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Dry eye disease and morphological changes in the anterior chamber in people with cystic fibrosis.

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Abstract:	Background: Cystic fibrosis (CF) is caused by variants in a gene that encodes a protein essential for water and ion transport in the epithelial cells of exocrine organs. Given the possible relationship of this protein and conjunctival and corneal epithelium, the aim of this study was to evaluate ophthalmologic alterations in people with CF. Methods. Forty-five people with CF underwent pulmonary evaluation including inflammatory score (IS). These people along with 98 sex-matched controls underwent ophthalmologic evaluation including dry eye disease (DED) testing, corneal topography using Pentacam™ and macular and peripapillary retinal nerve fiber layer (pRNFL) thickness with optical coherence tomography (OCT). Results. The CF group presented a higher percentage of pathologic tear break-up time (T-BUT) (55.6% vs 25%, p=0.001) and Schirmer's test 1 (40% versus 19.4%, p=0.009) than the control group. In the CF group, an inverse correlation was observed between T-BUT and IS (r=- 0.373, p=0.012), as well as T-BUT and peripheral eosinophilia (r=-0.338; p=0.023). People with CF presented lower values of central corneal thickness (p= 0.009), thinnest point (p =0.006), anterior chamber volume (p= 0.034), and anterior chamber angle (p= 0.011) than the control group and lower pRNLF thickness in the superior temporal sector (p=0.002). Conclusions. Our findings indicate a higher prevalence of dry eye disease (DED) among people with CF compared to controls. The severity of the condition increases with higher systemic inflammation. Additionally, CF may affect the anterior segment of the eye, leading to a reduction in the nerve fiber layer and early signs of glaucoma.

Málaga, 7th July 2024

Dr. Patrick Flume

Journal of Cystic Fibrosis

Editor in chief

Dear editor:

We submit the manuscript titled: “Dry eye disease and morphological changes in the anterior chamber in patients with cystic fibrosis” for consideration to be published as an original article in the Journal of Cystic Fibrosis.

The systemic manifestations caused by the mutation of the CFTR protein are numerous, but to the best of our knowledge, no powerful studies have been published regarding ocular involment in patients with cystic fibrosis. The development of modulator therapy has modified the mortality and morbidity in this severe disease, and efforts to improve the quality of life of these patients are increasing.

Our study shows that CF patients have dry eye disease, anatomical alterations in the anterior segment of the eye and optic nerve damage, which could be related to abnormal expression of the CFTR protein.

Therefore, protocols of ophthalmic care should be applied in the routine clinical practice of CF patients.

The collaboration of different professionals (pulmonologist/ophthalmologists) in a multidisciplinary, structured and protocolized way, as well as the dissemination of the knowledge studied, will improve the quality of care of people with CF and reduce variability in clinical practice.

This work is not currently under consideration in any other journal and has not been previously published. All authors have contributed significantly and have read and approved the manuscript and grant an exclusive license to the journal upon acceptance.

We greatly appreciate your consideration for our manuscript and hope that you find it suitable for publication in your prestigious journal.

Sincerely,

Dra. Casilda Olveira

Type of article: Original article.

Title: Dry eye disease and morphological changes in the anterior chamber in people with cystic fibrosis

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Declarations of Competing Interest

The authors declare not to have any conflicts of interest that may be considered to influence directly or indirectly the content of the manuscript.

All authors have contributed significantly and have read and approved the manuscript and grant an exclusive license to the journal upon acceptance.

Credit authorship contribution statement

Patricia Gutierrez: conceptualization, methodology, data curation, formal analysis, writing-original draft, writing-review & editing**. Laura Jiménez:** data curation, writing-review & editing. **Jessica Martínez:** data curation, writing-review & editing. **Carmen Alba:** writing-review & editing. **María Victoria Girón:** data curation, writing-review & editing**. Gabriel Olveira:** data curation, writing-review & editing**. Pedro Ruiz-Esteban:** formal analysis, writing-original draft, writing-review & editing**. Casilda Olveira:** conceptualization, data curation, formal analysis, writing-original draft, writing-review & editing, Supervision.

Aknowledgements

We would like to express our heartfelt gratitude to all the patients who participated in this study. We also thank Maria Repice for linguistic assistance in the preparation of the text.

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The authors acknowledge the use of AI-based tools (ChatGPT) for grammatical review of this manuscript.

TITTLE:

Dry eye disease and morphological changes in the anterior chamber in people with cystic fibrosis.

ABSTRACT:

Background: Cystic fibrosis (CF) is caused by variants in a gene that encodes a protein essential for water and ion transport in the epithelial cells of exocrine organs. Given the possible relationship of this protein and conjunctival and corneal epithelium, the aim of this study was to evaluate ophthalmologic alterations in people with CF.

Methods. Forty-five people with CF underwent pulmonary evaluation including inflammatory score (IS). These people along with 98 sex-matched controls underwent ophthalmologic evaluation including dry eye disease (DED) testing, corneal topography using Pentacam™ and macular and peripapillary retinal nerve fiber layer (pRNFL) thickness with optical coherence tomography (OCT).

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Conclusions. Our findings indicate a higher prevalence of dry eye disease (DED) among people with CF compared to controls. The severity of the condition increases with higher systemic inflammation. Additionally, CF may affect the anterior segment of the eye, leading to a reduction in the nerve fiber layer and early signs of glaucoma.

