Technical methods

Simplified screening procedure for detecting lupus inhibitor

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The lupus inhibitor is an autoantibody with anticoagulant properties, and is found in a high proportion of patients with systemic lupus erythematosus (SLE) as well as in patients with a wide variety of other disorders.¹⁻³ It is characterised by prolongation of phospholipid dependent coagulation tests due to its binding to phosphodiester linkages on the phospholipid portion of the factor Xa, factor Va, phospholipid calcium complex (prothrombinase).¹ The clinical importance of the lupus inhibitor lies in its strong correlation with recurrent venous and arterial thrombosis and fetal loss due to recurrent abortion.⁴ In contrast, it is not associated with abnormal bleeding unless a coincident haemostatic defect such as thrombocytopenia, abnormal platelet function, or hypofibrinogenaemia is present.

Historically, a lupus inhibitor was suspected when the activated partial thromboplastin (APTT) was prolonged and the prolongation was not corrected by mixtures of patient and normal plasma.⁵ More sensitive assays were, however, necessary following the demonstration that a small percentage of patients with lupus inhibitor had a normal APTT (or partial thromboplastin time with kaolin, PTTK).^{3,6,7} An example of such a test is the assay developed by Exner *et al*⁶ in which the kaolin clotting time using mixtures of pooled normal and test platelet poor plasma has proved a sensitive system for detecting lupus inhibitors. Like other sensitive tests, however, it is relatively time consuming and, as originally described, the distinction between samples positive and negative for lupus inhibitor depended on interpretation of a graph and was not precisely defined.

In view of the increasing number of requests for the lupus inhibitor test this survey was undertaken to re-evaluate the diagnosis of the lupus inhibitor in our laboratory. Our aim was to determine the rate of a positive test, the number of positive tests associated with prolongation of the APTT or PTTK, or both, and whether an abbreviated and sensitive screening procedure could be devised so that the laboratory work load could be reduced.

Material and methods

All samples referred for investigation over a period of 12 months (April 1986 to March 1987) were included in this survey. There were four main clinical indications for the lupus inhibitor test: suspected or diagnosed SLE or other connective tissue disorders; recurrent abortions; recurrent thromboses; and investigation of a prolonged APTT or PTTK. Blood was collected into sodium citrate anticoagulant and centrifuged at 1900 g for 10 minutes to produce platelet poor plasma which was then filtered through a 0.2 µm filter (Millipore). If the lupus inhibitor test was not performed immediately after filtering the sample was frozen at -20°C. Previous studies with paired samples had shown no loss of inhibitor activity following a single freezing and thawing. Samples from patients receiving anticoagulants were not included.

CLOTTING TESTS

APTT

This was performed in duplicate on a Coag-a-Mate 2001 (General Diagnostics, New Jersey, USA) using the supplied platelet factor reagent (rabbit brain phospholipids) and micronised silica as the particulate activator according to the manufacturer's recommendations. The normal range is 20-32 seconds.

PTTK

Platelet poor plasma (200 µl) was added to 100 µl platelet substitute (Diagnostic Reagents Ltd, Thame, Oxfordshire) as a source of phospholipid and 100 µl kaolin (20 mg/ml), and incubated at 37°C for three minutes. Prewarmed calcium chloride (200 µl) 0.025M was then added and the clotting time recorded. The normal range in our laboratory is 30-42 seconds.

Lupus inhibitor test

The kaolin clotting time was performed on mixtures of normal and test platelet poor plasma as previously described.⁶ Platelet poor plasma (200 µl) was incubated with 100 µl kaolin (20 mg/ml) at 37°C for three minutes. After addition of 200 µl 0.025M calcium chloride the clotting time was recorded. The ratios of pooled normal and test platelet poor plasma were 10:0, 9:1, 8:2, 5:5, 2:8 and 0:10. The results were graphed with kaolin clotting time on the ordinate and percentage mixtures on the abscissa (fig 1). A test was considered to be positive when small quantities of test plasma led to prolongation of kaolin clotting time and when the mixing curve was convex in the region near the left axis.

Technical methods

Fig 1 Representative lupus inhibitor mixing tests. Graphs (a) and (c) from positive patients: Δ kaolin clotting time 66 and 20 seconds, respectively. Graph (b) from heparinised patient: Δ kaolin clotting time eight seconds. Graph (d) normal: Δ kaolin clotting time five seconds.

