J Pediatr Res 2024;11(2):110-7
DOI: 10.4274/jpr.galenos.2024.09735

Original Article

# Investigation of Phagocyte Functions in Pseudomonas-Colonized Cystic Fibrosis Patients

Handan Duman Şenol[1], Meral Barlık[2], Ezgi Topyıldız[1], Figen Gülen[1,2], Güzide Aksu[1], Necil Kütükçüler[1], Esen Demir[1,2], Neslihan Edeer Karaca[1]

[1]Ege University Faculty of Medicine, Department of Pediatric Allergy and Immunology, İzmir, Turkey
[2]Ege University Faculty of Medicine, Department of Pediatric Pulmonology, İzmir, Turkey

**ABSTRACT**

**Aim:** Cystic fibrosis (CF) is an autosomal recessive disorder. Although it is considered as an epithelial disease due to impaired chloride transport, its pathogenesis remains unclear. CF is classified as a syndrome with congenital defects of phagocyte in recent human inborn errors of immunity phenotypic classifications. Neutrophils are the most effective cells in the eradication of bacterial infections such as *Pseudomonas aeruginosa*. The aim of the present study was to investigate the phagocyte functions in pseudomonas colonized CF patients.

**Materials and Methods:** A total number of 26 pseudomonas colonized CF patients and 21 healthy controls (gender and age matched) were included in this study. Absolute neutrophil counts (ANC), immunoglobulin values (Ig), the Migratest to evaluate chemotaxis in neutrophils and monocytes, CD11A/CD18/CD15 S (β2 integrin) adhesion molecules, and the Phagoburst test for intracellular bacterial killing were analyzed by flow cytometer.

**Results:** ANC, CD15S expression on neutrophils and IgG, IgA and IgM levels were higher in the CF patients than the control group ($p<0.01$). The neutrophils oxidative burst activity and the chemotactic ability of the CF patients did not differ from those of the controls. Patients with allergic bronchopulmonary aspergillosis and those with a mutation of 2183AA>G had significantly lower chemotaxis indexes than the others ($p=0.01$, $p=0.01$ respectively).

**Conclusion:** Our results from a small group of patients does not support impaired functions such as migration and phagocytosis of neutrophils in patients with CF. Further studies involving more CF patients are needed to make a definitive interpretation.

**Keywords:** Cystic fibrosis, chemotaxis, phagocytosis, neutrophil function, adhesion molecule

## Introduction

Cystic fibrosis (CF) is an autosomal recessive disorder caused by mutations in the gene encoding the transmembrane regulatory protein (CFTR). As a result of the defect in ion transport, a deterioration of chloride and fluid transport in the respiratory tract epithelium leads to mucus formation with increased viscosity, impaired mucociliary clearance, colonization of the airway epithelium with opportunistic infectious agents such as *Pseudomonas aeruginosa* (*P. aeruginosa*), which is one of the most damaging pathogens, and eventually the development of lung disorders seen in patients with CF (1). The pathogenesis of CF remains unclear. In recent years, abnormal increases in inflammation and defects in the clearance of pathogens are now thought to occur as a result of CFTR mutations

**Address for Correspondence**
Ezgi Topyıldız, Ege University Faculty of Medicine, Department of Pediatric Allergy and Immunology, İzmir, Turkey
**Phone:** +90 555 536 18 69 **E-mail:** ezgitopyildiz@gmail.com **ORCID:** orcid.org/0000-0002-9260-8157
**Received:** 19.06.2023 **Accepted:** 04.04.2024
***The manuscript has been presented as an orally in 10. National Pediatric Respiratory Diseases and Cystic Fibrosis Congress, 2-4 June 2022, Ankara, Turkey.**

in immune cells. In 2020, it was classified as a congenital defect of the phagocyte system (2).

