Association Between Respiratory Tract Methicillin-Resistant Staphylococcus aureus and Survival in Cystic Fibrosis

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Cystic fibrosis (CF) is the most common lethal autosomal recessive disorder in white individuals. Individuals with CF are living longer, and the median predicted survival age is now older than 37 years. The most common cause of death in CF is respiratory failure secondary to pulmonary infection. While Pseudomonas aeruginosa and Burkholderia cepacia complex are the pathogens most commonly associated with a shortened life span, there has been an increase in the prevalence of several potentially pathogenic microorganisms in CF.

Methicillin-resistant Staphylococcus aureus (MRSA) is one such important emerging pathogen. The prevalence of respiratory tract MRSA in CF patients is now more than 20%. Some MRSA strains contain virulence factors that may augment its ability to damage host tissue, such as that seen in CF patients with invasive lung infections associated with Panton-Valentine leukocidin–positive MRSA. The impact of MRSA on outcomes in CF remains unclear. We have previously shown in a large epidemiologic study that lung function decline is more rapid in CF children and adolescents with persistent MRSA compared with those without MRSA. However, this association was not seen in adults, and a recent smaller study concluded MRSA was not associated with more rapid lung function decline.

Context The prevalence of methicillin-resistant Staphylococcus aureus (MRSA) in the respiratory tract of individuals with cystic fibrosis (CF) has increased dramatically; however, its impact on outcomes in CF is unclear. Because the time between infection with bacteria in CF and death can be decades, observational studies with long periods of follow-up are well suited to address the current gap in knowledge.

Objective To determine whether isolation of MRSA from the respiratory tract of CF patients is associated with worse survival compared with patients who never have a culture positive for MRSA.

Design, Setting, and Participants Cohort study of 19,833 CF patients aged 6 to 45 years seen at centers accredited by the Cystic Fibrosis Foundation in the United States. Patients entered between January 1996 and December 2006 and were followed up through December 2008. Cox regression models with time-varying covariates were used to compare survival between CF patients with and without respiratory tract MRSA.

Main Outcome Measure Time from age at entry until age at death from any cause.

Results In 137,819 patient-years of observation (median, 7.3 years/patient), 2,537 CF patients died and 5,759 patients had MRSA detected. The mortality rate was 18.3 deaths (95% confidence interval [CI], 17.5–19.1) per 1000 patient-years in patients without MRSA and 27.7 deaths (95% CI, 25.3–30.4) per 1000 patient-years in those with MRSA. Among those with MRSA, the attributable risk percentage of death associated with MRSA was 34.0% (95% CI, 26.7%–40.4%). The unadjusted hazard ratio associated with MRSA was 1.47 (95% CI, 1.11–1.45). After adjustment for time-varying covariates associated with severity of illness, MRSA remained associated with a higher risk of death (1.27; 95% CI, 1.11–1.45).

Conclusion Detection of MRSA in the respiratory tract of CF patients was associated with worse survival.

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highlighted by a recent survey of US CF center directors, who identified MRSA as one of their top research priorities (Preston W. Campbell III, MD, Cystic Fibrosis Foundation, written communication, March 2010).

For these reasons we sought to address the association between MRSA and survival in CF. Since the time between infection and death due to lung disease in CF can be decades, observational studies with long periods of follow-up are well suited to evaluate this association. Therefore, we used the US Cystic Fibrosis Foundation Patient Registry (CFFPR) to test the hypothesis that CF patients who have a respiratory tract culture positive for MRSA have worse survival than CF patients who do not have a positive culture for MRSA.

METHODS

The CFFPR contains demographic and clinical data collected at centers accredited by the Cystic Fibrosis Foundation using a standardized data collection form. It captures 92% to 97% of CF deaths reported in the US Vital Statistics. The CFFPR added a specific field to record the presence or absence of respiratory tract MRSA in 1996. The Cystic Fibrosis Foundation recommends that a complete microbiologic assessment of respiratory cultures obtained from sputum, throat swab, and/or bronchoalveolar lavage fluid be performed at least on an annual basis and preferably on a quarterly basis. The performance at least on an annual basis and/or bronchoalveolar lavage fluid be obtained from sputum, throat swab, and/or bronchoalveolar lavage fluid be performed at least on an annual basis and preferably on a quarterly basis. The presence or absence of MRSA in respiratory cultures was reported annually from 1994 through 1997, quarterly from 1998 through 2002, and at each encounter since 2003. The microbiology laboratories at accredited centers use standardized protocols to recover and identify CF pathogens and are monitored by site visits by the Cystic Fibrosis Foundation. Patients undergo passive follow-up.

