

# An inflammatory Signature of Glucose Impairment in Cystic Fibrosis

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**Objective and Design:** Cystic fibrosis-related diabetes (CFRD) is a severe complication associated with increased morbidity and mortality in cystic fibrosis (CF) patients. Extensive inflammatory state in CF leads to pancreas damage and insulin resistance with consequent altered glucose tolerance and CFRD development. The aim of the present study was to identify circulating levels of inflammatory markers specifically associated with impaired glucose tolerance (IGT) and overt CFRD in a sample of young adults with CF.

**Materials and Methods:** Sixty-four CF outpatients, without evident active pulmonary exacerbation, infectious and autoimmune diseases, were enrolled in the study and the levels of 45 inflammatory serum mediators were measured through x magnetic bead panel multiplex technology.

**Results:** Serum levels of PDGF-AA, CCL20/MIP3 $\alpha$ , IFN $\alpha$ , CCL11/eotaxin, CXCL1/GRO $\alpha$ , GM-CSF, B7H1/PDL1, IL13, IL7, VEGF, and TGF $\alpha$  were all significantly ( $p < 0.05$ ) elevated in patients according to glycemic status and directly correlated with glycated hemoglobin and C-reactive protein levels.

**Conclusion:** Our findings suggest that increased levels of specific circulating inflammatory mediators are directly associated with impaired glucose tolerance in CF patients, thus, potentially implicating them in CFRD pathogenesis and warranting larger longitudinal studies to validate their monitoring as predictor of CFRD onset.

**Keywords:** cystic fibrosis-related diabetes, cytokines, growth factors, immune mediators, impaired glucose tolerance, inflammation

## Introduction

Cystic fibrosis (CF) is a genetic disease affecting more than 30,000 people in the US and 48,000 in Europe, of which 5531 are in Italy.<sup>1</sup> CF is caused by over 2000 different single mutations of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene,<sup>2</sup> which results in disruption of normal ion transport across the epithelial cell surface and leads to sticky mucus accumulation in the respiratory, reproductive, and digestive tracts including the pancreas.<sup>3</sup>

Persistent inflammation in CF patients promotes tissue injury, thus significantly contributing to the diffuse organ damage and failure seen in these patients.<sup>4</sup>

Lungs and pancreas are among the most affected organs and deterioration of their function highly affects CF morbidity and mortality.<sup>4</sup> In the pancreas, endocrine dysfunction may occur in parallel with exocrine insufficiency (pancreatic insufficiency, PI) leading to cystic fibrosis-related diabetes (CFRD), a unique form of diabetes, associated with worse clinical outcomes including faster deterioration of lung function evidenced by a decline of the FEV<sub>1%</sub> (forced expiratory volume in the first second).<sup>5</sup>

CFRD incidence increases with age. About 20% of adolescents present with overt diabetes and, by their forties, about half of the patients having PI will develop CFRD.<sup>5</sup> Interestingly, 10–15% of the CF patients maintain preserved exocrine pancreatic function (pancreatic sufficiency, PS) and most likely will not develop CFRD. Early diagnosis and treatment of diabetes improves overall prognosis and an annual screening with an oral glucose tolerance test (OGTT) is recommended starting at least by the age of 10.

The occurrence of PI and CFRD has been attributed to diverse causes including fibrosis and fatty infiltration in the pancreatic tissue followed by “beta cells strangulation”.<sup>6</sup> However, morphological study of pancreatic tissues from neonates, infants and very young children with CF found that in children with CF under four years of age, the relative beta-cell areas and percentage of beta cells were reduced to about 50% of controls, but this reduction was not associated with the severity of exocrine damage and fibrosis. Proliferating insulin-positive cells were scarce or absent in young CF pancreata whose islets were infiltrated with dispersed inflammatory cells suggesting smaller-sized beta-cell population present at birth and impaired beta-cell growth in CF children.<sup>7</sup> The more recent work by Hart et al<sup>8</sup> confirmed that PI and CFRD are characterized by beta cell loss and intra-islet inflammation in the setting of a complex pleiotropic disease and not by any intrinsic islet dysfunction from CFTR mutation. However, the incidence of diabetes and its severity does not even correlate with the residual beta cells. Glucose tolerance balance fails over time and patients may move in and out through stages of indeterminate glycemia, impaired glucose tolerance (IGT) and CFRD. The occurrence of pulmonary infections and the use of glucocorticoid therapy can also contribute to precipitate the IGT trend toward diabetes.<sup>5</sup>