Keywords:

Cystic Fibrosis

CFTR

Dry Eye Disease

Anterior chamber anatomy

Glaucoma

1. INTRODUCTION

Cystic fibrosis (CF) is a disease caused by variants in a gene that encodes the cystic fibrosis transmembrane conductance regulator (CFTR) protein [1]. CFTR plays an essential role in the transport of water, chloride ions and sodium reabsorption in the epithelial cells of organs with exocrine function. In people with CF, CFTR variants lead to altered mucociliary clearance, resulting in the development of chronic bronchial infections that generate an exaggerated inflammatory response [2].

Dry eye disease (DED) is a multifactorial condition characterized by ocular symptoms in which hyperosmolarity of the tear film and ocular surface inflammation play an etiological role [3]. Although the possible ocular involvement of cystic fibrosis is not well defined it is assumed that the disease affects all secretory epithelia including the eye. In this case, the associated ocular surface disease is primarily DED [4], [5] [6].

While the etiology of ocular surface changes in people with CF remains unclear, it is known that CFTR is expressed in the epithelial and endothelial cells of the conjunctiva, cornea, and lacrimal glands, contributing to the maintenance of tear film homeostasis. Several studies suggest that its activation could be considered an effective therapeutic target in DED [7] [8].
In recent years, cell counts in peripheral blood samples and the relationship between them have been considered indicators of systemic and ocular inflammatory conditions, and the possibility that DED may be a systemic disease has been discussed [9] .
It is well known that many factors are involved in the appearance of changes in the anatomical structure of the cornea, ocular surface and anterior segment of the eye, among them genetic, pharmacological and hormonal [10] [11]. In this sense, primary angle closure encompasses a group of disorders characterized by contact or closeness between the iris and the trabecular meshwork, which blocks proper drainage of the aqueous humor, generating an increase in intraocular pressure and reducing blood flow to the optic disc that can cause damage to the axons of the retinal ganglion cells.
This condition tends to develop in eyes with shallow anterior chambers, anteriorly positioned or displaced lenses, and angle narrowing [12] [13]. It is rare in young white people and its association with systemic diseases has not been described. There are no previous studies that associate it with CF.
No association between CF and glaucoma has been described to the best of our knowledge. However, recent studies have revealed a focal decrease in peripapillary nerve fiber layer (pRNFL) thickness in this group of people, similar to that seen in early stages of glaucoma [4]. While the exact pathophysiologic mechanism involved in glaucomatous damage in people with respiratory disease is not known, hypoxia has been proposed as a possible process involved [14].

Therefore, the aim of our study was to assess the presence of DED in people with CF and its association with biomarkers of systemic inflammation, as well as to compare morphologic changes in the anterior segment, ganglion cell complex (GCC) and pRNFL thicknesses in people with CF respect to a control group.

2. METHODS

2.1. Study design and population

This was a cross-sectional observational clinical study approved by the Provincial Ethics and Clinical Research Committee of Malaga (Protocol code: 1760-N-21 and date of approval: 28 October 2021). Only those subjects who agreed to participate in the study by informed consent were included. People recruitment, data collection and sample collection were conducted in accordance with the ethical principles of the most recent version of the Declaration of Helsinki and the standards of good clinical practice [15].

People with CF were recruited sequentially and prospectively from the CF unit when they attended their annual visit, in which pulmonology and analytical studies were performed, between the months of October 2021 and April 2022. The digitalized clinical history of each person was available, prepared prospectively from the time of diagnosis following European and national diagnostic and treatment regulations [16] [17]. A group of healthy controls (2:1) was selected for each CF person, matched for age and sex. Exclusion criteria were: age younger than 18 years or older than 60 years, absence of clinical stability in the previous 3 months (hospital admissions, respiratory exacerbations (requiring intravenous antibiotic therapy or weight change >3%), refractive error >5 diopters (D), ocular disease or previous corneal surgery and use of topical or systemic treatment contributing to the development of DED. People with CF and healthy controls were referred to the ophthalmology service of the Regional University Hospital of Malaga for a complete ophthalmologic evaluation.

2.2. Pulmonary evaluation: clinical, functional, radiological and analytical variables.

The pulmonary evaluation included demographic variables (age, sex, marital status). Anthropometric variables such as height (Holtain Limited, Crymych, UK) and weight (SECA, Hamburg, Germany) were measured. Body mass index was calculated. Severity criteria including age of onset of CF symptoms and number of respiratory exacerbations in the previous year were recorded in the database. We categorized the exacerbations based on their severity into two groups: mild to moderate, when patients only required oral antibiotics; and severe, when hospitalization or intravenous antibiotics were necessary [18]

On the day of the visit, simple and forced spirometry (Jaeger Oxycon Pro, Erich Jaeger, Germany) was performed, and values were expressed as a percentage of the predicted value in a reference population [19]. The 6-minute walk test (6MWT) was also performed [20].
To assess structural damage, computed tomography was requested and assessed with the modified Bhalla [21] and modified Reiff [22] scales.

Blood samples were collected for leukocyte count and acute phase reactants (alpha 1 antitrypsin, C-reactive protein, ferritin and fibrinogen). The samples were analyzed in the laboratory of the Regional University Hospital of Málaga following the protocols of the Andalusian Health System.

