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Blood platelet reduction after elexacaftor/tezacaftor/ivacaftor treatment in people with cystic fibrosis may depend on systemic inflammation reduction

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The elexacaftor/tezacaftor/ivacaftor (ETI) combination for cystic fibrosis transmembrane regulator modulators is a safe and effective treatment in both adults and children who are homozygous or compound heterozygous for the F508del variant. However, few cases involving a significant reduction in blood platelets and an increase in the alanine aminotransferase/platelet ratio have been described in adult and pediatric patients receiving ETI therapy. In the present study, we describe 272 people with cystic fibrosis (pwCF) (166 adult and 106 pediatric pwCF) who were independently followed at two centers; moreover, these individuals were homozygous or compound heterozygous for the F508del variant, were treated with ETI for at least one year, and exhibited monitored platelet and leukocyte counts (together with liver and inflammatory biochemical indices). As controls, 272 healthy subjects (HCs) matched for sex and age were evaluated. At baseline, both adult and pediatric pwCF demonstrated significantly ($p < 0.01$) greater blood platelet and leukocyte counts compared with HCs. One year of treatment significantly reduced blood platelet counts (adults: $248*10^3$/mmc vs. $288*10^3$/mmc, $p < 0.01$; children: $283*10^3$/mmc vs. $320*10^3$/mmc, $p < 0.01$) and leukocyte counts (adults: $6.5*10^3$/mmc vs. $7.6*10^3$/mmc, $p < 0.01$; children: $6.8*10^3$/mmc vs. $7.9*10^3$/mmc, $p < 0.01$). In addition, the serum C-reactive protein (CRP) level was significantly ($p < 0.01$) decreased after therapy, whereas the alanine aminotransferase (ALT) level and the ALT/platelet ratio were significantly increased ($p < 0.01$). After the second year of therapy, the laboratory parameters were not further altered in approximately half of the patients. The reduction in platelets was significantly correlated with a decrease in leukocytes (r_s: 0.352, $p < 0.001$), serum CRP levels (r_s: 0.392, $p < 0.001$) and exacerbations (oral antibiotic cycles, r_s: 0.241, $p = 0.002$; intravenous antibiotic cycles, r_s: 0.153, $p = 0.049$). These findings suggest that the normalization of platelets may be dependent on the reduction in systemic inflammation induced by ETI therapy.

Keywords Cystic fibrosis, Elexacaftor/tezacaftor/ivacaftor, Platelets, Inflammation

Abbreviations

CF	Cystic fibrosis
CFTR	Cystic fibrosis transmembrane conductance regulator
ETI	Elexacaftor/tezacaftor/ivacaftor

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SC	Sweat chloride
BMI	Body mass index
CFLD	CF liver disease
IQR	Interquartile range
LI	Lumacaftor/ivacaftor
HC	Healthy control subjects
ALT	Alanine aminostransferase
CRP	C-reactive protein
URL	Upper reference limit
CFHBI	CF hepatobiliary involvement
OAC	Oral antibiotic cycles
IAC	Intravenous antibiotic cycles

Outcomes for people with cystic fibrosis (pwCF) have greatly improved in the last decade due to the use of modulator drugs, which increase the amount and potentiate the activity of the cystic fibrosis transmembrane conductance regulator (CFTR)-mutated protein[1–3]. Various combinations of modulators are available; moreover, in vitro and ex vivo models, such as organoids[4] or nasal epithelial cells[5–7], can be used to predict the responsiveness of patients harboring different *CFTR* genotypes to modulators. Thus, the number of pwCF that have access to novel therapies has increased, including those with at least one allele harboring the most frequent F508del variant[8].

The combination of elexacaftor/tezacaftor/ivacaftor (ETI) enhances the amount and activity of the CFTR protein[7,9]. This specific treatment reduces sweat chloride (SC) levels and improves both lung function and imaging parameters[10,11], as well as body mass index (BMI) and glucose metabolism[12], in both adult[13,14] and pediatric[15,16] pwCF who are homozygous[14] or heterozygous[17,18] for F508del *in trans* with other variants.

