

TRANSLATIONAL ANDROLOGY AND UROLOGY

Peer Review File

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Reviewer A

-This study demonstrates perineural invasion is difficult to be certain on needle biopsy but more definite on RP. These limits the utility of using perineural invasion to stratify patients that are not undergoing RP

1. **REPLY:** We agree with the Reviewer. We emphasize that there is not a relevant correspondence between biopsy data and data on the whole specimen at RP. This is an important point, as it highlights that it is not possible to make decisions on the basis of PNI before surgery using only biopsy data.
2. **CHANGES IN THE TEXT:** this aspect is well underlined in Section 3.0 Results, lines 179-181 as well as in Discussion section 4, lines 238-248, and Conclusion Section 5.0, lines 290-293.

- while perineural invasion may be significant on univariate analysis, it approximates statistical significance on multivariate analysis.

This may be a function of sample size. and hence this should be discussed in detail.

Is perineural invasion a surrogate of other more prognostic factors and if so, why or why not?

If not, then what is other supporting evidence to show that it is an independent prognostic factor, and that the p value of marginal significance at multivariate analysis is likely a function of sample size.

1. **REPLY:** In our experience PNI was significantly correlated with biochemical progression after surgery. The risk of biochemical progression significantly increased in cases with PNI and in particular in cases with multifocal PNI. Non-significant results in multivariate analysis could be partially due to sample size. However, PNI was also significantly correlated with local T stage and ISUP grading. Therefore, this can explain why at multivariate analysis PNI did not remain a significant and independent predictor of biochemical progression. Thus, I do believe the reason of multivariate analysis results is predominantly determined by the fact that PNI can influence biochemical progression, but this influence is correlated also with local stage and grading. Therefore, PNI is a significant predictor of biochemical progression but not independent (as demonstrated at multivariate analysis) to T staging and ISUP grading. Another important point is the stratification of results on the basis of risk classes. In the intermediate risk class that remain more uncertain in terms of clinical indication, PNI can stratify cases at higher risk for biochemical progression.
2. **CHANGES IN THE TEXT:** All these aspects are well underlined in the text at: Section 3.2, lines 187-198; Section 3.3 lines 201-203; 207-212; 215-221; Section 3.4 lines 223-230;

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Section 4.0 lines 255-272.

Reviewer B

- 1) PNI in biopsy specimens is an indicator of unfavorable pathology at RP is a known fact, while PNI whether multifocal or unifocal at final RP specimen does not have any long term prognostic significance in multivariate analysis when other adverse pathological parameters are taken into account.

REPLY: In our experience biopsy determination of PNI is not a significant predictor of the real incidence of PNI in PC cases. We found strong differences between PNI rate at biopsy and at confirmation on the whole specimen obtained at surgery. We underline this significant clinical aspect that limit the possibility to use PNI as predictor of progression on the basis of the biopsy data and the need to consider it after surgery. The analysis on PNI obtained at surgery showed a significant correlation between PNI and biochemical progression that significantly increased, in particular in cases with multifocal PNI. Therefore, the determination of uni or multifocal PNI at surgery could predict biochemical progression risk as shown in our univariate analysis. However, PNI at surgery is also significantly associated with pathologic T stage and ISUP grading. This is the reason why at the multivariate analysis, after adjusting results for PNI with T stage and ISUP grading, PNI did not represent an independent predictor of biochemical progression

CHANGES IN THE TEXT: All these aspects are well underlined in: Section 3.0 Results, lines 179-181 and in Discussion section 4, lines 238-248; Conclusion Section 5.0, lines 290-293. Section 3.2, lines 187-198; Section 3.3 lines 201-203; 207-212; 215-221; Section 3.4 lines 223-230; Section 4.0 lines 255-272.

- 2) Furthermore, routine PNI testing on final pathological specimens is too time consuming and has an insignificant impact on prognostication when other factors such as ISUP score, pathological stage and margin status are considered.

REPLY: we in part agree with this consideration and we underline in the text that PNI is time consuming in the final pathological examination (Section 4 lines 236-238). In particular we believe that it is not useful and time consuming to describe PNI at biopsy level, considering the strong difference of results between biopsy and final pathology. At RP, several pathologists describe PNI and some prognostic indications in terms of biochemical progression risk can be obtained. It is true that PNI is not an independent predictor when T stage and ISUP grading are considered. We concluded our article with the same statement of the Reviewer.

CHANGES IN THE TEXT: All these aspects are underlined in: Section 3.0 Results, lines 179-181 and in Discussion section 4, lines 238-248; Conclusion Section 5.0, lines 290-298. Section 3.2, lines 187-198; Section 3.3 lines 201-203; 207-212; 215-221; Section 3.4 lines 223-230; Section 4.0 lines 255-272

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- 3) Therefore, routine PNI detection and testing is neither cost effective nor an efficacious tool to prognosticate biochemical failure or overall survival in patients with localized prostate cancer.

REPLY: in our experience PNI at surgery and not PNI at biopsy is a significant predictor of biochemical progression, in particular after stratification in terms of uni and multifocal PNI. PNI is not an independent predictor of survival after adjusting for T stage and ISUP grading and therefore cost effectiveness could be limited.

CHANGES IN THE TEXT: All these aspects are well underlined in: Section 3.0 Results, lines 179-181 and in Discussion section 4, lines 238-248; Conclusion Section 5.0, lines 290-298.

Section 3.2, lines 187-198; Section 3.3 lines 201-203; 207-212; 215-221; Section 3.4 lines 223-230; Section 4.0 lines 255-272.

- 4) This paper does not add any additional information to the existing literature on PNI significance.

REPLY: We agree with the Reviewer that PNI has been previously examined by several analyses. However, results remain not univocal. Pathologists continue to report PNI mainly at surgery, although the Reviewer underlines that PNI is not cost effectiveness. Therefore, it continues to be time in investigating on PNI. In our opinion, our analysis underlines some interesting points:

- Biopsy determination of PNI is not significant if you compare and verify results at surgery
- At surgery PNI and its stratification by focality (unifocal versus multifocal) is significantly associated with the risk of biochemical progression. Otherwise PNI is not an independent predictor of survival after adjusting for Stage and grading.
- In the intermediate risk group in which clinical indications are still uncertain, PNI can identify cases at higher risk of biochemical progression, helping stratification of cases.