The study design was a cohort study using longitudinal data from the CFFPR to compare patients positive for new respiratory cultures with MRSA with those who never had MRSA detected. Patients entered the study between January 1, 1996, and December 31, 2006, and were followed up until December 31, 2008. We excluded anyone younger than 6 years (unreliable pulmonary function data) and anyone older than 45 years (milder phenotype not representative of the CF population in general). Participants who had MRSA detected during their first 2 years of data collection or those who did not have at least 2 respiratory cultures and 2 years of observation were excluded.

We performed a descriptive analysis with calculation of means, standard deviations, and medians for continuous variables, and bivariate analyses were conducted using t tests. Categorical variables were measured using proportions and were compared using \( \chi^2 \) tests. For the baseline characteristics, at least a 5% difference in percent predicted forced expiratory volume in the first second of expiration (FEV\(_1\)) and at least a 0.5 difference in cultures, acute exacerbations, and outpatient visits between groups were considered clinically significant, regardless of statistical significance. Survival was assessed by the Kaplan-Meier product limit estimator and the log-rank test.

Cox regression models with time-varying covariates were used to account for possible differences between survival patterns that may be due to imbalances in severity of illness between the 2 groups. Examination of Schoenfeld residuals showed that the assumption of proportionality of hazards was reasonable. The primary outcome was the time from the age at entry into the cohort until age at death from any cause. In the cohort, 87% of deaths were related to cardiorespiratory failure; therefore, subgroup analyses investigating other etiologies of death were not performed. We used age as the time scale to minimize bias. Time at risk for the cohort began at age 7 years (because of inclusion/exclusion criteria, no patients could be observed prior to this age). To account for delayed entry into the study (ie, left-truncated data), the time at risk for an individual patient began at the age when he or she entered the cohort. For example, if a patient entered the cohort at age 15 years, the time from ages 7 to 15 years would be omitted. The 2-year observation period was not included in the analysis because no patient could have detectable MRSA during this time. Patients were censored at their last age at follow-up in 2008 (ie, right-censored data) and if they underwent organ transplantation (n=1401) or were lost to follow-up (n=1507).

Adjustments for clinical characteristics known to influence survival that may affect the relationship between respiratory tract MRSA and survival were performed. The models were constructed using confounders known to be associated with decreased survival. The confounders were identified a priori from clinical experience and a review of the literature; stepwise regression was not used. The covariates MRSA, P aeruginosa, and B cepacia complex were time-varying covariates and were recorded annually as present or absent. If respiratory culture results were missing in the year of death, then the culture result from the previous year was used. The following variables varied with time: (1) mean recorded value of FEV\(_1\) percent predicted in each year, calculated according to reference equations; (2) pancreatic status, which was a dichotomous variable defined by therapy with pancreatic enzymes; (3) CF-related diabetes mellitus, categorized according to the presence or absence of fasting hyperglycemia; and (4) socioeconomic status (SES), with Medicaid insurance as a proxy for low SES.

Several a priori subgroup and sensitivity analyses, adjusted for time-varying covariates, were performed. First, we investigated the impact of modeling MRSA as a time-lagged variable to ensure that MRSA did not occur as an association with aggressive treatment in the last year of life. Time-lagged MRSA resulted in the current year’s value of MRSA being substituted by the status of MRSA from the previous year. For example, an individual who entered the cohort in 2002 and died in 2005 would have the MRSA status in 2005 replaced by the 2004
value. In the main analysis, if that person was MRSA negative in 2002, 2003, and 2004 and only acquired MRSA in 2005 in the year of death, then that death would be associated with MRSA. However, in a 1-year lagged analysis, the death would instead be associated with the MRSA-negative group. None of the other variables besides MRSA status were lagged in the analysis. The competing risks of lung transplant were assessed by changing the definition for the primary outcome to death or lung transplant (from the original primary outcome of death); the hazard ratios (HRs) were unchanged from the main analysis.

