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Clinical findings of patients with cystic fibrosis according to newborn screening results

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Abstract

Background: Cystic fibrosis (CF) is a lethal recessive genetic disease caused by loss of function associated with mutations in the CF trans-membrane conductance regulator (CFTR). CF is highly prevalent (approximately 1 in 3500) in Caucasians. The aim of this study was to compare demographic and clinical features, diagnostic tests, treatments, and complications of patients with CF whose newborn screening (NBS) with twice-repeated immune reactive trypsinogen (IRT/IRT) testing was positive, normal, and not performed.

Methods: In this study, 359 of all 1,488 CF patients recorded in the CF Registry of Turkey in 2018, who had been born through the process of NBS, were evaluated. Demographic and clinical features were compared in patients diagnosed with positive NBS (Group 1), normal (Group 2), or without NBS (Group 3).

Results: In Group 1, there were 299 patients, in Group 2, there were 40 patients, and in Group 3, there were 20 patients. Among all patients, the median age at diagnosis was 0.17 years. The median age at diagnosis was higher in Groups 2 and 3 than in Group 1 (p=0.001). Fecal elastase results were higher in Group 2 (p=0.033). Weight z-score was lower and chronic *S. aureus* infection was more common in Group 3 (p=0.017, p=0.004, respectively).

Conclusions: Frequency of growth retardation and chronic *S. aureus* infection can be reduced with an early diagnosis with NBS. In the presence of clinical suspicion in patients with normal NBS, further analyses such as genetic testing should be performed, especially to prevent missing patients with severe mutations.

Keywords: clinical features, cystic fibrosis, immunoreactive trypsinogen, newborn screening, sweat chloride test

Introduction

Cystic fibrosis (CF) is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) protein. Irregular chloride transport causes thick, viscous secretions in the lungs, pancreas, liver, intestine, and reproductive system. Most patients develop a multi-system disease[1]. In Turkey the prevalence of the cystic fibrosis was estimated as 0.03%[2]. Although life expectancy and quality of life of patients with CF in Turkey are not known exactly, our oldest patient is now 43 years old[3]. Immunoreactive trypsinogen (IRT) is used in newborn screening (NBS) for CF as first-tier test in twenty-two European countries. While Austria, Portugal, Russia, Slovakia and Turkey using the IRT again as second-tier tests; in other countries DNA analysis and/or expanded or extended gene analysis and/or pancreatitis-associated protein are used[4]. NBS has been shown to reduce CF-related morbidity and mortality with early diagnosis and treatment[5]. After the implementation of NBS for CF in our country, patients have been diagnosed before the clinical features of CF emerged[6]. According to European Cystic Fibrosis Society (ECFS) recommendation, early experience with the two-stage IRT/IRT protocol showed that good sensitivity can be achieved[7]. In Russia, after the implementation of NBS for CF patients were diagnosed earlier. NBS had a positive effect on patients' growth, lung, gastrointestinal system findings, and pulmonary exacerbation numbers[8].

Patients diagnosed during NBS have been shown to have better body weight, height, body mass index (BMI), and respiratory function tests, and longer life expectancy[9,10]. There is also evidence that those with a late diagnosis of CF despite NBS have poorer outcomes such as poor lung function, more common chronic *P. aeruginosa* colonization, and increased hospitalization frequency[11]. Some of the neonates cannot be screened because of technical problems in taking of heel blood and parental rejection of NBS. CF diagnosis may be delayed due to the differences in the duration of admission to CF centers of patients with positive NBS, false-negative NBS, or failure to receive dried heel blood sample, and this may lead to disease-related morbidity and mortality. The low IRT values of patients with false-negative NBS were found to be associated with the low quality of the dried heel blood sample[12]. Receiving informed consent from parents for NBS across the globe is a controversial issue reflected in the diversity of NBS programs[9]. The World Health Organization (WHO) recommends measures to nullify parental rejection due to the importance of early diagnosis and treatment with NBS[10].