2.3. Ophthalmologic evaluation. Anatomical and functional variables.

Best corrected visual acuity (logMAR scale, Optotype ETDRS CHART2, ZeissMeditec AG, Germany), slit lamp evaluation of the anterior and posterior poles (SL 220, Zeiss, Spain), refraction (autorefractometer - keratometer, VISU REF, Zeiss, Spain) and intraocular pressure with an applanation tonometer with slit lamp support (Zeiss, Spain) were determined.
The DED evaluation included the ocular surface disease index score (OSDI questionnaire, Allergan, USA) [23], Schirmer's test type 1 (ST1) with sterile methylcellulose strips (Alcon, Spain), tear break-up time (T-BUT) and fluorescein staining with sterile 1% sodium fluorescein strips (Alcon, Spain) to evaluate the ocular surface following the Oxford Grading Scale [24]. Ocular Surface Disease Index (OSDI) uses a scale from 0 to 100 to assess the severity of symptoms related to ocular surface disease. A score on the OSDI above 13 is considered indicative of pathological ocular surface disease, meaning the patient has dry eye symptoms that should be treated.
The Oxford Grading Scale is used to assess the severity of ocular surface damage by evaluating staining on the cornea and conjunctiva. After applying fluorescein dye, the ocular surface is examined under cobalt blue light. The Oxford scale consists of 5 panels of reference images (graded from 0 to 5), each representing increasing severity of punctate epithelial erosions or

staining on the ocular surface. The scale ranges from Grade 0 (no staining) to Grade 5 (severe staining), with higher grades indicating more extensive surface damage. T-BUT values below 10 seconds and ST1 below 10 mm were considered pathological.
It also included the InflammaDry™ tear test, which detects MMP-9 (Matrix Metalloproteinase 9), an inflammatory marker that is consistently elevated in the tears of patients with DED [25]

The anterior segment of the eye (pachymetry, anterior chamber volume, angle) was assessed in all patients by Pentacam™ corneal tomography (Oculus Systems, Wetzlar, Germany) [26] For pachymetry, the central corneal thickness (CCT) was measured, with normal values ranging between 520 and 550 µm**.** Clinically, values below 500 µm are considered thin corneas [27] The anterior chamber volume (ACV), measured in cubic millimeters, has normal values ranging between 110 mm^3 and 160 mm^3. A value below 100-102 mm^3 is a key indicator of an occludable angle, as a reduced volume is associated with a higher probability of angle closure**.** The anterior chamber depth (ACD) was measured from the corneal endothelium to the lens. Normal ACD values range between 2.7 mm and 3.3 mm, with values bellow 2.5 mm indicating a higher risk of angle closure.

Spectral domain optical coherence tomography (SD-OCT Cirrus, model 500) was performed. Two acquisition protocols were performed: macular cube 512x125 and optic disc cube 200 x 200. Two analysis protocols were applied: pRNFL and GCC.

2.4. Statistical analysis

Data were analysed using the IBM SPSS 26.0 statistical program (IBM Corp., Armonk, NY, USA) for Windows. The results were expressed as percentages for qualitative data and as mean ± standard deviation or medians (interquartile ranges) for quantitative data according to the distribution of the data. The distribution of the data was analysed using the Kolmogorov-Smirnov test. Categorical variables were analysed by the chi-square test or Fisher's exact test and quantitative data by Student's *t*-test or the U-Mann-Whitney test, as appropriate.
A total inflammatory score was developed based on the statistical principles of Bonifaccio et al. for the INFLA-score in the Moli-sani study [28]. For this purpose, four biomarkers of systemic inflammation (eosinophils, neutrophils, C-reactive protein, and alpha 1-antitrypsin) were selected. Each variable was divided into deciles. The highest deciles received a score of 1 to 4, while the lowest deciles received a score of -4 to -1. The fifth and sixth deciles received a score of 0. The score obtained for each biomarker was summed in the total inflammatory score. Correlations between T-BUT and inflammatory score were performed by Pearson correlation coefficient. We performed multivariate linear regression analysis of factors associated with T-BUT. Significance was set at $p<0.05$.

3. RESULTS

This study included 45 people with CF and 98 healthy controls of the same age (33.4±9.7 years versus 32.5±9.6 years; p=0.573) and sex (male, 55.6% versus 50.0%; p=0.537).

Figure 1 shows the flow chart of the people studied.

