is responsible for the decrease in protein C and S during the procedures, although an increase in vascular permeability could have contributed to a limited extent (Figures 1K and 1L). The decrease in albumin was more pronounced in patients undergoing lung transplantation, suggesting hemodilution was more extensive during this procedure in comparison with CABG.

The combined results of our study provide evidence for the development of a hypercoagulable status in patients undergoing off-pump lung transplantation despite a pronounced prolongation of the conventional PT and APTT. The hypercoagulable status appears related to significant hemodilution, resulting in low levels of protein C and S, which are key endogenous anticoagulant proteins.

The finding that the results of the thrombin generation assay are not reflected by those of conventional laboratory tests suggests the latter should not be used to evaluate the hemostatic function of patients undergoing lung transplantation and CABG surgery. As a consequence, we do not recommend using these tests in the management of these patients, for example, to guide transfusion of blood products or antithrombotic treatment. These recommendations are in line with previous advice given by our group after studying the hemostatic function in patients undergoing a liver transplantation. In those studies, the thrombin-generating capacity in plasma was also increased despite a profoundly abnormal PT and APTT (7).

Our results also explain, at least in part, the high risk for systemic thromboembolic events in the postoperative period generally observed in this group of patients. In our center, patients undergoing lung transplantation routinely receive low-molecular-weight heparin prophylaxis shortly after surgery, often on the same day, and thereafter during the entire length of stay as part of our postoperative thromboprophylaxis protocol. This approach now seems justified by the results of this study. Moreover, the strong correlation between intraoperative resistance to thrombomodulin and length of stay in the hospital may suggest that attempts to avoid (hemodilution-related) thrombomodulin resistance are beneficial.

We propose further exploration of the implementation of a restrictive perioperative fluid management and routine postoperative anticoagulation as possible strategies to reduce the thrombotic risk or prevent thrombotic complications after lung transplantation. ■

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Measurement of Serum Calprotectin in Stable Patients Predicts Exacerbation and Lung Function Decline in Cystic Fibrosis

To the Editor:

Clinical tests that predict clinical outcomes in cystic fibrosis (CF) such as exacerbation risk and lung function decline are lacking. Calprotectin (MRP8/14, S100A8/A9) is an abundant neutrophil protein found in bronchoalveolar lavage fluid (1), sputum (2), and serum of patients with CF (3). We have demonstrated that serum calprotectin changes with treatment of exacerbation (3, 4), and predicts time to re-exacerbation (3). We hypothesized that stable patients with higher serum calprotectin at baseline would exacerbate sooner and have more rapid lung function decline.

Methods

Patients were recruited while clinically stable (no exacerbation treatment with oral, inhaled, or intravenous antibiotics in the preceding 2 wk). Exclusion criteria were previous lung

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This letter has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