Few severe side effects (such as liver injury) have been reported in adults and children treated with ETI[19]. However, mild effects such as hyperbilirubinemia[20] and hypertransaminasemia[21] are more commonly observed, particularly in adults[22]; additionally, these effects may be dependent on the accumulation of cholesterol in the liver, which triggers inflammation[23]. Although no trials focusing on ETI in adult[13] or pediatric pwCF[15,16,24–26] have reported a reduction in blood platelet count as a side effect, a reduction in platelet count was observed in 52 pwCF treated with ETI[27], and a significant increase in the alanine aminotransferase (ALT)/platelet ratio after ETI therapy has also been described[28]. Furthermore, few cases of severe reductions in blood platelets in pediatric pwCF during ETI therapy have been reported[29]. Moreover, the critical role of platelets in inflammation and the immune response, especially regarding their abilities to form aggregates with leukocytes[30] and secrete proinflammatory mediators[31], has been highlighted. Additionally, the ALT/platelet ratio has been reported to be a noninvasive marker of liver disease[32,33].

Therefore, the present dual-center study aimed to evaluate the effects of ETI therapy on platelets and leukocytes related to pulmonary exacerbations, as well as liver and inflammatory biochemical indices. Herein, we describe 272 pwCF (166 adult and 106 pediatric pwCF) who were homozygous or compound heterozygous for the F508del variant and who were treated with ETI.

Materials and methods

Patients

We followed the same criteria and methodology of our previous studies focusing on pwCF treated with ETI[21,22]. This prospective study (2023–2025) was approved by the Ethics Committees of the CF Regional Centre of Tuscany (Ethics Clearance number: 63/2023) and Campania (Ethics Committee number: 77/2021). The inclusion criteria included pwCF with the F508del mutation (either homozygous or *in trans* with another *CFTR* variant) and with at least one year of treatment with ETI. The exclusion criteria included mechanical ventilation, CF liver disease (CFLD)[34] and previous liver or lung transplantations. The patients were followed at the regional Centre of Campania and the regional Centre of Tuscany according to the CF standard of care[35]. The *CFTR* genotype was analyzed via *CFTR* gene sequencing[36]. Liver disease[34] was evaluated via clinical, biochemical or ultrasonographic assessments conducted in two consecutive examinations within a 3-month period in the absence of other causes of congenital or acquired chronic liver disease. CFLD refers to experiencing one (or more) of the following conditions: nodular liver, advanced fibrosis (F4), multilobular cirrhosis with or without portal hypertension, or noncirrhotic portal hypertension[34]. Pulmonary exacerbations were reported as oral antibiotic cycles/year (OAC) and intravenous antibiotic cycles/year (IAC).

Biochemical parameters

Alanine aminotransferase (ALT) and C-reactive protein (CRP) levels were evaluated in the serum, whereas blood platelets and leukocytes were evaluated in whole blood by using EDTA; these parameters were measured within one hour of blood sampling via automated analyzers by using standard procedures. For all of the subjects, the samples were collected before the initiation of ETI therapy and after one or two years of ETI therapy, with a maximum difference of one month from the exact expected date being observed. No blood samples were collected during the occurrence of pulmonary exacerbations. Furthermore, the samples were analyzed in the same laboratory.

Statistical analysis

Continuous data are reported as medians and IQRs. The Shapiro–Wilk test was utilized to evaluate the normality of the distributions. Comparisons between two groups of independent samples were evaluated via the Mann–Whitney U test. Paired comparisons were performed via the Wilcoxon test (between two groups) and the

Friedman test (between three groups). Categorical data are reported as frequencies (percentages) and were compared via the chi-square test. The correlations between variables were evaluated via Spearman correlation analysis. Statistical analyses were performed via SPSS (version 29, IBM SPSS Statistics). P values < 0.01 were considered to be statistically significant.

Results

Study population

We investigated 272 pwCF, 132 of whom were followed at the regional Centre of Campania, with 140 individuals being followed at the regional Centre of Tuscany (Table 1). Among the 272 pwCF, 166 adult patients (79 females [47.6%]; median age: 33 years, interquartile range [IQR]: 27–42) and 106 pediatric patients (58 females [54.7%]; median age: 14 years, IQR: 11–16) were identified. The 272 pwCF were treated with ETI for either one year (n = 175) or two years (n = 97). As a control group, we evaluated 272 healthy subjects (HCs) who were matched for sex and age with the 272 subjects with CF (Table 1).

