Sequelae of Donor-derived Mollicutes Transmission in Lung Recipients

To the Editor:

Hyperammonemia is a lethal syndrome in lung recipients characterized by progressive elevation in serum ammonia leading to mental status changes, cerebral edema, and death (1–3). It was previously speculated to result from an inborn error of urea metabolism, unmasked by calcineurin inhibition (3, 4). We recently demonstrated a strong association between *Ureaplasma* infection and hyperammonemia syndrome and reported that antimicrobial therapy targeting this organism reverses the clinical syndrome (5). Nevertheless, it remains unclear whether the pathogenic form of this organism is of donor or recipient origin. It also remains unknown whether *Ureaplasma* can lead to complications other than fatal hyperammonemia.

We prospectively evaluated consecutive lung recipients (n = 29) and donors (n = 28) at Northwestern Memorial Hospital from the inauguration of the lung transplant program in July 2014 until May 2016. The study was approved by the Institutional Review Board. Pretransplant urine and bronchoalveolar lavage fluid (BALF) from recipients and BALF from donors were tested for Mollicutes, as previously described using culture and polymerase chain reaction (5). The testing laboratory was blinded to the clinical status of the patients. One (3%) recipient was positive for *Ureaplasma urealyticum* in the urine before transplantation and was treated using levofloxacin and azithromycin for 2 weeks. Native lung BALF from all recipients was negative, whereas BALF from four donors (14%) was positive for Mollicutes (*Ureaplasma* alone = 3; *Ureaplasma* and *Mycoplasma hominis* = 1). Donors with Mollicutes were younger, at 23.3 versus 38.3 years ($P < 0.001$); were predominantly male ($P = 0.07$); all were sexually active with multiple sexual partners ($P = 0.1$); and all had a documented aspiration event before declaration of brain death ($P = 0.001$; Table 1).

Recipients received induction immunosuppression (methylprednisolone [500 mg], basiliximab [20 mg]), after which they were maintained on tacrolimus (target trough, 8–12 ng/ml), mycophenolate mofetil (1,000 mg twice daily), and prednisone (0.5 mg/kg daily). In addition, patients received empiric vancomycin and cefepime until intraoperative bronchial cultures were finalized. All recipients of *Ureaplasma*-positive donor lungs developed lung infiltrates and demonstrated systemic inflammatory response syndrome requiring vasopressors to maintain mean arterial pressures equal to or greater than 60 mm Hg on postoperative Day 1. In addition, *Ureaplasma*-positive, but not *Ureaplasma*-negative, donor lungs were associated with ammonia elevation in the first 3 days after transplantation, despite vancomycin and cefepime therapy, neither of which are active against Mollicutes because of their lack of cell walls. Recipients of allografts from *Ureaplasma*-positive donors demonstrated a trend toward greater incidence of grade 3 primary graft dysfunction, acute renal failure, acute rejection, and 60-day mortality (Table 1). In addition, two (50%) of these recipients developed bronchial dehiscence ($P = 0.02$).

The median time to obtaining Mollicutes test results was 5 days since it was sent out to the Diagnostic *Mycoplasma* Laboratory at the University of Alabama at Birmingham. Antimicrobial therapy was subsequently initiated in all recipients of *Ureaplasma*-positive donor lungs. The previously reported index patient developed recurrence of hyperammonemia during azithromycin monotherapy associated with a *Ureaplasma* strain harboring a A2058G mutation in the 23S ribosomal RNA gene operon 2, causing macrolide resistance (5). Therefore, subsequent patients were treated with a combination of macrolide and fluoroquinolone (Figure 1A). All recipients of *Ureaplasma*-positive lungs demonstrated improvement in serum ammonia and systemic inflammatory response syndrome within 24 hours of initiation of targeted antimicrobial therapy with complete recovery within 7 days. After 14 days of antimicrobial therapy the *Ureaplasma* cultures became negative, although polymerase chain reaction remained positive in two patients for up to 4 weeks. None of the treated patients developed relapse of hyperammonemia at a median follow-up of 8 months.