Neutrophils and monocytes are phagocytic cells which play a role in innate immunity. First, they migrate to the inflammatory site in response to chemoattractant. The other steps are recognition, adhesion, phagocytosis, and the killing of the target. Neutrophilia of the airway is a common feature seen in CF patients, even in the absence of positive bacterial cultures (3). However, as a result of defective CFTR expression in neutrophils, chloride transport into the phagolysosome is impaired, hypochlorous acid cannot be produced, and bacteria cannot be killed, and impaired phagolysosome activity contributes to airway damage by secreting proteins and enzymes such as elastase, which has been associated with bronchiectasis and severe lung damage (4).

Selectins and integrins are adhesion molecules which have different roles during the process of leucocyte-endothelial adhesion. While selectins mediate margination and slow rolling, integrins play a role in both the rolling and arrest on the vascular endothelium. The integrins CD11a/CD18 (lymphocyte function-associated antigen-1/LFA-1), which are expressed in leukocytes, help the leukocyte transmigration to the inflammation area, lymphocyte co-stimulatory signaling, and T-lymphocyte alloantigen-induced proliferation. De Rose et al. (5) reported that the high p-selectin and e-selectin levels they detected were caused by the defective process in neutrophil adhesion in patients with CF. Sorio et al. (6) reported that β1 and β2 integrin-related activation of chemotaxis are defective in monocytes in patients with CF, and CF is a monocyte-selective adhesion defect.

The disruption of the normal phagocytic defense of the host has been associated with biofilm formation, which is also the reason why CF patients are chronically infected with strains resistant to antibiotics. The conversion of *P. aeruginosa* from a non-mucoid phenotype to a mucoid phenotype usually causes a deterioration in respiratory functions and a worsening of the prognosis (7).

In conclusion, it is not clear whether the primary defect is neutrophil and monocyte function defect or an excessive response to over pro-inflammatory stimuli. The aim of the present study was to investigate phagocytic cell functions in pseudomonas colonized CF patients.

## Materials and Methods

### Patient Selection

A total number of 26 patients with CF who were pseudomonas colonized and 21 healthy controls were included in this study. Informed consent was obtained from all patients and parents. This study was approved by the Ege University Faculty of Medicine Medical Research Ethics Committee (approval no.: 20-12.1T/37, date: 17.12.2020) and sponsored by the University Office of Scientific Research Projects.

Patients aged 2-18 years of age were diagnosed by a positive sweat chloride (>60 mmol/L) test and/or a known genotype with compatible symptoms. *P. aeruginosa* colonization was defined as positivity for *P. aeruginosa* in at least four sputum samples in the prior 12 months and with more than a 50% positive growth of *P. aeruginosa*. The present study included clinically stable CF patients. Demographic information and laboratory data were recorded from the medical files.

The Shwachman-Kulczycki score was used for the evaluation of the clinical status of the patients. The score was calculated via the general activity, physical examination, nutritional status, and chest X-ray findings. Overall, 86 to 100 points were classified as "excellent", 71 to 85 as "good", 56 to 70 as "mild", 41 to 55 as "moderate", and ≤40 as "severe" (8).

### Laboratory Evaluation

### Whole Blood Count Assay

Whole blood count, leukocyte counts, absolute neutrophil and lymphocyte counts, and relative ratio were performed with a hemocounter Cell-Dyn 3700, Abbott Diagnostics, USA.

### Serum Immunoglobulin Assay

Serum immunoglobulins (IgG, IgA, IgM) were analyzed quantitatively via a Dade-Behring BN II Nephelometer, Siemens, Germany, and compared with age-related normal levels (9).

### CD11a, CD18 and CD15S Surface Expressions on Neutrophil Granulocytes and Monocytes

Surface expressions of CD11a, CD18, and CD 15s on monocytes and neutrophil granulocytes were analyzed by cell surface staining instructions using anti-CD11a FITC, anti-

CD18 PE moAb and HuCD15SBV510 (Becton-Dickinson, USA) in heparinized blood samples. Leukocytes were also analyzed for their intracellular expression of myeloperoxidase (MPO) by flow cytometry.