Results

INCIDENCE OF A POSITIVE LUPUS INHIBITOR TEST
Using the criteria defined by Exner *et al*, a positive lupus inhibitor was diagnosed in 40 of the 386 (10.0%) samples tested during the survey period. Typical positive and negative curves are shown in fig 1. The positive specimens were received from 33 patients who were all subsequently confirmed not to be receiving anticoagulants. A further nine samples (2.0%) were considered to be equivocal and retesting was requested. Of eight re-evaluated, two were confirmed to be positive and six negative.

INCIDENCE OF PROLONGED APTT AND PTTK IN POSITIVE PATIENTS

Thirty eight (95%) of those patients definitely considered to have a positive lupus inhibitor test had a prolonged PTTK, 33 (82.0%) had a prolonged APTT. In contrast, of the negative patients subsequently confirmed not to be receiving anticoagulants, 94 (29%) and 76 (23%) had prolonged PTTKs and APTTs, respectively.

Fig 2 Δ kaolin clotting time for patients negative and positive for lupus inhibitor. H = patient receiving heparin. E = equivocal lupus inhibitor mixing test.

DEVELOPMENT OF A LUPUS INHIBITOR SCREENING TEST

From the above data and as has been pointed out by other authors,³ it is obvious that a prolonged PTTK or APTT is not sufficiently sensitive to detect all positive patients and may also lead to erroneous diagnoses in others. We therefore evaluated an abbreviated lupus inhibitor screening test using only two points of the kaolin clotting time/platelet poor plasma mixture curve. The difference in seconds between the 8:2 point and the 10:0 point (Δ kaolin clotting time) was determined and plotted for samples positive and negative for lupus inhibitor. Fig 2 shows the 8:2–10:0 difference in all positive samples was 14 seconds or greater (mean SD Δ kaolin clotting time = 64.4 (54.9), range 14–270); only seven of the 336 negative or equivocal patients had a difference of greater than 14 seconds (mean (SD) Δ kaolin clotting time = 27.1 (14.7), range 14–56). Of these seven, three were

receiving full dose heparin (Δ kaolin clotting time; 56, 29, and 19 seconds) and four had been considered to have equivocal lupus inhibitor tests. On testing of subsequent samples from three of the equivocal patients, one with a Δ kaolin clotting time of 29 seconds was found to have a definite lupus inhibitor while the two others (19 and 14 seconds) were negative. The Δ kaolin clotting time in the two positive patients with normal PTTs was 14 and 34 seconds and in the positive patients with normal APTTs the Δ kaolin clotting time range was 14–150 seconds with a mean (SD) of 24.6 (21.6) seconds.

Discussion

This study was prompted by the increasing number of requests received by our laboratory for the lupus inhibitor test and the consequent drain on laboratory staff resources. Our policy had been to perform a full lupus inhibitor test on all requests, provided the patient was not receiving therapeutic anticoagulation. In our one year study this led to a lupus positivity rate of 10.4%. As already mentioned, if less sensitive assays such as the PTTK or APTT were used as screening tests a small but significant number (5 and 20%, respectively) of patients positive for lupus inhibitor would be missed.

In our attempts to develop a simplified yet sensitive screening test for the lupus inhibitor the Δ kaolin clotting time between the 8:2 and 10:0 points was evaluated. This is the most sensitive area of the curve for lupus inhibitor detection and least affected by heparin. If this assay alone had been used we would have detected all true lupus inhibitor positive samples (that is, no false negatives). In addition, however, there would have been seven "false" positive results in the 386 samples. Based on the results of this study we

intend to introduce the Δ kaolin clotting time as an initial screen. If the result is less than 14 seconds the sample is considered to be negative and if greater than or equal to 14 seconds a full test is performed. The full test would help exclude false positive results due to heparin and distinguish equivocal results which should be repeated. In addition, the use of the Δ kaolin clotting time will provide a rough quantitation for sequential studies and considerably reduce laboratory work load.

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Immunoturbidimetric assay for rheumatoid factor using an enzyme immunoassay microplate reader

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Several methods for detection of rheumatoid factors in serum are currently commercially available.^{1–3} The earliest assays developed, and those still most widely used, rely on the agglutination of latex particles coated with immunoglobulin G when exposed to sera con-

taining rheumatoid factors.¹ The assay described in this paper is turbidimetric, originally developed for use of chemical analysers (such as Cobas Bio, Kone CD),⁴ and modified for use with a microplate enzyme immunoassay reader.

Material and methods

Samples were received from patients being investigated for various arthritides. Fifty samples were tested and were shown by the Cobas assay to have rheumatoid factor activity between 50 and 500 IU/ml.

TURBIDIMETRIC ASSAY

The rheumatoid factor kit (Orion Diagnostica, Espoo, Finland) was supplied with all the reagents required to carry out the assay, including reference serum standar-