Low frequency of elevated prothrombin times in patients with lupus anticoagulants when using a recombinant thromboplastin reagent: implications for dosing and monitoring of oral anticoagulant therapy

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Accepted: 26 November 2004

ABSTRACT

Many patients with lupus anticoagulants (LA) are treated with oral anticoagulation and monitored using the international normalised ratio (INR) derived from the prothrombin time (PT). Recent reports have produced conflicting conclusions about the extent to which LA interferes with PT determination. The degree of anticoagulation may be overestimated in a patient whose LA affects the PT. A number of reports conclude that specific thromboplastin reagents containing recombinant tissue factor are sensitive to the presence of LAs and should not be used to monitor oral anticoagulant therapy in these patients. These studies were performed on orally anticoagulated patients. The present retrospective study on 400 patients with LAs who were not receiving therapeutic anticoagulation was performed to ascertain the frequency of prolonged PT in these patients when using Innovin recombinant thromboplastin. Only 17 (4.3%) out of 400 had prolonged PT in the presence of LA. As this is a low prevalence, and not all patients with LAs will require anticoagulant therapy, it is concluded that baseline INR determination should be used to highlight the need to monitor individual patients with LA-insensitive reagents. As the use of moderate-intensity oral anticoagulation for patients with LAs and previous thrombosis is receiving wider acceptance, an informed approach to anticoagulant monitoring will reduce the possibility of under-anticoagulating patients receiving this therapy.

KEY WORDS: Lupus anticoagulant.
Oral anticoagulant therapy.
Prothrombin time.
Recombinant thromboplastin.

Introduction

Lupus anticoagulants (LA) are autoimmune antiphospholipid antibodies that interfere with phospholipid-dependent coagulation reactions *in vitro*.[1] The reported prevalence of prothrombin time (PT) affected by LA varies between studies[2–4] and is largely attributed to reagent variability. The apparent variation in sensitivity to LA between thromboplastin reagents is considered to be due mainly to differences in phospholipid composition,[5,6] although antibody heterogeneity also contributes.

Many patients with LAs are treated with oral anticoagulation to prevent recurrence of thrombosis, which is monitored by periodic determination of the PT and subsequent generation of the international normalised ratio (INR). An LA antibody that prolongs the PT performed with a given thromboplastin will result in overestimation of the degree of anticoagulation.

It has been reported that Innovin thromboplastin, prepared from recombinant tissue factor and synthetic phospholipids, is sensitive to the presence of LAs.[5] Indeed, use of Innovin in the dilute PT has been shown to be more sensitive than brain-derived thromboplastins as a screening test for LAs.[6]

Here, a retrospective study is presented that evaluates the frequency of elevated PT using Innovin thromboplastin in LA-positive patients in a large cohort prior to oral anticoagulant therapy. If a patient's LA is known to interfere with the routine thromboplastin reagent prior to induction of anticoagulation, informed clinical decisions can be made about monitoring their oral anticoagulant therapy.

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Materials and methods

Blood collection, manipulation and storage

Blood was collected into a one-tenth volume of 0.105 mol/L trisodium citrate and double centrifuged to obtain plasma with a platelet count of less than 10 x 10^9/L, as described previously.[8] The platelet-poor plasma for LA testing was stored at –70°C for no longer than two months and thawed at 37°C for five minutes prior to analysis. Plasma for PT was analysed fresh, immediately after centrifugation.

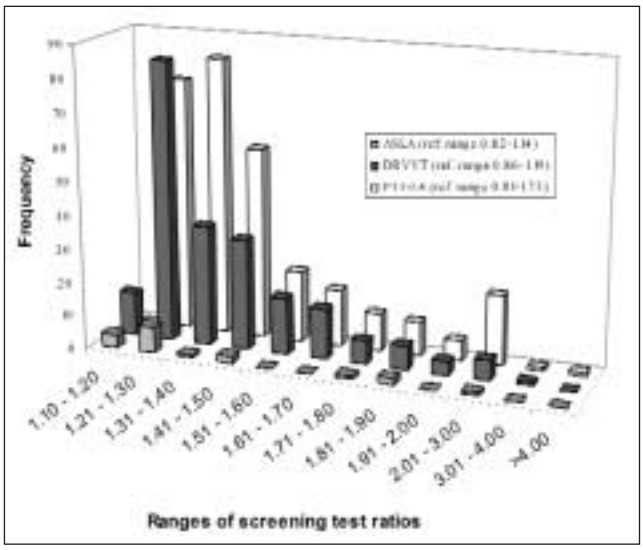

Fig. 1. Ranges and frequencies of elevated ASLA, DRVVT and PTT-LA screening test ratios in lupus anticoagulant-positive patients with normal INR results.

