Serum Iron Level Is Associated with Time to Antibiotics in Cystic Fibrosis

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Abstract

Background: Serum levels of hepcidin-25, a peptide hormone that reduces blood iron content, are elevated when patients with cystic fibrosis (CF) develop pulmonary exacerbation (PEx). Because hepcidin-25 is unavailable as a clinical laboratory test, we questioned whether a one-time serum iron level was associated with the subsequent number of days until PEx, as defined by the need to receive systemic antibiotics (ABX) for health deterioration.

Methods: Clinical, biochemical, and microbiological parameters were simultaneously checked in 54 adults with CF. Charts were reviewed to determine when they first experienced a PEx after these parameters were assessed. Time to ABX was compared in subgroups with and without specific attributes. Multivariate linear regression was used to identify parameters that significantly explained variation in time to ABX.

Results: In univariate analyses, time to ABX was significantly shorter in subjects with *Aspergillus*-positive sputum cultures and CF-related diabetes. Multivariate linear regression models demonstrated that shorter time to ABX was associated with younger age, lower serum iron level, and *Aspergillus* sputum culture positivity.

Conclusions: Serum iron, age, and *Aspergillus* sputum culture positivity are factors associated with shorter time to subsequent PEx in CF adults. Clin Trans Sci 2015; Volume 8: 754–758

Keywords: cystic fibrosis, exacerbation, iron, age, *Aspergillus*

Introduction

Several recent studies have sought to characterize biomarkers[1–3] and clinical features[4–6] that identify patients with cystic fibrosis (CF) who are at increased risk of antibiotic treatment. In all of these investigations, the need for antibiotics because of worsened health status was taken as evidence of pulmonary exacerbation (PEx). In lieu of a widely accepted definition for PEx,[7,8] linking this phenomenon to clinical judgment is reasonable but inherently fosters variability in care delivery,[9] and perhaps by extension, heterogeneous treatment outcomes. Because the morbidity associated with PEx is substantial and sometimes permanent,[10–12] salubrious interventions should be timely, customized, and as efficacious as possible. Biomarkers are intended to facilitate the achievement of these goals, but a biomarker needs to not only reflect biologically informative processes predictive of PEx but also be suitable for use as a routine diagnostic tool.[13] Presently, most blood and sputum biomarker candidates do not satisfy the latter criterion and/or have not been validated by testing their performance in large cohorts.[14]

Our group has observed distinctive changes in measures of iron homeostasis during PEx.[15] Treating CF patients with intravenous antibiotics for PEx significantly increased mean blood concentrations of iron but not hemoglobin while reducing serum levels of interleukin-6 (IL-6) and hepcidin-25, two mediators that lower blood iron content. IL-6 stimulates the liver to elaborate a small peptide called hepcidin-25 into the circulation[16] that promotes iron sequestration by mononuclear cells[17] and decreases iron uptake by enterocytes in the duodenum.[18] Thus, antibiotics improve blood iron levels in the context of PEx by modulating the hormonal axis that couples inflammatory pathways (IL-6) to iron absorption and utilization (hepcidin-25). We have also reported[19] that serum levels of hepcidin-25 are closely correlated with Akron Pulmonary Exacerbation Score, an inventory of signs, symptoms, and diagnostic test results that has been used to standardize antibiotic prescription in an academic pulmonary practice.[20]

Like other CF biomarker candidates,[1–3] serum hepcidin-25 is not yet available as a routine clinical laboratory test. However, given the aforementioned evidence that hepcidin-25 is elevated above baseline in patients during PEx and that its major physiological function is to lower serum iron levels,[21] we questioned in this retrospective study whether a single measurement of serum iron, which is available to most clinicians within a few hours from any modern clinical laboratory, was associated with the time it took for patients to subsequently experience a PEx, defined as any need for oral or intravenous ABX. We also examined whether other biochemical, clinical, and microbiological variables obtained concurrently with the serum iron readings could be used to explain variation in time to ABX.

