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

To the Editor:

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 when lung transplantation is indicated, resulting in a high mortality rate (5).

Recently, we found 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 hypothesized that removal of inhibitory antibody might restore host immune killing and improve patient health. Plasmapheresis is typically used to treat conditions in which injurious autoantibodies 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 man diagnosed with bronchiectasis at age 15 years, 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 owing to unrelated renal problems. PN3 was a 69-year-old woman with chronic multidrug-resistant *P. aeruginosa* infection since 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- to 21-day courses of intravenous piperacillin/tazobactam, meropenem, and ceftazidime. At time of plasmapheresis, PN1 and PN3 had FEV_1% 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, indicating the presence of inhibitory antibodies (Figure 1A). Complete killing was only restored when healthy control serum represented more than 70% and more than 90% of the PN1 and PN3 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 hypothesized that removing inhibitory antibody would ameliorate disease. On 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 4 hours daily for 5 days with albumin and electrolyte replacement. Commercial intravenous pooled immunoglobulin (IVIg) (Privigen; CSL Behring, Sussex, UK) was administered (0.4 g/kg body weight) for 5 days after plasmapheresis ended. Privigen did not inhibit serum-mediated killing of *P. aeruginosa*, nor did it confer bactericidal activity on patient serum in *in vitro* assays.

After treatment, both patients were discharged home. Within 2 weeks both were no longer housebound, although PN3 still required oxygen. Days in the hospital and intravenous antibiotic use dropped significantly ($P < 0.01$) for both patients (Figures 1B and 2A). A significant ($P < 0.001$) and sustained decrease in the inflammatory marker C-reactive protein was observed (Figure 1B). Sputa were *P. aeruginosa* negative for up to 3 months post-plasmapheresis, despite being cultured from more than 90% of sputum samples in the previous 18 months (Figure 2B). The FEV_1% predicted for PN1 did not improve significantly in the year post-treatment. In contrast, PN3's FEV_1% predicted improved significantly ($P < 0.001$) from 27.9% in the year pretreatment to 37.8% in the months after treatment.

Anti-LPS IgG2 titers dropped significantly with plasmapheresis but increased over 90 days such that sera were unable to kill the cognate *P. aeruginosa* (Figure 2B). Reemergence 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 titers 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 were 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 well-being; both patients reported greater independence and mobility than at any point in the previous 2 years, required fewer days in the hospital, and had a much reduced dependency on antibiotics.

Plasmapheresis is a nonselective 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 pretreatment levels within 3 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

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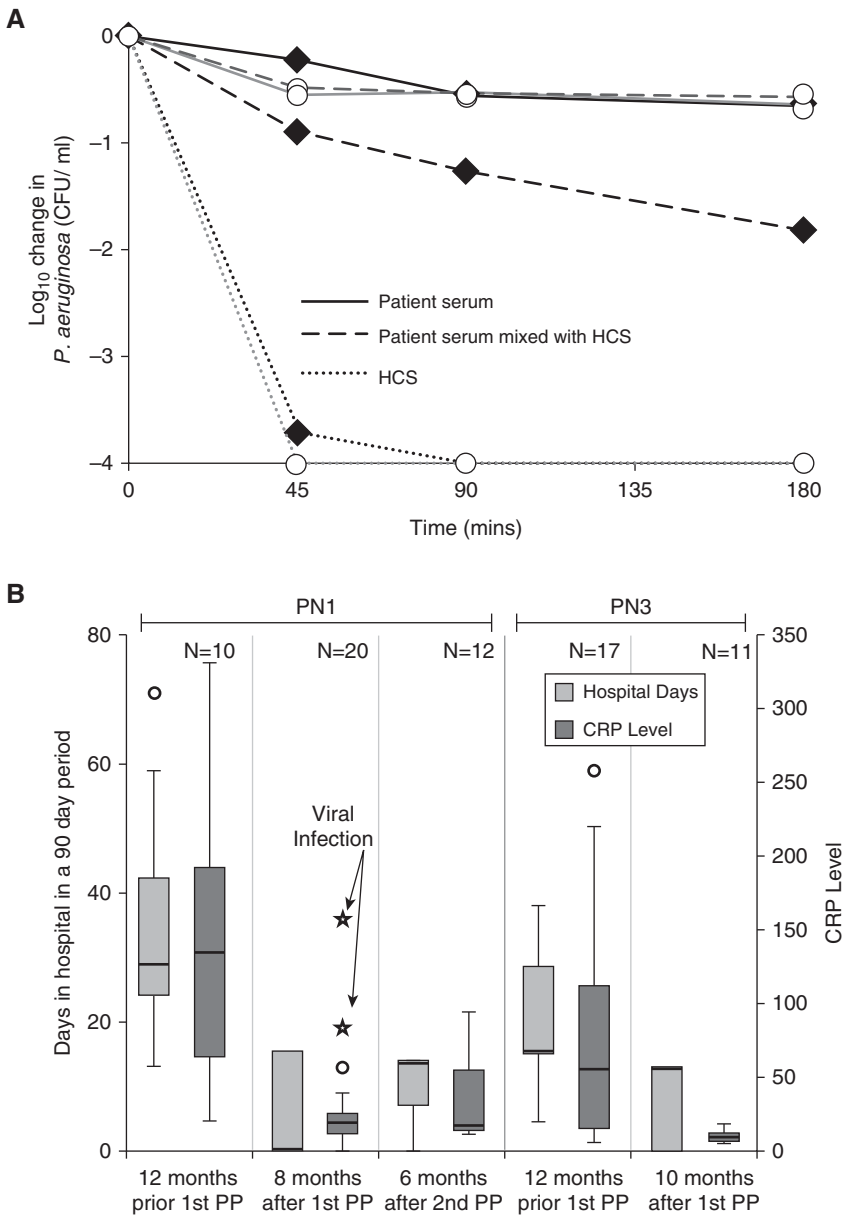

