ORIGINAL ARTICLE

Lung fibrosis in deceased HIV-infected patients with *Pneumocystis* pneumonia

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Background. Pneumocystis pneumonia (PcP) is one of the most common opportunistic infections found in patients with HIV. The prognosis if ventilation is required is poor, with mortality of 36 - 80%. Although more recent studies have shown improved survival, our experience has been that close to 100% of such patients die, and we therefore decided to investigate further.

Methods. All patients with confirmed or suspected PcP who died owing to respiratory failure were eligible for the study. Where consent was obtained, trucut lung biopsies were performed post mortem, stored in formalin and sent for histopathological assessment.

Results. Twelve adequate lung biopsies were obtained from 1 July 2008 to 28 February 2011 – 3 from men and 9 from women. The mean age was 34.7 years (range 24 - 46), and the mean admission CD4 count was 20.8 (range 1 - 68) cells/µl and median 18.5 cells/µl. All specimens demonstrated typical PcP histopathology; in addition, 9 showed significant interstitial fibrosis. Three had co-infection with cytomegalovirus (CMV), two of which had fibrosis present. There was no evidence of TB or other fungal infections.

Conclusion. The high mortality seen in this cohort of PcP patients was due to intractable respiratory failure from interstitial lung fibrosis. Whereas the differential includes ventilator induced lung injury, drug resistance or co-infections, we suggest that this is part of the disease progression in certain individuals. Further studies are required to identify interventions that could modify this process and improve outcomes in patients with PcP who require mechanical ventilation.

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Since the introduction of antiretroviral therapy (ART) for individuals who are HIV-infected with AIDS, there has been a dramatic decline in the number of these patients presenting with Pneumocystis jerovicii pneumonia (PcP) in the developed world. In South Africa, the antiretroviral (ARV) rollout was delayed for political reasons until 2004; consequently, significant numbers of patients are still presenting with PcP as a cause of respiratory failure. These patients are either unaware of their diagnosis or have not started ARVs for reasons that include poor access to medical facilities and drugs, denial and lack of education. If patients with PcP require mechanical ventilation, the prognosis is poor, with mortality ranging from 36 - 80%.[1,2] In fact, prior to the availability of ARVs, such patients were not mechanically ventilated in South Africa as no definitive therapy was available. Once these agents became available to all HIV-positive patients with CD4 counts <200 cells/µl, it became feasible for them to be considered for ventilation. At the Charlotte Maxeke Johannesburg Academic Hospital, it soon became apparent that few of these patients survived, despite early initiation of both ART and effective chemotherapy for PcP. Management included use of ARDSNET low tidal volume strategies,[3] conservative fluid protocols, adjunctive corticosteroids and minimal sedation. Despite these, mortality remained extremely high while other units were reporting 50 - 79% cure rates.[1,4] It was consequently decided to prospectively investigate the patients who had died in the unit, with the aim of determining the causes of failure of therapy. Possibilities that had been considered for this failure were concurrent infections including cytomegalovirus,[5,6] *Cryptococcus neoformans*, mycobacterial or bacterial infections such as *Streptococcus pneumonia*, drug resistance, as well as pulmonary Kaposi's sarcoma.[7]

Methods

This was a prospective study to investigate histological findings of patients who died from confirmed or suspected

PcP. All patients in these two categories, with respiratory failure, were considered for the study. PcP was suspected in patients with clinically advanced HIV presenting with hypoxic respiratory failure with typical chest radiograph changes, including bilateral diffuse alveolar infiltrates, granular opacities or, occasionally, unilateral or focal infiltrates.[7] *Pneumocystis* was confirmed *ante mortem* on sputum from 4 of the patients using the Giemsa stain; and 9 had organisms seen on histological samples. The remaining 3 had markedly elevated beta-D-glucan (BDG) levels >500 pg/ml.[8] Pre-mortem biopsies or bronchial washings were not possible owing to the severity of the hypoxia. With family consent, multiple trucut biopsies were taken from different regions of the lungs of each patient after death. The specimens were stored in formalin and subsequently stained with Grocott, Gordon and Sweets, Alcian blue, Ziehl-Neelsen and haematoxylin and eosin. Ethics approval was given by the University of the Witwatersrand Ethics Committee.

