Anticardiolipin, anti-β2-glycoprotein I and antiprothrombin antibodies in black South African patients with infectious disease

S Loizou, S Singh, E Wypkema, R A Asherson

Objectives: To investigate IgG, IgM, and IgA, antiphospholipid antibodies (aPL), against cardiolipin (aCL), β2-glycoprotein I (anti-β2GPI), and prothrombin (anti-PT), in black South African patients with infectious disease. Unlike patients with systemic lupus erythematosus (SLE) and the antiphospholipid syndrome (APS), raised levels of aPL in infectious diseases are not usually associated with thrombotic complications.

Patients and methods: Serum samples from 272 patients with a variety of infectious diseases (100 HIV positive, 112 leprosy, 25 syphilis, 25 malaria, and 10 HCV patients) were studied and compared with autoantibody levels in 100 normal controls. All three aPL were measured using commercial enzyme linked immunosorbent assay (ELISA) kits.

Results: Raised levels of all three aPL were found in all patient groups studied: aCL in 7%, anti-β2GPI in 6%, and aPT in 43% of 100 HIV patients, in 29%, 89%, and 21% of 112 patients with leprosy, in 8%, 8%, and 28% of 25 patients with syphilis, in 12%, 8%, and 28% of 25 patients with malaria, and in 20%, 30%, and 30% of 10 HCV patients studied, respectively.

Conclusions: The prevalence of aCL and anti-β2GPI in black South African HIV positive patients, or those with syphilis, malaria, or hepatitis C virus is lower than reported for mixed race or white populations. aPT were the most prevalent aPL detected in these patient groups, except in patients with leprosy, for whom anti-β2GPI was the most prevalent, and where the spectrum of aPL was similar to that seen in patients with SLE and APS.

Antiphospholipid antibodies (aPL) are a group of heterogeneous autoantibodies, which have been reported in many autoimmune diseases, and in the antiphospholipid syndrome (APS) which is characterised by raised levels of aPL, in association with thrombosis, recurrent fetal loss, thrombocytopenia, and a number of other less commonly found complications.\(^\text{1}\) aPL have also been found to be raised in a large number of infectious diseases, such as syphilis, HIV infection, malaria, leprosy, and viral infections, including hepatitis C (HCV), and B19 parvovirus infection, where they are not usually associated with the clinical complications attributed to them.\(^\text{2}\) Relatively recent studies, however, have indicated that, as well as cardiolipin, the phospholipid binding proteins β2-glycoprotein I and prothrombin can behave as real antigens as well as protein cofactors.\(^\text{3,4}\) Prothrombin and β2-glycoprotein I have also been reported to be implicated in lupus anticoagulant assays, which are rarely abnormal in infectious diseases.\(^\text{5, 6}\) In the course of many acute infections such as syphilis, HIV, hepatitis C, leprosy, and malaria, raised levels of anti-cardiolipin (aCL), anti-β2-glycoprotein I (anti-β2GPI), and anti-prothrombin (aPT) antibodies have been reported. They are often transient and can disappear after treatment of the infection.\(^\text{7,8}\) Although the aCL antibodies detected in infectious disorders were initially reported to be mainly β2GPI independent, β2GPI dependent aCL and antibodies against the protein antigens β2GPI and prothrombin itself have also been recently reported in many infections. The prevalence of aPL in infections has mainly been reported for the IgG and IgM isotypes, although a few recent studies have also investigated the IgA isotype in some infections.\(^\text{9–11}\)

In HIV infection aCL have been reported to be present in 0–94%, anti-β2GPI in 4–47%, and aPT in 2–12% of patients. The variations found in HIV are most probably due to the composition of the patient group and the disease stage studied—that is, asymptomatic to full blown AIDS, and the presence of complicating opportunistic infections.\(^\text{7–12}\) In syphilis aCL have been found to be raised in 18–100%, anti-β2GPI in 0–10%, and aPT in 4% of patients.\(^\text{3, 10–12}\) In patients with leprosy, aCL are reported to be raised in 37–98%, anti-β2GPI in 3–19%, and aPT in 6–45%.\(^\text{5, 10–12}\) For HCV and malaria infections, aCL are reported to be raised in 17–44% and 35–94% of patients respectively, and anti-β2GPI has been reported to be raised in <10% of patients infected with HCV.\(^\text{14–16}\)