Figure 1. Effect of plasmapheresis (PP) on patients with inhibitory antibodies. (*A*) Serum bactericidal assays using *Pseudomonas aeruginosa* isolated from the sputum of patient PN1 (*diamonds*) or patient PN3 (*circles*) with autologous patient serum, healthy control serum (HCS), or a 50:50 mix of patient serum and HCS; the patient serum was harvested before PP. Negative values correspond with a decrease in viable *P. aeruginosa* compared with initial concentration. (*B*) Box and whisker plot of mean number of days spent in the hospital (*left axis*) and C-reactive protein (CRP) levels (*right axis*). Days spent in the hospital calculated over 90 days for PN1 or PN3, recalculated monthly. CRP levels were measurements in the 3 months before PP and the months after the treatment. N indicates the number of CRP measurements used to make each box and whisker plot. The *horizontal lines* within the boxes indicate the medians, *boxes* show the interquartile range (IQR), and *whiskers* show the full range excluding outliers (*circles* or *stars*) defined as being more than ± 1.5 IQR outside the box. CFU = colony-forming unit.

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 (*1*) is found in plasma, (*2*) is associated with bacterial killing, (*3*) has a long half-life, and (*4*) accumulates so gradually after depletion by plasmapheresis. Other candidates, such as C-reactive protein 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 the production of blocking antibodies using rituximab was considered, but the

