

https://dspace.org/

Prevalence and clinical correlates of antiphospholipid antibodies in South Africans with systemic lupus erythematosus

Gould, Trevor John

2008-03-25T07:24:26Z
http://hdl.handle.net/10539/4708

Downloaded from a DSpace repository. https://dspace.org/

PREVALENCE AND CLINICAL CORRELATES OF ANTIPHOSPHOLIPID ANTIBODIES IN SOUTH AFRICANS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

Trevor Gould

PREVALENCE AND CLINICAL CORRELATES OF ANTIPHOSPHOLIPID ANTIBODIES IN SOUTH AFRICANS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

Trevor Gould

A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree:

Master of Medicine

In

the branch of Internal Medicine.

Johannesburg, 2007

DECLARATION

I, Trevor Gould declare that this research report is my own work. It is being submitted for the degree of Master of Medicine in the branch of Internal Medicine at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other university.

……

…….day of ……2007

PUBLICATIONS ARISING FROM THIS PROJECT

Gould T, Tikly M, Asherson R, Loizou S, Singh S. Prevalence and clinical correlates of anti-phospholipid antibodies in South Africans with systemic lupus erythematosus. Scand J Rheumatol 2006;35:29-34

ABSTRACT

OBJECTIVE: To determine the prevalence and clinical correlates of anti-phospholipid antibodies (aPL), including anti-cardiolipin antibodies (aCL), lupus anti-coagulant (LA), anti-β_2-glycoprotein 1 (aβ_2GP1) and anti-prothrombin (aPT) antibodies, in Black South African patients with systemic lupus erythematosus (SLE)

METHODS: A cross-sectional study of 100 SLE patients in whom clinical characteristics, including features of the anti-phospholipid syndrome (APS), disease activity, and damage were documented, and sera tested for aCL, aβ_2GP, and aPT of all isotypes, and LA.

RESULTS: Positive aCL, aβ_2GP1 and aPT and LA were found in 53, 84, 20, and 2 patients, respectively. Immunoglobulin (Ig)A aCL and IgG aβ_2GP1 were the commonest aCL (49.1%) and aβ_2GP1 (47%) isotypes, respectively. IgA aβ2GP1 were associated with both a history of thrombosis alone ($p<0.05$) and a history of any clinical feature, thrombosis and/or spontaneous abortion of the APS ($p<0.05$); IgA aCL were associated with a history of any clinical APS event ($p<0.05$); and aβ2GPI of any isotype were associated with a history of arthritis ($p<0.001$).

CONCLUSION: My findings provide further evidence that the screening for aβ_2GP1 and IgA aCL isotype may improve the risk assessment for APS in SLE patients of African extraction. Further prospective studies are warranted to determine the clinical utility of these tests and to elucidate the genetic basis for increased IgA aPL response in SLE patients of African extraction.

ACKNOWLEDGEMENTS

I wish to thank the following persons:

1. Professor M. Tikly. Not only for his guidance with this project, but his outstanding teaching and mentorship over the past 8-years.
2. Professor K.R.L.Huddle. An exceptionally gifted physician whose teaching I was fortunate to experience for 8-years. In addition, I am grateful for his persistent reminders, without which this project would not have been completed.
3. Dr R. Asherson, who sponsored the antiphospholipid antibody assays.
4. Mr S. Singh at Lancet laboratories who performed the antiphospholipid assays.
5. Dr S. Loizou for assisting with the statistical analysis of this project.

TABLE OF CONTENTS

	PAGE
DECLARATION	ii
PUBLICATIONS ARISING FROM THESIS	iii
ABSTRACT	iv
ACKNOWLEDGEMENTS	v
TABLE OF CONTENTS	vi
LIST OF TABLES	viii
ABBREVIATIONS	ix
1 INTRODUCTION	1
2 AIMS OF THE STUDY	3
3 PATIENTS AND METHODS	
3.1 Patients	4
3.2 Laboratory methods	4
3.3 Statistical methods	5
4 RESULTS	6

4.1 Prevalence and isotype distribution of anti-phospholipid antibodies 8

4.2 Clinical correlates 9

5 DISCUSSION 12

6 LIMITATIONS 15

7 CONCLUSION 16

8 APPENDICES 17

9 REFERENCES 22

LIST OF TABLES

Table		Page
TABLE 1	Details of patients fulfilling the clinical criteria of the Sapporo classification for the anti-phospholipid syndrome.	7
TABLE 2	Prevalence of IgG, IgM, and IgA isotypes of aCL, aβ_2GP1, and APL antibodies in 100 South African SLE patients.	8
TABLE 3	Clinical correlates with anti-phospholipid antibodies as continuous variables.	9
TABLE 4	Clinical correlates with anti-phospholipid antibodies as categorical variables.	11