The reported prevalence of these autoantibodies in infections is very variable, and this is probably due to methodological differences, such as the type of assay used, definition of cut off points for positivity, and heat inactivation of sera at 56°C; patient selection and ethnic composition of patient groups may also have a major role in the discrepancies of aPL positivity reported in infections, as for HIV, syphilis and HCV infection, studies were performed on predominantly white or mixed race populations.\(^\text{7, 10, 12}\) Only a few recent publications report the presence of IgA aPL in infections; the prevalence of aPL in exclusively black African patients with infectious disease has never been studied. This study aimed at determining the prevalence of IgG, IgM, and IgA, aCL, anti-β2GPI and aPT antibodies, in black South Africans with HIV infection, leprosy, syphilis, malaria and...
HCV infection, and comparing them with a group of normal black South African blood donors.

PATIENTS AND METHODS

Patients
We studied 272 black South African patients with infectious disease and compared them with a group of 100 black South African normal blood donors. The patients comprised 100 HIV positive patients, age range 19–52 years (mean age 33.49 women, 51 men); 112 patients with leprosy, age range 14–84 years (mean age 50, 59 women, 53 men); 25 patients with syphilis, age range 16–57 years (mean age 33, 20 women, 5 men); 25 patients with malaria, age range 3–61 years (mean age 34, 6 women, 19 men); 10 patients with HCV, age range 13–67 years (mean age 45, 5 women, 5 men). The control group consisted of 100 healthy blood donors, age range 21–64 years (mean age 36, 47 women, 53 men).

Methods
Antiphospholipid antibodies, IgG, IgM, and IgA, aCL, anti-β2GPI, and aPT, were measured using commercial enzyme linked immunosorbent assay (ELISA) kits (Cheshire Diagnostics Ltd, Ellesmere Port, Cheshire, UK), and expressed in units, according to the manufacturer’s instructions. For aPT the ELISA kit used was one where human prothrombin was directly coated onto activated polystyrene plates. IgG, IgM, and IgA aCL were expressed as GPL, MPL, and APL units, whereas anti-β2GPI and aPT were expressed as arbitrary ELISA units (AEU), according to the manufacturer’s instructions. Results for each of the three isotypes of the three types of aPL measured were considered positive when the optical density obtained for each patient exceeded that of the mean value plus 5SD, of the 100 sera from black South African normal healthy subjects.

Statistical analysis
Quantitative means were expressed as means and SD. Comparisons of values between the different patient groups and normal subjects were determined using Fisher’s exact test. Spearman’s rank correlations were used for comparisons of antibody levels between patient groups. The statistical analysis was performed using the GraphPad Prism software.

RESULTS
Table 1 shows the prevalences of the three different aPL studied, and their three isotypes, at 5SD above the mean of the 100 normal controls. Increased levels of aCL were found in 7/100 (7%) patients with HIV, in 32/112 (29%) patients with leprosy, in 2/25 (8%) patients with syphilis, in 3/25 (12%) patients with malaria, and in 2/10 (20%) patients with HCV. For anti-β2GPI raised levels were seen in 6 (6%) HIV, in 100 (89%) leprosy, in 2 (8%) syphilis, 3 (12%) malaria, and in 3 (30%) patients with HCV. aPT were raised in 43 (43%) HIV

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Frequencies of IgG, IgM, and IgA aCL anti-β2GPI and aPT positive patients with infectious disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV</td>
<td>aCL</td>
</tr>
<tr>
<td>Total any, No (%)</td>
<td>7 (7)</td>
</tr>
</tbody>
</table>
positive subjects, in 22 (20%) leprosy, in 7 (28%) syphilis, 7 (28%) malaria, and in 3 (30%) of the patients with HCV.