Methods

Spirometry, phlebotomy, and sputum cultures were performed at a single office visit in 54 CF adults who had participated in studies of iron homeostasis.[15,22] Most of the subjects (n = 42) had no evidence of recently worsened health, and the rest (n = 12) were evaluated at the end of PEx treatment. Only data collected at a single office visit were analyzed. No information was censored because a subject failed to receive antibiotics after a specific time period; in other words, every subject considered in this study was treated with systemic (i.e., oral or intravenous) ABX at some point after the aforementioned testing. Permission to use these data was granted by the Committee for the Protection of Human Subjects at Dartmouth College (CPHS #23311). Serum iron readings, complete blood counts, and sputum cultures with *in vitro* ABX susceptibility profiles were obtained, as previously

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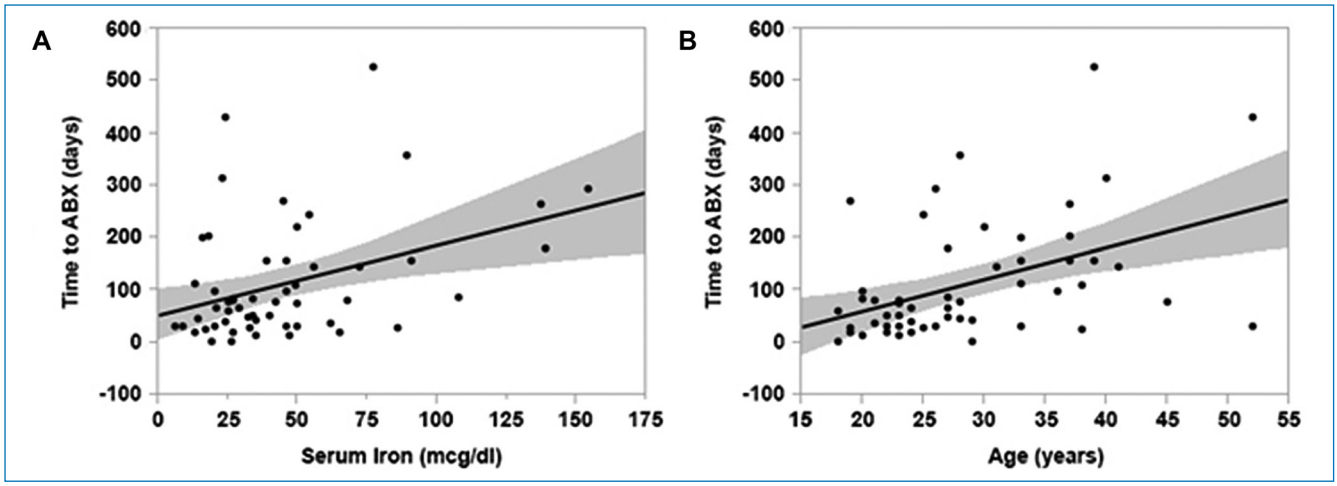

Figure 1. Linear regression models depicting the relationships between (A) time to ABX and serum iron and (B) time to ABX and age. Shaded regions represent the 95% CI for the best fit lines.

reported.[15,22] Data for each subject were included only if the most proximate date of ABX prescription after data acquisition was accurately identified in our electronic medical record (e-DH). This interval, measured in days, represented the time to ABX and was ascertained by reviewing notes and prescription orders for the 54 subjects, all of whom were followed exclusively at our CF center.

JMP 11.2.1 (SAS Institute, Inc., Cary, NC) was used to perform all calculations and to create the plots in *Figure 1*. Subjects were categorized by the presence or absence of attributes listed in *Table 1*. Then, based on these distinctions, mean differences between subgroups in the number of days that had elapsed between data collection and ABX were compared by unpaired Student's *t*-tests. For these univariate calculations, standard deviations (SD), 95% confidence intervals (CI) in the mean difference in time to ABX, and two-tailed *p*-values are given in *Table 1*. In *Figure 1*, simple linear regression was used to describe the relationships between serum iron and time to ABX (*Figure 1A*) and age and time to ABX (*Figure 1B*). Fisher's exact test was used to compare proportions of subjects with and without categorical features.

Multivariate linear regression was used to identify specific attributes that significantly predicted variation in the number of days between identification of these attributes and ABX. Model 1 (*Table 2*) included covariates listed in *Table 1* that satisfied a *p*-value threshold of 0.25 for entry into and 0.10 for exclusion from the model. Model 2 employed forward stepwise entry of *Aspergillus* sputum status as a covariate into model 1. In model 3, serum iron was entered as a covariate to model 2. The same *p*-value thresholds for inclusion and exclusion were used in models 2 and 3. A two-tailed p-value < 0.05 was considered statistically significant for all calculations.

