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Heliyon

Title: Image Analysis Uncovers Associations between Immune Landscape, Collagen Structure, and Neoadjuvant Chemotherapy in High-Grade Serous Ovarian Carcinomas

## Dear Reviewers,

Please find enclosed a revised version of our manuscript “**Image Analysis Uncovers Associations between Immune Landscape, Collagen Structure, and Neoadjuvant Chemotherapy in High-Grade Serous Ovarian Carcinomas**”. The modifications introduced in this new version have been color-coded to facilitate comparison with the original submission. Specific responses are in dark red italics while modifications to the manuscript are in blue.

We believe we have addressed the comments and have updated our manuscript accordingly. Detailed responses are outlined below. We hope that this new version satisfactorily addresses the concerns. Thank you for your consideration.

Best regards,

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**Reviewer #1 (Remarks to the Author)**  
“This study addresses a current topic.

The manuscript is quite well written and organized. English could be improved.

Figures and tables are comprehensive and clear.

The introduction explains in a clear and coherent manner the background of this study.

However, we think the authors should be acknowledged for their work. In fact, they correctly addressed an important topic, the methods sound good and their discussion is well balanced.

The main strengths of this paper are that it addresses an interesting and very timely question and provides a clear answer, with some limitations.”  
**- Comment 1:“**Introduction section: although the authors correctly included important papers in this setting, we believe the systemic treatment scenario for ovarian cancer should be further discussed in the Introduction section and some recently published papers added ( PMID: 35031442 ; PMID: 37535194; PMID: 32014900), only for a matter of consistency. We think it might be useful to introduce the topic of this interesting study.**”**

Response 1: We appreciate the reviewer's input on improving the introduction section of the paper. In response, we have updated the first paragraph of the Introduction section in the revised manuscript. We briefly mentioned the systemic treatment scenario for ovarian cancer and referenced the recently published papers as suggested by the reviewer.

*The standard treatment approach for high-grade serous ovarian carcinomas (HGSOC) typically involves either cancer-directed debulking surgery followed by adjuvant platinum-taxane chemotherapy or neoadjuvant chemotherapy (NACT) followed by interval cytoreductive surgery* (1)*. NACT, also known as preoperative chemotherapy, is widely used in managing HGSOC* (2)*. Furthermore, immunotherapy employing immune checkpoint inhibition has gained increasing popularity as a treatment option due to its durable effects and lower toxicity* (3–5)*.*

**- Comment 2:“**Discussion section: Very interesting and timely discussion. Of note, the authors should expand the Discussion section, including a more personal perspective to reflect on. For example, they could answer the following questions - in order to facilitate the understanding of this complex topic to readers: what potential does this study hold? What are the knowledge gaps and how do researchers tackle them? How do you see this area unfolding in the next 5 years? We think it would be extremely interesting for the readers.”

Response 2: We appreciate the reviewer’s comment on the Discussion section of the paper. We have expanded the Discussion section in the revised manuscript, as follows:

*Looking ahead, we anticipate rapid evolution in the field of computational pathology-based prognostic biomarkers for HGSOC over the next five years. Furthermore, integrating multi-omics data and immune profiling approaches will facilitate a more comprehensive characterization of the TME, laying the groundwork for personalized treatment strategies in HGSOC* (6)*. Overall, our study contributes to this evolving landscape and sets the stage for further exploration of the intricacies of the TME in HGSOC.*

