Peer Review File

<mark>Response to Reviewer A</mark>

This is a neat and well-written review. Just a small addition to the text, as suggested, then this paper is well worth publishing.

Yet I miss a small subchapter that could be added with reference to De Ritis Ratio (AST/ALT). I suggest adding such a subchapter.

Some literature:

1. Eriksson, V.; et al. A Retrospective Analysis of the De Ritis Ratio in Muscle Invasive Bladder Cancer, with Focus on Tumor Response and Long-Term Survival in Patients Receiving Neoadjuvant Chemotherapy and in Chemo Naïve Cystectomy Patients-A Study of a Clinical Multicentre Database. J Pers Med 2022 Oct 27;12(11):1769.

2. Ghahari, M.; et al. Association between Preoperative de Ritis (AST/ALT) Ratio and Oncological Outcomes Following Radical Cystectomy in Patients with Urothelial Bladder Cancer. Clin. Genitourin Cancer 2022, 20, e89–e93.

3. Ha, Y.S.; et al. Association between De Ritis ratio (aspartate aminotransferase/alanine aminotransferase) and oncological outcomes in bladder cancer patients after radical cystectomy. BMC Urol. 2019, 19, 10.

4. Yuk, H.D. et al. De Ritis Ratio (Aspartate Transaminase/Alanine Transaminase) as a

Significant Prognostic Factor in Patients Undergoing Radical Cystectomy with Bladder Urothelial Carcinoma: A Propensity Score-Matched Study. Dis. Markers 2019, 2019, 6702964.

Reply: In accordance with the reviewer's recommendation, we added a description and all of references as to De Ritis Ratio as follows:

"**3.9 The aspartate aminotransferase / alanine aminotransferase ratio**

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are critical regulators of cellular metabolism and cancer cell turnover, and have potential utility as blood biomarkers. De Ritis et al. were the first to show that the AST/ALT ratio (De Ritis ratio) was a useful indicator of etiology in patients with acute hepatitis (38). Since then, multiple studies have shown that the AST/ALT ratio is a prognostic indicator in cancer patients. Since an increased ratio is associated with a higher rate of anaerobic glycolysis, which is a hallmark of UC, it could be used as a prognostic indicator in this malignancy. Indeed, an elevated De Ritis ratio was significantly associated with worse prognosis and higher mortality in patients with UC who underwent radical cystectomy (39–42). " (in L222-L231)

<mark>Response to Reviewer B</mark>

Authors reviewed the peripheral blood parameters for UC treated with systemic therapy.

This review is the enumeration of the parameters which have been reported. No description of the mechanisms and the clinical application.

1. Authors searched the manuscripts with the terms including "nutrition", "urothelial", "bladder", "prognosis", "chemotherapy", "immunotherapy", and "urology. Why don't you use the term "biomarker"? Authors missed other biomarkers.

Reply: In accordance with the reviewer's suggestion, we added the term "prognostic biomarker, or predictive biomarker", and this increased the number of items that we found. Accordingly, we have significantly modified the manuscript as follows.

 "A comprehensive literature search was conducted using PubMed and Google Scholar databases up to October 2022. Search terms included "nutrition", "urothelial", "bladder", "prognosis", "chemotherapy", "immunotherapy", "urology", "prognostic biomarker", or "predictive biomarker". A summary of the search strategy is shown in Table 1. We also manually searched articles related to this topic. The studies were reviewed by three authors (T.N, T.N, and Y.S) to assess whether they were appropriate." (in L95-L100)

"**3.8 Globulin**

The difference between the amount of serum albumin relative to the total amount of serum protein is known as the 'gamma gap' or 'GG'. In dialysis patients and in patients dying from pulmonary complications, the GG has been used as an independent predictor of mortality (36,37). The globulin family includes α-globulin, β-globulin, and γ-globulins; the latter class includes immunoglobulins such as IgG and IgM. The globulins play an important role in immunity and chronic inflammation, and their levels reflect a cumulative exposure of different cytokines." (in L213-L220)

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"**4.10 Controlling Nutritional Status score**

The Controlling Nutritional Status score (CONUT) is a combined score calculated from total peripheral lymphocyte count, total cholesterol concentration, and serum albumin concentration (80). It has been reported as a prognostic factor for esophageal cancer and colorectal cancer (81). Alb score is calculated as 0 ($3.5 \leq$ Alb), 2 ($3 \leq$ Alb < 3.5), 4 ($2.5 \leq$ Alb < 3), and 6 (Alb < 2.5). The total peripheral lymphocyte count (TLC) score is calculated as 0 ($1800 \leq$ TLC), 1 ($1200 \leq$ TLC < 1800, 2 ($800 \leq$ TLC < 1200), and 3 (TLC < 800). The total cholesterol (T-cho) score is calculated as 0 ($180 \leq$ T-cho), 1 ($140 \leq$ T-cho < 180), 2 ($100 \leq$ T-cho < 140), and 3 (T-

cho < 100) respectively. The CONUT score is the sum of Alb, TLC, and T-cho scores, and is classified into 0–1 (normal), 2–4 (mild), 5–8 (moderate), and 9–12 (severe). A CONUT score of 4 or greater is associated with poor OS in urothelial carcinoma (82). " (in L359-L370)

"4.11 Albumin-globulin ratio

The albumin-to-globulin ratio (AGR) is the ratio of serum Alb to non-albumin proteins. Malnutrition and inflammatory cytokines can inhibit the production of Alb, resulting in low serum Alb concentration, which could stimulate cell proliferation and weaken immune defense mechanisms. Globulin contains several immune-related proteins, such as complement components, fibrinogen and serum amyloid A, which are involved in regulating immunity and inflammation. However, using Alb and/or globulin alone produces unstable results, and its measurement is susceptible to interference by external confounders. AGR has a higher predictive value when combined with measurements of globulin and serum albumin compared to measurements of either of these parameters in isolation (83). Although the exact cut-off value for AGR has not been determined, aUC patients with a low AGR and who were treated with pembrolizumab had shorter PFS and OS than patients with a high AGR (84)." (in L372-L384)

2. Are these parameters prognostic or predictive biomarkers? Authors stated many parameters, but it is unclear if these parameters are good or poor prognostic biomarkers.