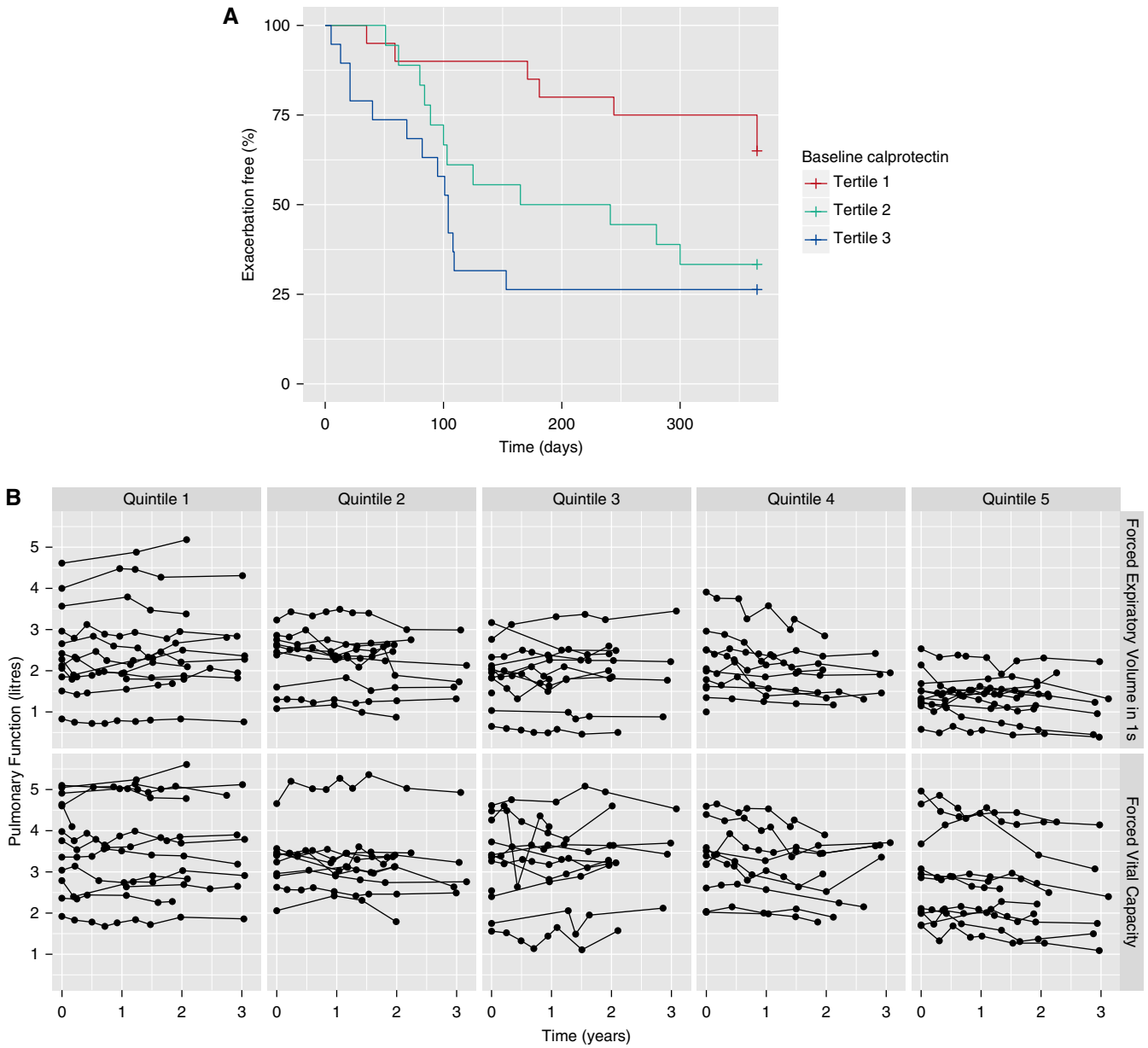

Figure 1. (*A*) Kaplan–Meier plot of time to first exacerbation. Subjects were divided into three groups based on median calprotectin level at baseline from low (tertile 1) to high (tertile 3). Median time to exacerbation from enrollment was lower in tertiles 2 and 3 compared with tertile 1 ($P < 0.001$). (*B*) Spaghetti plots of pulmonary function over time, by FEV_1 and FVC and according to baseline calprotectin. Subjects were divided into five groups based on median calprotectin level at baseline from low (quintile 1) to high (quintile 5). Baseline lung function was higher in the lower quintiles. Rate of pulmonary function decline was fastest in the higher quintiles. The association of calprotectin with decline in FEV_1 and FVC was significant at the conventional level (FEV_1, $P < 0.05$; FVC, $P < 0.01$) when corrected for other variables (*see* Table 2).

transplant, nonpulmonary chronic inflammatory condition, immunosuppressant therapy, or malignancy. The Lothian Research Ethics Committee approved all study procedures and protocols and patients gave written informed consent (LREC no. 09/S1101/43).

