Author's response to reviews

Title: Elevated preoperative peripheral blood monocyte count predicts poor prognosis for hepatocellular carcinoma after hepatic resection

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Author's response to reviews: see over
Dear Annie Lyn Bravo

I’m very glad to receive your letter.

We have made point-by-point response to the comments made by two reviewer(s) and revised our manuscript according to their suggestions. In addition, we have changed the arrangement of contents in discussion to make the manuscript more readable.

Thank you very much for your help.

Sincerely,

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Responses to Reviewer Megan McNally

Major Compulsory Revisions

1. What additional value does the monocyte value have in prognosis over the other independent predictors of survival (tumor size, encapsulation, multiple tumors)? (i.e. all of that information should be known postoperatively as well)

Prognostic factors identified in previous studies included tumor stage, serum alpha fetoprotein (AFP), vascular invasion, tumor size, poor differentiation and many novel immunological and histological biomarkers [1]. However, there is no consensus over their predictive values. Therefore, we can only try to find as more biomarkers or clinical and pathologic features as possible to help us discriminate patients at high risk of recurrence. Patients with more risk factors are more prone to recurrence and may benefit from post-operative adjuvant therapies. We couldn’t say that monocyte is superior to other independent predictors of survival. But it adds to the reservoir of the risk factors and gives us clue of recurrence.

2. Would a high preop monocyte value perhaps lead to consideration of neoadjuvant therapies?

HCC shows very low responsiveness to standard chemotherapeutic agents or radiotherapy as other tumors (such as osteosarcoma, lung cancer, and breast cancer), neoadjuvant therapies haven’t been proved to be useful and wouldn’t be taken into account [2-4]. Instead, post-operative adjuvant therapies might be considered.
3. **Would following the monocyte count postoperatively aid in identifying recurrence earlier?**

Surgical trauma produces alterations in the hemodynamic, metabolic, and immune responses of patients in the postoperative period. It causes an increase in the total number of white blood cells circulating in the body. Meanwhile, surgery can cause a variety of immunological disturbances. Decreases in peripheral lymphocyte numbers and impaired lymphocyte function are often observed after surgery. In addition, anesthesia and surgery also impair monocyte and macrophage functions, including chemotaxis and phagocytosis [5, 6]. In fact, proinflammatory cytokine production in the intraoperative and early postoperative periods is initiated by macrophages and monocytes at the initial site of injury as part of the acute-phase response. After surgery, too many factors will influence postoperative peripheral blood monocyte count, such as bleeding, shortage of liquid replacement therapy, sepsis and so on. Therefore, postoperative peripheral blood inflammatory cells haven’t been used quite often to predict prognosis as the preoperative counterparts, although there are a few such reports [7-9]. In fact, in Lee’s study, they found that monocyte significantly increased after surgery, but they did not detect significant effects for circulating monocytes and the survival [9].

Therefore, we think it might be unsuitable to use postoperative monocyte to predict prognosis for too many confounding factors. We have added this viewpoint in the manuscript.

4. **Would failure of the monocyte count to resolve indicate indolent disease?**

As mentioned above, there are too much confounding factors which may influence postoperative peripheral blood monocyte count. We find no such viewpoint from publications and
we couldn’t give a definitive answer here. If the monocyte count fails to resolve, we don’t think this indicates indolent disease.

5. **Does the author have any plans to assess patients in the future on a prospective basis or in a clinical trial looking at adjuvant therapies?**

Although we found that elevated monocyte predict early recurrence and these patients might benefit from postoperative adjuvant therapies, we haven’t been able to test this hypothesis, which we hope to prove in future clinical trial.

6. **I feel these questions should be addressed in the discussion to emphasize the clinical importance of this information.**

Thank you very much for your kind suggestions and we have tried to cover them in the discussion.

**Minor Essential Revisions**

1. **There are some spacing issues between numbers and %iles and words and the citations throughout the text.**

We have tried to correct these errors in the manuscript.

**Responses to Reviewer Michele Barone**

**Major Revision**

1. **The current study is not the first paper exploring the impact of high**
monocyte levels on outcomes after resection in HCC patients (See Sasaki et al, Surgery 2006). Therefore, concerns raise about the novelty of the work.

Sasaki et al. first reported that monocyte count was a useful prognostic indicator in HCC patients. However, there are great differences between our reports, which we think will add to our knowledge to HCC pathology, as cited in our article.

First, the selection of the cut-off value in our study is more convincing. The cut-off value for monocyte count was set based on a median value of circulating monocyte count in Sasaki’s report [10]. In our study, to exclude empirical bias, we used ROC curve to determine the optimal cut-off value.

Second, patients in our two studies had different hepatitis background of HCC. In Sasaki’s report, serum hepatitis C antibody was positive in 100 (65.4%) of the 153 tested patients and hepatitis B virus infection was positive in only 23.74% (47/198) patients, which was very different from our data (86 %, 302/351 patients).

Thirdly, the predictability of monocyte was different. From subgroup analysis we found that elevated monocyte predicted early recurrence whether cirrhosis was present or not, which was different from Sasaki’s report. Patients in these two studies have a different background of cirrhosis, which might account for the difference. In addition, we found that elevated monocyte count predicted poor prognosis in HBV positive HCC patients, while not in negative ones, which is also a novel finding.

2. It’s not clear what dependent parameter the authors used for ROC analysis.

Disease-free survival rate at 1 year? 3 years? 5 years? Moreover, for
prognostic purposes, the dependent variable should be overall survival (i.e. the primary endpoint in oncologic studies). Instead, in order to find a reliable cut-off point, authors stratified patients at high/low risk of recurrence. What does it mean recurrence? Disease-free survival? If so, a secondary endpoint has been erroneously taken into account.

