
Peer Review File

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Comment 1: Major critique: Several important issues are not entirely explained. In order to improve the clarity of text I suggest addressing more precision in certain details that are listed below. The flow of the article is not fluid and the English language needs a carefully revision.

Reply 1: We have described some of the details below in accordance with the reviewer's recommendations as following. English has also been revised. See the red font in the text. In addition, we will select medical writing service (AME Editing Service, <http://editing.amegroups.cn/#editing>) to edit the wordings of the main text and figures and tables.

Comment 2: Specific comments: Title: The authors focused mainly on liver injury due to CMV but in the title this aspect is not specified.

Reply 2: We have added "with human cytomegalovirus infection" to the title.

Changes in the text: Page 1 Line 2-3. Page 1 Line 16.

Comment 3: Introduction: Figure 1. It is not representative of the Figure legend "Schematic diagram of spleen aminopeptide oral lyophilized powder preparation". This "preparation" is not explained at all.

Reply 3: We delete "(Figure 1)" in the part of Introduction and add "Spleen aminopeptide oral lyophilized powder preparation" in the part of Methods.

Changes in the text: Page 3 Line14, Page 5 Line 18-22, Page 6 Line 1-11.

Comment 4: Methods: Patients: The authors must describe the selection of subjects: how and where they were recruited. It is not clear to me the etiology of liver injury. CMV seems that account for 99,1% of patients (according data of CMV-DNA in Table 4 of results, but this data it is not found in Patients section nor find in the Title). There is not shown how many patients are treated by GSH and /or UDCA. Moreover, the authors say that "According to the presence or absence of CMV infection including the positive CMV-IgM and CMV-DNA load values, ganciclovir was used in antiviral

therapy. (Page 3, lines 10-12). How many children were treated with ganciclovir? It is rather confusing and all these issues should be carefully explained because the aim of this study is to evaluate the efficacy of the therapy proposed. All these aspects can introduce bias in the interpretation of the results.

Reply 4: We describe the selection of subjects: how and where they were recruited as the following procedure-“From July 2018 to May 2020, 475 HCMV infected infants and children who were visited in the clinic outpatient and hospitalized in the Department of Pediatric Gastroenterology, Children's Hospital of Nanjing Medical University were enrolled in this study. 136 cases failed to take spleen aminopeptide oral lyophilized powder according to the protocol and 122 cases failed to perform liver function test at the 2nd or 4th week of follow-up. Therefore they withdrew from this study. Finally, 217 children were enrolled in this study.”

Changes in the text: Page4 Line8-13.

It is not clear to me the etiology of liver injury (according data of CMV-DNA in Table 4 of results, but this data it is not found in Patients section nor find in the Title): in this study, all infants and children with liver injury were caused by cytomegalovirus infection. Among 217 HCMV infected infants and children (the positive HCMV-IgM or more than and equal to 10^3 copies/ml of the HCMV-DNA load values) with liver injury before treatment, 125(57.6%) patients had the positive HCMV-IgM. 160 cases were 10^3 - 10^7 copies/ml (Table 4). Both HCMV-IgM positive and HCMV-DNA were found in 68 cases.

Changes in the text: Page4 Line13-15.

There is not shown how many patients are treated by GSH and /or UDCA. Total 217 cases were treated with GSH and 113 cases with jaundice were treated with GSH and UDCA.

Changes in the text: Page4 Line17.

According to the presence or absence of CMV infection including the positive CMV-IgM and CMV-DNA load values, ganciclovir was used in antiviral therapy. (Page 3, lines 10-12). How many children were treated with ganciclovir? It is rather confusing and all these issues should be carefully explained because the aim of this study is to evaluate the efficacy of the therapy proposed. The description was wrong here. Total 217 cases of HCMV infection were not given anti CMV drug therapy.

Changes in the text: Page4 Line18.

Changes in the text: Page4 Line8-13. Page4 Line13-15. Page4 Line18.

Comment 5: Inclusion an exclusion criteria: In addition, criteria for liver injury should be specified. Biochemical: What data and aminotransferase levels? Imaging: What criteria? And if pathology of the liver was obtained.

Reply 5: We have added “All of them met the diagnostic criteria of liver damage confirmed by serum alanine aminotransferase (ALT) or conjugated bilirubin (DB) increased to 2 times the upper limit of normal or serum aspartate aminotransferase (AST), alkaline phosphatase (ALP) and total bilirubin (TB) increased at the same time, and at least one of them increased to more than twice the normal upper limit.” to the inclusion as the criteria for liver injury. Imaging was not included in the diagnostic criteria of liver injury because enlargement of liver volume is one of the main imaging manifestations of liver damage in infants and children with cytomegalovirus infection, but it is not a sensitive evaluation index for infants and children with treatment course of only one month. Liver pathological examination was not included because the operation of liver puncture examination is traumatic and the parents of the children did not agree.

