**Author’s response to reviews**

**Title:** Association between tumour infiltrating lymphocytes, histotype and clinical outcome in epithelial ovarian cancer

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**Author’s response to reviews:**

We thank the reviewers for their time and effort in carefully evaluating our manuscript. We address their points below:

**Reviewer reports:**

Richard Flavin, MD PhD (Reviewer 1): In this study the authors examine the relationship between tumour infiltrating lymphocytes, histotype and clinical outcome in epithelial ovarian cancer.

The study is prospective in nature and is well powered.

**Major comments:**

Methods: More information on the evaluation of TILs is required - did the digital programme select areas for evaluation of TILs or was this done manually? At what magnification were 'low
and high powers' and at what power was the percentage category based on? How were the percentage categories selected?

Areas for lymphocyte evaluation were selected manually. The digital programme was used as a viewer for digital slides only. Selected areas were those with good preservation which contained viable tumour cells. ‘Low’ and ‘high’ powers represented 4x and 20x objective lens images respectively. TILs percent was determined using a 20x objective.

Our method for evaluation of TILs was stipulated by independent guidelines produced by an international working group in the context of breast cancer (since no equivalent guidelines have yet been produced for ovarian cancer). Percent categories were based on those stipulated in the guidelines:


How can the authors be certain that selected areas were purely of mononuclear lymphocyte infiltration - how did they account for background histiocytes, plasma cells and how were these cells masked? How were background tumour nuclei masked? Results need to be validated by a human visual read of the slides and by immunostaining for a generic marker such as CD45 for a select percentage of cases.

All mononuclear cells were included in the evaluation of TILs. This approach was designed to exclude polymorphonuclear cells, which are typically discernible based on morphology alone. This approach was adopted based on the guidelines produced by Salgado et al. It was our intention to evaluate the association between an estimate of TILs and clinical parameters based on morphology alone without use of ancillary tests. Comparable studies have made important contributions in breast cancer.

There is an element of selection bias in the study group with predominately high stage serous cancers and low stage for the remainder. The treatment regimens for such varying histotypes would invariably influence clinical outcomes and should be outlined and included in the regression analysis.

Unfortunately reliable and detailed treatment data is not available for this cohort. Regression models were adjusted for both FIGO stage and histotype. Since treatment regimens are highly correlated with these factors, the statistical effect of treatment is, to a large degree, accounted for in the model.

More images of the differing histotypes with varying TIL percentages need to be included and of much higher quality.
The quality of the images used was affected by the submission formatting process. They will be reviewed again at the proofing stage and, if necessary, higher quality images provided.

Minor: Please correct typographical errors.
This has been done.

Pashtoon Murtaza Kasi, M.D. (Reviewer 2): Dear Authors,

I would like to congratulate you on a job well done.

1. Manuscript very nicely written, easy to follow.

2. Incorporates a simple IHC technique with clinical meaning and easy to translate.

3. In an era of immunotherapy, it is of great value.

4. Larger sample size allows for analyses presented.

Out of curiousity, did you compare the TILs between 2 metastatic samples from the same patient to demonstrate inter-tumor heterogeneity in the same patient. That may be a separate project to look at.

We thank the reviewer for this suggestion. Unfortunately, there was not enough data to evaluate this reliably here but it is a question we will seek to address in other cohorts.

I would recommend accepting it without revisions.

Good wishes,

Pashtoon.

Many thanks and best wishes

Fiona James