



Plasma levels of chemokines decrease during elexacaftor/tezacaftor/ivacaftor therapy in adults with cystic fibrosis

Dirk Westhölter^{a,*}, Johannes Pipping^a, Jonas Raspe^a, Mona Schmitz^a, Sivagurunathan Sutharsan^b, Svenja Straßburg^b, Matthias Welsner^b, Christian Taube^a, Sebastian Reuter^a

^a Department of Pulmonary Medicine, University Hospital Essen- Ruhrlandklinik, Essen, Germany

^b Cystic Fibrosis Unit, Department of Pulmonary Medicine, University Hospital Essen- Ruhrlandklinik, Essen, Germany

ABSTRACT

Background: Cystic fibrosis (CF) is associated with dysregulated immune responses, exaggerated inflammation and chronic infection. CF transmembrane conductance regulator (CFTR) modulator therapies directly target the underlying protein defects and resulted in significant clinical benefits for people with CF (pwCF). This study analysed the effects of triple CFTR modulator therapy elexacaftor/tezacaftor/ivacaftor (ETI) on CF-associated inflammation, especially systemic chemokines.

Methods: A bead-based immunoassay was used to quantify proinflammatory chemokines (IL-8, IP-10, Eotaxin, TARC, RANTES, MIP-1 α , MIP-1 β , MIP-3 α , MIG, ENA-78, GRO α , I-TAC) in plasma samples from pwCF collected before, at three, and at six months after starting ETI therapy.

Results: Fifty-one pwCF (47 % female; mean age 32 \pm 10.4 years) were included. At baseline, 67 % were already receiving CFTR modulator therapy with tezacaftor/ivacaftor or lumacaftor/ivacaftor. After initiation of ETI therapy there was a significant improvement in percent predicted forced expiratory volume in 1 s (+12.7 points, $p < 0.001$) and a significant decrease in sweat chloride levels (-53.6 %, $p < 0.001$). After 6 months' treatment with ETI therapy there were significant decreases in plasma levels of MIP-3 α (-68.2 %, $p = 0.018$), GRO α (-17.7 %, $p = 0.013$), ENA-78 (-16.3 %, $p = 0.034$) and I-TAC (-3.4 %, $p = 0.032$). IL-8 exhibited a reduction that did not reach statistical significance (-17.8 %, $p = 0.057$); levels of other assessed cytokines did not change significantly from baseline.

Conclusions: ETI appears to affect a distinct group of chemokines that are predominately associated with neutrophilic inflammation, demonstrating the anti-inflammatory properties of ETI therapy.

1. Introduction

Exaggerated inflammation is a major driver of disease progression in cystic fibrosis (CF) that primarily, but not exclusively, affects the lungs [1]. CF-associated inflammation results from a complex interplay of dysregulated immune responses and chronic infection, contributing to airway obstruction, loss of lung function and progressive lung destruction [1,2]. The link between dysfunctional CF transmembrane conductance regulator (CFTR) protein and dysregulated immune responses in pwCF is not fully understood. CFTR modulator therapy directly targets the underlying protein defects and has shown clinical benefits in pwCF who have a wide range of different CFTR mutations. Elexacaftor/tezacaftor/ivacaftor (ETI) has been approved as a triple combination CFTR modulator therapy for pwCF with at least one copy of the CFTR variant F508del by the European Commission in 2020 and has been shown to improve quality of life and lung function to a greater extent than the previous generation of dual combination CFTR modulators [3,4]. Despite existing evidence for significant improvements in pulmonary function tests and in health-related quality of life during ETI therapy,

* Corresponding author. Department of Pulmonary Medicine University Hospital Essen- Ruhrlandklinik Tüschener Weg 40 45239 Essen Germany. E-mail address: dirk.westhoelter@uk-essen.de (D. Westhölter).

further research is needed to evaluate the effects of CFTR modulators on CF-associated inflammatory and immune processes. These effects on inflammatory processes are essential to our understanding of CF-associated inflammation and important to identify pwCF who might benefit from additional anti-inflammatory treatment strategies. Data from previous studies analysing the effects of mono and dual combination CFTR modulators on inflammatory markers in sputum and plasma from pwCF are inconsistent [5–8]. Recent studies have reported a normalisation of circulating neutrophil counts [9], improvements in monocyte/macrophage function [10,11], and reduction in airway and systemic proinflammatory cytokines [12–16] in pwCF receiving triple combination ETI. Overall, data on the immunological impact of CFTR modulator therapy are scarce and incomplete. Chemokines orchestrate immune responses and stimulate the migration of leukocytes [17], but have received limited attention in CF research. Chemokines are categorized into subfamilies, with CC chemokines being primarily responsible for the recruitment of monocytes/macrophages, eosinophils and specific lymphocyte subsets [18]. In bronchoalveolar lavage from children with CF compared with healthy controls, elevated levels of CC chemokines have been detected, even in the absence of apparent pulmonary infection [19]. Furthermore, CXC chemokines like IL-8 (CXCL8) attract neutrophils and are predominately associated with neutrophilic inflammation (19). Neutrophilic inflammation significantly contributes to the onset and progression of CF lung disease [18,20–22]. The study aimed to assess systemic levels of CC and CXC chemokine families during the initial 6 months of ETI therapy. We hypothesised that ETI therapy would have an effect on chemokine profiles in individuals with CF.

2. Methods

2.1. Study design and participants

This exploratory, observational study was started between June and November 2020 (T0, baseline). PwCF were invited to follow-up consultations at three (T1) and at six (T2) months after initiation of ETI therapy. Those presenting with acute respiratory infection or pulmonary exacerbation at baseline and/or follow-up were excluded from the final analysis. Routine clinical data were extracted from medical records at baseline and after three and six months of treatment with ETI. This included body mass index (BMI; kg/m²), forced expiratory volume in 1 s (FEV₁; L) and percent predicted (pp) FEV₁, C-reactive protein (CRP; mg/dL) and leukocytes (/nL).