Figures 1, 2, and 3 show the distribution and levels of the three autoantibodies studied for IgG, IgM, and IgA, respectively. The differences in prevalence of the three antibody isotypes were also investigated. IgG was found to be the most prevalent isotype of aCL in HIV, and aPT in HIV and patients with malaria; IgM was more prevalent, for anti-β2GPI in leprosy, and for aCL in malaria subjects, and IgA was the most prevalent isotype of aCL in leprosy; IgA anti-β2GPI was the sole isotype found increased in six patients with HIV. Different combinations of IgG, IgM, and IgA aPL were seen in a few patients with HIV, syphilis, malaria and patients with HCV; anti-β2GPI which was the most prevalent aPL in patients with leprosy was present in all the different combinations of the three aPL isotypes studied (table 1).

The prevalence of aPL positive patients was compared between the groups with different infectious diseases (table 2). Significant differences were seen between leprosy and HIV, for IgG anti-β2GPI and IgG aPT (p<0.0001, p<0.001, Fisher’s exact test), for IgM anti-β2GPI (p<0.0001), and for IgA anti-β2GPI and aCL (p<0.0001).
as well as for IgA aPT (p<0.007). Significant differences were also seen between patients with leprosy and those with malaria or HCV for IgG anti-β2GPI (p<0.0001, p<0.02); IgM anti-β2GPI levels were significantly different between patients with leprosy and those with malaria or HCV (p<0.0001), and IgA aCL levels were significantly different between patients with leprosy and those with malaria or HCV (p<0.002, p<0.05). The only other significant differences found were for IgG aPT between patients with HIV and those with syphilis or HCV (p<0.001, p<0.04).

Table 3 shows the relationships between the levels of the different aPL studied. Significant correlations were seen between IgG aCL, and/or anti-β2GPI, and aPT in HIV, leprosy, and patients with malaria, as well as between IgG anti-β2GPI and aPT, in patients with leprosy and malaria. For the IgM isotype, positive correlations were only seen in patients with leprosy between aCL and anti-β2GPI levels, whereas in the comparison of aCL and aPT, levels correlated significantly in patients with HIV, leprosy, and malaria. When IgM anti-β2GPI and aPT levels were compared, a correlation was seen in patients with leprosy and HCV. Finally, for IgA, correlations were found in patients with leprosy between aCL and anti-β2GPI; in patients with leprosy and HCV between aCL and aPT, and in HIV and patients with leprosy between anti-β2GPI and aPT.

**DISCUSSION**

It is well established that aPL (aCL and anti-β2GPI primarily) may be found in a large variety of infectious diseases. The frequency of antibodies to the IgA aPL isotype, and to prothrombin (aPT) only very recently investigated in this group of conditions is largely unknown, and this report is one of the first to document their association in several infectious diseases in a large group of black South African patients with infectious disease. In none of the patients studied was any clotting reported.

In infectious diseases, the prevalence of autoantibodies against the phospholipid binding proteins, β2GPI and prothrombin, is generally much lower than those seen in the APS and patients with other autoimmune diseases. The aPT have not been studied in infections to any degree, and it is only recently that their prevalence in some infections has begun to emerge. A review in 1999 first designed an ELISA for the detection of aPT, and found a high prevalence of aPT in patients with sera positive for the lupus anticoagulant (LA), in association with an autoimmune disease such as systemic lupus erythematosus (SLE) or APS. Puurunen et al in 1996, reported that 50% of their patients with SLE and thrombosis demonstrated aPT, and also found a strong correlation between aPT and anti-β2GPI. These results were subsequently confirmed by other authors. However, in a recent review, Galli et al did not confirm any significant correlation between aPT and thrombosis in both patients with SLE and those with primary APS. A recent paper by Salcido-Ochoa et al investigating aPT in patients with SLE and those with APS, found a higher frequency of aPT in patients with SLE or primary APS with thrombosis, but no patients had aPT as the only aPL antibody. They concluded that the measurement of aPT did not provide any additional information in clinical practice.

