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Pneumocystis carinii pneumonia

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Despite effective prophylaxis, *Pneumocystis carinii* pneumonia remains a common cause of respiratory disease in HIV infected patients.¹ Since the last update article in 1992² there have been further advances in our understanding of the molecular biology of this opportunistic fungus and there have been developments in the epidemiology, diagnosis, treatment, and prophylaxis of this infection.

In this article we review recent advances in the molecular biology of *P carinii* and clinical aspects of management of HIV infected patients with pneumocystis pneumonia.

Molecular biology

EVIDENCE THAT PNEUMOCYSTIS CARINII IS A FUNGUS

Despite the lack of ultrastructural features characteristic of protozoa, *P carinii* has, until recently, been regarded to be a member of that kingdom. In 1988 the sequence of an 18S ribosomal RNA and gene from rat-derived *P carinii* were found to closely resemble 18S ribosomal RNA sequences in certain fungi.³ At the time of this report there were only five other fungal sequences available for comparison, all from different ascomycetes species.⁴ There are now over 40 published fungal 18S sequences from 35 species including zygomycetes and basidiomycetes. A recently published evolutionary tree that compared rat-derived *P carinii* with 38 fungal sequences placed *P carinii* within the fungal kingdom on its own branch between the ascomycetes and the basidiomycetes.⁵

Although these data have strengthened the argument for classifying *P carinii* as a fungus, it does not eliminate the possibility that the 18S ribosomal RNA sequence from *P carinii* may not accurately reflect the evolutionary history of the organism. For example, the ribosomal RNA gene locus in *P carinii* might have been acquired via horizontal gene transfer from another (fungal) organism. If this were so, it would explain the unusual features of *P carinii* when compared with other fungi – for example, the lack of ergosterol in the plasma membrane⁶ or the lack of response to antifungal chemotherapeutic agents. However, other gene sequence data rule out the gene transfer hypothesis. Several protein encoding genes have been isolated from rat-derived *P carinii*. Trans-

lation elongation factor (EF3) has been found only in fungi. The genome of rat-derived *P carinii* contains a gene which encodes for a protein that is 57% identical in sequence to EF3 from *Saccharomyces cerevisiae*.⁷ The predicted sequences of two proteins dihydrofolate reductase (DHFR) and thymidylate synthetase (TS) have been used to construct phylogenetic trees that place *P carinii* on fungal branches. A number of other genes from rat-derived *P carinii* encode proteins that have close fungal homology. These include β tubulin,⁸ transcription factor IID – a P type cation translocating ATPase,⁹ and a 6.8 kilobase fragment of mitochondrial DNA (that encodes for apocytochrome b, NADH dehydrogenase subunits 1, 2, 3, and 6, cytochrome oxidase subunit II, and the small subunit of ribosomal RNA).¹⁰ The aromatic amino acid biosynthetic pathway is found in bacteria, plants and lower eukaryotes but is absent in mammals. Aromatic amino acid biosynthesis in fungi is dependent on the prechorismate pathway; steps 2 to 6 of this pathway are catalysed by a single penta-functional protein called AROM which is encoded by a single gene *arom*. The *arom* gene from *P carinii* has been cloned and characterised. Compared with that of *Saccharomyces cerevisiae* and *Aspergillus nidulans* it is highly homologous.¹¹

It has been suggested that *P carinii* is closely related to ustomycetous (basidiomycete) red yeast fungi, based upon a comparison of mitochondrial DNA from human, ferret, rabbit, and rat-derived *P carinii* and from a wide range of fungi spanning the seven phyla of the fungal kingdom.¹² The polymerase chain reaction (PCR) was used to amplify a segment of the mitochondrial large subunit ribosomal RNA gene; PCR product was obtained from *P carinii* and also from several (but not all) of the red yeast fungi. The PCR products were then sequenced and it was found that *P carinii* sequences were very similar to those from the ustomycetous red yeast fungi group that is widely distributed environmentally and which releases many widely dispersed airborne spores. This group includes urediniomycota (rusts) and ustomycota (smuts), well known plant pathogens. These data interestingly contrast with those of Van de Peer *et al*,⁵ who used nuclear ribosomal RNA sequences which placed *P carinii* on a unique branch between

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ascomycetes and the basidiomycetes red yeast cluster. This discordance is perhaps not surprising as *P. carinii* is phenotypically and genetically an atypical fungus. Analysis of the genes encoding 5.8S, 18S, and 26S ribosomal RNAs of rat-derived *P. carinii* show the gene cluster to be present in low copy numbers (1 or 2 per genome) and the gene clusters are widely spaced, at least 50 kilobase pairs apart, in contrast to other fungi and most eukaryotes where they are present in hundreds of copies and gene clusters are closely placed on the genome.

DIFFERENT SPECIES OF PNEUMOCYSTIS CARINII INFECT DIFFERENT ANIMAL HOSTS

Host specificity of *P. carinii* has been suspected for many years; organisms isolated from one host species fail to grow when inoculated into another. Characterisation of proteins and glycoproteins separated by electrophoresis and detected by binding of antibodies show that organisms obtained from different hosts display different band patterns and that antibodies raised against *P. carinii* from one host species commonly crossreact with protein and/or sugar epitopes in preparations of *P. carinii* from other species.^{13,14} These data support the view that *P. carinii* from different host species are related but antigenically distinct, although antigenic differences are not necessarily directly indicative of genetic differences.

Analysis of genetic relatedness amongst *P. carinii* from different host species has been carried out. Chromosome analysis by gel electrophoresis has shown that *P. carinii* karyotypes from rat and human hosts were similar but distinct.¹⁵ Sequence analysis of a portion of a mitochondrial genome encoding ribosomal RNA¹⁶ and a segment of gene encoding the β tubulin gene⁸ has shown that *P. carinii* from rats and humans are different.