2.2. Sample preparation and quantification of chemokines

Sample collection was supported by the West German Biobank. Whole blood was collected in VACUETTE® heparin tubes (Greiner Bio-One, Kremsmünster, Austria), underlaid with Biocoll® separating solution (Bio&Sell, Feucht, Germany) and centrifuged at 400 rcf for 25 min. Plasma samples for chemokine measurements were collected and stored at –80 °C until analysis. Concentrations (pg/mL) of twelve systemic chemokines (MIP-1α [CCL3], RANTES [CCL5], IP-10 [CXCL10], Eotaxin [CCL11], TARC [CCL17], MIP-1β [CCL4], MIG [CXCL9], MIP-3α [CCL20], ENA-78 [CXCL5], GROα [CXCL1], I-TAC [CXCL11] and interleukin [IL]-8 [CXCL8]) were quantified in thawed plasma samples using the bead-based immunoassay LEGENDplex® (LEGENDplex® Human proinflammatory chemokine panel 1, BioLegend, Koblenz, Germany) according to the manufacturer's instructions [23]. The selected panel contained

Table 1

Characteristics of people with cystic fibrosis (n = 51) receiving triple combination cystic fibrosis transmembrane conductance regulator modulator therapy, at baseline and at 3- and 6-month follow-up.

	Baseline	ETI		p-value (BL-3M)	p-value (BL-6M)	p-value (3M-6M)
		3 months	6 months			
Age, years, mean (SD)	32 (10.4)	–	–	–	–	–
Female sex, n (%)	24 (47)	–	–	–	–	–
ppFEV ₁ , mean (SD)	50.2 (21.6)	62.8 (22.8)	62.5 (23.2)	<0.001	<0.001	0.240
BMI, kg/m ² , mean (SD)	20.3 (2.5)	21.3 (2.6)	21.9 (2.6)	<0.001	<0.001	<0.001
Six-minute walk test, m, mean (SD)	521.8 (96.1)	594.8 (120.7)	606.2 (119.3)	<0.001	<0.001	0.327
Sweat chloride, mmol/L, mean (SD)	113.5 (73)	52.7 (19.2)	48.9 (17.6)	<0.001	<0.001	0.169
Leukocytes, /nL, median (Q1, Q3)	9.2 (7.1, 12.9)	6.7 (5.5, 8.2)	6.9 (5.7, 8.7)	<0.001	<0.001	0.082
CRP, mg/dL, median (Q1, Q3)	0.4 (0.4, 1.6)	0.4 (0.4, 0.4)	0.4 (0.4, 0.4)	<0.001	<0.001	0.875
<i>P. aeruginosa</i> infection, n (%)						
Chronic	22 (43)	–	–	–	–	–
Non chronic	29 (57)	–	–	–	–	–
CFTR genotype, n (%)						
Homozygous ΔF508	37 (72.5)	–	–	–	–	–
Heterozygous ΔF508	12 (23.5)	–	–	–	–	–
Other	2 (4)	–	–	–	–	–
Prior CFTR modulator therapy, n (%)						
Tezacaftor/ivacaftor	31 (60)	–	–	–	–	–
Lumacaftor/ivacaftor	3 (6)	–	–	–	–	–
None	17 (33)	–	–	–	–	–

Values are mean or median as indicated. 3 M, 3 months; 6 M, 6 months; BL, baseline; BMI, body mass index; CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; CRP, C-reactive protein; ETI, elexacaftor/tezacaftor/ivacaftor; ppFEV₁, percent predicted forced expiratory volume in 1 s; Q, quartile. Statistics: p-value describe changes between paired measurements.

both important members of the CC and the CXC chemokine subfamilies, which are associated with the attraction of lymphocytes, macrophages, eosinophils and neutrophils. In brief, plasma samples were incubated with capture bead/chemokine antibody conjugates followed by the addition of a biotinylated detection antibody cocktail and the streptavidin conjugated fluorochrome PE. Signal