ABBREVIATIONS

aPL	Anti-phospholipid antibodies
aCL	Anti-cardiolipin antibodies
LA	Lupus anti-coagulant
aβ_2GP1	Anti-β_2-glycoprotein 1
aPT	Anti-prothrombin antibodies
SLE	Systemic lupus erythematosus
APS	Anti-phospholipid syndrome
Ig	Immunoglobulin
ADP	Adenosine diphosphate
Anti-Sm	Anti-Smith antibodies
BSA	Black South African
ACR	American College of Rheumatology
ANA	Anti-nuclear antibody
SLEDAI	SLE Disease Activity Index
SLICC	Systemic lupus International Collaborating Clinics
ELISA	Enzyme-linked immunosorbent assay
AEU	Arbitrary ELISA units
RVVT	Russell's viper venom time
HTLV-1	Human T cell leukaemia virus type 1

1 INTRODUCTION

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterised by the presence of a variety of circulating autoantibodies, including those directed against phospholipids (aPL). Elevated levels of aPL are frequently, but not invariably, associated with the anti-phospholipid syndrome (APS), which manifests clinically as venous or arterial thrombosis and/or recurrent spontaneous abortions (1). The thrombophilic activity of aPL is mediated by binding to the natural anticoagulant β_2-glycoprotein 1 (β_2GP1), which inhibits prothrombinase activity of platelets and adenosine diphosphate (ADP)-induced platelet aggregation. To date, the immunoglobulin (Ig)G and IgM isotypes of anticardiolipin antibody (aCL) and the lupus anticoagulant (LA) are the only aPL included in the classification criteria for APS (2). An emerging issue is the clinical utility of the IgA isotype of aCL and antibodies of all isotypes directed against β_2GP1 (aβ_2GP1) and prothrombin (aPT) (3). Some experts argue that, as APS patients are unlikely to be negative for both aCL (IgG and IgM) and LA, it is superfluous to test for aβ2GP1 (4) and IgA aCL (5). The role of aPT as markers of thrombophilia in SLE appears to be even more tenuous, and is, at best, only partially supported by the published literature (6-8).

The spectrum and prevalence of both clinical features and autoantibodies, including aPL, show considerable inter-ethnic variation, which, in part, may account for ethnic differences in morbidity and mortality in SLE. The limited published experience from sub-Saharan Africa,

particularly South Africa, suggests that the prognosis of SLE is poor compared to Caucasian patients in industrialised countries (9). In addition, the prevalence of anti-RNP and anti-Smith (anti-Sm) antibodies in Black South Africans (BSA) has been found to be higher than that reported in Caucasians (10).

2 AIMS OF THE STUDY

I undertook a cross-sectional study to determine 1) the prevalence of IgG, IgM and IgA isotypes of aCL, LA and the newer aPL (aβ2GP1 and aPT) in BSA patients with SLE and 2) their relationship with thrombotic events and/ or spontaneous abortions as well as other clinical features of SLE. The study was approved by the Ethics Committee of the Faculty of Health Sciences, University of the Witwatersrand.

3 PATIENTS AND METHODS

3.1 Patients

One hundred unselected BSA patients, fulfilling at least 4 of the 11 American College of Rheumatology (ACR) criteria for SLE (11) and attending a tertiary care Lupus Clinic at Chris Hani Baragwanath Hospital, were studied. Patients known to be HIV positive were excluded from the study as HIV infection can be associated with aPL (12). Clinical and antinuclear antibody (ANA) results were obtained by a combination of a detailed history of pregnancy loss and thrombotic events, data extraction from case records, and clinical examination. Disease activity and damage were assessed using the systemic lupus erythematosus disease activity index (SLEDAI) (13) and systemic lupus international collaborating clinics (SLICC)/ACR damage score (14), respectively. The clinical features of APS, thrombosis and spontaneous abortions, were defined according to the Sapporo classification criteria for APS (2).

3.2 Laboratory Methods

The indirect immunofluorescence assay was used to screen for ANA and anti-dsDNA using HEp2 cell line and Crithidia lucillae as substrate (Diagnostic and Technical Services, Johannesburg, South Africa), respectively. aPL, IgG, IgM and IgA isotypes of the aCL, aβ2GP1 and aPT were measured using commercial enzyme linked immunoabsorbent assay (ELISA) kits (Cheshire Diagnostics Ltd, Ellesmere Port, Cheshire, UK), and expressed in units, according to the manufacturers 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 respectively, whereas aβ2GP1 and aPT were expressed as arbitrary ELISA units (AEU), according to the manufacturer's instructions. The upper limit of each of these tests was based on previously published work on 100 sera from the healthy controls of the same ethnic and geographic background (12). Thus, the upper limit of normal for IgG isotype of aCL, aβ_2GP1 and aPT were16 GPL, 6.7 and 13.8 AEU, for IgM isotype 9.6 MPL, 4.5 and 16 AEU, and for IgA isotype, 8.7 APL, 4.6 and 13.5 AEU, respectively. Lupus anticoagulant (LA) was detected using both the kaolin clotting time (16) and dilute Russell's viper venom time (RVVT) assays (Instrument Laboratories Spa. Milan, Italy).