We have used disease-free survival (from date of surgery to date of recurrence) as dependent parameter for ROC analysis, as done by many authors [11]. It’s true that overall survival (OS) is the gold standard for the demonstration of a clinical benefit in cancer trials. But replacement of OS by a surrogate endpoint allows reducing trial duration. In practice, surrogate endpoints have been used in digestive oncology. In Methy’s paper (BMC cancer 2010), they evaluated the potential surrogate endpoints for OS in digestive cancer trials, by way of a survey among clinicians and methodologists. They found that DFS, in the neoadjuvant settings or early stages, and PFS, in the non-operable or metastatic settings, were ranked first, with a frequency of more than 69% in 20 out of 22 settings. They suggested that the overall results should help priorities the endpoints to be statistically evaluated as surrogate for OS, so that trialists and clinicians can rely on endpoints that ensure relevant clinical benefit to the patient [12].

In addition, as suggested by Llovet et al, time to recurrence was recommended by the panel as the primary endpoint for HCC phase 2 and 3 studies that assess adjuvant therapies after resection or local ablation. [13, 14]. Our study was a retrospective study mainly evaluating the prognostic effect of preoperative monocyte count, therefore, we chose DFS as the primary endpoint. No secondary endpoint hasn’t been taken into account in the stratification or ROC analysis.
3. The definition of OS that authors give in Materials and methods is wrong. OS is the time from treatment to death for any cause (not only HCC-related death). See Llovet et al, Design and endpoints of clinical trials in hepatocellular carcinoma., J Natl Cancer Inst. 2008, for the details.

As written by Llovet et al, Cancer-specific survival is a related endpoint, in which only deaths due to cancer are considered for survival analysis and non–cancer-related deaths are censored. They concluded that “the time from treatment to death for any cause” is superior to cancer-specific survival when taking into account “intercurrent mortality” [13]. Our OS was cancer-specific survival. As suggested by the reviewer, we revised this definition. Since there were no Child C patients and no mortality from progressive liver failure without tumor recurrence, and no patients died of disease out of HCC recurrence. The revision of definition hasn’t changed our results.

Fig.1. The pyramid of endpoints represents a generally preferred hierarchy of study outcomes, going from those with the least clinical impact to the greatest import. It is often easier to obtain
endpoints from lower down in the pyramid, as they occur with greater frequency in a population; however, they are less definitive in establishing the ultimate clinical utility of a given intervention.[15]

(Croswell JM et al, J Hepatology, 2009)

4. ROC analysis is not the best way to find prognostic cut-off of a continuous parameter because OS and DFS are time-dependent variables. ROC can obviously be drawn but requires an artificial method (i.e. transforming OS or DFS in rates at predefined time periods). Such method could lead to biases because doesn’t encompass all study period but only a predefined limit (for example 1 or 3 years). Running log-rank test doesn’t present the aforementioned bias and should be performed in this setting. See Facciorusso et al. Serum Ferritin as a New Prognostic Factor in Hepatocellular Carcinoma Patients Treated with Radiofrequency Ablation. J Gastroenterol Hepatol. 2014 Apr 14. doi: 10.1111/jgh.12618, for the details.

Thank you very much for your suggestion. As you mentioned, receiver operating characteristic (ROC) curves may not be the best way to find prognostic cut-off of a continuous parameter, and could lead to biases because it doesn’t encompass all study period but only a predefined limit. However, ROC curves are widely used in medicine to determine a cutoff value, especially in the studies of tumors (including many papers published recently in BMC Cancer) [11, 16-19], which testify its rationality and effectiveness.

The goal of an ROC curve analysis is to determine the cutoff value. The determination of an
"ideal" cut-off value is almost always a trade-off between sensitivity (true positives) and specificity (true negatives). As both change with each "cut-off" value it becomes difficult to imagine which cut-off is ideal. The ROC curve offers a graphical illustration of these trade-offs at each "cut-off" for any diagnostic test that uses a continuous variable. Ideally, the best "cut-off" value provides both the highest sensitivity and the highest specificity. However, it is rare that this ideal can be achieved. So in most of cases, a higher sensitivity is chosen at the cost of lower specificity. Through ROC curve analysis, we determined the optimal cut-off value with best sensitivity and specificity.

We sincerely read the paper you have suggested [20]. Honestly, we felt it a little too difficult for us to use “the running log-rank test” for the present, which we hope we can use after we have fully understood this statistical method and its superiority over ROC curve.

5. **All 351 patients underwent a biopsy before surgery? In Methods the authors state “351 newly diagnosed, histologically proven HCC”. This procedure stands against current guidelines (both American and European) that consider biopsy only in case of discordance between CT and RMI. Please, better define the diagnostic algorithm followed in the study.**

We are sorry to make such a misunderstanding. In fact, no patient in this study underwent biopsy before surgery. We diagnosed HCC with CT/MRI combining with AFP before surgery. All the specimens were sent for pathological examination after surgery. We have made revision in the manuscript.
6. The study lacks a validation cohort, hence the results reported by the authors are weak and need further validation. Please develop this issue in Discussion.

For statistical validation, we have planned to split the data set into a training data set and a test data set, as done by Chiang et al. while we haven’t been able to provide so many cases with a solid result. Therefore, we hope we can have further validation in the future or from other centers [21]. We have added this issue in discussion.

Minor Revision

1. A careful language editing is needed. There are several typos and grammatical errors: for instance, page 11, raw 233 “the median monocyte count was two times higher Sasaki’s”. The word “than” should be added to this phrase.

We have revised this error and the manuscript has been reviewed and corrected by Professor Stephen Tomlinson from the Department of Microbiology and Immunology, Medical University of South Carolina.


We have added this part in the discussion as suggested.
References