Sterile inflammation in combination with recurrent infections contributes to loss of insulin production through beta-cell destruction, also by altering insulin sensitivity, making CFRD a unique form of diabetes.<sup>9</sup> While there is a clear association between pathogen-induced and sterile inflammation with IGT and development of CFRD, little is known on the inflammatory mediators directly involved in this process and whether their levels could be associated with the progression of glucose intolerance and diabetes onset in CF patients.

Here, we have performed a high throughput screening assessing circulating levels of different inflammatory mediators (cytokines, chemokines and growth factors) in a sample of CF young adults to investigate whether their expression profiles correlated with IGT and CFRD.

## Materials and Methods

### CF Patients

Sixty-four CF outpatients were enrolled at the CF Center of the Bambino Gesù Children’s Hospital of Rome, Italy, from July to September 2019. The CF center evaluates patients along childhood-adulthood transition for all disease comorbidities, including diabetes. The study was performed in accordance with the revised Declaration of Helsinki of 2000 and with the ethical standards of the Bambino Gesù Children’s Hospital Review Board that approved the study (approval no. 201802P004364). All the participants provided a written informed consent.

CF diagnosis was based on two positive sweat tests ( $\geq 60$  mmol/L) and/or genotyping. Pancreatic functionality was established on clinical observations (ie the presence of steatorrhea and malabsorption), clinical tests (determination of fecal elastase-1 and chymotrypsin and/or lipase) and pancreatic enzyme replacement treatment.

Inclusion criteria were age older than 18 and absence of evident active pulmonary exacerbations. Exclusion criteria were occurrence of infection (ie human Immunodeficiency Virus, Viral Hepatitis) and autoimmune diseases.

As routine investigation, patients underwent a standard OGTT for the diagnosis of IGT and diabetes; induced sputum or deep oropharyngeal swab to detect microbial infections and colonization and blood collection; spirometry with forced expiratory volume in the first second (FEV<sub>1%</sub>) quantification. In addition to routine exams, fasting sera from study participants were collected and stored at  $-20^{\circ}\text{C}$  in Eppendorf tubes until analysis. Patient’s history, genotype and follow-up data, including biochemistry and microbiology were retrieved from the electronic health records.

CFRD was diagnosed as 2 h blood glucose at T120’ of OGTT  $>200$  mg/dL ( $>11.1$  mmol/L), fasting blood glucose  $>126$  mg/dL ( $>7$  mmol/L) on two or more occasions, glycated hemoglobin (HbA1c)  $\geq 48$  mmol/mol; indeterminate hyperglycemia as random blood glucose  $>200$  mg/dL on two or more occasions with symptoms; and IGT as 2 h blood glucose at T120’ of OGTT  $>140$  and  $<199$  mg/dL during periods of stable health conditions.

Patients were grouped as follows: pancreatic sufficient patients with normal glucose tolerance (PS-NGT), exocrine pancreatic insufficient patients with normal glucose tolerance (PI-NGT) and exocrine pancreatic insufficient CF patients with impaired glucose tolerance or diabetes (PI-IGT/CFRD).

No patient had exocrine sufficiency and IGT/CFRD in our sample, being these patients very rare in the CF population.

## Luminex Assay

The levels of 45 mediators involved in inflammation and immune response (listed in [Table S1](#)) were measured through xMAP multiplex technology by a customized human magnetic luminex assay, (#FCSTM18-45, R&D Systems, Minneapolis, MN, USA). The analysis was carried out according to the manufacturer's instructions and quantification performed with a Bio-Plex® 200 System (Bio-Rad Laboratories, Hercules, CA, USA) equipped with magnetic workstation and Bio-Plex Manager Software version 6.1. All samples were run in duplicate (average % CV <10% for all analytes).

