Present day practice

False positive results with a rapid solubility test for haemoglobin S

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Sickledex (Ortho) detects the presence of haemoglobin S by precipitation of the insoluble haemoglobin variant creating a cloudy, turbid suspension in a prepared test solution. The test is performed by adding 0.02 ml of anticoagulated whole blood to the solution, or, if the haemoglobin concentration of the sample under test is 7 g/100 ml or less, by doubling the amount of whole blood used.

Several authors (Canning and Huntsman, 1970; Diggs, Schorr, Ascarl, and Reiss, 1968; Loh, 1968) have shown good reliability by failure to detect false positives or negatives in over 2000 cases. Diggs et al (1968) stated that multiple myeloma may cause confusing coarse flocculation, and Canning and Huntsman (1970) went further to say it may give rise to a false positive. They also commented on a false positive seen in one case of disseminated lupus erythematosus, concluding that this was due to the plasma, but did not enlarge upon this point.

Recently we reported the observation of a false positive result in a patient with renal failure who was shown to have only haemoglobin A present at a concentration of 7 g/100 ml (Lilleyman and Bills, 1971). This prompted us to test a number of other patients, and of 29 patients tested, nine gave positive results (Table). As may be seen, all the positive cases had haemoglobin concentrations of 7 g/100 ml or less, thus requiring a double volume (0.04 ml) of blood for the test. Of the 20 cases giving negative results, only four (with unrelated conditions) had haemoglobin values of less than 7 g/100 ml.

Four of the cases giving a false positive result were tested further, and it was shown that the test was negative if the haemoglobin concentration of the sample was raised above 10 g/100 ml by removal of plasma from the centrifuged specimen, thereby requiring a single volume of blood only for the test. From these observations it appears that the false positive results are associated with the increased amount of plasma present if 0.04 ml of blood is added. Support for this idea was obtained by demonstrating a 'positive' test on two normal samples containing haemoglobin A when diluted with autologous plasma to a concentration of less than 7 g/100 ml. The test was negative at higher concentrations.

Which plasma constituent is responsible for this phenomenon is less apparent. Biochemical profiles on the nine positive cases failed to reveal any consistent abnormality. The role of pure protein concentration was explored by performing tests with 0.04 ml volumes of an artificially produced medium containing physiological amounts of albumin, gamma globulin, and fibrinogen, and also a solution containing 5 g/100 ml of gamma globulin alone. These gave a slight opacity which would not cause confusion. Also, lipaemia was not observed to have any apparent effect in studies on two normal volunteers before and after a fatty meal.

We do not know the cause of the inconsistent false positives we have observed; a relation to the amount of plasma used in the test is indicated, though this does not appear to be simple.

<table>
<thead>
<tr>
<th>Case</th>
<th>Diagnosis</th>
<th>Haemoglobin (g per 100 ml)</th>
<th>Sickledex with Whole Blood (0.04 ml)</th>
<th>Sickledex Washed Cells</th>
<th>Hb Electrophoresis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chronic renal failure</td>
<td>6.4</td>
<td>Positive</td>
<td>Negative</td>
<td>Hb A only</td>
</tr>
<tr>
<td>2</td>
<td>Chronic renal failure</td>
<td>3.9</td>
<td>Positive</td>
<td>Negative</td>
<td>Hb A only</td>
</tr>
<tr>
<td>3</td>
<td>Chronic renal failure</td>
<td>4.6</td>
<td>Positive</td>
<td>Negative</td>
<td>Hb A only</td>
</tr>
<tr>
<td>4</td>
<td>Aplastic anaemia</td>
<td>2.3</td>
<td>Positive</td>
<td>Negative</td>
<td>Hb A only</td>
</tr>
<tr>
<td>5</td>
<td>Chronic renal failure</td>
<td>7.0</td>
<td>Positive</td>
<td>Negative</td>
<td>Hb A only</td>
</tr>
<tr>
<td>6</td>
<td>Chronic renal failure</td>
<td>4.5</td>
<td>Positive</td>
<td>Negative</td>
<td>(Not done)</td>
</tr>
<tr>
<td>7</td>
<td>Pernicious anaemia</td>
<td>4.4</td>
<td>Positive</td>
<td>Negative</td>
<td>(Not done)</td>
</tr>
<tr>
<td>8</td>
<td>Chronic renal failure</td>
<td>3.7</td>
<td>Positive</td>
<td>Negative</td>
<td>(Not done)</td>
</tr>
<tr>
<td>9</td>
<td>Chronic renal failure</td>
<td>3.9</td>
<td>Positive</td>
<td>Negative</td>
<td>(Not done)</td>
</tr>
</tbody>
</table>

Table False positive results using a rapid solubility test for haemoglobin S
These results show, however, that an erroneous diagnosis could be made in severely anaemic patients without dysproteinanaemia. Correction of this problem can be achieved simply by washing the cells under test, but we consider it important that this possible source of error should be recognized.

We should like to thank Mr C. K. Campbell of Ortho Diagnostics for his help and generous supplies of Sickledex.

Letters to the Editor

The Two Forms of Haemophilia A in a Population of Greek Haemophiliacs

Denson (1968) and Denson, Biggs, Haddon, Barret, and Cobb (1969) found that there are at least two populations of patients with haemophilia A. Those in the first group have neither factor VIII activity nor factor-VIII-like protein antigenically demonstratable. Those in the second group have little biological activity but in contrast have a factor-VIII-like protein capable of neutralizing a specific factor VIII inhibitor. The first group was termed haemophilia A- and the second haemophilia A+. Of 48 haemophilic patients only four belonged to the second group. Similar were the findings of Hoyer and Breckenridge (1968), Feinstein, Chong, Kasper, and Rapaport (1969) from the USA, and Meyer, Dray, and Larrieu (1970) from France.

Our findings in Greek haemophiliacs are

1. Forty haemophiliacs without inhibitor were investigated. The factor VIII activity was determined by the method of Pitney (1956). The distribution of factor VIII activity was <1% five patients, 2-5% 23 patients, and 5-10% 12 patients.

2. The activity of factor VIII inhibitor was quantitatively determined by the method of Biggs and Bidwell (1959). For the neutralization of the inhibitor by the test plasma the method proposed by Hoyer and Breckenridge (1968) was used.

Table I shows that the plasma of 37 haemophilic patients failed to neutralize the factor VIII inhibitor; thus these patients belong to haemophilia A-. The plasma of the other three patients behaved like normal plasma in the ability to neutralize the inhibitor, thus these patients belong to haemophilia A+.

As Table II shows, our findings are similar to those of the authors previously mentioned. The number of haemophiliacs in each series is relatively small, but it would seem that the distribution of the two forms of haemophilia is similar in various countries. More material is, however, needed, mainly of various ethnic origins.

Recently (1970) Bennett and Heuhns examined a population of haemophiliacs of British origin according to the ability of their plasma to neutralize a rabbit anti-serum against crude human factor VIII. The results were correlated with those of the biological assay of factor VIII activity of these samples. Three types of haemophilia were distinguished. In type 1 there was no biological activity and factor-VIII-like protein; in type 2 there was no biological activity but normal amounts of factor-VIII-like protein; and in type 3 there were low levels of factor VIII activity and normal amounts of factor-VIII-like protein.

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References