Results

Subjects were similar to the general CF population

Clinical, biochemical, and microbiological attributes of the population are listed in *Table 1*. We observed a relatively even distribution of patients based on sex, homozygous F508*del*-CFTR mutation status, and CF-related diabetes (CFRD). The proportions of subjects in our study who were homozygous for the F508*del*-CFTR mutation (56%) and carried a diagnosis of CFRD (55%) were similar to those seen in larger CF cohorts.[23,24] On average, subjects had measurements of percent-predicted forced expiratory volume in 1 second (FEV1%) that were typical for most CF patients in this age range.[25,26] The proportion of *Aspergillus*-positive sputum cultures (29%) in our study was identical to that reported in another single-center investigation.[27] Subjects with multidrug-resistant *Pseudomonas aeruginosa* (MDRPA) had a mean serum iron level (42.5 mcg/dL) that was similar to that of uninfected subjects (48.8 mcg/dL).

Sputum *Aspergillus* and CFRD were associated with shorter time to ABX

Of the categorical distinctions in *Table 1*, only isolation of *Aspergillus* species from a sputum culture sent to the microbiology laboratory at the time of serum iron measurement and having a diagnosis of CFRD at the time of serum iron measurement were associated with a shorter time to ABX. Subjects with CFRD received ABX an average of 67.1 days sooner than those without CFRD. Subjects with *Aspergillus*-positive sputum cultures received ABX an average of 64.9 days earlier than those without *Aspergillus* infection. Fisher's exact test revealed that subjects with *Aspergillus* were not significantly more likely to have CFRD (odds ratio = 3.3, 95% CI 0.9, 12.2, $p = 0.08$). F508*del*-CFTR homozygosity, female sex, FEV1%, and methicillin-resistant *Staphylococcus aureus* (MRSA) or MDRPA in sputum culture were not associated with differences in time to ABX. Importantly, these were univariate comparisons that did not address the issue of multiple factors being present in the same subject.

Serum iron and age are associated with time to ABX

We observed linear relationships between serum iron and time to ABX and between age and time to ABX (*Figure 1*). The relationship between serum iron and time to ABX (Panel A) can be expressed by the function, time to ABX = 1.34(serum iron) + 53.6. The correlation coefficient, r, for the fit line was 0.39 ($p = 0.004$). In Panel B, time to ABX = 6.1(age) – 60.2 with $r = 0.44$ ($p = 0.0008$). Serum iron and age were not correlated with each other ($r = -0.0003$, $p = 0.99$).

Factor	Subjects (% of cohort)	Mean days (SD)	Mean difference (95% CI)	*p*-Value
BMI (kg/m²)				
≥25.0 (referent)	11 (20)	163.0 (146.2)	–	–
18.1–24.9	35 (65)	114.9 (108.6)	−48.1 (−34.3, 130.4)	0.25
≤18.0	8 (15)	45.5 (28.6)	−117.5 (−228.9, −6.1)	0.04
FEV1%				
≥60 (referent)	20 (37)	111.3 (99.4)	–	–
41–59	17 (31.5)	144.9 (136.6)	33.6 (−112.6, 45.3)	0.39
≤40	17 (31.5)	87.7 (103.1)	−23.7 – 91.3, 44.0)	0.48
Hemoglobin (g/dL)				
≥13.0 (referent)	25 (46)	120.8 (129.3)	–	–
12.9–10.1	22 (41)	125.4 (109.8)	4.6 (−66.4, 75.6)	0.90
≤10.0	7 (13)	57.6 (25.0)	−63.2 (−164.6, 38.3)	0.21
F508*del*-CFTR homozygote				
No (referent)	24 (44)	124.5 (100.0)	−18.1 (−80.9, 44.8)	0.57
Yes	30 (56)	106.4 (124.6)		
Sex				
Male (referent)	26 (48)	111.1 (129.0)	6.4 (−56.3, 69.1)	0.84
Female	28 (52)	117.5 (99.6)		
CFRD				
No (referent)	24 (44)	151.7 (140.4)	−67.1 (−127.3, −6.9)	0.03
Yes	30 (56)	84.6 (76.2)		
Sputum MDRPA				
No (referent)	29 (54)	134.0 (124.9)	−42.2 (−104.0, 19.5)	0.18
Yes	25 (46)	91.8 (96.5)		
Sputum MRSA				
No (referent)	45 (83)	107.4 (111.3)	42.4 (−40.8, 125.6)	0.31
Yes	9 (17)	149.8 (125.6)		
Sputum *Aspergillus*				
No (referent)	38 (70)	133.7 (126.6)	−64.9 (−131.1, 1.3)	0.05
Yes	16 (30)	68.8 (54.2)		

CFRD = CF-related diabetes; BMI = body mass index; FEV1% = percent-predicted forced expiratory volume in 1 second; MDRPA = multidrug-resistant *Pseudomonas aeruginosa*; MRSA = methicillin-resistant *Staphylococcus aureus*; WBC = white blood cell count. Data are presented as mean ± standard deviation.