Because age was used as the time scale, we also wanted to evaluate whether left-truncated data (delayed entry) introduced bias into the results, so 2 separate analyses were undertaken using both calendar and follow-up time as the time scale. Both of these analyses resulted in a similar adjusted HR when compared with the main analysis (HR, 1.28; 95% confidence interval [CI], 1.12-1.46, and HR, 1.25; 95% CI, 1.09-1.42, respectively). To address bias from missing data (assumed missing at random), 2 additional methods were performed: last observation carried forward and the multivariate imputation by chained equations method of multiple imputation in Stata (5 data sets were imputed and analyzed).19,20 Finally, analyses for center effect and adjustments for exacerbations, mucoid P aeruginosa, weight, and height did not change the results; therefore, the main analysis is presented.

Table 1. Baseline Characteristics of Study Cohort

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>MRSA Detected During Follow-up (n = 5759)</th>
<th>MRSA Negative (n = 14 074)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), y</td>
<td>15.1 (8.2)</td>
<td>16.9 (9.5)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Female sex, No. (%)</td>
<td>2788 (48.4)</td>
<td>6669 (47.4)</td>
<td>.19</td>
</tr>
<tr>
<td>FEV1, mean (SD), % predicted</td>
<td>79.3 (24)</td>
<td>76.6 (27)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Low socioeconomic status, No. (%)a</td>
<td>2668 (46.3)</td>
<td>5702 (40.5)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Taking pancreatic enzymes, No. (%)a</td>
<td>5442 (94.5)</td>
<td>12 822 (91.1)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Positive culture, No. (%)b</td>
<td>3616 (62.8)</td>
<td>8219 (58.4)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>3104 (53.9)</td>
<td>6812 (48.4)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>CF-related diabetes mellitus, No. (%)</td>
<td>43 (0.75)</td>
<td>169 (1.2)</td>
<td>.009</td>
</tr>
<tr>
<td>F508 Homozygous, No. (%)</td>
<td>3081 (53.5)</td>
<td>6910 (49.1)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Cultures, mean (SD)b</td>
<td>2.2 (1.5)</td>
<td>2.2 (1.5)</td>
<td>.56</td>
</tr>
<tr>
<td>Acute exacerbations, mean (SD)b</td>
<td>0.90 (1.8)</td>
<td>0.91 (1.8)</td>
<td>.84</td>
</tr>
<tr>
<td>Outpatient visits, mean (SD)b</td>
<td>5.4 (2.1)</td>
<td>5.1 (2.3)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Abbreviations: CF, cystic fibrosis; FEV1, forced expiratory volume in the first second; MRSA, methicillin-resistant Staphylococcus aureus.

Statistical significance was defined as a 2-tailed α < .05. There was greater than 80% power to detect what we considered a clinically meaningful HR in the MRSA group that was 1.2 times greater than the HR in those without MRSA, assuming a sample size of 20 000 patients with an overall mortality rate of 13% during the study period. We performed analyses using Stata version 10.0 (StataCorp, College Station, Texas).

Patients in the CFFPR (or guardians for minors) gave informed consent permitting their deidentified records to be used for research purposes. The study was reviewed and approved by the institutional review board at Johns Hopkins School of Medicine and the Cystic Fibrosis Foundation.

RESULTS

Between 1996 and 2008, 30 658 individuals with CF aged 6 to 45 years entered the CFFPR. To be confident that the study cohort captured new respiratory cultures positive for MRSA, individuals were excluded if they had MRSA in the first 2 years of observation (n = 1712), less than 2 years of observation in the cohort (n = 4971), fewer than 2 cultures in the first 2 years (n = 3666), or no cultures or FEV1 measurements during follow-up (n = 476) (Figure 1). Thus, the study cohort comprised 19 833 individuals. During 137 819 patient-years of observation (median, 7.3 years/patient), there were 2537 deaths, and 5759 individuals had respiratory tract MRSA detected. The mean ages at entry and exit were 15.4 years (95% CI, 15.3-15.5) and 22.6 years (95% CI, 22.5-22.8), respectively. The median estimated survival age was 35.9 years (interquartile range [IQR], 25.4-52.5), and the median age at death was 24.7 years (IQR, 19.6-32.1).

Baseline characteristics between those who never had a culture positive for MRSA and those who would go on to have a culture positive for MRSA are described in Table 1. At entry into the cohort, those who would go on to have MRSA detected were younger (mean age, 15.1 years; 95% CI, 14.9-
MRSA AND SURVIVAL IN CYSTIC FIBROSIS

The mortality rate was 27.7 deaths (95% CI, 25.3-30.4) per 1000 patient-years in patients with MRSA and 18.3 deaths (95% CI, 17.5-19.1) in those without MRSA. Total observation time in patient-years was 113,967 (median, 5.7 years/patient) for those with negative respiratory tract cultures for MRSA, 16,318 (median, 2.1 years/patient) for those with positive cultures for MRSA, and 7,534 (median, 1.2 years/patient) for those with missing data about respiratory tract MRSA.