3.3 Statistical analysis

The Mann-Whitney U-test was applied to compare antibody levels between patient subgroups. The χ^2 test (with Yates' correction) or, where indicated, Fisher's exact test was used to compare frequencies of categorical variables between subgroups of patients. Statistical analysis was performed using Statistica v5.1 (StatSoft Inc) and Epinfo v6 software. A p-value <0.05 was defined as being significant.

4 RESULTS

The mean (±SD) age and disease duration of the 97 female and 3 male patients were 38.5 (11.3) and 4.8 (4.1) yrs, respectively. The cumulative frequencies of the key clinical features, as defined by the ACR classification criteria, were discoid rash and malar rash each in 40, arthritis in 62, nephropathy in 28, neuropsychiatric disease in 11, leucopaenia in 41, thrombocytopaenia in 9, positive Coomb's test in 19, positive ANA in 99 and anti-dsDNA antibodies in 47 patients. The mean (±SD) SLEDAI and SLICC/ACR damage scores were 5.2 (6.6) and 1.9 (1.4), respectively. Table 1 shows the 19 patients identified as having fulfilled the clinical criteria of APS: 5 had only thrombotic complications, 12 had only spontaneous abortions and 2 had both thrombotic complications and spontaneous abortions. At the time of the study, 50/89 (56.2%) patients were on chloroquine and 71/91 (78%) were on oral prednisone and/or on immunosuppressive agents, including azathioprine, methotrexate or cyclophosphamide.

TABLE 1. Details of patients fulfilling the clinical criteria of the Sapporo classification for antiphospholipid syndrome

Age	Sex	Thrombo-embolic event	No. of Miscarriages (trimester)	aCL isotype	aβ2GPI isotype	aPTisotype	LA
29	F	-	1 (2nd)	neg	IgG	neg	neg
39	F	CVA	2 (both 1st)	IgG, IgA	IgG, IgA	neg	neg
47	F	-	1 (2nd)	IgA	All	neg	neg
31	F	-	1 (2nd)	IgA	IgG	IgM	neg
31	F	DVT (leg)	-	IgA, IgM	All	IgG	pos
53	F	CVA, DVT (leg)	-	neg	IgG, IgM	neg	neg
46	F	-	1 (2nd)	All	All	All	neg
39	F	CVA	-	IgG, IgA	All	neg	neg
46	F	-	1 (3rd)	IgG, IgA	IgG, IgA	IgG	neg
67	F	-	1 (3rd)	IgA	IgA	neg	neg
39	F	-	3 (2nd, 2nd, 1st)	neg	IgA	neg	neg
54	F	-	1 (3rd)	IgA	IgG	neg	neg
30	F	DVT (leg)	-	IgA	IgG	neg	neg
34	F	-	1 (2nd)	IgA	IgG	neg	neg
27	F	-	1 (2nd)	neg	neg	neg	neg
53	F	-	1 (2nd)	neg	IgA	neg	neg
47	F	DVT (leg)	-	neg	IgG, IgA	neg	neg
43	F	-	1 (2nd)	neg	IgG, IgA	neg	neg
43	F	Leg thromboembolism	1 (1st)	IgG, IgA	IgG, IgA	IgG	neg

CVA – cerebrovascular accident; DVT – deep vein thrombosis

4.1 Prevalence and isotype distribution of antiphospholipid antibodies

The prevalence and distribution of the aPL and their 3 isotypes are shown in Table 2. Eighty six sera were positive for at least one aPL. In only 2 out of 89 (2.2%) were the LA assays positive (11 samples lost or invalid due to laboratory error). Positive aCL, aβ_2GP1 and aPT were found in 53, 84, and 20 of the patients. As the sole isotype, IgA was the commonest in 26 (49%) of the aCL positive sera, whereas IgG was the most prevalent sole isotype in 39 (47%) of the aβ_2GP1 positive sera and 14 (70%) of the aPT positive sera.

