Comparing substrates for the detection of ANAs

The article by Pollock and Toh about the detection of antinuclear antibodies (ANAs) using Hep2 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 (Quantafuor; 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 Hep2 cells. Indirect immunofluorescence was performed at a screening dilution of 1/40 anti-sera (1/1,250), and slides 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 Hep-2 cells. One patient with alcoholic liver disease was repeatedly negative on Hep-2 transfected 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 immunoassay (INNO-LIA ANA; Inogenetics NV, Ghent, Belgium) confirmed the absence of anti-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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Pathological investigation of deaths following surgery, anaesthesia, and medical procedures

We were concerned to find that Start and Cross’s otherwise comprehensive Best Practice article on the investigation of medical accidents’ failed to discuss the investigation of possible anaphylactic reactions. Fatal anaphylaxis can occur during approximately 1/100 000 general anaesthesia administrations. Anaphylactoid reactions, where IgE mediated allergy is not involved in reactions to—for example, contrast media, account for a further number of deaths. In some instances, an anaphylactic reaction might be obvious and postmortem examination may simply confirm the cause of death. In many other instances—for example, reactions to anaesthetic agents, antibiotics, or latex that occur during unconsciousness, the cause may be much less clear. A review of the patient’s history should be carried out, but will be unrewarding in most cases, which occur de novo. Repeated procedures are a well recognised risk factor and account for the high risk of latex anaphylaxis in children with spina bifida.

Macroscopic examination might reveal skin and airways angioedema, lung hyperinflammati-
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, and the useful information contained in their letter. It is obviously impossible to cover every aspect of the inves- tigation of death 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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