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Letter to the Editors-in-Chief

Antiphospholipid antibodies in Buerger's disease☆

Dear Editor

Buerger's disease (BD), or thromboangiitis obliterans, is an inflammatory, occlusive, peripheral vascular disease which usually occurs in young male smokers from low- or middle-income countries. The occlusion of small and medium-sized arteries may lead to tissue or limb loss. The aetiology and pathophysiology of the disease is not well understood. Although there is a close relationship between smoking and development or prognosis of the disease, smoking on its own cannot explain the low prevalence and geographical distribution of BD [1].

BD and antiphospholipid syndrome (APS) share some clinical manifestations, including arterial thrombosis, and, consequently, digital ulcers and gangrene or recurrent superficial thrombophlebitis [2]. Notably, the management of digital ulcers in APS remains challenging, as it is in cases of BD. However, as opposed to APS, BD is more prevalent in men, and deep vein thrombosis and involvement of the visceral vessels is uncommon in BD [1]. However, there have been a few case reports of increased anticardiolipin antibody titers in patients with BD [2,3]. It appears that BD patients with high antiphospholipid or anticardiolipin antibody titers tend to be younger and to suffer a significantly higher rate of major amputations than those within normal range of those autoantibodies [2,3].

In the current cross-sectional study, 41 male Caucasian patients with BD according to Shionoya's criteria [4] and computed tomography angiography and 30 healthy male smokers as a control group were included. Patients' written consent, demographic characteristics, and clinical manifestations were obtained during admission to Buerger's disease clinic from December 2016 through December 2018 and maintained in their medical records (Ethical code: MUMS-961484).

APS diagnosis was considered according to its latest criteria, including one clinical and one laboratory criterion [5]. Owing to the fact that all of the included patients showed evidence of arterial thrombosis, focus was placed only on the laboratory criterion for establishing APS diagnosis. According to the laboratory criteria, positive lupus anticoagulant antibodies or the presence of IgG and/or IgM anticardiolipin antibody more than 40 U/mL or the presence of IgG and/or IgM anti beta2glycoprotein 1 (β2GP1) more than 40 U/mL which could be detected on two or more occasions at least 12 weeks apart was considered for APS diagnosis [5]. Moreover, the co-existence of both positive anti β2GP1 (more than 20 U/mL for both IgG and IgM) and anticardiolipin (more than 10 U/mL for IgG and 7 U/mL for IgM) which could be detected on two or more occasions at least 12 weeks apart was also considered for APS diagnosis [5].

The serum levels of lupus anticoagulant antibodies (IgM, IgG) and anticardiolipin (IgM, IgG) were measured using the ELISA method (Orgentec Diagnostika, Mainz, Germany). Beta2glycoprotein 1 (β2GP1) (IgM, IgG) was also assessed using the ELISA method (EUROIMMUN, Luebeck, Germany). The test results were evaluated for any compatibility with clinical manifestation.

In this study, 41 male Caucasian BD patients with a mean age of 40.5 ± 1.7 years with a total of 106 follow-up visits were included, and APS autoantibodies were measured in their first report and follow-up.

The lupus anticoagulant antibody (IgM, IgG) was within the normal range in all BD samples and controls.

For four patients (8.8%), the anticardiolipin antibody (only the IgM type), with a mean of 8.06 ± 0.87 RU/mL was positive (normal range: less than 7RU/mL). The IgG type of anticardiolipin antibody was within the normal range for all patients. Both the IgM and IgG anticardiolipin antibodies were within normal ranges in the healthy smoker control group.

In 14 BD cases (35%), the anti β2GP1 antibody (only the IgM type), with a mean of 36.4 ± 13.8 RU/mL, was positive at the first visit or during at least one follow-up (normal range: less than 20RU/mL). Notably, a fluctuation in the level of anti β2GP1 (IgM) was found. The data are summarised in Table 1. However, the IgG type of the anti β2GP1 antibody was within the normal range in all patients. Both the IgM and IgG anti β2GP1 antibodies were within normal ranges in the healthy smoker control group.

Of particular note, the anti β2GP1 antibody was within the normal range in the symptom-free patients who were in the quiescent phase of their disease. Oddly, however, anti β2GP1 (IgM) was also within the lowest level of the normal range (7.1 ± 2.3RU/mL) during tissue gangrene. However, positive anti β2GP1 (IgM) was detected in all cases with progressive ulcers and progressive pain or paraesthesia. In addition, two patients underwent below-knee amputation during the study, and the level of anti β2GP1 (IgM) in both had decreased by the time of amputation (Fig. 1).

