Contribution of Uremia to *Ureaplasma*-Induced Hyperammonemia

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Abstract

Lung transplant recipients (LTRs) are vulnerable to unexplained hyperammonemia syndrome (HS) in the early post-operative period, a condition typically unresponsive to non-antibiotic interventions. Recently, we showed that HS in LTRs is strongly correlated with *Ureaplasma* infection of the respiratory tract. It is not well-understood what makes LTRs preferentially susceptible to this phenomenon, compared to other immunocompromised hosts. *Ureaplasma* species harbor highly active ureases that convert urea to ammonia and CO₂, utilizing the generated transmembrane potential to synthesize ATP. Post-operative LTRs commonly experience renal failure, resulting in uremia. We hypothesized that uremia could be a potentiating comorbidity to the development of HS secondary to *Ureaplasma* infection in LTRs by providing increased substrate for ureaplasmal ureases. We designed a novel dialyzed flow system to test the ammonia producing capacity of four isolates of *Ureaplasma parvum* and six isolates of *Ureaplasma urealyticum* in media formulations relating to normal and uremic host conditions. For all isolates, growth under uremic conditions resulted in significantly increased ammonia production over 24 hours, despite similar end-point bacterial quantities. Specifically, the isolates produced, on average, 1776.52 [standard deviation=263.98] µmol/L more ammonia when grown under uremic compared to normal conditions. This suggests that uremia, common in early post-operative LTRs, is a plausible contributing factor to the phenomenon of *Ureaplasma*-induced HS in this patient population.
Introduction

Thousands of lung transplants are performed every year in the United States, with numbers anticipated to grow as availability and survivability continue to improve. A primary reason for greater survivability in recent years has been incorporation of strategies to minimize mortality caused by post-transplant infections. A contributor that has plagued lung transplant recipient (LTR) survival is hyperammonemia syndrome (HS), which occurs in around 4% of LTRs (1, 2). Ammonia (NH$_3$) is a neurotoxin that readily transverses the blood-brain barrier and causes cerebral edema; chief mechanisms of NH$_3$ neurotoxicity are illustrated in Figure 1.

HS following lung transplantation typically progresses from early identification of elevated blood NH$_3$ levels or hyperammonemia (HA), as a cause of altered mental status resulting in confusion, lethargy, obtundation, and agitation, to eventual cerebral edema, resulting in seizure, coma, and often death (3-7). HS that presents in LTRs and, at lesser frequencies in other solid-organ transplant recipients (SOTRs), is atypical in that these patients do not have underlying liver disease or urea cycle disorders. Further,
non-targeted interventional efforts to suppress endogenous NH$_3$ production biochemically or physiologically and/or increase NH$_3$ excretion have had minimal impact.

Recently, *U. urealyticum* and *U. parvum* were linked with HS in LTRs (8), where the airways of every LTR presenting with unexplained HS studied \(n=13\) tested positive for *Ureaplasma* species, likely of donor origin. Further evidence that *Ureaplasma* species are causative agents of HS in LTRs has come by way of *in vivo* studies with murine models, where it was shown that intratracheal and intraperitoneal infection with either *U. urealyticum* or *U. parvum resulted in HA (9, 10). *Ureaplasma* species, which are normally considered commensal microbiota of the urogenital tract, produce a potent urease that splits urea into NH$_3$ and CO$_2$ as a means of ATP synthesis, powered by the generated NH$_3$ gradient across the membrane (11, 12). Interestingly, 95% of ATP generated by *Ureaplasma* species is urea dependent, making it a requirement for growth (13). The high level of NH$_3$ production from LTR *Ureaplasma* infection can exceed the capacity for detoxification by the host.

While unexplained HS has been described in non-LTR transplant patient populations (2, 14-28), the prevalence rate of ~4% seems highest, by far, in the LTR population. This suggests that the lung transplant scenario is specifically well-suited to microbial-driven HS. LTRs are also particularly vulnerable to uremia post-transplant (29, 30), potentially providing an abundance of substrate for ureaplasmal ureases, leading to NH$_3$ overproduction, overwhelming the detoxification capacity of the host (Figure 2).

