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Persistence of *Pseudomonas aeruginosa* in a pulmonary nodule with late relapse

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Abstract

Lung nodules are common diagnostic challenges in hematopoietic stem cell transplantation and solid organ transplantation. *Pseudomonas aeruginosa* is a known cause of lung abscess in these patients, but its ability to persist for months in a quiescent lung nodule and later cause recurrent infection is not well known or documented. A patient with a history of acute pre-B-cell lymphoblastic leukemia had enlargement and cavitation of a small right upper lobe pulmonary nodule 10 months after allogeneic hematopoietic stem cell transplantation. The nodule was the remnant of a presumed *P. aeruginosa* septic embolus that occurred 2.5 months after transplantation. With antibiotic treatment, the nodule had shrunk in size to <1 cm and remained stable. Transthoracic needle aspiration grew *P. aeruginosa* indistinguishable by molecular typing from isolates obtained 7.5 months earlier from blood and bronchoalveolar lavage fluid. Sub-centimeter pulmonary nodules attributable to previously treated *P. aeruginosa* may harbor viable organisms and lead to recrudescence infection.

Keywords

lung abscess; septic pulmonary embolus; hematopoietic stem cell transplant; *Pseudomonas aeruginosa*; recrudescence infection

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New or enlarging lung nodules with or without cavitation are a common challenge in hematopoietic stem cell and solid organ transplantation (1, 2). Myriad infectious and non-infectious etiologies with disparate treatments make diagnosis essential in the absence of a prompt response to empiric therapy. Bronchoalveolar lavage (BAL) has a variable yield and more invasive approaches are often necessary to establish a diagnosis.

Pseudomonas aeruginosa is a known cause of lung abscess in immune compromised hosts (2, 3) and other special populations such as injection drug users (4). However, the ability of this organism to persist in an otherwise quiescent lung nodule and recrudesce into an active infection is not generally appreciated or well documented. Here, molecular typing was used to demonstrate the long-term persistence of *P. aeruginosa* in a sub-centimeter pulmonary nodule.

Case report

A 48-year-old man with Philadelphia-chromosome positive acute pre-B-cell lymphoblastic leukemia in first complete remission had undergone a 6/6 human leukocyte antigen matched, sibling allogeneic stem cell transplantation after myeloablative conditioning. Voriconazole was empirically started before transplantation for scattered sub-centimeter pulmonary nodules seen on computed tomography (CT). The patient had a complicated post-transplant course notable for *Legionella* pneumonia, diffuse alveolar hemorrhage, acute steroid-refractory graft-versus-host disease of the skin and gut, and *Clostridium difficile* colitis. Graft function was poor, with absolute neutrophil ranging from 250 to 1500 cells/ μ L and lymphocyte counts from 50 to 760 cells/ μ L, as well as red cell and platelet transfusion dependence. Donor stem cell infusion for secondary graft failure had not improved cell counts. On routine follow-up 10 months after transplant, the patient was found to have enlargement and cavitation of a previously stable lung nodule.

During a blood transfusion 7.5 months before this presentation, the patient had developed fever to 39°C and pleuritic chest pain. Empiric antimicrobial therapy included meropenem, vancomycin, and liposomal amphotericin B. Hypotension prompted transfer to the intensive care unit and removal of an indwelling peripherally inserted central catheter. Blood cultures grew *P. aeruginosa*. Chest CT demonstrated a new peripheral wedge-shaped consolidation in the anterior segment of the right upper lobe consistent with a septic embolus (Fig. 1 A). BAL fluid also grew *P. aeruginosa*. Transthoracic echocardiogram showed normal heart valves and all blood cultures were negative after the start of antibiotics. The antibiotic regimen was ultimately narrowed to ceftazidime alone and the patient received 14 days of intravenous anti-pseudomonal therapy. Oral ciprofloxacin was then given to complete a 20-day total course. On serial CT scans, the presumed right upper lobe septic embolus gradually resolved to a residual, sub-centimeter nodule with indistinct borders (Fig. 1 B and C).

Two months after this episode, the patient developed new fever, relative leukopenia and a left upper lobe consolidation with air-bronchograms (Fig. 1 C). Cultures of BAL fluid grew *Mycobacterium abscessus* and *Candida glabrata*. Treatment for *M. abscessus* ultimately included imipenem/cilastatin, moxifloxacin, and inhaled amikacin. With this regimen, the left upper lobe infiltrate resolved and the small right upper lobe nodule remained stable (Fig.

