RESEARCH LETTER

blood advances

TO THE EDITOR:

Pseudomonas aeruginosa ExoU-associated virulence in HCT recipients and patients with hematologic malignancies

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Pseudomonas aeruginosa remains a significant pathogen in patients undergoing chemotherapy for hematologic malignancies (HMs) and hematopoietic cell transplant (HCT) recipients.[1,2] Studies on the outcome determinants of *P. aeruginosa* infections in these patients have largely focused on host factors, initiation of appropriate antibacterial therapy, and resistance to antibiotics.[1,3-7] In contrast, there is a relative paucity of data pertaining to the role of intrinsic *P. aeruginosa* virulence factors in disease severity and outcomes in these specific patient populations.

The *P. aeruginosa* ExoU enzyme is a phospholipase that causes cell membrane damage, leading to cell death, and is 1 of 4 enzymes delivered into the host cell by the type 3 secretion system, with the others being ExoS, ExoT, and ExoY.[8,9] Of these, ExoU plays the greatest role in *P. aeruginosa* pathogenesis and disease severity;[10,11] however, not all *P. aeruginosa* isolates contain *exoU*.[12] In vitro infection with *exoU*+ isolates results in greater cytotoxicity and increased virulence in mouse models of infection.[10,11,13-15]

In humans, the *exoU*+ genotype has been associated with increased early (5 day) mortality during bacteremia.[16,17] However, not all studies have recapitulated these findings.[18,19] Furthermore, although some studies have included oncology and patients with HCT as minority subsets of the overall study population,[16-19] no study, to our knowledge, has examined the role of ExoU in disease severity exclusively in patients with HMs and recipients of HCT, in whom unique host factors preclude the extrapolation of data from existing studies. Therefore, we performed this study to determine whether the *exoU*+ genotype is associated with early disease severity of *P. aeruginosa* in HCT recipients and patients with HMs.

We conducted a retrospective review of *P. aeruginosa* bloodstream infections (BSIs) and pneumonia that occurred between 1 October 2016 and 30 June 2020 in adult (age $\geq$ 18 years) patients with HMs and recipients of HCT at Oregon Health and Science University (OHSU). The study was approved by the OHSU institutional review board and conducted in accordance with the Declaration of Helsinki. The definitions and criteria for BSI episodes, pneumonia, appropriate empirical antimicrobial therapy, and multidrug resistant *P. aeruginosa* have been previously published.[20,21] At OHSU, levofloxacin is used for neutropenic prophylaxis, unless contraindicated. Cefepime is the first-line agent for the empirical treatment of febrile neutropenia, with piperacillin-tazobactam and meropenem being second- and third-line agents, respectively. Adjunctive empirical tobramycin is used at the discretion of medical teams, typically for patients with septic shock and/or suspicion of infection with multidrug resistant *P. aeruginosa*, while awaiting susceptibility data to guide definitive therapy; combination therapy for the duration of definitive therapy is not standard practice.

P. aeruginosa isolates from blood cultures and bronchoalveolar lavage fluid (BALF) from patients meeting the definition of pneumonia were obtained from the OHSU Clinical Microbiology Laboratory.

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Data are available on request from the corresponding author, Morgan Hakki (hakki@ohsu.edu).

Bacterial DNA preparation, whole-genome sequencing, and genome assembly of each isolate were performed as previously described.[22] GenBank accession numbers for the isolated whole-genome sequencing data have been previously published.[22] All genomes were inspected for the presence of *exoU* gene (NCBI reference sequence, WP_134607685.1).

The severity of infection was assessed based on the composite end point of death or need for intensive care unit (ICU) admission within 7 days of the initial infected blood or BALF culture. This composite end point was chosen on the basis that a simple survival outcome may not reflect infection severity given modern supportive care measures and for alignment with other studies evaluating the impact of the *exoU*+ genotype on early disease severity.[16-19] If an episode was associated with both a 7-day ICU admission and 7-day mortality, it was counted only once toward the composite end point.

Univariate analysis of categorical variables was performed using Fisher 2-tailed exact test. Factors with $P \leq .1$ in the univariate analysis were included in a multinomial logistic regression multivariable model (SPSS version 28 [IBM Corp, Armonk, NY]).

58 episodes of *P. aeruginosa* BSI and pneumonia were identified in 56 unique patients with HM or recipients of HCT. Two patients had 2 unique BSI episodes separated by 65 days and 120 days. Fifty-six of the 58 (96.6%) isolates obtained during the 58 episodes of infection were from the blood, and 2 (3.4%) were from the BALF. Patient characteristics at the time of infection are shown in Table 1. Cefepime or piperacillin-tazobactam was used as empirical therapy in 90% of the episodes, and most of the isolates were susceptible to these agents. Combination empirical therapy with tobramycin was infrequent (19%).

Eighteen isolates (31%) from 18 patients were *exoU*+, 16 (88.9%) were from blood cultures, and 2 (11.1%) were from BALF. Fourteen *exoU*+ isolates were sequence type (ST)-446, which is known to be 1 of the 2 dominant STs in our HM/HCT unit[22]; 3 were ST-308, and 1 ST-532. No significant differences were found when comparing the characteristics presented in Table 1 between infections caused by *exoU*+ and *exoU*- isolates (data not shown).

The composite end point of 7-day mortality or need for ICU transfer was met in 22 episodes (37.9%) of infection. Seventeen episodes (29.3%) required ICU transfer without 7-day mortality, and 5 episodes (8.6%) were associated with 7-day mortality.