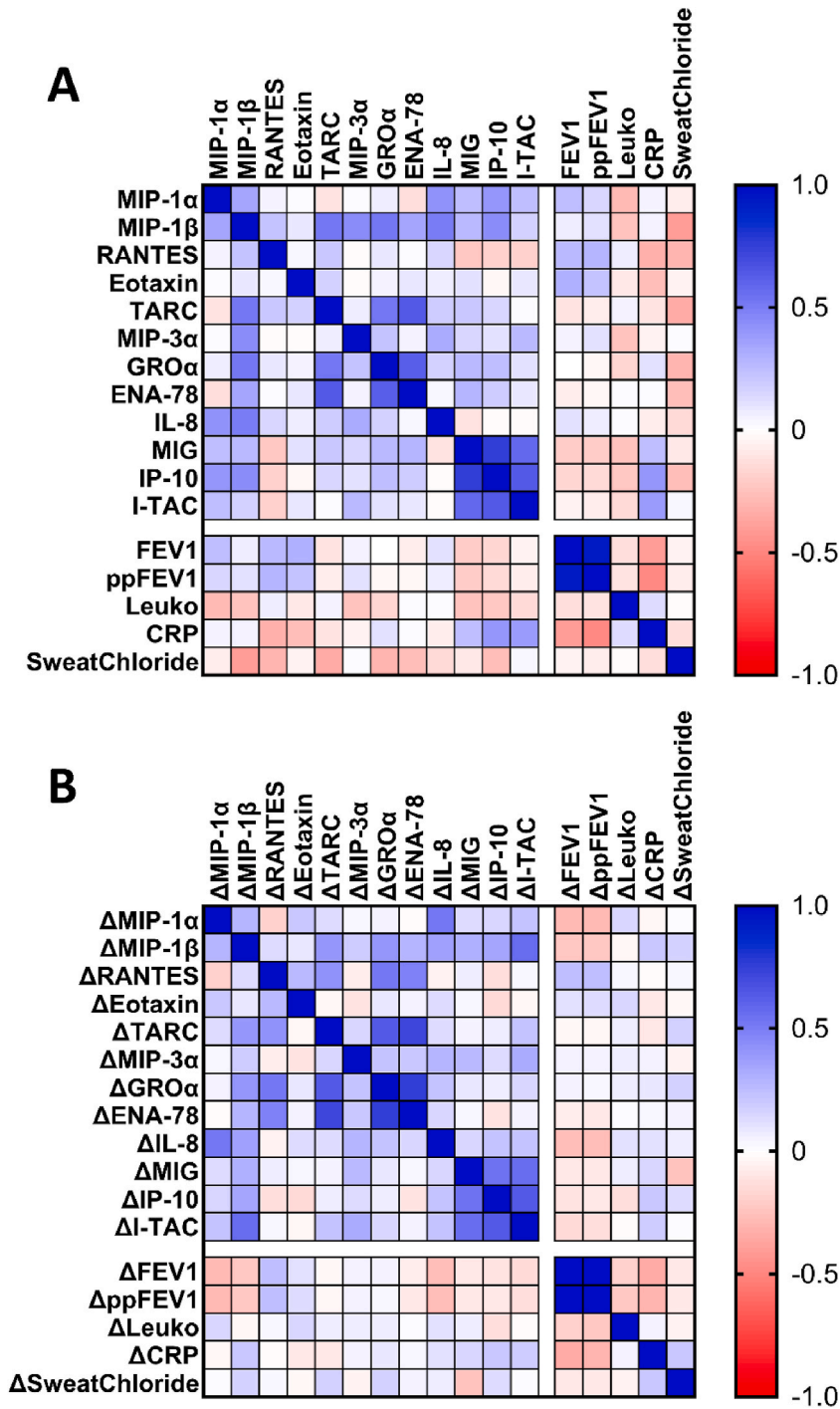


Fig. 1. Correlation analysis of plasma chemokine levels and clinical parameters, visualized by correlation matrices. Positive correlations are shown in blue, negative correlations in red. Colour intensity is proportional to the correlation coefficients. **A:** Correlation matrix of baseline chemokines and clinical parameters. **B:** Correlation matrix for change (%) in plasma chemokine levels and clinical parameters before and 6 months after receiving elexacaftor/tezacaftor/ivacaftor. BMI, body mass index; CRP, C-reactive protein; Leuko, leukocytes; ppFEV₁, percent predicted forced expiratory volume in 1 s. Statistics: Spearman correlation.

intensities were quantified by flow cytometry (CytoFLEX LX, Beckman Coulter). Standard curve dilution samples and test samples were analysed in duplicate (Table S1). Finally, chemokine concentrations were determined using the LEGENDplex 8.0 software package.

2.3. Statistical analyses

Statistical operations were performed using GraphPadPrism version 9 and/or R studio (version 2023.09.1+494). The normality of data was assessed through histogram analysis and the application of the Shapiro-Wilk test. Two-tailed (un)paired Student-t-test and Mann-Whitney *U* test were used to analyse normally distributed and nonparametric data, respectively. Nonparametric, paired data were analysed using the Wilcoxon signed-rank test. Pearson Chi-squared test was used to assess frequency distributions of categorical data. Correlations were analysed using the pairwise Spearman correlation test. Changes in chemokine levels were tested with a mixed-effects model with the participant as random effect. Correction for multiple testing was performed using the linear step-up procedure by Benjamini-Krieger-Yekutieli. Data are displayed as mean and standard deviation or median with first and third quartile, as indicated. Statistical significance was defined as $p < 0.05$. Chemokine results below the detection limit have been replaced with the detection limit divided by $\sqrt{2}$ in order to minimize censoring bias [24].

3. Results

3.1. Study population and clinical outcomes

A total of 51 pwCF were enrolled in this exploratory study. The study population included 24 females (47 %) and 27 males (53 %),