*We acknowledge several limitations in our study. Firstly, our small sample size is a notable constraint that could potentially impact the generalizability of our findings. Future work will focus on expanding the dataset to overcome the limitation of the small sample size. Another limitation was not considering different types of immune cells in the TME, such as tumor-associated macrophages and cancer-associated fibroblasts* (7)*. To address this, our future research will focus on investigating the effects of NACT on these distinct immune cell populations within the TME. Additionally, we did not distinguish tertiary lymphoid structures from stromal TIL clusters within the TME of HGSOC patients* (8)*. Further investigation into this aspect could be crucial, as tertiary lymphoid structures could reflect an active immune response, while stromal TIL clusters could signify a more chronic inflammatory process. Another notable limitation was the absence of specialized staining techniques, such as Mason's trichrome or Mallory's trichrome staining, which would have enabled a quantitative analysis of collagen fiber segmentation. This limitation stemmed from the unavailability of Mason's trichrome or Mallory's trichrome-stained whole-slide images for the cohort used in our study. Despite the absence of these specialized staining techniques, we validated the results of our method through input from pathologists. This validation helped ensure the accuracy and reliability of our findings.*

*In conclusion, while our study contributes to the growing body of knowledge on TME dynamics post-NACT in HGSOC, there is immense potential for further exploration and translation into clinical practice. By addressing knowledge gaps, leveraging advanced technologies, and fostering interdisciplinary collaborations, we envision a future where TME-focused therapies revolutionize the management of ovarian cancer and other solid tumors, leading to improved patient outcomes and survival rates.*

**- Comment 3*:* “**One additional little flaw: the authors could better explain the limitations of their work, in the last part of the Discussion.**”**

Response 3: We appreciate the reviewer’s comment. We have added the limitations of our work in the Discussion section as follows:

*We acknowledge several limitations in our study. Firstly, our small sample size is a notable constraint that could potentially impact the generalizability of our findings. Future work will focus on expanding the dataset to overcome the limitation of the small sample size. Another limitation was not considering different types of immune cells in the TME, such as tumor-associated macrophages and cancer-associated fibroblasts* (7)*. To address this, our future research will focus on investigating the effects of NACT on these distinct immune cell populations within the TME. Additionally, we did not distinguish tertiary lymphoid structures from stromal TIL clusters within the TME of HGSOC patients* (8)*. Further investigation into this aspect could be crucial, as tertiary lymphoid structures could reflect an active immune response, while stromal TIL clusters could signify a more chronic inflammatory process. Another notable limitation was the absence of specialized staining techniques, such as Mason's trichrome or Mallory's trichrome staining, which would have enabled a quantitative analysis of collagen fiber segmentation. This limitation stemmed from the unavailability of Mason's trichrome or Mallory's trichrome-stained whole-slide images for the cohort used in our study. Despite the absence of these specialized staining techniques, we validated the results of our method through input from pathologists. This validation helped ensure the accuracy and reliability of our findings.*

**- Comment 4: “**We suggest a linguistic revision and the addition of some references for a matter of consistency. Moreover, the authors should better clarify some points.***”***

Response 4: We thank the reviewer for their comment. The necessary changes regarding linguistic revision and addition of references have been highlighted in the revised manuscript, as detailed below:

The revisions in the Discussion section have been addressed in the earlier comments (Comments 2 and 3).

The updated summary section reads as follows:

*The changes in the tumor microenvironment (TME) of high-grade serous ovarian carcinomas (HGSOC) following neoadjuvant chemotherapy (NACT) are a complex area of study. Previous research underscores the importance of investigating the immune and collagen components within the TME for prognostic implications. In this study, we utilized computational pathology techniques with Hematoxylin and Eosin (H&E) whole-slide images (WSIs) to quantitatively characterize the immune and collagen architecture within the TME of HGSOC patients. Our analysis of 12 pre- and post-NACT H&E WSIs revealed an increase in immune infiltrate, primarily within the epithelial region. Additionally, post-NACT H&E WSIs exhibited chaotic collagen architecture compared to pre-NACT H&E WSIs. Importantly, features extracted from post-NACT H&E WSIs showed associations with overall survival, potentially aiding in the selection of patients for immunotherapy trials. These findings offer crucial insights into the TME changes in HGSOC patients following NACT and their potential implications for clinical outcomes.*

The updated first two paragraphs of the introduction section read as follows:

