyoid-type nodules, or fibroadenoma type nodules are often seen. We have added detail descriptions and representative histological photomicrographs of epithelial or stromal hyperplasia of BPH specimens in the revised manuscript.

**Changes in the text:**

We added relevant contents in the revised manuscript (see Supplemental figure 1).

**Reviewer's Comment 10:**

*Table 4: Need to use the inflammation grade, but not "Yes or No", of each inflammation pattern for the BMI comparison.*

**Reply 10:**

Thanks for pointing it out. We have classified the inflammation grade as high, moderate and mild for the BMI comparison.

**Changes in the text:**

We have modified the statement in the revised manuscript (see revised Table 4).

**Reviewer's Comment 11:**

*Supplement Table 3: This table does not make sense. Need to be revised.*

**Reply 11:**

We have eliminated the superfluous content in the revised draft.

**Changes in the text:**

We have eliminated the superfluous content in the revised draft (see in *Supplemental materials*).

**Reviewer's Comment 12:**

*Discussion, Page 7, line 10-15: It is described here that epithelial injury is one of the mechanisms inducing prostate inflammation. However, prostatic inflammation can occur by many other reasons. For example, in the case of periglandular inflammation, urinary reflux is a possible reason for prostatic inflammation. Thus, the authors need to expand the discussion including other mechanisms.*

**Reply 12:**

We appreciate the reviewer's attention to the mechanisms inducing prostate inflammation. We added relevant contents in *Discussion*: Stimuli such as infectious agents or urinary reflux, metabolic syndrome, aging processes, and autoimmune responses can also cause prostatic immune dysregulation through the development of multiple molecular pathways involved in inflammatory infiltration. Thanks for pointing it out.

**Changes in the text:**

We added relevant contents in *Discussion* (see Page 8, line 26-27; Page 9, line 1).

**Reviewer's Comment 13:**

*Discussion, Page 8, line 29-30: It is described here that the reason of BPH is the changes in the immune system and metabolism. However, these are not the only reasons for prostate inflammation. The authors need to revise this statement.*

**Reply 13:**

We appreciate the reviewer's attention to the reasons for prostate inflammation. We changed the statement in *Discussion* to make it more objective: changes in the immune system and metabolism may be part of the reasons for prostate inflammation and BPH. Thanks for pointing it out.

**Changes in the text:**

We added relevant contents in *Discussion* (see Page 10, line 18-19).

All the modifications in the manuscript were made by using the *track changes mode* of *Microsoft Word*.