Patients were monitored as part of their routine clinical care. Pulmonary function was measured as per guidelines (5). Exacerbation was defined as an acute requirement for intravenous antibiotics, determined by the CF clinical team, among patients who had an increase in symptoms and a decrease from baseline FEV_1. Exacerbation episodes that did not fulfill modified Fuchs criteria (6) were excluded from the analysis. Serum calprotectin and C-reactive protein (CRP) were measured by ELISA at baseline; complete blood counts were not obtained. Statistical analyses were performed in R (v2.15.2) (7).

Time to exacerbation by calprotectin was modeled in Cox proportional hazard models, using a penalized spline function

Table 1. Time to Exacerbation Prediction by Serum Calprotectin Level

	Tertile 1 (*n* = 20)	Tertile 2 (*n* = 18)	Tertile 3 (*n* = 19)	Per Twofold Increment in Baseline Calprotectin or CRP	*P* Value (*Compared with Tertile 1*)
Median calprotectin (range), μg/ml	5.4 (2.2–6.8)	10.9 (6.9–15.9)	21.2 (16.4–93.9)		
Exacerbations, n	7	12	14		
Median time to exacerbation, d	No median	203	104		
Hazard ratio, unadjusted	—	2.58 (1.01–6.59)	3.90 (1.56–9.75)	2.15 (1.45–3.18)	<0.001
Hazard ratio, adjusted*	—	2.79 (0.90–8.64)	4.21 (1.39–12.71)	2.05 (1.29–3.24)	0.002

Hazard ratio for time to first exacerbation in unadjusted analysis and adjusting for age, sex, previous exacerbation rate, and FEV_1 at baseline and for CRP at baseline.
*Note that three patients did not have a baseline high-sensitivity CRP model and were excluded from this model.

to examine for nonlinearity. Decline in FEV_1 and FVC was estimated using linear mixed models. Age, sex, previous exacerbation rate, symptom score, and high-sensitivity CRP were selected for inclusion in the regression models (in CRP analyses baseline calprotectin was substituted). There were no missing data for any of the baseline covariates, except for CRP, where three individuals with missing data were excluded in a complete case analysis. Because we used linear mixed models, missingness in pulmonary function measures was accommodated. Missing pulmonary function due to death was treated as missing at random for the main analyses. Calprotectin was treated as a continuous variable for the main analysis, but results are also presented by quantile. Tertiles are presented for time to exacerbation to ensure there were a reasonable number of events in each category. Rates of decline in lung function are presented by quintile rather than tertile as FEV_1 and FVC are continuous variables.

Results

The 57 patients enrolled had a mean (SD) age of 26.4 (7.7) years. Twenty-seven (47%) were male. Patients with higher baseline calprotectin were older and had more severe disease (*see* Table E1 in the online supplement). The sample was representative of our regional CF clinic population as also seen in Table E1. Calprotectin and CRP at baseline were moderately associated ($r = 0.35$, $P = 0.005$). Patients were monitored for exacerbations for 1 year with none lost to follow-up. Three patients died during lung function follow-up (calprotectin levels of 7.9, 15.3, and 17.7) with the remainder being censored at 3 years.

Thirty-three patients (58%) exacerbated within 1 year of baseline calprotectin. The median time to exacerbation was less in the upper (104 d) and middle (203 d) tertiles for calprotectin compared with the lower tertile (no median) (Figure 1A and Table 1). There was a 2.15-fold increase in hazard ratio (HR) for first exacerbation per twofold increment in calprotectin (95% confidence interval [CI], 1.45–3.18; $P < 0.001$). This association persisted after adjustment (HR, 2.05; 95% CI, 1.29–3.24; $P = 0.002$). CRP was similarly, but more weakly, associated with time to first exacerbation (HR, 1.40 per twofold increment in baseline CRP; 95% CI, 1.13–1.874; $P = 0.002$). This association was attenuated after adjustment (HR, 1.14 per twofold increment in baseline CRP; 95% CI, 0.90–1.46; $P = 0.28$).