Even after adjustment for FEV1 percent predicted, P aeruginosa, B cepacia complex, sex, SES, pancreatic insufficiency, and cultures positive for P aeruginosa and methicillin-sensitive S aureus (MSSA). There were no clinically significant differences in lung function, number of cultures performed, acute exacerbations, or outpatient visits between study groups during the baseline year. During subsequent follow-up, however, those with MRSA had more median cultures per year (3; IQR, 2-4; vs 2; IQR, 1-4) and acute exacerbations per year (1; IQR, 0-2; vs 0; IQR, 0-1) than those without MRSA. Total observation time in patient-years was 113,967 (median, 5.7 years/patient) for those with negative respiratory tract cultures for MRSA, 16,318 (median, 2.1 years/patient) for those with positive cultures for MRSA, and 7,534 (median, 1.2 years/patient) for those with missing data about respiratory tract MRSA.

The unadjusted and adjusted HRs in CF patients are shown in Table 2. All variables varied with time except sex. The unadjusted HR in individuals with MRSA was significantly higher when compared with those without MRSA (HR, 1.47; 95% CI, 1.32-1.62).

Table 2. Hazard Ratios of Mortality Calculated From Cox Regression Models With Time-Varying Covariates

<table>
<thead>
<tr>
<th>Covariates</th>
<th>Unadjusted HR (95% CI)</th>
<th>P Value</th>
<th>Adjusted HR (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRSA detected</td>
<td>1.47 (1.32-1.62)</td>
<td>&lt;.001</td>
<td>1.27 (1.11-1.45)</td>
<td>.001</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa detected</td>
<td>1.55 (1.37-1.76)</td>
<td>&lt;.001</td>
<td>1.03 (0.89-1.18)</td>
<td>.70</td>
</tr>
<tr>
<td>Burkholderia cepacia complex detected</td>
<td>2.84 (2.48-3.25)</td>
<td>&lt;.001</td>
<td>2.26 (1.95-2.62)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Female sex</td>
<td>1.19 (1.10-1.28)</td>
<td>&lt;.001</td>
<td>1.42 (1.29-1.57)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Low socioeconomic status&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.87 (1.73-2.03)</td>
<td>&lt;.001</td>
<td>1.38 (1.25-1.54)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Patients taking pancreatic enzymes</td>
<td>0.52 (0.47-0.58)</td>
<td>&lt;.001</td>
<td>1.12 (0.87-1.45)</td>
<td>.39</td>
</tr>
<tr>
<td>CF-related diabetes mellitus</td>
<td>1.73 (1.56-1.92)</td>
<td>&lt;.001</td>
<td>1.46 (1.28-1.67)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Calendar year, 5-y increments&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.78 (0.73-0.83)</td>
<td>&lt;.001</td>
<td>0.91 (0.83-0.99)</td>
<td>.04</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;1&lt;/sub&gt; % predicted, 10% increments&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.52 (2.43-2.62)</td>
<td>&lt;.001</td>
<td>2.45 (2.36-2.55)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Abbreviations: CF, cystic fibrosis; CI, confidence interval; FEV<sub>1</sub>, forced expiratory volume in the first second; HR, hazard ratio; MRSA, methicillin-resistant Staphylococcus aureus.

<sup>a</sup>Adjusted for all the covariates listed in the table. All variables varied with time except sex.

<sup>b</sup>Percentage of individuals with any state insurance, including Medicaid, used as a proxy.

<sup>c</sup>The HR changes for each 5-year increase in the cohort. For example, a patient in the cohort in 2005 had an unadjusted HR that is 0.78 times that of a patient in 2000.

<sup>d</sup>The HR changes for each 10% decrease in FEV<sub>1</sub> percent predicted. For example, if a patient’s FEV<sub>1</sub> decreased from 80% to 70%, the unadjusted HR increases by a factor of 2.02.

