EXTENDED REPORT

Anticardiolipin, anti-β_2-glycoprotein I and antiprothrombin antibodies in black South African patients with infectious disease

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Objectives: To investigate IgG, IgM, and IgA, antiphospholipid antibodies (aPL), against cardiolipin (aCL), β_2-glycoprotein I (anti-β_2GPI), 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 thee aPL were found in all patient groups studied: aCL in 7%, anti-β_2GPI 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-β_2GPI 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-β_2GPI 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.[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.[2] Relatively recent studies, however, have indicated that, as well as cardiolipin, the phospholipid binding proteins β_2-glycoprotein I and prothrombin can behave as real antigens as well as protein cofactors.[3 4] Prothrombin and β_2-glycoprotein I have also been reported to be implicated in lupus anticoagulant assays, which are rarely abnormal in infectious diseases.[2 5] In the course of many acute infections such as syphilis, HIV, hepatitis C, leprosy, and malaria, raised levels of anti-cardiolipin (aCL), anti-β_2-glycoprotein I (anti-β_2GPI), and anti-prothrombin (aPT) antibodies have been reported. They are often transient and can disappear after treatment of the infection.[6–9] Although the aCL antibodies detected in infectious disorders were initially reported to be mainly β_2GPI independent, β_2GPI dependent aCL and antibodies against the protein antigens β_2GPI 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.[10 11]

In HIV infection aCL have been reported to be present in 0–94%, anti-β_2GPI 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.[7 10–12] In syphilis aCL have been found to be raised in 18–100%, anti-β_2GPI in 0–10%, and aPT in 4% of patients.[8 10 13] In patients with leprosy, aCL are reported to be raised in 37–98%, anti-β_2GPI in 3–19%, and aPT in 6–45%.[6 10 14 15] For HCV and malaria infections, aCL are reported to be raised in 17–44% and 35–94% of patients respectively, and anti-β_2GPI has been reported to be raised in $<$10% of patients infected with HCV.[16–18]

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 to 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.[2 10] 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-β_2GPI and aPT antibodies, in black South Africans with HIV infection, leprosy, syphilis, malaria and

Abbreviations: aCL, anticardiolipin antibodies; AEU, arbitrary ELISA units; aPL, antiphospholipid antibodies; APS, antiphospholipid syndrome; aPT, antiprothrombin antobodies; aPTT, activated partial thromboplastin time; β_2GPI, β_2-glycoprotein I; ELISA, enzyme linked immunosorbent assay; HCV, hepatitis C virus; LA, lupus anticoagulant; SLE, systemic lupus erythematosus

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-β_2GPI, 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-β_2GPI 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-β_2GPI 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

