

The use of plasmapheresis in bronchiectasis patients with *Pseudomonas aeruginosa* infection and inhibitory antibodies

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10.15 MICROBIOLOGY AND PULMONARY INFECTIONS: Treatment

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Introduction

Chronic *Pseudomonas aeruginosa* lung infections commonly occur in patients suffering from bronchiectasis, leading to increased morbidity and mortality (1-4). Severe bronchiectasis often affects patients beyond the age where lung transplantation is indicated resulting in a high mortality rate (5).

Recently, we identified that ~20% of patients with bronchiectasis and chronic *P. aeruginosa* infection had excess IgG2 specific to the bacterial O-antigen (6). In contrast to the serum bactericidal effect normally associated with antibody, this IgG2 inhibited immune killing of the infecting strain (6). Crucially, patients with inhibitory antibody had worse lung disease (6).

We hypothesised that removal of inhibitory antibody might restore host immune killing and improve patient health. Plasmapheresis is typically used to treat conditions where injurious auto-antibodies arise (7, 8). Here we used plasmapheresis to remove inhibitory IgG2 from the serum of two critically ill patients with chronic *P. aeruginosa* infections (6).

Results

PN1 was a 64-year-old male diagnosed with bronchiectasis aged fifteen, after measles and pneumonia. *P. aeruginosa* was first detected in 2002. Increased morbidity was observed from 2011 when significant multilobar changes were observed. PN1 entered respiratory failure in 2012. He was unfit for a lung transplant due to unrelated renal problems. PN3 was a 69-year-old female had chronic multi-drug resistant *P. aeruginosa* infection since their childhood onset of bronchiectasis (pink disease). PN3 deteriorated rapidly in 2014 and entered respiratory failure.

Both patients were housebound, required long-term oxygen and nocturnal ventilation, and their disease was progressively refractory to treatment. Both failed to respond to multiple

courses of alternating broad spectrum antibiotics guided by sensitivity testing, including 14-21 day courses of IV piperacillin/tazobactam, meropenem and ceftazidime. At time of plasmapheresis, PN1 and PN3 had a FEV1% predicted of 19.8 and 27.9 respectively.

Previously, we demonstrated these patients' sera possessed inhibitory antibodies against our prototypical *P. aeruginosa*. Herein we determined both had impaired serum killing of their cognate *P. aeruginosa*, even when serum was mixed 50:50 with healthy-control serum (HCS), indicating the presence of inhibitory antibodies (Figure 1A). Complete killing was only restored when HCS represented >70% and >90% of the mixed sera, respectively (not shown). Both strains expressed high levels of O-antigen and patient sera had high IgG2 titers specific to their O-antigen serotype (Figure 2B).

We hypothesised that removing inhibitory antibody would ameliorate disease. Upon discussion with the hospital approvals board, in light of patient decline, and after patient consent, we conducted plasmapheresis as salvage therapy. Treatment was conducted for four hours daily for five days, with albumin and electrolyte replacement. Commercial intravenous pooled immunoglobulin (IVIg; Privigen®) was administered (0.4 g/kg body weight) for five days after plasmapheresis ended. Privigen did not inhibit serum-mediated killing of *P. aeruginosa*, nor did it confer on patient serum bactericidal activity.

After treatment, both patients were discharged home. Within two weeks both were no longer housebound, although PN3 still required oxygen. Days in hospital and i.v. antibiotic use dropped significantly ($P<0.01$) for both patients (Figure 1B, 2A). A significant ($P<0.001$) and sustained decrease in the inflammatory marker CRP was observed (Figure 1B). Sputa were *P. aeruginosa* negative for up to three months post-plasmapheresis, despite being cultured from over 90% of sputum samples in the previous 18 months (Figure 2B). The FEV1% predicted

for PN1 did not improve significantly in the year post treatment. In contrast, PN3's FEV1% predicted improved significantly ($P<0.001$) from 27.9% in the year pre-treatment to 37.8%

Anti-LPS IgG2 titres dropped significantly with plasmapheresis but increased over 90 days so that sera were unable to kill the cognate *P. aeruginosa* (Figure 2B). Re-emergence of inhibition correlated with the reappearance of *P. aeruginosa* in sputa, increased symptomatology and poorer responses to subsequent antibiotic courses. This prompted a second round of plasmapheresis for PN1. As before, the patient improved clinically (Figure 2), although again post treatment, titres of IgG2 eventually increased to a point where they inhibited serum-mediated killing. Further plasmapheresis is anticipated for both patients.

Discussion

The two patients described here had severe bronchiectasis with significant morbidity, were refractory to conventional treatment, and unsuitable for lung transplantation. Novel therapies are desperately needed for such patients. Plasmapheresis restored serum-mediated killing of their infecting strain *in vitro* and correlated with rapid improvements in patient health and wellbeing; both patients reported greater independence and mobility than at any point in the previous two years, required fewer days in hospital and had a much reduced dependency on antibiotics.

