Correspondence on 'Critical role of neutrophil extracellular traps (NETs) in patients with Behcet's disease'

We read with great interest the article by Le Joncour *et al*[1] on how neutrophil extracellular traps (NETs) contribute to the vascular manifestations in patients with Behcet's disease (BD). In their well-designed study, the authors found that neutrophils from BD patients were more prone to form NETs, which contributes to the elevated levels of circulating NETs.[1] The authors further identified that NETs were detected around affected blood vessels and NETs levels were significantly higher in patients with BD with vascular involvement, suggesting that these structures are implicated in the vascular manifestations in this disease.[1]

NETs are extracellular web-like structures composed of nuclear components bound to neutrophil-derived proteins.[2] NETs have been shown to induce vascular damage and are implicated in thrombosis.[3] An important source of NETs is a subset of proinflammatory neutrophils, the low-density granulocytes (LDGs). LDGs are found in the peripheral blood mononuclear cell (PBMCs) fraction following density-gradient separation of whole blood.[4] The levels of LDGs are increased in a number of autoimmune and autoinflammatory disorders, such as systemic lupus erythematosus (SLE), antineutrophil cytoplasmic antibody-associated vasculitis, and adult-onset Still's disease.[4–6] The pathogenic role of LDGs in these diseases is partially due to their significantly enhanced ability to form NETs.[4 7] As elevated levels of NETs are increased in BD,[1 8] we evaluated the levels of LDGs in patients with BD and assessed their correlations with clinical and laboratory parameters.

A total of 27 patients fulfilling the new international diagnostic criteria of BD[9] were enrolled in this study, including 18 patients with active disease and 9 patients with inactive disease. Active disease was defined as Behçet's Disease Clinical Activity Form (BDCAF)≥2.[10] Twenty-five sex-matched and age-matched volunteers with no evidence of autoimmune or other diseases were enrolled as healthy controls (HCs) (detailed materials and methods are included in online supplemental file). Among active patients with BD, 10 patients (10/18, 55.6%) had systemic involvement with vascular as the major involvement (8/10, 80%), including deep venous thrombosis, arterial aneurysm and superficial thrombophlebitis. The detailed demographic and clinical characteristics of patients with BD and HCs are depicted in online supplemental table 1.

Active patients with BD displayed significantly higher percentages of LDGs present in the circulation (median, 4.20%; P25 of 1.67% to P75 of 11.0%) compared with those with inactive BD (1.64%; 0.95% to 4.36%) ($p<0.05$) and with HCs (0.31%; 0.19% to 0.60%). ($p<0.001$) (figure 1A). Given the significantly increased number of LDG in active BD, we evaluated the utility of LDG-to-lymphocyte ratio (LLR) as a new predictor of inflammation. While neutrophil-to-lymphocyte ratio (NLR), a marker of systemic inflammation,[11] demonstrated a significant difference between active patients with BD and HCs ($p<0.001$), it did not significantly distinguish between active patients with inactive patients ($p>0.05$) (figure 1B). In contrast, LLR offered better resolution and separation between active patients and inactive patients ($p<0.05$) (figure 1C).

Patients with BD were further divided into two groups based on the presence/absence of systemic manifestations. The levels of LDGs were significantly higher in patients with vascular