## Statistical Analysis

Data are expressed as mean and standard deviation or number and percentage, unless differently stated. Comparisons among groups was done by ANOVA. Log mean was adopted to stabilize the variance. A nonparametric Dunn test was applied and the obtained *p*-values adjusted using the Benjamini–Hochberg false discovery rate (FDR) method. The F-tests were significant when *p*<0.05. Spearman rank correlation was used to investigate relationships among HbA1c as estimate of average glucose control, CRP as estimate of systemic inflammation, c-peptide as index of residual beta cell function, FEV<sub>1</sub> and the investigated serum mediators that resulted differently expressed among groups. Then, stepwise forward and backward regression models were run to identify molecules predicting values of HbA1c, CRP, c-peptide and FEV<sub>1</sub> values; ordinal regression model to identify predictors of PS and PI phenotypes; an exploratory factor analysis (with principal components approach) to identify inflammatory patterns associated with the abovementioned outcomes. Age, sex, BMI, use of drug were covariates in the analyses.

Analyses were performed with Stata IC/15.1 (StataCorp LLC, College Station, TX, USA). A *p*-value <0.05 was considered statistically significant.

## Results

### Study Sample

Out of the 64 CF patients enrolled in the study, four were excluded: ([Supplementary Figure 1](#); one patient with type 1 diabetes (T1D), one patient with a paresthesia of unidentified nature, one with leukocytosis and one with mononucleosis).

The final sample population included 60 patients, (40 females, 66.7%). Median age was 27.9±8.3 years. We stratified patients in three subgroups, according to pancreatic functionality and glycemic status as it follows: pancreatic sufficient patients with normoglycemia (PS-NGT, N=10), pancreatic insufficient patients with normoglycaemia (PI-NGT, N=26) and pancreatic insufficient patients with glucose impairment or overt diabetes (PI-IGT/CFRD, N=24). One patient had isolated IFG and two patients has indeterminate glycemia, all three were included in the PI-IGT/CFRD group. In this latter group, three patients presented with IGT and 18 had CFRD ([Supplementary Figure 1](#)). All the patients were Caucasian.

[Table 1](#) summarizes patients' anthropometric and metabolic characteristics. There were no significant differences in body weight, height and BMI among groups. The CF genotype distribution displayed the absence of the homozygosis for the F508del in the PS-NGT group and the highest percentage of patients carrying the allele F508del (either homozygosis or heterozygosis) in the PI-IGT/CFRD group. In [Table S2](#) the genotype of each patient is reported.

None of the patients of the PS-NGT group were using modulator drugs while five patients in the PI-NGT group and seven in the PI-IGT/CFRD group were following a medical treatment with modulator drugs ([Table 1](#)). As expected, glucose parameters were significantly different among groups with the PI-IGT/CFRD group displaying higher levels of glycemia at the different OGTT time-points and HbA1c. This group showed the highest value of CRP (*p*<0.05).

In contrast no difference was found in leukocytes count among the three groups. Finally, lowest mean FEV<sub>1</sub>% was found in the PI-IGT/CFRD group that also comprised the highest percentage of patients displaying severe pulmonary

**Table 1** Demographic and Metabolic Characteristics of the CF Patients Recruited in the Study

	PS-NGT (N=10; 16.7%)	PI (N=50; 83.3%)	
		PI-NGT (N=26; 52%)	PI-IGT/CFRD (N=24; 48%)
<b>Anthropometrics</b>			
Body weight (kg)	59.7±9.3	57.9±10.9	58.6±9.5
Height (cm)	164.1±6.3	162.7±9.6	162.8±8.5
BMI (kg/m <sup>2</sup> )	22.2±3.4	21.6±2.0	22.3±2.8
<b>Genotype, N (%)</b>			
F508del/F508del	–	5 (19.2%)	9 (37.5%)
F508del/others	4 (40%)	12 (46.2%)	12 (50%)
Others	6 (60%)	9 (34.6%)	3 (12.5%)
<b>CFTR Modulator drugs use, N</b>			
	–	5	7
<b>Metabolic parameters</b>			
Fasting glucose (mg/dL)	85.0±10.4	81.2±11.2	104.5±51.6 <sup>*b</sup>
Glucose 60' (mg/dl)	138.2±38.1	152.0±31.7	202.4±13.05 <sup>**a</sup>
Glucose 120' (mg/dL)	94±19.3	92.8±27.5	134.6±52.1 <sup>*b</sup>
Fasting insulin (mU/L)	5.63±2.9	5.66±2.7	4.87±3.3
Insulin 120' (mU/L)	22.8±5.6	30.2±23.8	31.9±29.3
Fasting C-peptide (ng/mL)	1.12±0.3	1.13±0.5	0.98±0.3
C-peptide 120' (ng/mL)	4.43±1.2	5.08±2.5	6.43±1.6
HbA1c (mmol/mol)	30.8±0.8	34.7±4.4	41.8±9.6 <sup>*b</sup>
HOMA-IR (dimensionless)	1.26±0.7	1.12±0.5	1.06±0.7
HOMA-B (%)	80.7±44.7	148.8±151.9	52.7±52.7
CRP (mg/dL)	0.38±0.4	0.59±0.6	1.68±2.5 <sup>*b</sup>
Leukocytes (10 <sup>6</sup> /μL)	6.34±2.4	7.64±2.5	6.8±2.9
<b>Respiratory parameters</b>			
FEV <sub>1</sub> %	79.5±26.7	78.6±24.6	72.5±27.8
FEV <sub>1</sub> % <40	1 (10%)	2 (7.6%)	3 (12.5%)
FEV <sub>1</sub> % 40–70	3 (30%)	7 (26.9%)	8 (33.3%)
FEV <sub>1</sub> % >70	6 (60%)	17 (65.5%)	13 (54.2%)