### Chemotactic Function of Granulocytes (Migratest)

The Migratest (Orpegen Pharma, Heidelberg, Germany) was used in order to evaluate the number of neutrophils which migrated through the cell culture toward chemoattractant fMLP. A percentage of activated granulocytes of more than 95% was accepted as normal. The chemotaxis index was calculated as follows;

**Chemotaxis index (10): Д: A-B**

A=Neutrophil count which migrated after FMLP/Beat

B=Neutrophil count which migrated before stimulation/ Beat

**Chemotaxis ratio (11):** Neutrophil count which migrated after FMLP/Neutrophil count which migrated before stimulation. A ratio greater than 1 was accepted as normal.

### Oxidative Burst Activity of Granulocytes (Phagoburst Test)

The Phagoburst commercial test kit (Orpegen Pharma, Heidelberg, Germany) was used for oxidative burst activity of monocytes and granulocytes in heparinized whole blood. Stimulants such as *Escherichia coli* (*E. coli*), *n*-formyl-methionine-leucine-phenylalanine (fMLP), and phorbol-12-myristate-13-acetate (PMA) were used to produce reactive oxidants and the percentage of phagocytic activity was determined by flow cytometry (Normal reference ranges; for monocytes by stimulant *E. coli*: 70-100%; for granulocytes by stimulant *E. coli*: 95-100%, fMLP: 1-20%, and PMA: 99-100%).

### Statistical Analysis

The data were evaluated using the Statistical Package for Social Sciences 25.0 (SPSS for Windows 25.0, Inc., Chicago, IL, USA) and by analyzing descriptive statistics (means, standard deviation). Student's t-test was used for the comparison of normally distributed variables. The chi-square, Mann-Whitney U test, and Kruskal-Wallis tests were used for non-normally distributed variables. A p-value <0.05 was considered as significant. Recurrent measurements of variant analysis were used for data during chemotaxis (measurements before and after fMLP).

## Results

### Patient Characteristics

A total of 26 CF patients [Male/Female (M/F): 14/12] with a mean age of 127.85±56.06 months and 21 healthy controls (M/F: 12/9) with a mean age of 122.07±70.2 months were included in the present study. There was no statistical significance for gender or age between the groups. All CF patients had bronchiectasis. All of the demographic and clinical characteristics of the patient group are summarized in Table I. In terms of CTFR mutations: nine (34.6%) patients were homozygous for F508, 10 (38.4%) were homozygous for other mutations and 7 were compound heterozygous.

### Laboratory Data

White-blood cell count and absolute neutrophil count were significantly increased in the patient group ($p<0.01$, respectively). Although five of the patients had hypogammaglobulinemia, mean IgG, IgA and IgM levels were significantly higher in the patient group ($p<0.01$). Among those patients with low IgG, the other immunoglobulin levels and lymphocyte subsets were normal and they had normal vaccine responses. There was no finding suggesting primary antibody deficiency accompanying CF. There were no differences for C-reactive protein (CRP) level or absolute lymphocyte count between the groups (Table II).

**Table I.** Demographic and clinical characteristics of cystic fibrosis patients

| | **n=26 (%)** |
|---|---|
| Age (months) (median) (IQR) (min.-max.) | 105.0 (143.2) (24-216) |
| Age at diagnosis (months) (median) (IQR) (min.-max.) | 3 (9.0) (1-192) |
| Age at symptom onset (months) (median) (IQR) (min.-max.) | 4 (1.0) (1-12) |
| Follow-up period (months) (median) (IQR) (min.-max.) | 86 (75.7) (17-216) |
| IRT Level (ng/ml) (mean ± SD) | 185.33±73.36 |
| Sweat test level (Cl) (mmol/L) (mean ± SD) | 77.66±17.21 |
| History of consanguinity | 15 (57.7%) |
| Number of hospitalizations per year (median) (min.-max.) | 7 (13) (0-48) |
| Failure to thrive | 24 (92.3%) |
| Concomitant colonization of *S. aureus* | 11 (42.3%) |
| Presence of ABPA | 4 (15.3%) |

IQR: Interquartile range, min.-max.: Minimum-maximum, ABPA: Allergic bronchopulmonary aspergillosis

The adhesion molecule percentages (CD11A, CD18, CD15s on neutrophils and monocytes) were similar for the patient and control groups except for CD15s levels on neutrophils, which were higher in the patient group (p=0.01) (Table III).

