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Plasma TGF-β_1 in pediatric cystic fibrosis: Potential biomarker of lung disease and response to therapy

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Abstract

Introduction—Transforming growth factor beta-1 (TGF-β_1) is an important genetic modifier of lung disease severity in cystic fibrosis (CF), yet the mechanism behind this disease association remains unknown. Initial steps in the investigation of the relationship between TGF-β_1 and CF lung disease include determining the most appropriate available biospecimen for TGF-β_1 protein measurement.

Hypothesis—In hospitalized pediatric CF patients, plasma TGF-β_1 is increased in association with clinical parameters of lung disease severity.

Methods—Serum and plasma were obtained pre- and post intravenous antibiotic therapy in pediatric CF patients hospitalized for a pulmonary exacerbation. Total TGF-β_1, measured via ELISA, was compared with markers of lung disease, including airway microbiology, lung function and response to therapy.

Results—Forty CF children were studied, 15 of whom underwent bronchoalveolar lavage (BAL) at the time of admission. Plasma TGF-β_1 positively correlated with BAL fluid TGF-β_1 ($r = .59$, $p < .05$). Admission plasma TGF-β_1 was increased in subjects positive for *Pseudomonas aeruginosa* ($p = .014$) and was inversely associated with diminished lung function ($p < .038$) after therapy. Treatment with antibiotics significantly decreased plasma TGF-β_1 ($p < .001$). Serum TGF-β_1 was not associated with plasma TGF-β_1, BALF TGF-β_1 or these clinical parameters of lung disease.

Conclusion—In pediatric CF, plasma (but not serum) TGF-β_1 is increased in association with *Pseudomonas* infection and lung disease, and is reduced in response to therapy. These findings emphasize the importance of optimizing biospecimen selection for future studies investigating the role of TGF-β_1 in CF lung disease.

Keywords

Genetic modifiers; airway remodeling; lung disease; bronchoalveolar lavage fluid; blood; serum

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Introduction

Cystic fibrosis (CF) is characterized by heterogeneous lung disease in children who share similar cystic fibrosis transmembrane regulator (CFTR) gene mutations. This observation has prompted significant interest in the identification of non-CFTR genetic modifiers of CF lung disease, potentially providing novel insight into the mechanisms of CF lung disease progression. Among candidate genetic modifiers, transforming growth factor beta 1 (TGF-β_1) has repeatedly been implicated[1,2,3,4], yet the mechanisms linking this genetic modifier with disease progression remains elusive[5]. An important early step in further understanding the association between TGF-β_1 and CF lung disease severity is to define a link between protein levels and clinically-important parameters of disease in a biospecimen that can be feasibly obtained in large numbers necessary for future multi-center studies.

TGF-β_1 is a pleiotropic cytokine that affects a wide array of cellular functions with relevance to CF, including lung development[6], immunomodulation[7] and airway remodeling[8]. In pulmonary tissue, TGF-β_1 is produced principally by bronchial epithelial cells and alveolar macrophages[9]. In the blood, the principle source of TGF-β_1 is from alpha granules of activated platelets involved in coagulation[10]. Thus, measurements of TGF-β_1 in blood are dependent upon the coagulation status of the specimen[11]. As serum is generated by triggering the coagulation cascade, serum TGF-β_1 levels are largely reflective of activated platelet contribution[12]. In contrast, plasma can be processed to limit *ex vivo* coagulation and thus potentially provide TGF-β_1 measurements more reflective of circulating levels[13]. Consequently, plasma TGF-β_1 may be more representative of circulating TGF-β_1 protein levels than serum, offering an improved opportunity to link a clinically-relevant and easily available biospecimen that may reflect clinical phenotype with both TGF-β_1 genotype and clinical parameters of lung disease.

Although TGF-β_1 has consistently been identified as a genetic modifier of CF lung disease, these reports have not universally agreed upon a common genetic polymorphism[1, 2,4,14] or the relationship of genetic polymorphism to protein measurement[2,4,15,16]. Potential explanations for this varied analysis includes inconsistent specimen choice, unclear links between protein concentration and clinically important parameters of CF disease, and varied genetic background of the reference populations.

The current study was conducted to explore whether relationships exist between protein levels of TGF-β_1 in plasma or serum and clinically-relevant parameters of pediatric CF lung disease. Specifically, we hypothesized that plasma TGF-β_1 is correlated with levels of TGF-β_1 in bronchoalveolar lavage fluid (BALF) and is associated with clinical parameters of CF lung disease.

Methods

Study Design

The study, approved by the University of North Carolina Biomedical Institutional Review Board (IRB), was designed as a prospective, one-year, single-center observational protocol to measure plasma and serum TGF-β_1 in pediatric CF patients admitted to the hospital for the initiation of intravenous (IV) antibiotics for treatment of a pulmonary exacerbation as defined by their CF care provider. Parents of all pediatric patients provided signed informed consent to participate in the study, with children assenting for the study when appropriate. Measurement of plasma and serum TGF-β_1 on admission were compared with three clinical parameters: 1) presence or absence of *Pseudomonas aeruginosa* (PsA) on admission respiratory culture; 2) FEV_1 obtained on admission and at the conclusion of therapy

compared to predicted values; and 3) recent/recurrent hospitalizations as defined as previous hospitalization in the past 12 months. A subgroup of admitted patients underwent clinically-indicated bronchoscopy with bronchoalveolar lavage at the time of admission (n=15). In these patients, serum and plasma TGF-β_1 were compared to bronchoalveolar lavage fluid (BALF) TGF-β_1. When available (n=24), specimens were additionally collected at the completion of antibiotic therapy and compared to admission values to evaluate the response to therapy.