TABLE 2. Prevalence of IgG, IgM and IgA isotypes of aCL, aβ2GPI and aPT antibodies in 100 Black South African SLE patients

Isotype	**aCL**	**aβ2GP1**	**aPT**
Total any	53	84	20
IgG only	3	39	14
IgM only	8	1	4
IgA only	26	16	0
IgG+IgM	3	1	1
IgG+IgA	9	20	0
IgM+IgA	3	1	0
IgG+IgM+IgA	1	6	1

4.2 Clinical correlates

Analysis of aPL as continuous variables (Table 3), revealed that patients with a history of thrombosis had significantly increased IgA aCL and IgG aβ_2GP1 levels compared to those without a history of thrombosis. Patients with a history of any clinical APS event (thrombosis and/or spontaneous abortion) had increased aβ_2GP1 IgG and IgA and aPT IgA levels compared to those without a history of an APS event. Patients with a history of arthritis had increased IgG aCL and IgA aβ2GP1 levels compared to those without a history of arthritis. Patients with a history of mouth ulcers had decreased levels of IgA aPT compared to those without a history of mouth ulcers.

TABLE 3. Clinical correlates with antiphospholipid antibodies as continuous variables

	Median (IQ)	**Median (IQ)**	
Clinical feature	***Present***	***absent***	***p value***
History of thrombosis	***n=7***	***n=94***	
IgA aCL	7.7 (5.2, 11.2)	14.6 (7, 24.3)	<0.05
IgG aβ2GPI	7.8 (5.8, 10.4)	12.6 (10.3, 12.8)	<0.01
History of any APS event	***n=19***	***n=82***	
IgG aβ2GPI	7.6 (5.9, 10.2)	10.5 (7.2, 12.8)	<0.05
IgA aβ_2GPI	3.5 (1.9, 6.6)	5.9 (3.3, 9.0)	<0.05
IgA aPT	3.4 (2.3, 4.9)	4.7 (3.4, 7.5)	<0.05
History of arthritis	***n=62***	***n=39***	
IgG aCL	7.5 (4.3, 11.4)	9.6 (6.1, 13.3)	<0.05
IgA aβ_2GPI	3 (1.7, 5.4)	4.7 (2.4, 9)	<0.05
History of mouth ulcers	***n=***	***n=***	
IgA aPT	4 (2.6, 5.9)	3 (2, 3.5)	<0.02

Values are median (IQ); IQ – 25, 75 interquartile values

Analysis of aPL as categorical variables (Table 4) showed that IgA aβ2GP1 were associated with both a history of thrombosis alone and a history any clinical APS event; IgA aCL were associated with a history of any clinical APS event; and aβ2GP1 of any isotype were associated with a history of arthritis. No aPL associations with thrombocytopaenia, a common laboratory finding in APS, or the SLEDAI and SLICC/ACR damage scores were observed.

TABLE 4. Clinical correlates with antiphospholipid antibodies as categorical variables

	Present		**Absent**		OR (95% CI)	p value
	positive	**negative**	**positive**	**negative**		
Arterial/venous thrombosis						
IgA aβ2GPI	6	1	37	56	9.1 (1.02-424.7)	<0.05
Any APS event						
IgA aCL	12	7	27	54	3.4 (1.1-11.4)	<0.05
IgA aβ2GPI	13	6	30	52	3.8 (1.2-13.2)	<0.03
Arthritis						
Any aβ2GPI isotype	59	3	26	13	9.1 (2.2-53.1)	<0.001

OR, odds ratio; CI, confidence interval

5 DISCUSSION

The high prevalence of IgA aCL isotype, found in 39/100 of patients, is noteworthy. It was the sole aCL isotype in 26 of patients and thus increased the overall sensitivity of aCL assay from 26% to 53%. Other studies have shown a similar trend of higher prevalence of IgA aCL in SLE patients of African extraction. Molina et al found that the IgA aCL was positive in all of the 26 Afro-Caribbean SLE patients studied and the sole isotype in 21 (81%) of patients (17). In African Americans with SLE, IgA aCL has been found to be the most prevalent isotype occurring in 24% of patients (15). The pathological basis for this relatively high prevalence of in IgA aCL in patients of African extraction is not known. Infection with human T cell leukaemia virus type 1 (HTLV-1) has been shown to induce an IgA aCL response in patients with tropical spastic syndrome (18). However, it is unlikely to account for this selective ethnic phenomenon, as HTLV-1 infections are not restricted to a specific ethnic group. From a clinical perspective, more importantly, IgA aCL levels were significantly raised in patients with a history of thrombosis and a positive IgA aCL result was associated with a history of any clinical APS event. Lopez et al, in an American population where the ethnic group was not specified, showed that IgA aCL correlated better with clinical APS events than thrombocytopaenia and IgG and IgM aCL isotypes (19). Diri et al reported that IgA aCL was most frequent isotype in 7 out of 8 African American patients with APS (15).