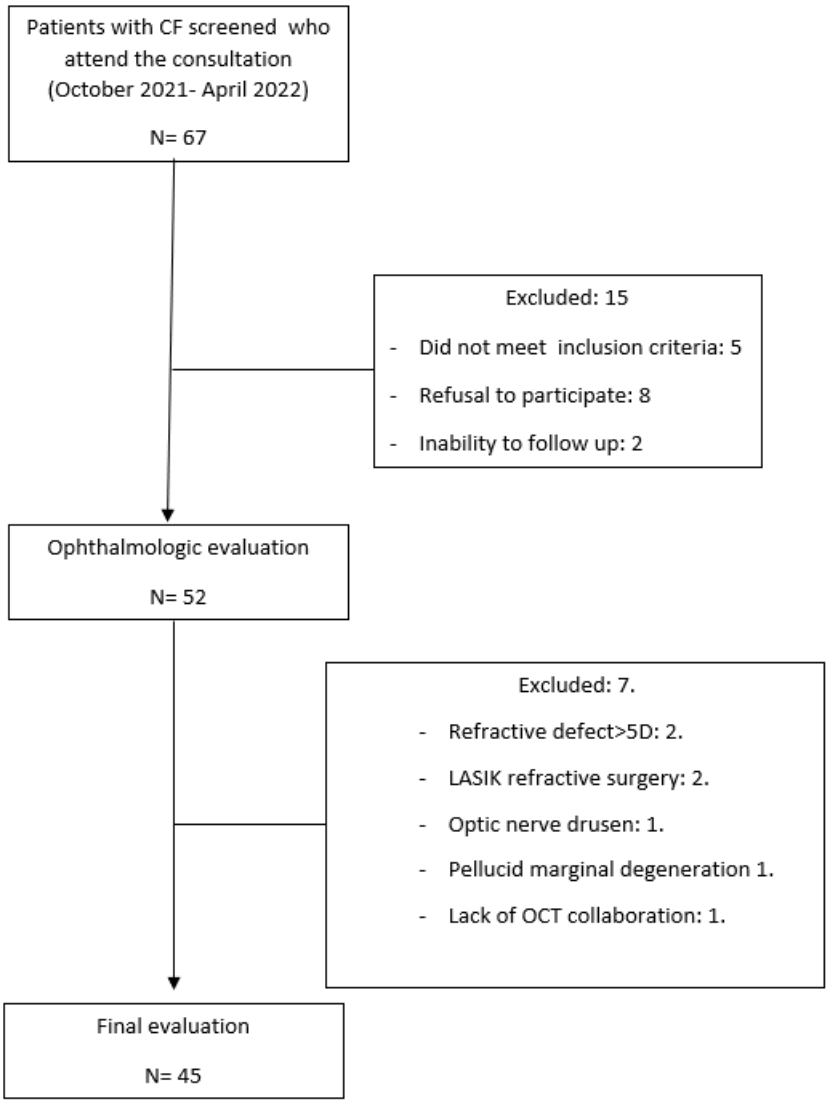


Figure 1. Flowchart of patients studied.
Abbreviations: CF: Cystic Fibrosis; LASIK: Laser-Assisted in Situ Keratomileusis; OCT: Optical Coherence Tomography.

Table 1 shows the clinical and demographic characteristics of the group of people with CF.

			CF (n=45)
Age (years)			33.4±9.7
Gender, M, n (%)			25 (55.6)
Height (cm)			166.0±9.8
Weight (kg)			66.2±19.1
BMI (kg/m^2)			23.7±5.8
Age of symptom onset (years)			5.8±11.1
Total exacerbations in the previous year			1.3±1.7
Severe	CF (n=9)	Mild/moderate	CF (n=22)
1 exacerbation	n=4	1 exacerbation	n=11
2 exacerbations	n=3	2 exacerbations	n=5
3 or more exacerbations	n=2	3 or more exacerbations	n=6
CFTR variant, n (%):			
• Other/Other			15 (33.3)
○ Minimal function variant			11 (24.4)
○ Residual function variant			3 (6.6)
○ Gating variant			1 (2.2)
• Heterozygous delta F 508			19 (42.2)
○ Residual function variant			5 (11.1)
• Homozygous delta F 508			11 (24.4)
Functional data:			
• FEV1 (%)			56.7±23.4
• FVC (%)			66.5±19.4
• FEV1/FVC (%)			81.6±15.5
• Oxygen Saturation at the beginning of the walking test (%)			96.9±2.0
• Oxygen Saturation at the end of the walking test (%)			94.0±6.2
Radiological data:			
• Reiff scale			5.3±5.0
• Bhalla scale			14.8±6.2
Analytical data:			
• White blood cell count			7548±2438
• Neutrophils (%)			55.4±12.0
• Eosinophils (%)			3.7±2.8
• Ferritin (ng/mL)			48.2±32.6
• Fibrinogen (mg/dL)			327.2±90.0
• CRP (mg/L)			9.5±14.1
• Alpha-1 antitrypsin (mg/dL)			138.3±27.8
Treatment:			
• Elexacaftor/tezacaftor/ivacaftor .			
○ n (%)			24 (53.3)
○ Treatment time (months)			4.44±6.3
• Tezacaftor/Ivacaftor.			
○ n (%)			9(20)
○ Treatment time (months)			4.17± 9.71
• Ivacaftor.			
○ n(%)			1(2,2)
○ Treatment time (months)			99
• Azithromycin, n (%)			25 (55.6)

Abbreviations: BMI: Body mass index; CFTR: Cystic fibrosis transmembrane conductance regulator; FEV1: Forced expiratory volume in 1 second; FVC: Forced vital capacity; CRP: C-reactive protein.

Normal values for FEV1 and FVC are typically 80-120% of the predicted value based on age, gender, height, and ethnicity. Normal FEV1/FVC ratio is typically ≥70%**.** Normal ranges for

laboratory parameters: White blood cell count: 4000-11000; Neutrophils (%): 40-70%, Eosinophils (%)1-4%; Ferritin(ng/mL: 20-500; CRP (mg/L): < 10 mg/L; Alpha-1 antitrypsin (mg/dL): 100-300.
The quantitative data is presented as mean ± standard deviation

Table 1. Clinical and demographic data of people with cystic fibrosis.