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The results for a priori subgroup and sensitivity analyses are in Table 3. The adjusted HR when MRSA was lagged 1 year was 1.54 (95% CI, 1.28-1.85). Similar results were found when MRSA was lagged 2 years (HR, 1.51; 95% CI, 1.24-1.82) and 3 years (HR, 1.44; 95% CI, 1.18-1.76). In addition, MRSA was associated with an increased risk of death when compared with MSSA (adjusted HR, 1.79; 95% CI, 1.51-2.12), and in comparisons of MRSA patients who never co-cultured *P aeruginosa* with those who were MRSA negative and *P aeruginosa* negative (adjusted HR, 1.96; 95% CI, 1.79-2.12), and patients who always had MRSA detected with those who were always MRSA negative (adjusted HR, 1.96; 95% CI, 1.80-2.15). Among individuals who ever had MRSA detected, HRs between those who cleared MRSA (n=1928) were compared with patients who had MRSA persistently detected (2 or more years with MRSA, n=3831). The adjusted HR of those with persistent MRSA compared with those who had MRSA on only 1 culture was 1.31 (95% CI, 1.07-1.60). There was no difference in the adjusted HR between patients who cleared MRSA and those who were always MRSA negative (HR, 0.99; 95% CI, 0.83-1.18).

Based on the results of our previous study using the CFFPR to determine the association between persistent MRSA detection and lung function decline, which suggested a lack of a relationship between MRSA and rate of FEV₁ decline in individuals with CF older than 22 years, we separately explored the relationship of MRSA on survival in adults. The adjusted HR in those older than 22 years was 1.37 (95% CI, 1.16-1.61). Finally, the association between MRSA and mortality from 1996 through 2002 (adjusted HR, 1.37; 95% CI, 1.04-1.82) was greater than the risk of death from 2003 to 2008 (adjusted HR, 1.24; 95% CI, 1.07-1.44), although this was not statistically significant (P = .19).

### Table 3. Sensitivity Analyses: Adjusted HRs of Mortality From MRSA Calculated From Cox Regression Models With Time-Varying Covariates Under Various Scenarios

<table>
<thead>
<tr>
<th>Defined MRSA Group</th>
<th>Comparator Group</th>
<th>Adjusted HR (95% CI)ᵃ</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time-lagged MRSA, 1 year</td>
<td>MRSA never detected</td>
<td>1.54 (1.28-1.85)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>MRSA detected</td>
<td>MSSA detected</td>
<td>1.79 (1.51-2.12)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>MRSA detected, <em>Pseudomonas aeruginosa</em> never detected</td>
<td>MRSA never detected, <em>P aeruginosa</em> never detected</td>
<td>1.96 (1.27-3.04)</td>
<td>.002</td>
</tr>
<tr>
<td>MRSA always detected</td>
<td>MRSA never detected</td>
<td>1.96 (1.38-2.79)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Persistent MRSA</td>
<td>MRSA clears</td>
<td>1.31 (1.07-1.60)</td>
<td>.008</td>
</tr>
<tr>
<td>MRSA clears</td>
<td>MRSA negative</td>
<td>0.99 (0.83-1.18)</td>
<td>.88</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; HR, hazard ratio; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-sensitive *S aureus*.

ᵃAdjusted for sex, cystic fibrosis-related diabetes mellitus, pancreatic status, *Burkholderia cepacia* complex, *P aeruginosa* (as appropriate), socioeconomic status, calendar year, and FEV₁ percent predicted. All variables varied with time except sex.

ᵇDetection of MRSA in only 1 year of follow-up.

In the study cohort, there were patients who had missing data or gaps in observation or were lost to follow-up. Data were not missing for pancreatic status, sex, SES, and calendar year. There were missing data for cultures (MRSA, *P aeruginosa*, and *B cepacia* complex, 4.4% each), CF-related diabetes (1.0%), and FEV₁ (2.5%). We performed 3 analyses to address potential bias from missing data (assumed at random), none of which changed the main results: (1) excluding all patients with missing respiratory cultures at the time of death (adjusted HR, MRSA, 1.30; 95% CI, 1.13-1.49) (2) imputing missing data using the last observation carried forward (adjusted HR, 1.28; 95% CI, 1.15-1.42), and (3) performing multiple imputation using multivariate imputation by chained equations (adjusted HR, 1.27; 95% CI, 1.13-1.43).

During follow-up, there were 5340 patient-years that were not observed (patients left the cohort for a year and then re-entered). These gaps in observation did not contribute to the time at risk. During this study, 184 patients in the MRSA group (3.2%) were lost to follow-up (ie, did not die, were not censored due to lung transplantation, or did not end follow-up in 2008); 9.4% were lost to follow-up in the MRSA-negative group (n=1323).