Raised levels of aPT have been reported in between 2 and 12% of patients with HIV, 6–45% with leprosy, 4% with

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**Table 2** Correlation between the total number of positive patients between the infectious patient groups

<table>
<thead>
<tr>
<th>Comparison between</th>
<th>IgA aCL</th>
<th>IgG aβ2GPI</th>
<th>IgM aβ2GPI</th>
<th>IgA aβ2GPI</th>
<th>IgG aPT</th>
<th>IgM aPT</th>
<th>IgA aPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leprosy and HIV</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>Leprosy and syphilis</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0002</td>
<td>NS</td>
<td>0.02</td>
<td>NS</td>
</tr>
<tr>
<td>Leprosy and malaria</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0002</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Leprosy and HCV</td>
<td>NS</td>
<td>0.02</td>
<td>0.0001</td>
<td>0.05</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>HIV and syphilis</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.001</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>HIV and HCV</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.04</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS, non-significant; p values of r^2 for Fisher’s exact test are shown.

*aPL isotypes where no correlation was found not shown.

**Table 3** Correlation between IgG, IgM, and IgA, aCL anti-β2GPI and aPT antibody levels in the groups of patients with infectious disease

<table>
<thead>
<tr>
<th>Correlation between</th>
<th>HIV</th>
<th>Leprosy</th>
<th>Malaria</th>
<th>HCV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>IgG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aCL and aβ2GPI</td>
<td>0.2436</td>
<td>0.0001</td>
<td>0.1451</td>
<td>0.0001</td>
</tr>
<tr>
<td>aCL and aPT</td>
<td>−0.2046</td>
<td>0.04</td>
<td>0.6359</td>
<td>0.0001</td>
</tr>
<tr>
<td>aβ2GPI and aPT</td>
<td>0.3837</td>
<td>0.0001</td>
<td>0.5140</td>
<td>0.03</td>
</tr>
<tr>
<td>IgM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aCL and aβ2GPI</td>
<td>NS</td>
<td></td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>aCL and aPT</td>
<td>0.2488</td>
<td>0.02</td>
<td>0.3585</td>
<td>0.0001</td>
</tr>
<tr>
<td>aβ2GPI and aPT</td>
<td>0.6328</td>
<td>0.0001</td>
<td>0.4166</td>
<td>0.04</td>
</tr>
<tr>
<td>IgA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aCL and aβ2GPI</td>
<td>NS</td>
<td></td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>aCL and aPT</td>
<td>0.2055</td>
<td>0.03</td>
<td>0.4315</td>
<td>0.0001</td>
</tr>
<tr>
<td>aβ2GPI and aPT</td>
<td>0.2313</td>
<td>0.03</td>
<td>0.7512</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

NS, non-significant; r, Spearman’s rank correlation; p value level of significance.

*Only infections where correlations were found are shown.
syphilis, and in <10% with HCV. As these studies have generally been performed on patients of mixed ethnic composition or in white patients, they do not reflect the prevalence in different ethnic groups. Additionally, the aPT ELISA, is a relatively new method, and not as yet fully standardised.

The first infection to be linked to aPL was syphilis and indeed the discovery of aCL, a phospholipid and one of the major antigens used in the Venereal Disease Research Laboratory Test, and their close association with the lupus anticoagulant in vitro test was a direct result of this finding. This resulted in the discovery of the APS and all its variants. Infections as a group are not commonly associated with any of the major clinical manifestations of the APS—antiphospholipid syndrome may be “triggered” by an infection in about 30% of these patients. “Molecular mimicry” has been invoked as a probable explanation for this occurrence. Support for this phenomenon has emerged from recent studies, where mice were immunised with short peptides found in a number of bacteria and viruses; these peptides shared a high sequence homology with the β2GPI binding site for aCL, and induced the production of anti-β2GPI antibodies. Some of these induced anti-β2GPI antibodies can be pathogenic, resulting in APS associated clinical features, manifested by increased percentage of fetal loss, thrombocytopenia, and prolonged activated partial thromboplastin time (aPTT). Furthermore, although the infection related aCL are usually non-pathogenic, there is also the possibility that these non-pathogenic “infectious” aCL might in some susceptible subjects with the right genetic HLA background, mutate at the CDR3 domain of the aCL binding site for β2GPI, which determines the pathogenicity of aCL antibodies.

