Differences in anti-phosphatidylserine–prothrombin complex antibodies and cutaneous vasculitis between regular livedo reticularis and livedo racemosa

Tamihiro Kawakami1, Masahide Yamazaki2, Masako Mizoguchi1 and Yoshinao Soma1

Objectives. We examined the prevalence of LAC, aCL antibodies (Abs), anti-β2-glycoprotein I (anti-β2GPI) Abs and anti-phosphatidylserine–prothrombin complex (anti-PS/PT) Abs in patients with regular livedo reticularis or with livedo racemosa to determine whether those Abs correlate with the clinical or serological features. Assuming that a correlation exists, early recognition of the serological features of the cutaneous manifestations may aid in the treatment and prediction of complications.

Methods. We examined the prevalence of LAC, aCL Abs, anti-β2GPI Abs and anti-PS/PT Abs in 143 Japanese patients who presented at our department with regular livedo reticularis or livedo racemosa between 2003 and 2008. LAC was determined according to the guidelines recommended by the Subcommittee on Lupus Anticoagulant/Phospholipid-Dependent Antibodies. Levels of anti-PS/PT, aCL and anti-β2GPI Abs in serum samples taken from patients were measured by specific ELISAs.

Results. Anti-PS/PT/PT Abs were detected in 94 (65.7%) of the livedo patients. Further, IgM anti-PS/PT/PT Abs were detected in 90 (62.9%) of the livedo patients. Serum IgM anti-PS/PT/PT Ab levels were significantly higher in livedo racemosa patients compared with regular livedo reticularis (19.2 ± 17.0 vs 8.93 ± 8.48 U/ml, \(P = 0.0013\)). Cutaneous vasculitis was significantly more prevalent among patients with livedo racemosa compared with regular livedo reticularis (\(P = 0.0014\)). Livedo racemosa patients had significantly higher CRP serum levels than regular livedo reticularis patients. Livedo racemosa has a stronger association with skin ulceration and arthralgia compared with regular livedo reticularis. Overall, we found a statistically significant association between cutaneous vasculitis and ischaemic cerebrovascular events in our livedo patients.

Conclusions. We speculate that IgM anti-PS/PT/PT Abs could be implicated in disease susceptibility for livedo racemosa. We further suspect that cutaneous vasculitis could be closely related to pathogenic factors that trigger the development of livedo racemosa. Early detection of cutaneous vasculitis in skin biopsies of livedo patients should be useful for prognostic evaluation, including ischaemic cerebrovascular events.

Key words: Cutaneous vasculitis, Regular livedo reticularis, Livedo racemosa, Anti-phosphatidylserine–prothrombin complex antibodies, Ischaemic cerebrovascular events.

Introduction

Livedo consists of macular violaceous connecting rings that form a net-like pattern. There are many potential causes, and this can make the evaluation of patients presenting with cutaneous manifestations very difficult [1]. Livedo reticularis is characterized by a reticular cyanotic cutaneous discoloration surrounding a pale central area. The term ‘livedo reticularis’ is often used indiscriminately [2, 3]. Livedo racemosa is characterized by a striking violaceous net-like pattern on the skin similar to livedo reticularis, from which it differs according to its shape (irregular or broken) (Fig. 1). Despite important clinical differences, in the English language literature the term ‘livedo reticularis’ is still used to describe all types of livedo, whereas the term ‘livedo racemosa’ is rarely used [4]. In a recent update of classification criteria for APS, livedo reticularis was defined as ‘persistent, not reversible with rewarming, violaceous, red or blue, reticular or motted pattern of the skin of trunk, arms or legs, consisting of regular unbroken circles (regular livedo reticularis) or irregular broken circles (livedo racemosa)’ [5]. The skin manifestations appear to be an important target organ for aPL antibodies (Abs), and in many cases APS may appear in association with the skin lesions [6]. This suggests the possibility that aPL Abs may influence the clinical patterns of livedo [7, 8].