Plasmapheresis is a non-selective intervention removing protective antibodies against *P. aeruginosa* and other pathogens. We mitigated against this by infusing IVIg pooled from healthy individuals. Levels of inhibitory antibodies returned to pre-treatment levels within three months post-plasmapheresis, coinciding with increased symptoms and *P. aeruginosa* in sputum. Therefore, repeated plasmapheresis may be necessary to maintain benefit.

As plasmapheresis was used as salvage therapy, optimal treatment controls were not available. Thus, although striking, the results are preliminary. The outcome could be a placebo effect but given the supporting *in vitro* data and the repeated efficacy of treatment in separate patients this seems unlikely. The removal of other serum components by plasmapheresis may contribute to the resolution of infection. However, we are unaware of a factor other than immunoglobulin that could account for these findings that is i) found in plasma; ii) associated with bacterial killing; iii) has a long half-life and iv) accumulates so gradually after depletion by plasmapheresis. Other candidates such as CRP or components of complement are more readily replaceable. Measuring levels of inhibitory IgG2 in sputa post-plasmapheresis would be relevant. The beneficial effects observed for these patients may be a consequence of administering IVIg. However, the potential role for IVIg alone was discounted by the clinical team reflecting the very high dilutional factors needed *in vitro* to suppress inhibitory antibodies. Suppressing blocking antibody production using rituximab was considered but the available evidence suggests that rituximab may increase respiratory infection rates in patients with bronchiectasis (9). Furthermore, the timescale for efficacy was felt to be months for rituximab therapy. To truly determine whether removal of anti-LPS IgG2 leads to health improvement, the ideal control would be to perform plasmapheresis on a patient with similar morbidity but no inhibitory antibody; this is ethically challenging. Ultimately, a randomized-blinded study of plasmapheresis in similar patient cohorts using standardised tests is essential to assess the efficacy and mechanism of action of this approach.

In conclusion, we have described the first use of plasmapheresis to improve infection-related symptomatology. Its use in pre-transplant and transplant-ineligible patients is of particular interest. Further multicentre studies of the prevalence of inhibitory antibody in bronchiectasis and other diseases with an infectious component will help us understand if plasmapheresis could be applied more widely.

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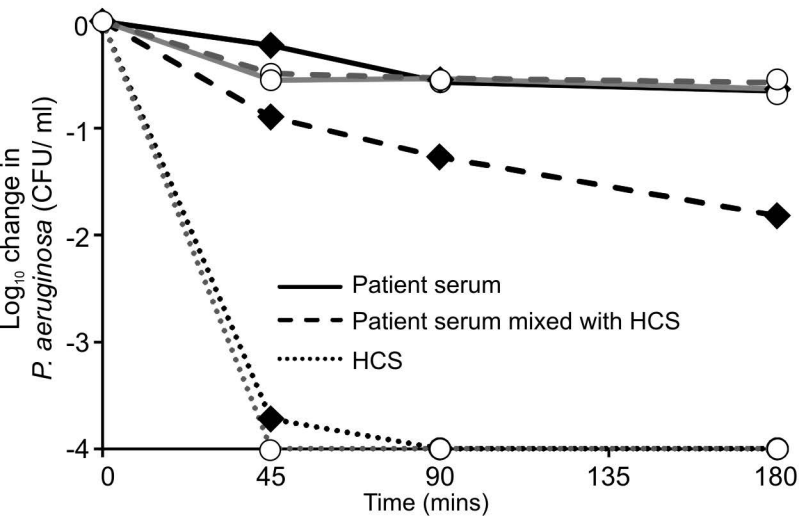
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Figure Legends

Figure 1: Effect of plasmapheresis on patients with inhibitory antibodies. (A) Serum bactericidal assays using *P. aeruginosa* isolated from the sputum of PN1 (diamonds) or PN3 (circles) with autologous patient serum, healthy control serum (HCS) or a 50:50 mix of patient serum and HCS; the patient serum was harvested before plasmapheresis. Negative values correspond with a decrease in viable *P. aeruginosa* compared with initial

concentration. (B) Box and whisker plot of mean of days spent in hospital (left axis) and CRP levels (right axis). Days spent in hospital calculated over 90 days for PN1 or PN3, recalculated monthly. CRP levels were measurements in the 3 months prior to plasmapheresis and the months after the treatment. N indicates the number of CRP measurements used to make each box and whisker plot.

Figure 2: Clinical data for patient's pre and post-treatment. (A) Moving average of i.v. antibiotic use over 90 days, recalculated monthly for PN1 (diamonds) or PN3 (circles) pre and post plasmapheresis. (B) Tracking patient LPS IgG2 titers. The titer of IgG2 specific for the LPS of the patients' cognate *P. aeruginosa* strain was measured by ELISA. ELISAs were done with purified LPS attached to a 96-well plate and dilutions of serum harvested from PN1 (diamonds) or PN3 (circles) at different dates. Patient sputum that cultured *P. aeruginosa* is indicated with an asterix (*). Points are coloured to indicate sera which was able (green) or unable (red) to kill the original patient isolate even when mixed 50:50 with HCS. The point of plasmapheresis is indicated by PP.

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