Lupus anticoagulant, anticardiolipin antibodies, and human immunodeficiency virus in haemophilia

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SUMMARY The prevalence of lupus anticoagulant, using the dilute Russell’s viper venom time (DRVT), was determined in 22 patients with mild to severe haemophilia A to see if there was any association with the presence of viral disease. Twelve haemophiliacs (58%) were lupus anticoagulant positive, with a mean patient:control ratio of 1.24 (range 1.15–1.52, normal range 0.84–1.06 which partially corrected with lysed, washed platelets). Nine of these patients were IgG or IgM, or both, anticardiolipin antibody positive and nine were human immunodeficiency virus (HIV) antibody positive, but associations between lupus anticoagulant, anticardiolipin antibodies, and HIV antibody positivity were not significant. Mixing studies of normal plasma and immune depleted factor VIII deficient plasma showed that the DRVT ratio increased when the factor VIII concentration fell below 0.15 IU/ml. There was no significant association between plasma factor VIII concentration and positive DRVT results in haemophiliacs. The addition of porcine factor VIII concentrate produced no correction in eight of the 12 with DRVTs indicative of lupus anticoagulant, suggesting that these were prolonged by antiphospholipid activity.

It is concluded that the presence of lupus anticoagulant and anticardiolipin antibodies in haemophiliacs may represent an antiphospholipid response to viral or challenge, not only to HIV but also to other viral antigens, and that a very low factor VIII concentration may produce a false positive DRVT result.

The lupus anticoagulant prolongs the clotting times of phospholipid dependent in vitro coagulation tests such as the activated partial thromboplastin time (APTT) and prothrombin time.1 This effect is due to the presence in plasma of variable populations of immunoglobulins, usually IgG or IgM, or both, which bind to and neutralise the negatively charged coagulant active phospholipids, such as phosphatidyl serine. The lupus anticoagulant often occurs in the plasma of patients with systemic lupus erythematosus (SLE) or other autoimmune disorders, but has also been detected in other diseases, as well as in apparently healthy subjects.

The presence of lupus anticoagulant is associated with an increased incidence of arterial and venous thrombosis, thrombocytopenia, and recurrent fetal loss.2 Patients with lupus anticoagulant often have antibodies against other phospholipids such as cardiolipin. Lupus anticoagulant and anticardiolipin antibodies have recently been reported in patients in various clinical phases of human immunodeficiency virus (HIV) infection.3–5 Anticardiolipin antibodies have also been observed in association with other infections including infectious mononucleosis,6 Pneumocystis carinii infection,7 syphilis and acute malaria.4

Haemophiliacs are a high risk group for virally transmitted diseases, including HIV infection, and may therefore be expected to show evidence of lupus anticoagulant or anticardiolipin antibodies. In the United Kingdom, about 32% of haemophiliacs tested (65% of the total registered) are HIV antibody positive, a small percentage are hepatitis B surface antigen (HBsAg) positive, and most are hepatitis B surface antibody (HBsAb) positive, and more than 90% have biochemical evidence of previous non-A, non-B infection. It is therapeutically important to distinguish between lupus anticoagulant and specific factor VIII inhibitors, which occur in about 10–15% of haemophiliacs, yet the APTT with a mixing test is usually used to screen for both activities. This study was undertaken using a more specific test for lupus anticoagulant.
anticoagulant, the dilute Russell's viper venom test (DRVT), to determine the prevalence of lupus anticoagulant in haemophiliacs and to examine correlations between lupus anticoagulant, anti-cardiolipin antibodies, and HIV seropositivity in haemophiliacs.

**Patients and methods**

Twenty and methods Patients haemophiliacs. haemophilia centrate positive. was negative. factor also patients serology, between terms of antibody 12 on liver disease non-B viral of liver hepatitis. Patients had normal hepatitis B virus serology, one patient was hepatitis B e antigen positive, 12 were hepatitis B surface antigen negative, but antibody positive, and in eight serology for hepatitis B was negative. Ten of the 12 HIV antibody positive patients also had positive hepatitis B serology. In terms of raised transaminase activities, most of the patients, on close scrutiny, showed evidence of non-A, non-B viral hepatitis. There was no clinical evidence of manifest liver disease in any of the patients, but no formal assessment of liver morphology by histological examination of liver biopsy specimens was undertaken. All patients had normal prothrombin times and fibrinogen concentrations, and none had detectable factor VIII inhibitor titres, as measured by the Bethesda assay.

