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Antiphospholipid antibodies and risk of myocardial infarction and ischaemic stroke in young women

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Abstract

Background Myocardial infarction and ischaemic stroke are major clinical manifestations of the antiphospholipid syndrome, which is an autoimmune disease found mostly in young women. Although the presence of circulating antiphospholipid antibodies in individuals who have a thrombotic event is a prerequisite for the diagnosis of the antiphospholipid syndrome, the risk of myocardial infarction and ischaemic stroke associated with antiphospholipid antibodies in the general population is unclear.

Methods We used the RATIO (Risk of Arterial Thrombosis In relation to Oral contraceptives), a large multicentre population-based case-control study including women aged under 50 years who were admitted to hospital at 16 centres with first myocardial infarction or ischaemic stroke and frequency-matched controls to measure markers of the antiphospholipid syndrome. Odds ratios and corresponding 95% confidence intervals were calculated as measures of rate ratios.

Results Blood samples of 175 patients with ischaemic stroke, 203 patients with myocardial infarction, and 628 healthy controls were used in the present study. Lupus anticoagulant was found in 30 (17%) patients with ischaemic stroke, six (3%) patients with myocardial infarction, and four (0.7%) in the control group. The odds ratio for myocardial infarction was 5.3 (95% CI 1.4–20.8), which increased to 21.6 (1.9–242.0) in women who used oral contraceptives and 33.7 (6.0–189.0) in those who smoked. The odds ratio for ischaemic stroke was 43.1 (12.2–152.0), which increased to 201.0 (22.1–1828.0) in women who used oral contraceptives and 87.0 (14.5–523.0) in those who smoked. In women who had anti-β2-glycoprotein I antibodies, the risk of ischaemic stroke was 2.3 (1.4–3.7), but the risk of myocardial infarction was not increased (0.9, 0.5–1.6). Neither anticardiolipin nor antiprothrombin antibodies affected the risk of myocardial infarction or ischaemic stroke.

Conclusion Our results suggest that lupus anticoagulant is a major risk factor for arterial thrombotic events in young women, and the presence of other cardiovascular risk factors increases the risk even further.

Introduction

Cardiovascular diseases are the second most common cause of death in developed countries. Although the incidence of myocardial infarction and ischaemic stroke in individuals younger than 50 years is low, the burden of disease in young people is large. Myocardial infarction and ischaemic stroke are usually associated with several risk factors for cardiovascular disease, such as smoking, hypertension, diabetes, obesity, hyperlipidaemia, or a family history of cardiovascular disease.[1]

The antiphospholipid syndrome is an acquired risk factor for thrombotic diseases. The antiphospholipid syndrome is more prevalent in young women than in the general population.[2] It is characterised by vascular thrombosis or complications during pregnancy and a repeatedly positive test for antiphospholipid antibodies.[3] There are several subpopulations of antiphospholipid antibodies; the coagulation inhibitor lupus anticoagulant and antibodies against the phospholipid cardiolipin or the plasma proteins β2-glycoprotein I and prothrombin are the antibody subpopulations most commonly tested for.

Owing to its non-specific clinical symptoms, diagnosis of the antiphospholipid syndrome is dependent on the detection of antiphospholipid antibodies. Data on the risk of a first thrombotic event in the presence of antiphospholipid antibodies are inconclusive[4] because most studies were done in selected populations of patients. Several studies have been done to assess the thrombotic risk associated with antiphospholipid antibodies in the general population.[5–11] Although there is a general consensus from the results of these studies that the presence of antiphospholipid antibodies is independently associated with increased risk of thrombotic diseases, there is still debate on the value of measuring the concentrations of different antiphospholipid antibody subpopulations in the general population.[12]

We investigated whether the presence of specific antiphospholipid antibody subpopulations (lupus anticoagulant, anticardiolipin, or anti-β2-glycoprotein I and antiprothrombin antibodies) affects the risk of myocardial infarction or ischaemic stroke in young women. Furthermore, we studied the effect of smoking, hyperlipidaemia, diabetes, use of oral contraceptives,[13,14] the factor V G1691A mutation (factor V Leiden),[15,16] the

prothrombin G20210A mutation,[16–18] and the factor XIII 204Phe allele[19,20] on the relation between antiphospholipid antibodies and myocardial infarction and ischaemic stroke.

