Detection of pneumocystis DNA in nasopharyngeal aspirates of leukaemic infants with pneumonia

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Abstract

The technique of polymerase chain reaction was used to detect *Pneumocystis carinii* DNA in nasopharyngeal secretions of three infants with leukaemia who had the clinical features of *P carinii* pneumonia. The use of this non-invasive technique allowed the early diagnosis and treatment of these infants whose protocols did not include the use of prophylactic co-trimoxazole

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The diagnosis of *Pneumocystis carinii* pneumonia in childhood has, in the past, usually depended on bronchoscopic or non-bronchoscopic alveolar lavage to identify *P carinii* by histochemical staining in the lower airway secretions. The technique of polymerase chain reaction has been shown to detect *P carinii* DNA effectively in upper airway secretions of infected adult patients.[1 2] We describe the use of polymerase chain reaction in the detection of *P carinii* DNA in nasopharyngeal aspirates from three infants, one with acute myeloid leukaemia (AML) and two with acute lymphoblastic leukaemia (ALL), who developed clinical features of *P carinii* pneumonia during treatment.

Patients

The patient details are shown in the table. Each patient developed acute respiratory distress in the early stages of chemotherapy, during a time of profound neutropenia (neutrophil count $<0{\cdot}5\times10^9$/l). The chest radiographs showed diffuse bilateral opacification consistent with interstitial pneumonia. Nasopharyngeal secretions were then collected.

Methods

DNA EXTRACTION

Samples were treated with proteinase K, 1 mg/ml final concentration (Boehringer Mannheim) in the presence of 10 mM EDTA, pH 8·0 and 0·5% sodium dodecyl sulphate, at 50°C for up to 18 hours. The samples were extracted with phenol:chloroform:isoamyl alcohol, and the DNA in the sample was recovered using a DNA binding resin (Magic DNA clean-up system, Promega), eluted in 50 μl sterile distilled water and stored frozen at −20°C.

DNA AMPLIFICATION

DNA amplification was carried out on the samples using the polymerase chain reaction, with the *P carinii* specific primers pAZ102-H and pAZ102-E.[1 2] The amplification reaction mixture contained 50 mM potassium chloride, 10 mM Tris, pH 9·0, 0·1% Triton X−100, 3 mM magnesium chloride, 400 μM dNTPs (each of the four deoxyribonucleoside triphosphates, Boehringer Mannheim), 1 μM oligonucleotide primer and 0·025 units/μl of Taq polymerase (Promega), with denaturation at 94°C for 1·5 min, annealing at 55°C for 1·5 min, and extension at 72°C for 2·0 min for 40 cycles. Negative controls, with no added template, were tested with each clinical sample. The amplification products underwent electrophoretic separation in 1·5% agarose gels, and the presence of a *P carinii* specific band (346 base pairs) was detected by visualisation with ultraviolet light after ethidium bromide staining.

Results

The polymerase chain reaction was carried out on total DNA extracted from the nasopharyngeal secretions from each patient and resulted in the synthesis of a *P carinii* specific amplification product, detected on ethidium bromide stained gels. Only 50 μl of secretions were required for this test. The detection of *P carinii* DNA allowed the non-invasive confirmation of the clinical diagnosis of *P carinii* pneumonia. However, in one infant (patient 1) who required ventilation, *P carinii* was also identified by microscopy after methamine silver staining of a sample of bronchoscopic lavage fluid. The three patients responded to high dose intravenous co-trimoxazole, in a manner consistent with the diagnosis of *P carinii* pneumonia.

Discussion

In the three infants with the clinical features of *P carinii* pneumonia, a test using the

Patient details

Patient No	*Diagnosis*	*Age (months)*	*Chemotherapy received*
1	ALL	7	MRC infant ALL protocol, week 8
2	ALL	11	MRC infant ALL protocol, week 7
3	AML	3	ADE 10+3+5*, ADE 8+3+5*

*ADE=cytarabine, daunorubicin, and etoposide. Numbers refer to days of treatment.

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polymerase chain reaction showed *P carinii* DNA to be present in their nasopharyngeal secretions. It is increasingly recognised that lower airway infection with *P carinii* is accompanied by the presence of the organism in upper airway secretions.[3] Access to lower airway secretions may be limited in children whose clinical state does not warrant intubation and ventilation. Bronchoscopic lavage may lead to respiratory deterioration in a patient already in respiratory distress. The alternative of induced sputum is impractical in infants and young children. Therefore, identification of *P carinii* in nasopharyngeal secretions may provide a simple and non-invasive means of diagnosis. A case report has previously described the identification of *P carinii*, using a fluorescent antibody technique, in the nasopharyngeal secretions of an HIV positive infant who had signs consistent with *P carinii* pneumonia.[4]

The polymerase chain reaction technique is highly specific and sensitive for *P carinii* DNA and has been shown to be diagnostically useful in bronchoalveolar lavage and induced sputum samples in adults with pneumonia.[1 2] Combining the sensitivity and specificity of *P carinii* DNA detection by the polymerase chain reaction with this non-invasive sample method offers a potentially valuable diagnostic tool that needs to be evaluated in a large group of children with suspected *P carinii* pneumonia.

None of the infants was on prophylactic co-trimoxazole at presentation. The current AML regimen does not prescribe it, nor is it included until week 16 of the current MRC-UKALL infant protocol once the late intensification has been completed, on the basis of the small risk to patients. The occurrence of pneumocystis pneumonia in infants early in their chemotherapy regimen suggests that infants with leukaemia are at particular risk of *P carinii* pneumonia and should therefore receive prophylactic co-trimoxazole early in their treatment.

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