Laboratory parameters in pwCF and HCs at baseline and after treatment with ETI

In adult pwCF, both the platelet and the leukocyte numbers at baseline were greater than those in HCs, although they were within normal limits (Table 2 A). After one year of treatment with ETI, the platelet and leukocyte counts of the pwCF were significantly lower (although they were still observed within normal limits) than those at baseline and in the HCs (Table 2 A). After one year of ETI therapy, most of the pwCF (including approximately 80% of the 166 adult pwCF and 75% of the 106 pediatric pwCF) demonstrated a blood platelet count reduction that was within 15% of the baseline values. However, the reduction in blood platelet count was observed to be so severe in one adult patient with CF that it caused an interruption in therapy (i.e., the blood platelet count decreased from $304*10^3$/mmc before therapy to $22*10^3$/mmc after one year of ETI). No significant differences in the serum ALT level or ALT/platelet ratio were observed between pwCF and HCs at baseline (Table 2 A). After one year of treatment with ETI, both of the aforementioned parameters were significantly greater in pwCF compared to both HCs and the parameter values of the pwCF at baseline (Table 2A).

In pediatric pwCF, the platelet number at baseline was significantly greater than that in HCs, although it was observed to be within normal limits (Table 2B). After one year of treatment with ETI, the platelet number significantly decreased compared with that at baseline and in the HCs (although this change was not statistically significant, Table 2B). At baseline, the leukocyte number was significantly greater ($p < 0.01$) than that in the HCs, although it was observed to be within normal limits (Table 2B). After one year of treatment with ETI, the leukocyte number was significantly lower than both the HC and pediatric pwCF values at baseline (Table 2B). Moreover, the serum ALT level was not significantly different between pediatric pwCF at baseline and HCs; additionally, it was not significantly altered by one year of treatment with ETI (Table 2B). Only one child discontinued therapy with ETI because of a severe increase in the ALT level (> 5 ×). The ALT/platelet ratio was not significantly different between pediatric pwCF at baseline and HCs, whereas it was significantly greater after one year of treatment than both HCs and pediatric pwCF at baseline (Table 2B). Finally, the serum CRP, OAC and IAC values (data not collected from the HCs) were significantly lower after one year of treatment compared to baseline values in both adult (Table 2A) and pediatric (Table 2B) pwCF.

Twenty-four adult and 73 pediatric pwCF completed two years of treatment with ETI. In adult pwCF, the platelet and leukocyte counts, as well as the serum CRP values and ALT/platelet ratios, after two years of treatment were not significantly different compared to the data obtained after one year of treatment, whereas the serum ALT values were significantly lower (although they were observed to be within normal limits) than those after one year of treatment (Supplementary Table 1). In pediatric pwCF, all of the data (including platelet numbers, leukocyte numbers, the serum ALT level, the ALT/platelet ratio and the CRP level after two years of treatment) were not significantly different compared to those obtained after one year of treatment with ETI (Supplementary Table 1).

	N	males	females	age	CFHBI
		n and (%)	n and (%)	median and IQR	
pwCF:	272	135 (49.6)	137 (51.4)	25 (15-36)	86 (31.6)
- from Campania	132	60 (45.5)	72 (54.5)	29 (17-38)	31 (23.5)
- from Tuscany	140	75 (53.6)	65 (46.4)	21 (19-33)	55 (39.3)
Adult subjects:	166	87 (52.4)	79 (47.6)	33 (27-42)	53 (31.9)
- F508del homozygous	61	31 (50.8)	30 (49.2)	32 (25-38)	19 (31.1)
- F508del double heterozygous	105	56 (53.3)	49 (46.7)	33 (28-47)	13 (12.3)
Pediatric subjects:	106	48 (45.3)	58 (54.7)	14 (11-16)	33 (31.1)
- F508del homozygous	45	20 (44.4)	25 (55.6)	13 (11-16)	13 (28.9)
- F508del double heterozygous	61	28 (45.9)	33 (54.1)	14 (12-16)	20 (32.8)
Adult healthy controls	166	87 (52.4)	79 (47.6)	33 (27-42)	
Pediatric healthy controls	106	48 (45.3)	58 (54.7)	14 (11-16)	

Table 1. Study population. IQR: interquartile range; pwCF: people with cystic fibrosis; CFHBI: CF hepatobiliary involvement.