The chemotaxis ratio was <1% in seven (26.9%) cases in the patient group and one (4.8%) in the control group. There was no difference between the patient and control groups in terms of low chemotaxis ratios. The case with the low chemotaxis ratio in the control group had no addition pathology and no history of acute infection or recent drug usage. Three of the four patients (75%) with allergic bronchopulmonary aspergillosis (ABPA) had a ratio <1, which was significantly higher than in those patients without ABPA (p=0.04). Among the three patients with ABPA, two of them had received azithromycin treatment. There was no difference for body-mass index in those patients with and those without ABPA. Although hospitalization rates per year were higher (5.6 per year) in those patients with low chemotaxis ratios, there was no significant difference when compared to those patients with normal ratios. The comparison of the patients with decreased/normal chemotaxis ratios is given in Table IV.

The chemotaxis index was significantly lower in those patients with a CTFR mutation homozygous for 2183AA>G than for the others (p=0.01).

According to recurrent measurement variant analysis during chemotaxis, there was a significant increment in chemotactic cells between before and after stimulation in both groups (p<0.01), but there was no difference between the groups (p>0.05).

There was no significant relationship between the frequency of hospitalization and the annual number of active infections, and the IgG level and chemotaxis index (p>0.05).

While phagocytic activity in response to PMA was lower in one CF patient and in one control, there was no significant difference between the groups. Also, no difference was found for phagocytic activity in response to *E. coli*. Myeloperoxidase levels were also similar (p>0.05) (Table III).

## Discussion

Patients with CF are susceptible to recurrent infections from bacteria, viruses, and fungi due to abnormally thick, sticky mucus trapping these germs in the airways. Additionally, immunologic abnormalities, such as decreased lymphocyte responsiveness, defects in opsonic activity and increased circulating immune complexes, predispose these individuals to recurrent and invasive infections and ongoing inflammations. In the present study, phagocytosis and chemotaxis abilities, which are important functions of neutrophils, were investigated by flow cytometry. No evidence for defective phagocytosis or chemotaxis were detected in those patients with CF. The present study only found an increased number of neutrophils and immunoglobulin levels in the CF patients. The increase in neutrophil and Ig counts was evaluated as a compensatory response to chronic infection and inflammation. Those patients with ABPA had significantly decreased neutrophil chemotaxis ratios, and the patients

**Table II.** Hemogram and immunoglobulin levels of the study groups

| | **Patients, n (26)** | **Control, n (21)** | **p value** |
|---|---|---|---|
| White-blood cell/mm3 (mean ± SD) | 11170±3938 | 7422.8±1677.7 | <0.01 |
| Absolute neutrophil count (mean ± SD)<br>Low ANC<br>Normal ANC | 6184.3±3119.2<br>0<br>26 (100%) | 3668.5±1151.9<br>1 (4.7%)<br>20 (95.2%) | <0.01 |
| Absolute lymphocyte count (median) (IQR) (min.-max.) | 2920 (2690) (1790-9540) | 2800 (1200) (1000-4100) | 0.27 |
| IgG (mean ± SD) (mg/dL) | 1173.4±481.6 | 759.4±143.8 | <0.01 |
| IgG values of patients<br>Low IgG<br>Normal IgG<br>High IgG | <br>(19.2%)<br>13 (50%)<br>8 (30.7%) | <br>1 (4.7%)<br>20 (95.2%)<br>- | <0.01 |
| IgM (mg/dL) | 120 (93) (59.7-852.0) | 80.0 (45.1) (50.2-130.9) | <0.01 |
| IgA (mg/dL) | 198.4±118.7 | 89.8±27.0 | <0.01 |
| IgE kU/L | 46.9 (167.8) (17-1410) | 41.3 (108.0) (17-810) | 0.64 |
| CRP (mg/L) | 3.3 (14) (0.3-31.5) | 3.0 (1) (1-15) | 0.45 |

