Monoclonal immunofluorescence compared with silver stain for investigating Pneumocystis carinii pneumonia

J Midgley, P A Parsons, D C Shanson, O A N Husain, N Francis

Abstract
Two hundred and eighty two specimens from 220 patients positive for HIV with respiratory tract symptoms, or febrile illness, or both, were examined for the presence of Pneumocystis carinii. Specimens were either induced sputum samples or bronchoalveolar lavage fluids. To establish the optimal method for laboratory diagnosis a comparison was made of detection of the organism by use of monoclonal antibody and immunofluorescence with conventional silver staining methods. Three commercially available reagents for immunofluorescence were also compared. Immunofluorescence was significantly more sensitive than the silver stain and the best results for immunofluorescence were obtained using Northumbria Biologicals Ltd reagents.

Most patients with AIDS have at least one episode of Pneumocystis carinii pneumonia. Clinical management of these patients is assisted by continued rational treatment once a prompt diagnosis is established, followed by appropriate prophylaxis.

Methods
Between November 1988 and July 1989, 282 specimens from the respiratory tract of patients positive for human immunodeficiency virus (HIV) with suspected Pneumocystis pneumonia were investigated for the presence of Pneumocystis carinii. Methods for obtaining induced sputum specimens and bronchoalveolar lavage (BAL) fluids were as described by Leigh et al. After a negative result for Pneumocystis on induced sputum the patient underwent bronchoscopy, if possible, within four days. Specimens were handled promptly in the laboratory; close cooperation between ward and laboratory was essential for efficient processing of this clinical material. Induced sputum samples were first homogenised with "Sputasol" for about 10 minutes. Occasionally BAL fluids were homogenised if the consistency was very viscous. Specimens at this stage were divided into several aliquots to investigate for other pathogens including Mycobacteria, Legionella, Cryptococcus, Candida and Aspergillus. The only viral pathogen sought was cytomegalovirus which was undertaken for us by the Virology Department at the Royal Free Hospital.

Results
Tables 1 and 2 give the results of the comparison of immunofluorescence with silver stain.

Discussion
No specimens were positive with silver staining and negative by immunofluorescence, as has been reported in the USA. There were two occasions, however, where a possible error in technique was indicated by the presence of cysts stained by silver alone, but these could clearly be seen by immunofluorescence when repeated. We do not therefore advocate the use of immunofluorescence alone. Monoclonal antibodies have been reported not to react with cysts from every case encountered, and some manufacturers state that their monoclonal antibody will react with trophozoites as well as with cyst forms. Non-immunological methods

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methods and newer monoclonal antibodies will continue to be included in our studies.

Positive immunofluorescence with a typical arrangement of clusters of cysts was similar with all three immunofluorescence reagents when large numbers of cysts—that is, greater than 20—were present, and this was found in only four of the 12 patients investigated by all three methods (table 3). Meridian state that the presence of two or more cysts should be considered positive for P carinii. We were not able to report confidently on such low numbers on any of the specimens stained by either Meridian or Dako. In neither of these methods was a counterstain used. With NBL reagents, however, low numbers of cysts, even in the absence of clusters, were distinctly visible under both low and high power magnification.

These reagents include the counterstain Evans Blue which may facilitate the recognition of single cysts from background material. The NBL reagents are available in kit form with full instructions, providing a technique which can be easily used by any laboratory with access to fluorescence microscopy. The remainder of our specimens were stained by NBL immunofluorescence reagents and our modified Grocott method only.

We found the immunofluorescence technique to be reliable, repeatable, easy to carry out, and to give well defined cyst fluorescence with minimal background staining. The method of preparation for the immunofluorescence method is straightforward with timed incubations. In contrast, the Grocott method is unreliable, needing considerable technical skill to judge when the slide is stained adequately. Over- or understaining may make a slide unreadable. Preparation time is less for the Grocott method as we speed up the incubation time by heating in a microwave oven, but screening time is considerably more than for the immunofluorescence stained slides. Care is needed to avoid confusion with yeasts which are often present in these specimens. The presence of P carinii could be shown in specimens from 63 patients in this study, 10 of these by immunofluorescence alone. In a further three patients the possibility of the presence of P carinii was indicated by equivocal immunofluorescence. Our results indicate an increase in sensitivity for the detection of P carinii in cytological preparations of induced sputum using the immunofluorescence technique.

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