Methods

Study design & participants The Risk of Arterial Thrombosis In relation to Oral contraceptives (RATIO) study is a multicentre, population-based, case-control study focussed on risk factors of myocardial infarction, ischaemic stroke and peripheral arterial disease in young women. Details of the study have been published previously. [13,14,21] The current study focuses on the two acute forms of arterial thrombosis i.e., myocardial infarction and ischaemic stroke. Eligible patients were recruited under all women under 50 presenting with one these two diseases at one of the 16 participating centres. Myocardial infarction was confirmed by symptoms, elevated concentrations of cardiac enzymes, and changes seen on electrocardiograph; ischaemic stroke without an overt cardioembolic source was confirmed by medical history, physical examination, and CT or MRI scans, which were assessed by experienced neurologists at the participating centres.[13,14] Exclusion criteria were transient ischaemic attack that lasted less than 24 h, haemorrhagic stroke, cerebral sinus venous thrombosis, carotid artery dissection, history of cardiovascular or cerebrovascular disease, aphasia or cognitive impairment that prevented completion of the questionnaire, or not speaking Dutch. Controls were approached by random digit dialling and frequency-matched to the cases on age, residence area, and index date of the event. The study was approved by the local medical ethics committee of all participating hospitals. Informed consent was obtained from all patients and controls, in accordance with the declaration of Helsinki.

Measurements All patients and controls completed a standardised, structured questionnaire on several topics such as their use of oral contraceptives, smoking status, alcohol intake, weight, height, family and medical history. All antiphospholipid antibody tests were done at a central laboratory. All samples were measured masked to case or control status. Presence of lupus anticoagulant was detected with dilute Russell's viper venom time (dRVVT) reagents (LA-screen and LA-confirm; Gradipore, Australia). This assay is based on a modification of the common pathway of coagulation. Plasma samples were thawed for the first time and diluted 1:1 (vol:vol) with pooled normal plasma from 173 healthy volunteers. Coagulation was initiated by adding an equal volume of LA-screen reagent to the mixed plasma, and coagulation times were recorded. In the event of a

prolonged coagulation time (LA-screen time >99th percentile of time recorded for 40 healthy volunteers), LA-confirm assays were done. Normalised ratios for LA-screen and LA-confirm coagulation times (ratios/c) were calculated with the following equation:

$$RATIO_{S/C} = \frac{LA_S / LA_{S\,normal}}{LA_C / LA_{C\,normal}}$$

where $LA_{s\ (normal)}$ is the mean LA-screen coagulation time of 40 healthy volunteers, and $LA_{c\ (normal)}$ is the mean LA-confirm coagulation time of 40 healthy volunteers. Samples were deemed positive for lupus anticoagulant when the $ratio_{s/c}$ was 1.15 or higher, on the basis of the 99th percentile of the value recorded for 40 healthy volunteers. This measurement is in accordance with current recommendations for testing for lupus anticoagulant.[3] Human plasma-derived β2-glycoprotein I was purified as previously described.[22] β2-glycoprotein I-coated 96-well microtitre plates (Nunc MaxiSorp U96; Nunc, Wiesbaden, Germany) were washed and incubated for 1 h with plasma samples diluted 1:100 in PBST (phosphate-buffered saline [10 mM phosphate, 140 mM NaCl, pH 7.35] with 0.1% Tween-20) at ambient temperature. After washing, plates were incubated with horseradish peroxidase-labelled goat anti-human-IgG antibodies (Southern Biotech, Birmingham, AL, USA) and developed with Amplex Red reagent (Invitrogen, Paisley, UK). Fluorescence was measured

Table 1. Characteristics of RATIO participants

	Myocardial infarction N=205	Ischaemic stroke N=175	Control N=638
Age (mean)	43	39	39
Caucasian ethnicity	195 (95%)	167 (97%)	602 (94%)
History of *			
Hypertension	53 (26%)	50 (29%)	40 (6%)
Diabetes	10 (5%)	7 (4%)	10 (2%)
Hypercholesterolaemia	21 (10%)	14 (8%)	19 (3%)
Oral contraceptives use *	81 (40%)	92 (53%)	213 (33%)
Smoking *	169 (82%)	101 (58%)	270 (42%)