IQR: Interquartile range, min.-max.: Minimum-maximum, SD: Standard deviation, ANC: Absolute neutrophil counts, Ig: Immunoglobulin, CRP: C-reactive protein

**Table III.** Comparison of adhesion molecule/burst test/MPO/L-Selectin levels of study groups

| | Patients (n=26) | Control (n=21) | p value |
|---|---|---|---|
| CD11A (monocyte) % | 99 (76-100) | 99 (90-100) | 0.31 |
| CD11A (neutrophil) % | 100 | 99.7 | 0.26 |
| CD15S (monocyte) % | 83 (23-98) | 85 (75-90) | 0.22 |
| CD15S (neutrophil) % | 100 (74-100) | 99 (82-100) | 0.01 |
| CD18 (monocyte) % | 99 (83-100) | 99 (95-100) | 0.23 |
| CD18 (neutrophil) % | 100 (100-100) | 100 (95-100) | 0.11 |
| Burst test<br>- PMA<br>- *E. coli* | <br>99.8±0.47<br>97.6±2.81 | <br>99.6±0.74<br>97.6±1.71 | <br>0.15<br>0.28 |
| MPO (monocyte) | 83.0±8.33 | 83.33±7.33 | 0.10 |
| MPO (neutrophil) | 98.6 ±1.43 | 99.0±1.94 | 0.06 |
| L-Selectin-Migratest | 95.2±5.4 | 92.5±6.3 | 0.06 |

PMA: Phorbol-12-myristate-13-acetate, MPO: Myeloperoxidase

**Table IV.** The comparison of the patients with decreased/normal chemotaxis ratio

| | Decreased CR, n (7) | Normal CR, n (19) | p value |
|---|---|---|---|
| Gender (F/M) | 4/3 | 8/11 | 0.66 |
| F508 Homozygous | 2 | 7 | 0.99 |
| ABPA | 3 (42.9) | 1 (5.3) | 0.04 |
| Age at pseud. colonization | 89.0±70.7 | 73.1±57.2 | 0.58 |
| Age at first pulmonary attack | 80.0±67.3 | 79.3±53.5 | 0.98 |
| Azithromycin | 3 (42.9) | 3 (15.7) | 0.29 |
| Hospitalization number/age (per year) | 5.6 (9.4) (0-12) | 0.6 (1.2) (0-8) | 0.18 |
| Sweat Test Cl (mmol/L) | 85.6±16.9 | 98.5±26.9 | 0.32 |
| Shwachman-Kulczycki score | 10 (9) (6-19) | 11 (2) (5-20) | 0.69 |
| BMI | 13.6 (5.8) (8.0-18.9) | 15 (3.1) (1.0-17.8) | 0.49 |
| White blood cell (mean ± SD) | 12,965.0±5,041.7 | 10,508.4±3,368.9 | 0.16 |
| Absolute neutrophil count (median) (IQR) (min.-max.) | 5,040 (7,590) (3,870-12,630) | 4,980 (4,840) (2,780-114,940) | 0.33 |
| Absolute lymphocyte count (median) (IQR) (min.-max.) | 2,950 (3,550) (1,790-9,540) | 2,865 (2,760) (2,760-8,630) | 0.91 |
| IgG (mean ± SD) | 1,135.4±391.4 | 1,190.1±527.2 | 0.80 |
| IgM | 117.0 (106.0) (104.0-229.0) | 119.0 (84.5) (59.7-288) | 0.53 |

F/M: Female/Male, IQR: Interquartile range, min.-max.: Minimum-maximum, SD: Standard deviation, Ig: Immunoglobulin, BMI: Body mass index, ABPA: Allergic bronchopulmonary aspergillosis

with a mutation of 2183AA>G had significantly lower chemotaxis indexes.