Sequence analysis of the mitochondrial ribosomal RNA gene from *P. carinii* in thoroughbred foals shows that it is distinct from rat, rabbit, ferret, and human *P. carinii*.¹⁷ In another study rat and human-derived *P. carinii* were compared by sequencing three genes.¹⁸ The sequences of 18S ribosomal RNA genes (441 bases) were 4% divergent in rat and human *P. carinii* – which is twice the divergence seen between two species of candida (*Candida albicans* and *Candida tropicalis*) and as great as that between ascomycetes from different genera such as *Podospora anserina* compared with *Neurospora crassa*. Analysis of two protein encoding genes, α tubulin and thymidylate synthetase, showed that rat and human *P. carinii* were 73% identical, an enormous degree of divergence given that the α tubulin gene from human *P. carinii* was also 73% identical to that from *Aspergillus nidulans*.

Although the relationship of genetic divergence to speciation is still not clear, the genetic divergence exhibited by these two kinds of *P. carinii* is of a degree typically seen amongst different species. These data and antigenic differences, together with apparent host specificity, clearly establish that human-derived

P. carinii is not the same organism as that from rats.

CAN DIFFERENT "SPECIES" OF PNEUMOCYSTIS CARINII INFECT THE SAME ANIMAL HOST?

Genetic divergence of *P. carinii* is not limited to *P. carinii* from different host species. Genetic variation within rat-derived *P. carinii* has been reported.¹⁵ Electrophoretic analysis of chromosomes from *P. carinii* prepared from individual rats from a given colony typically show a stable and specific electrophoretic karyotype containing 13–15 bands. The number of bands and their rates of migration in the gel vary between rat colonies but each rat within a given colony harbours *P. carinii* of the same karyotype.¹⁵ Some rats in some colonies contain *P. carinii* that shows an electrophoretic karyotype with 22 bands. It has subsequently been shown that the 22 bands are produced by two separate types of *P. carinii* that both infect the same rat¹⁹ (named prototype and variant). These two types differ not just in electrophoretic karyotype but also in the presence of a particular repeated DNA sequence in the presence of an intron in the 18S ribosomal RNA gene, and in the sequence of parts of the genes encoding 18S ribosomal RNA and transcription factor IID. Parasite populations from different co-infected rats contain prototype and variant in different proportions. The 18S ribosomal RNA genes of prototype and variant are 6.6% divergent and variant rat-derived *P. carinii* 18S ribosomal RNA gene is also 7.4% divergent from human-derived *P. carinii*. This large degree of divergence is significant when compared with divergence seen within this gene in other fungi. Changes in individual chromosome size have also been observed in isolates from the same rat colony over a period of time.^{15,20} The karyotype of *P. carinii* obtained from Sprague Dawley rats in Korea differs from the same strain of rats in Indianapolis, USA²¹ and the karyotype of Korean rat-derived *P. carinii* remains stable over a period of years.²¹

If rats can be infected by more than one species of *P. carinii*, sometimes at the same time, it might be that more than one species of *P. carinii* is capable of infecting humans, either singly or in combinations. If this was so then genetic variation may explain variations in serological response to *P. carinii* antigens and might account for differences in clinical presentation, treatment response, and geographical isolation.²² Studies of the nuclear ribosomal RNA operon from human-derived *P. carinii* from five HIV infected patients in the USA showed that, although the sequence of this gene was different from that of rat-derived *P. carinii*, each of the human-derived sequences was identical²³ and identical to that originally described.¹⁶ In another study of 12 patients the nucleotide sequences of large subunit mitochondrial ribosomal RNA of *P. carinii* were compared.²⁴ Five sequences (and five patients) were identical to the original description.¹⁶ Six of the sequences were different from the prototype sequence¹⁶ (but only showing polymorphism at

between one and three base positions). In one patient sequence analysis showed a marked difference from the prototype sequence and it appeared that this individual was infected with a hybrid of human and rat *P. carinii* sequences.²⁴ In contrast to this study suggesting that different strains may infect humans, analysis of a mitochondrial locus encoding the large subunit of ribosomal RNA from human-derived *P. carinii* obtained from four geographical locations (Memphis, USA; Porto Alegre, Brazil; Harare, Zimbabwe; and London, UK) in another study showed striking lack of sequence variation between these isolates with only a single base polymorphism (seen in the same position as that reported by Lee²⁴). In this study no individual was infected by more than one strain and there were no sequences identified which were hybrids of rat and human *P. carinii*.²⁵ This study also showed a lack of sequence variation between samples obtained from one geographical location (London) over a four and a half year period.²⁵ A similar lack of genetic variation is seen when the sequence of the *arom* gene is compared in isolates of human-derived *P. carinii* obtained from the same four geographical locations.²⁶ These data question the hypothesis that the relative prevalence of *P. carinii* in humans in different locations is due to strain differences but leave an unresolved paradox, that of apparent strain stability in the human host in contrast to the rat.

IS PNEUMOCYSTIS CARINII INFECTION DUE TO REACTIVATION OR REINFECTION?

If *P. carinii* grows only in mammalian hosts then transmission is likely to occur between hosts. *P. carinii* in laboratory rats exhibits these features. The organism is transmitted from rat to rat through inhalation and immunocompetent rats can harbour low numbers of *P. carinii* (the presence of which can be demonstrated by development of pneumocystosis if the rats are subsequently immunosuppressed in a contained environment with filtered air).²⁷ It seems also that a carrier state may exist within immunocompetent rats. *P. carinii* DNA can be detected by the PCR in the lungs of immunocompetent sentinel rats housed near immunosuppressed *P. carinii* infected rats and disappears rapidly from the lungs of these sentinel rats when they are isolated from the immunosuppressed infected animals.²⁷