Table 1. Clinical, microbiological, and biochemical characteristics of the study population.

Serum iron, age, and sputum *Aspergillus* predicted time to ABX in multivariate analyses

We first devised a multivariate regression model (*Table 2*) for time to ABX which incorporated age, F508*del*-CFTR homozygosity, CFRD, FEV1%, and MDRPA and MRSA sputum status. In this model, every year of life lived by the 54 subjects was associated with a mean increase in time to ABX of 6.4 days. Model 1 explained 32% of the variation in time to ABX ($p = 0.003$). Body mass index and FEV1% could not be simultaneously introduced into the model because of multicollinearity. Model 2 added *Aspergillus* in forward stepwise fashion. In model 2, age had the same effect as model 1 ($\beta = 6.4$, $p = 0.001$), but *Aspergillus* was not contributory. The variation in time to ABX accounted for by model 2 was 36% ($p = 0.0003$). Model 3 added serum iron to model 2 in forward stepwise fashion and accounted for 48% of variation in time to ABX with three significant terms. An increase in serum iron of 1 mcg/dL was associated with an increase in time to ABX of 1.4 days. Model 3 revealed that subjects who were positive for *Aspergillus* received ABX an average of 61.7 days earlier than those who were not infected. Age was retained as statistically significant covariate in the final model, with each of life lived being associated with a 5.8 day increase in time to ABX.

Discussion

The primary objective of this study was to determine if a single serum iron measurement in CF adults significantly explained subsequent time to ABX. We were interested in this question because we had previously observed that CF patients with worse lung function and more severe malnutrition, features that could plausibly increase their chances of receiving ABX, also generally

Factor	Model 1			Model 2 (Model 1 + *Aspergillus*)			Model 3 (Model 2 + Serum Iron)		
	α	95% CI	*p*-Value	α	95% CI	*p*-Value	α	95% CI	*p*-Value
Age	6.4	3.0, 9.8	0.0004	6.4	3.1, 9.7	0.001	5.8	2.9, 8.9	0.0003
CFRD	−58.5	−113.8, −3.2	0.04	−47.3	−102.9, 8.3	0.09	–	–	–
MRSA	62.8	−11.5, 137.2	0.09	67.2	−5.8, 140.1	0.07	–	–	–
Aspergillus	–	–	–	−53.2	−113.6, 7.1	0.08	−61.7	−115.6, −7.7	0.03
Serum iron	–	–	–	–	–	–	1.4	0.6, 2.1	0.0006

β = coefficient; CI = confidence interval.

Table 2. Parameter estimates for predictors of time to ABX from multivariate linear regression models incorporating data from all subjects ($n = 54$).

had lower circulating iron levels than healthier patients.[22] Moreover, we have found that serum concentrations of IL-6 and hepcidin-25, two humoral mediators that lower blood iron content[21] but are not readily quantifiable by clinical laboratories, are reduced by ABX, leading to relative improvements in serum iron.[15] In the current study, we identified a significant linear relationship between time to ABX and serum iron (*Figure 1A*) and that serum iron contributed uniquely to a multivariate linear regression model describing variation in time to ABX (*Table 2*). We also noted that age and sputum positivity for *Aspergillus* were significant covariates in this model.

The aforementioned relationships between serum iron and time to ABX suggest that this parameter varies with the health status of CF patients, perhaps under the influence of hepcidin-25.[28] Whether and to what extent serum iron levels fall during the onset of PEx are not yet known. However, the end-stage CF lung is replete with iron and associated binding proteins, such as hemoglobin and ferritin.[29] Thus, iron appears to accumulate in the lungs of CF patients as they age. If airway bleeding allows hemoglobin-bound iron to enter the CF lung during the earliest phases of PEx, this sequence might explain the shorter time to ABX in subjects with lower serum iron levels (*Figure 1A*). Further studies are needed to characterize the clinical ramifications of airway bleeding, including its potential contribution to the development of PEx.