The aims of this study were to compare demographic characteristics, clinical features, diagnostic tests, treatments, and complications of patients with CF whose NBS results were positive, normal, and not performed in the CF Registry of Turkey (CFRT), to evaluate the effects of NBS on patients' age of diagnosis, clinical features, treatments, and complications, and to evaluate the differences caused by NBS in patients at early diagnosis and follow-up in a country using the IRT/IRT protocol for NBS.

Material and Methods

All demographic and clinical data were obtained in 2018 from CFRT, which was generally created based on ECFS Patient Registry (ECFSPR) variable definitions[13]. In Turkey, NBS for CF was implemented by the Ministry of Health on January 1, 2015. In Turkey, IRT/IRT testing is used in dried heel blood sample as a screening method. IRT values are examined using the standardized fluorometric enzyme immunoassay method by the Public Health Institution of Turkey-Child and Adolescent Health Department in a single facility. If the first IRT is 90 μg/L and above which is taken at 72 hour of life, the second IRT is applied to the patient in 7-14 day of life. If the second IRT is 70 μg/L and above, the screening test is considered positive and the patient is referred to the CF centers for diagnostic tests[3].

According to the ECFS, the number of patients fulfilling the CF diagnostic criteria was recorded in CFRT. The inclusion criteria were two sweat chloride tests > 60 mmol/L or one sweat chloride test > 60 mmol/L and DNA analysis/genotyping-identified two CF-causing mutations. If the sweat chloride test result was ≤ 60 mmol/L, at least two of them needed to be fulfilled: 1) DNA analysis/genotyping—two identified CF-causing mutations and 2) Clinical presentation—typical features of CF[14].

Registries are epidemiological tools that provide continuous and comprehensive monitoring of individual data of patients suffering from any disease in a particular geographic area and aim to collect accurate information about their diseases. In the CF Registry, information about the health status of patients with CF is collected. This information is intended to be used to evaluate the health status of patients with CF, to reveal deficiencies related to care, to assist centers that monitor patients with CF, to guide centers' quality improvement initiatives, and to create care guidelines[15].

Patients with CF in the CFRT, who were born during the NBS process were included in the study. Patients with positive NBS, were classified as Group 1, patients with normal NBS as Group 2 and patients without NBS due to family rejection or technical reasons as Group 3. The data of age at diagnosis, current age, gender, weight, height, BMI and z-scores of weight, height, BMI of patients using the reference values given by the Disease Control Center, NBS, sweat chloride test and genetic tests, fecal elastase and fecal fat results, and history of meconium ileus were received[16]. Sweat test was performed with sweat conductivity or chloride titration by using ECFS quality control procedures[17]. All laboratories in Turkey accredited for sweat chloride test. WHO recommends that the growth of children is assessed with weight and height z-score below two years of age, and BMI z-score above two years of age[18].

The data of the presence of complications such as salt loss (Pseudo-Bartter syndrome), chronic liver disease, CF-related diabetes, allergic bronchopulmonary aspergillosis, pneumothorax, hemoptysis, malignancy, and osteoporosis were obtained. According to ECFSPR guideline, patients with primary

metabolic alkalosis with blood pH > 7.45, serum sodium < 130 mmol/l and serum chloride < 90 mmol/l were accepted as Pseudo-Bartter syndrome.[13] The presence of colonization results such as *Pseudomonas aeruginosa, Staphylococcus aureus, Burkholderia cepacia* complex*, Stenotrophomonas maltophilia,* non-tuberculous mycobacteria, and the presence of microorganisms in respiratory cultures such as mucoid *P. aeruginosa,* non-mucoid *P. aeruginosa, S. aureus,* methicillin-resistant *S. aureus*, *B. cepacia* complex*,* non-tuberculous mycobacteria*, S. maltophilia, Achromobacter* species, and *Haemophilus influenzae* was achieved. Chronic infection was defined as an infection that persists despite treatment and the immune or inflammatory response of the host[20].

