Short Communication

Anti-phospholipid syndrome in seven leprosy patients with thrombotic events on corticosteroid and/or thalidomide regimen: insights on genetic and laboratory profiles

Sebastian Vernal[1], Maria Jose Franco Brochado[1], Roberto Bueno-Filho[1], Paulo Louzada-Junior[2] and Ana Maria Roselino[1]

[1]. Divisão de Dermatologia, Departamento de CLínica Médica, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brasil.
[2]. Divisão de Imunologia, Departamento de Clínica Médica, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brasil.

Abstract

Introduction: Corticosteroids and/or thalidomides have been associated with thromboembolism events (TBE) in multibacillary (MB) leprosy. This report aimed to determine genetic and laboratory profiles associated with leprosy and TBE. Methods: Antiphospholipid antibodies (aPL), coagulation-related exams, prothrombin and Leiden’s factor V mutations, and ß2-glycoprotein-I (ß2GPI) Val247Leu polymorphism were assessed. Results: Six out of seven patients with leprosy were treated with prednisone and/or thalidomide during TBE and presented at least one positive aPL. All patients presented ß2GPI polymorphism, and one showed prothrombin mutation. Conclusions: Corticosteroid or thalidomide adverse effects and aPL and ß2GPI polymorphisms may cause TBE in patients with MB leprosy.

patients were diagnosed at the University Hospital of Ribeirão Preto Medical School, University of São Paulo, Southeastern Brazil, from 1997 to 2006. Data were collected from medical charts and electronic database system of the University Hospital. Leprosy was diagnosed and confirmed based on WHO's recommendations. Both WHO and Ridley-Joplin classifications were used to classify leprosy.

Anti-phenolic glycolipid 1 (Anti-PGL1) IgM was measured using in-house enzyme-linked immunosorbent assay (ELISA) as described previously; IgM and IgG aCL and anti-ß2P levels were determined using commercial ELISA kits (QUANTA Lite, INOVA, USA). Lupus anticoagulants (LA) were analyzed using Viperquik LA-Check test and Kaolin clotting time (KCT). The coagulation factors, i.e., anti-thrombin III, proteins C and S, and fibrinogen, were measured in the peripheral blood using chromogenic methods during thrombotic episodes.

Factor V Leiden and G20210A mutation of prothrombin gene were analyzed using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP). The Val247Leu polymorphism of the ß2-glycoprotein-I gene was determined as described previously.

Informed consent was obtained from all patients. This study was approved by the local Human Ethics Committee in accordance with the ethical standards of the Helsinki Declaration (1964, amended most recently in 2008) of the World Medical Association.

This report included seven leprosy patients with thrombotic events. Tables 1 and 2 and Figure 1 summarize the clinical, laboratory, and therapeutic data. Patients' median age was 48 years (youngest, 26 years; oldest, 70 years); six of them were males. The seven MB patients corresponded to the BB, BL, and LL classifications (one, four, and two, respectively). Five of the patients presented TR2 at the onset of leprosy, corresponding to BL and LL classifications; another BL patient was affected by TR1, as commonly described. The patient with BB leprosy did not present any associated reaction. The most common thrombotic events were deep venous thrombosis (DVT) that occurred in three patients and DVT associated with pulmonary thromboembolism (PTE) that occurred in the other three patients. One patient presented with PTE only. Among the six patients with leprosy reaction, five were treated with thalidomide plus corticosteroids during the thrombotic episode, whereas one patient was treated with corticosteroids alone. None of the patients were active smokers. Except for case 4, tests for thrombophilia did not show any genetic predisposition (Figure 1).

Herein, we will discuss the seven cases in more detail. Case 1 was a 45-year-old Caucasian male patient clinically diagnosed as having LL form of leprosy. He presented several ENL and multiform erythema outbreaks since the diagnosis. He developed DVT on the right limb in March 1999 when he was still under multi-drug treatment (MDT) for leprosy, including...
### TABLE 2: Laboratory data of seven patients with multibacillary leprosy who experienced thrombotic events.

<table>
<thead>
<tr>
<th></th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
<th>Patient 5</th>
<th>Patient 6</th>
<th>Patient 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Date) IgM anti-PGL1 (index) (NV &lt; 1.0)</td>
<td>(09.2000) 0.3</td>
<td>(09.2004) 4.6</td>
<td>(03.2002) 0.5</td>
<td>(01.2004) 1.5</td>
<td>(10.2004) 1.8</td>
<td>(11.2004) 1.5</td>
<td>(12.2006) 0.4</td>
</tr>
</tbody>
</table>

**IgM:** immunoglobulin M; **NV:** normal value; **PGL1:** Phenolic Glycolipid 1; **aCL:** anti-Cardiolipin; **GPL:** standard IgG cardiolipin units; **MPL:** standard IgM cardiolipin units; **ß2GPI:** ß2-glycoprotein-I; **Leu:** leucine; **Val:** valine.