**Notes:** Comparison between PI-NGT vs PS-NGT; <sup>a</sup>PI-IGT/CFRD vs PS-NGT and <sup>b</sup>PI-NGT vs PI-IGT/CFRD. Statistical significance of difference tested by t-test for quantitative variables and by Fisher's test for categorical variables. Level of significance: \**p*<0.05 \*\**p*<0.01.

**Abbreviations:** PS-NGT, normoglycemic exocrine pancreatic sufficiency; PI, exocrine pancreatic insufficiency; PI-NGT, exocrine pancreatic insufficiency with normoglycemia; PI-IGT/CFRD, exocrine pancreatic insufficiency with impaired glucose tolerance or diabetes; BMI, body mass index; CFTR, cystic fibrosis transmembrane conductance regulator gene; CRP, C-reactive protein; HbA1c, hemoglobin A1c; HOMA-IR and HOMA B, homeostatic assessment of insulin resistance and beta cell function; FEV<sub>1</sub>, forced expiratory volume in the first second.

insufficiency as defined by a  $FEV_{1\%} < 40$ . The majority of the patients showing preserved lung function ( $FEV_{1\%} > 70\%$ ) were found in the PS-NGT and PI-NGT groups.

## Luminex Assay Data Analysis and IGT/CFRD at Baseline

Serum levels of 45 molecules involved in inflammation and immune responses have been assessed using a customized human magnetic luminex assay. The list of the factors included in the assay and mean, minimum, and maximum values for each analyte (cytokines, chemokines, interferons and growth factors) in the three groups are reported in [Tables S3–S5](#). Serum levels of IL17A, IFN $\beta$  and basic FGF (FGFb) were undetectable in most of the patients whereas IL17A/IL25 and IL33 were undetectable only in the PS-NGT patients, therefore, these molecules were excluded from the analysis.

[Table 2](#) reports molecules whose geometric means were significantly different among the three groups.

[Table 3](#) reports correlation analysis among HbA1c as an estimate of glucose homeostasis, CRP as a marker of systemic inflammation, and C-peptide as an estimate of residual beta cell mass,  $FEV_1$  and the investigated serum mediators.