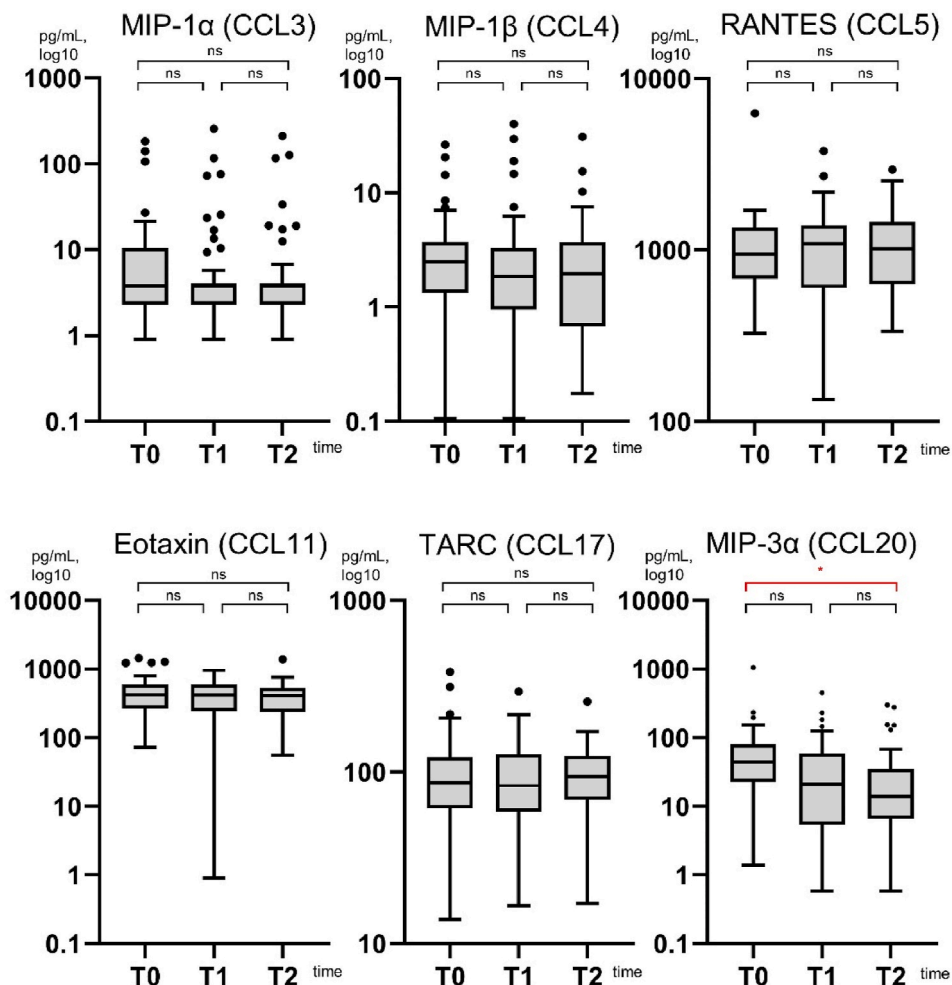


Fig. 2. Chemokine measurements (CC chemokines) in plasma from patients with cystic fibrosis receiving elixacaftor/tezacaftor/ivacaftor (in pg/mL, log₁₀ transformation). T0, baseline; T1, 3 months; T2, 6 months. Statistics: median, boxplots (Tukey style), mixed-effects model and correction for multiple comparisons: ns, not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

with a mean age of 32 years (Table 1). At baseline, 34 study participants (67 %) were being treated with dual combination CFTR modulator therapies tezacaftor/ivacaftor or lumacaftor/ivacaftor. Of these, 33/34 (97 %) were homozygous for the $\Delta F508$ mutation. Sweat chloride levels ($p = 0.843$) and percent predicted FEV₁ (ppFEV₁, $p = 0.147$) did not differ significantly between pwCF receiving or not receiving CFTR modulator therapy at baseline. Baseline CRP and leukocytes were initially above normal ranges in several pwCF. After 6 months' treatment with ETI, there was a significant increase in ppFEV₁ (+12.7 points, $p < 0.001$) and a significant reduction in sweat chloride levels (−53.6 %, $p < 0.001$). Average CRP and leukocyte levels were within the normal ranges at 3- and 6-month follow-up.

3.2. Systemic chemokine levels at baseline

Plasma samples for chemokine measurements were available from all included participants at baseline and follow-up. At baseline, measurable chemokine levels were detected in more than 75 % of the analysed plasma samples, except for MIP-1 α which had the lowest rate of detection (18/51, 35 %). Given that the baseline characteristics of our study population might have influenced levels of proinflammatory chemokines, we stratified baseline chemokine data for *Pseudomonas aeruginosa* infection status, CFTR genotype and prior CFTR modulator therapy (Table S2). Significantly lower eotaxin levels were measured in pwCF with prior CFTR modulator therapy (346.9 pg/mL vs. 543 pg/mL, $p = 0.015$). No significant differences in chemokine levels were observed between pwCF with and without chronic *P. aeruginosa* infection at baseline. Overall, correlations between chemokine levels and clinical parameters were weak. Levels of eotaxin and RANTES showed weak positive correlations with ppFEV₁ ($r = 0.224$, $p = 0.114$ and $r = 0.280$, $p = 0.046$; respectively; Fig. 1A). IP-10 and ITAC were associated with CRP ($r = 0.397$, $p = 0.005$ and $r = 0.379$, $p = 0.008$; respectively;