It seems, however, that healthy adult humans rarely carry *P. carinii* in numbers high enough to allow transmission of the organism.^{28,29} Leigh *et al* have shown that immunocompetent health care workers in an AIDS unit exposed to patients with *P. carinii* have higher serum antibodies against *P. carinii* than health care workers based in a care of the elderly unit. This suggests that asymptomatic carriage has occurred as a result of environmental exposure.³⁰ However, careful studies of lung tissue obtained at post mortem examination from HIV negative individuals have failed to reveal evidence of pneumocystis colonisation.²⁸ Alternatively, it is possible that *P. carinii* has an environmental source and is acquired by in-

halation from the air, a situation reminiscent of the opportunistic fungal pathogen *Histoplasma capsulatum*. It is known that *P. carinii* infection in HIV positive patients shows a seasonal variation, with peaks in the late spring and late summer/early autumn which appear to be related to variations in environmental temperature and humidity, factors that are important for growth and sporulation of fungi.³¹⁻³³ This assumes that there is an, as yet, morphologically unidentified environmental source of infectious particles and thus a third stage of the life cycle. Recent data confirm isolation of genetic material of *P. carinii* from spore trap samples and a cascade impactor placed in an orchard in a rural location in the United Kingdom. On sequence analysis the gene encoding the large subunit of mitochondrial ribosomal RNA from this material is found to be identical to *P. carinii*.³⁴ Preliminary observations in immunocompetent health care workers with variable exposure to patients with pneumocystis pneumonia, using DNA amplification on samples of induced sputum, suggest a carrier state may exist and that those having the closest contact with patients who have pneumocystis pneumonia have the greatest likelihood of carriage (Wakefield and Miller, unpublished observations).

In another study *P. carinii* colonisation was studied in induced sputum samples from 70 asymptomatic men using the PCR.³⁵ There were 10 heterosexual controls, 10 HIV negative homosexual controls, and 50 HIV positive homosexuals (20 had CD4 counts $>0.4 \times 10^9/l$, 20 had CD4 counts <0.4 but $>0.06 \times 10^9/l$, and 10 had CD4 counts $<0.06 \times 10^9/l$). *P. carinii* DNA was detected in none of the controls, in 10% of those with CD4 $>0.4 \times 10^9/l$, in 20% with CD4 $<0.4 \times 10^9/l$, and in 40% of those with CD4 $<0.06 \times 10^9/l$. This study clearly demonstrates *P. carinii* colonisation of bronchial secretions in asymptomatic HIV positive homosexuals in association with low CD4 counts.

CLINICAL APPLICATIONS OF MOLECULAR TECHNIQUES

Although DNA amplification by the PCR is still largely a research tool, it has already shown promise in studies of HIV positive immunosuppressed patients, being superior to conventional silver staining for diagnosis of *P. carinii* infection from bronchoalveolar lavage fluid and induced sputum,^{36,37} and showing higher sensitivity than immunofluorescence staining for *P. carinii* in samples of induced sputum from transplant patients receiving immunosuppressive drugs.³⁸ In a prospective comparison of DNA amplification with *P. carinii* specific primers and immunofluorescence (using a murine monoclonal antibody to the cyst wall of *P. carinii*) DNA amplification was between 10^4 and 10^6 times more sensitive than immunofluorescence for diagnosis of *P. carinii* infection in HIV positive men.³⁹ Interestingly, some patients in this study with obvious pneumocystis pneumonia (judged by typical presentations and response to treatment and

positive or equivocal immunofluorescence results) had negative DNA amplification results. All these patients had received several days of specific anti-pneumocystis treatment. This suggests that DNA amplification results may become negative soon after starting treatment for pneumocystis pneumonia. Ideally, samples for DNA amplification of *P. carinii*-specific DNA should be taken before or soon after starting treatment.³⁹

On samples of induced sputum DNA amplification provided a diagnostic yield for *P. carinii* of 95% (compared with 35% by microscopy after silver staining);³⁷ positive results were also obtained from sputum samples of poor quality regarded on microscopy as being unrepresentative of a lower respiratory tract specimen. In a preliminary prospective study DNA amplification was used to detect *P. carinii* in a saline mouthwash obtained from HIV positive patients with respiratory symptoms and signs and results were compared with silver staining and DNA amplification on paired bronchoscopic alveolar lavage.⁴⁰ This saline mouthwash was obtained just before bronchoscopy and lavage from 31 HIV positive patients. Eighteen patients had pneumocystis pneumonia, the diagnosis being made on typical clinical presentation, rapid response to specific treatment, and positive results of standard silver staining of bronchoalveolar lavage fluid. All 18 were also strongly positive by DNA amplification of bronchoalveolar lavage fluid. Thirteen patients had other diagnoses including *Mycobacterium tuberculosis*, non-Hodgkin's lymphoma, pulmonary Kaposi's sarcoma, bacterial pneumonia, and fungal pneumonia. Of the 18 patients with pneumocystis pneumonia nine had strongly positive DNA amplification results on analysis of the mouthwash and five others had less strongly positive results. All those without pneumocystis pneumonia had negative results for DNA detection giving an overall sensitivity of 78%. All those with pneumocystis pneumonia and negative DNA amplification results had received four or more days of intravenous co-trimoxazole and also had severe disease with markedly abnormal chest radiographs and hypoxaemia. It is not clear whether these negative results were, as originally reported by Leigh *et al.*,³⁹ due to the rapid disappearance of *P. carinii* DNA in response to treatment, or whether disease severity affects delivery of *P. carinii* organisms to the oropharyngeal space.

In a variant of *P. carinii* infection a granulomatous response occurs (see below). *P. carinii* cysts are enclosed by a palisade of histiocytes and only very small numbers of *P. carinii*, undetectable by silver staining, are present within the alveoli. Transbronchial biopsy samples are frequently negative because of the patchy nature of the disease, and a firm diagnosis may need to be established by open lung biopsy. DNA amplification on samples of bronchoalveolar lavage fluid from these patients has been found to be positive, although the amount of DNA detected is lower than that seen in patients with typical presentation of disease.⁴¹

Histology

Clinical infection with *P. carinii* most frequently results in a radiographically diffuse pneumonia which histologically consists of an eosinophilic intra-alveolar foamy exudate, associated with a mild plasma cell interstitial pneumonitis. By use of special stains routinely used for diagnosis such as Grocott's methenamine silver or toluidine blue O, the cysts of *P. carinii* are seen to lie within the foamy exudate. The exudate consists largely of the non-staining trophozoite forms of *P. carinii* which are best seen on electron microscopy.⁴² Unusually and atypically, pneumocystis pneumonia may present with radiological infiltrates limited to the upper lobes (particularly following prophylaxis with nebulised pentamidine⁴³), focal consolidation, nodular or cavitating lesions, or hilar lymphadenopathy. Pleural effusion is unusual and, if present, is small. The parasite infrequently extends beyond airspaces but rare cases of extrapulmonary pneumocystosis involving distant sites such as gut and liver have been reported, again associated with nebulised pentamidine prophylaxis.⁴⁴