Coagulation tests

Prothrombin times expressed as INRs were performed on a Sysmex CA1500 (Sysmex UK, Milton Keynes, UK) using Innovin thromboplastin (Dade, Marburg, Germany). Local calibration of the international sensitivity index gave an identical value to that provided by the manufacturer. Lupus anticoagulants were identified by dilute Russell's viper venom time (DRVVT),[8] using Gradipore LA Screen and Confirm (bioMérieux UK, Basingstoke, UK) and an LA-sensitive activated partial thromboplastin time (APTT) reagent, PTT-LA (Diagnostica Stago, Asnières, France) using Platelet Extract Reagent (Alpha Laboratories, Hampshire, UK) as a platelet neutralisation procedure (PNP) in the confirmatory step.

Samples negative for LA by DRVVT and APTT were analysed subsequently by the activated seven lupus anticoagulant (ASLA) assay,[9] using NovoSeven recombinant factor VIIa (Novo Nordisk A/S, Bagsvaerd, Denmark), Bell and Alton phospholipid (Diagnostic Reagents, Thame, UK) diluted 1 in 8 in 0.05 mol/L imidazole buffer (pH 7.2) in the screening test, and the platelet extract reagent in the confirmatory test. All LA assays were performed on the Sysmex CA1500. Platelet-poor plasma (Technoclone, Dorking, UK) was used as the normal plasma control for DRVVT, PTT-LA and ASLA, as it is prepared to be sufficiently platelet poor for use in LA detection assays. Equal-volume mixing tests (screening and confirmatory) were performed on all samples that produced elevated screening results in neat plasma.[10,11]

Interpretation criteria

Screen and confirm results (DRVVT, PTT-LA and ASLA) were converted to ratios by dividing the clotting time of the test by that of the normal control. Interpretation of the data for the presence of LAs was made by calculating the percentage correction of the screening test ratio by the confirmatory test ratio.

Test plasmas were defined as consistent with the presence of an LA if the screening test ratio was greater than the upper limit of normal, and this was corrected by ≥ 10%[8–10] provided that other causes of prolonged clotting time were excluded.[10,11] Reference ranges calculated as ± two standard deviations (SD) of the mean were previously derived locally[10,12] from 40 normal donors with normal clotting screens and no evidence of haemostatic disease.

Patient samples

Results from 400 patients with a thrombotic history who were referred for thrombophilia screening and shown to have LAs were evaluated retrospectively for the presence of elevated PT. None were coincidental findings of LA in asymptomatic patients. Results from patients receiving oral anticoagulation were excluded. Elevated INRs encountered in non-anticoagulated patients subsequently received a PT equal-volume mixing test with normal plasma to assess whether the abnormality could be due to an inhibitor or a factor deficiency.

Results

The relative numbers of samples that produced normal or elevated INRs and were positive for LA with each LA assay are shown in Table 1. Seventeen (4.2%) out of 400 had elevated INRs in the range 1.12–2.28 (mean: 1.29; median 1.21; locally derived reference range: 0.90–1.10). The INR and LA screening test ratios for these patients are shown in Table 2. There was no correlation between INR and DRVVT ratio and between INR and PTT-LA ratio.

The frequencies of degrees of elevation of LA screening test results in patients with normal INR results are detailed in Figure 1. Mean/median LA screening test ratios in these patients for PTT-LA, DRVVT and ASLA were 1.52/1.39, 1.42/1.33 and 1.38/1.26, respectively.

Discussion

It is inevitable that some LAs will interfere with PT determination. Studies addressing the effect of LAs on INR values in orally anticoagulated patients have drawn different conclusions about the extent to which LAs affect PT.[2–6, 13–16] Some studies conclude that specific recombinant thromboplastins are affected more than other reagents by LAs,[3,5,6,13,16] with Innovin being cited as LA-sensitive in two of them.[5,16] These studies evaluated results obtained with multiple thromboplastins but on relatively small numbers of patients.

The data presented here, on a large cohort of non-anticoagulated LA-positive patients, demonstrates that only a small percentage of LA-positive patients have antibodies that interfere with Innovin-derived INRs. Robert *et al*.[5] found that only a subset of their orally anticoagulated LA-positive population generated INRs with Innovin that diverged from those obtained with other reagents. From their experiments with monoclonal antibodies, Arnout and Vermylen have suggested that this may be due to the presence of LA that is exclusively β_2-glycoprotein I-dependent.[16]

As this was a retrospective study, it proved impossible to perform factor assays on the 17 samples with elevated INRs. It is routine practice in the authors' laboratory to perform equal-volume mixing studies on such results, and only samples that indicated inhibition were included in the study.