According to this study, although 35% of our patients had anti β2GP1 antibody (IgM) levels higher than 20RU/mL (positive), its serum level was less than 40 U/mL or back into normal range in less than 12 weeks and, thus, could not fulfil the laboratory criteria for APS diagnosis. Also, for four patients who had high levels of anticardiolipin antibody (IgM) during follow-up, the serum level of anticardiolipin antibody did not fulfil the APS diagnostic criteria and also returned to within the normal range within 12 weeks.

Notably, it appears that BD patients have their own pattern of antiphospholipid antibodies, which, as shown in this study, was a fluctuation in the level of anti β2GP1 antibody (IgM) during different stages of the disease. Therefore, it appears that β2GP1 may play a role in the pathogenesis of BD or may be a footprint from the underlying mechanism of BD. In particular, it has been demonstrated that β2GP1

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Table 1
Serum levels of β2GP1-IgM (RU/ml) in 14 BD patients who had at least one positive level of β2GP1 (IgM) during follow-up visits.

Anti β2GPI-IgM (RU/ml) at follow up visits	1st visit	2nd visit	3rd visit	4th visit	5th visit	6th visit	7th visit	8th visit
Case 1	25.44	10.92	–	–	–	–	–	–
Case 2	62.09	22.9	1.7	–	–	–	–	–
Case 3	21.23	59.59	43.88**	3.07	13.96	–	–	–
Case 4	56.08	13.16	3.95	–	–	–	–	–
Case 5	32.87	29.25	19.25	45.3	45.6	13.3	–	–
Case 6	21.8	10.8	–	–	–	–	–	–
Case 7	53.05	17.45	7.17	28.57	20.7	57.5	46	10.67
Case 8	11.5	19.4**	12.5	13	54.7	4.64	–	–
Case 9	2.28	22.2	6.37	–	–	–	–	–
Case 10	22.53	30	48.83	26.7	32.33**	27.4	–	–
Case 11	47	1.8	–	–	–	–	–	–
Case 12	27	19.5	4.92	–	–	–	–	–
Case 13	2.28	2.72	17.59	6	32	2.1	5.47	–
Case 14	9.69	12.07**	22.37**	17.3**	11.7	–	–	–

Grey cells: β2GP1 (IgM) positive values. **Anticardiolipin (IgM) positive values at that particular follow-up.