Specimen Collection and Processing

Blood specimens were collected within 72 hours of admission and at the conclusion of IV antibiotics. Specimens were preferentially collected through a central venous catheter such as a peripherally inserted central catheter (PICC) or Port-a-Cath. If samples were not able to be obtained via the central catheter at the end of therapy, the post-treatment specimen was omitted. For plasma collection, blood was collected in 2.6 ml glass Acid Citrate Dextrose (ACD) tubes (BD Vacutainer, Franklin Lakes, NJ) and centrifuged at 5000 g×10 minutes. For serum collection, blood was collected in 3.5 ml plastic Serum Separator (SST) tubes (BD Vacutainer, Franklin Lakes, NJ) and centrifuged at 1000 g×5 minutes. After centrifugation, plasma and serum samples were stored at −80°C until further analysis.

Bronchoalveolar lavage was performed per institutional clinical practice as previously described[17,18,19]. In brief, the location for lavage was at the bronchoscopist's discretion. Generally, lavage was directed at areas of either radiographic abnormalities or visibly heavy secretions. For each lavage, 1 ml/kg aliquots up to a maximum of 20 ml of normal saline were instilled and suctioned back through the bronchoscope. If more than one location was lavaged, the specimens were pooled for analysis. BAL fluid was centrifuged at 500 g×5 minutes with the cell-free supernatant stored at −80° C for subsequent assays.

TGF-β1 measurement

Total TGF-β_1 in plasma, serum and BALF was measured using commercially-available ELISA kits (R&D systems, Minneapolis, MN) as previously described19. Utilizing this assay, we had excellent reproducibility on duplicate testing (coefficient of variation = .044) and spike/recovery of rhTGF-β_1 (assay standard) into the plasma sample yielded acceptable results (94.8% of predicted values). In dilutional series, TGF-β_1 levels decreased predictably. The standard curve ranges from 31.2 pg/ml to 2000 pg/ml and the assay has a lower level of detection of approximately 5 pg/ml.. Serum specimens were diluted 40:1 and plasma specimens diluted 2:1 to fall within the range of the standard curve. This assay includes an acidification step per manufacturer’s recommendations which allows for the measurement of total TGF-β_1 protein within the biospecimen.

Microbiology

Respiratory cultures were processed by University of North Carolina McLendon Clinical Laboratories as previously described[20] for the presence of respiratory pathogens. The results of the admission respiratory culture defined the microbiologic classification (i.e. PsA positive or negative) of each study subject.

Lung function

Pulmonary function was performed per American Thoracic Society and European Respiratory Society criteria[21] and defined by the percent-predicted FEV_1 measured before the initiation and at the conclusion of antibiotic treatment for the pulmonary exacerbation during which the blood for TGF-β_1 was obtained. FEV_1 comparison to a healthy non-CF reference population utilized NHANES III reference equations[22] for children 8–18 years old

and Eigen's pre-school specific equations[23] for children less than 8 years old. For comparison of FEV_1 within the CF population, Kulich's CF-specific reference equations were utilized[24].

Statistical Analysis

Descriptive statistics, such as means and standard errors (SEMs) for continuous variables and frequencies and proportions for categorical variables, were computed for all study variables of interest. Continuous data were analyzed for normality using stem-and-leaf plots, normal probability plots, and the Kolmogorov-Smirnov test. All such variables were determined to follow an approximate normal distribution except for admission TGF-β_1, post-antibiotic plasma TGF-β_1, and admission BALF. A further look at the distributions of these variables indicated that one value for each of these three variables was an outlier. We confirmed that these values were outliers using the extreme studentized deviate single-outlier procedure[25] (which is the mathematical equivalent to the Grubbs test) and removed them from further analysis. After outlier removal, continuous data were determined to follow an approximate normal distribution. Relationships between continuous variables were examined using the Pearson correlation analysis, and comparisons were performed using the usual two-group t test, or the two-group t test assuming unequal variances (Satterthwaite's method) where needed. The paired t test was used to perform comparisons between pre-and post-antibiotic therapy plasma, serum, and FEV_1 values, and between pre- and post-Kulich FEV_1 values. The exact version of the McNemar test was used to examine the association between change in lung function and change in plasma TGF-β_1 (as illustrated in Figure 4b). All statistical tests were two-sided and were performed using a significance level of 0.05. Graphical analyses were performed using GraphPad Prism (version 5.0; GraphPad Software, Inc., San Diego, CA) and statistical analyses were performed using SAS (version 9.2; SAS Institute, Inc., Cary, NC).

Results

Clinical characteristics of research participants

Table 1 summarizes the demographics of the study participants. Blood from 40 subjects hospitalized from January 2007 to March 2008 was analyzed. The mean age of study subjects (±SEM) was 12.2 ± 0.70 years with an admission FEV_1 of 71.8 ± 3.6% predicted that improved to 84.2 ± 3.3% predicted at the conclusion of treatment. Plasma samples were available in all 40 subjects and serum samples were available in 39 subjects on admission. BALF TGF-β_1 was available in 15 subjects on admission. Post-antibiotic blood samples were available in 24 patients, with plasma available in all 24 subjects and serum available in 23 subjects.