**COMMENT**

We performed a survival analysis on 19833 patients with CF to determine whether MRSA is associated with shortened survival. The most important finding from this study was that detection of MRSA in the respiratory tract of CF individuals, even in the most conservative analyses controlling for factors associated with severity of illness, was associated with a risk of death that was 1.27 times that of individuals who never had MRSA detected. In CF patients with MRSA, one-third of the attributable risk of death may be associated with the presence of MRSA. These findings suggest that MRSA may be a potentially modifiable risk factor for death in CF.

The question arises as to whether MRSA is independently contributing to worse survival or a marker of disease severity. By Kaplan-Meier analysis, the unadjusted impact of MRSA on survival is a life span that is a median 6.2 years shorter in those with MRSA than those without (Figure 2). However, because Kaplan-Meier estimations do not account for multiple confounders that influence survival, we used Cox regression models with time-varying covariates to adjust for factors known to be associated with worse survival. Even after these adjustments, MRSA remained an independent risk factor for death. This finding was further strengthened by the robustness of the results of several sensitivity analyses. Because the study cohort included 19833 patients (5759 with MRSA), there were adequate numbers of pa-
tients to perform several sensitivity analyses adjusted for confounders (Table 3).

Our a priori sensitivity analyses also support the idea that MRSA is an independent contributor to worse survival in CF and not just a marker of severity. In our analysis, detection of MRSA was associated with worse outcomes in CF compared with MSSA; individuals with MRSA were 1.79 times more likely to die than those with MSSA. This finding is consistent with our previous FEV₁ data showing more rapid decline in individuals with MRSA compared with MSSA² and suggests that further studies are needed to investigate associations between virulence factors in CF MRSA strains and exacerbation of CF lung disease.

Additionally, the decreased survival associated with MRSA is not due to MRSA being more commonly linked with P. aeruginosa infection. There is a significant increase in the adjusted HR even in MRSA-positive CF patients who never have a positive culture for P. aeruginosa.

Time-lagged analyses of our results also suggest that MRSA is an independent risk factor for death and not just an “end-of-life” marker. There is a risk that MRSA could falsely appear to be associated with worse survival because hospitalizations and antibiotic courses, known risk factors for MRSA in non-CF populations, occur more frequently during a CF patient’s last year of life and increase the risk of MRSA acquisition. However, if MRSA is simply appearing as a marker of the end of life, one would expect the increased risk of death to be significantly reduced by a time-lagged analysis. In the 1-year lagged model, the adjusted HR associated with MRSA was 1.54 times the HR of those who were MRSA negative. This increased hazard remained consistent whether MRSA was present 1, 2, or 3 years before death.

Another analysis supporting the idea that MRSA is an independent contributor to worse survival in CF and not a marker of severity is that transient MRSA detection that clears within 1 year is not associated with an increased risk of death. If MRSA is only a marker of severity, those individuals who have MRSA but subsequently clear the organism should also have an increased risk of death (because MRSA would not be responsible for the increased risk of death, but only a marker of the general severity of illness of the patient). We found the opposite: CF individuals who clear MRSA within 1 year have the same risk of death as those who never have a positive culture for MRSA, while those who persistently test positive for MRSA (2 or more years with MRSA detected) have a significantly increased risk of death.

Finally, persistence of MRSA in respiratory cultures is associated with an increased risk of death. When individuals in the study cohort who always had MRSA detected are compared with those who never had MRSA detected, the risk of death was significantly increased, with those always MRSA positive being almost 2 times as likely to die. All of these analyses suggest that MRSA is more than a marker of severity and may be a potentially modifiable risk factor for death.

In addition to sensitivity analyses, several subgroup analyses were performed. We found that MRSA increases mortality risk in both children and adults with CF; in contrast to our previous findings that MRSA increases rate of decline of FEV₁ only in children with CF. This is consistent with the observation that rate of decrease in FEV₁ significantly slows at lower FEV₁ and does not demonstrate as much dynamic change with exacerbations. This observation has been previously reported as the primary finding in an analysis of the Epidemiologic Study of Cystic Fibrosis database and is seen in the practice of many CF centers that rely on symptoms more than FEV₁ when making treatment decisions in severe CF lung disease. The association between MRSA and the clinical status of CF adults with lower FEV₁ is likely better reflected by end points such as exacerbations and mortality than by rate of decline in FEV₁ and may explain the contrast in findings for adults between our previous FEV₁ and current mortality analysis.