Prednisone (60mg/day) and thalidomide (300mg/day), due to ENL. Laboratorial tests were performed 5 years after the DVT episode and yielded negative results for aCL and anti-ß2GPI. LA was negative in 2004, but turned positive in 2005. LA has been a stronger risk factor for thrombosis than positive aCL or anti-ß2GPI in purely obstetrical APS. Protein C was the only abnormal laboratory test result related to coagulation. The primary effect of activated protein C is to inactivate the Va and VIIIa coagulation factors, which are necessary to efficiently generate thrombin and activate factor X. Genetic deficiency of protein C has been related to DVT; however, the protein C level was above the normal range in this case.
FIGURE 1: Timeline overview of thrombotic events and laboratory data of the seven patients with leprosy.
Case 2 was a 54-year-old Caucasian male patient diagnosed as having BL in 2002. He had been presenting multiform bullous erythema and ENL outbreaks since the diagnosis. In 2003 and 2004, he presented DVT in the left limb associated with PTE in the upper and lower lobes of the left lung, respectively. Both episodes occurred during the use of MDT plus prednisone (10–60 mg/day) and thalidomide (100–200 mg/day). During the PTE episode in 2004, IgM and IgG aCL increased. In infectious diseases, aPL antibodies tend to be IgM type because the infectious agent might trigger the autoimmune response. However, IgG aCL has also been detected in patients with leprosy. The only laboratory test related to abnormal coagulation was the KCT. This was expected from the positive aPL result, which would interfere with the accumulation of prothrombinase complex.

The third patient was a 26-year-old Caucasian female diagnosed as having LL in 2001. In the last two years, she had been chronically using prednisone due to misdiagnosis of lupus erythematosus. In 2001, ENL outbreaks occurred when she used prednisone and an alternative MDT scheme. Thalidomide was introduced in May 2003. In July 2003, she presented PTE while using prednisone (40 mg/day) and thalidomide (200 mg/day). She was not using any other medications at the time, not even contraceptive pills. aPL screening during PTE revealed high IgM and IgG aCL and anti-ß2GPI levels, and LA tests yielded positive results.

The fourth patient was a 69-year-old mulatto male who had been diagnosed as having BB leprosy in 2003. In January 2004, he experienced a DVT in the right limb and bilateral PTE while using MDT and hydrochlorothiazide. High IgG aCL (18.8 GPL) was confirmed 18 months after the thrombotic event. He also presented high levels of fibrinogen (800 mg/dL), which may be elevated in response to inflammation or tissue injury during leprosy. Heterozygosis for the prothrombin G20210A gene was demonstrated.

The fifth patient was a 31-year-old Caucasian male diagnosed as having BL in 2003 and who had been experiencing ENL outbreaks since the diagnosis. In 2004, he had DVT in the right limb while using MDT associated with prednisone (30 mg/day) and thalidomide (300 mg/day). High IgM and IgG aCL were confirmed at the time of DVT. Six months later, IgM and IgG aCL turned and remained negative in 2005. High levels of IgM and IgG anti-ß2GPI antibodies were also observed 1.5 years after the DVT episode. Unfortunately, laboratory tests related to coagulation were not performed. Assessment for thrombophilia did not show any genetic predisposition.

The sixth patient was a 48-year-old Caucasian male who was diagnosed as having BL and ENL in 2001. In 2004, he developed DVT in the left limb while using thalidomide (100 mg/day). IgM and IgG aCL and anti-ß2GPI levels were within the normal limits and LA was negative during and after the thrombotic episode, but LA became positive 1 year later. Like the fourth case, this patient had high fibrinogen levels as expected in an inflammatory scenario. KCT was also positive due the presence of aPL, as expected.

The last patient was a 70-year-old Caucasian male diagnosed as having BL in 2006, with TIR. In 2007, he developed DVT in the left limb and PTE during an alternative MDT scheme associated with prednisone (60 mg/day). IgM and IgG aCL and aPL were normal. Regrettably, LA and laboratory tests related to coagulation were not performed.

Regarding the β2GPI genetic polymorphism evaluated in haplotypes 1 and 4, three patients presented Val/Val homozygosis (cases 4, 6, and 7), and the other three presented Leu247Val heterozygosis (cases 1, 2, 3, and 5). High frequency of β2GPI Val/Val homozygosis has been described in Brazilian leprosy patients as compared to controls. Furthermore, MB patients with high IgM anti-ß2GPI levels presented higher β2GPI Val/Val homozygosis frequency than the controls. β2GPI Val/Val homozygosis seems to represent a higher risk factor than Leu247Val heterozygosis for the development of APS in the general population. However, this has not been confirmed yet, and larger studies attributing higher risk of APS to Val/Val homozygosis should be conducted.

Only one patient (case 4) had mutated prothrombin G20210A gene, which independently conferred a 2.8-fold increased risk of DVT in both sexes and in all age groups. This patient presented a thrombotic event without leprosy reaction outbreaks even though he was not treated with thalidomide and/or corticosteroid. Interestingly, he was the only patient in this case series who had genetic polymorphism for thrombophilia. Therefore, genetic thrombophilia could explain the development of a thrombotic event even without using thalidomide and/or corticosteroids. Moreover, the presence of aCL and β2GPI Leu247Val heterozygosis may have contributed to the development of DVT in this patient.

Some reports of thrombotic events due to the use of thalidomide and/or corticosteroids in MB leprosy patients with ENL are notorious. However, serum tests or assessment of patients’ genetic background regarding the diagnosis of intrinsic thrombophilia was not conducted in these studies. Here, we analyzed several pro-thrombotic factors in seven MB patients from Southeastern Brazil, not only to determine acquired risk factors due to leprosy and AEs of the drugs but also the manifestation of APS and β2GPI Leu247Val polymorphism. Based on our results, the Brazilian Ministry of Health has recommended the use of aspirin (100 mg/day) during the treatment of ENL, which could also be recommended in other countries where thalidomide and/or corticosteroids are the first-line therapy for T2R.

Acknowledgments

We thank the biologists Flávia Vieira and Sandra Silva Rodrigues Santos for their technical laboratory support.

Conflict of interest

All authors declared to have no conflicts of interest.

Financial support

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. SV received PhD scholarship from CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior).
REFERENCES