	HCs	CF	
		Baseline	1y of ETI
A			
Platelets (N*10^3/mmc)	254 (245-273)	288 (230-347)[a]	248 (201-287)[a,b]
Leucocytes (N*10^3/mmc)	7.1 (6.6-7.7)	7.6 (6.3-9.6)[a]	6.5 (5.4-7.7)[a,b]
ALT (U/L)	22 (17-26)	22 (14-30)	27 (20-44)[a,b]
ALT/platelets	0.08 (0.06-0.10)	0.07 (0.04-0.13)	0.13 (0.07-0.22)[a,b]
CRP (mg/dL)	N.A.	0.37 (0.33-0.92)	0.33 (0.10-0.33)[b]
OAC (N/year)	N.A.	1.8 (2.0)	0.3 (0.6)[b]
IAC (N/year)	N.A.	0.5 (1.0)	0.01 (0.1)[b]
B			
Platelets (N*10^3/mmc)	298 (261-322)	320 (261-400)[a]	283 (234-332)[b]
Leucocytes (N*10^3/mmc)	7.1 (6.7-7.7)	7.9 (6.8-10.1)[a]	6.8 (5.5-7.7)[a,b]
ALT (U/L)	21 (19-24)	22 (18-34)	23 (17-31)
ALT/platelets	0.07 (0.06-0.08)	0.07 (0.05-0.11)	0.08 (0.05-0.14)[a,b]
CRP (mg/dL)	N.A.	0.29 (0.06-0.47)	0.20 (0.06-0.33)[b]
OAC (N/year)	N.A.	1.7 (1.6)	0.7 (1.0)[b]
IAC (N/year)	N.A.	0.6 (1.6)	0.1 (0.3)[b]

Table 2.. Laboratory parameters and exacerbations in 166 adult (**A**) and in 106 pediatric (**B**) pwCF at baseline and after 1 year of ETI therapy and in age/sex-matched healthy controls. Data are reported as median (interquartile range) or average (standard deviation) as appropriate. [a] $p < 0.01$ vs CTRL, Mann-Whitney U test; [b] $p < 0.01$ vs baseline, Wilcoxon test. HCs: healthy control subjects; pwCF: people with CF; ETI: elexacaftor/tezacaftor/ivacaftor; ALT: alanine aminotransferase; CRP: C reactive protein; N.A : not available; OAC: oral antibiotic cycles; IAC: intravenous antibiotic cycles.

A	Δ% platelets		Δ% leucocytes		Δ% CRP		Δ% OAC		Δ% IAC	
	r_s	p value	r_s	p value	r_s	p value	r_s	p value	r_s	p value
Δ% platelets	-	-	0.352	**< 0.001**	0.392	**< 0.001**	0.241	**0.002**	0.153	**0.049**
Δ% leucocytes			-	-	0.177	**0.023**	0.148	0.057	0.301	**<0.001**
Δ% CRP					-	-	0.112	0.152	0.274	**<0.001**
B										
Δ% platelets	-	-	0.400	**< 0.001**	0.382	**< 0.001**	0.056	0.565	0.196	**0.044**
Δ% leucocytes			-	-	0.192	0.051	0.141	0.150	0.191	0.050
Δ% CRP					-	-	−0.010	0.917	0.195	**0.047**

Table 3.. Spearman correlation analysis of platelets, leucocytes, CRP and exacerbations delta percentage (Δ%) after 1 year of ETI therapy in adult (**A**) and pediatric (**B**) pwCF. CRP: C reactive protein; OAC: oral antibiotic cycles; IAC: intravenous antibiotic cycles.

Thus, we calculated the differences between the values obtained after one year of ETI therapy and the values at baseline for blood platelet, leukocyte, serum CRP, OAC and IAC values for all of the adult and pediatric pwCF (including delta-platelets, leukocytes, CRP, OAC and IAC). We correlated the values of delta-platelets with those of delta-leukocytes, delta-CRP, delta-OAC and delta-IAC in both adult (Table 3A) and pediatric (Table 3B) pwCF. Delta-platelets were significantly and positively correlated with delta-leukocytes, delta-CRP values, delta-OAC and delta-IAC in both adult and pediatric pwCF (except for delta-OAC in pediatric pwCF) (Table 3A and 3B).