Overall, aβ_2GP1 were the commonest aPL, with the IgG isotype as the sole isotype in almost half the patients who were aβ_2GP1 positive. My findings (tables 3 and 4) suggest that both the IgG and IgA isotypes may be of value in risk assessment for APS. As in the Afro- Americans, the isotype appears to be particularly associated with APS events in my study patients (15). Most studies suggest that, although aβ_2GP1 are associated with APS events, testing for them has no additional value compared to the established aPL, aCL and LA (20-22). However, in the present study, in almost a third (6/19) of the patients with clinical features of APS, aβ_2GP1 were the sole aPL. In a Canadian study, Bruce et al similarly found that aβ_2GP1 were present in some patients with clinical features of APS who were negative for both aCL and LA (23). These findings suggest that although testing for aβ_2GP1 isotypes does not supersede the established aPL, they might be of additional value in detecting patients with APS who are negative for aCL and LA.

Unlike aβ_2GP1, measuring aPT appears to be of little clinical value in the context of APS . Apart from the higher IgA aPT levels in patients with a history an APS event, I found no significant associations. Moreover, in neither the overall cohort or in the subgroup of patients with a history of an APS event were aPT the sole positive aPL. While some studies have suggested measuring aPT might be of value in the context of APS (7, 24), several studies have shown otherwise (25-27). In a large study of primary and secondary APS, von Landenberg et al found that IgG aPT were particularly associated with early pregnancy loss (24). My findings are consistent with a recent Mexican study showing that both SLE and APS patients

with thrombosis had a higher prevalence of aPT, but no patients demonstrated aPT as the sole aPL (28).

The strong association of a history of arthritis with aβ_2GP1 is of interest, especially since it has not been reported previously. Although elevated levels aPL have been found in other inflammatory arthritides such as rheumatoid arthritis (29) and juvenile idiopathic arthritis (30), it is not known whether these antibodies have a role in the pathogenesis of inflammatory arthritis.

6 LIMITATIONS

Some of the limitations to a cross-sectional study of this nature include, firstly, that aPL levels are known to fluctuate over time (31, 32) and may be suppressed by immunosuppressive drugs. In the present study, more than three-quarters of the patients were on such agents. Second, the cross-sectional design of the study did not allow me to determine with certainty as to how many of the 19 patients with clinical features of APS met the Sapporo classification criteria for APS, according to which aPL have to be positive on at least 2 occasions 6 weeks apart.

7 CONCLUSION

My findings provide further evidence that, at least in patients of African extraction, measuring IgA aCL and all isotypes of aβ_2GP1 might be of value in risk assessment and diagnosis of APS. Prospective studies are needed to confirm these findings. The possible role of genetic factors, particularly class II HLA genes, in the expression of IgA aPL response in SLE patients of African extraction also needs to be elucidated.

8 APPENDICES

APPENDIX A

SLE-DAI SCORE

Weighted score	SLE-DAI score	Descriptor	Definition
8		Seizure	Recent onset -exclude metabolic, infectious or drug causes
8		Psychosis	Altered ability to function in normal activity due to severe disturbance in perception of reality. Includes hallucinations; incoherence; marked loose associations; impoverished thought content; marked illogical thinking; bizarre, disorganised , or catatonic behaviour - exclude uraemia or offending drugs
8		Organic brain syndrome	Altered mental function with impaired orientation or impaired memory / other intellectual function, with rapid onset and fluctuating clinical signs. Includes clouding of consciousness with a reduced capacity to focus and an inability to sustain attention on environment and at least two of the following: perceptual disturbance, incoherent speech, insomnia or daytime drowsiness, increased or decreased psychomotor activity -exclude metabolic, infectious or drug causes
8		Visual	Retinal changes from SLE: cytoid bodies, retinal haemorrhages, serous exudate or haemorrhage in the chorold, optic neuritis (not due to hypertension, drugs or infection)
8		Cranial nerve	New onset of a sensory or motor neuropathy involving a cranial nerve
8		Lupus headache	Severe persistent headache; may be migrainous; non-responsive to narcotic analge
8		CVA	New syndrome -exclude arteriosclerosis
8		Vasculitis	Ulceration, gangrene, tender finger nodules, periungal infarction, splinter haemorrhages- vasculitis confirmed by biopsy / angiography
4		Arthritis	More than 2 joints with pain and signs of inflammation (such as tenderness, swelling, or effusion)
4		Myositis	Proximal muscle aching / weakness associated with elevated CPK / aldolase levels, EMG changes, or biopsy showing myositis
4		Casts	Haem granule or red cell cast
4		Haematuria	> 5 RBC / hpf - excluding other causes (stones, infection)
4		Proteinuria	> 0.5g protein / 24 hrs. New onset or recent increase of > 0.5g / 24 hrs
4		Pyuria	> 5 leucocytes / hpf - exclude infection
2		Malar rash	New onset or recurrence of an inflammatory type of rash
2		Alopecia	New or recurrent/ A patch of abnormal, diffuse loss of hair
2		Oral / nasal ulcers	New onset or recurrence of oral or nasal ulcers
2		Pleurisy	Pleuritic chest pain with pleural rub or effusion, or pleural thickening
2		Pericarditis	Pericardial pain with at least one of the following: rub, effusion -confirmation by ECG or Echocardiography
2		Low complement	A decrease in C3 or C4 (to less than lower limit of reference range)
2		anti-dsDNA	anti-dsDNA > 1/20 titre
1		Fever	> 38 C after exclusion of infection
1		Thrombocytopenia	< 100 000 platelets
1		leucopaenia	WCC < 3 000 (not due to drugs
TOTAL SLE-DAI			Enter the weighted score for each descriptor in the SLE-DAI column if descriptor present at the time of visit or in the preceding 10 days