**Table 2** Molecules Whose Mean Values Were Significantly Different in the Three Groups

Serum (pg/mL)	PS-NGT	PI-NGT	PI-IGT/CFRD
<b>PDGF-AA</b>	9945.9 (9200.4–14,404.2)	15,057.4* (12,574.1–20,816.9)	13,932.9* (11,324.6–20,288.4)
<b>CCL20/MIP3<math>\alpha</math></b>	14.4 (5.4–32.0)	54.6** (26.6–88.1)	51.7** (28.3–89.3)
<b>IFN<math>\alpha</math></b>	0.14 (0.06–0.3)	1.4* (0.6–1.7)	0.8* (0.5–1.7)
<b>CCL11/eotaxin</b>	215.7 (155.1–259.4)	458.4** (244.4–815.3)	416.3** (312.5–593.6)
<b>CXCL1/GRO<math>\alpha</math></b>	192 (135.6–349.0)	433.2** (327.2–612.7)	392.3* (278.0–588.9)
<b>GMCSF</b>	2.4 (1.7–4.7)	8.5* (4.3–12.6)	9.9* (5.8–20.0)
<b>B7H1/PDL1</b>	36.2 (31.8–62.0)	63.1 (42.8–87.2)	75.6* (49.8–104.8)
<b>IL13</b>	28.2 (21.0–29.1)	39.5* (28.1–55.0)	36.5* (27.6–48.8)
<b>IL7</b>	5.9 (3.6–9.0)	7.3 (5.6–13.2)	9.8* (7.6–13.8)
<b>VEGF</b>	301.5 (193.8–519.0)	471.5* (308.8–721.6)	525.8* (441.1–701.0)
<b>TGF<math>\alpha</math></b>	12.3 (9.6–14.0)	21.2* (12.3–33.8)	26.4* (12.1–63.5)

**Notes:** Data are expressed as geometric mean (calculated using values  $\neq 0$ ) and (first Q – third Q). Comparison between PS-NGT vs PI-NGT and PS-NGT vs PI-IGT/CFRD. Statistical significance of difference tested by t-test for quantitative variables and by Fisher's test for categorical variables. Level of significance: \* $p < 0.05$  \*\* $p < 0.01$ .

**Abbreviations:** PS-NGT, exocrine pancreatic sufficiency with normoglycemia; PI-NGT, exocrine pancreatic insufficiency with normoglycemia; PI-IGT/CFRD, exocrine pancreatic insufficiency with impaired glucose tolerance or diabetes; PDGF-AA, platelet-derived growth factor AA; VEGF, vascular endothelial growth factor; CCL20/MIP3 $\alpha$ , C-C motif chemokine ligand 20; CCL11/eotaxin, C-C motif chemokine 11; CXCL1/GRO $\alpha$ , growth-regulated alpha protein; B7H1/PDL1, programmed death ligand 1; GMCSF, granulocyte macrophage growth factor.

**Table 3** Correlation Analysis (Spearman's Rho) Among FEV<sub>1</sub>%, HbA1c, CRP, C-Peptide and the Investigated Serum Mediators

	FEV <sub>1</sub>	HbA1c	CRP	C-Pep	PDGF AA	CCL20/ MIP3α	IFNα	CCL11/ Eotaxin	CXCL1/ GROα	GM CSF	B7H1/ PD-L1	IL13	IL7	VEGF	TGFα
FEV <sub>1</sub>	I														
HbA1c	-0.37*	I													
CRP	0.66***	0.38*	I												
C-pep	-0.27	0.54*	0.35	I											
PDGF AA	-0.11	0.21	0.21	0.14	I										
CCL20/MIP3α	0.002	0.27	0.21	0.11	0.18	I									
IFNα	-0.15	0.15	0.57**	-0.30	0.58**	0.48*	I								
CCL11/eotaxin	0.07	0.19	0.096	-0.22	-0.02	0.36**	0.27	I							
CXCL1/GROα	-0.15	0.23	0.23	-0.23	0.40**	0.36**	0.58**	0.39**	I						
GMCSF	-0.003	0.23	0.23	-0.10	0.18	0.54**	0.66**	0.36	0.32	I					
B7H1/PDL1	-0.19	0.29	0.26	0.28	0.38**	0.11	0.45*	0.11	0.3009*	0.38	I				
IL13	-0.002	0.36*	0.24	0.11	0.22	0.40**	0.40	0.66***	0.51***	0.25	0.22	I			
IL7	-0.19	0.35*	0.26*	0.06	0.28*	0.47***	0.66**	0.31*	0.33**	0.507**	0.22	0.55	I		
VEGF	-0.09	0.33*	0.28*	0.05	0.48***	0.41***	0.504*	0.24	0.52***	0.30	0.45***	0.39**	0.36**	I	
TGFα	-0.19	0.13	0.27	0.17	0.36*	0.503***	0.502*	0.15	0.604***	0.49*	0.31*	0.21	0.15	0.46**	I

Notes: \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ,   rho  $< 0.30$  "poor",    $0.30 \leq \text{rho} < 0.60$  "fair",   rho  $\geq 0.80$  "very strong",   rho = 1 "perfect",   n.s.