The impact of the CFTR genotype and CFHBI on laboratory parameters

Our group of pwCF included both patients who were homozygous for the F508del variant and patients who were compound heterozygous for F508del *in trans* with another *CFTR* variant. Thus, we evaluated laboratory parameters at baseline and after one year of treatment with ETI in the two subgroups (Table 4). In adult pwCF (both at baseline and after one year of treatment), we observed significantly higher values of serum ALT and the ALT/platelet ratio (although they were observed within normal limits) and lower values of OAC in patients who were homozygous for the F508del variant compared to compound heterozygous pwCF (Table 4A). The other parameters (including platelet and leukocyte counts, as well as the serum CRP level) were not significantly different between the two subgroups at baseline or after one year of treatment with ETI (Table 4A). In pediatric pwCF (both at baseline and after one year of therapy with ETI), no parameters were significantly different between pwCF who were homozygous for F508del and pwCF who were compound heterozygous for F508del *in*

	Baseline			After one year of ETI therapy		
A	homozygous	heterozygous	p value	homozygous	heterozygous	p value
	(n = 61)	(n = 105)		(n = 61)	(n = 105)	
Platelets (N*10^3/mmc)	281 (217-345)	288 (237-353)	n.s.	248 (197-287)	248 (202-289)	n.s.
Leucocytes (N*10^3/mmc)	7.7 (6.6-9.7)	7.4 (6.0-9.4)	n.s.	6.1 (5.2-7.3)	6.7 (5.7-7.9)	n.s.
ALT (U/L)	25 (18-38)	20 (12-29)	0.003	37 (24-53)	24 (18-36)	<0.001
ALT/platelets	0.09 (0.06-0.14)	0.06 (0.04-0.12)	0.003	0.16 (0.10-0.26)	0.10 (0.07-0.18)	0.001
CRP (mg/dL)	0.33 (0.29-0.75)	0.47 (0.33-1.0)	n.s.	0.32 (0.07-0.33)	0.33 (0.11-0.33)	n.s.
OAC (N/year)	1.3 (1.6)	2.1 (2.1)	0.012	0.2 (0.5)	0.3 (0.7)	n.s.
IAC (N/year)	0.5 (1.0)	0.5 (1.0)	n.s.	0.0 (0.0)	0.02 (0.1)	n.s.
B						
	(n = 45)	(n = 61)		(n = 45)	(n = 61)	
Platelets (N*10^3/mmc)	292 (258-384)	327 (270-420)	n.s.	271 (209-317)	301 (246-335)	n.s.
Leucocytes (N*10^3/mmc)	8.1 (7.2-9.6)	7.6 (6.3-10.7)	n.s.	6.8 (5.7-7.8)	6.8 (5.2-7.6)	n.s.
ALT (U/L)	24 (19-45)	20 (16-29)	n.s.	22 (18-29)	24 (17-32)	n.s.
ALT/platelets	0.09 (0.05-0.16)	0.07 (0.04-0.11)	n.s.	0.09 (0.06-0.14)	0.08 (0.05-0.13)	n.s.
CRP (mg/dL)	0.29 (0.06-0.33)	0.33 (0.10-0.63)	n.s.	0.06 (0.06-0.33)	0.33 (0.06-0.33)	n.s.
OAC (N/year)	1.3 (1.3)	2.0 (1.7)	0.027	0.7 (1.2)	0.8 (0.9)	n.s.
IAC (N/year)	0.4 (1.7)	0.7 (1.5)	n.s.	0.0 (0.0)	0.1 (0.3)	n.s.

Table 4.. Comparison of laboratory parameters and exacerbations between homozygous and compound heterozygous for F508del variant adult (**A**) and pediatric (**B**) pwCF at baseline and after one year of ETI therapy. Data are reported as median (interquartile range) or average (standard deviation) as appropriate. pwCF: people with cystic fibrosis; ALT: alanine aminotransferase; CRP: C reactive protein; n.s.: not significant; OAC: oral antibiotic cycles; IAC: intravenous antibiotic cycles.