Coincidentally, the sole recipient with *Ureaplasma* in the urine pretransplantation received *Ureaplasma*-positive donor lungs. Her native lung BALF and urine at the time of transplant were free of Mollicutes, and the organism identified in her BALF post-transplant was *Ureaplasma parvum*, the same as detected in the donor BALF. Further, pretransplant urine had *U. urealyticum*. These findings suggest that the hyperammonemia, and possibly systemic inflammatory response syndrome, were attributable to donor-derived organisms. To further establish the causality, we injected donor *Ureaplasma* isolates into immunocompetent C57Bl/6 mice intravenously, which resulted in hyperammonemia at 8 hours (Figure 1B). Hyperammonemia was prevented by treatment with antimicrobial therapy. These results fulfill Koch's third postulate, causally linking *Ureaplasma* with the defining feature of hyperammonemia syndrome.

U. urealyticum and *U. parvum* are underrecognized human pathogens that lack cell walls and do not grow in routine culture media (6, 7). *Ureaplasma* species are commensals of the urogenital tract and are found in up to 40–80% of sexually active men and women (8). Pneumonias are common in patients with end-stage lung disease and are usually treated with fluoroquinolones and macrolides. Therefore, the low incidence in pretransplant urine was likely related to antimicrobial therapy in this cohort. Intriguingly, *Ureaplasma* species are not part of the lung microbiome (9). As all four of the *Ureaplasma*-positive donors in our series had a documented aspiration event, we speculate that *Ureaplasma* species colonizing the oral cavity, perhaps from sexual contact with their partners, were introduced into the airway during aspiration. Urinary testing for *Ureaplasma* was negative in two kidney recipients of the *Ureaplasma*-positive donors immediately after transplantation. It therefore seems unlikely that *Ureaplasma* from the donor's urogenital tract gained access to the lung. However, this needs to be validated in further prospective studies.

Since the identification of *Ureaplasma* species as pathogens for hyperammonemia, we have initiated a protocol to screen all donors and recipients and treat microbiologically proven infections. Given that macrolides are bacteriostatic for *Ureaplasma* species and that monotherapy can lead to resistance, we use a combination of a

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Table 1. Donor Variables and Associated Recipient Complications

	Ureaplasma Positive (*N* = 4)	*Ureaplasma* Negative (*N* = 24)	All* (*N* = 28)	*P* Value
Donor variables				
Age, yr, mean ± SD	23.3 ± 3.2	38.3 ± 10.7	36.2 ± 8.2	<0.001
Sex, n (%)				0.07
Male	3 (75)	14 (58)	17 (61)	
Female	1 (25)	10 (42)	11 (39)	
Race, n (%)				0.8
White	2 (50)	12 (50)	14 (50)	
Black	1 (25)	8 (33)	9 (32)	
Hispanic	1 (25)	3 (13)	4 (14)	
Asian	—	1 (4)	1 (4)	
Sexually active, n (%)	4 (100)	12 (50)	16 (57)	0.1
Cause of death, n (%)				0.4
Traumatic brain injury	3 (75)	14 (58)	17 (61)	
Stroke/intracranial hemorrhage	1 (25)	10 (42)	11 (39)	
Aspiration, n (%)†	4 (100)	3 (13)	7 (25)	0.001
Recipient complications, n (%)				
Hyperammonemia‡	4 (100)	0	4 (14)	<0.001
Primary graft dysfunction, grade 3	1 (25)	1 (4)	2 (7)	0.3
Acute renal failure	3 (75)	5 (20)	9 (31)	0.5
Bronchial dehiscence	2 (50)	0 (0)	2 (7)	0.02
Acute rejection	1 (25)	1 (4)	2 (7)	0.2
Mental status changes	4 (100)	1 (4)	2 (7)	0.2
60-d mortality	1 (25)	1 (4)	2 (7)	0.3

*One donor was used for two recipients of single-lung transplant; therefore, the number of donors is one less than the total recipients.
†Aspiration was diagnosed during bronchoscopy by the Organ Procurement Organization at the time of initial donor evaluation and documented into the donor records before acceptance of the organs by the recipient centers.
‡Hyperammonemia was diagnosed if two consecutive levels of serum ammonia were elevated.

macrolide with a quinolone or doxycycline. Despite that, we noted an increase in morbidity and mortality in recipients of *Ureaplasma*-positive donor lungs. Because hyperammonemia develops rapidly, the prevalence of *Ureaplasma* was high in donors (14%), and the historical mortality of hyperammonemia approaches 100%, we suggest that trials of universal prophylaxis pending negative cultures and pathogen-directed therapy against *Ureaplasma* infection are warranted. Azithromycin was previously shown to reduce the incidence of chronic lung allograft rejection (10). After this, some centers have administered azithromycin routinely immediately after transplantation, and this has indirectly resulted in prevention of hyperammonemia (personal communications with Marie Budev, Cleveland Clinic Foundation, Cleveland, OH, and Rajat Walia, Norton Thoracic Institute, Phoenix, AZ).

