Reviewer's report

Title: Estimation of groin recurrence risk in patients with squamous cell vulvar carcinoma by the assessment of marker gene expression in the lymph nodes.

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Reviewer: Matthias Choschzick

Reviewer's report:

Dear Mr. Danrolf de Jesus,

the study above analyzed the gene expression profiles of four vulvar carcinomas with positive and negative lymph nodes. There were seven genes significantly overexpressed in positive lymph nodes in comparison to negative lymph nodes of the same patient. The results were evaluated in 20 patients and a predictive value for groin recurrence was found.

There are still a number of issues with this manuscript:

In the discussion section the authors added the sentences “Importantly, our microarray data were obtained for HPV-negative patients in order to exclude the interference of the infection with the expression results and to focus on metastasis-associated genes’ selection. However, patients with HPV infection should also be enrolled in future studies to assess the influence of HPV on the markers’ performance.” Indeed, HPV-association of vulvar carcinomas surely influences the gene expression profile. For instance, HPV positivity of vulvar cancer is negative connected to the p53 mutation status and the occurrence of EGFR amplification. The authors should at least state throughout the manuscript including the title, the abstract and the conclusions that there results are only reliable for HPV negative patients. Furthermore, in 4 cases the HPV status was not determined and one HPV16 positive tumor was included in the study. These cases should be eliminated from the study if the authors think that the HPV status is important for the gene expression profile.

The authors state in their answer: “The results only mirror the known adverse prognostic effect of lymph node status in vulvar cancer: This is most probably true.” In my view the same “prognostic” results with can be achieved with other tumor-associated genes like keratins or p53 without the laborious gene selection process in the presented study. Beside this, the number of examined cases is probably (surely) too low for prognostic statements.

In their conclusions the authors write “…may provide a promising tool for intra-operative sentinel LN evaluation in VC patients.” All these statements about the value of the applied methods for detection of lymph node metastases in comparison to histopathological examination should be eliminated from the study because the authors provide no data that their method is more reliable than pathology. Otherwise they have to provide a detailed analysis of sensitivity and specificity of their analytical method in comparison to pathological examination of
lymph nodes especially the ultra-staging of groin lymph nodes. The proposal (see conclusions) to apply these methods intra-operative is also not justified by the presented results.

I agree with the comment of the first reviewer (Omer Devaja) “My oppinion is that title is a bit to far fetch. I am not convinced that expression of described marker genes can relaialble predict lymph nodal recurrence.”

May be, there are other issues with this paper more closely connected to the methodology and the complex statistics. But I do not feel qualified to form a reliable opinion in terms to these topics.

Best regards,

M Choschzick, MD

**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

'I declare that I have no competing interests'