*The standard treatment approach for high-grade serous ovarian carcinomas (HGSOC) typically involves either cancer-directed debulking surgery followed by adjuvant platinum-taxane chemotherapy or neoadjuvant chemotherapy (NACT) followed by interval cytoreductive surgery* (1)*. NACT, also known as preoperative chemotherapy, is widely used in managing HGSOC* (2)*. Furthermore, immunotherapy employing immune checkpoint inhibition has gained increasing popularity as a treatment option due to its durable effects and lower toxicity* (3–5)*.*

*Recent studies have shed light on changes in the tumor microenvironment (TME) composition in HGSOC patients after NACT. This insight arises from analyzing paired samples collected before and after NACT administration in the same patient* (9,10)*, raising crucial questions about the dynamic changes within the TME of HGSOC patients post-NACT that could potentially influence clinical outcomes (Figure 1).*

**Reviewer #2 (Remarks to the Author)**  
**- Comment 1:“**It is unclear why the authors conducted this study on only a sample of 12 patients, since this oncopathology is quite common**”**

Response 1: We appreciate the reviewers' feedback and thank them for their constructive comments. Our study contributes to the growing body of knowledge on TME dynamics post-NACT in HGSOC. While we recognize the importance of expanding the sample size, it's essential to note the challenges inherent in obtaining matched slides for analysis in oncopathologies. We also acknowledge the pilot nature of our study, constrained by tissue accessibility and availability. Importantly, our findings demonstrate a significant association of the developed signature with clinical outcomes, indicating its potential for further validation and utilization in subsequent studies on treatment response assessment.

**- Comment 2:“**The authors used classical hematoxylin and eosin staining, which is not a specific stain for detecting collagen fibers. For example, the use of Mason's trichrome or Malory's trichrome staining would have given a better result. Moreover, these are affordable stains that are often used in pathology. In addition, you can use picrosirius red, which will allow you to differentiate collagen fibers in the stromal component of neoplasms with even greater efficiency.**”**

Response 2: It's important to note that while there are alternative staining techniques available, they aren't universally employed in every study. Our choice to utilize the standard H&E staining method was deliberate, as it's commonly applied across various studies. Additionally, it's pertinent to highlight that this study was retrospective in nature, meaning we relied on previously collected samples. It's worth acknowledging that obtaining and re-staining past samples can pose challenges due to limitations within laboratory settings.

We would like to emphasize that the ultimate validation of our collagen fiber segmentation method hinges upon its capacity to predict clinically relevant outcomes. Our current work demonstrates the feasibility of achieving this validation, where we show the association of features extracted from the quantitative characterization of collagen components within the tumor microenvironment of high-grade serous ovarian carcinomas from post-NACT H&E WSIs and survival outcomes.

To evaluate the effectiveness of the method for segmenting collagen fibers from H&E WSIs, we conducted a visual assessment involving two pathologists. They independently reviewed one randomly selected 3000x3000-pixel tile from 12 different patients. The tiles were marked with collagen fibers captured by our approach. The two pathologists examined the tiles and categorized them into one of three categories (good, fair, or poor). For the collagen fiber segmentation, the first pathologist ranked 90% of the tiles as good or fair, while the second pathologist assigned a good or fair ranking to 92% of the tiles (**Table 1**).

Table 1.Quality check results of Collagen fiber segmentation

|  |  |  |
| --- | --- | --- |
| **Task** | **Pathologist 1**  **(% of tiles belonging to**  **good/fair category)** | **Pathologist 2**  **(% of tiles belonging to**  **good/fair category)** |
| Collagen fiber segmentation | 90% | 92% |

We have included this limitation in the discussion section of the revised manuscript, as follows:

*Another notable limitation was the absence of specialized staining techniques, such as Mason's trichrome or Mallory's trichrome staining, which would have enabled a quantitative analysis of collagen fiber segmentation. This limitation stemmed from the unavailability of Mason's trichrome or Mallory's trichrome-stained whole-slide images for the cohort used in our study. Despite the absence of these specialized staining techniques, we validated the results of our method through input from pathologists. This validation helped ensure the accuracy and reliability of our findings.*

**- Comment 3:“**The method of the evaluation of tumor-infiltrating lymphocytes (TILs) was used in the study, it was recommended by an International TILs Working Group in 2014. This method involves determining the area occupied by lymphocytes in samples stained with H&E.