	Adult			Pediatric		
A	no CFHBI	CFHBI	p value	no CFHBI	CFHBI	p value
	(n = 113)	(n = 53)		(n = 73)	(n = 33)	
CRP (mg/dL)	0.38 (0.33-0.89)	0.36 (0.33-1.0)	n.s.	0.33 (0.07-0.55)	0.29 (0.06-0.33)	n.s.
Platelets (N*10^3/mmc)	288 (236-345)	272 (215-365)	n.s.	322 (265-404)	312 (248-397)	n.s.
Leucocytes (N*10^3/mmc)	7.5 (6.4-9.4)	7.7 (6.0-10.0)	n.s.	8.1 (6.8-11.0)	7.6 (6.8-9.1)	n.s.
ALT (U/L)	20 (14-28)	25 (18-37)	n.s.	21 (18-29)	23 (19-53)	n.s.
ALT/platelets	0.07 (0.04-0.12)	0.09 (0.05-0.14)	n.s.	0.07 (0.04-0.09)	0.09 (0.05-0.18)	n.s.
OAC (N/year)	1.6 (1.8)	2.3 (2.3)	n.s.	1.8 (1.5)	1.6 (1.8)	n.s.
IAC (N/year)	0.4 (0.9)	0.7 (1.0)	0.037	0.6 (1.7)	0.6 (1.3)	n.s.
B						
	(n = 113)	(n = 53)		(n = 73)	(n = 33)	
CRP (mg/dL)	0.33 (0.30-0.33)	0.22 (0.06-0.33)	n.s.	0.20 (0.06-0.33)	0.06 (0.06-0.33)	n.s.
Platelets (N*10^3/mmc)	248 (201-286)	246 (197-295)	n.s.	288 (234-332)	279 (228-328)	n.s.
Leucocytes (N*10^3/mmc)	6.5 (5.5-7.4)	6.6 (5.3-8.1)	n.s.	6.9 (5.7-7.8)	6.3 (5.2-7.2)	n.s.
ALT (U/L)	26 (20-42)	30 (21-48)	n.s.	23 (17-28)	28 (18-55)	n.s.
ALT/platelets	0.12 (0.07-0.19)	0.14 (0.07-0.24)	n.s.	0.08 (0.05-0.11)	0.11 (0.06-0.22)	n.s.
OAC (N/year)	0.3 (0.6)	0.4 (0.7)	n.s.	0.8 (1.1)	0.6 (0.8)	n.s.
IAC (N/year)	0.02 (0.1)	0.0 (0.0)	n.s.	0.04 (0.2)	0.06 (0.3)	n.s.

Table 5.. Comparison of laboratory parameters and exacerbations between pwCF with and without CFHBI at baseline (**A**) and after one year of ETI therapy (**B**). Data are reported as median (interquartile range) or average (standard deviation) as appropriate. pwCF: people with cystic fibrosis; ALT: alanine aminotransferase; CFHBI: CF hepatobiliary involvement; CRP: C reactive protein; n.s.: not significant; OAC: oral antibiotic cycles; IAC: intravenous antibiotic cycles.

trans with another *CFTR* variant (except for OAC, which was lower in patients who were homozygous for the F508del variant) (Table 4B).

Moreover, our group of pwCF included both patients with CFHBI at baseline and patients without this complication. Thus, we evaluated laboratory parameters at baseline and after one year of treatment with ETI in the two subgroups (Table 5). In both adult (Table 5A) and pediatric (Table 5B) pwCF, laboratory parameters were

not significantly different at baseline or after one year of treatment with ETI (except for IAC, which was greater in adult pwCF with CFHBI) (Table 5A).

Discussion

One year of treatment with ETI in 166 adult and 106 pediatric pwCF who were either homozygous for F508del or heterozygous for F508del *in trans* with another *CFTR* variant resulted in a significant reduction in blood platelet counts, although these values were observed to be within normal limits. This reduction was significantly correlated with decreases in blood leukocyte counts, serum CRP levels and pulmonary exacerbations. Moreover, ETI treatment significantly increased the serum ALT level and ALT/platelet ratio.

None of the previous large trials that evaluated the effects of ETI have reported changes in platelet number, including the first phase III trial[13] and a long-term registry-based study conducted in adults[37], as well as a phase III study conducted in children aged 6–11 years[15], a larger study performed in children of the same age[24] and a phase III trial conducted in children aged 2–5 years[25]. This lack of change in platelet number could be due to the fact that the platelet reduction that we observed in most of the patients (including approximately 80% of adult and 75% of pediatric pwCF) was within 15% of the baseline values. However, only one individual with CF demonstrated a reduction in platelet number that was sufficiently relevant to interrupt the treatment, and this result aligned with a previous study reporting of only one pwCF exhibiting a severe reduction in blood platelet count after ETI therapy[29]. Our data also matched the significant reduction in blood platelets reported in 52 pediatric pwCF treated with ETI[27]. Another study reported a significant reduction in the ALT/platelet ratio in 74 pwCF after one year of therapy with ETI[28]; however, in this study, the values of ALT and platelets were missing. We confirmed these data in our larger population of adult and pediatric pwCF, demonstrating that the increase in the ALT/platelet ratio was likely due to both the significant increase in ALT, which we previously described in adult pwCF after one year of therapy[21,22], as well as due to a significant reduction in platelet number. The ALT/platelet ratio is a noninvasive biomarker of liver fibrosis, and it has been used to assess the severity of liver disease[38] and liver fibrosis[39] in pwCF by using carefully defined cutoff values[34].