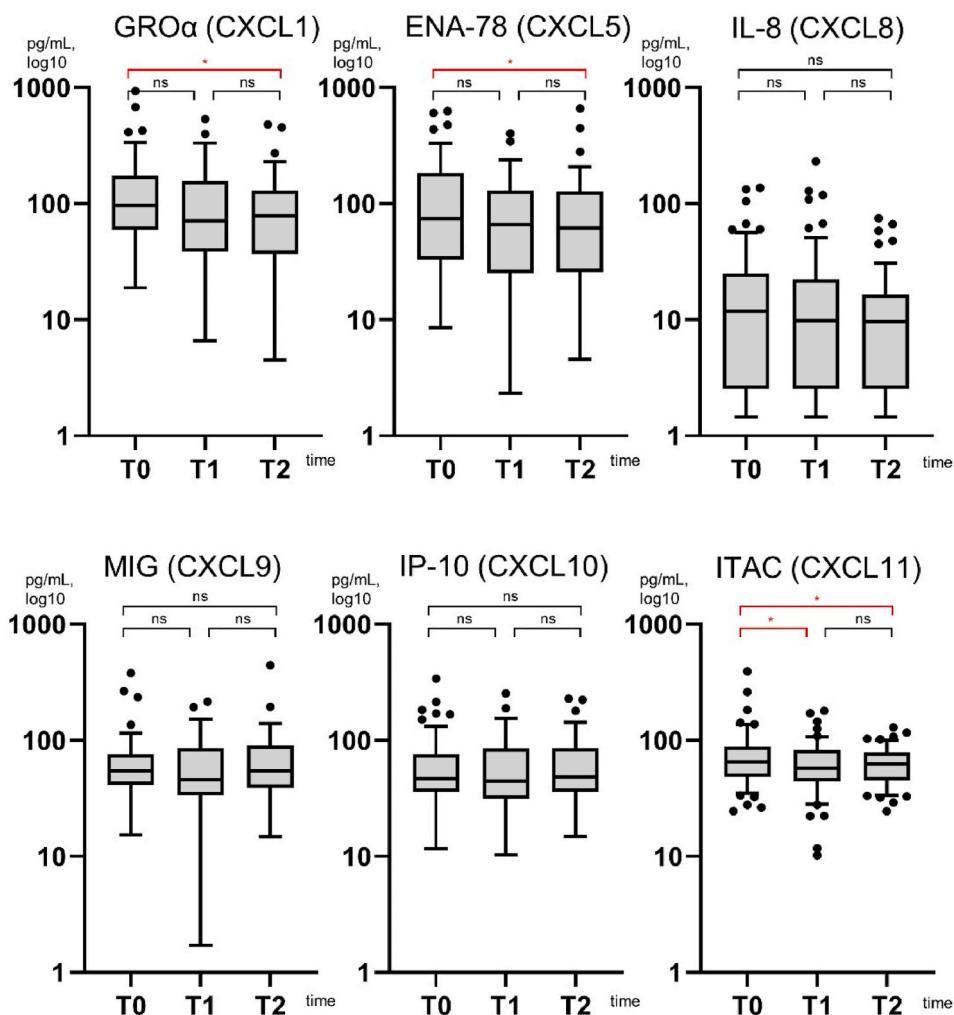


Fig. 3. Chemokine measurements (CXC chemokines) in plasma from patients with cystic fibrosis receiving elxacaftor/tezacaftor/ivacaftor (in pg/mL, log₁₀ transformation). T0, baseline; T1, 3 months; T2, 6 months. Statistics: median, boxplots (Tukey style), mixed-effects model and correction for multiple comparisons: ns, not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Fig. 1A), and there was a negative correlation between RANTES levels and CRP ($r = -0.320$, $p = 0.027$).

3.3. ETI alters systemic chemokine levels

There were significant reductions in plasma levels of MIP-3 α , ENA-78 and GRO α at 6 months and of I-TAC at 3 and 6 months after initiation of ETI (Figs. 2–3; Table S3). In addition, plasma levels of IL-8 were reduced from baseline at the 3- and 6-month follow-up, but changes did not reach statistical significance after correction for multiple testing (Figs. 2–3; Table S3). Plasma levels of all other analysed chemokines remained stable during the 6-month follow-up period (Figs. 2–3; Table S3). At baseline, eotaxin levels were lower in pwCF with prior CFTR modulator therapy, but were not reduced further after initiation of ETI in the overall study population ($p = 0.073$). However, in the subgroup of pwCF without prior CFTR modulator therapy ($n = 17$), plasma eotaxin levels were significantly decreased after 6 months of ETI therapy (median 543.0 pg/mL at baseline versus 459.75 pg/mL at 6 months, $p < 0.001$). Decreases in IL-8 levels were significantly associated with improved ppFEV₁ ($r = -0.277$, $p = 0.049$). In addition, there were associations between decreases in MIP-1 α and MIP-1 β levels and improved ppFEV₁ ($r = -0.289$, $p = 0.040$; $r = -0.228$, $p = 0.107$; respectively, Fig. 1B).

4. Discussion

ETI improves lung function and reduces pulmonary exacerbations in pwCF, but its potential to attenuate CF inflammation remains insufficiently investigated. We hypothesised that levels of chemokines, particularly those associated with neutrophilic inflammation, would decrease in response to ETI. The results of the current study showed that ETI affected three out of six analysed CXC chemokines, whereas only one of the analysed CC chemokines was found reduced. The role of chemokines in the pathogenesis of cystic fibrosis has not been studied in detail. However, the introduction of highly effective CFTR modulator therapy provides an opportunity to study the effects of CFTR restoration on chemokines and to identify chemokines with potential pathological expression in untreated CF disease.

CXC chemokines are powerful in both attracting and activating neutrophils [18]. Among CXC chemokines, GRO α and ENA-78 exhibit structural and functional homologies and were significantly downregulated in pwCF receiving ETI in the present study [17]. In addition, we observed moderate downregulation of the CXC chemokines I-TAC and IL-8. Decreased IL-8 levels were also linked to improvements in FEV₁. In particular, the role of IL-8 has been studied in CF. IL-8 significantly contributes to the dysregulated and hyperabundant attraction of neutrophils within CF lungs [22]. It has also been found to be elevated in both bronchoalveolar lavage fluid and blood from pwCF compared with healthy controls [12,25,26]. To our knowledge, IL-8 is the only chemokine that has been studied in pwCF receiving CFTR modulator therapy [6,12,14]. Consistent with our data, Sheikh et al. and Schaupp et al. reported reductions in plasma and sputum levels of IL-8 in pwCF receiving ETI [12,14]. However, the reduction in IL-8 observed was relatively modest in our study. Conflicting and supporting data exist concerning the role of GRO α in CF. Experimental restoration of CFTR also suppressed GRO α secretion in human CF epithelial models [27]. However, plasma levels of GRO α were not been elevated in untreated pwCF compared with healthy controls in a previous study [28]. There is limited evidence about the proinflammatory role of ENA-78 and I-TAC in cystic fibrosis from experimental studies [29,30]. However, to our knowledge ENA-78 and I-TAC concentrations have not previously been analysed in plasma or sputum from pwCF. Our findings indicate that not only IL-8, but also three other CXC chemokines are downregulated in response to ETI, suggesting mainly a decline of neutrophilic inflammation. Overall, these results are in line with previous studies reporting a normalisation of circulating neutrophil counts [9], reduced neutrophil-associated systemic cytokines [12,13] and reduced inflammatory airway markers such as neutrophil elastase in pwCF receiving ETI [14,15].

Among CC chemokines, plasma levels of MIP-3 α were reduced in pwCF receiving ETI in this study. MIP-3 α attracts memory T-lymphocytes, neutrophil subsets and dendritic cells [31,32]. In the context of CF and chronic lung diseases, MIP-3 α has been identified as a product of the human airway epithelium that is stimulated by proinflammatory cytokines such as IL-1 β and TNF- α [33]. Furthermore, MIP-3 α concentrations have been found to be significantly elevated in bronchoalveolar lavage fluid from pwCF compared with healthy controls [19,33]. Anti-inflammatory/antibacterial therapy with azithromycin was also associated with a decline of MIP-3 α in pwCF [34]. Other chemokines of the CC chemokine family (MIP-1 α , MIP-1 β) have also been found to be elevated in bronchoalveolar lavage fluid and blood from pwCF [19,35], but plasma levels of these chemokines were not significantly altered by treatment with ETI in the present study. However, we did find weak associations between reductions in MIP-1 α and MIP-1 β levels and improvements in lung function during ETI therapy. Eotaxin has been evaluated in eosinophilic airway diseases, but little is known about its function in CF. The reduction of MIP-3 α in response to ETI, alongside with stable levels of several other CC chemokines, implies an important role of MIP-3 α in CF-associated inflammation. In contrast to the other analysed CC chemokines, MIP-3 α also attracts neutrophils in addition to lymphocytes and other immune cells, in line with the earlier mentioned decline of neutrophilic inflammation observed in pwCF receiving ETI [31,32].