The data of using a continuous antibiotic, recombinant human DNase (rhDNase), antibiotics, bronchodilator, azithromycin, ursodeoxycholic acid, pancreatic enzyme replacement therapy (PERT), proton pump inhibitors, multivitamins, calcium, bisphosphonate, insulin, CFTR modulators, enteral nutrition, oxygen therapy, and non-invasive mechanical ventilation were received. The pancreatic sufficiency/insufficiency status of patients was determined according to fecal elastase levels. According to the CFRT data, fecal elastase level is classified as ≥ 200 μg/g and < 200 μg/g as also implented in the ECFSPR guideline[13]. When the fecal elastase level is below 200 μg/g, it is defined as low and indicates pancreatic insufficiency.

The CFTR genotype was recorded and both mutations were classified as severe if classes I, II, or III, and as mild if ≥ 1 mutation were classes IV or V according to previously published classifications[21,22].

Age at diagnosis, current age, gender, weight, height, BMI, and z-scores of weight, height, BMI, NBS, sweat chloride test, genetic test, fecal elastase and fecal fat results, history of meconium ileus, medications, presence of colonization, and complications were compared in patients between Group 1, Group 2, and Group 3.

IBM SPSS Statistics version 22.0 (IBM, Armonk, NY, USA) was used for the statistical analyses. In the descriptive statistics section, categorical variables are presented with numbers, percentages, and continuous variables with mean ± standard deviation and median (minimum–maximum value). The Pearson chi-square test and Fisher's exact test were used to evaluate categorical variables. The Mann–Whitney U test was used for comparative analysis between two independent variables in the data that did not conform to the normal distribution, and the independent sample t-test was used in the data matching the normal distribution. In comparison of three and more variables, one-way variance analysis (ANOVA) was performed where parametric test conditions were ensured, and the Kruskal-Wallis H test was performed where parametric test conditions were not ensured. The relationship between the data that did not conform to the normal distribution was evaluated by Spearman's correlation test, and the data that fit the normal distribution was evaluated by Pearson's correlation test. P-values less than 0.05 were considered statistically significant.

Data entry to the registry has been approved by the local ethics committee and written consent for data entry has been obtained from all patients and/or their parents. All procedures performed in studies involving human participants were prepared in accordance with the ethical standards of the institutional and/or national research committee (Hacettepe University Ethics Board, date: April 12, 2007, reference number: HEK 07/16-21 and date: June 5, 2018, reference number: GO 18/473-31) and the Declaration of Helsinki and subsequent amendments or comparable ethical standards.

Results

Of the 1,488 patients involved in CFRT in 2018, 359 patients who were born after the implementation of NBS for CF between 2015–2018 were included in the study. One hundred and seventy-nine (49.9%) patients were females and 180 (50.1%) were males. Among all included patients, the median age of patients was 2.0 years (0.08- 3.5). The median age at diagnosis was 0.17 years (0.08- 3) among those born during the NBS process. The median age of diagnosis of patients born before the NBS process was 0.5 years (0.08- 41), statistically higher than that of patients born during the NBS process ($p = 0.001$). The median-weight z-score of 168 patients aged below two years was -1.3 (-3.2- 1.7), the height z-score was -0.89 (-3.52- 2.4), and the BMI z-score of 191 patients aged above two years was -0.92 (-3.48-1.9).

There were 299 (83.3%) patients in Group 1, 40 (11.1%) patients in Group 2, and 20 (5.6%) patients in Group 3. The number of females was statistically significantly higher in Group 3 ($p = 0.022$). The median ages of patients were 2.0 years (0.08–3.5) in Group 1, 1.7 years (0.08–3.5) in Group 2, 3.2 years (1.17–3.5) in Group 3 and there was no statistically significant difference between the groups ($p = 0.163$). The median ages at diagnosis were 0.17 years (0.08-2) in Group 1, 0.5 years (0.08-3) in Group 2, and 0.21 years (0.08-2) in Group 3. The median age at diagnosis was significantly higher in Groups 2 and 3 than in Group 1 ($p = 0.001$). Although the weight z-score was significantly lower in Group 3, there was no significant difference between the groups in terms of height and BMI z-score ($p = 0.017$, $p = 0.138$, $p = 0.911$, respectively). A comparison of the demographic characteristics of the groups is given in Table 1.