3.1. Cornea, ocular surface and anterior segment of the eye in CF vs control

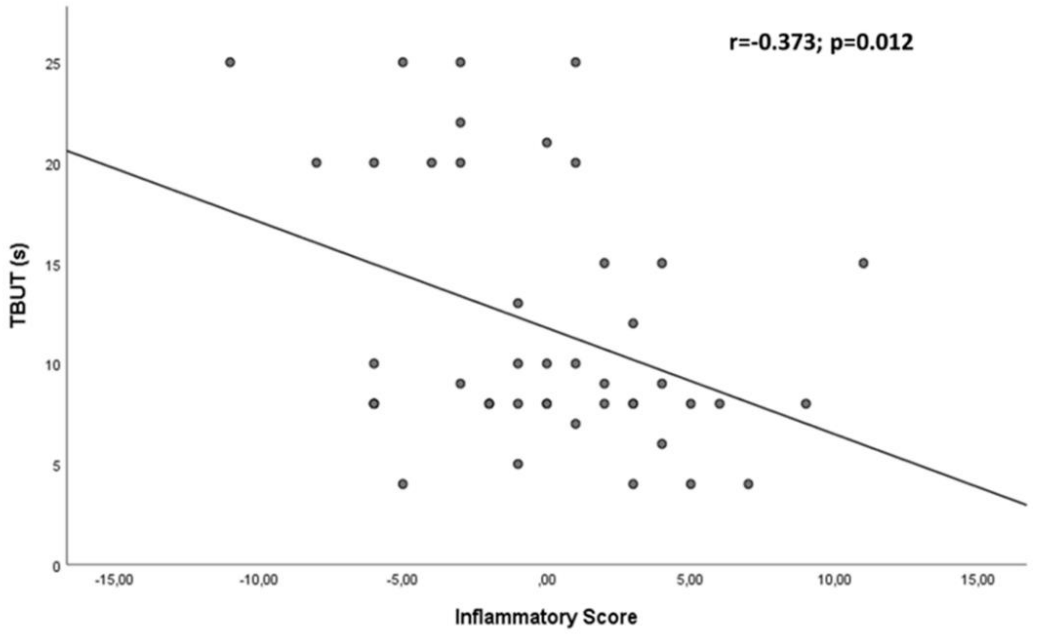
Table 2 shows ophthalmologic characteristics among people with cystic fibrosis compared to healthy controls in dry eye disease.

	CF (n=45)	Control (n=98)	P value
Sphere (D)	-0.90±2.17	-1.27±1.97	0.318
Cylinder (D)	-0.75±0.69	-0.76±0.71	0.947
Cylinder axis (degrees)	**82.8±52.6**	**110.1±58.7**	**0.011**
SE (D)	-1.17±2.16	-1.66±2.00	0.191
BCVA (log MAR)	**1.01±0.06**	**1.04±0.10**	**0.041**
IOP (mmHg)	17.6±2.8	17.6±2.6	0.926
T-BUT<10s, n (%)	**25 (55.6)**	**25 (25.5)**	**0.001**
T-BUT (s)	**9 [8-17.5]**	**17 [9.5-21]**	**0.001**
ST1<10, n (%)	**18 (40.0)**	**19 (19.4)**	**0.009**
ST1 (mm)	**11 [4.5-24]**	**18.5 [11-29.3]**	**0.001**
OSDI>13, n (%)	9 (20.0)	30 (30.6)	0.186
OSDI (punctuation)	7.6±8.7	11.9±13.8	0.026
Visual symptoms, n (%)			**<0.001**
• None or mild episodic fatigue	26 (57.8)	86 (87.8)	
• Episodic, bothersome, and/or limiting	14 (31.1)	9 (9.2)	
• Chronic and/or constant, limiting	5 (11.1)	2 (2.0)	
• Constant and/or possibly disabling	0 (0.0)	1 (1.0)	
Oxford	1.33±0.64	1.29±0.67	0.691
Oxford>0, n (%)	11 (24.4)	17 (17.3)	0.321
Inflammadry test positive (MMP 9 >40ng/ml) (%)	24 (60)	-	-

Abbreviations: BCVA: best-corrected visual acuity; CF: cystic fibrosis; IOP: intraocular pressure; Kmax: Maximal keratometry; OSDI: Ocular Surface Disease Index; SE: spherical equivalent; T-BUT: tear break-up time; ST1: Schirmer's test 1; MMP 9: Matrix Metalloproteinase 9.
The quantitative data is presented as mean ± standard deviation or medians (interquartile ranges).
Table 2. Ophthalmologic characteristics among people with cystic fibrosis compared to healthy controls in dry eye disease.

Figure S1 illustrates the differences observed between the CF group and the control group in relation to tests assessing dry eye disease, along with the percentage of individuals with pathological results in each group.

In the group of people with CF, there was a significant inverse correlation between T-BUT and the inflammatory score (Figure 2). Likewise, there was a significant inverse correlation between T-BUT and eosinophils (r=-0.338; p=0.023).



Abbreviation: T-BUT: tear break-up time.

Figure 2. Correlation between inflammatory score and T-BUT.

