

The Use of Plasmapheresis in Bronchiectasis Patients with *Pseudomonas aeruginosa* Infection and Inhibitory Antibodies

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2

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

5

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27

28 **Introduction**

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

33 Recently, we identified that ~20% of patients with bronchiectasis and chronic *P. aeruginosa*
34 infection had excess IgG2 specific to the bacterial O-antigen (6). In contrast to the serum
35 bactericidal effect normally associated with antibody, this IgG2 inhibited immune killing of
36 the infecting strain (6). Crucially, patients with inhibitory antibody had worse lung disease
37 (6).

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

42 **Results**

43 PN1 was a 64-year-old male diagnosed with bronchiectasis aged fifteen, after measles and
44 pneumonia. *P. aeruginosa* was first detected in 2002. Increased morbidity was observed from
45 2011 when significant multilobar changes were observed. PN1 entered respiratory failure in
46 2012. He was unfit for a lung transplant due to unrelated renal problems. PN3 was a 69-year-
47 old female had chronic multi-drug resistant *P. aeruginosa* infection since their childhood
48 onset of bronchiectasis (pink disease). PN3 deteriorated rapidly in 2014 and entered
49 respiratory failure.

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

52 courses of alternating broad spectrum antibiotics guided by sensitivity testing, including 14-
53 21 day courses of IV piperacillin/tazobactam, meropenem and ceftazidime. At time of
54 plasmapheresis, PN1 and PN3 had a FEV1% predicted of 19.8 and 27.9 respectively.

55 Previously, we demonstrated these patients' sera possessed inhibitory antibodies against our
56 prototypical *P. aeruginosa* (6). Herein we determined both had impaired serum killing of
57 their cognate *P. aeruginosa*, even when serum was mixed 50:50 with healthy-control serum
58 (HCS), indicating the presence of inhibitory antibodies (Figure 1A). Complete killing was
59 only restored when HCS represented >70% and >90% of the mixed sera, respectively (not
60 shown). Both strains expressed high levels of O-antigen and patient sera had high IgG2 titers
61 specific to their O-antigen serotype (Figure 2B).

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

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

75 for PN1 did not improve significantly in the year post treatment. In contrast, PN3's FEV1%
76 predicted improved significantly ($P < 0.001$) from 27.9% in the year pre-treatment to 37.8%
77 Anti-LPS IgG2 titres dropped significantly with plasmapheresis but increased over 90 days so
78 that sera were unable to kill the cognate *P. aeruginosa* (Figure 2B). Re-emergence of
79 inhibition correlated with the reappearance of *P. aeruginosa* in sputa, increased
80 symptomatology and poorer responses to subsequent antibiotic courses. This prompted a
81 second round of plasmapheresis for PN1. As before, the patient improved clinically (Figure
82 2), although again post treatment, titres of IgG2 eventually increased to a point where they
83 inhibited serum-mediated killing. Further plasmapheresis is anticipated for both patients.

84

85 **Discussion**

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

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

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

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

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125 anonymization and distribution of clinical data.

126

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151

152 Figure Legends

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

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

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