Venous blood was taken into 0·106 M trisodium citrate, one part anticoagulant to nine parts blood, or into plain glass tubes, centrifuged at 2000 g for 15 minutes to obtain platelet poor plasma or serum and stored in plastic at −20°C until assay.

**DILUTE RUSSELL’S VIPER VENOM TIME (DRVT)**

The DRVT was performed with the following modifications: Russell's viper venom (Diagnostic Reagents Ltd, Thame, Oxfordshire, or Sigma Chemical Co, Poole, Dorset) and “Bell and Alton” platelet substitute (Diagnostic Reagents Ltd), were used, and optimal concentrations of the phospholipid were determined for each batch. A platelet neutralisation procedure was also performed, using an optimal concentration of washed normal platelets lysed by repeated freezing and thawing instead of phospholipid reagent. Pooled normal plasma was tested with each batch of samples as a control and results were calculated as a ratio of patient to control clotting time. The normal range was established in 20 healthy laboratory staff and expressed as the mean ratio ± 2 SD. Reproducibility tests showed a correlation coefficient (CV) of < 5%.

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Patients were defined as lupus anticoagulant positive if the DRVT ratio with phospholipid reagent was greater than 1·06 but decreased when lysed washed platelets were substituted.

**INFLUENCE OF THE FACTOR VIII CONCENTRATION**

The DRVT with the platelet neutralisation procedure was performed on varying proportion mixtures of normal plasma and immune depleted factor VIII deficient plasma (Diagnostic Reagents Ltd). The factor VIII activity of the normal plasma was determined by a one-stage clotting assay standardised with the British Standard 86/546 (NIBSC, South Mimms, Potters Bar, Hertfordshire).

The DRVT with the platelet neutralisation procedure was repeated in all haemophiliacs as well as in normal controls, and several patients with systemic lupus erythematosus known to have lupus anticoagulant, following the addition of polyelectrolyte purified porcine factor VIII concentrate (Hyate:C, Speywood Laboratories, United Kingdom) to correct the factor VIII concentration to greater than 1·00 IU/ml.

**ANTICARDIOLIPIN ANTIBODIES**

Anticardiolipin assays were performed by an enzyme linked immunosorbsent assay (ELISA) using 10% adult bovine serum (Sigma Chemical Co) as a blocking agent and sample diluent. The assay was standardised using reference sera (The Lupus Research Laboratory, Rayne Institute, St Thomas’s Hospital, London), calibrated by an International Workshop. Results were expressed as IgG antiphospholipid (GPL) and IgM antiphospholipid (MPL) units, where 1 GPL unit was the binding activity of 1 µg/ml IgG anticardiolipin antibody and 1 MPL was the activity of 1 µg/ml IgM anticardiolipin antibody, affinity purified from reference sera. Normal values were taken as < 5 GPL and < 3 MPL which also corresponded to the mean ± 2 SD of 25 normal subjects studied locally.

Associations between lupus anticoagulant, IgG or IgM anticardiolipin antibodies, or both, and HIV seropositivity were examined using Fisher's exact probability test. The DRVT results using phospholipid or lysed washed platelets were compared using a paired t test.

**Results**

Ten of the haemophiliacs were lupus anticoagulant negative, having either normal DRVT ratios, or high ratios which showed no correction at all with lysed washed platelets. The other 12 were lupus anticoagulant positive (table 1) with a mean ratio of 1·24 (range 1·15–1·52), which partially corrected with lysed washed platelets (mean 1·14, p < 0·0001). Nine
Association between viral disease and positivity for lupus anticoagulant and antiphospholipid antibodies

Table 1  Lupus anticoagulant positive haemophiliacs (n = 12): DRVT ratios and HIV status