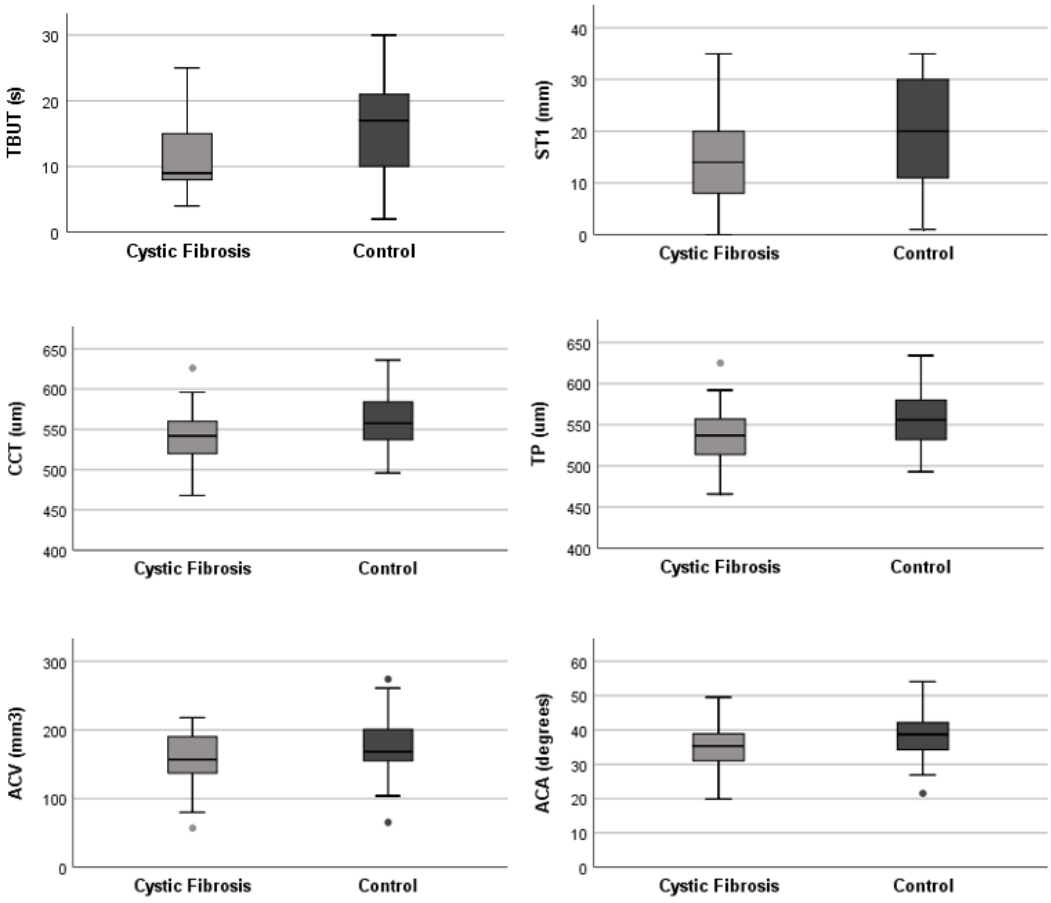
Additionally, linear regression analysis again showed a significant correlation between T-BUT and inflammatory score adjusted for confounding variables such as age, gender, treatment with Elexacaftor/tezacaftor/ivacaftor and treatment with azithromycin [-0.869 - (-0.052); 95% CI, p= 0.028] (Table S1).

The characteristics of the cornea and anterior eye segment differed between the CF group and the control group, highlighting the statistically significant differences found in the central corneal thickness (542.2 µm vs 557.5, p= 0.009), thinnest point (538.2 µm vs 554.4, p= 0.006), anterior chamber volume (160.2 mm^3 vs 175.1, p = 0.034), anterior chamber depth (2.9 mm vs 3.1, p=0.051) and anterior chamber angle (35.4 degrees vs 38.3, p=0.011) (Table S2)

Figure S2 provides a graphical representation of anterior segment measurements obtained from Scheimpflug images using the Pentacam™. It compares people with cystic fibrosis to a control group, highlighting differences in corneal thickness, anterior chamber depth, and anterior chamber angle.

Figure S3 presents Pentacam™ Scheimpflug images comparing the anterior segment structures of 6 people with cystic fibrosis and 6 individuals from the control group. These images provide a detailed visual comparison of key ocular parameters, including the iridocorneal angle.

Figure 3 shows the distribution of ocular parameters (TBUT, ST1, CCT, TP, ACV, ACA) in people with cystic fibrosis vs. controls.



Abbreviations: T-BUT: tear break-up time; ST1: Schirmer's test 1; CCT: central corneal thickness, TP: thinnest point (corneal thickness at the thinnest location); ACV: anterior chamber volume; ACA: anterior chamber angle.
Figure 3. Ocular Parameters in People with Cystic Fibrosis and Controls.

3.2 Macular and optic disc OCT in CF versus control

People with CF presented a larger disc area compared to the control group (1.9 mm^2 vs 1.7 mm^2, p= 0.036), smaller minimum GCC thickness (79.3 µm vs 82.1, p= 0.020) and a smaller peripapillary superior temporal retinal nerve fiber layer (117.1 µm vs 130.6 p=0.002) (Table S3).

Figure S4 shows **t**he OCT Optic Disc Cube scan, illustrating a decrease in RNFL thickness in a person with cystic fibrosis (a) compared to a healthy control matched for age and sex (b).

4. DISCUSSION

The findings in our sample suggest a higher prevalence of DED in people with CF than healthy control subjects, which is consistent with the limited data published to date [4].
In our group of people with CF, both T-BUT and tear secretion volume were significantly lower than in the control group, pointing to both an evaporative and an aqueous-deficient mechanism as the primary cause of DED. Considering this findings, genetic dysfunction of CFTR, expressed

in the lacrimal gland as well as in the cornea and conjunctiva, could generate lower tear secretion volume and tear film instability and promote the process of inflammation and chronic damage to the ocular surface. This fact is supported by the high percentage of people who had a positive InflammaDry™ test in the group of people with CF.