Early in the AIDS epidemic transbronchial biopsies were routinely carried out, in addition to bronchoalveolar lavage, at fibreoptic bronchoscopy for diagnosis of pneumocystis pneumonia and other pulmonary conditions. Transbronchial biopsies are no longer routinely performed as they do not add to the diagnostic yield for pneumocystis pneumonia and other conditions (such as bacterial and mycobacterial infection) compared with bronchoalveolar lavage, and are associated with morbidity from pneumothorax and haemorrhage.⁴⁵ Transbronchial biopsy samples are required to establish the diagnosis of non-specific pneumonitis, lymphoid interstitial pneumonitis, or lymphoma. Open lung biopsies are rarely carried out because of the high yield from bronchoalveolar lavage.

A retrospective review of 138 episodes of pneumocystis pneumonia in an institution where transbronchial biopsy was not performed showed eight episodes (6%) with atypical histological appearances.⁴² In six episodes the diagnosis was made by lung biopsy (open in five and percutaneous in one) after negative bronchoscopic investigations and in two the diagnosis was made at post mortem examination having been suspected clinically before death. Of the six diagnosed before death, the decision to perform a biopsy was made because of failure to respond to anti-pneumocystis treatment in three cases, or because of atypical radiographic appearance in three cases. Atypical histological appearances were granulomatous inflammation in four cases, two cases having a "pneumocystoma" consisting of foamy eosinophilic material surrounded by fibroblasts; Grocott's methenamine silver staining showed *P. carinii* cysts within the foamy material (one case also had typical histological pneumonia elsewhere in the lungs, together with *P. carinii* in hilar lymph nodes – that is, extrapulmonary pneumocystosis). One case (who had evidence of co-infection with cytomegalovirus, with both intranuclear and intracytoplasmic inclusions)

had diffuse alveolar damage, subpleural emphysema, patchy intrapulmonary calcification and fibrosis, and extrapulmonary pneumocystosis. The final case, in addition to typical plasma cell interstitial pneumonia, had bronchiolitis obliterans organising pneumonia (BOOP).

It is possible that the prevalence of atypical histological appearances is even higher and that some cases are missed because biopsies are not performed. In a retrospective review from the National Institutes of Health, Bethesda, Maryland, USA, where transbronchial biopsies are routinely performed, atypical histological features were identified in 123 biopsy specimens (117 transbronchial biopsies, six open lung biopsies) from 76 HIV infected patients.⁴⁶ The findings were interstitial fibrosis in 77 specimens (63%), intraluminal fibrosis in 44 (36%), granulomatous inflammation in six (5%), parenchymal cavities and interstitial patchy calcification each in three (2%), and vascular invasion/vasculitis caused by *P. carinii* in one. More than one histological abnormality was identified in some specimens. Overall six (8%) patients had co-infection with cytomegalovirus.

In a third study three cases of variant pneumocystis pneumonia were described.⁴⁷ The patients all had long clinical histories, chest radiographs showing focal abnormalities and negative results from bronchoscopic investigations, including transbronchial biopsy. The diagnosis was made in these patients by open lung biopsy. Histological examination revealed extensive interstitial fibrosis, occasional giant cells, and honeycombing.

These two studies^{42,47} emphasise that open lung biopsy still retains an important role in a small proportion of HIV positive patients with respiratory disease. In a recent retrospective analysis of 19 HIV positive patients with negative bronchoscopic findings who underwent open lung biopsy, seven patients had non-specific interstitial pneumonitis (one also had BOOP), two had Kaposi's sarcoma, two had BOOP, one patient each had pulmonary haemorrhage, lymphoma, necrotic lung without infection, and normal lung.⁴⁸ This study demonstrated, not only the range of diagnoses made by open lung biopsy, but also the very important fact that the diagnoses made had an important bearing on subsequent management; either appropriate treatment was instituted or inappropriate treatment could be withdrawn once the correct diagnosis had been made.

Investigation of *Pneumocystis carinii* pneumonia

EMPIRICAL TREATMENT

The optimal management strategy in an HIV positive patient with respiratory symptoms, an abnormal chest radiograph, and suspected pneumocystis pneumonia remains controversial. Some groups advocate microbiological/cytological confirmation of the diagnosis in all cases, whereas others have suggested that empirical treatment (without bronchoscopy) can safely be used for patients with typical symptoms, chest radiographic ab-

normalities, and hypoxaemia.⁴⁹ The revised Centres for Disease Control (CDC) Surveillance case definition criteria and the Communicable Disease Surveillance Centre (CDSC) criteria both allow a presumptive diagnosis of pneumocystis pneumonia (and therefore a diagnosis of AIDS) if an HIV positive patient presents with dyspnoea on exertion or non-productive cough of recent onset, chest radiographic evidence of diffuse bilateral interstitial lung disease, arterial hypoxaemia, and no evidence of a bacterial pneumonia.⁵⁰ In these patients the need to confirm a diagnosis has been questioned and there are now many physicians who treat empirically patients with classic presentations of pneumocystis pneumonia, with bronchoscopy reserved only for those who fail to respond to empirical treatment.⁵¹

A recent study, using a decision analysis model constructed with baseline probabilities derived from published data and expert opinion, set out to determine whether early bronchoscopy for all patients or empirical treatment with co-trimoxazole or pentamidine and steroids for pneumocystis pneumonia, with delayed bronchoscopy at five days in non-responders, was the superior management strategy in patients with CDC criteria for presumptive pneumocystis pneumonia.⁵² The expected one month survival rate (with and without quality of life adjustment) was found to be essentially the same for the two strategies. The authors of this study suggested that, as early bronchoscopy does not offer any additional survival benefits and is associated with greater costs and manpower resources, empirical treatment appeared to be the superior management strategy.