1 D and E). However, after 3 months of therapy, imipenem/cilastatin was discontinued because of abdominal pain and diarrhea.

Five weeks after stopping imipenem/cilastatin, a CT scan showed enlargement of the right upper lobe nodule (Fig. 1 F). Imipenem/cilastatin was restarted, but 1 month later, a repeat chest CT showed further enlargement of the nodule, from 1.1 to 1.7 cm (Fig. 1 G), and cavitation (Fig. 1 H). The patient felt well and denied fevers, chills, night sweats, cough, or weight loss. BAL was non-diagnostic and did not demonstrate airway colonization with *P. aeruginosa*. Transthoracic fine needle aspiration (FNA) of the nodule grew *P. aeruginosa* resistant to imipenem and meropenem, but sensitive to ceftazidime and fluoroquinolones. The patient was continued on inhaled amikacin, and started on intravenous ceftazidime and levofloxacin. Surgical resection of the nodule was considered but not undertaken, because of poor functional status and pulmonary function tests showing a severe diffusion defect as measured by lung diffusing capacity for carbon monoxide.

Methods

Pulsed-field gel electrophoresis assay (PFGE) (Fig. 2)

P. aeruginosa isolates were subcultured onto sheep blood agar plates and incubated at 35°C in 5% CO₂ overnight. Colonies were resuspended in 1 M NaCl, 10 mM Tris-Cl (pH 7.6) solution and genomic DNA was prepared in 0.8% InCert agarose plugs (Cambrex Corp., East Rutherford, New Jersey USA) using standard methods (5, 6) including 3h treatment in a solution of 0.5 M EDTA (pH 9–9.5), 1% Nalauoylsacrosine, and 0.1 mg/mL proteinase K. DNA was digested with 50 U of SpeI restriction enzyme at 37°C for 2h. PFGE was performed in a CHEF DR III gel chamber (Bio-Rad Laboratories, Hercules, California USA) with ramped pulse times from 5s – 35s at 6 V/cm for 24h. Lambda DNA ladder (Lonza Rockland Inc., Rockland, Maine USA) was used as a molecular weight standard.

Repetitive element palindromic polymerase chain reaction assay (rep-PCR) (Fig. 3)

Genomic DNA was extracted with the Mo Bio Laboratories UltraClean[®] microbial isolation kit (Bacterial Barcodes, Inc/bioMérieux, Athens, Georgia, USA). PCRs were performed with 90–140 ng of purified bacterial DNA and reagents in the DiversiLab[™] DNA fingerprinting kit (Bacterial Barcodes, Inc.) (5). PCR was performed using an initial denaturation of 94°C for 120s, followed by 35 cycles of 94°C for 30s, 60°C for 30s, and 70°C for 90s, with a final extension at 70°C for 180s. PCR products were separated in the Agilent 2100 BioAnalyzer[®] (Agilent Technologies, Palo Alto, California, USA) using a microfluidics DNA chip according to the protocol provided by Bacterial Barcodes, Inc. The rep-PCR fingerprint profiles were compared with DiversiLab[™] v3.4 software (Bacterial Barcodes, Inc.) using the Pearson correlation coefficient.

Results

Comparison of bacterial isolates

Patient isolates included those recovered from blood and BAL fluid at the time of his initial bacteremia and presumed catheter-related septic embolus, and the FNA specimen from the

newly enlarging pulmonary nodule. Comparator *P. aeruginosa* strains included 2 unrelated blood isolates from different patients, and BAL and urine isolates from 2 other patients, all obtained from concurrent inpatients at the National Institutes of Health Clinical Center. All patient isolates had been recovered from primary cultures by standard laboratory methods and identified by MALDI-TOF MS (Bruker Daltonics, Billerica, Massachusetts, USA). Susceptibility testing was performed on patient isolates using a Phoenix automated instrument (BD, Franklin Lakes, New Jersey, USA). The ATCC reference *P. aeruginosa* strain #27853 was included in the analysis.