Despite their involvement in inflammatory processes, plasma levels of several CC and CXC chemokines remained stable and therefore appear to be unaffected by ETI therapy. Potentially, the expression of certain chemokines is directly associated with CFTR function or induced by CFTR-dependent mediators. Estell et al. have shown that RANTES expression depends on the presence of CFTR in plasma membranes of cultured human epithelial cells [36,37]. Furthermore, the CXC chemokines IP-10 and MIG are induced by interferon- γ , which in turn has been found reduced in airways of pwCF [38–40]. Thus, CFTR restoration by CFTR modulator therapy might even paradoxically enhance or at least prevent a reduction of some proinflammatory chemokines. Other chemokines may be equally distributed among pwCF with and without CFTR modulator therapy and healthy individuals.

This study of plasma chemokines has limitations. First, our study was limited by missing sputum/bronchoalveolar lavage fluid samples. The composition of chemokines in the airways might differ from that in plasma. Second, no samples from healthy controls were available. Hence, estimating the degree of chemokine reduction in relation to baseline levels from healthy controls was not

possible. Our baseline cohort was heterogeneous regarding infection status, genotype and prior CFTR modulator therapy, which may affect the study findings. Further, our cohort included pwCF with mild to severe lung disease. However, the average ppFEV1 was lower than in the approval study for ETI. In addition, pwCF homozygous for the dF508 mutation were overrepresented. Therefore, our results might not be directly applicable to pwCF who have milder disease and/or other disease-causing mutations. We analysed a medium-sized cohort of 51 pwCF during the first six months of ETI therapy. The results from our single-center study necessitate validation from future, long-term studies.

5. Conclusion

This is the first study to analyse CC and CXC chemokine levels in pwCF receiving ETI therapy. Overall, our study highlights differential effects of ETI therapy on systemic chemokines in pwCF. We observed significant downregulation of the CC chemokine MIP-3 α and of the CXC chemokines I-TAC, GRO α , and ENA-78 primarily suggesting a decline of neutrophilic inflammation. Levels of several other proinflammatory chemokines were unchanged or even increased during treatment with ETI. Targeting chemokines could potentially be a future therapeutic option for pwCF who display a persistent inflammatory phenotype.

Ethics declaration

This study was reviewed and approved by the ethics committee of the University of Duisburg-Essen, with the approval number: 17-7365-BO. All participants provided informed consent to participate in the study.

Funding

Supported by Deutsche Forschungsgemeinschaft (DFG, German Research Foundation): FU 356/12–2 (clinician scientist program, DW), INST 58,219/68-1 FUGG (major research instrumentation funding), TA 275/8–1 (CT), BE 7517/3–1 (CT).

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Dirk Westhölter: Writing - review & editing, Writing - original draft, Visualization, Validation, Investigation, Formal analysis, Data curation, Conceptualization. **Johannes Pipping:** Validation, Investigation, Data curation. **Jonas Raspe:** Validation, Investigation, Formal analysis, Data curation. **Mona Schmitz:** Investigation, Formal analysis. **Sivagurunathan Sutharsan:** Supervision, Data curation. **Svenja Straßburg:** Data curation. **Matthias Welsner:** Supervision, Data curation. **Christian Taube:** Validation, Supervision, Conceptualization. **Sebastian Reuter:** Writing - review & editing, Writing - original draft, Validation, Supervision, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

English language editing assistance was provided by Nicola Ryan, independent medical writer.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.heliyon.2023.e23428>.

References

- [1] A.M. Cantin, D. Hartl, M.W. Konstan, J.F. Chmiel, Inflammation in cystic fibrosis lung disease: pathogenesis and therapy, *J Cyst Fibros Off J Eur Cyst Fibros Soc* 14 (4) (2015) 419–430, <https://doi.org/10.1016/j.jcf.2015.03.003>.
- [2] E.A. Roesch, D.P. Nichols, J.F. Chmiel, Inflammation in cystic fibrosis: an update, *Pediatr. Pulmonol.* 53 (S3) (2018) S30–S50, <https://doi.org/10.1002/ppul.24129>.
- [3] H.G.M. Heijerman, E.F. McKone, D.G. Downey, et al., Efficacy and safety of the elxacaftor plus tezacaftor plus ivacaftor combination regimen in people with cystic fibrosis homozygous for the F508del mutation: a double-blind, randomised, phase 3 trial, *Lancet (London, England)* 394 (10212) (2019) 1940–1948, [https://doi.org/10.1016/S0140-6736\(19\)32597-8](https://doi.org/10.1016/S0140-6736(19)32597-8).