The OSDI scores obtained in the cystic fibrosis (CF) cohort were within the normal range, indicating minimal or negligible symptoms of dry eye disease (DED) in this population. This finding suggests that, despite the potential presence of subclinical ocular alterations, the impact of DED on the quality of life in these patients is minimal. The absence of overt clinical symptoms may partly explain why routine screening protocols for DED have not been widely implemented in clinical practice for people with CF. However, the early identification of DED remains critical, particularly prior to ocular surgeries, as undiagnosed DED could negatively impact postoperative recovery and outcomes [29]. Notably, healthy controls exhibited higher OSDI scores compared to the CF cohort. It is plausible that, in patients with severe systemic diseases such as CF, the perception of ocular surface symptoms may be overshadowed by more severe or debilitating systemic symptoms, including respiratory impairment, chronic fatigue, or recurrent infections. These results underscore the importance of thorough ocular assessments in people with CF, even in the absence of significant DED symptoms.

In the lung, CFTR dysfunction generates an alteration in mucociliary clearance that favors chronic colonization by potentially pathogenic microorganisms and produces an exaggerated inflammatory response [30] [2].

The association between systemic inflammation and eosinophilia has been previously studied in people with CF, indicating a wider role of inflammatory pathways in this condition[31]. Additionally, the link between systemic inflammation and DED has been described, and some studies have observed the presence of inflammatory mediators in the tears of people with CF [9] [32] [33]. However, we found no studies relating systemic inflammation to DED in people with CF. The significant association between T-BUT and inflammatory markers such as eosinophils suggests that systemic inflammation may play a direct role in the pathophysiology of DED in this population. The interest of this finding lies in the possible involvement of systemic inflammation in the pathophysiology of DED in CF and in the use of these biomarkers for its diagnosis and monitoring. This opens the door to considering anti-inflammatory treatments, such as CFTR modulators, as a means of mitigating ocular surface disease in people with CF.

The advent of new imaging technologies such as Pentacam™ have greatly contributed to the identification and understanding of the pathways involved in angle-closure glaucoma [34]. The association between primary angle-closure and anatomical factors such as narrow anterior chamber, hyperopia and genetic factors has been previously described [35] [10]. Our sample is the first in which an anterior segment evaluation by Pentacam™ is performed in people with CF.

The observed values in the corneal and anterior eye segment characteristics revealed significant differences between the cystic fibrosis (CF) group and the control group. Notably, central corneal thickness (CCT) and the thinnest corneal point were significantly reduced in the CF group compared to the control. These findings suggest that, while both groups remain within clinically normal ranges, people with CF may exhibit thinner corneas. This reduction in corneal thickness may have important implications for corneal biomechanical resistance, potentially affecting the accuracy of intraocular pressure (IOP) measurements and increasing the risk of developing glaucoma. This knowledge is critical, especially for patients who may have normal IOP**,** as observed in our CF cohort, but are still at high risk due to altered corneal biomechanics. Thin corneas have been associated with increased susceptibility to optic nerve damage, even in the absence of elevated IOP[36].

The anterior chamber volume (ACV) and anterior chamber angle (ACA) also showed significant reductions in people with CF. Lower ACV and ACA values are associated with an increased risk of angle-closure glaucoma, indicating a potential predisposition in this population. This finding is particularly noteworthy given their myopic condition, which is typically linked to wider angles and a lower risk of angle closure. These results highlight the importance of close monitoring, as anatomical changes in the anterior chamber may not be evident in initial evaluations.

These anatomical characteristics may facilitate the blockage of aqueous humor flow, leading to temporary but recurrent increases in intraocular pressure (IOP). While the IOP values in our people with CF remain within the normal range, these IOP fluctuations can still cause cumulative damage to the optic nerve over time

On the other hand, although the anterior chamber depth (ACD) did not reach statistical significance, the observed trend towards a shallower depth in people with CF is consistent with other findings related to reduced anterior chamber volume and angle.

Previous studies have suggested a possible relationship between glaucomatous optic neuropathy and CF, noting a reduction in pRNFL thickness and visual field alterations in these people. Hypoxemia has been proposed as a possible pathophysiological mechanism underlying these changes, similar to the mechanisms observed in patients with obstructive sleep apnea/hypopnea syndrome and chronic obstructive pulmonary disease (COPD) [4] [37]. However, our people with CF did not have respiratory insufficiency measured with the 6-minute walk test, suggesting that in our cohort hypoxemia is not a determining factor. It is known that in early stages of glaucoma, local defects in the pRNFL are more frequently observed in the superior and inferior quadrants, with the temporal half of these sectors being more vulnerable [38]. In our group of people with CF, a decrease in pRNFL thickness was found in the superior temporal quadrant with respect to the control group.

Taking these findings into account**,** angle closure and corneal biomechanical alterations, rather than hypoxemia, may be the primary factors responsible for the structural changes in the optic nerve observed in people with CF. This underscores the importance of close ophthalmologic monitoring in people with CF, as subtle ocular changes, even with normal intraocular pressure (IOP) values, may increase the risk of glaucoma progression.

