To the Editor,

We have read with interest the letter of Marini et al, 2020 about the percentages and absolute numbers of double positive T cells (DPTs) in peripheral blood of a normal Italian population. In a previous article by our group (1), the DPTs population was evaluated in one hundred suitable donors from a Colombian blood bank using flow cytometry. Our main findings showed a median of DPTs of 2.6% and a higher percentage in women.

In the Italian cohort, they found an increase of DPTs with age and no difference by gender. In our study, we did not test donors over 61 years old to corroborate if age is associated with the marked increased level of DPTs above this age as shown in the Italian population. We reanalyzed our data and did not find a difference in the percentages of DPTs when comparing age groups (Figure 1).

In the Spanish and German cohorts, although there is no significant difference of DPTs according to gender, women showed a tendency to have more DPTs when compared to men (2,3).

The flow cytometry panel (monoclonal antibodies and fluorochromes) used in our work detected and discriminated the DPTs through manual gating as shown in the publication (1). Previous studies show that the antibody cocktail and the gating strategy (manual versus automated) are sources of variability in the results (4). Also, according to the flow cytometry analysis showed (1), it was possible to determine the subpopulations of CD4^{high}CD8^{low} and CD4^{low}CD8^{high} in healthy donors as described by other authors (5,6).

In order to understand the differences found in the publications, future studies must include a more diverse population, larger samples, and increased age range.

Keywords: T lymphocytes, flow cytometry

Conflict of interests: The authors declare no conflict of interest.
References

![Box plot showing median and interquartile rank of donors according to age group. Kruskal-Wallis p=0.83.](https://via.placeholder.com/150)

**Figure 1** Median and interquartile rank of donors according to age group. Kruskal-Wallis p=0.83.