LAC, aCL Abs or anti-β2-glycoprotein I (anti-β2GPI) Abs constitute one of the criteria for the classification of primary APS [9]. Detection of anti-phospholipid cofactor Abs, in addition to the classic aCL Abs and LAC, seems to be of considerable clinical importance. Prothrombin (PT) is another possible antigenic target of APS [10]. Some authors have suggested that anti-phosphatidylserine–PT complex (anti-PS/PT) Abs rather than anti-PT Abs alone are associated with the symptoms of
APS [11, 12]. In a previous study, we suggested that cutaneous vasculitis could be dependent associated with the presence of anti-PS/PT Abs [13]. These findings further suggest that aPL Abs are closely related to pathogenic factors that trigger the development of cutaneous vasculitis.

In the present study, we examined the prevalence of LAC, aCL Abs, anti-PS/PT Abs and anti-β2GPI Abs in 143 Japanese patients with regular livedo reticularis or with livedo racemosa to determine whether those Abs correlate with the clinical or serological features. Assuming that a correlation exists, early recognition of the serological features of the cutaneous manifestations may aid in the treatment and prediction of complications.

Patients and methods

We retrospectively examined 143 Japanese livedo patients (30 men, 113 women; mean age 46.4 ± 20.8 years) who had been seen at the Department of Dermatology, St Marianna University School of Medicine, between 2003 and 2008. Fifty healthy persons with comparable sex and age distributions were recruited as normal controls (10 men, 40 women; mean age 40.8 ± 9.3 years). In accordance with the literature, when irregular broken circles were present, the livedo pattern was considered as livedo racemosa (Fig. 1A). On the other hand, when the regularity of the fishnet reticular pattern led to unbroken circles, the livedo was considered as regular livedo reticularis (Fig. 1B). Cutaneous vasculitis was diagnosed based on the presence of leucocytoclastic vasculitis, such as fibrinoid degeneration, nuclear dust, neutrophilic infiltration and erythrocyte extravasation in the dermis to the subcutaneous fat.

All plasma and serum samples were collected and immediately centrifuged at 1500 g for 30 min at 4°C. After filtration, aliquots of platelet-free plasma were stored at −70°C until used for the LAC clotting tests. All serum samples were stored at −70°C prior to assay. According to the guidelines recommended by the Subcommittee on Lupus Anticoagulant/Phospholipid-Dependent Antibodies, LAC was screened by measuring diluted Russell’s viper venom time and kaolin clotting time and was confirmed by mixing studies and the demonstration of phospholipid dependence [14]. IgG and IgM isotypes of anti-PS/PT Abs and IgG and IgM aCL Abs were measured with a specific ELISA (Medical & Biological Laboratories, Nagoya, Japan) according to the manufacturer’s protocol. Briefly, serum samples diluted to 1:101 were added to 96-well plates coated with PS/PT or CL, and were incubated for 1 h at 20°C. Polyclonal goat anti-human IgG and IgM Abs labelled with horseradish peroxidase were used as conjugate solutions to recognize the two isotypes of anti-PS/PT or aCL Abs. Colour was developed with 3,3′,5,5′-tetramethylbenzidine and H2O2, and the plates were read at 450 nm. IgG and IgM anti-β2GPI Abs were determined according to the standardized anti-β2GPI Ab ELISA (Diagnostica Stago, Asnières, France). The cut-off points were established using the 99th percentile of data for the 50 normal controls. The following cut-off values were used: IgG (12 U/ml) and IgM (10 U/ml) anti-PS/PT Abs, IgG (10 U/ml) and IgM (10 U/ml) aCL Abs, IgG (10 U/ml) and IgM (10 U/ml) anti-β2GPI Abs. Serum CRP levels were assayed using laser nephelometry (Nihon Kohden Corp., Tokyo, Japan).

Statistical analysis was performed using the Mann-Whitney U-test for comparison of age. The Mann–Whitney U-test was also used to determine the relationship between blood parameters and the various clinical symptoms (skin ulcers, arthralgia, myalgia, mononeuritis, arterial systemic hypertension, miscarriages, cardiac valvular abnormalities and ischaemic cerebrovascular events); the level of significance was set at P < 0.05 in all cases. The correlation between the two livedo patterns, gender, location, cutaneous vasculitis and IgM anti-PS/PT Ab titre was assessed by Spearman’s rank correlation test. All data are expressed as means ± s.d. The whole study was approved by the St Marianna University Ethics Committee, and informed consent was obtained from all patients (No. 1117).