Comparisons among plasma, serum and BALF TGF-β_1

TGF-β_1 was measureable in all plasma, serum and BALF specimens. Utilizing the definition of an outlier as defined in the methods section, three outlier values were observed: One admission plasma TGF-β_1 value (21, 900 pg/ml), one post-antibiotic plasma value (11,750 pg/ml) and one admission BAL fluid value (844 pg/ml). Excluding these values from further analysis, plasma TGF-β_1 on admission averaged 2450 ± 228 pg/ml. As expected, serum TGF-β_1 levels were more than an order of magnitude higher (72, 800 ± 5390 pg/ml) than plasma TGF-β_1 on admission. BALF TGF-β_1 averaged 116.4 ± 23.7 pg/ml. Age was not associated with admission plasma, serum, or BALF TGF-β_1 ($p > .20$ for all comparisons). Both admission plasma ($r = .33$, $p = .043$) and serum TGF-β_1 ($r = .47$, $p = .003$) were associated with admission platelet count, while admission BALF TGF-β_1 was not significantly associated with admission platelet count ($r = .43$, $p = .12$).

Plasma TGF-β_1 was positively correlated with BALF TGF-β_1 (r = .59, p = .035) as shown in Figure 1. Serum and plasma TGF-β_1 lacked correlation (r = .06, p = .72). Serum TGF-β_1 similarly lacked correlation with BALF TGF-β_1 (r = −0.001, p = .99).

Association between plasma TGF-β_1 and clinical parameters of disease

a) Microbiology—The relationship between airway microbiology (PsA culture result) and plasma TGF-β_1 is shown in Figure 2. Twenty-four of 40 (60%) children had a positive culture for PsA either in BALF or sputum on admission. On admission, plasma TGF-β_1 was significantly increased in patients with an admission respiratory culture positive for PsA (PsA+: 2880 ± 327 pg/ml; PsA−: 1840 ± 229 pg/ml, p = .014). In contrast, PsA status was not related to serum TGF-β_1 (PsA+: 74, 700 ± 8360 pg/ml; PsA-: 70,100 ± 5590 pg/ml, p = .65). In addition, neither the presence of *Staphylococcus aureus* nor Methicillin-resistant *Staphylococcus aureus* (MRSA) was associated with elevated plasma or serum protein levels.

b) Lung function—The relationship between lung function and plasma TGF-β_1 is shown in figure 3. To determine if TGF-β_1 was increased in subjects with more advanced lung disease, subjects were dichotomized to those who maintained an FEV_1 above or equal to 80% predicted values on admission for a pulmonary exacerbation (“mild impairment”) and those whose lung function was below 80% on admission (“moderate to severe impairment”). Mean FEV_1 in the “mild impairment” group was 93.2 ± 2.5% (n=16) compared to 55.4 ± 2.7% (n=21) in the “moderate to severe impairment” group, p < .001. Plasma TGF-β_1 was significantly increased in the “moderate to severe impairment” as compared to the “mild impairment” group (2940 ± 373 pg/ml vs. 1920 ± 201 pg/ml, p = .023). In contrast, serum TGF-β_1 was actually increased in the “mild impairment” group [91,000 ± 8000 pg/ml vs. 63,500 ± 6800 pg/ml, p = .013); the subject with the highest serum TGF-β_1 (164,000 pg/ml) had an FEV_1 of exactly 80% predicted].

In an attempt to categorize subjects based upon the relative status of CF lung disease, subjects were also stratified utilizing the Kulich CF-specific reference equations[23] for age, height and FEV_1 (L) developed for subjects with CF between 6–40 years of age. In contrast to non-CF, non-diseased reference equations in which 100% is considered “normal”, Kulich values >50% are considered above normative values and Kuhlich values <50% are considered below normative values. Kulich FEV_1% was 41.8 ± 4.9 % on admission that improved to 59.9 ± 4.8 % after completion of therapy (p < 0.001).

Research subjects were subsequently dichotomized according to whether their lung function returned to above or below the CF-specific normative value (50^{th} percentile) after the completion of therapy. Admission plasma TGF-β_1 was significantly higher (3580 ± 597 pg/ml vs. 2085 ± 215 pg/ml, p = .038) in subjects whose lung function *remained* below normative values after the completion of antibiotic therapy compared to those whose lung function returned to above normative values. Utilizing this same lung function stratification, serum TGF-β_1 did not differ between subject groups (p = .48).

c) Recurrent hospitalization—Slightly more than half (20/39, with information missing on one individual) of research participants were hospitalized in the 12 months prior to the current exacerbation. Neither plasma nor serum TGF-β_1 was significantly increased in those recently hospitalized compared to those who had not been hospitalized in the last year (p = 0.10 and 0.38, respectively).

d) Response to antibiotic therapy—Post-antibiotic blood specimens were available for analysis in 24/40 subjects. Reasons for missed samples at the conclusion of antibiotic