CF is a well-known neutrophil dominant inflammatory disease. There are numerous studies showing neutrophilia in the airways of these patients even in the absence of infection (3,12). Spontaneous apoptosis of neutrophils is a necessary event which protects the host from the harmful effects of inflammation. It has been shown in CF patients that they have defective neutrophil apoptosis leading to long survival of neutrophils. Gray et al. (13) studied blood neutrophil apoptosis by flow cytometry in CF piglets and CF patients. They showed that, because of decreased apoptosis, the life of CF neutrophils was longer. They also showed neutrophil extracellular trap (NET) formation (NETosis), which helps bacterial killing, was excessive in CF patients causing more inflammation in the airways. The present study found an increased number of neutrophils in the absence of active

infection (CRP levels were similar in the CF and control groups), which is consistent with the literature.

Serum immunoglobulin levels were high among the patients, especially those with the mutation F508. One study reported hypergammaglobulinemia among CF patients and commented that this was a result of chronic infections and inflammation, and that it was related with poor prognosis (14). A recent study found Hypo-IgG levels among 66 CF children with a low number of pulmonary exacerbations and duration of antibiotic therapy (15). Five of our patients with higher rates of hospitalization had hypogammaglobulinemia, but this difference was not significant. The total protein/albumin levels were normal, and there was no malabsorption or protein lose that could explain hypogammaglobulinemia in these patients. Their body-mass index was low but there was no difference from those patients with normal globulin levels. There was no other finding such as inadequate vaccine responses which would suggest primary antibody deficiency. In addition, two patients with initial hypogamaglobulinemia showed gradual IgG elevation during follow-up. It was suspected that the chronic inflammation might be the cause of transient hypogammaglobulinemia. In recent years, studies have reported on the upregulation of mucosal IgA and its receptor, pIgR, as well as serum IgA (16). Also, a strong upregulation of IgA and pIgR was achieved after pseudomonas infection (17).

Similar to the present study, Leuer et al. (18) found no inability of phagocytosis both in neutrophils and monocytes in 35 CF adult patients in comparison to 12 healthy controls. In contrast to our findings, Aslanhan et al. (11), who compared the neutrophil functions of CF patients with pseudomonas colonized (n=8), non-colonized (n=8) and a control group (n=8), found increased phagocytosis, oxidative burst and chemotaxis in the CF patients. They reported that, although the phagocytosis capacity in peripheral blood is more prominent, there was a failed elimination of the colonized bacteria in the lung bronchi.

Three of the four patients (75%) with ABPA in our study had a chemotactic ratio below <%1, which was significantly higher than those patients with normal ratios. ABPA, which is described as hypersensitivity to aspergillus fumigatus, is seen in nearly 10% of patients with CF. Low body-mass index, long-term medication with macrolides, and prophylactic antibiotics for colonization with *Staphylococcus aureus* and *P. aeruginosa* have been reported as risk factors for ABPA (19,20). It has been reported that macrolide treatment has immunomodulatory effects in CF patients; the inhibition of neutrophil chemotaxis to the airways and the production of chemotactic factors (IL8 and LTB4) (21,22). Among our three patients with low chemotactic ratios (<1) with ABPA, two of them used macrolide treatment. As the patient number was very low in the present study, it was not possible to propose that macrolides play a role in neutrophil chemotaxis. *In vitro* functional studies are needed to prove the inhibitory role of macrolides on neutrophil chemotaxis.

Hardisty et al. (23) reported that ivacaftor treatment improved neutrophil markers of adhesion and activation in patients with R117H residual function CFTR mutations. Bratcher et al. (24) showed that ivacaftor normalized CD11b on neutrophils and CD63 on monocytes in patients with mutations of G551D. Therefore, it can be said that modulator therapies achieve mutation specific leucocyte modulation. We found that the chemotaxis index was significantly lower in those patients with a mutation of 2183AA>G than in the others. There might be differences of neutrophil functions depending on the mutations and manifestations of the disease.