However, the general brief of *P. carinii* has extended and the diagnosis should not just be considered in the case of HIV positive patients with late stage disease and low CD4 lymphocyte counts. During primary infection with HIV there is often profound and transient reduction in circulating CD4 lymphocytes and it is during this phase, which coincides with "sero-conversion", that pneumocystis pneumonia can occur. This has recently been described in three patients; a good clinical recovery occurred and CD4 counts rose spontaneously.⁵³ Furthermore, recent reports document pneumocystis pneumonia occurring in eight patients with no known underlying immune deficit with normal immunological laboratory functions who were repeatedly seronegative for HIV.^{54,55}

GENERAL DIAGNOSTIC STRATEGY

Of the non-invasive tests available for evaluation of these patients, decline in lung function measurements, in particular the carbon monoxide transfer factor (TLCO) remains an extremely sensitive, but rather non-specific, measure of pulmonary abnormality in HIV infected individuals.⁵⁶ A low value for TLCO therefore does not mean that opportunist lung infection is present,⁵⁷ but is of value as it is a test with a good negative predictive value – that is, a normal TLCO value virtually excludes the presence of pneumocystis pneumonia.^{56,58} Serial measurements are also of some value in

determining the presence of new organic disease when a reduction in TLCO occurs. Serum lactate dehydrogenase (LDH) levels are raised in pneumocystis pneumonia and have been advocated as being of diagnostic use.⁵⁹ Widespread use of anti-pneumocystis prophylaxis generally (and aerosolised pentamidine prophylaxis specifically) has raised concerns that relapse, when it occurs, may be clinically and radiographically atypical. In this situation the finding of a raised serum LDH level may have diagnostic use.⁶⁰ Exercise induced oxygen saturation is probably the most sensitive and specific non-invasive test for diagnosis of pneumocystis pneumonia.^{61,62} An exercise induced desaturation of three points was found to be 100% sensitive and 77% specific for pneumocystis pneumonia.⁶¹ This test is cheap and simple to carry out, and a normal exercise test virtually excludes the presence of pneumocystis pneumonia. In addition, the test is not affected by the use of anti-pneumocystis prophylaxis. An exercise induced desaturation to 90% or below, taken with typical bilateral interstitial shadowing on the chest radiograph, is highly predictive for the presence of pneumocystis pneumonia.⁶²

INDIUM-111 LABELLED HUMAN POLYCLONAL IMMUNOGLOBULIN SCANNING

Human polyclonal immunoglobulin (HIG) labelled with indium-111 (¹¹¹In) localises sites of infection in immunocompetent and immunodeficient/suppressed patients. In a prospective study ¹¹¹In-HIG scanning was used to investigate 55 HIV positive patients presenting with acute respiratory episodes. Overall, 17 patients were found to have pneumocystis pneumonia, 20 had other infections (seven bacterial pneumonia, six *Mycobacterium avium-intracellulare*, five cytomegalovirus, two fungal), eight had tumours (five Kaposi's sarcoma, three intrapulmonary lymphoma), and in 10 patients extrapulmonary causes for the presentation were confirmed.⁶³ Of those with pneumocystis pneumonia 14 had diffuse intrapulmonary accumulation of ¹¹¹In-HIG (of these six had diffuse chest radiographic changes, two had focal abnormalities, and six were normal) and three had focal accumulation; all had focal chest radiographic abnormalities. Diffuse accumulation of ¹¹¹In-HIG was also seen in four with other infections; all had normal chest radiographs. The remainder had focal ¹¹¹In-HIG accumulation and chest radiographic abnormalities. No intrapulmonary accumulation of ¹¹¹In-HIG occurred in those with Kaposi's sarcoma or lymphoma, nor in nine of the 10 in whom an extrapulmonary cause was found, the exception being a patient with acute renal failure.

Like gallium-67 (⁶⁷Ga) citrate scanning, ¹¹¹In-HIG can distinguish between infection and Kaposi's sarcoma but, unlike gallium, it can also distinguish infection from lymphoma.⁶⁴ ¹¹¹In-HIG is particularly useful in differentiating pulmonary Kaposi's sarcoma from pulmonary Kaposi's sarcoma with co-infection in patients presenting with abnormal

chest radiographs and respiratory symptoms. Compared with ⁶⁷Ga the imaging protocol for ¹¹¹In-HIG is shorter (48 hours versus 72 hours) and the dose of radiation to the patient is lower.

¹¹¹In-HIG scanning should be considered before proceeding to open lung biopsy for confirmation of the diagnosis in patients with suspected pneumocystis pneumonia and negative results from bronchoscopy and bronchoalveolar lavage or no response to specific therapy, in whom tumour (mimicking infection) may non-invasively be distinguished from pneumocystis pneumonia.

Prognosis of *Pneumocystis carinii* pneumonia

Once the diagnosis of pneumocystis pneumonia has been established a number of clinical features have prognostic significance, including the degree of hypoxaemia and respiratory failure, extent of radiographic shadowing, duration of history, level of elevation of serum LDH enzyme, the presence of neutrophils in bronchoalveolar lavage fluid, and the degree of interstitial oedema on transbronchial biopsies. It has recently been shown that a pneumocystis pneumonia severity score based on serum LDH enzyme levels, the alveolar arterial oxygen gradient, and the percentage of neutrophils in bronchoalveolar lavage fluid has very high prognostic significance for survival, with the highest score carrying very high negative predictive values for survival.⁶⁵ Furthermore, the quantity of *P. carinii* in transbronchial biopsy samples correlated strongly with overall interstitial inflammation and oedema and tissue accumulation of neutrophils, lymphocytes, and macrophages.⁶⁶ The *P. carinii* cyst burden in bronchoalveolar lavage fluid correlated with oedema formation, but not with the percentage of neutrophils, lymphocytes, or macrophages in bronchoalveolar lavage fluid. Both the *P. carinii* cyst burden and neutrophil count in bronchoalveolar lavage fluid correlated with PaO₂ and serum LDH enzyme levels, but the short and long term survival times were not affected by the quantity of *P. carinii* or inflammatory cells in either transbronchial biopsy tissue or bronchoalveolar lavage fluid.⁶⁶

