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Platelet microparticle levels: a biomarker of thromboangiitis obliterans (Buerger's disease) exacerbation

Dear Editor:

Buerger's disease, or thromboangiitis obliterans (TAO), is a rare inflammatory vascular disease affecting the small- and medium-sized arteries and superficial veins of the extremities. It typically occurs in young male smokers with an age of onset before 40–45 years. TAO is characterized by chronic and acute phases, the latter often being associated with the occurrence of ischemic ulcerations of the toes and severe pain requiring hospitalization [1]. Amputation of finger or toes occurs in 46% of the cases after 15 years of evolution. Various investigations have been carried out with the aim of identifying an autoimmune mechanism responsible for TAO. Hypersensitivity to types I and III collagen associated with the presence of anti-collagen or anti-elastin antibodies has been shown [2]. Nevertheless, these abnormalities have been proven to be non-specific and have not been confirmed. Increased levels of anti-endothelial cell or antiphospholipid antibodies have been reported in patients with active disease but the significance of such findings remains to be established. Exposure to tobacco is strongly associated with occurrence of TAO. This suggests that tobacco plays a role in the pathogenesis of the disease or, at least, that tobacco is its major contributor. However, the association between tobacco and TAO is unclear. Previous studies showed that platelet aggregability could be modified in smokers [3] but platelet activation has never been suggested in TAO. However, platelet activation in inflammation or autoimmune context has been recently proposed to contribute to the amplification of thrombotic complications in cardiovascular disease and cancer through the expression of surface adhesion molecules inducing a prothrombotic state [4, 5]. Moreover, platelet apoptosis or activation lead to the release of platelet's membrane particles called platelet microparticles (PMPs) [6–8]. PMPs are procoagulant as they expose negatively charged phospholipids. PMP levels are increased in several pathological conditions such as the antiphospholipid syndrome, heparin-induced thrombocytopenia, chronic renal failure, sickle cell disease, cancer, sepsis, paroxysmal nocturnal haemoglobinuria and cardiovascular diseases including peripheral vascular disease [9].

Because of the potential link between Buerger's disease, tobacco and platelet activation, the aim of this study was to evaluate PMP levels in patients with TAO. Indeed, PMPs could be a marker of platelet activation on damaged vessel wall and a putative implication of PMPs in the clinical manifestation of the disease could lead to the use of antiplatelet agents in TAO. We conducted a prospective study in which 15 consecutive patients with a median age of 41 (min–max, 32–52 years) and a sex ratio M/F of 14/1, were included. All patients fulfilled Adar's criteria for TAO [10]. Among these 15 patients, 9 were admitted in emergency situations because of acute exacerbation of trophic ischemic lesions and 6 were outpatients with a stable disease. We compared PMP levels in TAO with a control group of 15 patients with peripheral artery disease with a median age of 63 (min–max, 27–78 years). Whole blood PMPs were determined by flow cytometry, as previously described [11]. Blood samples were collected into Vacutainer tubes (Becton-Dickinson, Le Pont-de-Claix, France) containing 0.105 M sodium citrate (1 vol/9 vol). Briefly, a 1/10 dilution of whole blood was analysed within 2 hrs of blood collection. Fifty microlitres of these blood dilutions were incubated during 20 min with 10 μl of platelet-specific antibody (CD41a-PE) or isotype control (BD BioScience, Becton-Dickinson, Le Pont-de-Claix, France). Calibrated polypropylene beads in tru-count tube (BD BioScience) were used for quantification. The reaction was stopped by 1 ml of phosphate-buffered saline solution. The sample was immediately analysed by using a FACS Calibur flow cytometer with CellQuest software (BD BioScience). PMPs were defined as events with sizes less than 1 μm and positive for CD41. Results were expressed as total number of PMPs per microlitre of whole blood. The nonparametric Mann-Whitney *U*-test was used to check for differences between PMP levels in the different groups.