All data are in respect to the year of event (cases) or index date (controls). Data on ethnicity are missing for 1 ischaemic stroke case & 4 controls, on history of hypertension for 4 controls, on history of Diabetes for 1 myocardial infarction case & 3 controls, on hyperlipidaemia for 1 myocardial infarction case & 5 controls, on oral contraceptive use for 6 controls, on smoking for 6 controls and on alcohol use for 1 ischaemic stroke case & 6 controls.

in a FluoSTAR OPTIMA reader (BMG Labtech, Offenburg, Germany). PBST was used for all washing procedures. Values are reported as percentage of a positive control. Cut-off was set at the 90th, 95th, or 99th percentile of the value recorded for the control group. IgG anticardiolipin antibody concentrations were measured in plasma samples with a commercially available kit (Corgenix, Broomfield, CO, USA) in accordance with the instructions of the manufacturer. Measurements are reported as concentrations of anticardiolipin IgG (GPL). Cut-off was set at the 90^{th}, 95^{th}, or 99^{th} percentile of the concentration recorded for the control group. Concentrations of IgG antiprothrombin antibodies were measured as described previously[23] and values were reported as a percentage of the value of a positive control group. Cut-off was set at the 90^{th}, 95^{th}, or 99^{th} percentile of the control group.

Statistical analyses Rate ratios of myocardial infarction and stroke associated with antiphospholipid antibodies were estimated with odds ratios and 95% CIs with unconditional logistic regression. ORs were adjusted for the stratifying variables: age (continuous variable), residence area (four categories), and index year (six categories). Furthermore, we calculated the risk of myocardial infarction and ischaemic stroke in the presence of combinations of antiphospholipid antibody subtypes. To assess the contribution of cardiovascular risk factors to the risk of myocardial infarction and ischaemic stroke in patients with antiphospholipid antibodies, we calculated ORs to compare exposure to a cardiovascular risk factor, antiphospholipid antibodies, or both with the absence of both.

Results

Blood samples from 203 cases with myocardial infarction, 175 cases with ischaemic stroke and 628 control women were available for the current analyses. Table 1 shows the characteristics of the study population. As expected, patients with myocardial infarction or ischaemic stroke had a higher prevalence of cardiovascular risk factors, such as hypertension, diabetes, hyperlipidaemia, oral contraceptive use, and smoking, than did the controls. All oral contraceptives were combinations of an oestrogen (ethinylestradiol) and a progestin (levonorgestrel, desorgestrel, lynestrenol, or cyproterone acetate).

We analysed the effect of different antiphospholipid antibody subpopulations on the risk of myocardial infarction or ischaemic stroke after adjustment for the matching variables (table 2). Lupus anticoagulant was found in six (3%) patients with myocardial infarction and

Table 2. Antiphospholipid syndrome markers and the risk of myocardial infarction and ischaemic stroke

				Odds Ratio (95% CI)[§]	
	MI	**IS**	**Controls**	**Myocardial Infarction**	**Ischaemic stroke**
Lupus anticoagulant					
(N)	202	175	627		
$Ratio_{s/c} \geq 1.15$	6	30	4	5.3 (1.4 – 20.8)	43.1 (12.2 – 152)
$Ratio_{s/c} \geq 1.20$	5	19	2	11.7 (2.1 – 65)	48.3 (8.2 – 284)
$Ratio_{s/c} \geq 1.30$	4	10	0	NA	NA
Anticardiolipin IgG, cut-off (GPL)					
(N)	201	169	623		
≥14.6[*]	26	12	62	1.6 (0.9 – 2.6)	0.8 (0.4 – 1.7)
≥16.9[†]	16	8	31	1.8 (0.9 – 3.4)	0.9 (0.4 – 2.2)
≥24.5[‡]	2	6	6	1.5 (0.3 – 8.4)	1.9 (0.5 – 7.6)
Anti-β_2-Glycoprotein I IgG, cut-off (% of positive control)					
(N)	203	175	628		
>27.9[*]	18	39	62	0.9 (0.5 – 1.6)	2.3 (1.4 – 3.7)
>37.7[†]	11	24	31	1.2 (0.6 – 2.6)	2.8 (1.5 – 5.3)
>74.3[‡]	3	4	6	2.2 (0.5 – 10.3)	1.7 (0.4 – 7.9)
Anti-prothrombin IgG, cut-off (% of positive control)					
(N)	203	175	628		
>31.8[*]	14	21	62	0.7 (0.4 – 1.2)	1.3 (0.7 – 2.4)
>47.4[†]	7	13	31	0.8 (0.3 – 1.9)	1.8 (0.8 – 4.2)
>85.9[‡]	3	4	6	1.8 (0.4 – 7.4)	2.6 (0.6 – 11.1)