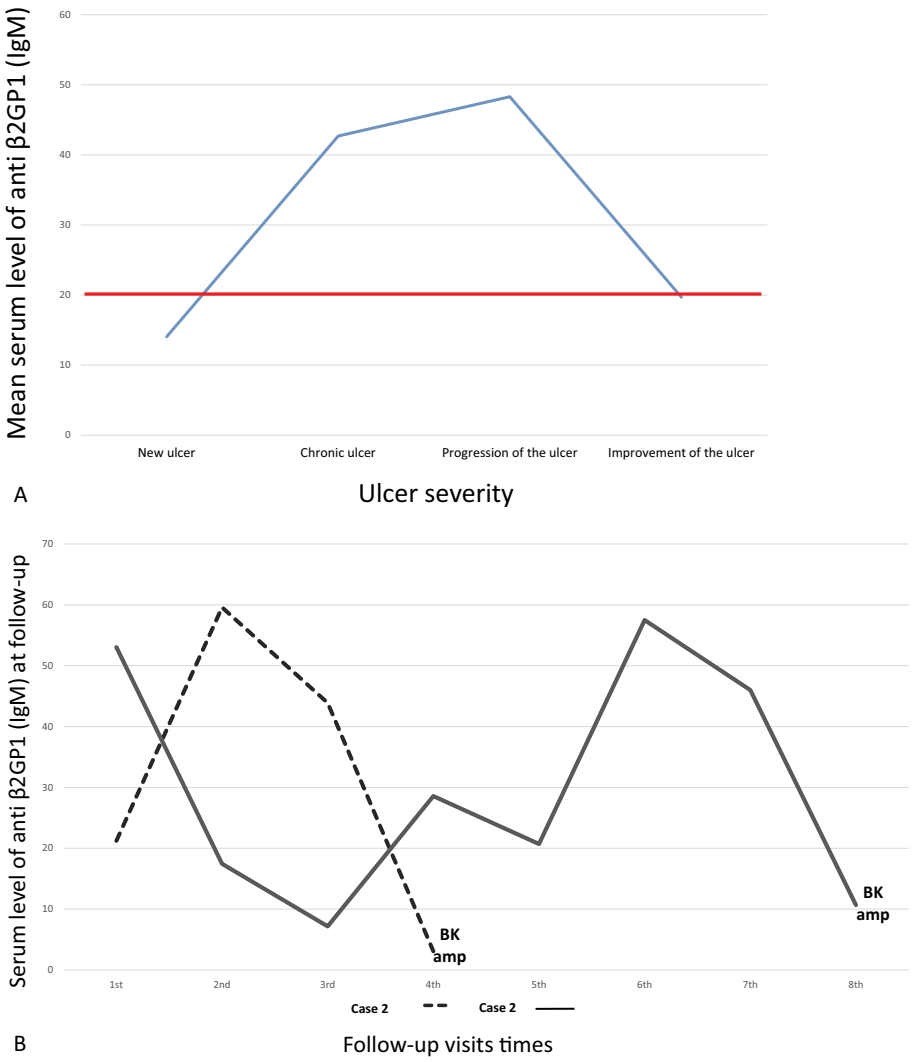

Fig. 1. A: Fluctuations in the mean serum levels of anti β2GP1 (IgM) according to severity of chronic ulcer (ranged from 0.1 to 62.19RU/ml). Red line shows the normal range cut off that is less than 20RU/ml. B: Fluctuations in the level of anti β 2GP1 during the natural history of two patients who underwent below-knee amputation (BK amp). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

plays a role in angiogenesis, apoptosis, NETosis and thrombosis.

β2GP1 is produced in the liver, and the main proportion of β2GP1 is soluble in serum. It has been demonstrated that β2GP1 serum level rises in chronic inflammation [6]. However, in acute inflammation, it behaves as a negative phase protein and its serum level decreases [6]. Moreover, free β2GP1 can inhibit angiogenesis by inhibiting the phosphorylation of the receptor of vascular endothelial cell growth factor (VEGFR) and endothelial nitric oxide synthase [6].

Inhibition of angiogenesis could lead to tissue hypoxia and, consequently, tissue damage and cell apoptosis or necrosis, which may present as an ischemic ulcer. Next, β2GP1 will attach to the apoptotic or necrotic cells as a defence mechanism of innate immunity to help the macrophages to phagocyte the necrotic cells, since macrophages have receptors for β2GP1. It has been noted that the antibody against β2GP1 develops when β2GP1 binds to apoptotic or necrotic cells [7] or when it becomes oxidised under the influence of high oxidative stress, which can be expected in ischemic tissue, and these oxidative forms of β2GP1 are immunogenic forms that can trigger plasma cell [8]. The production of anti β2GP1 antibody by plasma cell can be influenced by the free soluble β2GP1 as soon as the immunologic response for producing the antibody against it develops.

As soon as the anti β2GP1 antibody is produced, the antibody can bind the soluble β2GP1 in the serum and make an immune complex. The β2GP1-anti-β2GP1 immune complex can adhere to platelets, monocytes/macrophages and endothelial cells and ultimately lead to thrombosis and NETosis [9].

The free soluble of β2GP1 might be altered during the natural history of BD and thereby lead to fluctuation in the serum level of the anti β2GP1 antibody and inhibit switching of antibody production into the IgG isotype. However, in contrast to APS, in which the higher titer of autoantibodies leads to increased risk of thrombotic events [10], in this study, a higher titer of anti β2GP1 was observed along with progression of the pain or ulcer, whilst the anti β2GP1 antibody was within the normal range in all of the patients with the complication of gangrene. This could be explained by the β2GP1 binding to a mass of necrotic cells, at which point its serum level and, consequently, the trigger for anti β2GP1 plasma cells to produce antibodies would decrease.

According to this study, none of the patients fulfilled the diagnostic criteria for APS. However, a high level of the IgM type of anti β2GP1 was determined in approximately 35% of the patients and may play a role in the pathogenesis of BD. Further studies evaluating the anti β2GP1 antibody as a potential prognostic marker are highly recommended.

Declaration of Competing Interest

The authors declare that there is no conflict of interest in this study.

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