APPENDIX B

SYSTEMIC LUPUS INTERNATIONAL COLLABORATING CLINICS/AMERICAN COLLEGE OF RHEUMATOLOGY DAMAGE INDEX FOR SYSTEMIC LUPUS ERYTHEMATOS IS

Non reversible change, not related to active inflammation, occurring since diagnosis of lupus, ascertained by clinical assessment and present for at least 6 months unless otherwise stated. Repeat episodes must occur at least 6 months apart to score 2. The same lesion cannot be scored twice.

OCULAR SCORE (either eye, by clinical assessment)	
Any cataract ever	0/1
Retinal change or optic atrophy	0/1
NEUROPSYCHIATRIC	
Cognitive impairment (memory deficit, difficulty with spoken or written language, impaired performed level)	0/1
Major psychosis	0/1
Seizures requiring therapy for 6 months	0/1
Cerebrovascular accident level (score 2 if>1)	0/1/2
Cranial or peripheral neuropathy (excluding optic)	0/1
Transverse myelitis	0/1
RENAL	
Estimated or measured glomerular filtration rate <50%	0/1
Proteinuria >3.5mg/24 hour's	0/1
End-stage rental disease (regardless of dialysis or transplantation)	0/3
PULMONARY	
Pulmonary hypertension (right ventricular prominence, or loud P2)	0/1
Pulmonary fibrosis (physical and radiograph)	0/1
Shrinking lung (radiograph)	0/1
Pleural fibrosis (radiograph)	0/1
Pulmonary infarction (radiograph)	0/1
CARDIOVASCULAR	
Angina or coronary artery bypass	0/1
Myocardial infarction ever (score 2 if>1)	0/2
Cardiomyopathy (ventricular dysfunction)	0/1
Valvular disease (diastolic murmur or systolic murmur >3/6)	0/1
Pericarditis for 6 months or pericardiectomy	0/1

PERIPHERAL VASCULAR	
Claudication for 6 months	0/1
Minor tissue loss (pulp space)	0/1
Significant tissue loss ever (e.g. loss of digit or limb) score 2 if >1 site)	0/1/2
Venous thrombosis with swelling, ulceration, or venous stasis	0/1
GASTROINTESTINAL	
Infarction resection of bowel below duodenum, spleen, liver, or gall bladder ever, (score 2 if> 1 site)	0/1/2
Mesenteric insufficiency	0/1
Chronic peritonitis	0/1
Stricture or upper gastrointestinal tract surgery ever	0/2
MUSCULOSKELETAL	
Muscle atrophy or weakness	0/1
Deforming or erosive arthritis (including reducible deformities, excluding avascular necrosis)	0/1
Osteoporosis with fracture or vertebral collapse (excluding avascular necrosis)	0/1
Avascular necrosis (score 2 if>1)	0/1/2
Osteomyelitis	0/1
SKIN	
Scarring alopecia	0/1
Extensive scarring or panniculitis other than scalp and pulp space	0/1
Skin ulceration (excluding thrombosis) for >6 months	0/1
Premature gonadal failure	0/1
Diabetes (regardless of treatment)	0/1
Malignancy (excluding dysplasia) (score 2 if >1 site)	0/1/2

APPENDIX C

Sapporo criteria for the diagnosis of APLS (1999)

Wilson et al. Arthritis Rheum
1999;42:1309

CLINICAL	LABORATORY
Vascular thrombosis: 1 or > arterial, venous or small vessel thrombosis in any organ/tissue	Anticardiolipin antibody (aCL) IgG or IgM in moderate to high titer on 2 or more occasions at least 6 weeks apart
Pregnancy morbidity:	
•1 or > unexplained fetal deaths at or beyond 10th week	Lupus anticoagulant (LA) on 2 or more occasions at least 6 weeks apart
• 1 or > premature births of normal neonate due to preeclampsia, eclampsia or placental insufficiency	
• 3 or > unexplained spontaneous abortions before 10th week	