therapy included completion of antibiotics at home, extension of antibiotics beyond the follow-up clinic visit and inability to obtain an adequate sample from the central PICC line. In the subset of subjects with follow-up specimens available, mean age was 13.0 ± 0.8 years with 16/24 subjects positive for PsA on admission. Correlation between pre-therapy and post-therapy TGF-β_1 was strong for plasma ($r = .90$, $p < .001$) and weak for serum ($r = .21$, $p = .33$). In the subset of subjects with both pre-therapy and post-therapy samples, FEV_1 improved from 64.3 ± 3.7 % predicted to 81.2 ± 3.8% predicted ($p < .001$) after treatment of their pulmonary exacerbation. In association with this improvement in pulmonary function, plasma TGF-β_1 decreased from 2600 ± 360 pg/ml to 1770 ± 227 pg/ml ($p < .001$). Plasma TGF-β_1 was reduced in 83% of subjects after antibiotic therapy ($p < .001$). In contrast, serum TGF-β_1 was unchanged before and after therapy (Pre: 59,570 ± 7060 pg/ml, Post: 56,160 ± 5390 pg/ml, $p = .67$). Similar to admission plasma TGF-β_1, post-therapy plasma TGF-β_1 tended to be increased in subjects whose lung function remained below normative CF-specific values after the completion of therapy. In these patients with persistently depressed lung function, post-therapy plasma TGF-β_1 was 2660 ± 525 pg/ml compared to 1430 ± 167 pg/ml in subjects with above normative values ($p = .059$). Serum TGF-β_1 was not significantly different between groups. The relationships between improved lung function and diminished plasma TGF-β_1 are shown in Figure 4.

e) Genotype—Although this study was not designed nor sufficiently powered to evaluate for significant associations between TGF-β_1 genotype and plasma TGF-β_1 protein concentrations, all subjects were genotyped for TGF-β_1 codon 10 polymorphisms. Consequently, we report the data to help with future sample size calculations.

On admission, no significant difference was detected in plasma TGF-β_1 by genotype (TT: 2300 ± 395, n=17; CT: 2580 ± 358, n=16; CC: 2565 ± 315, n=6; $p = .84$ by ANOVA). Combining the subjects with one C polymorphism, we similarly did not see a significant difference by genotype (TT: 2300 ± 395, n=17; CT/CC: 2580 ± 270, n=22; $p = .55$ by t-test analysis). These analyses do not include the one outlier in admission plasma TGF-β_1 measurement (21,900, CT genotype).

At follow-up, no significant difference was detected by ANOVA across the three genotypes although the sample size was even more limited (TT: 1520 ± 333, n=13; CT: 1930 ± 311, n=8; CC: 2740 ± 450, n=2; $p = .31$ by ANOVA). Comparing subjects with one C polymorphism, we similarly did not find significance (TT: 1520 ± 333, n=13; CT/CC: 2090 ± 276, n=11; p=.22). These analyses do not include the one outlier in follow-up plasma TGF-β_1 measurement (11,750, CT genotype). Of note, we performed these analyses utilizing parametric techniques as described in the methods section.

Discussion

We have found that in pediatric CF, plasma TGF-β_1 is increased in association with PsA infection and diminished lung function, and is reduced in association with clinically-effective treatment for a pulmonary exacerbation. Furthermore, our data underscore that TGF-β_1 protein measurements are not interchangeable between platelet-free plasma and serum. Specifically, serum TGF-β_1 levels were >20-fold higher than plasma levels with no correlation between serum TGF-β_1 and either plasma or BALF TGF-β_1. In contrast, plasma TGF-β_1 was significantly correlated with BALF TGF-β_1 with a consistent decrease in plasma TGF-β_1 associated with a clinically-meaningful response to therapy. Our data thus support exploration of platelet-free plasma TGF-β_1 as a peripheral biomarker of CF lung disease in childhood, and suggest a negative relationship between elevated TGF-β_1 protein levels and lung function in pediatric CF lung disease.

When plasma is utilized for studies of TGF-β_1, careful attention must be placed on the method of plasma processing to limit the contribution of activated platelet degranulation *ex vivo*. In our experience, important technical steps include prevention of coagulation (immediate processing), collection through a central venous catheter if available, selection of the appropriate specimen tube (citrate preferred as both heparin and EDTA can activate TGF-β_1), adequate centrifugation (to segregate a platelet-free fraction) and use of a sensitive and specific TGF-β_1 detection assay. Utilizing these techniques, the plasma levels of TGF-β_1 in this study of CF patients were consistent with those reported in the literature from optimally collected plasma specimens[10,11,13,26]. Our results prompt critical review of previous literature linking TGF-β_1 genotype with TGF-β_1 protein measurements in blood. Our data suggest that serum TGF-β_1 is of limited relevance to CF lung disease phenotype compared to plasma. Moreover, for studies that evaluate the association of TGF-β_1 genotype and plasma TGF-β_1 protein, the methods of plasma acquisition and processing should be carefully considered.

While aberrant inflammatory cytokine profiles are well-documented in CF BALF specimens[17,27,28,29], parallel findings of altered cytokine protein levels in the peripheral circulation have been limited. To our knowledge, this is the first study which has demonstrated a significant correlation between plasma and BALF TGF-β_1 in the pediatric CF population. Additionally, the correlation between plasma and BALF TGF-β_1 in the context of TGF-β_1 as disease modifier of CF lung disease severity[1,2,4,30] suggest that plasma TGF-β_1 may hold promise as a relatively noninvasive biomarker to assess CF lung disease. While this study indicates that plasma TGF-β_1 meets the classic definition of a biomarker as an objectively-measureable biologically-relevant characteristic[31], future studies as delineated by Mayer-Hamblett et al[32] will be necessary to evaluate its full utility as a CF biomarker. These include assessment of its clinical and therapeutic relevance, sensitivity and specificity across a variety of clinical scenarios, reproducibility within the same patient and across CF centers, and feasibility for wide-spread collection.

The potential utility of plasma TGF-β_1 to track CF lung disease is further underscored by the association of increased plasma TGF-β_1 with PsA infection and diminished lung function (Figs 2 and 3). While the mechanisms of these associations are beyond the scope of this study, our data point towards relationships between increased TGF-β_1 levels and disease severity. It is not clear whether elevated TGF-β_1 levels represent a cause or consequence of CF lung disease, but the reduction of TGF-β_1 plasma levels in response to treatment may identify a useful biomarker for CF therapeutic interventions, and possibly a target for disease modification.