Reply: All biomarkers in this article were reviewed from the perspective of predicting prognosis before treatment, and to select patients for particular treatments. To reiterate, they are prognostic biomarkers. However, several articles use the terms interchangeably, and we may have failed to make clear distinctions in the previous draft of the review. To avoid confusion, we have added a new figure that highlights biomarkers associated with better or worse prognosis.

3. Can we select patients who received platinum-based chemotherapy or immune-checkpoint inhibitors with these biomarkers?

Reply: This reviewer's question highlights a very important point. However, all biomarkers introduced in this article were prognostic biomarkers. Therefore, it is impossible to select which treatment should be performed in the pretreatment stage using these biomarkers.

4. I recommend to change the paragraphs to describe biomarkers for each systemic treatments.

Reply: In accordance with the reviewer's suggestion, we tried to modify the manuscript. However, the systemic treatment section consisted of only two items: platinum-based chemotherapy or immune checkpoint inhibitor. Division by these categories caused a lot of duplication in the text which would have been confusing for the reader. Therefore, we subdivided based on each of the biomarkers separately.

5. I recommend authors to add figures to illustrate their conclusions.

Reply: In accordance with the reviewer's suggestion, we have added a new figure for designating better or worse prognostic biomarkers. We believe this has significantly improved clarity.

6. What is the mechanisms of these parameters associated with the predictive or prognostic of UC patients?

Reply: In accordance with the reviewer's question, we have now discussed the biological mechanisms and physiological processes that are pertinent to the review as follows.

"There is increasing evidence that pro-inflammatory cells in the tumor microenvironment actively contribute to tumor progression (4). Furthermore, the elevated levels of pro-inflammatory cytokines that accompany tumorigenesis often lead to cachexia, which is the process of involuntary loss of muscle and adipose tissue. Markers of cachexia including high blood cell counts and increased levels of C-reactive protein (CRP) in the serum of patients. Given the close association between systemic inflammation and cachexia, there is intense basic and clinical research into the optimal combination of blood-based markers that should be used to monitor these pathologies in cancer patients. Indeed, assessment of such markers in combination with other clinical data such as body mass can be used to predict prognosis." (in L80-L89)

Response to Reviewer C

The authors provide a review focusing on serum markers in mUC. The paper was very readable, but we believe that more detailed explanations in some parts would improve the paper.

We leave our comments below.

L58.

The author does not discuss the cut-off values of the markers much in the paper, and I think this sentence is unnecessary.

Reply: In accordance with the reviewer's suggestion, we deleted the sentence.

L65 It is difficult to tell whether this "Various" refers to the previous sentence or all cancers.

Reply: In accordance with the reviewer's suggestion, we modified the description.

L69 While I agree that the prognosis for mUC is generally poor, I do not agree that most cases treated are 'palliative'.

Reply: In accordance with the reviewer's suggestion, we modified the description. (in L65-L67)

L99 Please tell us who the three authors are. Please provide initials.

Reply: In accordance with the reviewer's suggestion, we added the description to the Methods. (in L95-L100)

L141-142 Please add references on the treatment of cachexia.

Reply: In accordance with the reviewer's recommendation, we have added the references.

L166 Neutrophils suppress lymphocytes, is this just a consequence? Please provide your opinion.

Reply: In accordance with the reviewer's recommendation, we modified the description as below:

"Moreover, suppression of lymphocyte function by activated neutrophils could be mediated by the secretion of myeloperoxidase, further contributing to cancer progression by dampening the immune response (21)." (in L165-L168)

Regarding the order of items (3).

Would the order of hemoglobin, neutrophils, monocytes, lymphocytes, platelets, and CRP be more acceptable to the reader?

Reply: In accordance with the reviewer's recommendation, we modified the order.

L186 CRP in mUC has been the subject of much research and needs to be explained in more detail on page

Reply: In accordance with the reviewer's recommendation, we modified the description as below:

"**3.6 CRP**

CRP is an acute phase proteins (APP) produced by hepatocytes during the inflammatory response. Its production and secretion are stimulated by the cytokines IL-6, TNF, and IL-1 (30,31). Although not a cancer-specific marker, CRP levels correlate with those of IL-6 and can therefore be used as an indicator of inflammation in cancer patients. Two main hypotheses exist regarding CRP elevation in patients with cancer. First, tumor cells may increase the levels of CRP indirectly during the induction of inflammation. Alternatively, tumor cells may themselves secrete factors that lead to increased CRP levels. The value of CRP alone as a prognostic biomarker for patients with UC has been assessed in multiple clinical trials. For aUC patients, it has been reported that elevated CRP was associated with both worse PFS and OS (32). As CRP measurement is simple and inexpensive, it can serve as a non-specific biomarker of response for initial treatment." (in L192-L204)

L217, L225 The authors define 'predictive' and 'prognostic' in text L104. According to that definition, is the NLR not a predictive marker?

Reply: All biomarkers in this article were reviewed for their utility as pre-treatment prognostic markers rather than as indicators of the most suitable treatment modality. In other words, they are prognostic biomarkers. However, several articles use the terms interchangeably, and we may have failed to make clear distinctions in the previous draft of the review. In fact, we could not use NLR status to determine whether patients should receive cisplatin-based chemotherapy or ICIs, and therefore conclude that NLR should only be considered as a prognostic biomarker.