MI denotes myocardial infarction; IS denotes ischaemic stroke; CI denotes confidence interval; NA denotes not applicable; ref denotes reference category. All odds ratios are adjusted for age, residence area and index year. value corresponding to the 90th percentile of controls. † value corresponding to the 95th percentile of controls. ‡ value corresponding to the 99th percentile of controls.*

30 (17%) patients with ischaemic stroke, whereas only four (0.6%) controls had lupus anticoagulant. The OR for myocardial infarction was 5.3 (95% CI 1.4–20.8) and the OR for ischaemic stroke was 43.1 (12.2–152.0) in women with lupus anticoagulant compared with women without. 23 patients with myocardial infarction and 22 patients with ischaemic stroke received vitamin K antagonists; after these patients were excluded, the OR for myocardial infarction was 4.6 (1.1–19.5) and the OR for ischaemic stroke was 45.7 (12.4–169.0). When we used a more stringent cut-off for lupus anticoagulant ($ratio_{s/c}$ ≥1.20), the ORs for myocardial infarction and ischaemic stroke were higher. None of the control women had lupus anticoagulant with the $ratio_{s/c}$ ≥1.30 as the cut-off. Neither anticardiolipin nor antiprothrombin antibodies affected the risk of myocardial infarction or ischaemic stroke. In women with anti-β2-glycoprotein I antibodies, the risk of ischaemic stroke was 2.3 (1.4–3.7) compared with women without anti-β2-glycoprotein I antibodies; however, anti-β2-glycoprotein I antibodies did not affect the risk of myocardial infarction. The presence of lupus anticoagulant and any additional antiphospholipid antibody

Table 3. Risk of myocardial infarction or ischaemic stroke in relation to the presence of lupus anticoagulant and the presence or absence of cardiovascular risk factors

	No lupus anticoagulant					Lupus anticoagulant*				
	Distribution, N			Odds Ratio (95% CI)		Distribution, N			Odds Ratio (95% CI)	
	MI	IS	CONT	MI	IS	MI	IS	CONT	MI	IS
Oral contraceptives										
No	120	71	409	1 [ref]	1 [ref]	2	12	3	3.5 (0.5 – 22.6)	33.6 (6.8 – 167)
Yes	76	74	208	2.3 (1.6 – 3.4)	2.9 (1.8 – 4.6)	4	18	1	21.6 (1.9 – 242)	201 (22.1 – 1828)
Smoking										
No	36	57	354	1 [ref]	1 [ref]	0	13	2	NA	47.2 (8.1 – 276)
Yes	160	88	264	6.4 (4.2 – 9.7)	2.2 (1.5 – 3.4)	6	17	2	33.7 (6.0 – 189)	87 (14.5 – 523)
Factor V G1691A										
No	184	126	587	1 [ref]	1 [ref]	6	24	3	6.6 (1.5 – 29)	57 (13 – 251)
Yes	12	11	33	1.1 (0.5 – 2.2)	1.9 (0.9 – 4.2)	0	1	1	NA	11 (0.5 – 225)
Prothrombin G20210A										
No	192	139	605	1 [ref]	1 [ref]	6	27	4	5.3 (1.4 – 21)	42 (12 – 153)
Yes	4	3	15	0.7 (0.2 – 2.5)	0.7 (0.2 – 2.8)	0	1	0	NA	NA
FXIII 204Phe allele										
No	186	91	582	1 [ref]	1 [ref]	6	17	3	6.5 (1.5 – 28)	51.8 (9.9 – 270)
Yes	10	44	35	0.9 (0.4 – 1.9)	8.8 (4.9 – 5.9)	0	8	1	NA	81.4 (8.9 – 739)

MI denotes myocardial infarction; IS denotes ischaemic stroke; CI denotes confidence interval; NA denotes not applicable; ref denotes reference category. All odds ratios are adjusted for age, residence area and index year.

subpopulation did not affect the risk of myocardial infarction or ischaemic stroke, compared with the risk in patients with only lupus anticoagulant. Adjustment for hypertension, diabetes, and hyperlipidaemia did not affect the relative risks for myocardial infarction or ischaemic stroke in women with antiphospholipid antibodies.