Treatment of *Pneumocystis carinii* pneumonia

A wide range of antimicrobial agents is effective against *P. carinii* (table 1). These have been reviewed in detail elsewhere.⁶⁷ It is helpful to stratify cases of pneumocystis pneumonia into mild, moderate, and severe.^{2,67} High dose co-trimoxazole remains the agent of first choice for pneumocystis pneumonia and is given for three weeks, intravenously in most patients until clinical improvement occurs followed by oral therapy to complete the 21 day course. Mild cases can be treated with oral therapy from the outset. Pentamidine has to be given intravenously and is more toxic than co-trimoxazole; it appears to be about as effective.^{68,69} Dapsone and trimethoprim appear to be as effective as co-trimoxazole for mild to moderate

Table 1 Treatment of *Pneumocystis carinii* pneumonia

First choice	Co-trimoxazole
Second choice	Pentamidine Dapsone and trimethoprim Clindamycin and primaquine Atovaquone
Adjuvant therapy	Glucocorticoids
Rarely used	Trimetrexate Difluoromethyl ornithine (eflornithine) Nebulised pentamidine

pneumocystis pneumonia and have fewer side effects.⁷⁰

CLINDAMYCIN AND PRIMAQUINE

This combination is now widely used in the major AIDS centres but there are few data from large prospective studies comparing its efficacy with other drugs such as co-trimoxazole. There is no licence for use of clindamycin and primaquine for the treatment of pneumocystis pneumonia in the United Kingdom. The combination has been evaluated in small pilot comparisons and appears to be as effective as co-trimoxazole for treatment of mild to moderate pneumonia.⁷¹ It is used in doses of clindamycin given intravenously (600 mg four times daily) or by mouth (300–450 mg four times daily, usually after initial intravenous treatment) and primaquine by mouth (15 mg daily). Approximately half of patients develop rash and a quarter develop diarrhoea; if this side effect does occur, samples of stool should be analysed for the presence of *Clostridium difficile* toxin. Primaquine should be avoided in patients with glucose-6-phosphate dehydrogenase deficiency because of the risk of haemolysis.

ATOVAQUONE

In laboratory animals atovaquone (previously called BW566C800), a 1,4-hydroxynaphthoquinone, is at least as effective as co-trimoxazole in the prevention of treatment of murine pneumocystis pneumonia. The exact mechanism of action is not known; in *Plasmodium falciparum* infection hydroxynaphthoquinones interfere with the electron transport chain, indirectly inhibit the activity of dihydro-orotate dehydrogenase, and stop de novo synthesis of pyrimidines.

A double blind multicentre prospective study has compared oral atovaquone 750 mg three times daily with oral co-trimoxazole 1920 mg three times daily, both given for 21 days, in HIV positive patients with mild to moderately severe pneumocystis pneumonia.⁷² Of 322 patients studied 160 received atovaquone and 162 received co-trimoxazole. Twenty percent of patients given atovaquone and 7% given co-trimoxazole did not respond. Treatment limiting side effects (nausea, vomiting, constipation, fever, and rash) were more frequent in patients treated with co-trimoxazole (20%) than in those given atovaquone (7%). Within four weeks of completion of treatment there were 11 deaths in the atovaquone group (four

due to pneumocystis pneumonia) and one death in the co-trimoxazole group. Patients with diarrhoea at entry to the study had lower plasma drug concentrations, a higher therapeutic failure rate, and greater risk of death in the atovaquone group, but not in the co-trimoxazole group. Atovaquone is less effective than co-trimoxazole for the treatment of mild to moderate pneumocystis pneumonia but has fewer treatment limiting side effects.⁷² It is available in the UK.

ADJUVANT STEROIDS

The role of high dose corticosteroids in patients with respiratory failure associated with pneumocystis pneumonia is now clearly established, with reductions in both morbidity and mortality for patients with a Pao₂ of ≤9.3 kPa or an alveolar-arterial oxygen gradient of ≥4.7 kPa on admission.²⁶⁷

DRUGS NOT ROUTINELY USED TO TREAT PNEUMOCYSTIS CARINII PNEUMONIA

Both trimetrexate and difluoromethyl ornithine have been given as salvage therapy in patients who have not responded to, or could not tolerate, co-trimoxazole or pentamidine. Neither is licensed in the UK and their use is not widespread. Nebulised pentamidine enjoyed a vogue several years ago as first line treatment for mild to moderate pneumocystis pneumonia. Success rates ranged from 60% to 85%, which are comparable to co-trimoxazole. However, there is a slow response to treatment and both upper lobe relapse and extrapulmonary disseminated pneumocystosis have caused concern.⁴⁴

INTENSIVE CARE FOR SEVERE PNEUMOCYSTIS CARINII PNEUMONIA

In the first few years of the AIDS epidemic the decision to admit HIV infected patients to the intensive care unit was rarely questioned. It soon became apparent that patients with severe progressive pneumocystis pneumonia and respiratory failure had a very poor prognosis, with survival rates to hospital discharge of 0–14%. As a result the number of admissions to intensive care units decreased markedly in North America. More recently survival rates of 35–55% have been reported; the reasons for this are not immediately clear but it has resulted in a threefold increase in intensive care unit bed usage by AIDS patients in the United States. A similar trend appears to be emerging in the United Kingdom. The improvement in short term survival prospects for individuals with severe pneumocystis pneumonia is occurring on a background of a slowly improving long term survival for individuals infected with HIV. Primary and secondary prophylaxis for pneumocystis pneumonia may also be playing a part.⁷³

A recent study from Paris of HIV positive patients with severe pneumocystis pneumonia and respiratory failure requiring transfer to the intensive care unit for intubation and mech-

anical ventilation found that no prognostic factors identified survivors from non-survivors, apart from the interval between the start of specific anti-pneumocystis therapy and the need for mechanical ventilation and the duration of known HIV seropositivity (survivors having a shorter HIV history).⁷⁴ The mortality rate was 50% in those patients in whom mechanical ventilation was started early in the course of pneumocystis related respiratory failure (all patients received co-trimoxazole and corticosteroids for less than five days); in those who had received co-trimoxazole and steroids for five or more days mortality was 96%. From this study it appears that the prognosis of HIV positive patients with pneumocystis pneumonia and respiratory failure requiring mechanical ventilation remains poor, particularly if the mechanical ventilation follows the failure of anti-pneumocystis and steroid treatment. It is probable that the early use of steroids has changed the course of pneumocystis associated respiratory failure. Firstly, despite increasing numbers of HIV positive patients with pneumocystis pneumonia, a smaller proportion of patients are admitted to intensive care units for treatment of respiratory failure. Secondly, it has selected out patients in whom anti-pneumocystis treatment has failed and who have more severe pneumocystis pneumonia.