A

	Pathogen	Antimicrobial susceptibility		
		Erythromycin	Tetracycline	Levofloxacin
Donor A	*U. urealyticum*	R	S	S
Donor B	*U. urealyticum* +*M. salivarum*	S	S	S
Donor C	*U. urealyticum*	S	S	S
Donor D	*U. parvum*	S	S	S
Recipient D	*U. urealyticum*	S	S	S

B

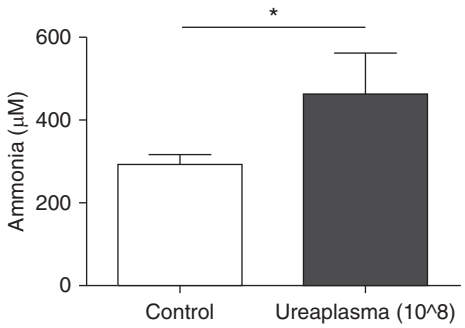

Figure 1. *Ureaplasma* infection and hyperammonemia. (*A*) Antimicrobial susceptibilities of Mollicutes-positive donor lung bronchoalveolar lavage fluid cultures and recipient D pretransplant urine. (*B*) The *Ureaplasma* isolates were inoculated (count 10^8) into immunocompetent mice, which resulted in hyperammonemia. n = 5; *$P < 0.05$. *M. salivarum* = *Mycoplasma salivarum*; R = resistant; S = susceptible; *U. parvum* = *Ureaplasma parvum*; *U. urealyticum* = *Ureaplasma urealyticum*.

In summary, we found that *Ureaplasma* infection in donor lungs was associated with increased morbidity after lung transplantation. We isolated *Ureaplasma* species from humans in pure culture and showed that these organisms are sufficient to increase serum ammonia levels when injected into normal mice but not in those treated with antimicrobials. The relatively high prevalence of these organisms in donor lungs and associated mortality warrant the need for future studies to investigate the optimal strategies for *Ureaplasma* treatment and prophylaxis. ■

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Ramiro Fernandez, M.D.
Monica Chi, B.S.
Michael G. Ison, M.D.
Northwestern University
Chicago, Illinois

Ken B. Waites, M.D.
Donna M. Crabb, M.T.
Amy E. Ratliff, M.S.L.
University of Alabama at Birmingham
Birmingham, Alabama

Hector Cajigas, M.D.
Malcolm M. DeCamp, M.D.
David Odell, M.D.
G. R. Scott Budinger, M.D.
Ankit Bharat, M.D.
Northwestern University
Chicago, Illinois

ORCID ID: 0000-0002-0590-3773 (R.F.).

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Effect of Hydroxyurea Therapy on Pulmonary Function in Children with Sickle Cell Anemia

To the Editor:

Sickle cell disease (SCD) affects approximately 100,000 Americans and 1 in 365 African Americans (1). Most SCD-related deaths are associated with irreversible end-organ damage (2), and pulmonary disease is a common cause of morbidity and mortality. It is well established that children with SCD have progressive changes in pulmonary function testing (PFT), with decreased lung volumes and flows (3). Airflow limitation and airway hyperresponsiveness are associated with increased morbidity and premature death (4, 5). Hydroxyurea (HU) is now widely recommended for the treatment of individuals with SCD of the SS and S/β^0 genotypes and should be offered to children regardless of clinical severity of SCD (3). However, HU is underused because of low prescription rates, fear of toxicities, and poor patient compliance (6). HU has demonstrated favorable effects on vaso-occlusion through multiple mechanisms, the most significant of which is the stimulation of fetal hemoglobin (HbF) (3). HU therapy might lessen the severity of airway hyperreactivity in children with SCD (7), but its effects on longitudinal lung function decline are currently unknown.

We examined the effect of HU therapy on longitudinal PFT changes in children with sickle cell anemia (SCA). Institutional research ethics board approval was obtained for this retrospective study (REB# 1000027385). Some of the results of this study were previously reported in the form of an abstract (8). Study participants were initiated on HU therapy from 2000 to 2011. The decision to initiate HU was made by the treating hematologist, based on the clinical course of the individual patient. After HU initiation, participants were monitored clinically over a 4-year period, with scheduled clinic visits every

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