We assessed the joint effect of additional cardiovascular risk factors in women with lupus anticoagulant compared with women without lupus anticoagulant and each cardiovascular risk factor (table 3). In women without lupus anticoagulant, the risk of myocardial infarction was 2.3 (95% CI, 1.6–3.4) in users of oral contraceptives and 6.4 (4.2–9.7) in smokers. In women with lupus anticoagulant, the OR for myocardial infarction for those who used oral contraceptives was 21.6 (1.9–242.0) and for those who smoked was 33.7 (6.0–189.0). The factor V G1691A variant, the prothrombin G20210A variant, and the factor XIII 204Phe variant did not affect the risk of myocardial infarction.

In women without lupus anticoagulant, the OR for ischaemic stroke was 2.9 (95% CI 1.8–4.6) in users of oral contraceptives, 2.2 (1.5–3.4) in smokers, and 8.8 (4.9–15.9) in women with the factor XIII 204Phe variant. In women with lupus anticoagulant, the OR for ischaemic stroke was 201 (22–1828) in users of oral contraceptives, 87 (14.5–523) in smokers, and 81 (8.9–739) in women with the factor XIII 204Phe variant. The factor V G1691A and prothrombin G20210A variants had no effect on the risk of ischaemic stroke.

Discussion

We found that lupus anticoagulants were associated with an increased risk of myocardial infarction (OR 5.3) and ischaemic stroke (OR 43.1) in women younger than 50 years. Increased concentrations of anti-β2-glycoprotein I antibodies were associated with an increased risk of ischaemic stroke (OR 2.3) but not myocardial infarction. Neither anticardiolipin nor antiprothrombin antibodies alone increased the risk of myocardial infarction or ischaemic stroke. There were no indications that the presence of more than one subpopulation of antiphospholipid antibodies affected the risk of myocardial infarction or ischaemic stroke. Additional cardiovascular risk factors, such as use of oral contraceptives, smoking, or the factor XIII 204Phe variant, increased the risk of myocardial infarction or ischaemic stroke further in women who had lupus anticoagulant. The frequency of the factor V G1691A or the prothrombin G20210A mutations was too low in women with lupus anticoagulant to ascertain whether they had an effect on the risk of myocardial infarction or ischaemic stroke.

The RATIO study compared the characteristics of a large group of women who had had myocardial infarction or ischaemic stroke with those of a large population-based control group obtained through random-digit dialling. Diagnoses and the location of all ischaemic strokes were verified with CT or MRI, which reduces the risk of misclassification. The random-digit dialling procedure and the high response rate in the control group, which was not informed about the risk factors assessed in this study, such as oral contraceptive use or smoking, minimises the risk of participation bias in the control group.

Our study has several limitations. All patients were survivors of a major arterial event who were admitted to hospital. If there is different survival rate for those with the antiphospholipid syndrome, our analyses which are limited to patients who survived potentially could lead to an over or underestimation of the true risk. This is, however, unlikely to have a major effect on our findings because ischaemic stroke and myocardial infarction are rarely fatal in young women.[24,25] Furthermore, we cannot completely exclude recall bias, although structured questionnaires were used and colour photographs of contraceptive preparations were provided to help women recall the oral contraceptive they had used. Data on hypertension, diabetes, and hyperlipidaemia at the time of the event were collected with standardised questionnaires completed several years later.

Correct handling of samples is important for the detection of lupus anticoagulant. Because plasma samples were obtained after only one centrifugation step, we cannot exclude the possibility that residual platelets were present in the sample. The handling of blood samples was standardised to exclude any possible effects of differences in sample handling between cases and controls. Another potential source of bias is anticoagulant treatment with vitamin K antagonists, which might interfere with the detection of lupus anticoagulant. Because lupus anticoagulants are phospholipid-dependent coagulation inhibitors, only longer than normal coagulation times that are corrected by the addition of excess phospholipids qualify as positive for lupus anticoagulant. Although all samples were mixed with normal pooled plasma to correct for possible deficiencies in coagulation factors, an international normalised ratio greater than 3·0 might not be corrected by phospholipids and might cause false-negative values for lupus anticoagulant rather than false-positive values. To exclude any effect of the use of vitamin K antagonists on the risk of myocardial infarction or ischaemic stroke, we repeated the analyses