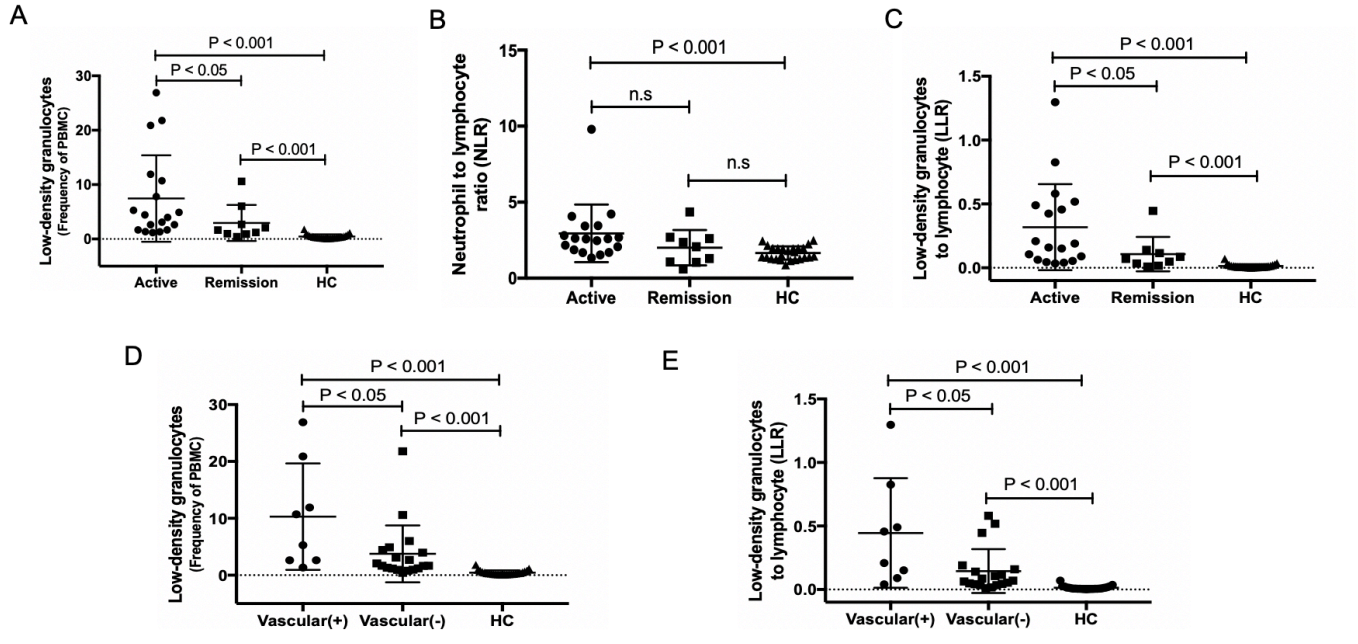

Figure 1 Low-density granulocytes (LDGs) are elevated in Behcet's disease (BD) and are associated with vascular involvement. (A) Frequency of LDGs in patients with active BD, inactive BD and healthy controls (HCs). (B) Neutrophil-to-lymphocyte ratio (NLR) in patients with active BD, inactive BD and HCS. (C) LDG-to-lymphocyte ratio (LLR) in patients with active BD, inactive BD and HCs. (D) Frequency of LDGs in BD patients with vascular involvement, in BD patients without vascular involvement and HCs. (E) LLR in BD patients with vascular involvement, in BD patients without vascular involvement and HCs. PBMC, peripheral blood mononuclear cell.

involvement than those without vascular involvement (7.99, 2.63 to 18.65 vs 1.67, 1.17 to 4.44, p<0.05) (figure 1D). In addition, patients with vascular involvement displayed a significantly higher level of LLR than those without vascular involvement (0.33, 0.11 to 0.74 vs 0.07, 0.04 to 0.16, p<0.05) (figure 1E). In contrast, the levels of NLR were comparable between patients with vascular involvement and those without vascular involvement (2.61, 2.17 to 3.26 vs 2.17, 1.33 to 2.81, p>0.05).

Associations between NLR, LDG or LLR with clinical parameters were assessed (online supplemental table 2). No significant difference was observed between the disease activity index of BDCAF with NLR, LDG or LLR. In contrast, a significant positive correlation between LDGs and vascular involvement was identified (r=0.437, p<0.05). In line with this, LLR, but not NLR, was also positively correlated with vascular involvement (r=0.427, p<0.05). Further, a positive correlation between LLR and erythema nodosum were observed (r=0.402, p<0.05). Based on these data, which may suggest LDGs or LLR could be a predictor for vascular involvement in patients with BD.

Endothelial dysfunction and neutrophil-mediated vascular inflammation are key drivers in thrombosis in BD.[12 13] With growing acknowledgement of the heterogeneity among granulocytes, the role of LDGs have been well-characterised.[7] LDGs are associated with endothelial damage as well as with induction of abnormal endothelial differentiation, with potential implications in the deleterious effects to the vasculature.[6 14 15] In SLE, LDGs are independently associated with the presence of increased vascular inflammation.[14] Importantly, LDG-derived NETs contain matrix metalloproteinases-9 (MMP-9), which activates endothelial MMP-2 and subsequently leads to endothelial damage and vascular dysfunction.[15] Our findings that the levels of LDGs were significantly correlated with vascular involvement, therefore are consistent with the findings by Le Joncour *et al*.[1] It is likely that the LDGs are the major source of NETs that are implicated in vascular damage in BD. Thus, our findings provide a rationale for targeting this pathogenic neutrophil subset. Our data also suggest that LLR may be more sensitive than NLR in evaluating disease activity as well as vascular involvement in BD.

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