Table 1. Frequencies of normal and elevated prothrombin times in 400 patients positive for lupus anticoagulant by DRVVT, PTT-LA and ASLA testing.

Test(s) positive for LA	Number with normal INR (%)		Number with elevated INR (%)	
DRVVT only	104	26.0	4	1.0
PTT-LA only	144	36.0	4	1.0
DRVTT and PTT-LA	115	28.8	8	2.0
ASLA only	20	5.0	1	0.2
Totals	383	95.8	17	4.2

All 17 samples subsequently demonstrated phospholipid-dependent inhibition in the LA assays.

While it is theoretically possible that some of the elevated INRs were due to the immune-mediated hypoprothrombinaemia that can be encountered in patients with LA,[11] rather than direct interference by the LA, it is unlikely as this is a rare phenomenon and none of the patients presented with bleeding symptoms. Equally, some of the patients could have had co-existing non-phospholipid-dependent antibodies that prolonged the INR. If this was the case, however, an elevated baseline INR would still be relevant to patient management if oral anticoagulation was required.

It is possible that the 17 patients identified here would have generated normal INR results with alternative thromboplastins. However, the aim of the present study was to assess prevalence of elevated INR results with Innovin thromboplastin, as it has previously been identified as sensitive to LAs. Although INR calculation was introduced originally to monitor oral anticoagulation, some laboratories and studies now adopt it as a method of reporting PT results for diagnostic purposes, and it is used as such in the authors' department.[17–20]

There was a wide spread of degrees of prolongation in LA screening test ratios in patients with and without elevated INRs. This indicates that it was not just the more potent antibodies that caused elevated INRs but that they were also due to other properties such as common antibody specificity.

It has been practice in some institutions to anticoagulate patients with LAs to a target INR range of 3.0–4.0, based, at least in part, on the rationale that the INR can underestimate the degree of anticoagulation when LA is present,[21] although this may raise the risk of bleeding. The optimal therapy for patients with LAs and previous thrombosis is controversial.[21–24] However, recent prospective clinical data support the view that high-intensity warfarin (INR: 3.0–4.0) is not superior to moderate-intensity warfarin (INR: 2.0–3.0) for thromboprophylaxis in patients with antiphospholipid antibodies and previous thrombosis.[25]

In view of the low percentage of cases of LA affecting Innovin-derived INRs and the fact that Innovin is the most popular thromboplastin reagent in the UK,[26] a pragmatic anticoagulant monitoring approach that does not alter routine PT analysis for all patients would seem appropriate, bearing in mind that not all patients with LAs require anticoagulant therapy. If a patient's LA is known to interfere with a particular thromboplastin reagent prior to induction of anticoagulation, they can be monitored with a suitable LA-insensitive thromboplastin for the duration of oral anticoagulant therapy.

Table 2. Lupus anticoagulant screening test results in 17 lupus anticoagulant-positive non-anticoagulated patients with elevated INR results.

Patient	INR (0.90–1.10)	PTT-LA ratio (0.81–1.23)	DRVVT ratio (0.86–1.19)	ASLA ratio (0.82–1.14)
FM	1.12	1.30	1.25	ND
PY	1.14	3.96	2.81	ND
MP	1.15	N	1.36	ND
FM	1.15	1.76	1.57	ND
PC	1.16	1.54	N	ND
EP	1.17	3.47	2.75	ND
CS	1.18	N	1.32	ND
EP	1.18	1.30	N	ND
JG	1.21	1.72	1.27	ND
AA	1.23	1.58	N	ND
JJ	1.24	N	N	2.58
EJ	1.24	1.29	1.63	ND
AA	1.27	2.56	2.10	ND
AK	1.33	N	1.22	ND
JJ	1.34	5.83	3.16	ND
BO	1.53	1.34	N	ND
BS	2.28	N	1.38	ND

Reference range for each assay in parentheses.
N: result within reference range
ND: not done

Choice of an appropriate reagent for monitoring oral anticoagulant therapy has the potential to improve the safety of moderate-intensity anticoagulation by reducing the likelihood of overestimation. This is the first study to assess INR results in a non-anticoagulated LA-positive population and provides formal evidence of the prevalence of abnormal PT results in these patients.

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