4.1. Study limitations.

As this was a study of people with the rare disease cystic fibrosis and it was a single-center project, the number of subjects recruited was small, so greater statistical power would be achieved with a larger population. When we divided the cystic fibrosis patients into three genotype groups (Homozygous delta F508, Heterozygous delta F508, Other/Other), we did not find any statistically significant differences in the ophthalmological parameters analysed, such as dry eye disease , anterior segment parameters assessed via Pentacam™ or in peripapillary retinal nerve fiber layer thickness (pRNFL). It is important to note that the small sample size in each group may have limited the statistical power of the analysis, and larger studies will be needed in the future to better understand these potential relationships

In addition, since it was a cross-sectional study, no valid conclusions can be drawn regarding causal relationships.
An important limitation of the study is the relatively short duration of ETI exposure at the time of data collection. As this was a real-world study initiated when access to highly effective CFTR modulators (ETI) became available, only half of the participants were receiving ETI, and for a relatively short duration. This further reduced the sample size within subgroups, limiting the

ability to detect statistically significant differences. Longitudinal studies are needed to assess the long-term effects of extended ETI exposure on the ocular surface.
However, despite these limitations, some of the strengths of the study are that it is the first published study to use various eye tests (DED evaluation, corneal topography, OCT) to understand the impact of cystic fibrosis (CF) on eye health. A control group of 98 healthy subjects is included for meaningful comparisons, highlighting differences in eye conditions between people with CF and healthy individuals. Additionally, the study examines the relationship between eye health and other clinical variables, such as systemic inflammation, offering insights into how CF affects the eye in the context of overall inflammation.

5. CONCLUSIONS

Subclinical DED is more common in people with CF than in healthy patients and tear film instability may be correlated with the extent of systemic inflammation.
It is possible that angle closure is the mechanism involved in the early glaucomatous optic neuropathy observed in our people with CF, although further studies are needed to assess ophthalmologic involvement in this group of patients.

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Data sharing statement

Data are available on request due to privacy restrictions. The data presented in this study are available on request from the corresponding author. In compliance with Spanish Organic Law 15/1999, the data are not publicly available.

Declarations of Competing Interest

The authors declare not to have any conflicts of interest that may be considered to influence directly or indirectly the content of the manuscript.

Credit authorship contribution statement

Patricia Gutiérrez: conceptualization, methodology, data curation, formal analysis, writing-original draft, writing-review & editing**. Laura Jiménez:** data curation, writing-review & editing. **Jessica Martínez:** data curation, writing-review & editing. **Carmen Alba:** writing-review & editing. **María Victoria Girón:** data curation, writing-review & editing**. Gabriel Olveira:** data curation, writing-review & editing**. Pedro Ruiz-Esteban:** formal analysis, writing-original draft, writing-review & editing**. Casilda Olveira:** conceptualization, data curation, formal analysis, writing-original draft, writing-review & editing, Supervision.

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HIGHLIGHTS

1. People with CF (pwCF) had a higher prevalence of dry eye disease (DED) compared to healthy controls.

2. Systemic inflammation is associated with DED in pwCF and may contribute to worse ocular surface condition.

3. PwCF had anterior eye segment and optic nerve abnormalities, indicating glaucomatous changes.

4. Compared to controls, pwCF had smaller anterior eye volumes and angles as measured by tomography.

5. Regularly scheduled eye exams are crucial for early disease detection in pwCF.

Response to Reviewers JCF-D-24-00330R1

Title: Dry eye disease and morphological changes in the anterior chamber in people with cystic fibrosis.

Journal of Cystic Fibrosis

Dear Patrick A. Flume:

Thank you for reviewing our manuscript again and for considering the revisions we've made based on the reviewer's comments. We appreciate your additional feedback, as it will help us improve our work. Below, we summarize our responses and the changes we have made to the manuscript. We are including a revised version with all edits highlighted in red, as well as a detailed response to all of the reviewer's points and a complete list of modifications for your reference.

I look forward to hearing from you.

Yours sincerely,

Editor comment:

Thank you for your responsiveness to the reviewers' questions and suggestions.

	Editor comment	Answer	Changes made
E_1	In the first line if the results section, please change CF people to people with CF.	We apologize for this minor error.	A change has been made in the manuscript.
E_2	Additionally, please further improve the readability of the highlights. For example, you can use the abbreviation pwCF (people with CF). #4 could read "Compared to controls, pwCF had smaller anterior eye volumes and angles as measured by tomography". Consider changing #5 to simply read (in order to drive home the most important point) "Regularly scheduled eye exams are crucial for early disease detection in pwCF"	We sincerely appreciate your valuable feedback. We have implemented the suggested changes in the highlights to improve their readability, ensuring clearer communication of the manuscript's key points.	We have implemented the suggested changes in the highlights.

Reviewer 1 comment:

Reviewer #1: Thank you for the opportunity to review the revised manuscript by Gutierrez and colleagues. I welcome the revisions and the answers to the reviewers' points which have greatly improved the readability of this manuscript for a non-ophthalmologist. The authors have addressed all the comments I have made and either altered them or have acknowledged the limitation. I have one small comment to make.

	Reviewer comment	Answer	Changes made
R1_1	Highlights: Highlight 2 as currently worded suggests systemic inflammation has a causative effect on T-BUT in CF, which may be true but only association can be proved from the current work.	Thank you very much for your input. We fully agree with your observation and will make the necessary adjustments to reflect the association without suggesting a causal effect.	We have implemented the suggested change in the highlights: 2. Systemic inflammation is associated with DED in pwCF and may contribute to worse ocular surface condition.

Figure(s)

Figure 1. Flowchart of patients studied.