<table>
<thead>
<tr>
<th>DRVT (PL)</th>
<th>DRVT (LWP)</th>
<th>DRVT (PL-VIII)</th>
<th>HIV antibody positivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.17</td>
<td>1.06</td>
<td>0.86</td>
<td>Yes</td>
</tr>
<tr>
<td>1.20</td>
<td>1.06</td>
<td>0.89</td>
<td></td>
</tr>
<tr>
<td>1.21</td>
<td>1.09</td>
<td>1.18</td>
<td></td>
</tr>
<tr>
<td>1.15</td>
<td>1.09</td>
<td>1.06</td>
<td></td>
</tr>
<tr>
<td>1.52</td>
<td>1.44</td>
<td>1.32</td>
<td></td>
</tr>
<tr>
<td>1.15</td>
<td>1.06</td>
<td>0.88</td>
<td>No</td>
</tr>
<tr>
<td>1.30</td>
<td>1.11</td>
<td>0.89</td>
<td></td>
</tr>
<tr>
<td>1.26</td>
<td>1.13</td>
<td>1.10</td>
<td>Yes</td>
</tr>
<tr>
<td>1.23</td>
<td>1.14</td>
<td>1.14</td>
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</tr>
<tr>
<td>1.29</td>
<td>1.19</td>
<td>1.13</td>
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</tr>
<tr>
<td>1.23</td>
<td>1.19</td>
<td>1.10</td>
<td></td>
</tr>
<tr>
<td>1.21</td>
<td>1.13</td>
<td>0.99</td>
<td>No</td>
</tr>
</tbody>
</table>

Normal range

0.84 - 1.06

DRVT: (PL) using phospholipid; (LWP): using lysed washed platelets; (PL-VIII): using phospholipid after addition of porcine factor VIII concentrate.

Eight of the 12 abnormal DRVTs (lupus anticoagulant positive) remained abnormal after correlation of the factor VIII deficiency with porcine factor VIII concentrate; nine of 12 lupus anticoagulant positive patients were HIV antibody positive.

of these lupus anticoagulant positive patients were HIV positive, but this association was not significant, and nine were antiphospholipid antibody positive (one antiphospholipid antibody positive patient was HIV negative and vice versa) (table 2). Similar DRVT results were obtained using two different commercial Russell’s viper venom preparations.

Because Russell’s viper venom activates factor X both directly and indirectly (through factors VIII and IXa following factor IX cleavage),14 the possible contribution of varying degrees of factor VIII deficiency to the prolonged DRVTs was investigated.

Normal plasma, serially diluted in plasma, immune depleted of factor VIII, retained a normal DRVT ratio until the factor VIII concentration fell to below 0.15 IU/ml. There was no correction of the ratio with lysed washed platelets (figure). The addition of porcine factor VIII concentrate to normal or systemic lupus erythematosus plasmas had no effect on the DRVT result.

There were no significant associations between the factor VIII concentrations or lupus anticoagulant/antiphospholipid antibodies positivity in the haemophiliacs. When the DRVTs were repeated after correcting the patients’ plasma factor VIII concentrations to >1.00 IU/ml with porcine factor VIII concentrate, eight of the 12 previously abnormal DRVTs remained abnormal. Seven of these eight were HIV antibody positive as were two of the four patients in whom the DRVT corrected with the addition of factor VIII (table 1). Lupus anticoagulant positivity did not correlate closely with antiphospholipid antibody positivity. There were no significant associations between factor VIII concentrate usage and lupus anticoagulant or antiphospholipid antibody positivity, and the porcine as well as human factor VIII concentrates used in the patients studied contained no detectable antiphospholipid antibodies.

Discussion

We found evidence of lupus anticoagulant in 12 of 22 (58%) patients with mild to severe haemophilia A and an increased prevalence in the HIV antibody positive group. This is in accordance with previous studies suggesting an association between lupus anti-

Table 2  Lupus anticoagulant and antiphospholipid antibody results in 22 haemophiliacs

<table>
<thead>
<tr>
<th></th>
<th>Lupus anticoagulant positive</th>
<th>Lupus anticoagulant negative</th>
<th>Anticardiolipin antibody (IgG)</th>
<th>Anticardiolipin antibody (IgM)</th>
<th>Anticardiolipin antibodies negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV positive</td>
<td>(n = 12)</td>
<td>9</td>
<td>3</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>HIV negative</td>
<td>(n = 10)</td>
<td>3</td>
<td>7</td>
<td>4</td>
<td>2</td>
</tr>
</tbody>
</table>

Three of the HIV antibody positive and 1 of the HIV antibody negative haemophiliacs were both IgG and IgM ACA positive.
coagulant, anticardiolipin antibodies, and HIV infection in homosexuals and between anticardiolipin antibodies and HIV infection in haemophiliacs. Recognition of lupus anticoagulant in haemophiliacs and its differentiation from a specific factor VIII inhibitor may have important clinical and laboratory implications.