The 3 patient *P. aeruginosa* isolates from the initial blood and BAL specimens, and from the FNA obtained >7 months later, had the same SpeI banding patterns by PFGE (Fig. 2; Lanes 1–3) (5, 6) and would be considered indistinguishable by the criteria of Tenover et al. (7). The 4 comparator *P. aeruginosa* strains recovered from concurrent inpatients were different from the patient's isolates and from each other, indicating strain diversity within the hospital. The ATCC reference strain of *P. aeruginosa* produced a band pattern unique from all other isolates.

Analysis of the same set of strains with rep-PCR, a PCR typing technique based on the amplification of repetitive element DNA, demonstrated very similar results (Fig. 3). DiversiLab™ software (Bacterial Barcodes, Inc.) calculated that the 3 isolates from our patient were 98.7–99.3% similar to each other by this technique, but dissimilar to the comparator *P. aeruginosa* isolates (55.9–74.7%).

Discussion

Using molecular diagnostics, a previously stable, sub-centimeter pulmonary nodule was found to harbor viable *P. aeruginosa* for >7 months. Ultimately, this putative dormant nidus recrudesced into an active infection. Three sequential patient *P. aeruginosa* isolates from blood, BAL fluid, and finally from the recrudescent pulmonary abscess, obtained much later by FNA, were all found to be indistinguishable by PFGE (5, 6). In contrast, 4 comparator *P. aeruginosa* strains from concurrent inpatients were different from these isolates and from each other (i.e., unique by the criteria of Tenover et al. [7]). A second strain-typing method, rep-PCR, also indicated that the *P. aeruginosa* isolate obtained by FNA was indistinguishable from organisms recovered from blood and BAL fluid >7 months previously, but distinct from comparator strains carried by concurrent inpatients. Notably the remnant nodule, which developed signs of active infection (enlargement and cavitation), corresponded anatomically to an earlier wedge-shaped consolidation that first appeared in the setting of a catheter-related bloodstream infection caused by *P. aeruginosa*. An important point is that repeated bronchoscopies never demonstrated chronic airway colonization with *P. aeruginosa*.

The sudden development of pleuritic chest pain during a blood transfusion, subsequent isolation of *P. aeruginosa* from blood and BAL fluid, and findings on CT scan led to a presumptive diagnosis of septic pulmonary embolus. Although *P. aeruginosa* has been reported to cause vasculitis with infarction that can mimic pulmonary embolus (8), an infected blood clot originating from the central catheter best explains this patient's clinical

presentation. Transthoracic echocardiogram did not find evidence of endocarditis, and blood cultures became immediately negative with antibiotics and catheter removal. Nonetheless, septic pulmonary emboli alone may warrant 4–8 weeks of antibiotic therapy (9, 10). The patient presented here received only 20 days of treatment. Although the area of consolidation continued to resolve into a sub-centimeter nodule, even after the discontinuation of antibiotics, the relatively short course of initial therapy may have allowed the organism to persist. However, *P. aeruginosa* continued to survive in this remnant despite an additional 3 months of imipenem/cilastatin and moxifloxacin subsequently administered for *M. abscessus* pneumonia. Of note, the original *P. aeruginosa* isolates from blood and BAL fluid were fully susceptible to these antibiotics. Prolonged relative neutropenia may have been another contributor to bacterial persistence. With survival of *P. aeruginosa* in the nodule, the FNA isolate taken >7 months later remained sensitive to ceftazidime, but had become resistant to carbapenems. The change in sensitivity suggests selection due to the presence of hetero-resistance, or less likely, *de novo* mutation driven by imipenem exposure.

Carbapenem resistance in *P. aeruginosa* has been linked to multiple mechanisms including down-regulated porin OprD expression, efflux pump and AmpC overexpression, and the acquisition of carbapenemase encoding genes (11–14). Here, resistance arose in an otherwise sterile site that appeared to harbor only *P. aeruginosa*. This scenario makes horizontal transmission of a carbapenemase gene unlikely. Furthermore, resistance to both imipenem and meropenem, but sensitivity to ceftazidime suggests a combination of mechanisms involving OprD and AmpC (imipenem) and efflux pumps (meropenem). However, the relationship between genotype and phenotype in carbapenem-resistant *P. aeruginosa* is complex (15, 16), and mechanisms cannot be deduced simply based on antibiotic sensitivity patterns. Nonetheless, the persistence of this organism and the development of antibiotic resistance *in situ* underscore the difficulty of treating a pulmonary nodule or abscess caused by *P. aeruginosa* without definitive drainage or surgical resection.

