

## IMPACT OF CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR MODULATORS ON RESPIRATORY, NUTRITIONAL, AND INTESTINAL PARAMETERS IN CHILDREN WITH CYSTIC FIBROSIS: A SINGLE CENTER OBSERVATIONAL STUDY

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IMPACT OF CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR MODULATORS ON RESPIRATORY, NUTRITIONAL, AND INTESTINAL PARAMETERS IN CHILDREN WITH CYSTIC FIBROSIS: A SINGLE CENTER OBSERVATIONAL STUDY (Abstract): Highly effective cystic fibrosis transmembrane conductance regulator (CFTR) modulators have substantially changed the course of pediatric cystic fibrosis (CF) by improving CFTR function, respiratory outcomes, and nutritional status. The extent to which modulator therapy reverses severe exocrine pancreatic insufficiency and chronic low-grade intestinal inflammation in children remains incompletely defined. **Aim:** To characterize the multidimensional impact of CFTR modulator therapy on CFTR function, respiratory morbidity, nutritional status, exocrine pancreatic function, and intestinal inflammation in a real-world pediatric CF cohort. **Materials and methods:** In this prospective, single-center observational study (April 2024-January 2026), we included children aged 1-17 years with confirmed CF. The main treatment cohort comprised 32 genetically eligible children who initiated and maintained CFTR modulator therapy (ETI) for at least 12 months. Sweat chloride, anthropometric z scores, respiratory microbiology, liver function, and vitamin D status were assessed at predefined timepoints. In a digestive sub cohort of 31 treated children, fecal elastase, fecal calprotectin, and fecal neutrophil gelatinase-associated lipocalin (NGAL) were measured according to treatment duration and compared with values in 22 age matched healthy controls without digestive disease. Respiratory morbidity was evaluated from the frequency of infectious pulmonary exacerbations, airway microbiology, and lung function; spirometry was performed in children aged  $\geq 6$  years and ventilatory impairment was categorized as normal, mild, moderate, or severe. **Results:** After 12 months of CFTR modulator therapy, mean sweat chloride decreased from 114.1 to 78.3 mmol/L, indicating a marked improvement in CFTR function at cohort level. Weight for age and body mass index (BMI) z scores improved significantly, whereas height for age z scores remained essentially unchanged, consistent with a predominantly weight-driven short-term nutritional recovery. The mean annual rate of infectious pulmonary exacerbations declined from 4.72 at baseline to 0.41 at 6 months, reflecting a rapid and sustained reduction in respiratory morbidity. Diagnosis before 6 months of age was associated with a higher risk of severe exocrine pancreatic insufficiency and vitamin D deficiency, delineating an early onset, severe digestive phenotype. **Conclusions:** In children

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with CF, CFTR modulator therapy confers major clinical and biological benefits in terms of CFTR function, respiratory morbidity, and weight-related nutritional recovery. However, established severe exocrine pancreatic insufficiency and persistent low-grade intestinal inflammation appear only partially reversible, suggesting that the digestive phenotype of CF is less amenable to correction once structural damage has occurred. Incorporating fecal elastase and fecal inflammatory biomarkers into routine follow-up, alongside anthropometry, respiratory outcomes, and hepatic monitoring, may enhance multidimensional disease surveillance and more precisely guide long-term nutritional and hepatodigestive management in the era of highly effective CFTR modulators. **Keywords:** CYSTIC FIBROSIS, CFTR MODULATORS, FECAL CALPROTECTIN, ELEXACAFTOR, TEZACAFTOR, IVACAFTOR, SWEAT CHLORIDE, NGAL, PANCREATIC INSUFFICIENCY.

## INTRODUCTION

Cystic fibrosis (CF) remains one of the most common autosomal recessive multi-system genetic disorders in childhood. It is caused by mutations in the CFTR (cystic fibrosis transmembrane conductance regulator) gene, which impair epithelial chloride and bicarbonate transport, leading to dehydrated secretions and a complex respiratory, digestive, nutritional, and hepatopancreatic phenotype (1, 2). Over the last decade, pediatric CF care has entered a new therapeutic era with the development of CFTR modulator therapies, particularly highly effective combinations such as elexacaftor / tezacaftor / ivacaftor (ETI), which target the basic molecular defect rather than acting solely at the symptomatic level (1, 2). Current standards of care acknowledge that these therapies have profoundly changed the prognosis of CF and have required a redefinition of how clinical response, long-term monitoring, and residual disease phenotypes are evaluated in the modulator era (1).

The respiratory benefits of CFTR modulators are well established. Pivotal trials and their extensions have shown that ETI leads to marked improvement in CFTR function, reflected by a significant reduction in sweat chloride concentration, to-

gether with improved lung function, fewer pulmonary exacerbations, and better overall clinical status (2). These effects were initially demonstrated in adolescents and adults with eligible genotypes. They were subsequently confirmed in children aged 6-11 years and, more recently, in those aged 2-5 years, supporting the concept that early intervention may alter disease trajectory before irreversible structural damage is fully established (3-5). In particular, phase 3 pediatric studies of ETI have shown favorable efficacy and safety profiles, reinforcing the central role of modulator therapy in contemporary pediatric CF management (3-5).

Beyond pulmonary improvement, increasing attention has been directed toward the impact of modulators on nutritional status. Malnutrition and growth failure remain major determinants of prognosis in pediatric CF and are driven by maldigestion, chronic inflammation, recurrent respiratory infection, and increased energy expenditure (6). In the CFTR modulator era, several studies and recent reviews have consistently reported improvements in body weight and body mass index, suggesting that partial restoration of CFTR activity may reduce the metabolic burden of disease and improve nutrient utilization (3, 5, 6).