Patients with peripheral arterial disease (PAD) had a significant increase in PMP levels compared to patients with TAO (12,276 ± 3069 PMPs/μl, $*P = 0.035$) (Fig. 1). PMP levels were significantly higher in patients with acute episode of TAO ($n = 9$) (9863 ± 3731/μl) than in patients with stable TAO ($n = 6$) (5152 ± 576/μl, $P = 0.013$). Subgroup analysis showed that elevated PMP levels remained significantly higher than in patients with stable TAO but not in patients with exacerbation. Patients have been classified into acute TAO when they were hospitalized in emergency for ischemic necrosis with rest pain and acute ischemic ulcerations of the toes or feet. This difference between exacerbation and stable disease appears in the Adar classification [10] and has been previously used by Maslowski *et al.* to evaluate modification of biological parameter like antiphospholipid antibodies in TAO [12].

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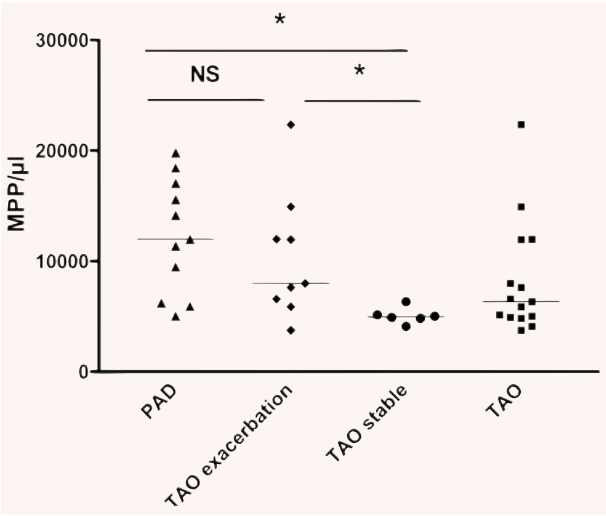

Fig. 1 Level of PMPs in Buerger's disease (TAO) compared to PAD. NS = not significant.

Circulating PMPs are released from platelets after their activation. Beside their role as a marker of platelet activation, they also exhibit pro-coagulant activity and are thought to play a role in the inflammatory process. Increased PMP levels are found in patients with intermittent claudication and are further increased in critical limb ischemia, thereby showing that PMPs are related to the severity of peripheral artery disease [13]. Yano *et al.* measured PMP levels in three patients with TAO and found increased levels in a patient without antiplatelet therapy [14]. Influence of chronic antiplatelet therapy on PMP levels is unknown [15], except for cilostazol that decreases PMP levels [16]. Apart from tobacco cessation, no efficient treatment is currently available for patients affected by Buerger's disease and almost 40% of them will experience amputation after 10 years of follow-up. Iloprost has demonstrated its efficiency in several vascular disorders, including Buerger's disease. In the TAO study, iloprost demonstrates a positive effect on ulcer healing and relief of ischaemic pain, compared with the aspirin-treated group; after 6 months of treatment the response rate was 88% in patients treated with iloprost compared to 21% in patients treated with aspirin [17]. In our study, two of three patients with a low dose of aspirin had high levels of PMPs, suggesting that aspirin has no effect on PMPs in TAO; furthermore all the patients in the PAD groups with higher levels of PMPs received antiplatelet agents such as aspirin or clopidogrel. Iloprost, a potent PGI2 mimetic which promotes vasodilatation and reduces platelet aggregation, also modifies platelet and endothelial microparticle formation [18]. One of the effects of Iloprost administrated during TAO could be mediated through a reduction of platelet activation and PMP formation. A prospective follow-up study may be of interest to evaluate the role of PMP levels as a predictive marker of disease activity and a biomarker of Iloprost efficiency. TAO is a very rare disease, so one of the limitations of our study is the low number of patients studied.

Altogether this study suggests an association between platelet activation and exacerbation of TAO. However, PMP increase observed in our study is probably more a reflection of the disease evolution than a risk factor of TAO. However, this PMP increase could represent an interesting biomarker associated with disease evolution and/or response to treatment that deserves to be confirmed in future prospective follow-up studies.

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