12th May 2021

Dear Editor in Chief

World Journal of Clinical Oncology

Re: Manuscript NO: 65716, Carcinosarcoma of Gallbladder: A World Review

Many thanks for seeking peer reviews for our manuscript. We appreciate the time and expertise of peer reviewers and submit point by point response to the comments and have made edits in the manuscript.

Comment 1: The discussion part feels a bit unstructured when reading. It could be more clearly structured in subsections related to e.g., diagnostics (imaging, tumor markers etc.), therapy, prognosis, etc.

Response 1: We have taken your suggestion and have split our discussion into several subsections with subheadings for clarity. The subheadings added are incidence, signs and symptoms, biochemical investigations, imaging, histological diagnosis, surgical management, adjuvant treatment, prognosis, role of tumor markers, and comparison to gallbladder adenocarcinoma.

Comment 2: Extended cholecystectomy could be explained in more detail, also maybe giving a recommendation for clinical practice (conventional cholecystectomy with frozen section and extension of resection after that if positive vs. two-time surgical approach, etc.) given the importance of surgical therapy for these tumors.

Response 2: We have provided recommendations for clinical practice extrapolating from the evidence from carcinomas of the gallbladder management. In the subsection on surgical management, we have revised the paragraph and text and following is added - Completion liver resection with or without lymphadenectomy and/or bile duct resection is an accepted standard for post simple cholecystectomy discovered GBC with T_{1b} and higher stage. This approach not only

involves two surgeries, but also increases the risk of cutting through the tumor with potential for tumor seeding and dissemination. Yip VS et al in a series of 40 patients with incidental GBC reported that majority of patients were not amenable for further curative resection. A report from Memorial Sloan-Kettering Cancer Centre involving 116 patients showed that survival of patients with residual disease is not different than survival of patients with stage IV disease and neither group of patients benefit from reoperation. Thus, single surgery may be better. Radical cholecystectomy has higher morbidity as compared to simple cholecystectomy; and thus, the concept of something intermediate i.e., extended cholecystectomy is attractive. Fujisaki S et al. reported a case describing the concept of Laparoscopic extended cholecystectomy (LEC) with 1cm liver margin; however, they proposed open conversion when intraoperative histology showed GBC invading subserosal layer. With current advancements, LEC was noted to have lesser intraoperative and postoperative complications than open extended cholecystectomy. The key differences between a 'radical' and 'extended' cholecystectomy are restricting the liver parenchyma transection to the 2cm wedge of liver tissue and also performing regional lymphadenectomy and choledochectomy only in selected patients. Radical cholecystectomy can be done by open, laparoscopic or robot assisted approach, with comparable short-term outcomes.

Please note that 3 citations are added.

Comment 3: In my opinion the similarities/commonalities between gallbladder-carcinoma and -carcinosarcoma are not clearly outlined. Many of the sections in the discussion part are equally true for any gallbladder-malignancy.

Response 3: Thank you for the comment. This is indeed true due to rarity of CSGB condition. Most authors have extrapolated the principles of diagnosis, treatment and prognosis from the evidence and data that is reported from gallbladder adenocarcinoma. We agree that in general, principles will remain the same and CSGB will be a histological surprise in a patient suspected to have gallbladder adenocarcinoma. However, since the focus is CSGB, we have added a final paragraph to summarize the differences in a bid to compare and contrast the two malignancies more clearly. The following is added in the subsection on comparison - There is substantial overlap of risk factors, diagnosis and treatment of CSGB with gallbladder adenocarcinoma. Thus, majority authors extrapolate the clinical characteristics of gallbladder adenocarcinoma to determine best approach to diagnosis and management for CSGB. From this review, we can determine three key

differences between CSGB and gallbladder adenocarcinoma. Firstly, tumor markers have limited utility in patients with CSGB. In a study of 55 cases by Shukla et al, it is noted that the combination of CA 125 and CA 19-9 helped detect gallbladder malignancy in patients with gallstones (80.7%). Secondly, the prognosis of CSGB may be marginally better compared to carcinoma of the gallbladder. In the meta-analysis by Zhang et al., it was noted that the survival rate was slightly better (16±5% 5-year survival) compared to carcinoma of the gallbladder (0-10% 5-year survival) (3). Thus, the identification of CSGB will be useful to determine the prognosis for patients, albeit with only a small variation between the two. Thirdly, immunohistochemistry markers like vimentin and cytokeratin are associated with diagnosis of CSGB

One citation is added.

Comment 4: Where statements are made regarding the worse prognosis of carcinosarcoma they are not supported by meaningful referencing.

Response 4: We have supported the statements by citing meta-analysis article by Zhang et al. who corroborates our statement of poor prognosis of carcinosarcoma of the gallbladder.