The DRVT is the method of choice for detection of lupus anticoagulant in many laboratories because it has good sensitivity and specificity. In theory this test should not be influenced by decreased concentrations of factor VIII, IX, XI, or XII as Russell’s viper venom directly activates factor X. Indeed, Thiagarajan and coworkers found that plasmas rendered deficient of these factors by the addition of antibodies have normal DRVT values. The results presented here suggest that the factor VIII concentration can influence the DRVT result, possibly because our method is very sensitive due to the use of optimal reagent concentrations and a different phospholipid reagent. The effect of very low factor VIII concentrations on the DRVT suggests that the Russell’s viper venom is causing factor Xa generation through its action on factor IX, as well as by cleaving factor X directly, under the conditions used. Decreased concentrations of factor VIII, and by implication factor IX, in patients’ samples might therefore be expected to lead to false positive results in the DRVT. Mixing studies of normal plasma and immune depleted factor VIII deficient plasma (non-haemophiliac) showed that the DRVT ratio was increased only when the factor VIII concentration fell below 0.15 IU/ml, and that there was no correction in the platelet neutralisation procedure. The DRVT should therefore be carried out with extreme caution in patients with severe haemophilia or type III von Willebrand’s disease.

There was no correlation between the factor VIII concentration and positive DRVT results in the haemophiliacs. The addition of factor VIII to normal or lupus anticoagulant positive systemic lupus erythematosus plasmas had no effect on the DRVT and addition to the haemophiliac plasmas did not result in a correction in eight of the 12 samples with DRVTs indicative of lupus anticoagulant. This suggests that the DRVT was not prolonged by the low factor VIII concentrations but by antiphospholipid activity. The porcine factor VIII concentrate may contain some antiphospholipid neutralising activity which would swamp a weak lupus anticoagulant and account for the correction with factor VIII addition in four of the 12 haemophiliacs with abnormal DRVTs. Notably, there was a high incidence of anticardiolipin antibody positivity in the 12 patients with positive DRVT results.

There have been no reports of thrombotic episodes in patients with lupus anticoagulant, anticardiolipin antibody, and HIV infection, or in the patients in this study, in contrast to the increased thrombotic risk in patients with lupus anticoagulant associated with systemic lupus erythematosus. The observation that systemic lupus erythematosus sera, unlike syphilitic sera, exhibit a low antiphosphatidyl ethanolamine: phosphatidyl serine binding ratio, has led to the proposal that the thrombotic potential in systemic lupus erythematosus may be related to phospholipid epitope specificity. After platelet activation phosphatidyl serine, a negatively charged coagulant active platelet membrane phospholipid, is translocated from the inner to the outer half of the phospholipid layer where it acts as a binding surface for the factor X and prothrombin activation complexes with subsequent thrombin generation. Platelet bound antiphosphatidyl serine may promote aggregation, or activation and degranulation of platelets, and thus facilitate thrombus formation.

The clinical importance of the association between infection with various micro-organisms and antiphospholipid antibodies is unclear. Low titre anticardiolipin and anti-single stranded DNA antibodies have been reported in acute Klebsiella and other bacterial infections and in ankylosing spondylitis, which is associated with raised anti-Klebsiella activity, as well as in patients with chronic neurological diseases associated with disorders of immunity such as multiple sclerosis, myasthenia gravis, and the Lambert-Eaton myasthenic syndrome. The observations that antiphospholipid antibodies have been detected in connective tissue diseases, chronic neurological disorders, as well as in infections, raises the possibility that common membrane antigens and ubiquitous bacterial antigens have structural similarities, and this may lead to a breakdown of immunological tolerance.

Haemophiliacs have been exposed to many different viral agents as a result of the use of large pools of plasma as starting material for factor VIII concentrate production as well as copurification of viral particles because of their size. Our findings of lupus anticoagulant and anticardiolipin antibodies in HIV antibody positive haemophiliacs may represent a response to viraemic challenge. There may be cross reactivity between HIV or other viral antigens and coagulant active phospholipids during the immune response to infection, leading to expression of normally down-regulated antibodies. Alternatively, viruses may cause disruption of cell membranes with exposure of phospholipids or modification of phospholipids, such that they become antigenic.

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References


Requests for reprints to: Dr H Cohen, Department of Haematology, University College and Middlesex School of Medicine, Mortimer Street, London W1N 8AA, England.
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Limited space, however, is devoted to epidemiological studies and only a few chapters discuss the link between DNA adduct detection and risk assessment. Furthermore, the absence of reports on strategies of cancer prevention was most disappointing. It would have been of great interest, as anticipated in the title, to learn how the existing techniques could be applied to the prevention of some environmental cancers.

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