However, the magnitude of these benefits is hetero-geneous, and nutritional recovery is not uniform across all anthropometric parameters, with linear growth often responding more slowly than weight gain (6). These observations support the need for an integrated longitudinal evaluation of nutritional response under modulator therapy, particularly in children, for whom growth and developmental windows are of major clinical relevance (6).

In contrast to the robust respiratory and weight-related response, the extrapulmonary digestive benefits of CFTR modulators remain incompletely defined. The exocrine pancreas is one of the earliest and most severely affected organs in CF, and exocrine pancreatic insufficiency often develops in the first months of life (7, 8). Although emerging evidence suggests that modulator therapy may increase fecal elastase levels and allow partial recovery of pancreatic function in a small subgroup of patients, especially those treated early and with less advanced residual damage, most children with severe exocrine pancreatic insufficiency do not achieve full pancreatic sufficiency (7,8). A recent review emphasized that, although the paradigm of exocrine pancreatic disease is beginning to shift in the era of highly effective modulator therapy, meaningful functional recovery remains limited to a minority of patients, and objective monitoring with fecal elastase continues to be recommended in clinical practice (8).

Another insufficiently clarified domain is persistent intestinal inflammation in CF. A growing body of evidence supports the existence of a CF-associated enteropathy characterized by dysbiosis, altered intestinal permeability, maldigestion, and low-grade mucosal inflammation (9-11). Earlier

studies demonstrated that fecal inflammatory markers, particularly fecal calprotectin, may be elevated in children with CF, and this intestinal inflammation has been associated with more severe clinical phenotypes and impaired growth (9, 10). More recent systematic reviews, however, indicate that the interpretation of fecal calprotectin in CF remains complex, as it may reflect both intestinal inflammation and a broader systemic inflammatory burden (11). In parallel, newer biomarkers such as neutrophil gelatinase-associated lipocalin (NGAL) have attracted interest for the assessment of epithelial and neutrophil-driven inflammation, although experience in pediatric CF remains limited (12, 13). Emerging data suggest that modulator therapy may only partially improve intestinal inflammation, while complete normalization is not consistently observed, particularly in patients with longstanding or advanced digestive disease (12, 13).

In this context, real-world studies in pediatric cohorts remain essential. Clinical trials of CFTR modulators often include highly selected populations, relatively limited follow-up periods, and underrepresentation of severe or complex digestive phenotypes (2-5). In everyday clinical practice, individual response to modulator therapy is influenced by age, genotype, pre-existing pulmonary and digestive structural damage, baseline nutritional phenotype, and duration of treatment exposure (1, 6, 8). Therefore, a multidimensional evaluation of children treated with CFTR modulators—including parameters of CFTR function, respiratory morbidity, growth, exocrine pancreatic function, and fecal inflammatory biomarkers—is needed to better understand which components of disease are reversible and which persist as residual phenotypes in

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the era of highly effective modulators (1, 7, 8, 12, 13).

Against this background, the present study aimed to assess the impact of CFTR modulator therapy on respiratory, nutritional, and intestinal parameters in a monocentric pediatric CF cohort under real-world conditions, integrating a longitudinal before-and-after treatment analysis with a cross-sectional comparison against healthy controls.

## MATERIALS AND METHODS

We conducted a prospective, single-center observational study at a regional pediatric CF referral center between April 2024 and January 2026. The study combined a longitudinal component, assessing repeated measurements before and after CFTR modulator initiation, with a cross-sectional comparison of digestive biomarkers between children with CF and healthy controls.

**Inclusion criteria:** children aged 1-17 years with confirmed CF were consecutively enrolled during routine clinical follow-up. The diagnosis of CF was established based on a compatible clinical phenotype, an abnormal or intermediate sweat chloride test confirmed according to current standards, and the identification of two pathogenic or likely pathogenic CFTR variants. **Exclusion criteria** included non-CF gastrointestinal disease, known inflammatory bowel disease, malignancy, other major comorbidities unrelated to CF, and incomplete follow-up during the study period.

The main treatment cohort consisted of 32 genetically eligible children with CF who initiated and maintained CFTR modulator therapy and had available longitudinal follow-up data. A digestive sub-cohort of 31 treated children was included in the

analysis of fecal biomarkers. For comparison of inflammatory and pancreatic fecal markers, 22 apparently healthy age-matched children without a history of inflammatory bowel disease, chronic malabsorption, or chronic gastrointestinal infection were enrolled as controls.

Eligible patients received CFTR modulator therapy according to national recommendations, based on age and genotype. Treatment regimens included corrector/potentiator combinations, with most children receiving elexacaftor/tezacaftor/ivacaftor (ETI). Dosing was adjusted for age and body weight. Participants were evaluated at baseline, before treatment initiation, and at regular follow-up visits, for at least 6-12 months for systemic outcomes, with a longer observation period available for selected digestive and hepatic parameters.

At each visit, the following variables were recorded: demographic and genetic characteristics; anthropometric data (weight, height, BMI, and corresponding age and sex adjusted z scores); respiratory outcomes (number of pulmonary exacerbations, hospitalizations, and antibiotic courses); respiratory microbiology; selected biochemical parameters (including liver enzymes and vitamin status); and sweat chloride concentration. Sweat chloride was measured using standardized procedures at baseline and after at least 12 months of CFTR modulator therapy. Infectious pulmonary exacerbations were recorded longitudinally at baseline and during follow-up and were defined in accordance with European CF working group recommendations and criteria used in recent real-world ETI studies.