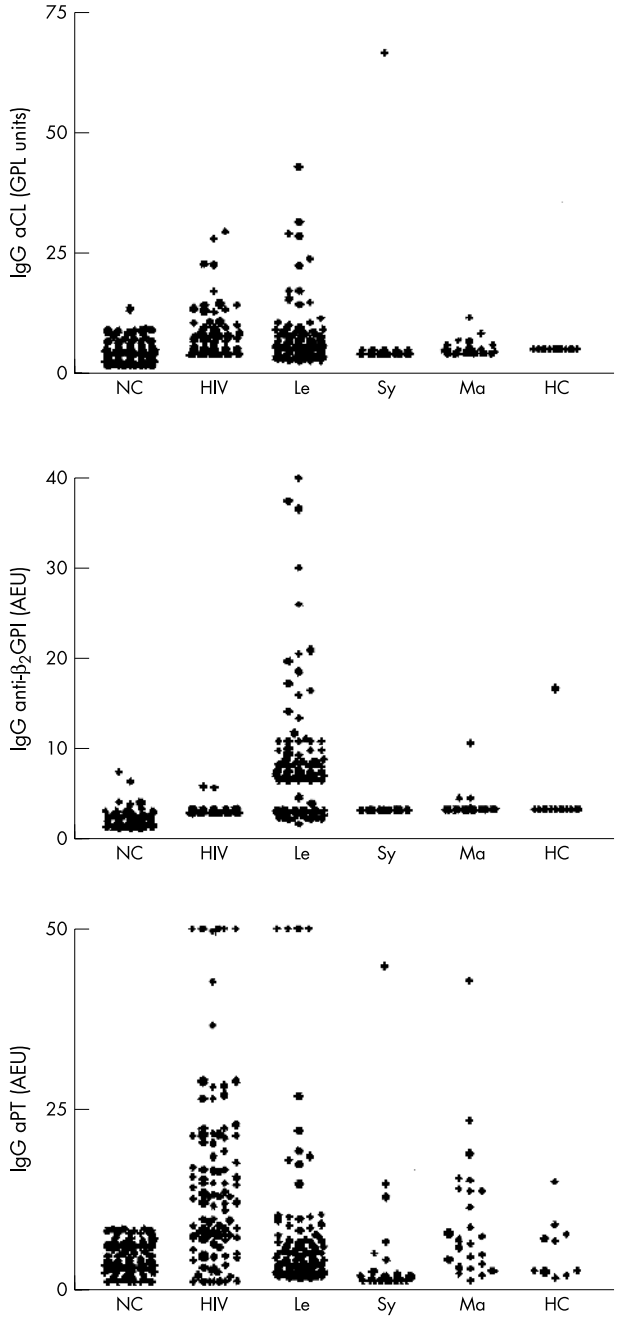

Figure 1 IgG aCL, anti-β_2GPI and aPT antibody levels in normal controls (NC) and in patients with HIV, leprosy (Le), syphilis (Sy), malaria (Ma), hepatitis C (HC). The upper limit of normal, set at 5SD above the mean level of the normal controls, was 16 GPL for aCL, 6.7 AEU for anti-β_2GPI, and 13.8 AEU for aPT.

Table 1 Frequencies of IgG, IgM, and IgA aCL anti-β_2GPI and aPT positive patients with infectious disease

	HIV			Leprosy			Syphilis			Malaria			Hepatitis C		
	aCL	aβ_2GPI	aPT	aCL	aβ_2GPI	aPT	aCL	aβ_2GPI	aPT	aCL	aβ_2GPI	aPT	aCL	aβ_2GPI	aPT
Total any, No (%)	7 (7)	6 (6)	43 (43)	32 (29)	100 (89)	22 (20)	2 (8)	2 (8)	7 (28)	3 (12)	3 (12)	7 (28)	2 (20)	3 (30)	3 (30)
IgG only	5	0	34	7	2	6	0	0	2	0	0	4	0	1	0
IgM only	1	0	0	3	20	2	1	0	5	3	0	0	2	1	1
IgA only	0	6	0	19	5	8	0	2	0	0	2	2	0	1	1
IgG+IgM	0	0	7	0	25	1	1	0	0	0	0	0	0	0	1
IgG+IgA	0	0	1	1	7	3	0	0	0	0	1	1	0	0	0
IgM+IgA	1	0	0	2	12	1	0	0	0	0	0	0	0	0	0
IgG+IgM+IgA	0	0	1	0	29	1	0	0	0	0	0	0	0	0	0