Prophylaxis of *Pneumocystis carinii* pneumonia

Primary prophylaxis against pneumocystis pneumonia (to prevent the first episode) should be offered to any patient with a CD4 (T helper) lymphocyte count below $0.2 \times 10^9/l$ (normal range = $0.35-2.2 \times 10^9/l$) or a CD4:total lymphocyte count ratio below 1:5, and to any HIV positive patient with oral thrush or unexplained fever, regardless of CD4 count. Those with other AIDS-defining diagnoses such as Kaposi's sarcoma, cerebral toxoplasmosis, or cryptococcal meningitis should also receive primary prophylaxis regardless of CD4 count. Secondary prophylaxis (to prevent subsequent episodes) should be offered to those who have already had an episode of pneumocystis pneumonia.⁶⁷

In a recent study from Canada the influence of anti-pneumocystis prophylaxis on hospital admission patterns in HIV positive patients was assessed.⁷⁵ Widespread use of aerosolised pentamidine for prophylaxis was introduced in 1989. Prior to this, in 1988, 68% of admissions of HIV positive patients were for infections and, following the introduction of prophylaxis, this percentage remained the same at 68%. Before prophylaxis of those admitted with infection, 48% had pneumocystis pneumonia and 52% other forms of infection, whereas after prophylaxis was introduced the number had fallen to 29% with pneumocystis pneumonia and 71% with other forms of infection. Similar changes have been reported from London.¹ Admissions for respiratory illness accounted for 34% of all admissions before the introduction of anti-pneumocystis prophylaxis and 27% following its introduction. Of those respiratory

admissions before prophylaxis 68% had pneumocystis pneumonia, 14% had bacterial infections, and the rest had other respiratory disease, whereas after introduction of prophylaxis the proportion with pneumocystis pneumonia had fallen to 48%, 23% had bacterial infections, and the rest had other respiratory disease (including 12% who had pulmonary Kaposi's sarcoma).

These studies suggested that prophylaxis for pneumocystis pneumonia is effective. It is, perhaps, surprising that more dramatic falls in the number of cases of pneumocystis pneumonia have not been seen in view of the proven efficacy of prophylaxis. This raises issues of compliance which may be poor for a number of reasons. Firstly, there are various cultural trends amongst individuals at high risk of acquiring HIV infection that tend to mitigate against traditional allopathic medicine and favour alternative or natural remedies. Secondly, many agents used routinely in HIV infections such as anti-retroviral drugs have received a bad press in recent years and this may cast a general scepticism towards taking any regular medication; this may also apply to anti-pneumocystis prophylaxis. Finally, toxic drug reactions are common in HIV infected individuals and adverse drug reactions may be a further reason for poor compliance with prophylactic medication.

CO-TRIMOXAZOLE

Co-trimoxazole 960 mg (800 mg sulphamethoxazole and 120 mg trimethoprim) once daily or three times a week is the most effective regimen for both primary and secondary prophylaxis^{76,77} (table 2). Rash, which occurs in approximately 20%, is the commonest side effect. Co-trimoxazole does not appear to interact with zidovudine at these doses.⁷⁶

NEBULISED PENTAMIDINE

Nebulised pentamidine (300 mg once per month via a Respigard II nebuliser) is less effective than oral co-trimoxazole^{76,77} but has fewer side effects (cough and bronchoconstriction are the most frequent). As nebulised pentamidine is not systemically absorbed, it is unlikely to prevent extrapulmonary pneumocystosis.⁴⁴

DAPSONE AND PYRIMETHAMINE

Dapsone (50 mg daily) and pyrimethamine (50 mg once per week) with folic acid (25 mg once per week) is as effective as nebulised pentamidine (dose as above) for primary prophylaxis, but is less well tolerated.⁷⁸ Dapsone (100 mg daily) and pyrimethamine (25 mg per

Table 2 Prophylaxis of *Pneumocystis carinii* pneumonia

First choice	Co-trimoxazole
Second choice	Nebulised pentamidine
	Dapsone
	Dapsone and pyrimethamine
	Sulfadoxine and pyrimethamine

week) is less effective than co-trimoxazole given three times a week.⁷⁹

Conclusions

Molecular biological techniques have clearly demonstrated that *P. carinii* is a fungus and that there is genetic diversity with different strains infecting different animals.

An improved understanding of the epidemiology of the natural history of *P. carinii* suggests that clinical disease arises by re-infection and not reactivation. Frequent re-infection results in colonisation in some healthy individuals and colonisation, with subsequent disease, in the immunodeficient.

An empirical approach to diagnosis for patients with typical presentations remains an appropriate management strategy, but other diagnoses may be missed. For those with atypical presentation or lack of response to treatment bronchoscopy, bronchoalveolar lavage, transbronchial biopsy, or open lung biopsy may be necessary to secure a diagnosis.

Indirect diagnostic tests are of little diagnostic use except to confirm the presence of organic disease (in contrast to a common cold) in a symptomatic HIV positive patient. With further calibration DNA amplification on saliva/mouthwash samples may become a highly sensitive and specific non-invasive diagnostic tool for diagnosis of *P. carinii*.

There has been little change in recommendations for treatment over the last two years: co-trimoxazole remains the agent of choice, and is so effective that it will be difficult to displace it as first line therapy. Nebulised pentamidine should not be used to treat pneumocystis pneumonia. The overall death rate from pneumocystis pneumonia in the UK is now about 5%, somewhat less than that of hospitalised patients with community acquired pneumonia.

Anti-pneumocystis prophylaxis is effective, the drug of first choice being oral co-trimoxazole. Clearly, significant numbers of HIV positive patients with low CD4 lymphocyte counts are not taking prophylaxis as many continue to present with pneumocystis pneumonia.