Integrins and selectins play a role in leukocyte transmigration to the inflammation area. Sorio et al. (6) reported diminished activation of β1 and β2 integrins and chemotaxis in the mononuclear cells of CF patients. They reported that neutrophil adhesion and chemotaxis was normal, and defined CF as a monocyte-selective adhesion deficiency. The present study found no differences for adhesion molecules, CD11B and CD18, between the CF and control groups on either neutrophils or monocyte. We only found increased levels of CD15s on neutrophils. CD15s is a ligand for selectins playing a role in neutrophil aggregation and slow rolling (25). Markic et al. (26) reported CRP, procalcitonin and CD15s percentages to be predictors of severe bacterial infection. Although our patients did not have acute infection at the time of the study, chronic inflammation in CF patients could be the reason.

**Study Limitations**

The inclusion of more patients, both pseudomonas colonized and non-colonized, the analysis of the same patient in both their stable and active infectious periods, and performing Netosis assays and functional analyses on the phagocytic cells obtained from bronchoalveolar lavage material could provide more meaningful results. Unfortunately, only basic tests could be carried out with the available project budget on a limited number of patients.

## Conclusion

In conclusion, although our data showed no evidence for defective phagocytosis or chemotaxis in those patients with CF, we found decreased chemotactic ability in those patients with ABPA and mutations of 2183AA>G. Our results might provide an insight into the neutrophil function differentiation of different mutations. Since our study had a small sample size, further studies involving more CF patients are needed to make a definitive interpretation.

**Acknowledgements:** The authors acknowledge Gulden Hakverdi for her help with statistical analysis and Ege University Faculty of Medicine, Department of Medical Genetics, and Department of Pediatric Clinical Genetics for their help with genetic analysis.

### Ethics

**Ethics Committee Approval:** This study was approved by the Ege University Faculty of Medicine Medical Research Ethics Committee (approval no.: 20-12.1T/37, date: 17.12.2020).

**Informed Consent:** Informed consent was obtained from all patients and parents.

### Authorship Contributions

Surgical and Medical Practices: M.B., F.G., E.D., N.E.K., Concept: H.D.Ş., M.B., G.A., N.K., E.D., N.E.K., Design: H.D.Ş., E.T., F.G., N.K., E.D., N.E.K., Data Collection and/or Processing: H.D.Ş., M.B., E.T., N.K., Analysis and/or Interpretation: H.D.Ş., E.T., G.A., E.D., N.E.K., Literature Search: E.T., F.G., G.A., Writing: H.D.Ş., F.G., G.A., N.K., E.D.**Conflict of Interest:** The authors declare that there is no conflict of interest regarding the publication of this article.