Host and bacterial factors were evaluated for their association with the composite end point (Table 2). The *ExoU*+ genotype and pneumonia were associated with the composite end point in a univariate analysis, and both were independently associated with the composite end point when analyzed in a multivariable model. Of the 5 episodes associated with 7-day mortality, 4 (80%) were caused by *exoU*+ isolates ($P = .03$). A trend toward an association of an *exoU*+ genotype with 14-day mortality (N = 11) was observed (6 of 11 [54.5%] vs 12 of 47 [25.5%]; $P = .08$); no association with 30- or 60- day mortality was observed (data not shown).

To our knowledge, the finding that an *exoU*+ genotype was associated with early disease severity is the first such demonstration specifically in patients with HMs and recipients of HCT and is consistent with the data from studies involving other patient populations.[16,17] This finding also represents, to the best of our knowledge, the first to associate an intrinsic *P. aeruginosa* pathogenicity factor with disease severity in these patients who are highly vulnerable, in whom the focus has traditionally been on unique host factors. The *exoU*+ genotype was a more important predictor of disease severity than host factors such as neutropenia (including profound neutropenia), steroid receipt, and age. The only other factor associated with early disease severity was pneumonia, which has been previously identified as an independent risk factor for poor outcomes in similar patients.[1,7]

The relatively small sample size and clonal relatedness of *exoU*+ isolates limit the generalizability of our findings, which requires

Table 1. Patient characteristics at time of infection onset

Characteristic	N (%)*
Sex†	
Male	37 (66)
Female	19 (34)
Age, median (range)	62.5 (21-80)
HCT recipient	25 (43.1)
Underlying disease†	
AML	27 (45)
ALL	9 (20)
MDS	8 (14.3)
Lymphoma	8 (14.3)
Other‡	4 (7.1)
Neutropenia§	44 (75.9)
Profound neutropenia‖	38 (65.5)
Empiric anti-*Pseudomonal* antibiotic	
Cefepime	37 (63.7)
Piperacillin-tazobactam	15 (26)
Meropenem	3 (5.2)
Ceftolozane-tazobactam	3 (5.2)
Adjunctive empirical tobramycin¶	11 (19)
Antipseudomonal antibiotic susceptibility	
Cefepime	54 (93.1)
Piperacillin-tazobactam	50 (86.2)
Meropenem	19 (34)
MDR *P. aeruginosa*	8 (13.8)
AEAT	51(87.9)
Pneumonia	18 (31.0)
Copathogen	14 (24.1)
Steroid receipt#	21 (36.2)
exoU+ genotype	18 (31)

The time of infection onset is defined as the date of first infected blood or BALF culture.
AEAT, appropriate empirical antibiotic therapy; AML, acute myeloid leukemia; ALL, acute lymphocytic leukemia; MDS, myelodysplastic syndrome; MDR, multidrug resistant.
*Total N = 58 episodes, except where indicated.
†Out of 56 unique patients.
‡Multiple myeloma (n = 3); acute promyelocytic leukemia (n = 1).
§Absolute neutrophil count < 500 cells per mm^3.
‖Absolute neutrophil count < 100 cells per mm^3.
¶Used in combination with an antipseudomonal β-lactam or meropenem.
#Receipt of steroids at any dose at the time of infection onset.

Table 2. Factors associated with the composite outcome of 7-day mortality or ICU admission

	Composite outcome, N (%)		Univariate analysis			Multivariable analysis		
Factor	Y (n = 22)	N (n = 36)	*P*-value	OR	95% CI	*P*-value	OR	95% CI
exoU+ genotype	11 (50)	7 (19.4)	.02	4.1	1.3-13.4	.02	5.2	1.4-19.7
Neutropenia	17 (77.2)	27 (75)	.72					
Profound neutropenia	15 (68.2)	23 (63.9)	.91					
Pneumonia	12 (54.5)	6 (16.7)	.004	6	1.7-20.2	.003	7.3	1.9-27.6
MDR isolate	2 (9.1)	6 (16.7)	.66					
Steroid receipt	8 (36.4)	13 (36.1)	.66					
IEAT	2 (9.1)	5 (13.9)	.88					
Male sex	14 (63.6)	25 (69.4)	.82					
HCT recipient	10 (45.5)	15 (41.7)	.53					
Copathogen	5 (22.7)	9 (25)	.71					
Age, y (median)	64	61	.73					

CI, confidence interval; IEAT, inappropriate empiric antibiotic therapy; MDR, multidrug resistant; OR, odds ratio.

confirmation in larger studies. If supported by such studies, strategies to rapidly detect *exoU* in *P. aeruginosa* isolates and to develop interventions to mitigate its impact[8] are justified. Intriguingly, tobramycin disrupted type-III secretion system expression and reduced ExoU-mediated cytotoxicity,[23] suggesting a potential role for adjunctive tobramycin targeted to the treatment of *exoU*+ isolates. Because of the limitations inherent to our study and our institutional approach to tobramycin use in these patients, we were unable to evaluate the impact of tobramycin administration on the outcomes. Our findings also highlight the need to focus future efforts on defining the contributions of other *P. aeruginosa* virulence factors, in addition to *exoU*,[24] to the severe disease that characterizes *P. aeruginosa* infections in highly unique and vulnerable patients.

Contribution: M.H. designed the study, performed the research, analyzed the data, and wrote the manuscript; L.F. performed the research, analyzed the data, and reviewed the manuscript; and L.S. performed the research and reviewed the manuscript.

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