In 1990 it was found that the binding of the aPL to phospholipid was enhanced by the cofactor β2GPI in autoimmune conditions such as SLE and the “primary” APS. Although the “non-thrombogenic” aPL did not require this cofactor to enhance the binding. The two types of aPL were then referred to as “autoimmune” or β2GPI dependent and “infectious” or β2GPI independent. However, this distinction has not been found to be absolute. A recent study of 35 lepromatous patients found only one with anti-β2GPI activity, whereas other investigators found increased levels of anti-β2GPI in a significant proportion of their patients’ sera. A very thorough recent study, which examined IgG and IgM, aCL, anti-β2GPI, and aPT, as well as LA (measured by aPTT and dilute Russell’s viper venom time), reported a prevalence of 61% for aCL, 57% for anti-β2GPI, 45% for aPT, and 69% positive for LA; interestingly, most of the plasmas from LA positive patients with leprosy were also positive for antibodies to β2GPI and/or prothrombin. In our group of patients with leprosy, we measured IgG, IgM, and IgA aPL, and we confirmed the high prevalence of anti-β2GPI (89%), as opposed to a much lower prevalence of aCL (29%), and an intermediate prevalence of aPT (21%). Additionally, we found that IgA aCL was the predominant isotype in our patients with leprosy, whereas IgM was the most prevalent isotype of anti-β2GPI. The prevalence of these antibodies in our patients with leprosy, was often found to be significantly higher than that seen in our HIV, syphilis, malaria, and HCV patient groups (table 2). Furthermore we have observed strong correlations, between aCL, anti-β2GPI and aPT antibody levels, for all three aPL isotypes studied (table 3).

During the mid-1980s to the early 1990s, numerous studies, investigated the prevalence of LA and aCL in HIV infected patients. The prevalence reported has varied from 0 to 53.5% for LA, and from 0 to 94% for IgG and/or IgM aCL. The very broad range in the reported positivity for these two autoantibodies in these earlier studies was due to differences in the populations studied, which varied from asymptomatic HIV positive subjects, to patients with or without opportunistic infections, or other HIV associated complications, so patients with the full AIDS syndrome and due to the aCL assays used at the time, which were not fully validated. More recently, aCL in HIV infection were shown to be of the “infectious” type, binding being reduced if β2GPI was added. Anti-β2GPI was detected in only 5% of a series by Petrovas et al, a finding which was confirmed by Gonzales et al. More recent studies investigating all three aPL in HIV-1 infection have reported aCL to be positive in 36–88%, anti-β2GPI in 4–27%, and aPT in 2–12% of patients. In our series of 100 patients with HIV, there was a low prevalence of anti-β2GPI (6%), all exclusively belonging to the IgA isotype, as well as aCL (7%), which were mainly positive for IgG. However, a prevalence of 43% (mainly IgG) aPT was found. Black South Africans therefore do not appear to exhibit similar prevalences of aCL and aPT, as Caucasians. This is probably due to infection with HIV-1 subtypes B or C, which is the virus infecting black South Africans, as opposed to HIV-1 subtype B, which is the predominant HIV virus found in white subjects. We have also compared the number of patients positive for aPL, between HIV and the other infectious diseases studied here; significant differences were seen between HIV and leprosy, for IgG anti-β2GPI and aPT, for IgM anti-β2GPI, and for IgA aCL, anti-β2GPI, and aPT antibodies. A statistically significant correlation was also seen, for IgG aPT between HIV and syphilis or patients with HCV (table 2). When a correlation was sought between aPL levels for all the three aPL isotypes investigated here, significant correlations were seen between IgG aCL and anti-β2GPI or aPT, between IgM aCL and aPT, and between IgA anti-β2GPI and aPT (table 3).

In syphilis, early studies reported no LA positivity, and a prevalence of 45–50% for aCL. More recently, aCL was reported to be positive in 21–67% of patients, anti-β2GPI in 1–11%, and aPT in 4% of patients with syphilis. In the present study, we found a prevalence of 8% for aCL and anti-β2GPI or aPT, between IgM aCL and aPT, and between IgA anti-β2GPI and aPT (table 3).
secondary consequence of the infectious disease process; alternatively, their induction might result from the exposure of cell wall phospholipids, after the breakdown on damaged body cells, as a consequence of inflammation due to the infection. The current hypothesis therefore is that infections may be a “trigger” for the induction of “pathogenic” aPL in predisposed or compromised subjects.

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