Results

Sixteen lung biopsies were obtained from 1 July 2008 to 28 February 2011. Table 1 lists patient demographics and laboratory characteristics. Four were inadequate samples and therefore not included. The final 12 were from 3 male and 9 female patients. Mean age of patients was 34.7 years (range 24 - 46 years). Mean admission CD4 count 20.8 (range 1 - 68) cells/µl, and the median CD4 was 18.5 cells/µl.

ICU details

All 12 patients were admitted to the intensive care unit (ICU), where 10 were mechanically ventilated; none developed pneumothoraces. All received appropriate high-dose trimethoprim-sulfamethoxazole (TMP-SMX) with high-dose corticosteroids as primary management. None were on ART at the time of presentation.

Histopathology

All 12 of the final specimens demonstrated the typical histopathological pattern of PcP, including alveoli filled with frothy material, type 2 cell hyperplasia and pneumocystis organisms. In addition, 9 of the 12 showed evidence of interstitial fibrosis with expansion of the interstitium by fibroblasts and collagen of varying degrees of severity. There was significant destruction and distortion of the lung architecture, resulting in a marked decrease in available alveolar-endothelial surface area for diffusion (Fig. 1). Three had evidence of CMV co-infection with intracellular inclusion bodies, and 2 of these also showed evidence of fibrosis. One of the latter 2 had a super-added bacterial infection that was not evident in any of the other specimens. There was no evidence of TB or other fungal infection in any of the specimens.

Discussion

Pneumocystis pneumonia is still one of the most common opportunistic infections found in patients infected with HIV.[9] *Pneumocystis* is primarily an alveolar pathogen that does not invade the pneumocyte to which it tightly adheres. The histopathological changes that are seen are explained by the exuberant host inflammatory response to the organism, which promotes pulmonary injury in only some patients during infection. Severe *pneumocystis* pneumonia can result in a significant neutrophilic response that leads to diffuse alveolar damage, impaired gas exchange and respiratory failure.[10] *P. jirovecii* has specific proteases that have the ability to damage the lung interstitium, and endogenous host proteases including matrix metalloproteinases (MMP) are also secreted in response to an influx of pro-inflammatory mediators (Interleukin-6 (IL-6), Interleukin-8 (IL-8), monocyte chemotactic protein-1 (MCP-1), and tumour necrosis factor alpha (TNF-α)) from alveolar epithelial cells.[11,12] This can explain the extensive capillary leak and frothy hyaline material that fills the alveolus in typical PcP. It is possible that the extensive effacement of normal alveolar architecture with fibrosis demonstrated in these biopsies is part of a reparative process that may occur only in those individuals genetically predisposed to the development of fibrosis, so that not all patients with PcP behave similarly.

In our sample, 75% of the patients who died of refractory respiratory failure revealed varying degrees of interstitial fibrosis resulting in obliteration of the alveolar capillary interface and loss of surface area for diffusion with the remainder, demonstrating unresponsive PcP. The pattern of the former is similar to that of the fibrotic stage of acute respiratory

Table 1. Patient demographics and laboratory characteristics

Patient	Sex	Age (years)	CD4 cells/µl	PcP on sputum	BDG pg/ml	PcP on histo	CMV on histo	Fibrosis
SM	♂	27	11	N/A	N/A	Yes	No	Present
NM	♀	46	19	Yes	N/A	Yes	Yes	None
MS	♂	40	31	Yes	402	Yes	No	Present
DM	♀	33	1	Yes	>500	Yes	Yes	Present
NS*	♀	44	29	Yes	>500	No	No	Present
XD*	♀	24	n.a.	N/A	>500	Yes	No	Present
NM	♀	28	68	N/A	>500	Yes	No	None
TR	♀	24	22	N/A	>500	Yes	No	Present
NM	♀	25	7	N/A	>500	No	No	Present
AM	♀	46	18	N/A	n.a.	Yes	No	None
TM	♀	41	n.a.	N/A	>500	No	No	Present
JN	♂	38	2	N/A	51	Yes	Yes	Present

*Not ventilated.
N/A=not available.