Lung function was assessed by spirometry in children aged  $\geq 6$  years, in line with international and national guidelines that

recommend spirometry as the standard for objective evaluation of ventilatory function from this age, when cooperation is generally reproducible. The main parameter analyzed was forced expiratory volume in 1 second (FEV<sub>1</sub>), expressed as a percentage of the predicted value for age, sex, and height. FEV<sub>1</sub> was recorded at baseline and at scheduled follow-up visits, and the severity of ventilatory impairment was categorized as normal, mild, moderate, or severe, according to current recommendations.

Fecal samples were collected from spontaneously passed stool during periods of clinical stability, in the absence of acute gastroenteritis and without recent exposure to antibiotics likely to substantially alter the intestinal microbiota. Samples were stored refrigerated, transported promptly to the laboratory, and frozen until analysis.

The following fecal biomarkers were measured: fecal elastase ( $\mu\text{g/g}$ ) as a marker of exocrine pancreatic function; fecal calprotectin ( $\mu\text{g/g}$ ) as a marker of intestinal inflammation; and fecal lipocalin 2/NGAL ( $\text{ng/g}$ ) as an additional biomarker of mucosal inflammation and barrier dysfunction. Measurements were performed using commercially available ELISA kits validated for pediatric use, according to the manufacturers' instructions; all samples were analyzed in duplicate, and results were expressed per gram of stool.

**Study Objectives.** The primary objectives of the study were: (1) to assess changes in sweat chloride following initiation of CFTR modulator therapy; (2) to evaluate changes in nutritional status, as reflected by weight for age, height for age, and BMI z scores; and (3) to characterize changes over time in the frequency of infectious pulmonary exacerbations and, where available, FEV<sub>1</sub>, given its role as a

major prognostic marker in CF.

Secondary objectives were: (1) to describe fecal elastase, calprotectin, and NGAL levels in children treated with CFTR modulators; (2) to compare fecal biomarkers between children with CF and healthy controls; and (3) to explore associations between digestive biomarkers, exocrine pancreatic insufficiency, vitamin D status, respiratory profile (including FEV<sub>1</sub>, when available), and hepatic markers.

**Statistical Analysis.** Statistical analyses were performed using Python (Pandas and SciPy libraries). Continuous variables were summarized as mean  $\pm$  standard deviation or median and interquartile range (IQR), according to their distribution. Categorical variables were described as frequencies and percentages. Normality was assessed using the Kolmogorov-Smirnov, Anderson-Darling, D'Agostino-Pearson, and Shapiro-Wilk tests.

Paired comparisons for longitudinal outcomes were performed using paired t tests or corresponding non-parametric tests, as appropriate. Comparisons between the CF and control groups were carried out using independent samples t tests or Mann-Whitney tests, depending on data distribution. Repeated measures ANOVA was used to evaluate changes in pulmonary exacerbation frequency over time. Associations between categorical variables were analyzed using Fisher's exact test, with relative risks and 95% confidence intervals reported where relevant. A two-sided p value  $<0.05$  was considered statistically significant.

**Ethical Considerations.** The study was approved by the institutional ethics committee and conducted in accordance with the Declaration of Helsinki, national regulations

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governing biomedical research in minors, respectively, by the Ethics Committee of the Sf. Maria Emergency Children Hospital, Iasi (approved number 31600/26.10.2023) and by the Research Ethics Committee of the Grigore T. Popa University of Medicine and Pharmacy Iasi (approved number 434 /24.04.2024). Written informed consent was obtained from the legal guardians of all participants, and assent was additionally obtained from older children and adolescents, where appropriate. All data were anonymized before analysis.

**RESULTS**

**Baseline Characteristics.** The study included 32 children with CF treated with CFTR modulators and 22 healthy controls, who were used for digestive biomarker

comparisons. The mean age at CF diagnosis in the treated cohort was 29.63 months (95% CI: 15.63-43.62), and the median age at evaluation was approximately 12 years. Most treated children carried severe CFTR variants functionally classified as class I/II, including 18 patients (56.3%) homozygous for F508del. More than half of the treated patients were diagnosed before 6 months of age, mainly in the context of abnormal newborn screening or meconium ileus. All children receiving modulators had exocrine pancreatic insufficiency, and approximately 90% had severe pancreatic insufficiency (fecal elastase <100 µg/g). At modulator initiation, 61% of patients met criteria for malnutrition. Baseline demographic and clinical characteristics of the cohorts are summarized in first table.

TABLE I.

**Baseline demographic, genetic, and clinical characteristics of the study population**

Variable	CF modulator group (n = 32)	Healthy controls (n = 22)
Age at evaluation, years	~12 (median)	~12 (median)
Age at diagnosis, months	29.63 (95% CI: 15.63-43.62)	
Female sex, n (%)	18 (56.3%)	11 (50.0%)
508del homozygous, n (%)	18 (56,3%)	-
Diagnosis before 6 months of age, n (%)	~54%	-
Exocrine pancreatic insufficiency, n (%)	32 (100%)	0
Severe EPI (fecal elastase <100 µg/g), n (%)	~90%	0
Malnutrition at treatment initiation, n (%)	61%	0

The median age at evaluation in the CF modulator group was approximately 12 years, comparable to the age range of the control group, and the sex distribution was similar (56.3% vs. 50% female), making a major confounding effect of these variables

on subsequent comparisons unlikely. The mean age at diagnosis in the treated group was 29.63 months, although more than half of the patients were identified before 6 months of age, reflecting early diagnosis, often in the context of abnormal newborn

screening or suggestive digestive manifestations.