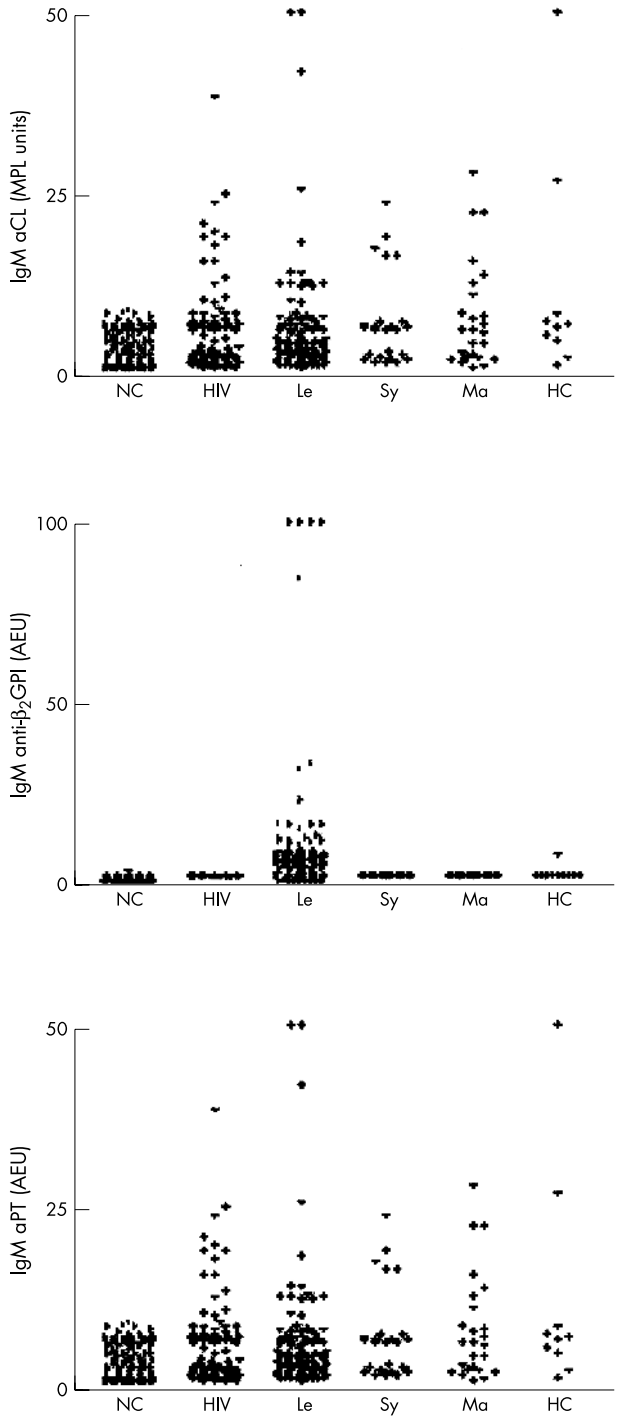

Figure 2 IgM aCL, anti-β_2GPI, and aPT antibody levels in normal controls (NC), and in patients with HIV, leprosy (Le), syphilis (Sy), malaria (Ma), hepatitis C (HC). The upper limit of normal set at 5SD above the mean level of the normal controls, was 9.6 MPL for aCL, 4.5 AEU for anti-β_2GPI, and 16 AEU for aPT.

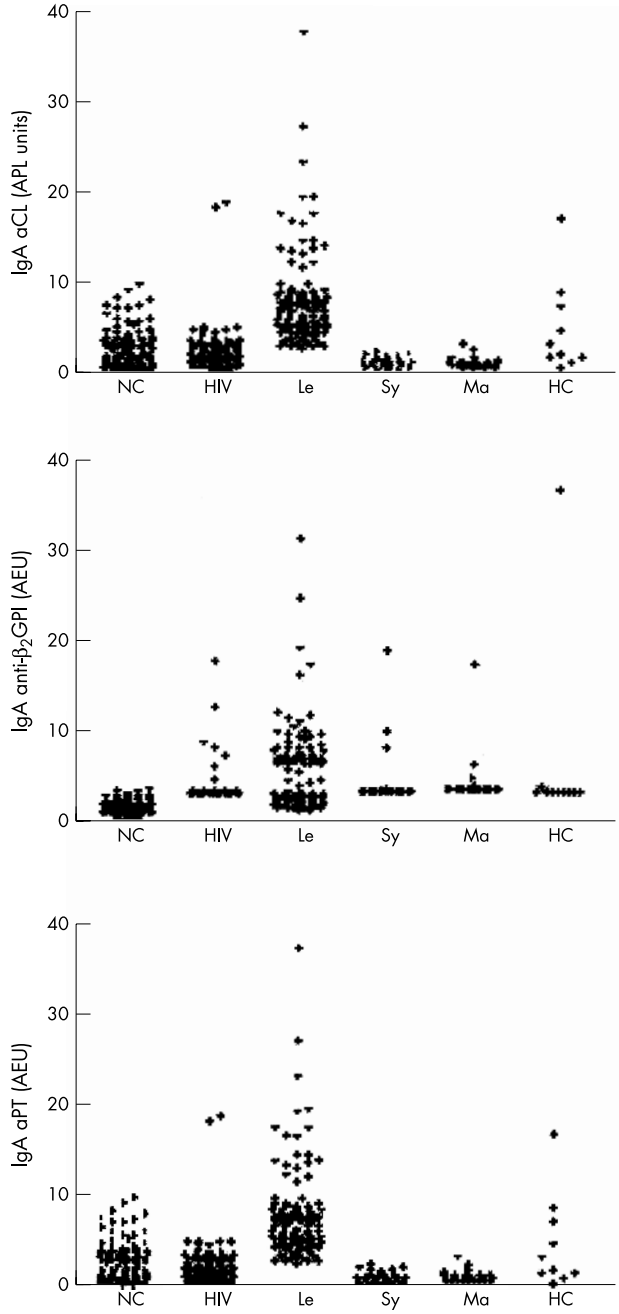

Figure 3 IgA aCL, anti-β_2GPI, and aPT antibody levels in normal controls (NC), and in patients with HIV, leprosy (Le), syphilis (Sy), malaria (Ma), hepatitis C (HC). The upper limit of normal set at 5SD above the mean level of the normal controls, was 8.7 APL for aCL, 4.6 AEU for anti-β_2GPI, and 13.5 AEU for aPT.