**Financial Disclosure:** Sponsored by the University Office of Scientific Research Projects.

## References

1. Riordan JR, Rommens JM, Kerem B et al. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. Science 1989; 245:1066-73.
2. Tangye SG, Al-Herz W, Bousfiha A, et al. Human Inborn Errors of Immunity: 2019 Update on the Classification from the International Union of Immunological Societies Expert Committee. J Clin Immunol 2020;40:24-64.
3. Balough K, McCubbin M, Weinberger M, Smits W, Ahrens R, Fick R. The relationship between infection and inflammation in the early stages of lung disease from cystic fibrosis. Pediatr Pulmonol 1995;20:63-70.
4. Kelly E, Greene CM, McElvaney NG. Targeting neutrophil elastase in cystic fibrosis. Expert Opin Ther Targets 2008; 12:145-57.
5. De Rose V, Oliva A, Messore B, Grosso B, Mollar C, Pozzi E. Circulating adhesion molecules in cystic fibrosis. Am J Respir Crit Care Med 1998; 157:1234-9.
6. Sorio C, Montresor A, Bolomini-Vittori M, et al. Mutations of Cystic Fibrosis Transmembrane Conductance Regulator Gene Cause a Monocyte-Selective Adhesion Deficiency. Am J Respir Crit Care Med 2016; 193:1123-33.
7. Govan JR, Brown AR, Jones AM. Evolving epidemiology of Pseudomonas aeruginosa and the Burkholderia cepacia complex in cystic fibrosis lung infection. Future Microbiol 2007; 2:153-64.
8. SHWACHMAN H, KULCZYCKI LL. Long-term study of one hundred five patients with cystic fibrosis; studies made over a five- to fourteen-year period. AMA J Dis Child 1958; 96:6-15.
9. Aksu G, Genel F, Koturoğlu G, Kurugöl Z, Kütükçüler N. Serum immunoglobulin (IgG, IgM, IgA) and IgG subclass concentrations in healthy children: a study using nephelometric technique. Turk J Pediatr 2006; 48:19-24.
10. Hertwig L. The implication of natural killer cells and neutrophils in autoimmune disorders of the central nervous system. 2016.
11. Aslanhan U, Cakir E, Pur Ozyigit L, et al. Pseudomonas aeruginosa colonization in cystic fibrosis: Impact on neutrophil functions and cytokine secretion capacity. Pediatr Pulmonol 2021; 56:1504-13.
12. Khan TZ, Wagener JS, Bost T, Martinez J, Accurso FJ, Riches DW. Early pulmonary inflammation in infants with cystic fibrosis. Am J Respir Crit Care Med 1995; 151:1075-82.
13. Gray RD, Hardisty G, Regan KH, et al. Delayed neutrophil apoptosis enhances NET formation in cystic fibrosis. Thorax 2018; 73:134-44.
14. Garside JP, Kerrin DP, Brownlee KG, Gooi HC, Taylor JM, Conway SP. Immunoglobulin and IgG subclass levels in a regional pediatric cystic fibrosis clinic. Pediatr Pulmonol 2005; 39:135-40.
15. Hanssens LS, Cellauro S, Duchateau J, Casimir GJ. Immunoglobulin G: A useful outcome marker in the follow-up of cystic fibrosis patients? Immun Inflamm Dis 2021; 9:608-14.
16. Gohy S, Moeremans A, Pilette C, Collin A. Immunoglobulin A Mucosal Immunity and Altered Respiratory Epithelium in Cystic Fibrosis. Cells 2021; 10:3603.
17. Collin AM, Lecocq M, Noel S, et al. Lung immunoglobulin A immunity dysregulation in cystic fibrosis. EBioMedicine 2020; 60:102974.
18. Leuer L, Krill A, Wilkens H, et al. The Phagocytosis of Blood Leukocytes from Cystic Fibrosis Patients is not Impaired in General. Lung 2020; 198:235-9.
19. Jubin V, Ranque S, Stremler Le Bel N, Sarles J, Dubus JC. Risk factors for Aspergillus colonization and allergic bronchopulmonary aspergillosis in children with cystic fibrosis. Pediatr Pulmonol 2010; 45:764-71.
20. Bargon J, Dauletbaev N, Köhler B, Wolf M, Posselt HG, Wagner TO. Prophylactic antibiotic therapy is associated with an increased prevalence of Aspergillus colonization in adult cystic fibrosis patients. Respir Med 1999; 93:835-8.
21. Tamaoki J, Kadota J, Takizawa H. Clinical implications of the immunomodulatory effects of macrolides. Am J Med 2004; 117 Suppl 9A:5S-11S.

22. Tsai WC, Rodriguez ML, Young KS, et al. Azithromycin blocks neutrophil recruitment in Pseudomonas endobronchial infection. Am J Respir Crit Care Med 2004; 170:1331-9.

23. Hardisty GR, Law SM, Carter S, et al. Ivacaftor modifies cystic fibrosis neutrophil phenotype in subjects with R117H residual function CFTR mutations. Eur Respir J 2021; 57:2002161.

24. Bratcher PE, Rowe SM, Reeves G, et al. Alterations in blood leukocytes of G551D-bearing cystic fibrosis patients undergoing treatment with ivacaftor. J Cyst Fibros 2016; 15:67-73.

25. Mulligan MS, Warner RL, Rittershaus CW, et al. Endothelial targeting and enhanced antiinflammatory effects of complement inhibitors possessing sialyl Lewisx moieties. J Immunol 1999; 162:4952-9.

26. Markic J, Jeroncic A, Polancec D, et al. CD15s is a potential biomarker of serious bacterial infection in infants admitted to hospital. Eur J Pediatr 2013; 172:1363-9.