**Abbreviations:** FEV<sub>1</sub>, forced expiratory volume in the first second; CRP, C-reactive protein; PDGF-AA, platelet-derived growth factor AA; CCL20/MIP3α, C-C motif chemokine ligand 20; CCL11/eotaxin, C-C motif chemokine 11; CXCL1/GROα, growth-regulated alpha protein; B7H1/PDL1, programmed death ligand 1; GMCSF, granulocyte macrophage growth factor; VEGF, vascular endothelial growth factor.



Regression analyses did not find any molecule from [Tables 2](#) and [3](#) predicting phenotypes of PS, PI and IGT/CFRD as an ordinal outcome and HbA1c, C-peptide, PCR and FEV<sub>1</sub> values as continuous outcomes.

## Discussion

Glucose intolerance and overt diabetes in CF patients are clinically significant conditions associated with functional decline and increased mortality.<sup>10</sup> Despite strict monitoring (OGTT testing) required by age 10, progression to overt diabetes in CF patients is still not well predictable or defined. As inflammation, both sterile and pathogen-related, has been identified as a major player in CFRD onset and progression,<sup>11,12</sup> a correlation of inflammatory mediators with different levels of glucose tolerance could provide new diagnostic tools and open the path to innovative immunomodulatory therapeutic interventions. The results of our high throughput study identified 11 molecules of which serum levels were significantly augmented in the PI-IGT/CFRD group compared to those with normal endocrine and exocrine pancreatic function (PS-NGT) ([Table 2](#)).

These upregulated factors included: CXCL1/GRO $\alpha$ , VEGF, CCL11/eotaxin, PDGF-AA, IFN $\alpha$ , CCL20/MIP3 $\alpha$ , and B7H1/PDL1, IL7, IL13, TGF $\alpha$  and GM-CSF. The PI-IGT/CFRD group displayed higher CRP values that directly correlated with poor metabolic glucose control (HbA1c) and reduced pulmonary function. Increased VEGF, IL7, IL13 expression levels were correlated with HbA1c ([Table 3](#)).

CXCL1/GRO $\alpha$  is a chemokine known to play a key role in recruitment and activation of neutrophils. In addition, this chemokine is involved in vascular complications and inflammation of pancreatic islets in T2D<sup>13,14</sup> and in airways inflammation in non-CF subjects.<sup>15</sup> VEGF is known for its ability to promote migration and proliferation of endothelial cells, monocytes/macrophages chemotaxis and vasodilatation through the release of nitric oxide.<sup>16</sup> However, increased VEGF levels have been reported in patients affected by prediabetes and T2D<sup>17</sup> and are involved in the pathogenesis of diabetic microvascular complications.<sup>18</sup> Most importantly, VEGF is also pivotal for lung maturation and surfactant production<sup>19</sup> and its levels are increased in pulmonary diseases.<sup>20</sup> As to PDL1, a B7 family co-stimulatory protein expressed on antigen presenting cells, its engagement within its receptor inhibits T cell proliferation, induces apoptosis and promotes differentiation of CD4<sup>+</sup> T cells into Foxp3<sup>+</sup> regulatory T cells. PD1/PDL1 system is a critical immune checkpoint<sup>21</sup> that plays a pivotal role in the pathogenesis of type 1 diabetes. In the attempt at counteracting the host immune response, the beta cells of patients with T1D overexpress the molecule on their surface.<sup>22</sup> However, elevated concentrations of PD-1 have been also implicated in the pathogenesis and progression of IPF.<sup>23</sup> While GRO $\alpha$  and VEGF might be involved in pancreatic damage, PDL1 elevation is likely to have a counter-regulatory role and could represent a protective marker.

Increased levels of VEGF, GRO $\alpha$  and PDL1, in the PI-IGT/CFRD group are likely associated with the pro-fibrotic lung environment. The fibrocystic lung represents a peculiar microenvironment characterized by severe oxygen depletion. Hypoxia/inflammation could promote activation of transcription factors, such as the signal transducers and activator of transcription 3 (STAT3) that is a known transcriptional inducer of VEGF, GRO $\alpha$  and PDL1.<sup>24–26</sup> Interestingly, we also found higher levels of IL7, a mediator supporting the proliferation of multiple immune cells and activating a signaling cascade, which also includes the JAK-STAT pathway, further enhancing the signaling mechanisms involved in pulmonary and endocrine pancreas inflammation and impairment.