In the subgroup analysis exploring the temporal association between MRSA and mortality, there was a trend toward a higher risk of death in the years 1996 through 2002 compared with 2003 through 2008. There were significant improvements in models of care over that time period, which might explain the trend toward an improvement in mortality. One other possible contributor is that since the early 2000s, there has been increasing concern about MRSA as a pathogen and availability of new oral MRSA therapy, which may have made practitioners more likely to treat MRSA. Unfortunately, treatment data for MRSA is not available in the CFFPR, and this hypothesis will need to be examined in future studies.

Although this study demonstrates a clear association between MRSA and worse survival in individuals with CF, epidemiologic studies can be affected by a number of biases that may influence results. First, misclassification of individuals within the study cohort is a risk. The strict inclusion and exclusion criteria limited initial misclassification bias by requiring multiple MRSA-positive cultures over a 2-year period prior to entering the cohort. However, if misclassification bias is present and sicker MRSA patients are mixed with the MRSA-negative group, the result would be to minimize and potentially underestimate the association between MRSA and mortality.

Second, sampling bias can result in an apparent increased association between survival and MRSA, because sicker individuals may have more cultures and thus be more likely to have MRSA detected. This is unlikely a significant factor in the study because the difference in the number of cultures between the groups was similar at baseline, and during follow-up there was only a small difference (median, 1 culture/year). Furthermore, an analysis including the number of cultures as a variable did not change the results (adjusted HR, 1.33; 95% CI, 1.15-
A third potential bias is that more patients were lost to follow-up in the MRSA-negative group than the MRSA group (9.4% vs 3.2%). This is unlikely to have a significant influence on the primary outcome because (1) 92% to 97% of CF deaths are captured by the registry; (2) at the time of the last visit, MRSA-negative patients lost to follow-up had a median age of 21.1 years (IQR, 16.2-27.9) and a median FEV\textsubscript{1} percent predicted of 72.6% (IQR, 47.3%-91.2%), suggesting they were healthier patients who chose not to follow-up as opposed to patients who had died; and (3) analyses treating as deaths all patients lost to follow-up who had an FEV\textsubscript{1} less than 30% did not change the results. This differential in follow-up actually results in a bias toward finding no difference between the groups and suggests the true HR may be greater than our estimate.

A fourth limitation is that the CFPPR data did not include detailed information on MRSA treatment for individuals in the study cohort. This prevents analysis of treatment effect on outcomes and may bias results to an underestimation of MRSA effect. Finally, as with all observational studies, unmeasured confounders may contribute to the results. As described in the Methods section, we used robust Cox regression models adjusted for numerous confounders and performed multiple sensitivity analyses to decrease this possibility.

The applicability of our estimates to all CF patients with MRSA merits discussion. Based on the inclusion and exclusion criteria, the study findings are generalizable to CF patients who have cultures performed and who are seen at accredited CF centers at least annually. Because strains and prevalence of MRSA vary from country to country, generalizability of these results outside the United States should be done with caution, and similar large-cohort studies will be required to determine reproducibility in these populations.

The results of this study in conjunction with previous data further establish MRSA as a significant CF pathogen and provide impetus for more aggressive treatment of CF patients who are persistently MRSA positive. Ideally this treatment will be conducted in the context of clinical trials, because optimal therapeutic approaches for MRSA, both persistent and new, are not yet known. The study results also reinforce the importance of following current CF infection control guidelines to minimize transmission of MRSA, particularly in outpatient clinics with high CF patient volume.

CONCLUSION

This study demonstrates that individuals with CF who have respiratory tract cultures positive for MRSA have worse survival compared with those who never have MRSA, even after adjusting for severity of illness.

Author Contributions: Dr Dasenbrook had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Statistical analysis: Dasenbrook, Checkley, Merlo, Lechtzin, Boyle.

Acquisition of data: Dasenbrook, Boyle.

Analysis and interpretation of data: Dasenbrook, Checkley, Merlo, Konstan, Lechtzin, Boyle.

DRAFTING OF THE MANUSCRIPT: Dasenbrook, Boyle.

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RECIPIENT OF FUNDING: Dasenbrook, Boyle.

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Study supervision: Boyle.

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