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-β_2GPI in leprosy, and for aCL in malaria subjects, and IgA was the most prevalent isotype of aCL in leprosy; IgA anti-β_2GPI 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-β_2GPI 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-β_2GPI and IgG aPT ($p<0.0001$, $p<0.001$, Fisher's exact test), for IgM anti-β_2GPI ($p<0.0001$), and for IgA anti-β_2GPI and aCL ($p<0.0001$),

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

Comparison between	IgA aCL	IgG aβ_2GPI	IgM aβ_2GPI	IgA aβ_2GPI	IgG aPT	IgM aPT	IgA aPT
Leprosy and HIV	<0.0001	<0.0001	<0.0001	<0.0001	<0.001	NS	<0.007
Leprosy and syphilis	<0.001	<0.001	<0.0001	<0.0002	NS	<0.02	NS
Leprosy and malaria	<0.001	<0.0001	<0.0001	<0.002	NS	NS	NS
Leprosy and HCV	NS	<0.02	<0.0001	<0.05	NS	NS	NS
HIV and syphilis	NS	NS	NS	NS	<0.001	NS	NS
HIV and HCV	NS	NS	NS	NS	<0.04	NS	NS

NS, non-significant; p values of χ^2 for Fisher's exact test are shown.
*aPL isotypes where no correlation was found not shown.

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-β_2GPI ($p<0.0001$, $p<0.02$); IgM anti-β_2GPI 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 malaria ($p<0.001$). IgA anti-β_2GPI levels were also 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-β_2GPI, and aPT in HIV, leprosy, and patients with malaria, as well as between IgG anti-β_2GPI 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-β_2GPI levels, whereas in the comparison of aCL and aPT, levels correlated significantly in patients with HIV, leprosy, and malaria. When IgM anti-β_2GPI 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-β_2GPI; in patients with leprosy and HCV between aCL and aPT, and in HIV and patients with leprosy between anti-β_2GPI and aPT.

DISCUSSION

It is well established that aPL (aCL and anti-β_2GPI primarily) may be found in a large variety of infectious diseases.[10 19] 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, β_2GPI 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. Arvieux in 1995[4] 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-β_2GPI.[20] 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.[21] 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.[22] 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

Table 3 Correlation between IgG, IgM, and IgA, aCL anti-β_2GPI and aPT antibody levels in the groups of patients with infectious disease

	HIV		Leprosy		Malaria		HCV	
Correlation between*	r_s	p	r_s	p	r_s	p	r_s	p
IgG								
aCL and aβ_2GPI	0.2436	0.0001	0.4151	0.0001	0.5186	0.008	NS	
aCL and aPT	−0.2046	0.04	0.6359	0.0001	0.4504	0.03	NS	
aβ_2GPI and aPT	NS		0.3837	0.0001	0.5140	0.03	NS	
IgM								
aCL and aβ_2GPI	NS		0.4889	0.0001	NS		NS	
aCL and aPT	0.2488	0.02	0.3585	0.0001	0.4166	0.04	NS	
aβ_2GPI and aPT	NS		0.6328	0.0001	NS		0.7006	0.0001
IgA								
aCL and aβ_2GPI	NS		0.2055	0.03	NS		NS	
aCL and aPT	NS		0.4315	0.0001	NS		0.6617	0.05
aβ_2GPI and aPT	0.2313	0.03	0.7512	0.0001	NS		NS	

NS, non-significant; r_s, Spearman's rank correlation; p value level of significance.
*Only infections where correlations were found are shown.

