Comparing substrates for the detection of ANAs

The article by Pollock and Toh1 about the detection of antinuclear antibodies (ANAs) using Hep-2 cells transfected with Ro/SS-A raises the important question of the optimal method for screening of sera from patients with suspected autoimmune connective tissue disease. We have directly compared the performance of the same Hep-2 transfected cells (Hep2000; Immuno Concepts, California, USA) with Hep-2 untransfected cells (Quantflour; Sanofi Diagnostics Pasteur Inc, Minnesota, USA). The results from our study combined with a reassessment of Pollock and Toh’s data cast doubt on their conclusion that Hep-2 transfected cells are more reliable than other substrates for detecting clinically meaningful ANAs.

Sera from 258 patients referred to our laboratory for ANA testing were analysed for the presence, titre, and pattern of ANAs using Ro/SS-A transfected and untransfected Hep-2 cells. Indirect immunofluorescence was performed at a screening dilution of 1/40, and titres were reviewed independently by at least two scientists. In general, the correlation between the two substrates for detection of ANAs was good (table 1); discrepancies that are unlikely to be of clinical relevance were found for low titre positives. The only significant difference between Hep-2 transfected and Hep-2 untransfected cells was that the latter were more sensitive for ANAs at high titres.

Pollock and Toh report that seven of 110 Ro/SS-A positive sera did not have a positive ANA pattern in the background non-hyperexpressing cells; in contrast, we found speckled ANA staining using the untransfected cells in 10 of 11 anti-Ro/SS-A positive samples. One patient with alcoholic liver disease was repeatedly negative on Hep-2 untransfected cells but Ro/SS-A positive on Hep-2 transfected cells. This patient’s serum was analysed for antibodies to extractable nuclear antigen (ENA). Enzyme linked immunosorbent assay (ELISA) (ENA RELISA; Immuno Concepts) detected anti-SS-A antibodies at a concentration of 33 ENA units. However, the patient does not have any features of an autoimmune connective tissue disease and testing of his serum by counterimmunoelectrophoresis and line immunosay (INNO-LIA ANA; Innogenetics NV, Ghent, Belgium) confirmed the absence of anti-Ro/SS-A antibodies.

Pollock and Toh also conclude that the positive predictive value and specificity of detection of anti-Ro/SS-A antibodies on Hep-2 transfected cells is 100% and 91%, respectively, by comparing detection by immunofluorescence with detection by ELISA. This conclusion is questionable, because the specificity and predictive value of the test should depend on a correlation with the clinical diagnosis rather than another diagnostic test. Although it is useful to know the concordance between Ro/SS-A detected by Hep-2 transfected cells and ELISA, the true clinical usefulness of the test lies in its specificity and positive predictive value for the associated clinical conditions. In fact, the true specificity of the test can be calculated from the data provided in table 3 of their paper, and is only 77% (64 of 83 patients positive for Ro/SS-A) for the diagnosis of systemic lupus erythematosus and Sjögren’s syndrome, with a sensitivity of 89% (64 of 72 patients).

Together with our data, these figures indicate that the Hep2000 substrate in fact performs suboptimally, and like ELISAs that detect anti-Ro/SS-A antibodies in up to 17% of normal sera, appears too sensitive for reliable diagnosis in autoimmune connective tissue disease. Furthermore, the advantage of using the transfected cell line for initial screening is likewise questionable, because not only do anti-Ro/SS-A positive sera detected by immunofluorescence need to be checked for additional ENAs by other methods, but 15 sera testing positive for anti-Ro/SS-A antibodies by immunofluorescence were negative by immunofluorescence in Pollock and Toh’s study.

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**Table 1** Results of indirect immunofluorescence at dilutions of 1/40 and 1/160 using Hep2 and Hep2000 as substrates for sera from 258 patients*

<table>
<thead>
<tr>
<th></th>
<th>Hep2</th>
<th>Hep2000</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1/40</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1/160</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1/60</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>120</td>
<td>130</td>
</tr>
<tr>
<td>Specified**</td>
<td>64</td>
<td>78</td>
</tr>
<tr>
<td>Homogenous</td>
<td>55</td>
<td>33</td>
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<tr>
<td>Centromere</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Other</td>
<td>9</td>
<td>6</td>
</tr>
</tbody>
</table>

*Serum from some patients had more than one ANA pattern.
**12% difference (95% confidence interval, 2% to 22%; p = 0.03).
***Includes Ro/SS-A pattern on Hep2000.

ANA, antinuclear antibody.
flation, or the consequences of hypovolaemia. Blood may be taken in the first two to three days after death to confirm raised mast cell tryptase, which is released during anaphylactic or anaphylactoid reactions. Caution is required in interpreting mast cell tryptase concentrations because they can be increased after exposure to opiates. IgE against specific allergens can be sought in postmortem blood samples. The absence of specific IgE cannot rule out allergy as the cause of death, but confirmation of sensitivity to specific agents may add weight to a diagnosis of anaphylaxis. Unfortunately, specific IgE testing is not available to many anaesthetic or antibiotic drugs. We advise collecting samples of clotted and EDTA anticoagulated blood as soon as possible after death and guidance from an expert laboratory.

Is postmortem testing for anaphylaxis important? We believe that collecting important data on drugs and other interventions requires such examinations to be carried out. For example, collection of data on a series of mishaps related to desensitisation led to improved guidelines for this procedure and a reduction in mortality. We are unaware of litigation arising from alleged anaphylaxis, but again in this situation, assiduous collection of data may form an important part of a legal defence.

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The authors reply

We thank Drs Helbert and Robinson for pointing out this area that we did not cover in our review,1 and the useful information contained in their letter. It is obviously impossible to cover every aspect of the investigation of deaths following anaesthesia but anaphylaxis, although rare, is important and we will include it in future versions of the ACP Best Practice Guidelines.

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International Society for Diagnostic Quantitative Pathology
XIIIth International Congress
6–20 October 2000
Hilton International Hotel, Adelaide, Australia

Quantitative diagnostic pathology is a field of applied science addressing the problems of diagnosis and prognosis of disease by producing robust objective solutions. The conference theme “Quantitative diagnostic pathology in the information age” reflects the maturity of the discipline and the need to standardise the various methods now used. The sessions will reflect the latest thinking and research in the area as well as introducing the use of quantitative methods, data processing techniques, and signal processing in the area of molecular pathology. The programme of scientific presentations and poster sessions outlining new and evolving work is supported by a series of lectures on the state of the art and the importance of new directions. The keynote speakers are leading figures in the fields of quantitative pathology, molecular pathology, applied mathematics and statistics, and sensor and information signal processing, as well as clinicians providing important problems in diagnostic pathology that need to be resolved from their perspective.

Over the course of the week there will be several scientific sessions concentrating on specific themes. These include the following: standardisation and quality control in quantitative pathology; quantitative genetic/molecular pathology; data evaluation and analysis; signal processing; data evaluation “workshops”; prostatic pathology; implementation of quantitative pathology in routine diagnostic pathology; gynaecological and breast pathology; gastrointestinal pathology—diagnosis and predicting outcomes; angiogenesis; quantitative pathology and lymphomas.

Two special symposia will also be organised focusing on “new advances in quantitative pathology” and “web based environments for supporting the learning of pathology”.

Registration fee: before 31 July, $A500 (students registrars $A350); after 31 July and before 6 September, $A580 (students registrars $A400).

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