In addition, we added “HCMV antibody titer and HCMV-DNA load values was positive, and no biliary malformation, metabolic liver disease, drug poisoning hepatitis, etc. were found; hepatitis virus, EB virus, respiratory virus and other common viruses were negative” to the inclusion as the criteria for cytomegalovirus infection.

Changes in the text: Page 5 Line 3-7. Page5 Line 8-12.

Comment 6: Regimen protocols: A flow chart indicating number of children treated with GSH/UDCA/ganciclovir should be grateful. Also, the composition of the product administered. That could be obvious for the authors, but many readers do not know this product.

Reply 6: A flow chart indicating number of infants and children with GSH/UDCA/Spleen aminopeptide was completed in Figure 2 (Page 19). The composition of the product was introduced-Its main component is polypeptide with relative molecular weight of 1000-10000, Most of them are peptides with relative molecular weight of 2000-5000, including thymosin β 4 (Page6 Line 8-10) .

Changes in the text: Page 19. Page6 Line 8-10.

Comment 7: Detection of liver damage and immune function: The authors should specify the criteria for consider that the therapy ameliorates liver function (i.e. normality of ALT or diminution of levels? And what rate of CMV-DNA load values reduction?)

Reply 7: In this study, the diagnostic criteria of liver damage confirmed by serum alanine aminotransferase (ALT) or Direct bilirubin (DB) increased to 2 times the upper limit of normal or serum aspartate aminotransferase (AST) and total bilirubin (TB) increased at the same time, and at least one of them increased to more than twice the normal upper limit. After 1 month of spleen aminopeptide oral lyophilized powder combined with GSH and/or UDCA, the above indicators decreased sharply than those without aminopeptide oral lyophilized powder, suggesting that aminopeptide oral lyophilized powder was remarkable in the curative effects. The anti-HCMV effect is the same. Therefore, it is not necessary to specify the criteria for consider that the therapy ameliorates liver function.

Changes in the text: Page 5 Line 4-14

Comment 8: Statistical analysis: I suppose that the comparisons are not paired.

Reply 8: The data of this study were not conducted by paired t test. **The statistical methods were as follows:** Continuous variables were expressed as median (IQR) and compared with the Mann-Whitney U test. Categorical variables were expressed as number (%) and compared by χ^2 test or Fisher's exact test between with spleen aminopeptide and without spleen aminopeptide.

Changes in the text: Page 7 Line 14-17

Comment 9: Results: it is redundant to show the results in the Tables and in the text. It should be specified how many children normalized liver function and negativized the CMV infection according the initial therapy (GSH/UDCA/ganciclovir)

Reply 9: The content of the text is a detailed explanation of the chart. In this study, it was defined that the positive HCMV-IgM or more than and equal to 10^3 copies/ml of the HCMV-DNA load values as cytomegalovirus infection positive. Total 217 children were cytomegalovirus positive before treatment, and 125 of them were IgM positive, 160 cases were 10^3 - 10^7 copies/ml. Both HCMV-IgM positive and HCMV-DNA were

found in 68 cases.

Changes in the text: Page 10 Line 1-4

Comment 9: Discussion: The authors should emphasize what is the novelty in the results of the present investigation with respect the findings of different previous studies. Why cardiovascular risk is higher in black subjects? In this sense, the authors should state their interpretation of the findings and try to explain why this product ameliorates liver injury

Reply 9: Previous studies have not shown that spleen aminopeptide can improve liver injury. Our research confirms that spleen aminopeptide oral lyophilized powder can effectively enhance the liver protective effects and jaundice clearance rates of GSH or UDCA, reduce liver damage and promote bile excretion in coordination with GSH or UDCA, which might be a way of immunoregulation and different from the modes of of GSH or UDCA action. Subsequently, we further proved the anti HCMV infection effect of spleen Aminopeptide and the enhancement of immunity by the increases of CD4⁺ cells.

Changes in the text: Page 12 Line 7-10, Line 13-14, Line 20-22,Page 13,Line 4-7

Comment 10: Tables: All abbreviations should be explained at the feet (TB, DB...)

Reply 10: All abbreviations were explained in the Tables and Figures.

Changes in the text: Page 21 Line 4-5, Page 19 Line 2-4.

Comment 11: References: There are some references rather general (i. e.number 6, 7, 12,13..) and not specifically related to the subject of this paper. Please revise carefully.

Reply 11: We deleted the reference 6,7,12,13 and added reference 24.

Changes in the text: Page 15-16