syphilis, and in <10% with HCV.[10] 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[2], 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.[1] Infections as a group are not commonly associated with any of the major clinical manifestations of the APS—for example, clotting. However a minority of patients have in fact been documented who have manifested such complications, and this has become one of the major topics of recent interest to researchers in this field as well as being the subject of several major reviews.[10 19 24] Not only have several infections (mainly viral) been accompanied by clinical events resembling those seen in patients with APS but also it has recently been shown that an often fatal but thankfully uncommon complication of the APS termed the ''catastrophic antiphospholipid syndrome''[25] may be ''triggered'' by an infection in about 30% of these patients. ''Molecular mimicry'' has been invoked as a probable explanation for this occurrence.[26] 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 β_2GPI binding site for aCL, and induced the production of anti-β_2GPI antibodies. Some of these induced anti-β_2GPI 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).[27 28] 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 β_2GPI, which determines the pathogenicity of aCL antibodies.[29]

In 1990 it was found that the binding of the aPL to phospholipid was enhanced by the cofactor β_2GPI in autoimmune conditions such as SLE and the ''primary'' APS, whereas 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 β_2GPI dependent and ''infectious'' or β_2GPI independent. However, this distinction has not been found to be absolute.[3] A recent study of 35 lepromatous patients found only one with anti-β_2GPI activity,[14] whereas other investigators found increased levels of anti-β_2GPI in a significant proportion of their patients' sera.[15 30 31]. A very thorough recent study, which examined IgG and IgM, aCL, anti-β_2GPI, 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-β_2GPI, 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 β_2GPI and/or prothrombin.[15] In our group of patients with leprosy, we measured IgG, IgM, and IgA aPL, and we confirmed the high prevalence of anti-β_2GPI (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-β_2GPI. 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-β_2GPI, 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, to patients with the full AIDS syndrome, and also due to the aCL assays used at the time, which were not fully validated.[19] More recently, aCL in HIV infection were shown to be of the ''infectious'' type, binding being reduced if β_2GPI was added. Anti-β_2GPI was detected in only 5% of a series by Petrovas *et al*,[11] a finding which was confirmed by Gonzales *et al*.[32] More recent studies investigating all three aPL in HIV-1 infection have reported aCL to be positive in 36–88%, anti-β_2GPI in 4–27%, and aPT in 2–12% of patients.[7 11 12 33 34] In our series of 100 patients with HIV, there was a low prevalence of anti-β_2GPI (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.[35] 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-β_2GPI and aPT, for IgM anti-β_2GPI, and for IgA aCL, anti-β_2GPI, 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-β_2GPI or aPT, between IgM aCL and aPT, and between IgA anti-β_2GPI 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-β_2GPI in 1–11%, and aPT in 4% of patients with syphilis.[8 11 36] In the present study, we found a prevalence of 8% for aCL and anti-β_2GPI , and a higher prevalence of 28% for aPT (table 1). We found no correlation in our syphilis patient group between any of the three aPL, for any of the three immunoglobulin isotypes studied.

A few studies have reported on aCL in malaria.[18 19 37] Our patients with malaria demonstrated a prevalence of 28% for aPT, as opposed to a low frequency for anti-β_2GPI (8%), and an intermediate frequency for aCL (12%). Significant correlations were seen between IgG aCL, anti-β_2GPI and aPT levels, and between aCL and aPT IgM levels (table 3). In HCV infection, anti-β_2GPI independent aCL are reported to be raised in 17–44% of patients, whereas raised anti-β_2GPI and aPT are seldom found.[10 12 16 17] In our small cohort of HCV patients, studying all three aPL isotypes, we found that 20% of patients were positive for aCL, and 30% were positive for anti-β_2GPI and aPT, respectively.

The aPL, which are often present in infectious diseases, are not usually associated with thrombotic and other complications attributed to them in patients with SLE and APS. These infectious aPL could be induced by subtle disturbances of the regulation of cellular and humoral immunity, which are a

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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REFERENCES