From a genetic standpoint, most children carried class I/II CFTR variants, and F508del homozygotes accounted for 56.3% of the cohort, indicating a mutation profile dominated by minimal function genotypes. All patients receiving modulators had exocrine pancreatic insufficiency, and approximately 90% had severe EPI (fecal elastase <100 µg/g), in contrast to the complete absence of pancreatic involvement in the control group. At treatment initiation, 61% of children with CF were malnourished, whereas none of the healthy controls met malnutrition criteria. Taken together, these data show that although the groups are comparable in terms of age and sex, the CF modulator cohort is characterized by a severe genotype, early disease onset, and a profoundly abnormal digestive phenotype with severe EPI and nutritional deficit, justifying careful assessment of the response to modulator therapy in this high-risk population.

#### **CFTR Function and Nutritional Response to Modulator Therapy.**

Changes in CFTR function, anthropometric parameters, lung function, and respiratory morbidity following initiation of CFTR modulators are summarized in second table. After 12 months of treatment, a robust pharmacodynamic response was observed, reflected by a marked reduction in sweat chloride concentration. Mean sweat chloride decreased from 114.1 mmol/L (95% CI: 105.4-122.8) at baseline to 78.3 mmol/L (95% CI: 69.8-86.8) at 12 months, corresponding to a mean change of -35.78 mmol/L (95% CI: -46.00 to -25.57;  $t(31) = 7.144$ ;  $p < 0.001$ ). This magnitude of response is consistent with

values reported in clinical trials and real-world studies of ETI and indicates substantial correction of CFTR dysfunction at the cohort level.

Nutritional recovery during the first year of treatment was predominantly driven by weight gain. Weight for age z scores improved from -1.03 to -0.17, and BMI for age z scores from -1.01 to 0.13, with both changes being highly statistically significant ( $p < 0.0001$ ). In contrast, height for age z scores remained virtually unchanged (-0.65 at baseline *vs.* -0.65 at 12 months;  $\Delta = +0.057$  SD;  $p = 0.717$ ), suggesting that early nutritional benefit is expressed mainly through weight restoration, whereas linear growth likely requires longer observation intervals to show detectable improvement.

In accordance with current recommendations for pediatric lung function testing, standardized spirometry was performed only in children aged  $\geq 6$  years, in whom cooperation is generally reproducible. In this subgroup with spirometry available at 12 months, mean FEV<sub>1</sub> increased from 62.4% to 77.4% of the predicted value, corresponding to a mean gain of 14.9 percentage points (95% CI: 8.5-21.3;  $p = 0.0001$ ). This improvement in ppFEV<sub>1</sub> falls within the range of increases reported in clinical and observational ETI studies in children and adolescents (approximately 9-14 percentage points) and confirms that, in our pediatric cohort, modulator therapy is associated with a substantial functional gain in lung function.

In parallel, the mean number of infectious pulmonary exacerbations declined from 4.72 episodes per year at baseline to 0.41 episodes during the first 6 months of treatment, a reduction that was highly statistically significant ( $p < 0.0001$ ). The

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concomitant decrease in sweat chloride, improvement in weight-related z scores, increase in ppFEV<sub>1</sub>, and abrupt fall in exacerbation frequency support a major and rapid impact of CFTR modulators on CFTR function, nutritional status, and respiratory morbidity. Overall, under real-world conditions, CFTR modulator therapy

in children with CF is associated with a rapid and clinically meaningful improvement in CFTR function, a substantial correction of weight deficit, and a relevant enhancement of lung function, whereas the trajectory of statural growth appears, at least in the short term, to be less clearly influenced.

TABLE II.  
**Changes in CFTR function, anthropometry, and respiratory outcomes following CFTR modulator therapy.**

Outcome	Baseline	Follow-up	Effect estimate	p value
Sweat chloride (mmol/L)	114.1	78.3 at 12 months	-35.78 mmol/L (95% CI: -46.00 to -25.57)	<0.001
Weight-for-age z-score	-1.03	-0.17 at 12 months	+0.86 SD	<0.0001
BMI-for-age z-score	-1.01	0.13 at 12 months	+1.06 SD equivalent	<0.0001
Height-for-age z-score	-0.65 SD	-0.65 SD	+0.057 SD	0.717
Infectious pulmonary exacerbations	4.72/yr.	0.41 at 6 months	Marked reduction over time	<0.0001
pp FEV <sub>1</sub> % predicted	62.4 %	77.4 at 12 months	+14.9 percentage points (95% CI: +8.5 to +21.3)	p= 0.0001

\*ppFEV<sub>1</sub> calculated in the subgroup of 20 children aged ≥6 years with complete FEV<sub>0</sub>-FEV<sub>12</sub> data

**Respiratory Parameters and**

**Microbiological Profile.** Changes in respiratory morbidity after initiation of CFTR modulator therapy are detailed in Table 2 and in this subsection. Infectious pulmonary exacerbations showed an abrupt reduction during the first 6 months of treatment, with the mean number of episodes decreasing from 4.72 ± 1.20 episodes per year at baseline (95% CI: 4.29-5.15) to 0.41 ± 0.76 episodes in the first 6 months (95% CI: 0.13-0.68; p < 0.0001). Repeated measures ANOVA (timepoints: baseline, 1, 3, and 6 months) demonstrated a highly significant overall effect of time on exacerbation frequency (Greenhouse - Geisser

corrected F(1.692, 52.44) = 140.7; p < 0.0001), indicating a strong and sustained therapeutic impact of modulators on respiratory morbidity.

