Letters to the Editor

TPHA compared with cardiolipin tests for serological detection of early primary syphilis

The requirement for syphilis screening of blood before it is transfused continues to stimulate debate and was the subject of a review in Vox Sanguinis in 1981. In this "International Forum" the advantages of Treponema pallidum haemagglutination (TPHA) over cardiolipin reagents were noted by several participants.

At the North London Transfusion Centre we have been using TPHA routinely for over two years. We use a modification which is both economical and sensitive. Diluted reagents (Fujizoki, Diamed Diagnostics, Liverpool) are added to serum dilutions in microtitre plates. After centrifugation the plates are inclined at 70°. Positive samples form "buttons" of cells whereas negative samples form "streaks". Other laboratories have employed economical modifications in Terasaki plates.

One criticism of TPHA tests has been that they may be less sensitive than cardiolipin reagents for detecting early primary syphilis, though they detect infections from many years ago; the latter are presumably less significant in the context of transmission by transfusion.

Serum samples from 15 patients with early primary syphilis were therefore collected for us by Dr Johnston at the Venereal Diseases Reference Laboratory, Whitechapel. Also serum from each of 10 rabbits inoculated with a treponemal suspension was obtained at 3, 7, 10, 14 and 21 days after inoculation. The rabbits were inoculated to provide TPI reagents and had been pre-tested to avoid any false-positive reactions due to rabbit syphilis, caused by T. cuniculi.

The sera were tested by a cardiolipin test (RPR, Becton and Dickinson, Wembley) and by Fujizoki TPHA. TPHA titrations on the human sera were by standard as well as modified methods.

The three-day serum samples from all 10 rabbits were negative with cardiolipin and modified TPHA tests. However, by seven days (when orchitis is just beginning) and thereafter, all 10 were positive with both cardiolipin and modified TPHA tests. There was sufficient serum for standard Fujizoki TPHA testing from only two of the rabbits. The sera of both these rabbits were TPHA positive using the standard method by day 7.

Nine of the 15 human sera were positive by both cardiolipin and Fujizoki tests (standard or modified). The patterns of reaction for the remaining six sera are shown in the Table.

As a result of these studies (both in experimental animals and using human sera) we feel that the standard TPHA method has similar success in detecting early primary syphilis as the cardiolipin test. Furthermore, the modified TPHA is, if anything, slightly better for detecting early primary syphilis than the cardiolipin test and is certainly no worse.

We gratefully acknowledge the generous help of Dr NA Johnston of the Venereal Diseases

Technical method

13 Strauss W. Imidazole increases the sensitivity of the cytochemical reaction for peroxidase with diaminobenzidine at neutral pH. J Histochem Cytochem 1982;30:491-3.

Requests for reprints to: Dr JB Matthews, Immunology Laboratory, Department of Oral Pathology, The Dental School, St Chad's Queensway, Birmingham B4 6NN, England.

Pattern of reactions in six sera using cardiolipin and TPHA

<table>
<thead>
<tr>
<th>Serum No</th>
<th>Cardiolipin result</th>
<th>Standard Fujizoki TPHA</th>
<th>Modified Fujizoki TPHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>±</td>
<td>-</td>
<td>±</td>
</tr>
<tr>
<td>2</td>
<td>±</td>
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</tr>
<tr>
<td>5</td>
<td>±</td>
<td>+</td>
<td>+</td>
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<tr>
<td>6</td>
<td>±</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

± = weakly positive reaction.
Serum gamma-glutamyltransferase and alkaline phosphatase in rheumatoid arthritis

The paper by Spooner and colleagues in the June issue prompts us to recall our own observations on patients with rheumatoid arthritis. In a consecutive series of 46 patients (34 females, 12 males), all with positive serology, serum gamma-glutamyltransferase (GGT) activity was increased in eight patients (17%) and alkaline phosphatase (AP) in six (13%). This incidence of raised GGT is slightly lower than that found by Spooner et al, whereas the incidence of raised AP is much less.

On all patients showing raised serum enzyme activity we carried out isoenzyme examination by electrophoresis. GGT-2 was the principal GGT isoenzyme in six of the eight patients showing raised GGT activity, GGT-3 was present in all, and markedly increased in three. This pattern is typical of, but not exclusive to, patients with liver disease, and is especially found with intrahepatic cholestasis. In all six patients with increased total AP activity there was increased activity of the liver AP isoenzyme. In four of these, this was accompanied by increased “biliary” isoenzyme, and in one by additional increase of the bone isoenzyme.

Our studies indicate that in patients with rheumatoid arthritis showing raised activities of GGT or AP, this is likely to be of hepatic origin.

References


Serum gamma-glutamyltransferase and alkaline phosphatase in rheumatoid arthritis

In collaboration with the International Committee for Standardisation in Haematology (ICSH), the European Community Bureau of Reference (BCR) has produced three certified reference materials for the standardisation of commercial or laboratory-made human, bovine and rabbit thromboplastins, respectively. These reference materials have been calibrated against the WHO international reference preparation (IRP 67/40). By using the appropriate BCR reference material (human, bovine or rabbit) a sensitivity index can be assigned to any thromboplastin working preparation which will thus be directly related to the WHO primary reference preparation.

In clinical practice a prothrombin ratio obtained by means of thromboplastin reagent with an assigned sensitivity index can then be converted to an international normalised ratio (INR) by a simple equation: INR = antilog of (log prothrombin x sensitivity index).

Manufacturers are being encouraged to establish the sensitivity indices of their thromboplastin reagents and to provide an appropriate Table of INRs. A therapeutic range for INR of 2-0-4-0 has been recommended.

Details of the scheme have recently been described. Information of the availability of BCR Certified Reference Materials, a report of the certification protocol and recommended methodology for calibration of working preparations are available from the European Community Bureau of Reference, Rue de la Loi 2000, Brussels B-1049, Belgium.

SM LEWIS
Chairman, International Committee for Standardisation in Haematology

Reference


Direct evidence of localised immunological damage in vulvar lichen sclerosus et atrophicus

Lichen sclerosus et atrophicus (LSA) is known to be associated with an increased incidence of organ-specific autoantibodies and autoimmune diseases. In addition a raised incidence of HLA-B40 in this disease has recently been reported and has led to the suggestion that this antigen may be in linkage disequilibrium with immune response genes controlling the susceptibility to both LSA and their autoimmune diseases.

The present study has been designed to determine whether LSA is associated with immunological phenomena. A search has been undertaken for immunohistochemical evidence of deposition of immunoglobulin, complement (C3) and fibrin in the vulvar lesion and using adjacent normal skin as a control. In addition, sera from these patients have been screened for autoantibodies.

PATIENTS AND METHODS

Biopsies from 16 Caucasian women (age range 36-78 yr, mean 64-6 yr) with vulvar LSA were collected over a period of six years. The histopathological diagnosis was confirmed by two independent observers and was based on the presence of hyperkeratosis, epidermal atrophy, homogenisation of the collagen of the upper dermis and an underlying chronic inflammatory cell infiltrate. Wedge biopsies of the affected and non-affected skin were snap-frozen and then examined by a standard direct immunofluorescence technique for the presence of immunoglobulin (IgG, IgA, IgM and IgE), complement (C3) and fibrin with commercially prepared fluorescein-labelled antisera (Wellcome Foundation and Hoechst Pharmaceuticals). Serum samples from 14 patients were obtained and screened routinely in the immunopathology laboratory for the presence of organ-specific and non-organ-specific antibodies.
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J Barbara, R Salker, F Lalji and P Mochnaty

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