However, it has been 10 years, and for precise carcinoma diagnostics and identification of leukocyte subtypes, the implementation of molecular methods is now necessary. Using standard methods alone is no longer sufficient for classifying the TILs component; instead, immunohistochemical reactions should be conducted.

Although the use of modern methods of digital pathology may allow us to look at this from a new perspective.**”**

Response 3:

The reviewer is right about the need for a nuanced approach in discussing this topic. It's true that while molecular studies and immunohistochemical (IHC) methods are recommended for precise carcinoma diagnostics and leukocyte subtype identification, they are not universally implemented in practice as standard as H&E staining. This discrepancy can sometimes create challenges in accurately classifying tumor-infiltrating lymphocytes (TILs) and understanding their roles in the tumor microenvironment.

Expanding on this, future research should indeed focus on analyzing subtypes of lymphocytes such as CD4, CD8, and CD20, alongside other immune cells like macrophages and fibroblasts. This comprehensive analysis will provide a more detailed understanding of the immune landscape within tumors and help tailor therapeutic strategies accordingly. Integrating modern digital pathology methods can also offer new perspectives and insights into these complex interactions.

**- Comment 4:“**The section "Clinicopathologic variables of the cohort" should be moved to the Materials and Methods section”

Response 4: We have moved the section “Clinicopathologic variables of the cohort” to the STAR Methods section in the revised manuscript.

**- Comment 5:“**The article should include a detailed clinical description of all 12 patients in the form of a table (can be presented as additional materials)”

Response 5: We thank the reviewer for their comment. We have included the table in the Supplementary material file as follows:

Supplementary Table 1. Summary of clinical features for the patients in dataset

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Patient (ID)** | **Age**  **(in years)** | **Death** | **Tumor Stage** | **Overall survival (in months)** | **Debulking status** | **Chemo cycles** |
| 1 | 61 | True | 3 | 16.83333333 | Suboptimal | 6 |
| 2 | 61 | True | 4 | 12.43333333 | Optimal | 3 |
| 3 | 71 | True | 3 | 17.8 | Optimal | 4 |
| 4 | 75 | True | 3 | 19.86666667 | Optimal | 6 |
| 5 | 47 | True | 4 | 42.93333333 | Optimal | 6 |
| 6 | 77 | True | 3 | 28.93333333 | Optimal | 5 |
| 7 | 67 | True | 3 | 55.1 | Optimal | 3 |
| 8 | 80 | True | 3 | 46.56666667 | Optimal | 5 |
| 9 | 67 | False | 3 | 52.46666667 | Optimal | 5 |
| 10 | 58 | False | 3 | 50.56666667 | Optimal | 3 |
| 11 | 59 | False | 3 | 42.5 | Optimal | 4 |
| 12 | 73 | False | 3 | 39.86666667 | Optimal | 4 |

**- Comment 6:“**The article also lacks a provision on the review of the study by a bioethics committee, as well as information on the consent of patients to provide their own clinical material for scientific purposes”

Response 6: We thank the reviewer for their comment. We have added the Ethical Statement section in the STAR Methods section of the revised manuscript, which reads as follows:

***Ethical statement***

*This study was performed under the Emory University Institutional Review Board (IRB) protocol STUDY00005888, which was approved as a non-human study and all relevant ethical regulations were followed. De-identified human samples were obtained from UPMC, collected under the same IRB approved protocol STUDY00005888. UPMC collected specimens with participants’ informed consent.*

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