There were a total of 341 spirometry measures with a median (interquartile range) of 6 (5–8), obtained over a median of 2.26 (1.96–3.17) years of follow-up. Decline in pulmonary function was associated with baseline calprotectin (Figure 1B and Table 2). Patients with higher baseline calprotectin had lower baseline FEV_1 and FVC and more rapid decline in FEV_1 and FVC. Overall, the mean (SD) decline in FEV_1 and FVC was 55 (126) and 65 (91) ml/yr, respectively. The rate of decline was faster per twofold increment in baseline calprotectin, with the relationship being strongest for FVC (51 ml/yr; 95% CI, −21 to 81 ml/yr; $P < 0.001$, $P = 0.009$ adjusted), with FEV_1 following a similar trend (33 ml/yr; 95% CI, −2 to 68 ml/yr; $P = 0.06$, $P = 0.03$ adjusted). CRP was

Table 2. Decline in Lung Function Based on Baseline Calprotectin Level

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	Per Twofold Increment in Baseline Calprotectin	*P* Value
Median calprotectin, μg/ml	4.9	6.6	10.9	16.7	24.2	—	—
FEV_1, ml							
Unadjusted	−35	84	0	186	68	33 (–2 to 68)	0.06
Adjusted*	−59	66	−51	195	65	44 (3 to 84)	0.03
FVC, ml							
Unadjusted	−14	54	−22	134	175	51 (21 to 81)	<0.001
Adjusted*	−78	−17	−122	88	89	50 (12 to 87)	0.009

The rate of lung function decline per year increases with increasing baseline calprotectin. Data are presented for both unadjusted and adjusted (for age, sex, previous exacerbation rate, symptom score, and high-sensitivity CRP).
*Note that three patients did not have a baseline high-sensitivity CRP model and were excluded from the analysis for this model.

not associated with decline in FEV_1 or FVC in analyses adjusting for age, sex, previous exacerbation rate, and symptom score (per twofold increment in CRP: −5 ml/yr; 95% CI, −31 to 20 ml/yr; $P = 0.68$ decline in FEV_1 and 15 ml/yr; 95% CI, −10 to 39 ml/yr; $P = 0.24$ decline in FVC).

Discussion

This study demonstrates that serum calprotectin levels are significantly associated with key future clinical events in CF including pulmonary exacerbations and lung function decline. Exacerbations are coupled with declines in lung function, which may not return to baseline after treatment (8, 9), and are associated with mortality (10). One study has suggested that severity and quality of life scoring predict risk of future exacerbation but that inflammatory biomarkers only predict re-exacerbation (11). Sputum high mobility group box 1 (HMGB1) levels may predict CF exacerbation (12), but this requires specialized sample processing that is not available in most clinics. The lung clearance index, a physiological measurement, also predicts exacerbation frequency and onset in children (13), but again requires specialized equipment. In contrast, calprotectin may be measured in serum with relative ease, underlined by its use in a pediatric study of azithromycin (14).

Rate of decline in lung function is also a marker of disease severity and disease progression. No serum biomarker has been identified that can predict lung function decline, although Sagel and colleagues have demonstrated a strong association between sputum neutrophil elastase levels (measured at multiple time points) and more rapid declines in lung function (15). We demonstrate that the rate of decline in FVC is approximately 50 ml/yr faster per twofold increment in baseline serum calprotectin (Table 2), and this association is independent of all other variables tested.

In conclusion, single measurement of serum calprotectin in stability predicts changes in disease activity manifest by time to next exacerbation and decline in lung function. Measurement of calprotectin in the clinic may allow the identification of patients at high risk of exacerbation and/or lung function decline and allow appropriate tailoring of therapy. Larger multicenter studies are now required to confirm these findings. ■

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