Trend analysis suggested a predominantly linear downward pattern, with a rapid decline in exacerbations immediately after treatment initiation and subsequent stabilization at very low values. This profile is consistent with real-world ETI data in adolescents and adults and confirms the major early impact of modulator therapy on exacerbation burden in the pediatric population. Together with the increase in ppFEV<sub>1</sub> and improvement in nutritional parameters, these findings support an im-

portant overall respiratory benefit in the cohort analyzed.

Concerning respiratory microbiology, chronic colonization with *Pseudomonas aeruginosa* and/or *Staphylococcus aureus* showed clinically plausible associations with disease severity and bronchiectasis burden; however, these relationships did not reach statistical significance in the present cohort, likely due to the relatively small sample size and limited number of events. Nevertheless, the observed trends are consistent with our previous data demonstrating a correlation between *S. aureus* and *P. aeruginosa* colonization and bronchiectasis development in the extended center cohort, suggesting that the microbiological profile remains an important determinant of respiratory severity even in the modulator era.

#### **Exocrine Pancreatic Function and Intestinal Inflammation.**

The profile of digestive biomarkers in children with CF treated with CFTR modulators and in healthy controls is presented in table III. Compared with healthy controls, children with CF on modulator therapy had significantly higher levels of fecal inflammatory biomarkers. Median fecal calprotectin was 92.4  $\mu\text{g/g}$  (IQR: 30.7-196) in the CF group versus 18.8  $\mu\text{g/g}$  (IQR: 5-40) in controls ( $p = 0.0028$ ), with approximately half of the CF children having values above 100  $\mu\text{g/g}$ . Median fecal NGAL was also higher in children with CF than in controls (1.685  $\mu\text{g/g}$  vs. 0.915  $\mu\text{g/g}$ ;  $p = 0.0008$ ), whereas values in the control group were often very low or undetectable.

Fecal elastase remained profoundly reduced in the CF cohort, with a median value of 1.2  $\mu\text{g/g}$  (IQR: 0.5-20), while all controls had values  $\geq 200$   $\mu\text{g/g}$  ( $p <$

0.0001). This confirms the persistence of severe exocrine pancreatic insufficiency despite exposure to highly effective modulator therapy, in contrast to isolated reports of partial pancreatic function recovery in a small subset of patients.

Longitudinally, neither fecal calprotectin nor fecal NGAL showed a clear trend towards normalization under treatment. Median calprotectin values remained broadly similar across exposure categories (approximately 108  $\mu\text{g/g}$  at 0-12 months, 69  $\mu\text{g/g}$  at 12-24 months, and 91  $\mu\text{g/g}$  at  $>24$  months;  $p = 0.73$ ), and fecal NGAL exhibited a similar pattern ( $p = 0.72$ ). Although several individual patients demonstrated decreases in calprotectin over time, none achieved complete normalization below 50  $\mu\text{g/g}$ , suggesting the persistence of concomitant low-grade intestinal inflammation in most children, in line with published data on CF-associated enteropathy.

Exocrine pancreatic function also remained essentially unchanged over the course of longitudinal follow-up. Fecal elastase values remained below 100  $\mu\text{g/g}$  in almost all patients, and no child reached the threshold for pancreatic sufficiency ( $>200$   $\mu\text{g/g}$ ); only one patient shifted from severe to moderate exocrine pancreatic insufficiency. Fecal calprotectin and NGAL correlated positively with each other and inversely with fecal elastase, supporting the presence of a persistent digestive phenotype characterized by low-grade intestinal inflammation and severe exocrine pancreatic insufficiency. Figures 1-3 graphically illustrate these between-group differences and the inter-individual variability of fecal biomarkers, liver function, and intestinal inflammation in CF patients treated with modulators.

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TABLE III.  
**Digestive biomarker profile in children with CF treated with CFTR modulators and in healthy controls.**

Biomarker	CF group	Healthy controls	p value
Fecal calprotectin (µg/g)	92.4 (IQR: 30.7-196)	18.8 (IQR: 5-40)	0.0028
Fecal NGAL (µg/g)	1.685	0.915	0.0008
Fecal elastase (µg/g)	1.2 (IQR: 0.5-20)	≥200 in all controls	<0.0001

**Vitamin D and Hepatic Profile**

The evolution of hepatic parameters during CFTR modulator therapy is presented in table IV. Children diagnosed before 6 months of age had a significantly higher risk of severe exocrine pancreatic insufficiency and vitamin D deficiency, suggesting that an early and severe digestive phenotype is closely linked to minimal function CFTR genotypes and early pancreatic involvement.