- [4] S. Sutharsan, E.F. McKone, D.G. Downey, et al., Efficacy and safety of elexacaftor plus tezacaftor plus ivacaftor versus tezacaftor plus ivacaftor in people with cystic fibrosis homozygous for F508del-CFTR: a 24-week, multicentre, randomised, double-blind, active-controlled, phase 3b trial, *Lancet Respir. Med.* 10 (3) (2022) 267–277, [https://doi.org/10.1016/S2213-2600\(21\)00454-9](https://doi.org/10.1016/S2213-2600(21)00454-9).
- [5] D. Westhölter, H. Beckert, S. Strabburg, et al., *Pseudomonas aeruginosa* infection, but not mono or dual-combination CFTR modulator therapy affects circulating regulatory T cells in an adult population with cystic fibrosis, *J Cyst Fibros Off J Eur Cyst Fibros Soc.* Published online (May 2021), <https://doi.org/10.1016/j.jcf.2021.05.001>.
- [6] K.B. Hisert, S.L. Heltshe, C. Pope, et al., Restoring cystic fibrosis transmembrane conductance regulator function reduces airway bacteria and inflammation in people with cystic fibrosis and chronic lung infections, *Am. J. Respir. Crit. Care Med.* 195 (12) (2017) 1617–1628, <https://doi.org/10.1164/rccm.201609-1954OC>.
- [7] P. McNally, D. Butler, Y.V. Karpievitch, et al., Ivacaftor and airway inflammation in preschool children with cystic fibrosis, *Am. J. Respir. Crit. Care Med.* 204 (5) (2021) 605–608, <https://doi.org/10.1164/rccm.202012-4332LE>.
- [8] H.H. Jarosz-Griffiths, T. Scambler, C.H. Wong, et al., Different CFTR modulator combinations downregulate inflammation differently in cystic fibrosis, in: S. Rath, J.W.M. van der Meer, R. Coll, S. Bekkering (Eds.), *Elife* 9 (2020), e54556, <https://doi.org/10.7554/eLife.54556>.
- [9] T. Dhote, C. Martin, L. Regard, et al., Normalization of circulating neutrophil counts after 12 months of elexacaftor-tezacaftor-ivacaftor in patients with advanced cystic fibrosis, *Eur Respir J.* Published online January 1 (2022), 2202096, <https://doi.org/10.1183/13993003.02096-2022>.
- [10] S. Zhang, C.L. Shrestha, F. Robledo-Avila, et al., Cystic fibrosis macrophage function and clinical outcomes after elexacaftor/tezacaftor/ivacaftor, *Eur Respir J.* Published online January 1 (2022), 2102861, <https://doi.org/10.1183/13993003.02861-2021>.
- [11] L. Cavinato, F.R. Luly, V. Pastore, et al., Ellexacaftor/tezacaftor/ivacaftor corrects monocyte microbicidal deficiency in cystic fibrosis, *Eur. Respir. J.* 61 (4) (2023), <https://doi.org/10.1183/13993003.00725-2022>.
- [12] S. Sheikh, R.D. Britt Jr., N.A. Ryan-Wenger, et al., Impact of elexacaftor-tezacaftor-ivacaftor on bacterial colonization and inflammatory responses in cystic fibrosis, *Pediatr. Pulmonol.* (2022), <https://doi.org/10.1002/ppul.26261>.
- [13] D. Westhölter, J. Raspe, H. Uebner, et al., Regulatory T cell enhancement in adults with cystic fibrosis receiving Ellexacaftor/Tezacaftor/Ivacaftor therapy, *Front. Immunol.* 14 (2023), 1107437, <https://doi.org/10.3389/fimmu.2023.1107437>.
- [14] L. Schaupp, A. Addante, M. Völler, et al., Longitudinal effects of elexacaftor/tezacaftor/ivacaftor on sputum viscoelastic properties, airway infection and inflammation in patients with cystic fibrosis, *Eur. Respir. J.* 62 (2) (2023), <https://doi.org/10.1183/13993003.02153-2022>.
- [15] M. Casey, C. Gabillard-Lefort, O.F. McElvaney, et al., Effect of elexacaftor/tezacaftor/ivacaftor on airway and systemic inflammation in cystic fibrosis, *Thorax* 78 (8) (2023), <https://doi.org/10.1136/thorax-2022-219943>, 835 LP-839.
- [16] A. Lepissier, A.S. Bonnel, N. Wizla, et al., Moving the dial on airway inflammation in response to Trikafta® in adolescents with cystic fibrosis, *Am. J. Respir. Crit. Care Med.* 4 (2023), <https://doi.org/10.1164/rccm.202210-1938LE>. Published online January.
- [17] M. Vareille, E. Kieninger, M.R. Edwards, N. Regamey, The airway epithelium: soldier in the fight against respiratory viruses, *Clin. Microbiol. Rev.* 24 (1) (2011) 210–229, <https://doi.org/10.1128/CMR.00014-10>.
- [18] D.T. Groves, Y. Jiang, Chemokines, a family of chemotactic cytokines, *Crit. Rev. Oral Biol. Med.* 6 (2) (1995) 109–118, <https://doi.org/10.1177/10454411950060020101>.
- [19] S. Brennan, P.D. Sly, C.L. Gangell, et al., Alveolar macrophages and CC chemokines are increased in children with cystic fibrosis, *Eur. Respir. J.* 34 (3) (2009), <https://doi.org/10.1183/09031936.00178508>, 655 LP-661.
- [20] S.D. Sagel, B.D. Wagner, M.M. Anthony, P. Emmett, E.T. Zemanick, Sputum biomarkers of inflammation and lung function decline in children with cystic fibrosis, *Am. J. Respir. Crit. Care Med.* 186 (9) (2012) 857–865, <https://doi.org/10.1164/rccm.201203-0507OC>.
- [21] J. Laval, A. Ralhan, D. Hartl, Neutrophils in cystic fibrosis, *Biol. Chem.* 397 (6) (2016) 485–496, <https://doi.org/10.1515/hsz-2015-0271>.
