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Comment on: Increased inflammation and disease activity among current cigarette smokers with rheumatoid arthritis: a cross-sectional analysis of US veterans: reply

Sɪʀ, We were pleased to read the letter by Dr Wilke in response to our recent article addressing the association of smoking with disease activity in RA [1]. Dr Wilke points out that depression and anxiety are associated with worse patient-related outcomes in RA. Indeed, our group has previously published, for the same cohort, the association of depression and/or anxiety with disease activity measures in RA, demonstrating robust associations with more patient-reported measures such as pain, tender joint counts, Multidimensional Health Assessment Questionnaire score and patient global assessment, and less striking associations with DAS-28 [2]. Using the same diagnostic codes that were used in this prior effort, we have now examined the associations of smoking with both DAS28 and cytokine score after accounting for confounding due to depression and/or anxiety. We found that the associations of current smoking (referent to former or never smoking) with DAS-28 and cytokine score were not changed following adjustment for depression and/or anxiety.

Jeremy Sokolove[1,2], Harlan Sayles[3,4] and Ted Mikuls[3,4]

[1]*Veterans Affairs Palo Alto Health Care,* [2]*Department of Medicine, Division of Immunology and Rheumatology, Stanford University School of Medicine, Palo Alto, CA,* [3]*Veterans Affairs Nebraska Western-Iowa Health Care System and* [4]*Nebraska Arthritis Outcomes Research Center, University of Nebraska Medical Center, Omaha, NE, USA*
Accepted 14 March 2017
Correspondence to: Jeremy Sokolove, Veterans Affairs Palo Alto Health Care System, Mail Stop 111G, 3801 Miranda Avenue, Palo Alto, CA 94304, USA.
E-mail: sokolove@stanford.edu

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

We read the article written by Lood *et al.* [1] with great interest. In this article we noticed that Fig. 1A shows the flow cytometry analysis of isolated platelets. Specifically,

Fɪɢ. 1 Representative platelet gating criteria for platelet detection
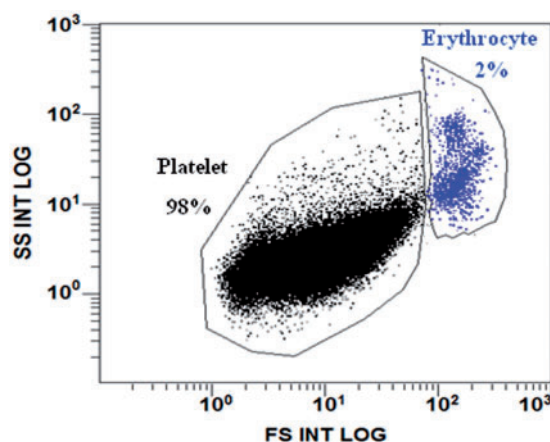
In this gating strategy, the discrimination value was set to zero; platelets (98%) and erythrocytes (2%) are displayed.

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.

Yanxia Chen[1] and Jinlin Liu[2,3]

[1]*Department of Rheumatology*, [2]*Flow Cytometer and Immunology Unit, Department of Clinical Laboratory, Zhejiang Provincial People's Hospital, People's Hospital of Hangzhou Medical College* and [3]*Key Laboratory of Tumour Molecular Diagnosis and Individualized Medicine of Zhejiang Province, Hangzhou, China*
Accepted 25 April 2017
Correspondence to: Jinlin Liu, Department of Clinical Laboratory, Zhejiang Provincial People's Hospital, People's Hospital of Hangzhou Medical College, 158 Shangtang Road, 310014 Hangzhou, China. E-mail: liujinlinhz@163.com

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1 Lood C, Tyden H, Gullstrand B *et al.* Decreased platelet size is associated with platelet activation and anti-phospholipid syndrome in systemic lupus erythematosus. Rheumatology 2017;56:408–16.

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