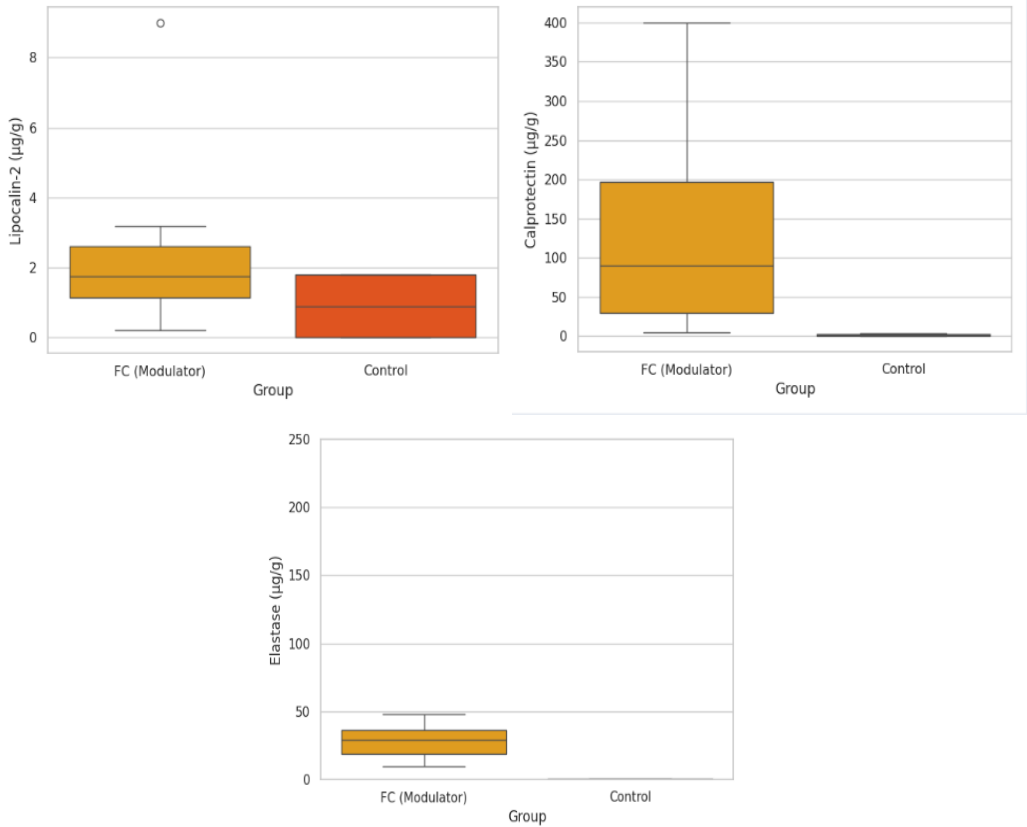
Under CFTR modulator therapy, the hepatic profile remained globally stable, with only modest and transient changes in liver enzymes. Median ALT increased slightly at 6 months, followed by a decline to values below baseline after more than 24 months (29.3 (23.1-41.4) U/L at baseline vs. 24.9 (21.5-30.5) U/L at >24 months; p = 0.009), with a similar pattern observed for

AST (28.2 (21.8-38.3) U/L at baseline vs. 22.8 (19.4-28.5) U/L at >24 months; p = 0.006). GGT, total bilirubin, and INR remained stable and within normal limits throughout follow-up, in line with studies reporting minimal or no hepatic changes under ETI.

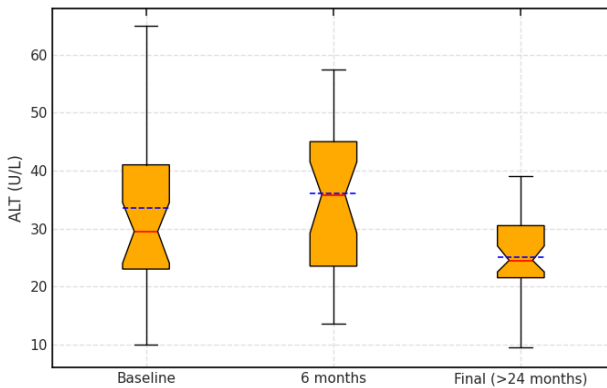
No significant correlations were identified between peak ALT values and fecal calprotectin or NGAL levels, suggesting that mild variations in transaminases were largely independent of intestinal inflammatory burden. However, all cases of mild hepatic abnormalities occurred in children with severe exocrine pancreatic insufficiency and severe CFTR genotypes, whereas no child with residual pancreatic function exhibited hepatic changes, supporting a close relationship between a severe digestive phenotype and hepatic vulnerability.

TABLE IV.  
**Evolution of hepatic parameters during CFTR modulator therapy.**

Parameter	Baseline median (IQR)	6-month median (IQR)	>24-month median (IQR)	p-value (baseline vs. >24 months)
ALT (U/L)	29.3 (23.1-41.4)	35.6 (23.8-45.0)	24.9 (21.5-30.5)	0.009
AST (U/L)	28.2 (21.8-38.3)	34.6 (20.4-42.3)	22.8 (19.4-28.5)	0.006
GGT (U/L)	17.4 (15.2-22.5)	18.0 (15.0-25.0)	18.2 (15.2-21.5)	0.270
Total bilirubin (mg/dL)	0.51 (0.36-0.63)	0.50 (0.40-0.70)	0.54 (0.39-0.64)	0.920
INR	1.02 (0.98-1.03)	1.01 (0.99-1.04)	1.01 (0.98-1.04)	0.150

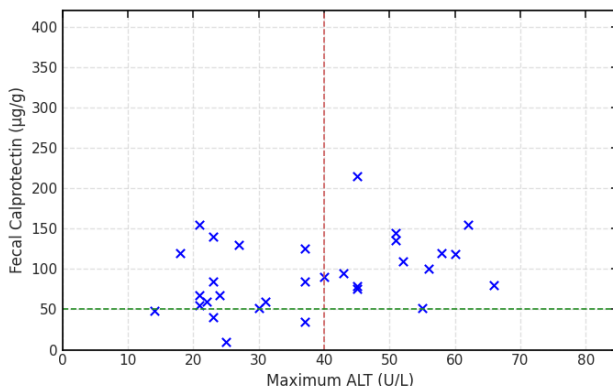


**Fig. 1.** Fecal digestive biomarkers in children with CF receiving CFTR modulator therapy compared with healthy controls.



**Fig. 2.** ALT evolution at baseline, 6 months, and >24 months of CFTR modulator therapy, illustrating a transient increase at 6 months followed by a decline below baseline values ( $p < 0.01$ ), suggestive of a modest improvement in hepatic profile.

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**Fig. 3.** Correlation between liver function and intestinal inflammation in CF patients treated with modulators.