without the results from the patients treated with anticoagulants. This resulted in a slight decrease in the risk of myocardial infarction, but the risk of ischaemic stroke was not affected. We do not have data on vitamin K antagonist use in the control population; however, because the controls were women without a history of cardiovascular disease, it is highly unlikely that any of these women were on anticoagulant drugs. All blood samples were obtained several years after the thrombotic event. We can therefore only assume that the presence of antiphospholipid antibodies is associated with the event. Other researchers have shown

the presence of antiphospholipid antibodies within hours after an arterial thrombotic event,[26] although the development of antibodies generally takes days. Therefore, our data indicate a causal relation between antiphospholipid antibodies and thrombosis. Because only one blood sample was taken for each patient, we cannot exclude the possibility that a proportion of the anticardiolipin antibodies are transient infection-related antibodies.

Two other studies compared the effects of antiphospholipid antibodies on the risk of ischaemic stroke[5] or myocardial infarction[6] in an unselected population of young women. In line with our results, each study reported an increased risk of an arterial thrombotic event associated with antiphospholipid antibodies, although the risks were attributed to different antiphospholipid antibody subpopulations. One of these studies[5] reported a two-fold increase in the risk of ischaemic stroke in the presence of lupus anticoagulant or anticardiolipin antibodies, whereas the presence of anticardiolipin antibodies was not associated with an increased risk of ischaemic stroke in our study. High titres of anticardiolipin antibodies (>40 GPL) were not detected in our study. Inclusion of patients with unconfirmed or cardioembolic strokes in this study might explain the low risk of stroke associated with lupus anticoagulant in this particular study. Another explanation is that the less specific aPTT-based lupus anticoagulant assay was used; this assay depends on the intrinsic pathway of coagulation and is therefore more sensitive for phospholipid independent coagulation inhibitors than is the dRVVT.[27]

One study in young women[6] reported a two-fold increased risk of myocardial infarction if anti-β2-glycoprotein I antibodies, but not anticardiolipin antibodies, were detected. The presence of anti-β2-glycoprotein I antibodies did not increase the risk of myocardial infarction in our study.

The lupus anticoagulant subpopulation of antiphospholipid antibodies is a heterogeneous pool of antibodies that have several antigenic targets. Insight into the mechanism behind the prothrombotic effects of antiphospholipid antibodies has increased steadily over the years. Antiphospholipid antibodies, particularly those with lupus anticoagulant activity, cause platelet activation and lead to a procoagulant endothelial phenotype.[28] Prothrombotic effects on the coagulation system, such as antiphospholipid antibody-related acquired activated protein C resistance,[29] are also seen in patients with the antiphospholipid syndrome. All of these prothrombotic effects of antiphospholipid antibodies might be responsible for the increased risk of myocardial infarction and ischaemic stroke described in this study. In view of the effects of antiphospholipid antibodies on the haemostatic system, it is not surprising that smoking, which causes endothelial dysfunction,[30] and oral contraceptive use, which affects the anticoagulant protein C axis,[31] increase the effect of lupus anticoagulants on the risk of ischaemic stroke or myocardial infarction. The reason why the effect of lupus anticoagulant on ischaemic stroke is more pronounced than its effect on myocardial infarction remains to be established, but might reflect a true difference in the aetiology of the two diseases. Although antiplatelet drugs are recommended over oral anticoagulants as secondary thromboprophylaxis for non-cardioembolic ischaemic stroke, the use of oral anticoagulants in patients with antiphospholipid syndrome, rather than treatment with antiplatelet drugs, is preferred by some experts.[32,33] Therefore, screening for lupus anticoagulant in young women with ischaemic stroke might be warranted.

Conclusions From the tested markers of the antiphospholipid syndrome, LAC proved to evoke the largest increase in risk for both myocardial infarction and ischaemic stroke. However, the was a striking difference between these two diseases in the magnitude of the effect. This, together with the observation that anti-β2-glycoprotein I antibodies were associated with ischaemic stroke but not with myocardial infarction, shows that the role of the antiphospholipid syndrome differs between these two diseases.

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