platelets were gated based on their forward scatter (FSC) and side scatter (SSC) properties, as well as the expression of CD42a, before comparing the median FSC value between healthy controls and SLE patients. All the conclusions in the study were drawn based on this gating strategy. However, the percentage of gated cells was only 72.5%, leaving 27.5% of cells unaccounted for: were these platelets or cell debris? Because of the gating strategy adopted in the study, and considering that the FSC is a measure of cell size, we wonder whether the 27.5% of ungated cells were actually small-size platelets. For platelet detection in the cytometry unit of our clinical laboratory, we usually display a minor subset of erythrocytes (Fig. 1), set the discrimination value to zero, display all the platelets and then use a mAb to confirm the identity of the gated platelets. In this scenario, all the platelets are investigated regardless of their size (Fig. 1). We suggest that the authors report the FSC and SSC in terms of their logarithmic value to amplify the dynamic range of the signal and display all the platelets.

We believe that Lood *et al.* did not include the small platelets and drew the conclusion that 'decreased platelet size is associated with platelet activation and anti-phospholipid syndrome in systemic lupus erythematosus'. Thus we suggest that the findings of the study be further evaluated to include all the platelets.

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Comment on: Decreased platelet size is associated with platelet activation and anti-phospholipid syndrome in systemic lupus erythematosus: reply

Sir, In the comments raised by Chen and Liu [1], the authors argue that the data presented in our recent publication [2] are based on incorrect gating strategies excluding small cell debris and that the cell debris population would contain a large number of small platelets, thus affecting the outcome of our analyses. We would like to address this critique by providing some new re-analysed data as well as re-emphasizing some of the key results from our prior publication.

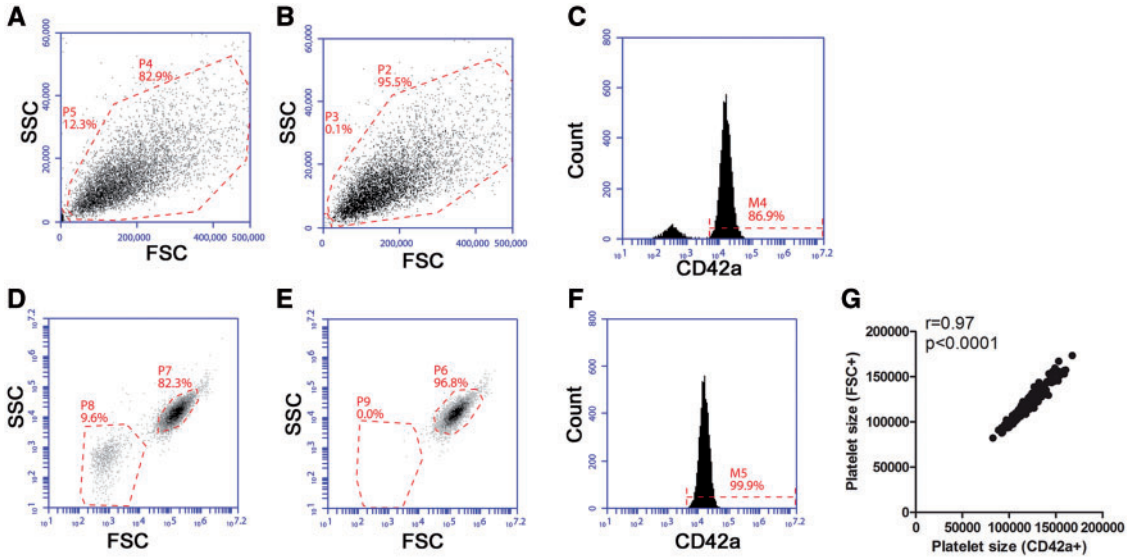
First and foremost, as described in our previous publication, our flow cytometry–derived data on platelet size were in high concordance with the gold standard analysis of mean platelet volume as measured by an automatic cell counter ($r = 0.79$, $P < 0.0001$), thus validating our method and confirming the decreased platelet size in SLE patients [2].

In the flow cytometry figure presented in the previous publication, 72.5% of the total events are included in the platelet gate. This percentage was usually higher (mean 83.4%), as also illustrated in Fig. 1A and D. Based on analysis of >300 blood samples, we know that >99% of the events included in this gate are $CD42a^+$ platelets. Thus, using our gating strategies, we will capture a very pure population of platelets. However, as stated by the authors, not every event was included. However, in contrast to the assumption by the authors, only a small proportion of the non-gated events were found in the small debris (mean 3.17%), whereas the remaining events were found as outliers surrounding the platelet gate. Within the debris only a small percentage of the particles were $CD42a^+$ (mean 3.81%). Given the low percentage of debris, as well as platelets within the debris, the inclusion of small debris would only account for 0.15% of the total platelet count not already included in our original gating strategy.

Consistent with the low presence of platelets within the excluded debris, exclusion of all non-$CD42a^+$ particles prior to assessing the platelet size (Fig. 1B and E) almost completely removed the small cell debris population. Importantly, we found that the median platelet size was very similar independent of gating strategies, that is, if gating on platelet size or CD42a positivity in the initial gate ($r = 0.97$, $P < 0.0001$; Fig. 1G). Further, all of our analyses also remained statistically significant with the $CD42a^+$ gating strategy. Of note, several of the analyses even got higher odds ratios and decreased *P*-values using the $CD42a^+$ gating strategy.

As proposed by the authors, we also displayed our data using a logarithmic scale (Fig. 1D–E). As can be seen, the debris is still apparent, with similar numbers independent on using linear or logarithmic scale (Fig. 1A and D). We would argue that the flow cytometry settings, including thresholds, discriminations and forward scatter and side scatter values all account for the ability to detect or

Fig. 1 Platelet gating strategies



Platelets were gated based on forward scatter (FSC) and side scatter properties (**A** and **D**) or positivity for the platelet marker CD42a (**B** and **E**) and displayed on linear (**A** and **B**) and logarithmic (**D** and **E**) flow plots. The flow cytometry plots are representative of > 300 blood samples included in the study [2]. The $CD42a^+$ frequency was analysed in non-gated (**C**) and gated (**F**) platelets. P2 and P4 denote the main platelet gate, whereas P3 and P5 denote the lower left cell debris gate. (**G**) Platelet size was determined by flow cytometry using two different gating strategies: including all $CD42a^+$ events ($CD42a^+$) or selecting the platelet population based on FSC settings (FSC^+), and correlation was analysed by Spearman correlation.

exclude small cell debris. Further, our flow cytometer has a much broader resolution ($10^{6.2}$) as compared with the one used by the authors (10^4), allowing us to detect much smaller particles. Had we omitted the first two logs of the scale, most of the debris would not have been included in the analysis. Thus we have no reason to doubt the validity of our results and/or the gating strategy. Using the gating strategy adapted in the published paper, we are able to capture almost all of the platelets with very high purity, and we have now ascertained, using the CD42a gating strategy, that the population captured by us is truly representative for the full platelet population. In conclusion, our results remain firm, with SLE patients having a decreased platelet size related to the presence of aCL and APS.

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