Sweat test was performed with sweat conductivity test in 147 (40.9%) patients and with chloride titration in 212 (59.1%) patients. Sweat test was performed with sweat chloride titration in 90 patients (30.1%) in Group 1, 13 (32.5%) in Group 2 and 3 (15%) in Group 3. The means of the first and second sweat chloride test results were 77.1±19.5 and 78.3±18.6 mmol/L in Group 1, 62.2±27.0 and 46.9±21.5 mmol/L in Group 2, and 74.2±19.5 and 69.6±9.6 mmol/L in Group 3. There was no significant difference in the first and second sweat chloride test results in all groups ($p = 0.159$, $p = 0.087$, $p = 0.236$ respectively). In Group 1, the first and second sweat chloride test results were similar to Group 3 and significantly higher than Group 2 ($p = 0.009$, $p = 0.001$, respectively). In Group 2, the sweat chloride test of two patients was below 30

mmol/L, eight patients were between 30–60 mmol/L, and two CF-causing mutations were present in their CFTR gene analysis.

Seventeen (5.6%) patients in Group 1, two (5%) patients in Group 2, and five (25%) patients in Group 3 had a history of meconium ileus. The history of meconium ileus was higher in Group 3 than in the other groups ($p = 0.018$) and 45.8% of patients with a history of meconium ileus had severe mutations. Pseudo-Bartter syndrome was present in 20.8%, pancreatic insufficiency in 100%, the use of rhDNase in 79.1%, and chronic *S. aureus* infection was present in 16.6% of patients with a history of meconium ileus.

Fecal elastase levels were low in 69 (23.8%) patients in Group 1, two (5.4%) patients in Group 2, and five (25%) patients in Group 3. Fecal elastase results were statistically significantly higher in Group 2 compared with other groups ($p = 0.033$). Fecal fat was detected in 65 (21.7%) patients in Group 1, 14 (35%) patients in Group 2, and one (5%) patient in Group 3. A comparison of the diagnostic findings of the groups is shown in Table 2.

One-hundred and eight different mutations and polymorphisms were detected in 500 alleles in 268 patients. No mutation has been detected or yet concluded in the genetic tests of 91 patients. F508del was the most common mutation in 119 alleles in patients born during the NBS process and was homozygous in 34 patients. Other common mutations were G542X, D110H, G85E, and L997F. A homozygous F508del mutation was detected in 31 (10.3%) patients in Group 1, two (5%) patients in Group 2, and one (5%) patient in Group 3. Mild mutations were detected in 19 (6.3%) patients in Group 1, 11 patients (27.5%) in Group 2, and two (10%) patients in Group 3. Severe mutations were detected in 92 (30.7%) patients in Group 1, nine (22.5%) patients in Group 2, and four (20%) patients in Group 3. The sweat chloride test results were normal in 44% of patients with severe mutations in Group 2. Other patients had only polymorphisms or unclassified mutations. The frequency of severe mutations in Group 1 was significantly higher than in other groups ($p = 0.001$).

The number of patients using antibiotics for more than three months was 19 in Group 1 and one in Group 2. The use of inhaler bronchodilators was present in 23 patients in Group 1, two patients in Group 2, and two patients in Group 3, and there was no statistically significant difference between the groups ($p = 0.768$). The use of rhDNase was present in 245 (81.9%) patients in Group 1, 25 (62.5%) patients in Group 2, and 17 (85.0%) patients in Group 3, and statistically significantly lower in Group 2 ($p = 0.024$). The use of azithromycin was present in only 10 patients in Group 1. The number of patients using ursodeoxycholic acid was 34 patients in Group 1, three patients in Group 2, and one patient in Group 1. Proton pump inhibitor use was present in 24 patients in Group 1, two patients in Group 2, and one patient in Group 3. The number of patients using PERT was 266 (88.9%) in Group 1, 26 (65%) in Group 2, and 18 (90%) in Group 3, and statistically significantly lower in Group 2 ($p = 0.001$). CFTR modulator use was only in

Group 2 and in one patient. In Group 1, four patients had oxygen supplementation, and two patients used non-invasive mechanical ventilators. A comparison of the groups in terms of their treatments is given in Table 3.