Ethics

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

COMMITTEE FOR RESEARCH ON HUMAN SUBJECTS (MEDICAL)
Ref: R14/49 Gould

CLEARANCE CERTIFICATE **PROTOCOL NUMBER** M01-11-21

PROJECT Antiphospholipid Antibodies In Black South Africans With Systemic Lupus Erythematosus

INVESTIGATORS Dr TJ Gould

DEPARTMENT School of Clinical Medicine, CH Baragwanath Hospital

DATE CONSIDERED 01-11-09

DECISION OF THE COMMITTEE *

Approved unconditionally

DATE 01-11-30 **CHAIRMAN** .....(Professor P E Cleaton-Jones)

* Guidelines for written "informed consent" attached where applicable.

c c Supervisor: Prof M Tikly
Dept of School of Clinical Medcine, CH Baragwanath Hospital

Works2\lain0015\HumEth97.wdb\M 01-11-21

==========

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10001, 10th Floor, Senate House, University.

I/~~we~~ fully understand the conditions under which I am/~~we are~~ authorized to carry out the abovementioned research and I/~~we~~ guarantee to ensure compliance with these conditions. Should any departure ~~to~~ be contemplated from the research procedure as approved I/~~we~~ undertake to resubmit the protocol to the Committee.

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

9 REFERENCES

1. Marai I, Zandman-Goddard G, Shoenfeld Y. The systemic nature of the antiphospholipid syndrome. Scand J Rheumatol 2004;33:365-72.

2. Wilson WA, Gharavi AE, Koike T, Lockshin MD, Branch DW, Piette JC, et al. International consensus statement on preliminary classification criteria for definite antiphospholipid syndrome: report of an international workshop. Arthritis Rheum 1999;42(7):1309-11.

3. Marai I, Tincani A, Balestrieri G, Shoenfeld Y. Anticardiolipin and anti-beta-2-glycoprotein 1 antibodies. Autoimmunity 2005;38:33-8.

4. Petri M. Clinical and management aspects of the antiphospholipid antibody syndrome. In: Wallace DJ, Hahn BH, editors. Dubois' Lupus Erythematosus. 6th ed. Philadelphia: Lea and Febiger, 2000:1093-1128

5. Bertolaccini ML, Atsumi T, Escudero Contreras A, Khamashta MA, Hughes GR. The value of IgA antiphospholipid tecting for the diagnosis of antiphospholipid (Hughes) syndrome in systemic lupus erythematosis. J Rheumatol 2001;28:2637-43.

6. Bertolaccini ML, Roch B, Amengual O, Atsumi T, Khamashta MA, Hughes GR. Multiple antiphospholipid tests do not increase the diagnostic yield in antiphospholipid syndrome. Br J Rheumatol 1998;37(11):1229-32.

7. Munoz-Rodriguez FJ, Reverter JC, Font J, Tassies D, Cervera R, Espinosa G, et al. Prevalence and clinical significance of antiprothrombin antibodies in patients with

systemic lupus erythematosus or with primary antiphospholipid syndrome. Haematologica 2000;85(6):632-7.

8. Galli M, Lucani D, Bertolini G, Barbui T. Anti-beta 2-glycoprotein !, antiprothrombin antibodies, and the risk of thrombosis in the antiphospholipid syndrome. Blood 2330;102:2717-23.

9. Mody GM, Parag KB, Nathoo BC, Pudifin DJ, Duursma J, Seedat YK. High mortality with systemic lupus erythematosus in hospitalized African blacks. Br J Rheumatol 1994;33(12):1151-3.

10. Tikly M, Burgin S, Mohanlal P, Belligan A, George J. Autoantibodies in black South Africans with systemic lupus erythematosus: spectrum and clinical associations. Clin Rheumatol 1996;15:261-5.

11. Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982;25(11):1271-7.

12. Loizou S, Singh S, Wypkema E, Asherson RA. Anticardiolipin, anti-beta(2)-glycoprotein I and antiprothrombin antibodies in black South African patients with infectious disease. Ann Rheum Dis 2003;62(11):1106-11.

13. Bombardier C, Gladman DD, Urowitz MB, Caron D, Chang CH. Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. Arthritis Rheum 1992;35(6):630-40.

14. Gladman D, Ginzler E, Goldsmith C, Fortin P, Liang M, Urowitz M, et al. The development and initial validation of the Systemic Lupus International Collaborating Clinics/American College of Rheumatology damage index for systemic lupus erythematosus. Arthritis Rheum 1996;39(3):363-9.

15. Cucurull E, Gharavi AE, Diri E, Mendez E, Kapoor D, Espinoza LR. IgA anticardiolipin and anti-beta2-glycoprotein I are the most prevalent isotypes in African American patients with systemic lupus erythematosus. Am J Med Sci 1999;318(1):55-60.