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look at first glance as though ICI 118 551 has no effect on exercise-induced hyperkalaemia compared with placebo. However, the baseline potassium concentration was significantly lower in the ICI 118 551 group than in the placebo group, and this may explain the apparent lack of effect on exercise response. Furthermore, in that study atenolol had no effect on the exercise-induced potassium response, suggesting that β_1 receptors are not involved. Indeed, in our own study salbutamol had no effect on the exercise heart rate response, showing that β_1 blockade did not occur. It has also been shown that a low dose of nadolol (5 mg), which has a minimal β_1 blockade, exhibits marked β_2 blockade as assessed by exercise-induced hyperkalaemia.³ Thus, the fact that in our study both salbutamol and propranolol significantly augmented the exercise-induced potassium response is in keeping with a β_2 receptor mediator mechanism linked to the Na/K-ATPase pump. This hypothesis is further supported by the enhancement of exercise-induced hyperkalaemia by digitalis which directly inhibits the Na/K-ATPase pump.⁴

We therefore remain of the firm opinion that, in the presence of high adrenergic tone, salbutamol exhibits β_2 antagonist activity as demonstrated by augmentation of exercise-induced hyperkalaemia. This would be in keeping with the known pharmacological properties of salbutamol as a partial β_2 agonist along with in vitro data showing salbutamol to antagonise the relaxant effect of isoprenaline in the presence of carbachol-induced bronchoconstriction.⁵ Whilst we agree that salbutamol is of great value in treating acute bronchospasm, it may be that a β_2 agonist with greater intrinsic activity may be preferable in certain situations where airway tone is increased.

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Paradoxical vocal cord adduction in cystic fibrosis

Woe to the authors who claim a first (June 1995;50:694-5), and to the editor and referees who allow the claim to be published! Paradoxical vocal cord adduction has indeed been described before in cystic fibrosis¹ and cited in a recent review.² It is not exclusively seen in girls; our own experience in asthma (in preparation) and that of others³⁻⁵ includes boys with the condition. Useful pointers in

the history include the disappearance of symptoms when asleep, and, in the investigations, a significant disparity between the results of bedside spirometric tests and those obtained by a really experienced lung function technician. We agree that the possibility of this condition should be considered in patients with known proven airway disease, preferably at an early stage, before too many unnecessary investigations and potentially toxic treatments have been inflicted on the sufferer.

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BOOK NOTICES

ABC of Asthma. 3rd edition. John Rees and John Price. (Pp 49; £8.95). London: BMJ Publishing Group, 1995. 0 7279 0882 0.

This book is one of a series of ABC publications produced by the BMJ Publishing Group, and is the third edition devoted to asthma in adults and children. Some chapters have been reproduced in recent editions of the *British Medical Journal*.

The book is divided into two sections, the first and longer one pertaining to adult asthma and the second to asthma in children, although there is of course some overlap between the two. Chapters cover the definition, diagnosis, prognosis, and treatment of acute and chronic asthma – the latter based on guidelines recently produced by the British Thoracic Society. Subsections within chapters are clearly labelled, short for easy reading, and each has an associated illustration.

Recent interesting insights into risk factors for childhood asthma and hence prospects for prevention are discussed, as are other topical issues in asthma management such as the safety of β_2 agonists and CFC-free metered dose inhalers, although gastro-oesophageal reflux is not mentioned in an otherwise excellent chapter on precipitating factors of asthma. The possibility of further reading into these subject areas is limited, however, by the absence of a bibliography.

One minor criticism is that some inconsistencies in terminology may be confusing for those unfamiliar with the subject. For example, the authors use the term "airway responsiveness" interchangeably with "bronchial reactivity", similarly β_2 agonists are variably described as β stimulants, β_2 adrenergic agonists (and even β antagonists at one point).

Overall, the book provides a simple over-

view of asthma which medical students, junior doctors, nurses, and allied professionals will find up to date and easy to read. – HB

Patient Education and Chronic Obstructive Pulmonary Disease. Annelees H S van den Broek. (Pp 158; £12.00). Netherlands: Section of Clinical and Health Psychology, Leiden University, 1995. 90 802379 2 2.

This little book is laid out and reads like a PhD thesis. Essentially it consists of a literature review followed by detailed presentation of the results from a study of the effects of a patient education group programme during pulmonary rehabilitation. It contains useful references and a few stimulating ideas, but it lacks maturity. Many of the data are presented uncritically with no attempt at a distinction between clinically important and statistically significant findings. The book cannot serve either as an authoritative review of the field or as a primer for those wishing to set up a programme. Someone experienced in the field might find useful nuggets contained within it, however. The potential reader is advised to examine it before purchase.

The book comes from Holland and reflects the powerful influence of "the Dutch hypothesis" about the underlying processes in asthma and COPD. In places, where convenient, it treats the two as part of the same disease – that is, chronic non-specific lung disease (CNSLD). However, this approach is frequently abandoned and training programmes specifically for "asthma" or "COPD" are described. This ambiguity is not the fault of the author who is a non-clinician. It does raise a question of the practical value of a term that encompasses two conditions that, by implication, may be sufficiently well distinguished to demand different educational approaches.

No price was attached to my review copy, but it should be cheap. The translation from the Dutch is idiosyncratic in places, although perfectly comprehensible. If non-English work such as this can be made readily available in English at a modest price, then I think that imperfect grammar is acceptable. No matter how cheap the product, however, there is no excuse for a publisher to produce a book with a large number of spelling mistakes that could easily have been corrected using any word processor in a few minutes. – PJ

CORRECTION

Inadvertent duplicate publication

Parts of the review article by Drs R F Miller and D M Mitchell that appeared in *Thorax* 1995;50:191-200 on *Pneumocystis carinii* pneumonia were the same as a previously published review article by Professor J R Stringer in *Infectious Agents and Disease* 1993; 2:109-117. This was not known to the Editor of *Thorax* as the responsibility for producing and editing the review article was that of the series editors. The journal and the responsible author (Dr R F Miller) sincerely regret this breach of editorial ethics.