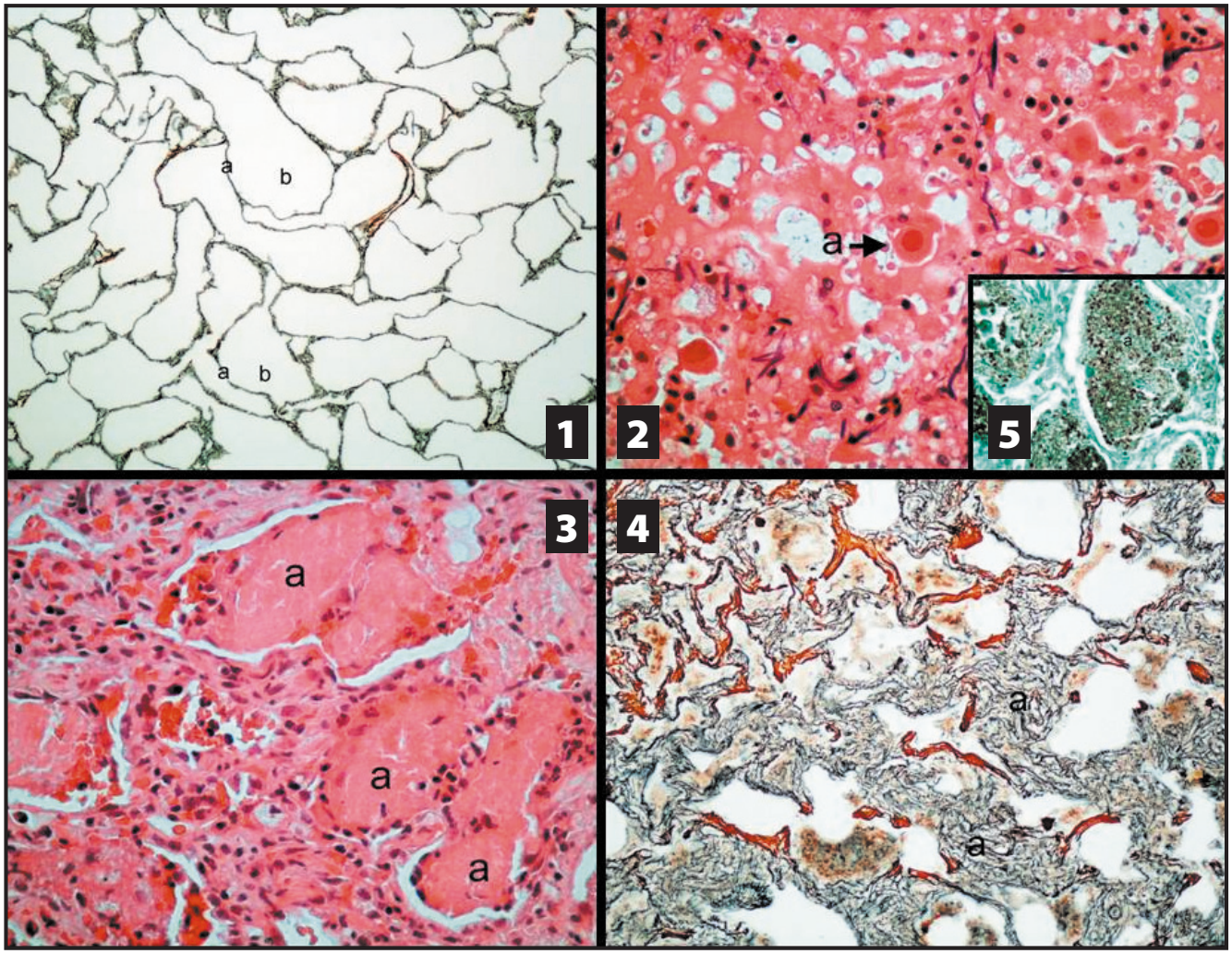

Fig. 1. Low magnification of alveoli showing normal interstitium of (a) alveolar walls and (b) alveolar spaces (Gordon and Sweet stain).
Fig. 2. High magnification showing an enlarged pneumocyte with (a) an intranuclear CMV inclusion (haemotoxylin and eosin stain).
Fig. 3. Low magnification showing (a) frothy exudate filling the alveolar spaces (haemotoxylin and eosin stain).
Fig. 4. Low magnification showing (a) marked expansion of the interstitium by fibrous tissue (Gordon and Sweet stain).
Fig. 5. Higher magnification showing pneumocystis organisms within frothy intra-alveolar exudates (Grocott stain).

distress syndrome (ARDS); and, whereas it might be argued that this could be consistent with ARDS following an infection by a more virulent organism such as *Streptococcus pneumoniae*, this organism was not cultured *in vivo*, and patients all received standard empiric therapy for community-acquired pneumonia. Importantly, none presented with the secondary organ dysfunction or systemic inflammatory response syndrome (SIRS), more typical of severe infections with this organism. In addition, all the patients received corticosteroids as a component of therapy for PcP that may be effective in the therapy of refractory ARDS owing to other causes.[13]

Another factor that could be responsible for the fibrotic injury is ventilator-induced lung injury (VILI). However, 2 of the 13 patients were not ventilated, and their biopsies showed similar interstitial fibrotic changes to those who were, and the other 11 were ventilated with tidal volumes ≤6ml/kg, and were recruited and maintained on appropriate PEEP, making this explanation unlikely.

