

Dear editor:

Thank you for your letter and guidance! We have carefully revised the manuscript as required. The research objectives have been added to the abstract, and PMID or DOI numbers have been added to the references. All modified text is marked in green font. If some text has been deleted, mark a small amount of normal text nearby with green font. In addition, we have provided detailed one-on-one responses to the comments and questions raised by the reviewers.

Best regards

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### **Reply to the content of the reviewer.**

**1. Question:** Introduction is quite general regarding describing the findings in lymphocyte subsets from previous research works where this aspect has been analyzed in a deeper way. Some works should be included to enrich the introductory section. Fohner AE, Sitlani CM, Buzkova P, Doyle MF, Liu X, Bis JC, Fitzpatrick A, Heckbert SR, Huber SA, Kuller L, Longstreth WT, Feinstein MJ, Freiberg M, Olson NC, Seshadri S, Lopez O, Odden MC, Tracy RP, Psaty BM, Delaney JA, Floyd JS. Association of Peripheral Lymphocyte Subsets with Cognitive Decline and Dementia: The Cardiovascular Health Study. *J Alzheimers Dis.* 2022;88(1):7-15. doi: 10.3233/JAD-220091. PMID: 35527553; PMCID: PMC9277688. Speciale L, Calabrese E, Saresella M, Tinelli C, Mariani C, Sanvito L, Longhi R, Ferrante P. Lymphocyte subset patterns and cytokine production in Alzheimer's disease patients. *Neurobiol Aging.* 2007 Aug;28(8):1163-9. doi: 10.1016/j.neurobiolaging.2006.05.020. Epub 2006 Jun 30. PMID: 16814429.

**Reply:** We fully accept the comments of the reviewers. In the preface of the revised manuscript, we have added these two references. Please note that the order of references has been changed later in the text. All newly modified parts of the manuscript are marked with green font (the same below).

**2. Question:** Method section should be greatly improved: Describing detailed the process for T, B and NK staining, adquisition, and reagents used. Representative image of the flow cytometry analysis should be included.

**Reply:** Thank you very much for the reviewer's advice. In the revised version of the manuscript, we have added more detailed methods of lymphocyte detection in the research methods section. An image of the lymphocyte subsets measured by flow cytometry was also included (see Figure 2).

**3. Question:** Regarding, this point, since this is a retrospective study i wonder why lymphocyte subsets was performed to dementia patients, did these patients presented another inflammatory, or immunological concomitant disease?

**Reply:** There is increasing evidence that the occurrence of Alzheimer's disease is closely related to slow immune inflammation, and the changes of lymphocytes in the blood may be directly related to this slow immune inflammation. Therefore, in this study, data from lymphocyte subsets were collected and analyzed.

4. **Question:** In the other hand, analysis must be improved by comparing groups according to main general characteristics such as Alzheimer vs other dementia and according to the HIS or MMSE scale.

**Reply:** This study mainly analyzes the correlation between the existing data groups; There are a lot of contents about the comparison between the groups, and we are preparing another paper manuscript. Such content, if included in this article, may dilute the existing discussion topic.

5. **Question:** The results section also must be improved greatly. I strongly suggest: a) representing the results in graphs when possible besides the tables,

**Reply:** We agree with the reviewer's viewpoint, as most high-quality papers are accompanied by high-quality images of original experimental results. However, in this retrospective study, some results only had data and no images. As a modification supplement, we have added a structural diagram (See Attachment Fig 1).

6. **Question:** “ b) Including representative images for MRI and Flow cytometry counts. Clarify the units in which Lymphocytes subsets is represented, and how calculus were made, ( specify proportion from full blood sample, from leucocytes, or lymphocytes?) “

**Reply:** Sincerely accept the suggestions of the reviewers. In the revised manuscript, we have added one flow cytometry image and one nuclear magnetic resonance spectroscopy images (see Fig 3.). Regarding lymphocyte subpopulations, this study only conducted count analysis and only presented relative percentages, without any medical measurement units.

7. **Question:** Also verify or explain detailed the concordance between text and tables numbers since the text mentions 55% but table its expressed as 0.55 T cell. Include the ROC for T cell count.

**Reply:** For the convenience of table layout, 55% was written as 0.55; It is equivalent.

8. **Question:** Finally, adapt the discussion according to the new finds this new analysis could provide to the manuscript. Include references for discussing Th subsets such as Th 17 and T regulatory cells.

**Reply:** Fully accept the opinions of the reviewers. In the discussion section of the revised manuscript, we have appropriately added some content related to regulatory T cells