However, we excluded the possibility that the significant increase observed in the ALT/platelet ratio was dependent on liver fibrosis due to ETI therapy for various reasons. First, both adult and pediatric pwCF included in our study exhibited significantly greater numbers of platelets compared to HCs before treatment, whereas after one year of treatment, the number of platelets in pwCF was not significantly different from that of HCs. Furthermore, after the second year of treatment with ETI, the ALT/platelet ratio was not further altered compared with that in the first year of treatment, thus leading to the progression of liver fibrosis being an unlikely scenario, which aligned with the results of a previous study demonstrating a reduction in liver stiffness in pwCF treated with ETI[40]. Moreover, the reduction in blood platelets observed after one year of ETI therapy in our included pwCF was significantly correlated with a decrease in blood leukocytes and serum CRP levels (although these parameters were observed within normal limits), thereby suggesting that the reduction in blood platelets is related to reduced systemic inflammation in response to ETI. Moreover, treatment with ETI significantly reduces leukocyte and serum Ig levels[41]; moreover, it resulted in a reduction in inflammatory cytokines in the sputum of 76 adolescent pwCF after one month of ETI treatment[42]. Furthermore, ETI can reduce ATP/P2X7R-induced inflammasome activation in circulating monocytes[43], along with significantly downregulating the expression of proinflammatory genes in airway epithelial cells[44] and reducing platelet activation[45]. In addition, after one year of ETI therapy, we detected a significant reduction in pulmonary exacerbation, which aligns with the literature[17] and is correlated with a decrease in the platelet count. It has been reported that an increase in the platelet count may be associated with an increase in inflammation markers during exacerbations in patients with chronic obstructive pulmonary disease[46]. Therefore, the decrease in platelet count observed in our cohort may be dependent on the reduction in systemic inflammation induced by ETI therapy. Finally, although pwCF with CFLD were excluded from treatment with ETI, 86 pwCF who were included in the present study (including 53 adult and 33 pediatric pwCF) had CFHBI, and no differences were observed for the platelet number or the ALT/platelet ratio between pwCF with and without CFHBI both before and after treatment with ETI.

One limitation of this study involves the reduction in platelet counts, leukocyte counts, ALT levels and CRP levels to within normal ranges, which represents an unclear result (if clinically meaningful). Furthermore, ALT fluctuations should be considered to occur over time (even during regular follow-ups) due to infections[47], nutritional status[48] and the use of other medications, such as antibiotics[49].

In conclusion, therapy with ETI causes a significant reduction in blood platelet count both in adult and pediatric pwCF who are homozygous or compound heterozygous for the F508del variant. However, rather than a reduction being demonstrated, the platelet count seems to normalize. Moreover, our included pwCF demonstrated a significantly greater number of platelets before treatment and after the treatment, and the values returned to levels that were comparable with those of healthy subjects. This reduction is correlated with a significant decrease in leukocyte counts (which were observed to be higher before treatment), serum CRP levels and pulmonary exacerbations. Thus, when considering the idea that few cases of severe thrombocytopenia are induced by treatment, we suggest monitoring the platelet count during ETI therapy, thereby extending our investigation to populations of pediatric and adolescent pwCF who are receiving treatment for more than two years.

Data availability

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

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Author contributions
Design of the work: VC, VR and VT; Methodology, investigation and data analysis: AC, CC, CF, PI, MG, AS and AT; Manuscript writing and validation: AC, MG and AT. All authors read and approved the final manuscript.

Declarations

Competing interests
The authors declare no competing interests.

Ethics approval and consent to participate
The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethical committee of the CF regional Centre of Tuscany (Ethics Clearance number 63/2023) and of Campania (Ethics Committee number 77/2021). Informed consent was obtained from all participants or the legal guardian/next relative of the participants.

Additional information
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