One important finding of this study was the significant association between an elevated admission plasma TGF-β_1 and lung function that remains below normative values despite antibiotic therapy. Increased cytokine measurement during the time of exacerbation has been felt to reflect airway inflammation, and we have previously shown an association between neutrophilic inflammation and BALF TGF-β_1[19]. However, the link between elevated TGF-β_1 (a potent pro-fibrotic mediator) at the time of admission and lung function at the conclusion of conventional therapy suggests that TGF-β_1 may play a role in pathogenic airway remodeling that is refractory to traditional therapy. Supportive of this hypothesis is data from Hilliard et al[29] that reveals a significant association between BALF TGF-β_1 and reticular basement membrane thickness demonstrated by endobronchial biopsy. Thus, we speculate that TGF-β_1 may bridge the inflammatory and remodeling pathways in CF, serving as one potential mechanism through which TGF-β_1 may modify CF lung disease progression.

An important caveat of this study is that we measured and correlated total TGF-β_1 protein levels rather than the transient, biologically active form in tissue compartments. Total and active TGF-β_1 are not always highly correlated and local activation is a tightly regulated process that independently influences biologic effect[33].

We additionally recognize the limitation that this study was not designed to identify the sources of plasma TGF-β_1. The positive correlation between both BALF TGF-β_1 and platelet count suggests both a pulmonary and platelet contribution to plasma TGF-β_1. While an association is to be expected as platelets are reported to be a major source of circulating TGF-β_1[10], future studies could consider the use of a specific marker of *ex vivo* degranulation such as Platelet Factor 4 (PF4) to insure that measured protein levels represent circulating levels rather than an artifact of specimen processing.

Finally, the relatively small sample size limited our ability to perform multivariable analyses, such as multiple regression analyses with inflammatory cytokines, as well as limiting our ability to form conclusions about TGF-β_1 genotype-phenotype relationships. Using an odds ratio for disease of 2.2 and a predicted prevalence of the codon 10 CC genotype of 13–17%[2,3], a study population of several hundred subjects would be necessary to detect significant associations between TGF-β_1 genotype, protein concentration and disease severity. Instead, we chose to focus this single-center study on emphasizing the importance of TGF-β_1 biospecimen selection and processing by emphasizing relationships with key clinical parameters. Our results help lay the foundation for larger multi-center studies to examine TGF-β_1 genotypes, their relationship with TGF-β_1 protein levels and clinical outcome measures.

Despite these considerations, our findings suggest that plasma TGF-β_1 should be included in future CF biospecimen profiles with further investigation of the mechanisms through which TGF-β_1 may modulate CF lung disease progression. The results of this study provide evidence that plasma is the preferred blood biospecimen for measuring TGF-β_1 protein levels in CF, as plasma TGF-β_1 correlates with BALF levels and fluctuates in association with several important parameters of CF lung disease severity. Whether plasma TGF-β_1 is a potentially useful biomarker of disease progression or response to therapy merits further investigation.

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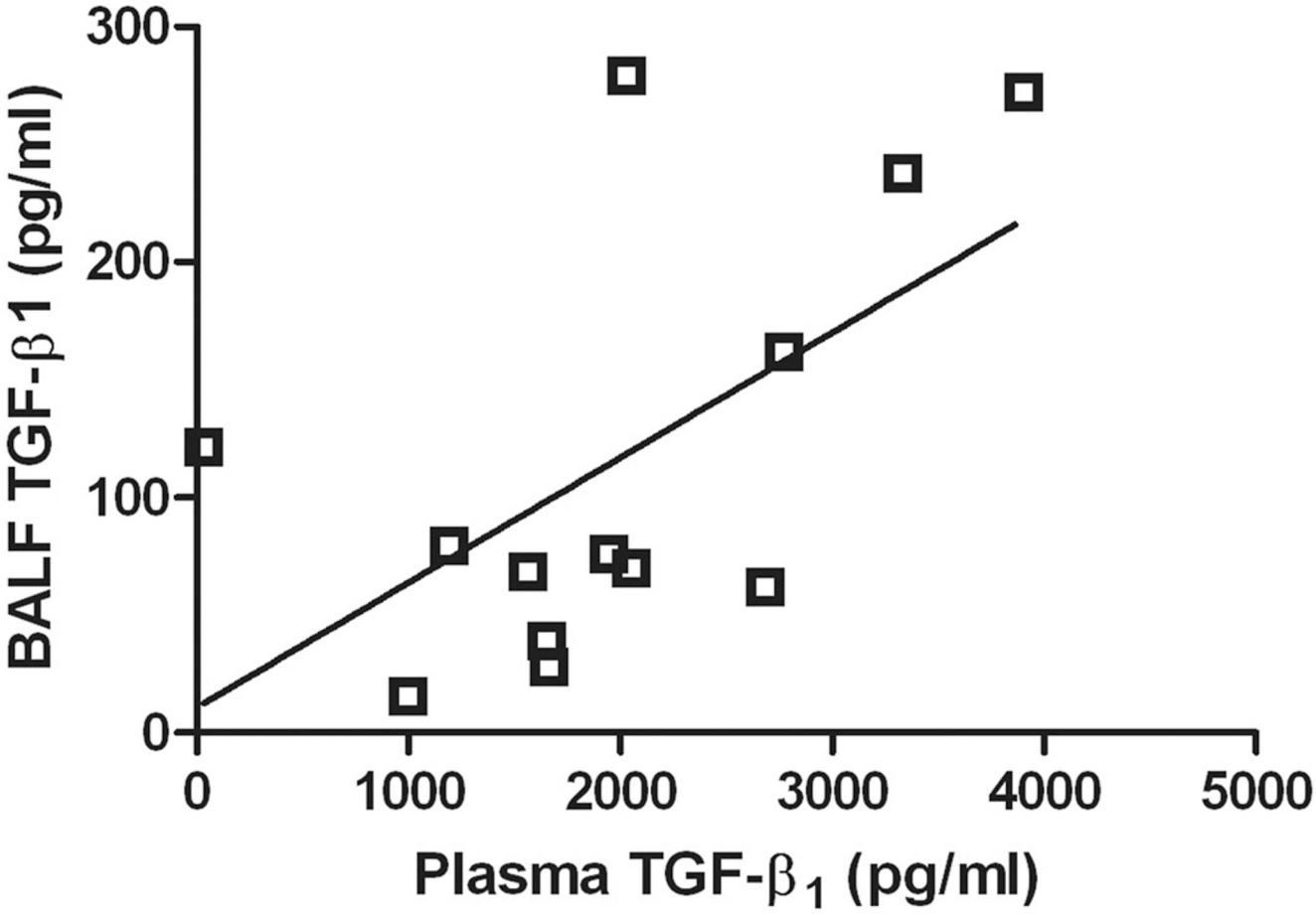


