Peer Review File

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Reviewer A

Comment 1: Hemophagocytic lymphohistiocytosis is a serious immune disorder, which leads to immune-mediated organ damage. What is the status of immunosuppressive agents in the treatment of HLH?

Reply 1: Thank you for the comment. According to the literature, the immunosuppressive agents in the treatment of HLH mainly include glucocorticoids, cyclophosphamide, cyclosporine, etoposide and doxorubicin. Firstly, glucocorticoids are almost admitted as initial treatment and used in almost regimens. Secondly, cyclophosphamide and doxorubicin are from CHOP, and widely used for lymphoma-associated HLH. Thirdly, etoposide and cyclosporine are from HLH-97/2004 protocol. There is currently no standard treatment for the HLH secondary to solid tumor, so we chose the most widely used solution for secondary HLH after consultation from hematologists. At last, the DEP regime including doxorubicin, etoposide and methylprednisolone is used as a salvage therapy for the patients who did not achieve partial response to a standard therapy. We have modified our text as advised to add this part of content (see Page 9, line 19).

Changes in the text: There is a lack of relevant trials guiding the treatment of cancer patients with HLH. The immunosuppressive agents used in the treatment of HLH mainly include glucocorticoids, cyclophosphamide, cyclosporine, etoposide and doxorubicin. Among these agents, glucocorticoids are almost admitted as initial treatment and used in almost regimens, and others are from HLH 94 and 2004 protocols, CHOP regimens and DEP regimen. CHOP is mostly used for lymphoma-associated HLH, and DEP is used as a salvage therapy. In the treatment for case 2, we apply the regimen including etoposide and dexamethasone mainly based on the HLH 2004 protocol.

Comment 2: In some reports, it is estimated that in Italy, Sweden and the United States, the annual incidence rate of HLH is 1 per 800 thousand children and less than 10 per thousand children. Is there an epidemiological survey of HLH in Asian population?

Reply 2: Thank you for your comment. As we described in the text, the epidemiology data of HLH is limited. Unfortunately, HLH in gastroenterological diseases is rarely reported. The incidence of people including adults and children is from a nationwide survey in Japan, and it is 1 per 800,000 people. We have modified our text as advised (see Page 7,line 16).

Changes in the text: In some reports, HLH has an estimated yearly incidence of one per 800,000 people in Japan and less than ten per million children in Italy, Sweden, and the USA.

Comment 3: In the treatment of HLH, early diagnosis and intervention are very important. What are the main methods of early diagnosis at present?

Reply 3: Thank you for the comment. A patient can be diagnosed as HLH when he meets at least 5 from 8 criteria according the HLH-2004 or he gets a molecular diagnosis. Among these criteria, fever, splenomegaly, hypertriglyceridemia and hypofibrinogenemia are not rare in

cancer patients, and cytopenia is common in patients undergoing chemotherapy. Giving test of sCD25, ferritin, NK-cell activities and biopsy on every patients is not viable and necessary. So we recommend to apply sCD25 and ferritin test, and bone marrow biopsy at an early stage on the such patients with symptoms to diagnose HLH as soon as possible. We have modified our text as advised to expound our idea clearly (see Page 9,line 15).
Changes in the text: When these symptoms are present in a cancer patient, especially in patients undergoing chemotherapy and immune checkpoint inhibitors therapy, screening tests including sCD25, ferritin, NK-cell activities, and bone marrow biopsy need to be considered at an early stage in order to diagnose HLH as soon as possible.

Comment 4: The exact mechanism of HLH secondary to solid tumors has not been elucidated. What are the main potential mechanisms?
Reply 4: Thank you for the comment. A defect in granule mediated cytotoxicity, which is important in killing cells, is the underlying common mechanism in both primary and secondary forms of HLH. Key gene mutations for these functions have been implicated in primary HLH, while secondary HLH do not have a known genetic cause. The secondary HLH occurs as a complication in children and adults in the setting of immunodeficiency or an underlying malignant, infectious, or autoimmune disorder. A number of possible mechanisms of secondary HLH have been identified. As for HLH secondary to solid tumors, there are two potential mechanisms. First, it is postulated that the hyperinflammation is triggered by the neoplasm due to an excessive secretion of pro-inflammatory cytokines and persistent antigen stimulation by the tumor cells. Second, the combined immunodeficiency generated by the underlying malignancy and the loss of immune homeostasis due to chemotherapy further aggravates T-cell dysfunction that lowers the threshold for triggering HLH in these patients. We have modified our text as advised (see Page 8,line 21).
Changes in the text: The exact underlying mechanism of HLH secondary to a solid tumor has not yet been elucidated. It is assumed that the hyperinflammation is triggered by the secretion of pro-inflammatory cytokines and persistent antigen stimulation by tumor cells. In addition, the unbalance of immune homeostasis induced by chemotherapy and the underlying malignancy would lower the threshold for triggering HLH.

Comment 5: Etoposide can clear the activated immune cells to calm down the HLH cytokine storm. Long term use of etoposide may cause bone marrow suppression. What are the drugs that can inhibit the storm of inflammatory factors? Can it be used in the treatment of HLH?
Reply 5: Thank you for the comment. Calming down the over reactivated immune system is the goal of the therapy in HLH. The regimens mentioned in the text almost all aim at this goal. In addition to these regimens, novel therapies for hypercytokinemia of HLH has become an attractive alternative therapeutic target, including IFN-γ inhibitor NI-0501 and JAK inhibitor ruxolitinib. These two drugs could block single or multiple cytokines and be beneficial, but its increased toxicity is also a possibility. Although these drugs may be applied on HLH patients triggered by cancer, studies comparing these strategies are still lacking. So, we did not put them in this report.

Comment 6: The incidence rate of solid tumor associated HLH is low and the mortality is high.

Are there biomarkers for predicting the prognosis of HLH?

Reply 6: Thank you for the comment. According to Professor Alison M. Schram and Nancy Berliner from Harvard Medical School, the response to the treatment is mainly used to predict the prognosis, and it could be determined by clinical and laboratory evidence of resolution, including improved splenomegaly, lymphadenopathy, fever curve, ferritin, sIL2R, liver function tests, and fibrinogen. In our experience, the ferritin level during and after treatment seems to correlate with disease activity. Although ferritin level used as a monitoring marker has been proposed as an experience in several reports, there is still a lack of studies on it. So, we have modified our text as advised (see Page 10,line 21).

Changes in the text: Also, monitoring the treatment effect is crucial. In our study, we monitored the fever curve, ferritin and sCD25 levels, liver function tests, and fibrinogen in case 2 to determine the treatment response. Our data showed that ferritin levels correlated with disease activity (Figure 3), and it matches the experience of other reports.

Reviewer B

Comment 1: I would be interested to see if the tumors could be profiled - any of DNA / RNA seq, or IHC to look at immune infiltration, or ctDNA; these might give insight into what made these gastric tumors different from the vast majority that don't produce HLH.

Reply 1: Thank you very much for your comment. Your comment does broaden our horizons and enlighten our thoughts. DNA/RNA seq and ctDNA were nor performed on the patients. However, tests for immune infiltration by IHC could be possibly done in case 1. The problem is that the tumor specimen in case 1 has been paraffin-embedded for years and the patient has passed away, we have to seek consent from his family member. Since then, it still takes weeks to search for the specimen and finish the test. As for the patient 2, her tumor is unresectable, and after HLH diagnosed she could not tolerate gastroscopy anymore, so we did not get enough sample for further analyze through biopsy. After catching your points, we are going to perform these analyses on the next patients in future, and we would summarize the results of DNA/RNA seq and IHC from all the patients and publish these data. Thank you very much!