The number of patients with chronic *P. aeruginosa* infection was 29 in Group 1, three in Group 2, and one in Group 3. Chronic *S. aureus* infection was present in 48 (16.0%) patients in Group 1, three (7.5%) patients in Group 2, and eight (40.0%) patients in Group 3, and there was a significant difference between the groups ($p = 0.004$). Chronic *H. influenzae* infection was present in seven patients in Group 1, two patients in Group 2, and one patient in Group 3. The number of patients with chronic methicillin-resistant *S. aureus* infection was 11 in Group 1, two in Group 2, and three in Group 3, and there was no statistically significant difference between groups ($p = 0.105$). In Group 1, non-tuberculous mycobacteria were present in one patient and *S. maltophilia* in 14 patients. *Achromobacter* species were present in two patients in Group 1 and one patient in Group 3. A comparison of chronic infection status and respiratory tract cultures of the groups is shown in Table 4.

Pseudo-Bartter syndrome was present in 40 patients in Group 1, eight patients in Group 2, and four patients in Group 3, and there was no statistically significant difference between the groups ($p = 0.408$). There was no patient with liver disease in Group 3, while liver disease was present in 12 patients in Group 1 and two patients in Group 2. None of the patients had CF-related diabetes, allergic bronchopulmonary aspergillosis, pneumothorax, hemoptysis, malignancy, or osteoporosis.

Discussion

This study showed that in the presence of clinical findings, patients with a normal NBS can be diagnosed as having CF even if their sweat chloride test results are normal. Furthermore, severe mutations can be detected in these patients. Although CF patients with a positive NBS are diagnosed at the earliest, the age of diagnosis is delayed more in patients with normal NBS. Growth retardation and chronic *S. aureus* infection were more common in patients without NBS. The frequency of these complications can be reduced in CF patients with an early diagnosis of NBS.

Castellani et al.[7] emphasized that to minimize CF-related morbidity and mortality, CF should be diagnosed in the first two months, preferably the first month. Patients with normal NBS had an older age at diagnosis. Age at diagnosis with NBS was significantly younger than patients who were born before the NBS process. Sims et al.[23] compared patients diagnosed with NBS in the first two months after birth and patients diagnosed late with their clinical findings and showed that the group diagnosed with NBS had a higher height z-score and Shwachman-Kulczyki score and fewer treatment requirements for longer than three months. In our study, growth retardation, which may lead to decreased respiratory function and poor

prognosis, was more common in patients without NBS. NBS can positively affect weight gain due to early diagnosis, treatment, and regular follow-up of patients, especially before clinical signs occur.

In our study, sweat chloride levels of patients with normal NBS were significantly lower. The patients with normal NBS and sweat chloride tests were diagnosed by proving the presence of two disease causing CF mutations in CFTR gene analysis and typical features of CF. The number of mutations designated as CF causing is not fixed and likely to increase in numbers over time[24]. Therefore CFTR gene analysis of patients with suspected CF should be evaluated intermittently. Nearly half of patients with normal sweat chloride tests and NBS had severe mutations. The normal result of NBS and the sweat chloride test does not exclude the diagnosis of CF. In the presence of clinical features strongly suggesting CF, even if the results of NBS and sweat chloride tests are normal, patients should be evaluated by CFTR gene analysis or, if available, nasal potential difference. Sweat chloride and IRT levels can be normal in patients with mild mutations whose CFTR function is partially preserved.