This is the second study to show that *Aspergillus* infection is associated with PEx (*Table 2*), which is noteworthy because fungi are not routinely targeted for treatment, except in cases of allergic bronchopulmonary aspergillosis (ABPA).[30] Without adjusting for FEV1% (which our study suggests is not necessary), Amin et al.[6] calculated a relative risk of 1.94 for PEx necessitating hospital admission among CF children with ≥2 *Aspergillus*-positive sputum cultures in the same year compared to those without this finding. In a young cohort (mean age: 17.2±9.2 years), Milla et al.[31] noted that older age but not recovery of *Aspergillus* from sputum more completely accounted for variation in negative outcomes. The prevalence of *Aspergillus* infection in our study (29.1%) was higher than that (12.4%) reported by Milla et al.,[31] perhaps offering an explanation for this discordant finding. Regardless, the clinical ramifications of incident and prevalent *Aspergillus* airway infection in CF patients warrant further investigation.

Increasing age had a seemingly protective effect against antibiotic use (*Figure 1*B). Block et al.[5] prospectively followed 249 adult and adolescent CF patients who were infected by multidrug resistant bacteria for up to 4.5 years. Applying Fuchs criteria,[32] these authors defined PEx as "an acute exacerbation of pulmonary symptoms that, in the opinion of the patient's CF physician, was severe enough to require intravenous antibiotics." Similar to our observation that younger patients were at higher risk of receiving antibiotics (*Figure 1B*), Block et al.[5] noted that older age was independently associated with lower PEx risk, both in a Cox proportional-hazards model (HR 0.98, $p = 0.03$) and a multivariate logistic regression model of factors predicting PEx during the first year of follow-up (OR 0.93, $p = 0.002$).

This phenomenon has not been explained, but it might reflect an influence of patient age on decision making by CF care providers. Kraynack et al.[9] surveyed CF centers using a series of vignettes and showed that even modest variations in clinical presentation substantially impacted antibiotic treatment decisions. One might also postulate that younger CF patients are more likely than older ones to access the healthcare system, perhaps because of more concerted advocacy from caregivers, fewer lifestyle responsibilities, and/or more frequent exposure to PEx triggers, including respiratory viruses.[33] However, the mean age of subjects with PEx in our study was 29.1±8.8 years and was 30.1±13.8 years in that of Block et al.,[5] diminishing the likelihood of these possibilities. Having affirmed that age alone somehow modifies risk of antibiotic use, we feel that contributory factors in adults and children should be sought and contrasted.

We acknowledge several limitations to our study. First, given its retrospective nature, we cannot exclude the possibility that subjects obtained antibiotics outside our CF center. This could confound our analyses because antibiotics increase serum iron levels,[15] and we would not have known exactly when they were prescribed relative to data collection. By only including data from subjects who were in their usual state of health, we attempted to minimize the effects of antecedent antibiotic exposure on parameters of interest. However, 12 of 54 subjects (22%) had recently completed antibiotic courses. Because it is not yet known how rapidly and to what extent serum iron levels fall between antibiotic courses, we cannot explain how this subset might have influenced our calculations, except to offer that their symptoms may have waxed and waned more frequently, thus predisposing them to be treated with antibiotics more often.

Second, it is not yet known how inhaled antibiotics and macrolide antibiotics affect iron homeostasis in CF patients. Participants in the current study had been prescribed these classes of medications, but our models for time to ABX do not account for their use because we could not be completely confident about treatment adherence and documentation of their use.

Third, our work reflects the practice patterns of a single CF center. Although we could tell from the electronic health record when subjects were prescribed antibiotics, we cannot make any inferences about the clinical rationale behind their use and whether knowledge of serum iron or other attributes influenced

the decision making process. Despite the limited size of our cohort, it was similar in many ways to larger CF patient populations. However, the lower burden of MRSA[34,35] and higher prevalence of MDRPA[36] in our study are idiosyncrasies that might affect not only PEx frequency but also trends in biomarkers of iron homeostasis.

Finally, we were unable to report herein whether serum hepcidin-25 alone predicted variation in time to ABX. The cost associated with performance of the assay was prohibitive. As this was a retrospective study, we did not have archived serum from all 54 subjects. This would have been necessary to appropriately introduce serum hepcidin-25 into the multivariate models (*Table 2*).

Conclusion

In summary, we demonstrate that serum iron, age, and sputum *Aspergillus* positivity are factors associated with time to ABX in CF and may be useful in determining the risk that a patient will soon require antibiotics. Paradigms for risk stratification and risk factor modification have been described for acute coronary syndromes,[37] acute kidney injury,[38] and acute pulmonary embolism[39] but are not yet defined for PEx. Devising the most useful paradigm for PEx might require, as our data suggest, using multiple parameters to better explain variation in outcomes like antibiotic use. This approach also addresses the need for individualized treatment strategies for CF patients.

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