Figure 1.
Correlation between plasma and BALF TGF-β_1 at admission (r = .59, p = .035) by Pearson coefficient analysis.

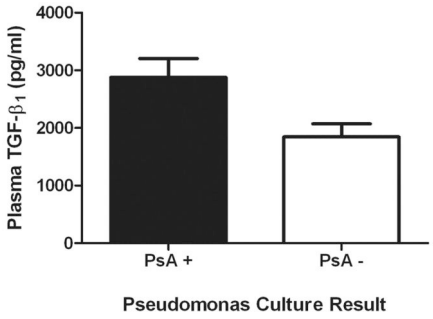


Figure 2.

Association between plasma TGF-β_1 levels and *Pseudomonas aeruginosa* positive (PsA+) respiratory culture at admission. TGF-β_1 is significantly increased in PsA+ subjects (2880 ± 327 pg/ml vs. 1840 ± 229 pg/ml, p = .014) admission.

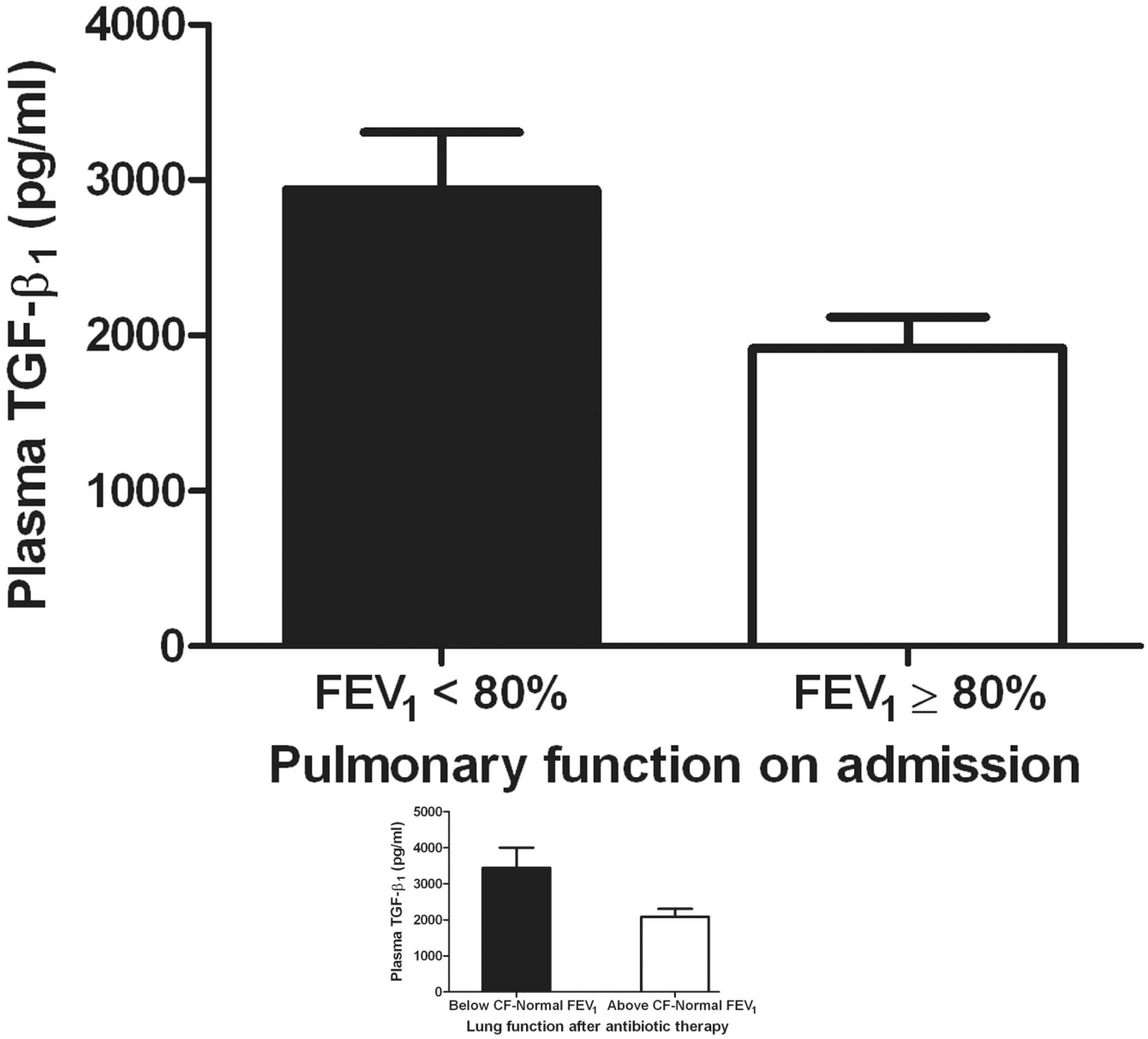
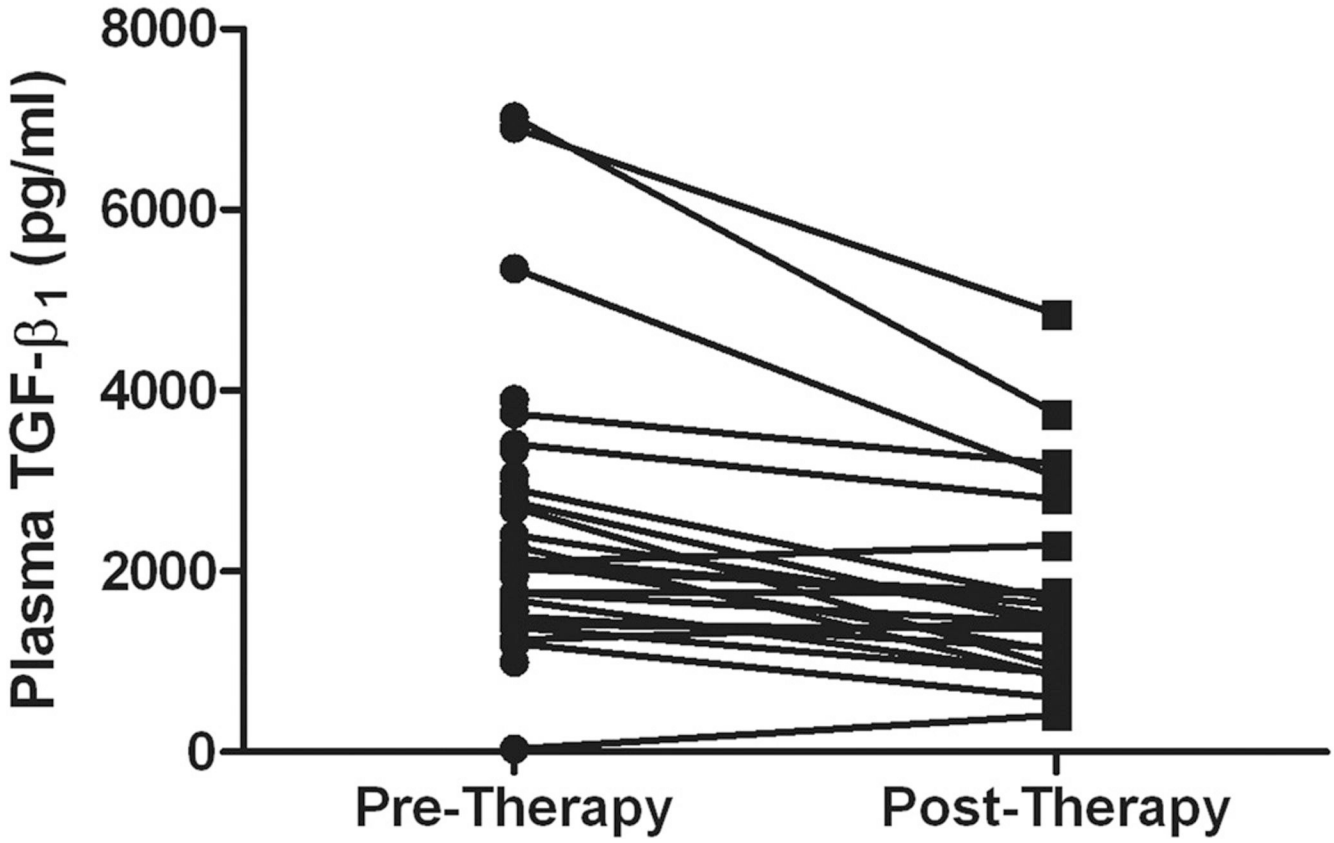


Figure 3.