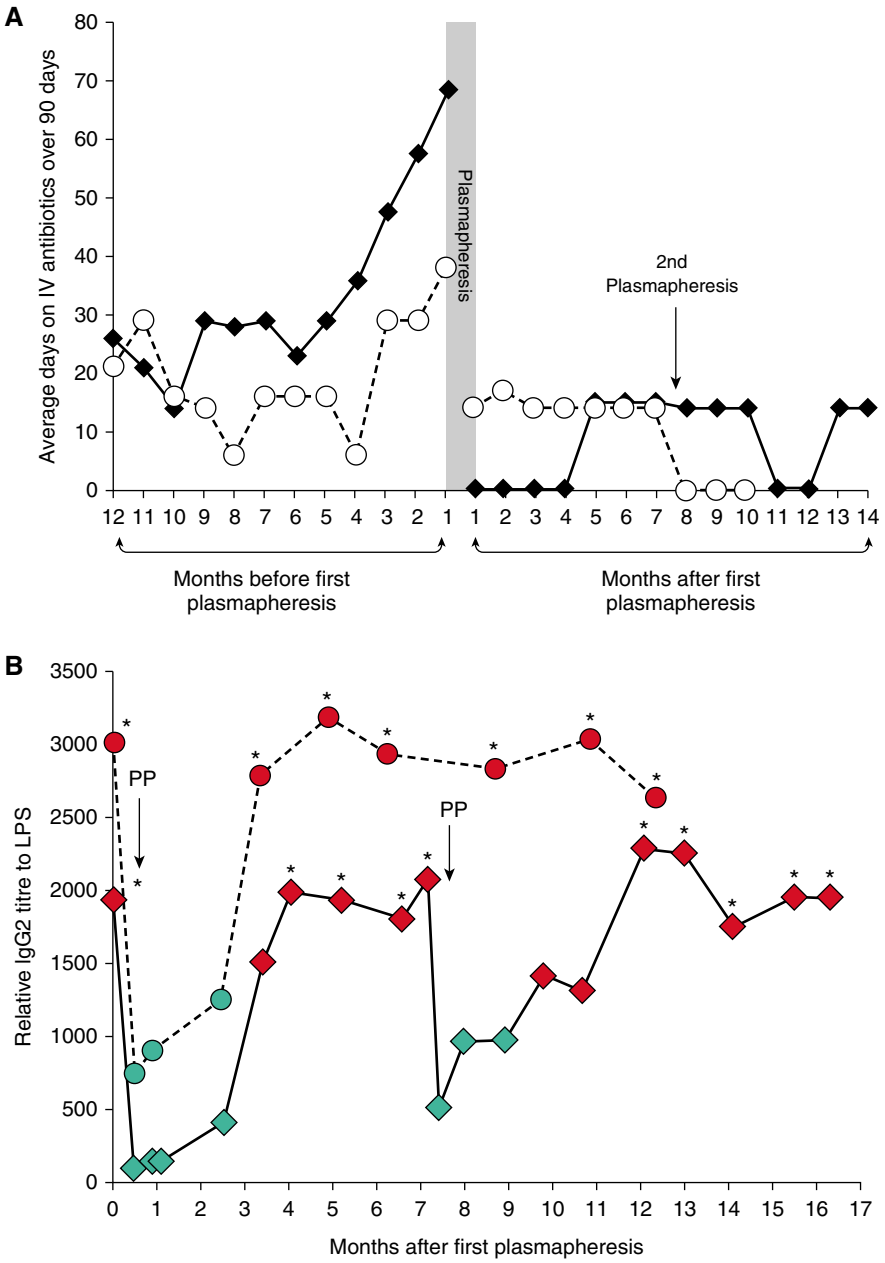

Figure 2. Clinical data for patients, before and after treatment. (*A*) Moving average of intravenous (IV) antibiotic use over 90 days, recalculated monthly for patient PN1 (*diamonds*) or patient PN3 (*circles*) before and after plasmapheresis. (*B*) Tracking patient LPS IgG2 titers. The titer of IgG2 specific for the LPS of the patients' cognate *Pseudomonas 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 *asterisk*. Points are colored to indicate sera that were able (*green*) or unable (*red*) to kill the original patient isolate even when mixed 50:50 with healthy control serum. The point of plasmapheresis is indicated by *PP*.

available evidence suggests that rituximab may increase respiratory infection rates in patients with bronchiectasis (9). Furthermore, the time scale for efficacy was believed 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 standardized 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 pretransplant and transplant-ineligible patients is of particular interest. Further multicenter 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. ■

Author disclosures are available with the text of this letter at www.atsjournals.org.

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Prolonged Apnea Supported by High-Frequency Noninvasive Ventilation: A Pilot Study

To the Editor:

Respiratory movements cause motion artifacts during image acquisition of the thorax and upper abdomen, which limit the clinical use of magnetic resonance imaging in the visualization of lung parenchyma and thoracic vascular structures (1, 2), reduce the accuracy of positron emission tomography (3, 4), and increase the toxicity of radiation therapy, directly influencing the amount of normal tissue included in the irradiated volumes (5, 6).

Suppressing respiratory movements during imaging acquisition and radiation therapy may, therefore, improve image quality and reduce healthy tissue irradiation while maximizing radiation dose to the tumor. The suppression of thoracic movement has been previously obtained in invasively ventilated subjects under general anesthesia using high-frequency ventilation (HFV), which ensures oxygen delivery and carbon dioxide (CO_2) clearance (7–9).

We performed an interventional, crossover, randomized, open-label study, applying for the first time HFV using a noninvasive interface (HF-NIV) to obtain prolonged apnea (absence of thoracoabdominal respiratory movements) in 10 nonsedated healthy adults with normal spirometry and no known cardiopulmonary disease (median age, 30 yr; range, 26–56 yr; 6/10 men). The study was conducted in accordance with the Declaration of Helsinki and the local ethics committee (Protocol 225/14 CHUV-DO-PART).

HF-NIV was performed using a Monsoon III ventilator (Acutronic Medical Systems, Hirzel, Switzerland) and a noninvasive patient interface (Phasitron; Percussionaire, Sagle, ID). This setting allowed constant monitoring of airway pressures and the application of ventilation with an open airway, protecting against overpressure, and allowing the resumption of spontaneous breathing at any moment during HFV. Each participant performed three breath hold attempts after 1 minute of self-induced hyperventilation: a maximal spontaneous (unassisted) apnea and two HF-NIV–assisted attempts with respiratory rate (RR) of 250/min and 500/min, in a randomized sequence. The working pressure of the ventilator was set to obtain a lung volume between the end-inspiratory lung volume and the total lung capacity, and a mean airway pressure of 15 to 20 cm H_2O, adapted according to volunteers' comfort. To minimize the risk of barotraumas, the ventilator safety pressure relief valve was set at 40 cm H_2O. Inspired oxygen fraction was