In South Africa, where medical resources are limited, the majority of patients with PcP and respiratory failure (most of whom have PaO_2/FiO_2 ratios <200) are treated with oxygen via a re-breathing mask and appropriate pharmacological therapy in the general wards. Only the most severely hypoxic patients or those who fail therapy are considered for ventilation. This observation highlights a weakness in our sample, with a selection bias for those with a worse prognosis. Patients who were not considered candidates for ICU admission might have developed respiratory failure and died in the general medical wards, or more usually might have made a full recovery despite initial low P/F ratios. The latter, who in more resource-rich environments would have been admitted to ICU, could account for the high survival rates in other studies. Those admitted to ICU in South Africa are preselected, with most having already received and failed appropriate pharmacotherapy. It has previously been reported in the pre-ART era that patients who required ventilation despite adequate and appropriate therapy, have a poor prognosis.[4]

The reasons for the failure of therapy and the failure to benefit from mechanical ventilation have not previously been well described. Why some patients and not others develop fibrosis has also not been adequately elucidated. It could be argued that these patients might have had 2 disease processes: PcP superimposed on a more chronic condition or that this was an acute exacerbation of a more chronic underlying inflammatory process similar to

those that occur in the idiopathic interstitial pneumonias.[14] However, this does not explain why these patients had elevated β-D glucan levels in the absence of fungal infection elsewhere, and X-ray features not compatible with the interstitial pneumonias; and in the latter case, why the histological features were typical of PcP. CMV has been postulated to be more than a 'fellow traveller' in patients with PcP, and treatment with gancyclovir has been reported to improve outcome.[5,6] It is conceivable that infection by both organisms could be synergistic regarding the fibro-proliferative effects. However, in only 3 of our cohort was there evidence of CMV co-infection, 2 of whom did have fibrosis.

An important consideration for treatment failure is the possibility of resistance to sulfa drugs owing to mutations of *Pneumocystis* dihydropteroate synthase (DHPS) gene due to increased drug pressure from the widespread use of TMP-SMX prophylaxis. DHPS, the enzyme responsible for folate synthesis and the target of TMP-SMX, has undergone gene mutations that have been identified in 56% of *P. jirovecii* strains in South Africa.[15] However, as human strains of PcP cannot be cultured *in vitro*, it is difficult to prove that these mutations confer drug resistance. A number of studies have evaluated the effect of these mutations on clinical outcomes with conflicting results. Helweg-Larsen *et al*. demonstrated that patients infected by organisms with a DHPS mutation had a threefold increased risk of death.[16] Navin and colleagues, however, found no association with mortality at 6 weeks nor with treatment failure.[17] In fact, they found that 85% of patients with DHPS mutations treated with TMP-SMX responded to treatment.

A limitation of our study is the small sample size. In view of our resource-limited setting, these patients are not often viewed as good ICU candidates. Therefore, even though the PCP burden in South African hospitals is high, the available ICU PcP population is restricted. We feel that these are important data and will add to the understanding of the clinical course of these patients, even taking into account the small sample size.

Interstitial fibrosis has previously been demonstrated in patients who have survived an episode of PcP, as well as on previous necropsy studies.[4,18] There have also been histological reports of cryptogenic organising pneumonia, granulomatous inflammation and diffuse alveolar damage.[19] Our cohort, however, was unusual in that the majority of patients with PcP, most of whom were ventilated, had evidence of extensive pulmonary fibrosis – which was associated with an extremely poor prognosis. This phenomenon has been described previously; however, it has not been highlighted as the probable underlying cause for treatment failure and death. We suggest that, if we want to improve the dismal outcome of these patients, we need to consider the state of the underlying lung, and realise that treatment of the organism alone is insufficient. Primarily, we need to expand the rollout of ARVs and, failing this, try to both recognise and treat the condition sooner, prior to the development of fibrosis. Ideally, we should also develop a management protocol that addresses the lung fibrosis once it has occurred.

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