A) Association between plasma TGF-β_1 and lung function on hospital admission. Plasma TGF-β_1 is significantly increased in CF subjects with forced expiratory volume in 1 second (FEV_1) of less than or equal to 80% predicted values (Low FEV_1: 2940 ± 373 pg/ml; High FEV_1: 1920 ± 201 pg/ml, p = .023)

B) Association between plasma TGF-β_1 and lung function after the completion of therapy. Plasma TGF-β_1 on admission is significantly increased in CF subjects whose FEV_1 remains below CF-specific normative values at the conclusion of antibiotic therapy (Low post-therapy FEV_1: 3580 ± 597 pg/ml; High post-therapy FEV_1: 2085 ± 215 pg/ml, p = .038)



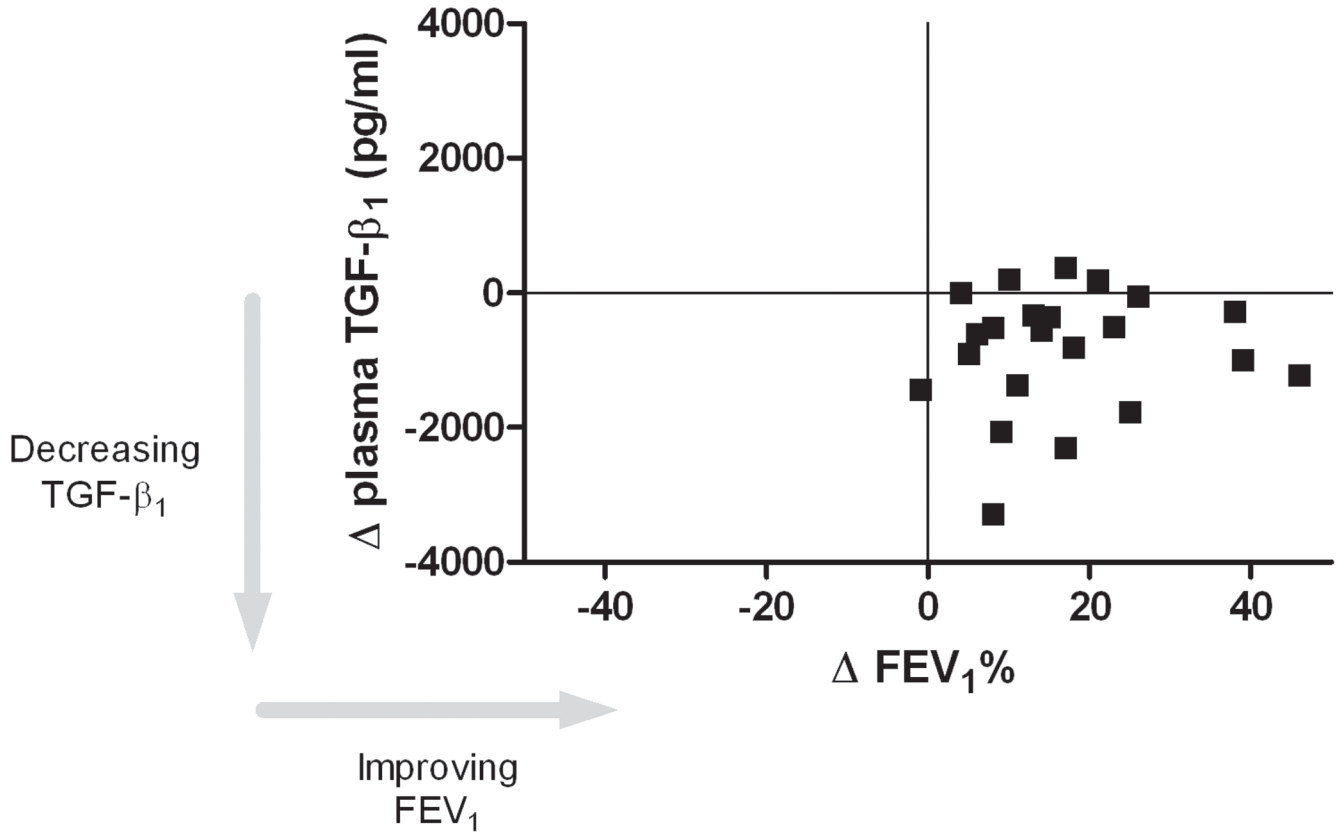


Figure 4.

A) Response of plasma TGF-β_1 to treatment of a pulmonary exacerbation. After the conclusion of clinically-directed treatment, plasma TGF-β_1 decreases from 2600 ± 360 pg/ml to 1770 ± 227 pg/ml ($p < .001$).

B) Association between improved lung function (FEV_1) and reduced plasma TGF-β_1 during treatment of a pulmonary exacerbation. After standard clinically-directed therapy, plasma TGF-β_1 is reduced in 83% of subjects ($p < .001$).

Table 1

Demographics and clinical characteristics of study subjects. Data are presented as mean ± SEM or as fraction of subset (%).

Age (Range)	12.2 ± 0.7 years (1–19 years)
Gender, (female/total)%	(19/40) 47.5%
BMI percentile	35.6 ± 4.7%
Genotype	
CFTR	
%ΔF508/ΔF508	(16/39) 41.0%
%ΔF508/other	(20/39) 51.3%
%other/other	(3/39) 7.7%
TGFB1 codon 10 genotype	
%TT	(17/40) 42.5%
%CT	(17/40) 42.5%
%CC	(6/40) 15.0%
Lung function: FEV_1 (% predicted)	
Pre-antibiotics	71.8 ± 3.6 %
Post-antibiotics	84.2 ± 3.3%
Microbiology,	
% positive bacterial respiratory culture	36/40 (90%)
% polymicrobial	28/36 (78%)
% *Pseudomonas aeruginosa*	23/36 (64%)
% mucoid phenotype	14/23 (61%)
% Staphylococcus aureus	23/36 (64%)
% methicillin-resistant	16/23 (70%)
Hospitalizations	
Frequency	0.87 ± .17 hospitalizations/year
% hospitalized in last 12 months	(20/39) 51.3%

Abbreviations: FEV_1% (Forced expiratory volume in 1 second as a percentage of predicted values in healthy non-CF subjects, BMI (Body Mass Index)