Results

Clinical and serological findings in patients with livedo patterns

Livedo patterns were divided into two categories, livedo racemosa (irregular broken circles) and regular livedo reticularis. One hundred and eleven (77.6%) of the 143 patients were diagnosed with livedo racemosa and 32 (22.4%) were diagnosed with regular livedo reticularis. Livedo patterns were localized on the lower extremities alone in 100 patients (70.0%) and on the extremities and/or trunk in 43 (30.0%). Of the 143 patients, 109 patients underwent skin biopsies. Of those patients, we found cutaneous vasculitis in 83 (76.1%). LAC was prevalent in 57 (40.0%) of the 143 patients, whereas the prevalence of IgG anti-PS/PT Abs was 15 (10.5%) and that of IgM anti-PS/PT Abs was 90 (62.9%). Anti-PS/PT Abs were detected in 94 (65.7%) of the livedo patients. Of those 94 patients, IgM anti-PS/PT Abs alone were present in 79 (84.0%) patients, IgG anti-PS/PT Abs alone were present in 4 (4.3%) patients and both were present in 11 (11.7%). The mean IgM anti-PS/PT Ab level (16.9 ± 16.0 U/ml) in patients with livedo was significantly higher than in the normal controls (P < 0.01). Sixteen (11.2%) of the patients with livedo had positive titres for IgG aCL Abs and 13 (9.9%) for IgM aCL Abs. IgG anti-β2GPI Abs were found in eight (5.6%) of the 143 patients, and IgM anti-β2GPI Abs in six (4.2%).

Differences in clinical and serological findings between livedo racemosa and regular livedo reticularis

The livedo racemosa group was significantly younger than the regular livedo reticularis (P = 0.012; Table 1). The ratio of women in the livedo racemosa group was not significantly higher than in the livedo reticularis group. There was a significantly higher frequency of skin ulcers in patients with livedo racemosa compared with regular livedo reticularis (P = 0.0031). Similarly, cutaneous vasculitis was significantly more prevalent among patients with livedo racemosa than regular livedo reticularis (P = 0.0014). There was also a significantly higher incidence of arthralgia associated with the livedo racemosa patients than in regular livedo reticularis. There was no significant difference in the incidence of myalgia, mononeuritis, hypertension, miscarriage, cardiac valvular abnormalities or ischaemic cerebrovascular events between the two groups. Livedo racemosa patients had significantly higher CRP serum levels than regular livedo reticularis patients (P = 0.0062). Further, the IgM anti-PS/PT Ab titre was significantly higher in livedo racemosa patients than in regular livedo reticularis patients (19.2 ± 17.0 vs 8.93 ± 8.48 U/ml, P = 0.0013). Similar trends were seen with respect to IgG anti-PS/PT Ab levels (P = 0.0056), whereas no significant differences were observed in the LAC, aCL Ab or anti-β2GPI Ab levels.

Characteristics of anti-PS/PT IgM-positive and anti-PS/PT IgM-negative livedo patients

The demographic, clinical and serological characteristics of our patients are described in Table 2, according to the presence or absence of IgM anti-PS/PT Abs. The presence of IgM anti-PS/PT Abs was positively correlated with age (P = 0.0027). The positive group was significantly younger than the negative group. The prevalence of IgM anti-PS/PT Abs differed significantly between livedo racemosa and regular livedo reticularis (P = 0.0009, r² = 0.574). We found a significant correlation between serum IgM anti-PS/PT Abs and cutaneous vasculitis in livedo patients (P = 0.0122). Arthralgia was also frequently observed in patients who were IgM anti-PS/PT Ab positive. In contrast, there was not a significant positive correlation between IgM anti-PS/PT Ab levels and IgG anti-PS/PT Ab levels. Levels of serum aCL Abs and anti-β2GPI Abs did not correlate significantly between IgM anti-PS/PT Ab-positive and -negative.
Clinical and serological findings in livedo patients with or without cutaneous vasculitis

The demographic, clinical and serological characteristics of our patients are described in Table 3, according to the presence of absence of cutaneous vasculitis. We performed skin biopsies on the livedo of 109 patients and detected cutaneous vasculitis in 83 of them. We found a significant correlation between cutaneous vasculitis and livedo racemosa in our patients ($P=0.0028$, $r^2=0.726$). Skin ulcerations were significantly more prevalent among patients with cutaneous vasculitis compared with patients without cutaneous vasculitis ($P=0.0016$). Patients with cutaneous vasculitis also showed a significantly higher frequency of ischaemic cerebrovascular events compared with patients without cutaneous vasculitis ($P=0.0408$). The presence of cutaneous vasculitis was associated with higher levels of IgM anti-PS/PT Abs compared with those without ($P=0.014$). Similar trends were seen with respect to IgG anti-PS/PT Ab-positive findings ($P=0.032$). In contrast, levels of LAC, aCL Abs or anti-$\beta_2$-GPI Abs did not differ significantly between patients with or without cutaneous vasculitis.