Here, we investigated whether blood urea nitrogen (BUN) levels in LTRs could potentially contribute to production of pathological levels of NH$_3$ by *Ureaplasma* species.
Materials and Methods

Study Isolates

The isolates of *U. parvum* and *U. urealyticum* investigated in this study are listed in Table 1. They include three respiratory isolates of *U. parvum* and five respiratory isolates of *U. urealyticum*, as well as one commercially available urogenital isolate of each species (from ATCC). Patient respiratory isolates are stored at the Mayo Clinic Infectious Disease Research

Figure 2. Hypothetical mechanism of *Ureaplasma*-induced hyperammonemia syndrome secondary to acute kidney failure in early post-operative lung transplant recipients. 1. Acute kidney failure (AKF) due to renal hypoperfusion results in decreased elimination of urea from the blood via urine excretion. 2. Increased blood urea availability provides additional substrate for ureaplasmal (purple cells) ureases in the infected respiratory tract, leading to greater ammonia (blue and grey NH₃ molecule) production in comparison to normal blood urea concentrations. 3. Elevated serum ammonia levels overwhelm the detoxification capacity of liver urea cycle enzymes, leading to hyperammonemia. 4. Excess serum ammonia diffuses into astrocytes and other glial cells in the brain, causing them to swell and burst, resulting in cerebral edema and hyperammonemia syndrome. Created using BioRender.
Laboratory (IDRL). Isolates were grown to $10^7$ color changing units (CCU) using a *Ureaplasma* bioreactor, as previously described (31). 500 µL aliquots in U9 media (Hardy Diagnostics) buffered with 100 mM 2-ethanesulfonic acid (MES) at pH 6.0 were frozen at -80°C until use.

**Table 1. Ureaplasma isolates studied**

<table>
<thead>
<tr>
<th>Species</th>
<th>Isolate #</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>U. parvum</em></td>
<td>IDRL-10774</td>
<td>Bronchoalveolar Lavage Fluid</td>
</tr>
<tr>
<td><em>U. parvum</em></td>
<td>IDRL-11887</td>
<td>Bronchoalveolar Lavage Fluid</td>
</tr>
<tr>
<td><em>U. parvum</em></td>
<td>IDRL-11264</td>
<td>Sputum</td>
</tr>
<tr>
<td><em>U. parvum</em></td>
<td>ATCC-27815</td>
<td>Urethritis</td>
</tr>
<tr>
<td><em>U. urealyticum</em></td>
<td>IDRL-10763</td>
<td>Bronchial Washings</td>
</tr>
<tr>
<td><em>U. urealyticum</em></td>
<td>IDRL-10612</td>
<td>Bronchoalveolar Lavage Fluid</td>
</tr>
<tr>
<td><em>U. urealyticum</em></td>
<td>IDRL-10611</td>
<td>Bronchoalveolar Lavage Fluid</td>
</tr>
<tr>
<td><em>U. urealyticum</em></td>
<td>IDRL-11235</td>
<td>Tracheal Secretions</td>
</tr>
<tr>
<td><em>U. urealyticum</em></td>
<td>IDRL-12698</td>
<td>Bronchoalveolar Lavage Fluid</td>
</tr>
<tr>
<td><em>U. urealyticum</em></td>
<td>ATCC-27816</td>
<td>Urethritis</td>
</tr>
</tbody>
</table>

Growth conditions

10 ml cultures of $10^5$ CCU/mL for each *Ureaplasma* isolate were encased in dialysis tubing (Specta/Por® Float-A-Lyzer®G2 1000kD Dialysis Device; G235073) and submerged in 250 ml of 100 mM MES-buffered U9, allowing measurement of NH$_3$ levels over time. The entire
device was incubated at 37°C, with fresh broth added and spent media removed, via flow at 2 ml/hour. Urea concentrations in the growth media were varied to mimic normal and high BUN levels (10 and 50 mg/dL, respectively) in the flow chamber and inflow. Samples were taken from cell-free portions of the flow chamber (outside the dialysis tube) and collected into microcentrifuge tubes at 0 and 24 hours, and NH$_3$ concentrations tested using an NH$_3$ Assay Kit (Abcam; ab102509). *Ureaplasma* cells in the dialysis tubing were quantified at each collection time.