Chronic pseudomonal lung infections have only rarely been described in patients without cystic fibrosis. A single case report in the literature followed a solitary pulmonary nodule for >20 years in an immune competent host, which was ultimately determined to be a *P. aeruginosa* abscess on complete excision (17). The paucity of similar cases in the literature suggests that *P. aeruginosa* persistence in lung nodules or small abscesses is unusual, but also possibly under recognized. Of note, *P. aeruginosa* bacteremia in patients with neoplastic disease has been reported to have a high recurrence rate 1.5–17 months after an initial episode (18). Among patients with acquired immunodeficiency syndrome and lung abscesses, 11/28 were due to *P. aeruginosa*, and *P. aeruginosa* was recovered from 4/11 recurrences, at 2–16 months after initial treatment (3). While late recurrences have also been reported in injection drug users with right-sided *P. aeruginosa* endocarditis and septic pulmonary emboli (4, 19), ongoing risk factors and the lack of molecular epidemiology complicate assessment of whether these cases represent true relapses or new infections.

This case demonstrates that a septic embolus to the lung may function as a sheltered nidus for *P. aeruginosa*. Reactivation many months later and selection of resistant phenotypes may occur within a sub-centimeter remnant of a prior septic embolus. In post-transplant patients, tracing pulmonary lesions back in time to their origin and the judicious use of

molecular techniques can provide clinical context that is diagnostically important and can help guide future management.

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Fig. 1.

Serial computed tomography (CT) scans are shown. (A) Initial CT scan (7.5 months earlier) showing right upper lobe consolidation from a presumed septic embolus; (B) 1 week after completion of anti-pseudomonal antibiotics (4 weeks after the onset of infection); (C) 2.5 months after the septic embolus (5 months ago), a new left upper lobe pneumonia was attributed to *Mycobacterium abscessus*; (D) 4 months ago (3.5 months after the septic embolus); (E) 2.5 months ago (5 months after the septic embolus), just before imipenem/cilastatin was discontinued for diarrhea; (F) 1 month ago (6.5 months after the septic embolus), showing enlargement of the pulmonary nodule to 1.1 cm; (G) 7.5 months after the septic embolus, the nodule now measures 1.7 cm; and (H) a different slice from the same CT scan, showing a small cavity.

Fig. 2.

Pulsed-field gel electrophoresis (PFGE) demonstrated that the patient's *Pseudomonas aeruginosa* isolates were indistinguishable from each other, but different from the isolates of concurrent inpatients. Lanes 1–3 are isolates from the patient and lanes 4–7 are comparator isolates from concurrent inpatients. Lane 1: Patient initial blood isolate; Lane 2: Patient initial bronchoalveolar lavage (BAL) isolate; Lane 3: Patient lung fine-needle aspiration isolate > 7 months later; Lane 4: Comparator blood isolate 1 from concurrent inpatient; Lane 5: Comparator blood isolate 2 from concurrent inpatient; Lane 6: Comparator BAL isolate from inpatient concurrent with the patient's relapsed infection; Lane 7: Comparator urine isolate from inpatient concurrent with the patient's relapsed infection; Lane 8: ATCC 27853 *P. aeruginosa* strain; Lane 9: Ladder.

Fig. 3.

Repetitive element palindromic polymerase chain reaction assay (rep-PCR) isolate fingerprint profiles analyzed with Diversilab™ v3.4 strain typing software also indicated that the patient's *Pseudomonas aeruginosa* isolates were indistinguishable from each other, but different from the isolates of concurrent inpatients. (Left) The left panel shows the software-generated similarity dendrogram based on rep-PCR. (Right) The right panel displays the numerical similarity calculations. Sample 1: Kit negative control; Sample 2: Comparator blood isolate 1; Sample 3: Comparator urine isolate; Sample 4: Comparator bronchoalveolar lavage (BAL) isolate; Sample 5: Kit positive control; Sample 6: Comparator blood isolate 2; Sample 7: ATCC 27853 *P. aeruginosa* strain; Sample 8: Patient BAL isolate; Sample 9: Patient blood isolate; Sample 10: Patient lung fine-needle aspiration isolate.