Comment 5: The (adjuvant) chemotherapeutic options (and the lack of high evidence data) could also be discussed in the light of gallbladder-carcinoma (where I believe the options and evidence are the same)

Response 5: Thanks for this suggestion. We have enhanced the section on adjuvant therapy. The following is added - The adjuvant treatment reduces recurrence risk and improves survival outcomes by eliminating or controlling the micrometastatic disease. A meta-analysis of retrospective studies including 6712 gallbladder cancer patients reported that lymph node positive patients enjoyed the survival benefit.

Adjuvant radiotherapy is shown to be of value in reducing local recurrence in selected patients with gallbladder cancer. In a study including 4180 patients with resected gallbladder cancer diagnosed from 1988 to 2003 from the Surveillance, Epidemiology, and End results database, Wang J et al. reported that adjuvant radiotherapy provides survival benefit in node positive or T2 and higher stage disease. A single arm phase II study conducted by South West Oncology Group reported that gemcitabine plus capecitabine, followed by radiation (45 Gy to regional lymphatics,

54-59.4 Gy to tumor bed) and capecitanine resulted in 56% 2-year survival rate for patients with gallbladder cancer. Based on this results, American Society of Clinical Oncology guidelines recommend chemotherapy plus radiation in gallbladder cancer patients with R1 resection.

Overall, three citations are added.

Comment 6: So, in the end: there should be a clear conclusion in the end of the manuscript what of the compiled and presented data makes gallbladder-carcinosarcoma a clinically relevant diagnosis (apart from other gallbladder malignancies) as opposed by only an academic one.

Response 6: We noted your comment and have established links between what is clinically relevant to distinguish carcinosarcoma of the gallbladder from other gallbladder malignancies. The following is added - While most features of CSGB parallel that of carcinomas of the gallbladder clinically, identification of CSGB specifically allows clinicians to determine overall prognosis. Due to paucity of reported cases, more evidence is required before meaningful and valid evidence-based patient-centric recommendations can be made. This review serves to educate and raise awareness among the clinicians dealing with gallbladder malignancies. It is likely that there are more clinical differences between CSGB and common forms of gallbladder cancer; and active reporting of cases will help enhance understanding of this rare cancer.

Comments inside the word document:

Comment 1. What is the basis for 5cm cut off?

Response 1: Tumour size is not determinant of gallbladder cancer TNM stage. The AJCC 8th edition does include size cut-off 5cm to differentiate stage T1b and above intra-hepatic cholangiocarcinoma. As the previous meta-analysis suggested that tumors below 5cm had better survival, we decided to validate this. We have rephrased the statement as - Kaplan-Meier survival curves were compared between lesions larger than 5cm and those smaller than 5cm as data by Zhang et al. suggested that tumors smaller than 5cm had better survival(3).

Comment 2. Case report should not be the basis for citing that CSGB is aggressive biliary malignancy.

Response 2: We agree. The case reports do indicate that prognosis is universally poor. This is also considering the reporting bias and our basis to suggest that CSGB has poor prognosis is based on the previous meta-analysis. This study also confirms poor survival outcomes as evident from data tabulation. Thus, we have rephrased the statement and citations as – CSGB is considered the most aggressive biliary tract malignancy, usually discovered at late stages, and has poor prognosis(3).

Comment 3: Incidence vs. risk of gallstones.

Response 3: This is correct. We cannot make assumption about the risk based on retrospective data and thus the cause-effect assumption needs to be rephrased as incidence and a possible association. Thus, we have revised the 'risk of gallstones' as - In our study too, the incidence of gallstones was high (83%).

Comment 4: Some minor comments are added by help of the tracked changes function in the word file.

Response 4: We have noted your comments in the word file and have made the appropriate changes to the phrasings of certain statement.

Comments from Science Editor:

Comment 1. The "Author Contributions" section is missing. Please provide the author contributions.

Response 1: We have included the following for author's contributions: **Author's contributions:** Teng TZJ, BQY Chua and VG Shelat contributed to the conception of the idea and writing of the paper.

Comment 2. The authors did not provide original pictures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor

Response 2: We have provided the original figure documents in PowerPoint format titled "65716 Figures.pptx"

Comment 3. PMID and DOI numbers are missing in the reference list. Please provide the PubMed numbers and DOI citation numbers to the reference list and list all authors of the references. Please revise throughout.

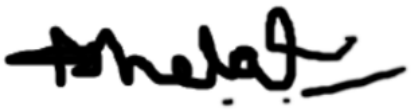
Response 3: We have formatted the reference list to include the DOI citation numbers and PubMed numbers.

Additional edits:

We have included "Not Available" abbreviated as "NA" in Table 1 where information was not reported by the literature for the relevant columns.

Thanking you

Sincerely



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