**Results**

For all *U. parvum* and *U. urealyticum* isolates tested, growth in media containing 50 mg/dl urea (uremic conditions) resulted in significantly greater NH$_3$ production than in media containing 10 mg/dl urea (normal conditions) over 24 hours ([Figure 3](#figure3)). Isolates grown under uremic conditions produced, on average, 1776.52 [standard deviation (SD)=263.98] µmol/L more NH$_3$ than those grown in normal conditions. Differences in NH$_3$ production between species was not significantly different, with *U. parvum* and *U. urealyticum* isolates producing an average of 1854.20 µmol/L (SD = 66.84) µmol/L and 1731.30 µmol/L (SD=131.23) more NH$_3$, respectively, under uremic conditions. There was no...
noticeable difference between patient respiratory isolates and commercially available urogenital isolates. The average difference in NH₃ production between uremic and normal conditions for all patient respiratory isolates was 1794.26 µmol/L (SD=291.40) versus 1800.95 µmol/L (SD=72.38) and 1655.44 µmol/L (SD=118.96) for the U. parvum (ATCC 27815) and U. urealyticum (ATCC 27816) urogenital isolates, respectively. 24-hour CCU counts between uremic and normal conditions was not significantly different (Supplemental Figure 1), indicating that NH₃ production was not a result of different numbers of bacteria.

Discussion

Results of this study show that conditions representative of uremia resulted in elevated production of ammonia by all Ureaplasma isolates tested. The discovery that Ureaplasma respiratory infections were the cause of the previously unexplained phenomenon of non-hepatic HS in early post-operative LTRs (8) has led to an improvement in patient care and a reduction in mortality rates. Still, what makes LTRs particularly vulnerable to this phenomenon remains unknown. Here we investigated the potential impact of uremia as a product of acute kidney failure in the early postoperative period as a potentiating comorbidity. It has been estimated that as many as 75% of LTRs experience acute kidney failure post-operation due to renal hypoperfusion brought on by several factors, including 1) decreased circulating blood volume resulting from diuretic use to prevent pulmonary edema from leaky capillaries, 2) nephrotoxic effects of calcineurin inhibitors, and 3) reduced renal oxygenation due to post-operation hypoxia (29, 30, 32-34). We hypothesized that, among patients harboring a post-transplant Ureaplasma respiratory infection, elevated blood urea concentrations would provide greater substrate availability for ureaplasmal ureases, leading to the production of NH₃ at sufficient levels to overwhelm the host detoxification capacity.
Using a novel dialyzed flow system, we measured the NH$_3$ production of 10 isolates of *U. parvum* and *U. urealyticum* (8 clinical respiratory isolates and 2 commercially available urogenital isolates) over 24 hours under conditions representative of normal (10 mg/dl) and elevated (50 mg/dl) BUN. We found that, for all isolates tested, more ammonia was produced under uremic than normal conditions. Further, endpoint CCU counts were not significantly different for normal compared to uremic conditions, indicating that the greater ammonia production of uremic conditions can be credited to increased ureaplasmal urease activity. These results provide strong *in vitro* support for the hypothesis that post-transplant acute kidney failure is a contributing factor to *Ureaplasma*-induced HS in LTRs, serving as a reminder that the effects of specific comorbidities on disease of microbial metabolite production/overproduction should be more readily considered.

**Acknowledgements**

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**Conflict of Interest Statement**

Dr. Patel reports grants from ContraFect, TenNor Therapeutics Limited, Hylomorph, BioFire and Shionogi. Dr. Patel is a consultant to Curetis, Specific Technologies, Next Gen Diagnostics, PathoQuest, Selux Diagnostics, 1928 Diagnostics, PhAST, Torus Biosystems, Mammoth Biosciences and Qvella; monies are paid to Mayo Clinic. Dr. Patel is also a consultant to Netflix. In addition, Dr. Patel has a patent on *Bordetella pertussis/parapertussis* PCR issued, a patent on a device/method for sonication with royalties paid by Samsung to Mayo Clinic, and a patent on
an anti-biofilm substance issued. Dr. Patel receives an editor’s stipend from IDSA, and honoraria from the NBME, Up-to-Date and the Infectious Diseases Board Review Course.

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References


Supplemental Figure 1. Differences in endpoint *Ureaplasma* quantities between normal and uremic conditions were insignificant. Isolates of *U. parvum* or *U. urealyticum* (N=3 per isolate per condition) were grown in the dialyzed flow system under normal (10 mg/dL urea) or uremic (50 mg/dL urea) conditions for 24 hours. Endpoint color changing units (CCUs) were quantified via serial dilution in 10B broth (Remel). Significance between conditions was determined via a two-tailed unpaired t-test.