Discussion

In the present study, anti-PS/PT Abs were detected in 94 (65.7%) of the 143 livedo patients examined. The IgM isotype of anti-PS/PT Abs was detected in 90 (62.9%) of those patients. Serum IgM anti-PS/PT Ab levels were significantly higher in livedo racemosa patients compared with patients with regular livedo reticularis. We suggest that the presence of IgM anti-PS/PT Abs plays
some role in the pathogenesis of livedo racemosa. Interestingly, in the anti-PS/PT IgM-positive group, we observed a higher frequency of cutaneous vasculitis compared with the anti-PS/PT IgM-negative group. In addition, we found that patients with cutaneous vasculitis showed a significantly higher titre of IgM anti-PS/PT Abs compared with patients without cutaneous vasculitis. We previously proposed that IgM anti-PS/PT Abs could be implicated in disease susceptibility for livedo racemosa [15]. These conditions, i.e. elevated IgM anti-PS/PT Abs and comprising cutaneous vasculitis, may be closely related to the pathogenic factors that trigger the development of livedo racemosa.

PS is a regular constituent of the inner leaflet of the cell membrane, which is only exposed on the outside of cells during apoptosis or by damaged endothelial cells [16]. Some studies have shown that PT binds specifically to the surface of apoptotic cells [17, 18]. Livedo racemosa is significantly associated with skin ulceration and arthralgia compared with regular livedo reticularis. The serum CRP titres in patients with livedo racemosa were significantly higher than in regular livedo reticularis patients. CRP is an inflammatory marker and an elevated CRP titre likely contributes to the aggressive clinical condition. We found that patients with livedo racemosa seem to have more severe clinical symptoms compared with patients with regular livedo reticularis. Based on these findings, we believe that PT binds to apoptotic and/or damaged endothelial cells via an inflammatory response and combines with CRP in livedo racemosa. The presence of IgM anti-PS/PT Abs in skin biopsy specimens from patients with livedo racemosa could serve as a marker for livedo patients and should be monitored as an indicator of livedo activity.

Sneddon syndrome is characterized by the association of livedo and ischaemic cerebrovascular events, including stroke and transient ischaemic attack. Livedo was noted before cerebrovascular events in more than half of patients with Sneddon syndrome [4, 19]. A statistically significant association between cutaneous vasculitis and ischaemic cerebrovascular events was found in our livedo patients. These findings indicate that cutaneous vasculitis may be a useful parameter to define the risk of ischaemic cerebrovascular events. We further propose that performing a skin biopsy to determine the presence of cutaneous vasculitis could be an important prognostic indicator of the risk of ischaemic cerebrovascular events in livedo patients. These findings may shed further light on how to treat patients with livedo, especially those who present with cutaneous vasculitis. We suggest that anticoagulation and/or anti-aggregant therapies should be administered in such cases, if cutaneous vasculitis is detected as a manifestation of livedo.

In conclusion, we found high titres of serum IgM anti-PS/PT Abs in livedo patients and there was a significant association between IgM anti-PS/PT Abs and livedo racemosa. We speculate that anti-PS/PT Abs and cutaneous vasculitis could be implicated in disease susceptibility for livedo racemosa. Finding cutaneous vasculitis in skin biopsies from livedo patients should be useful for prognostic evaluation including cerebrovascular events. Further studies are required to confirm the pathogenesis of the livedo appearance and establish the importance of skin biopsy in treatment pathways.

Rheumatology key messages

- IgM anti-PS/PT Abs could be implicated in disease susceptibility for livedo racemosa.
- Cutaneous vasculitis could be closely related to pathogenic factors that trigger the development of livedo racemosa.
- Cutaneous vasculitis in skin biopsies from livedo patients appears to be useful for the prognosis of ischaemic cerebrovascular events.

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References