16. O'Neill AI, Ibrahim KM, Des Parkin J. Automation of the Kaolin Clotting Time. Pathology 1992;24(1):12-14.

17. Molina JF, Gutierrez-Urena S, Molina J, Uribe O, Richards S, De Ceulaer C, et al. Variability of anticardiolipin antibody isotype distribution in 3 geographic populations of patients with systemic lupus erythematosus. J Rheumatol 1997;24(2):291-6.

18. Faghiri Z, Wilson WA, Taheri F, Barton EN, Morgan OS, Gharavi AE. Antibodies to cardiolipin and beta2-glycoprotein-1 in HTLV-1-associated myelopathy/tropical spastic paraparesis. Lupus 1999;8(3):210-14.

19. Lopez LR, Santos ME, Espinoza LR, La Rosa FG. Clinical significance of immunoglobulin A versus immunoglobulins G and M anti-cardiolipin antibodies in patients with systemic lupus erythematosus. Correlation with thrombosis, thrombocytopenia, and recurrent abortion. Am J Clin Pathol 1992;98(4):449-54.

20. Cuadrado MJ, Tinahones F, Camps MT, de Ramon E, Gomez-Zumaquero JM, Mujic F, et al. Antiphospholipid, anti-beta 2-glycoprotein-I and anti-oxidized-low-density-lipoprotein antibodies in antiphospholipid syndrome. Q J Med 1998;91(9):619-26.

21. Lynch A, Byers T, Emlen W, Rynes D, Shetterly SM, Hamman RF. Association of antibodies to beta2-glycoprotein 1 with pregnancy loss and pregnancy-induced hypertension: a prospective study in low-risk pregnancy. Obstet Gynecol 1999;93(2):193-8.

22. Lee RM, Emlen W, Scott JR, Branch DW, Silver RM. Anti-beta2-glycoprotein I antibodies in women with recurrent spontaneous abortion, unexplained fetal death, and antiphospholipid syndrome. Am J Obstet Gynecol 1999;181(3):642-8.

23. Bruce IN, Clark-Soloninka CA, Spitzer KA, Gladman DD, Urowitz MB, Laskin CA. Prevalence of antibodies to beta2-glycoprotein I in systemic lupus erythematosus and their association with antiphospholipid antibody syndrome criteria: a single center study and literature review. J Rheumatol 2000;27(12):2833-7.

24. von Landenberg P, Mathias T, Zaech J, Schultz M, Lorber M, Blank M, et al. Antiprothrombin antibodies are associated with pregnancy loss in patients with the antiphospholipid syndrome. Am J Reprod Immunol 2003;49:51-56

25. Galli M, Barbui T. Antiprothrombin antibodies: detection and clinical significance in the antiphospholipid syndrome. Blood 1999;93(7):2149-57.

26. Galli M. Should we include anti-prothrombin antibodies in the screening for the antiphospholipid syndrome? J Autoimmun 2000;15(2):101-5.

27. Swadzba J, De Clerck LS, Stevens WJ, Bridts CH, van Cotthem KA, Musial J, et al. Anticardiolipin, anti-beta(2)-glycoprotein I, antiprothrombin antibodies, and lupus anticoagulant in patients with systemic lupus erythematosus with a history of thrombosis. J Rheumatol 1997;24(9):1710-5.

28. Salcido-Ochoa F, Cabiedes J, Alarcon-Segovia D, Carbral AR. Anti-prothrombin antibodies in patients with systemic lupus erythematosus or with primary antiphospholipid syndrome. J Clin Rheumatol 2002;8:251-255.

29. Cervera R, Asherson RA. Clinical and epidemiological aspects in the antiphospholipid syndrome. Immunobiology 2003;207(1):5-11.

30. Avcin T, Ambrozic A, Bozic B, Accetto M, Kveder T, Rozman B. Estimation of anticardiolipin antibodies, anti-beta2 glycoprotein I antibodies and lupus anticoagulant in a prospective longitudinal study of children with juvenile idiopathic arthritis. Clin Exp Rheumatol 2002;20(1):101-8.

31. Out HJ, de Groot PG, Hasselaar P, dan Vliet M, Derksen RH. Fluctuations of anticardiolipin antibody levels in patients with systemic lupus erythematosus: a prospective study. Ann Rheum Dis 1989;48(12):1023-8.

32. Perez-Vazquez ME, Villa AR, Drenkard C, Cabiedes J, Alarcon-Segovia D. Influence of disease duration, continued followup and further antiphospholipid testing on the frequency and classification category of antiphospholipid syndrome in a cohort of patients with systemic lupus erythematosus. J Rheumatol 1993;20(3):437-42.