1 **Asherson RA**, Cervera R, Piette JC, Shoenfeld Y. The antiphospholipid syndrome: History, definition, classification and differential diagnosis. In: Asherson RA, Cervera R, Piette JC, Shoenfeld Y, eds. *The antiphospholipid syndrome*. Boca Raton, Florida, USA: CRC press, 1996:3–12.
2 **Roubey RAS**. Immunology of the antiphospholipid antibody syndrome. *Arthritis Rheum* 1996;**39**:1444–54.
3 **Roubey RAS**, Eisenberg RA, Harper MF, Winfield JB. 'Anti- cardiolipin' autoantibodies recognize β_2-glycoprotein I in the absence of phospholipid. Importance of antigen density and bivalent binding. *J Immunol* 1995;**154**:950–60.
4 **Arvieux J**, Darnige L, Caron C, Reber G, Bensa JC, Colomb MG. Development of an ELISA for autoantibodies to prothrombin showing their prevalence in patients with lupus anticoagulants. *Thromb Haemost* 1995;**74**:1120–5.
5 **Galli M**, Finazzi G, Bevers EM, Barbui T. Kaolin clotting time and dilute Russell's viper venom time distinguish between prothrombin- and β_2-glycoprotein I-dependent antiphospholipid antibodies. *Blood* 1995;**86**:617–23.
6 **Guedes Barbarosa LS**, Gilbrut B, Shoenfeld Y, Scheinberg MA. Autoantibodies in leprosy sera. *Clin Rheumatol* 1996;**15**:26–8.
7 **Abuaf N**, Laperche S, Rajoely B, Carsique R, Deschamps A, Rouquette AM, *et al*. Autoantibodies to phospholipids and to the coagulation proteins in AIDS. *Thromb Haemost* 1997;**77**:856–61.
8 **de Laranaga GF**, Forastiero RR, Carreras LO, Alonso BS. Different types of antiphospholipid antibodies in AIDS: a comparison with syphilis and the antiphospholipid syndrome. *Thromb Res* 1999;**96**:19–25.
9 **Carreras LO**, Forastiero RR, Martinuzzo ME. Which are the best biological markers of the antiphospholipid syndrome. *J Autoimmun* 2000;**15**:163–72.
10 **Zandman-Goddard G**, Blank M, Shoenfeld Y. Antiphospholipid antibodies and infections-drugs. In: Asherson RA, Cervera R, Piette JC, Shoenfeld Y, eds. *The antiphospholipid syndrome II*. Amsterdam: Elsevier Science, 2002:343–60.
11 **Petrovas C**, Vlachoyiannopoulos PG, Kordossis T, Moutsopoulos HM. Antiphospholipid antibodies in HIV infection and SLE with and without antiphospholipid syndrome; comparisons of phospholipid specificity, avidity and reactivity with β_2-GPI. *J Autoimmun* 1999;**13**:347–355.
12 **Gugliemone H**, Vitozzi S, Elbarcha O, Fernandez E. Cofactor dependence and isotype distribution of anticardiolipin antibodies in viral infections. *Ann Rheum Dis* 2001;**60**:500–4.
13 **Forastiero RR**, Martinuozo Me, Kordich LC, Carreras LO. Reactivity to beta2glycoprotein I clearly differentiates anticardiolipin antibodies from antiphospholipid syndrome and syphilis. *Thromb Haemost* 1996;**75**:717–20.
14 **Elbeialy A**, Strassburger-Lorna K, Atsumi T, Bertolaccini ML, Ameng Hanafi M, Khamashta MA, *et al*. Antiphospholipid antibodies in leprotic patients: a correlation with disease manifestations. *Clin Exp Rheumatol* 2000;**18**:492–4.
15 **de Laranaga GF**, Forastiero RR, Martinuzzo ME, Carreras MO, Tsariktsian G, Surno MM, *et al*. High prevalence of antiphospholipid antibodies in leprosy: evaluation of antigen reactivity. *Lupus* 2000;**9**:594–600.
16 **Leroy V**, Arvieux J, Jacob MC, Maynard-Muet M, Baud M, Zarski JP. Prevalence and significance of anticardiolipin, anti-β_2 glycoprotein I and anti-prothrombin antibodies in chronic hepatitis C. *Br J Haematol* 1998;**101**:468–74.
17 **Harada M**, Fujisawa Y, Sakisaka S, Kawaguchi T, Taniguchi E, Sakamoto M, *et al*. High prevalence of anticardiolipin antibodies in hepatitis C virus infection: lack of effects on thrombocytopenia and thrombotic complications. *J Gastroenterol* 2000;**35**:272–7.