CCL11/eotaxin, CCL20/MIP3 $\alpha$ , PDGF-AA, IFN $\alpha$  were also upregulated in CF patients with altered glucose metabolism and are likely to mediate both lung and pancreas damage owing to their pro-fibrogenic/pro-angiogenic activity and ability to trigger the immune system as found in various lung fibrotic diseases and diabetes conditions (i.e CCL11/eotaxin).<sup>27–30</sup>

Our every attempt at identifying patterns of molecules predicting phenotypes of interest that considered clinical features such as age, comorbidities, exacerbation rates has failed. An explanation for this might be the small sample size. [Supplemental Figure 2](#) resumes signaling mechanisms potentially involved in pulmonary and endocrine pancreas inflammation and impairment.

Indeed, we are aware of caveats of our study including the sample size that, however, is reasonably large when considering that CF is a rare condition and with different sex severity among groups,<sup>31</sup> and the cross-sectional design that does not allow us to draw any causative relationship between inflammatory phenotypes and occurrence of 2conclusion, our high throughput analysis has revealed the existence of a potential signature of inflammation associated with altered glucose homeostasis in CF. Whether these markers can be diagnostic/prognostic of the CFRD risk, hence complementing the OGTT screening, must be further investigated in longitudinal studies of larger cohort. Currently, glucose testing remains an easy, fast, and inexpensive method to track occurrence of altered glucose homeostasis, but patients may

fluctuate for a long time between normal and altered glucose metabolism before a diagnosis of IGT or CFRD is made. Identification of early biomarkers of CFRD development in an already compromised patient population might be informative to establish early therapies that, targeting definite inflammatory paths, ie the PD-1/PD-L1, reduce the burden of CFRD-related complications, including more rapid lung deterioration and increased mortality, with a more personalized medicine approach.

## Abbreviations

B7H1/PDL1, programmed death ligand 1; CCL2/MCP1, C-C motif chemokine ligand 2; CCL3/MIP1 $\alpha$ , macrophage inflammatory protein 1 alpha; CCL4/MIP1b, macrophage inflammatory protein 1-beta; CCL5/RANTES, C-C motif chemokine ligand 5; CCL11/eotaxin, C-C motif chemokine 11; CCL19/MIP3b, macrophage inflammatory protein 3 beta; CCL20/MIP3 $\alpha$ , C-C motif chemokine ligand 20; CD40L, CD40 ligand; CFRD, cystic fibrosis-related diabetes; CFTR, cystic fibrosis transmembrane conductance regulator gene; CRP, C-reactive protein; CXCL1/GRO $\alpha$ , growth-regulated alpha protein; CXCL2/GRO $\beta$ , C-X-C motif chemokine ligand 2; CXCL10/IP10, C-X-C motif chemokine ligand 10; CX3CL1/fractalkine, C-X3-C motif chemokine ligand 1; FEV<sub>1</sub>, forced expiratory volume in the first second; FGF basic, fibroblast growth factor basic; Flt3 L, fms-related tyrosine kinase 3 ligand; GCSF, granulocyte colony stimulating factor; GM-CSF, granulocyte macrophage growth factor; IGT, impaired glucose tolerance; Il1ra/IL1F3, interleukin 1 receptor antagonist; PDGF-AA, platelet-derived growth factor AA; PDGF-BB, platelet-derived growth factor BB; PI, exocrine pancreatic insufficiency; PI-IGT/CFRD, exocrine pancreatic insufficiency with impaired glucose tolerance or diabetes; PI-NGT, exocrine pancreatic insufficiency with normoglycemia; PS-NGT, normoglycemic exocrine pancreatic sufficiency; STAT3, signal transducers and activators of transcription 3; TRAIL, TNF-related apoptosis-inducing ligand; VEGF, vascular endothelial growth factor.

## Data Sharing Statement

Clinical data are available from the last author upon reasonable request in accordance with the General Data Protection Regulation (EU) 2016/679.

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## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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## Disclosure

The authors report no conflicts of interest in this work.

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