### DISCUSSION

This pediatric real-world study shows that CFTR modulator therapy elicits a strong, yet organ-specific, therapeutic response in children with cystic fibrosis. The most pronounced benefits were observed in CFTR function, respiratory morbidity, and weight-related nutritional recovery, whereas severe exocrine pancreatic dysfunction and low-grade intestinal inflammation were only minimally reversible in most patients, which is consistent with other studies in the literature. (7, 8, 12, 14).

The marked reduction in sweat chloride confirms a robust pharmacodynamic effect of CFTR modulators under routine clinical conditions. Although the magnitude of sweat chloride decline in this cohort was somewhat lower than that reported in tightly selected pediatric ETI trials, the change remained clinically meaningful and likely reflects real-world heterogeneity in genotype, baseline disease severity, preexisting structural damage, and treatment adherence (2-5, 14, 15). Similar variability has been reported in observational studies, in which a substantial proportion of ETI-treated patients retain sweat chloride values above

60 mmol/L despite overall improvement, underscoring that CFTR correction is heterogeneous outside trial settings (14-16). Collectively, these findings support the role of sweat chloride as a useful, but not fully deterministic, biomarker of treatment response in the modulator era.

Respiratory improvement in our cohort was substantial. The abrupt decline in infectious pulmonary exacerbations during the first six months of therapy supports the concept that highly effective modulators interrupt the cycle of mucus obstruction, infection, and inflammation that drives CF lung disease progression (2-5, 14). This pattern is consistent with observational cohorts and registry data showing marked reductions in pulmonary morbidity after the introduction of ETI, including fewer exacerbations, hospitalizations, and antibiotic courses (3-5, 14, 15). The gain in ppFEV<sub>1</sub> in the subgroup with spirometry data was also in line with real-world pediatric series, which report meaningful improvements in lung function sustained over 12 months and beyond (3-5, 14-16). Nevertheless, persistent chronic airway colonization in some children indicates that established infection

and bronchiectatic structural lung disease are not fully reversible through CFTR correction alone, and that airway microbiology remains an important determinant of long-term respiratory outcomes (14-16).

From a nutritional standpoint, treatment was associated with substantial improvements in weight and BMI for age z scores, whereas height for age remained essentially unchanged during the first year of follow-up. This dissociation suggests that improved energy balance and reduced inflammatory burden may drive early weight catch-up, while linear growth likely requires longer follow-up and may remain constrained by prior disease burden, longstanding exocrine pancreatic insufficiency, and cumulative nutritional deficits. A similar early divergence between weight and height trajectories has been reported in longitudinal registry analyses, including recent Danish data showing that height z scores remained largely unchanged two years after ETI initiation despite significant gains in weight and BMI (17). These observations support the notion that statural deficits represent a more fixed component of the CF phenotype once established and may not fully normalize even in the context of highly effective CFTR modulation.

The digestive findings are among the most clinically relevant aspects of this study. Severe exocrine pancreatic insufficiency persisted in nearly all children despite modulator exposure, and there was no meaningful recovery of fecal elastase in the vast majority of patients. This observation supports the concept that, once acinar destruction and fibrosis are established, restoration of epithelial ion transport alone is insufficient to reestablish exocrine pancreatic function. (18) Reports of pancreatic recovery under CFTR modulators have

been largely confined to infants and very young children treated early, before advanced structural damage is present. For example, extension data from ivacaftor trials in children aged 2-5 years showed sustained improvements in fecal elastase, suggesting that early modulation may delay or mitigate pancreatic deterioration in selected genotypes (18-20). In older patients with established structural disease—such as our cohort with a median age of 12 years—exposure to elexacaftor/tezacaftor/ivacaftor appears insufficient to restore exocrine function, in line with PROMISE GI findings showing no clinically significant change in fecal elastase after six months of ETI in patients aged  $\geq 12$  years (18-21).

Similarly, fecal calprotectin and NGAL remained elevated compared with healthy controls and did not normalize over time, despite an excellent respiratory and weight response. In line with previous work, fecal calprotectin levels in children with cystic fibrosis are frequently above normal and often reflect overall disease burden rather than overt gastrointestinal symptoms (22). The inverse relationship between fecal inflammatory markers and fecal elastase in our cohort suggests that severe exocrine pancreatic insufficiency and intestinal inflammation may represent interconnected components of a common digestive phenotype rather than independent abnormalities (18, 20). Prospective multicenter data show that CFTR modulators reduce intestinal inflammation, with a significant fall in fecal calprotectin—particularly under elexacaftor / tezacaftor/ ivacaftor—yet a substantial proportion of patients retain abnormal values after 6-12 months of treatment, indicating only partial resolution of CF-associated enteropathy (12).

The pattern of liver enzyme dynamics

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observed in this cohort - a modest early increase followed by longer-term normalization - is consistent with emerging evidence on the hepatic effects of elxacaftor/tezacaftor/ivacaftor. In pediatric cohorts evaluated with shear wave or transient elastography, initiation of elxacaftor/tezacaftor/ivacaftor has been associated with mild, mostly transient elevations in transaminases and a subsequent reduction in liver stiffness among children with cystic fibrosis and hepatobiliary involvement (23). In our study, GGT, total bilirubin, and INR remained stable and within normal limits, and late ALT and AST values fell below baseline, supporting a favorable hepatic safety profile overall that is in line with systematic reviews of CFTR modulator therapies and their impact on liver biochemistry and non-invasive liver disease markers (24). Persistence of vitamin D deficiency in children with severe exocrine pancreatic insufficiency further underscores that CFTR modulators do not eliminate the need for structured digestive and hepatobiliary management. Pancreatic enzyme replacement, supplementation with fat-soluble vitamins, and close nutritional and hepatic monitoring should therefore not be relaxed solely based on respiratory or anthropometric improvement (24).