- [22] K. Jundi, C.M. Greene, Transcription of interleukin-8: how altered regulation can affect cystic fibrosis lung disease, *Biomolecules* 5 (3) (2015) 1386–1398, <https://doi.org/10.3390/biom5031386>.
- [23] J.S. Lehmann, A. Zhao, B. Sun, W. Jiang, S. Ji, Multiplex cytokine profiling of stimulated mouse splenocytes using a cytometric bead-based immunoassay platform, *J. Vis. Exp.* 129 (2017), 56440, <https://doi.org/10.3791/56440>.
- [24] D.J. Handelsman, L.P. Ly, An accurate substitution method to minimize left censoring bias in serum steroid measurements, *Endocrinology* 160 (10) (2019) 2395–2400, <https://doi.org/10.1210/en.2019-00340>.
- [25] T.P. Dean, Y. Dai, J.K. Shute, M.K. Church, J.O. Warner, Interleukin-8 concentrations are elevated in bronchoalveolar lavage, sputum, and sera of children with cystic fibrosis, *Pediatr. Res.* 34 (2) (1993) 159–161, <https://doi.org/10.1203/00006450-199308000-00010>.
- [26] T.L. Bonfield, J.R. Panuska, M.W. Konstan, et al., Inflammatory cytokines in cystic fibrosis lungs, *Am. J. Respir. Crit. Care Med.* 152 (6 Pt 1) (1995) 2111–2118, <https://doi.org/10.1164/ajrccm.152.6.8520783>.
- [27] G. Veit, F. Bossard, J. Goepff, et al., Proinflammatory cytokine secretion is suppressed by TMEM16A or CFTR channel activity in human cystic fibrosis bronchial epithelia, *Mol. Biol. Cell* 23 (1) (2012) 4188–4202, <https://doi.org/10.1091/mbc.e12-06-0424>.
- [28] H.A. Wyatt, A.P. Sampson, I.M. Balfour-Lynn, J.F. Price, Production of the potent neutrophil chemokine, growth-related protein alpha (GROalpha), is not elevated in cystic fibrosis children, *Respir. Med.* 94 (2) (2000) 106–111, <https://doi.org/10.1053/rmed.1999.0725>.
- [29] C.R. Bozic, N.P. Gerard, C. Gerard, Receptor binding specificity and pulmonary gene expression of the neutrophil-activating peptide ENA-78, *Am. J. Respir. Cell Mol. Biol.* 14 (3) (1996) 302–308, <https://doi.org/10.1165/ajrcmb.14.3.8845182>.
- [30] M. Adib-Conquy, T. Pedron, A.F. Petit-Bertron, et al., Neutrophils in cystic fibrosis display a distinct gene expression pattern, *Mol. Med.* 14 (1) (2008) 36–44, <https://doi.org/10.2119/2007-00081.Adib-Conquy>.
- [31] K. Hirota, H. Yoshitomi, M. Hashimoto, et al., Preferential recruitment of CCR6-expressing Th17 cells to inflamed joints via CCL20 in rheumatoid arthritis and its animal model, *J. Exp. Med.* 204 (12) (2007) 2803–2812, <https://doi.org/10.1084/jem.20071397>.
- [32] P. Scapini, C. Laudanna, C. Pinardi, et al., Neutrophils produce biologically active macrophage inflammatory protein-3alpha (MIP-3alpha)/CCL20 and MIP-3beta/CCL19, *Eur. J. Immunol.* 31 (7) (2001) 1981–1988, [https://doi.org/10.1002/1521-4141\(200107\)31:7<1981::aid-immu1981>3.0.co;2-x](https://doi.org/10.1002/1521-4141(200107)31:7<1981::aid-immu1981>3.0.co;2-x).
- [33] T.D. Starner, C.K. Barker, H.P. Jia, Y. Kang, P.B.J. McCray, CCL20 is an inducible product of human airway epithelia with innate immune properties, *Am. J. Respir. Cell Mol. Biol.* 29 (5) (2003) 627–633, <https://doi.org/10.1165/rcmb.2002-0272OC>.
- [34] K. Dong, A. Singh, R.T. Ng, et al., Proteomic profiling to identify blood biomarkers predictive of response to azithromycin in children and adolescents with cystic fibrosis, *Chest* 156 (4) (2019) 667–673, <https://doi.org/10.1016/j.chest.2019.05.017>.
- [35] S. Rao, A.K.A. Wright, W. Montiero, L. Ziegler-Heitbrock, J. Grigg, Monocyte chemottractant chemokines in cystic fibrosis, *J Cyst Fibros Off J Eur Cyst Fibros Soc* 8 (2) (2009) 97–103, <https://doi.org/10.1016/j.jcf.2008.09.009>.
- [36] K. Estell, G. Braunstein, T. Tucker, K. Varga, J.F. Collawn, L.M. Schwiebert, Plasma membrane CFTR regulates RANTES expression via its C-terminal PDZ-interacting motif, *Mol. Cell Biol.* 23 (2) (2003) 594–606, <https://doi.org/10.1128/MCB.23.2.594-606.2003>.
- [37] L.M. Schwiebert, K. Estell, S.M. Probst, Chemokine expression in CF epithelia: implications for the role of CFTR in RANTES expression, *Am J Physiol Physiol* 276 (3) (1999) C700–C710, <https://doi.org/10.1152/ajpcell.1999.276.3.C700>.
- [38] H. Takada, Y. Takahata, A. Nomura, S. Ohga, Y. Mizuno, T. Hara, Increased serum levels of interferon-gamma-inducible protein 10 and monokine induced by gamma interferon in patients with haemophagocytic lymphohistiocytosis, *Clin. Exp. Immunol.* 133 (3) (2003) 448–453, <https://doi.org/10.1046/j.1365-2249.2003.02237.x>.
- [39] F. Liao, R.L. Rabin, J.R. Yannelli, L.G. Koniaris, P. Vanguri, J.M. Farber, Human Mig chemokine: biochemical and functional characterization, *J. Exp. Med.* 182 (5) (1995) 1301–1314, <https://doi.org/10.1084/jem.182.5.1301>.
- [40] D. Hartl, M. Griese, M. Kappler, et al., Pulmonary T(H)2 response in *Pseudomonas aeruginosa*-infected patients with cystic fibrosis, *J. Allergy Clin. Immunol.* 117 (1) (2006) 204–211, <https://doi.org/10.1016/j.jaci.2005.09.023>.