18 **Soni PN**, De Bruyn CC, Duursma J, Sharp BL, Pudifin DJ. Are anticardiolipin antibodies responsible for some of the complications of severe acute Plasmodium facliparum malaria? *S Afr Med J* 1993;**83**:660–2.
19 **Loizou SA**, Walport MJ, Davies KA. The antiphospholipid syndrome in infectious diseases. In: Asherson RA, Cervera R, Piette JC, Shoenfeld Y, eds. *The antiphospholipid syndrome*. Boca Raton, Florida, USA: CRC Press, 1996:267–84.
20 **Puurunen M**, Vaarala O, Julkunen H, Aho K, Paluoso T. Antibodies to phospholipid-binding plasma proteins and occurrence of thrombosis in patients with systemic lupus erythematosus. *Clin Immunol Immunopathol* 1996;**80**:16–22.
21 **Galli M**, Dlott J, Norbis F, Ruggeri L, Cler L, Triplett DA, *et al*. Lupus anticoagulants and thrombosis: clinical association of different coagulation and immunologic tests. *Thromb Haemost* 2000;**84**:1012–16.
22 **Salcido-Ochoa F**, Cabiedes J, Alarcon-Segovia D, Cabral AR. Antiprothrombin antibodies in patients with systemic lupus erythematosus or with primary antiphospholipid syndrome. *J Clin Rheumatol* 2002;**8**:251–5.
23 **Michaelis L**. Precipitin reaction bei syphilis. *Berl Klin Wochenschr* 1907;**44**:1477–8.
24 **Asherson RA**, Cervera R. Infections, Antiphospholipid antibodies and syndromes. *Ann Rheum Dis* 2003;**62**:3988–93.
25 **Asherson RA**. The catastrophic antiphospholipid syndrome. *J Rheumatol* 1992;**19**:508–12.
26 **Asherson RA**, Shoenfeld Y. The role of infection in the pathogenesis of catastrophic antiphospholipid syndrome–molecular mimicry? *J Rheumatol* 2000;**27**:12–14.
27 **Gharavi AE**, Pierangeli SS, Harris EN. Origin of antiphospholipid antibodies. *Rheum Dis Clin North Am* 2001;**27**:551–63.
28 **Blank M**, Krause I, Fridkin M, Keller N, Kopolovic J, Goldberg I, *et al*. Bacterial induction of autoantibodies to β_2-glycoprotein-I accounts for the infectious aetiology of antiphospholipid syndrome. *J Clin Invest* 2002;**109**:797–804.
29 **Asherson R**, Cervera R, Piette JC, Schoenfeld Y, Epsinosa G, Petri MA, *et al*. Catastrophic antiphospholipid syndrome: clues to the pathogenesis from a series of 80 patients. *Medicine (Baltimore)* 2001;**80**:355–77.
30 **Hojnik M**, Gilburd B, Ziporen L, Blank M, Tomer Y, Scheinberg MA, *et al*. Anticardiolipin antibodies in infections are heterogeneous in their dependency on β_2-glycoprotein I: analysis of anticardiolipin antibodies in leprosy. *Lupus* 1994;**3**:515–21.
31 **Fiallo P**, Travaglino C, Nunzi E, Cardo PP. β_2-glycoprotein I dependence of anticardiolipin antibodies in multibacillary leprosy patients. *Lepr Rev* 1998;**69**:376–81.
32 **Gonzalez C**, Leston A, Garcia-Berrocal B, Sanchez-Rodriguez A, Martin-Oter B. Antiphosphatidylserine antibodies in patients with autoimmune diseases and HIV-infected patients: effects of Tween 20 and relationship to β_2-glycoprotein I. *J Clin Lab Anal* 1999;**13**:59–64.
33 **Guerin J**, Feighery C, Sim RB, Jackson J. Antibodies to β_2-glycoprotein I – a specific marker for antiphospholipid syndrome. *Clin Exp Immunol* 1997;**109**:304–9.
34 **Guerin V**, Ryman A, Couchouron A. Transitory anti-β_2-glycoprotein I antibodies in infections. *Lupus* 1999;**8**:490–491.
35 **Hu DJ**, Dondero TJ, Rayfield MA, Richard George J, Schochetman G, Jaffe HW, *et al*. The emerging genetic diversity of HIV. The importance of global surveillance for diagnostics, research, and prevention. *JAMA* 1996;**275**:210–16.
36 **Santiago MB**, Stellin R, Gaburo JRN, Nueno C, Viana VS, Cossermelli W, *et al*. Antiphospholipid antibodies in syphilis. *Braz J Med Biol Res* 1990;**23**:397–402.
37 **Facer CA**, Agiostradidou G. High levels of anti-phospholipid antibodies in uncomplicated and severe Plasmodium falciparum and in P. vivax malaria. *Clin Exp Immunol* 1994;**95**:304–9.