This study has several strengths. It prospectively evaluates a real-world pediatric cohort, integrates systemic and digestive outcomes, incorporates healthy controls for fecal biomarker comparison, and addresses a clinically relevant but insufficiently explored question: whether highly effective CFTR modulators fully reverse the digestive phenotype of CF. However, several limitations must be acknowledged. The study was single-center and included a modest sample size, limiting statistical

power for subgroup analyses, and the number of untreated CF comparators was too small to support robust parallel inferences. Follow up duration varied across outcomes, and some analyses were restricted to subgroups with available longitudinal digestive data. In addition, the observational design precludes causal inference, and the study did not include microbiome sequencing or standardized imaging of structural gastrointestinal disease.

Despite these limitations, the present findings have important clinical implications. In pediatric CF, treatment success should not be defined solely by improved respiratory outcomes and weight gain. The persistent burden of digestive disease remains clinically relevant and warrants dedicated longitudinal evaluation. A multidimensional follow-up model incorporating fecal elastase, fecal inflammatory biomarkers, vitamin status, and hepatic surveillance, alongside respiratory outcomes, may better capture overall disease activity in the era of highly effective CFTR modulators and support more precise long-term nutritional and hepato-digestive management strategies.

**Future research directions.** The introduction of highly effective CFTR modulators has fundamentally altered the natural history of cystic fibrosis, transforming the prognosis of pediatric patients from one defined by progressive functional decline into an on-treatment prognosis that is conditioned by the degree of individual response and the magnitude of residual risk. Building on the observations from this study, several priority avenues for future research emerge.

The persistence of exocrine pancreatic insufficiency and intestinal inflammation,

reflected by elevated fecal calprotectin and NGAL, indicates that CF-associated enteropathy is not fully reversible in patients with established structural lesions (12, 16-18). Future studies should incorporate longitudinal fecal microbiome sequencing to elucidate the mechanisms underlying persistent dysbiosis and its relationship with intestinal inflammation in the modulator era. In parallel, prospective cohorts of infants diagnosed through newborn screening are needed to determine whether ultra-early initiation of CFTR modulators can prevent the development of this residual digestive phenotype and avert irreversible pancreatic acinar destruction (12, 18, 19-22).

Although the rapid recovery of weight and BMI for age *z* scores represents a major therapeutic success, this accelerated trajectory requires a re-evaluation of traditional nutritional strategies. The shift from a historical risk of undernutrition to an emerging phenotype characterized by a risk of overweight and obesity warrants detailed investigation, including the potential long-term cardiometabolic consequences. Future research should therefore longitudinally assess body composition (lean mass versus fat mass), lipid profile, and the risk of cystic fibrosis-related diabetes (CFRD), to tailor enzyme replacement, caloric intake, and dietary counselling to the new treatment-induced metabolic trajectories (17).

The mild and transient fluctuations in liver enzymes (ALT/AST) observed under elxacaftor/tezacaftor/ivacaftor in this cohort underscore the importance of rigorous pharmacovigilance. Future studies should correlate serum biomarkers (e.g., APRI) with noninvasive structural assessments such as transient elastography (Fibro Scan) to differentiate drug-related hepatotoxicity from progression of CF-associated liver

disease and to define evidence-based surveillance protocols. In the same long-term safety domain, further work is needed to standardize baseline and follow-up ophthalmologic assessment in children treated with CFTR modulators, in light of pediatric reports of cataract development following early ivacaftor or ETI exposure in utero and during breastfeeding (23-26).

As respiratory morbidity, particularly pulmonary exacerbations, declines dramatically under modulator therapy, classical endpoints progressively lose their ability to discriminate long-term prognosis (23, 24, 27). Future research should validate dynamic prognostic models that integrate sensitive functional markers of small airway disease, such as the Lung Clearance Index, together with high-resolution imaging scores (e.g. chest CT bronchiectasis and air-trapping indices) (24, 26). Such a multidimensional approach may unmask silent structural lung disease and the persistence of niche chronic infections, thereby guiding individualized treatment decisions under real-world conditions, from the newborn period until adulthood (24, 26-29).

## CONCLUSIONS

In this real-world pediatric cohort, CFTR modulator therapy was associated with marked clinical benefits, including substantial reductions in sweat chloride, infectious pulmonary exacerbations, and improvements in ppFEV<sub>1</sub> and weight/BMI-for-age *z* scores, indicating a rapid shift from progressive functional decline toward improved respiratory stability and nutritional recovery. However, severe exocrine pancreatic insufficiency and low-grade intestinal inflammation persisted in almost all children, with fecal elastase remaining profoundly reduced and fecal calprotectin

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and NGAL only minimally improved, suggesting that the digestive phenotype of cystic fibrosis is only partially reversible once structural damage has occurred. The hepatic profile with elexacaftor/tezacaftor/ivacaftor was generally favorable, with only mild, transient ALT/AST elevations and stable GGT, bilirubin, and INR. Yet, enzyme fluctuations in children with severe exocrine pancreatic insufficiency and high-risk genotypes underscore the need for ongoing hepatobiliary surveillance. Overall, pediatric cystic fibrosis care in the modulator era should be judged not solely by gains in lung function or weight, but by sustained, multidimensional control of

respiratory, pancreatic, intestinal, hepatic, and nutritional health across the life course, supported by early initiation of CFTR modulators and lifelong, multidisciplinary follow-up.

### CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest.

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