The most important problem of conductivity testing is that it measures not only chloride ions, but also lactate, bicarbonate and sodium chloride. Therefore, it is not recommended for use in the diagnosis of CF[25]. In our study, sweat test was performed with sweat conductivity test in 40.9% of the patients. Physicians should not forget that there are different reference values from the sweat chloride test when evaluating the result of conductivity method. For the diagnosis of CF, conductivity results must be confirmed by sweat chloride test.

In a multicenter retrospective study in Italy, of the 85 CF patients with a history of meconium ileus, 41 had positive NBS and nine had normal NBS results. NBS was not performed to 35 patients. The age at diagnosis ranged from birth to 386 days, with an average of 31 days[26]. Low levels of IRT can be detected in the presence of meconium ileus. In our study, two patients with a history of meconium ileus had low IRT levels, while 17 patients had high IRT levels. The history of meconium ileus was more common in patients without NBS. The diagnosis of CF is more probable if there is evidence of meconium ileus. The history of meconium ileus has been shown to have adverse effects on the lungs and growth of patients[27]. In our study, growth retardation and chronic *S. aureus* infection were more common in patients without NBS. These poor outcomes may be related to the higher number of meconium ileus in patients without NBS. About half of the patients with a history of meconium ileus had severe mutations. Pseudo-Bartter syndrome was found in about a quarter of patients. The use of rhDNase was present in the vast majority of patients. Chronic *S. aureus* infection was present in about one in five patients. Pancreatic insufficiency was present in all patients. Meconium ileus causes early diagnosis of CF, but it may also be a sign of poor prognosis.

In the meta-analysis in which 40 studies and eight reviews were evaluated, F508del was the most common mutation in patients with positive NBS and was present in more than half of the patients[28]. Sherman et al.[29]

found F508del as the most common mutation in their study comparing 86 patients with positive NBS and 45 patients without NBS, and their frequency was 24% and 44%, respectively. Lumertz et al.[30] reported two patients with normal NBS and homozygous F508del mutation. One of these patients was diagnosed early with meconium ileus, but in follow-up, pancreatic insufficiency and chronic *S. aureus* infection had appeared. The second patient was diagnosed with growth retardation, pancreatic insufficiency, recurrent pneumonia, and chronic *S. aureus* infection at the age of three. In the study of Bozdogan et al., F508del was detected in 5.9% of the patients with positive NBS in Turkey[31]. In our study, 91% of all patients with homozygous F508del mutations were patients with positive NBS. Severe mutation frequency was higher in NBS positive patients. However, homozygous F508del mutation was detected in two patients with normal NBS. One of our patients had pancreatic insufficiency and the use of rhDNase. The other patient had pancreatic insufficiency, chronic MRSA infection, liver disease and the use of rhDNase. Early diagnosis of patients with severe mutations can affect their prognoses. Although F508del is considered a severe mutation, recently it has been classified as Classes II, III, and VI, according to the extended classification[32]. NBS results can be normal in patients with minimal or residual CFTR function. Therefore, the prognosis of patients may differ as expected.

Chronic *S. aureus* infection is detected more frequently in the first three years of age[33]. In a study conducted in Russia with national registry data between 2012–2015, chronic *S. aureus* infection was detected in 38 (84.4%) of 45 patients with a median age at diagnosis of 1.17 months without NBS and 69 (80.2%) of 86 patients with a median age at diagnosis of 0.19 months with positive NBS[29]. In a study evaluating national registry data in Canada, between 2008–2013, chronic *S. aureus* infection was detected in 79 (77.5%) of 102 patients with a median age at diagnosis of 4.9 months without NBS and in 127 (63.2%) of 201 patients with NBS positive a median age of 0.7 months[34]. Similar to the literature, in our study chronic *S. aureus* infection was detected more often in patients without NBS. In children aged below two years, chronic *S. aureus* infections are associated with deterioration in lung function[35]. In the study of Vernooij-van Langen et al.[36], *S. aureus* was found in 25% of NBS-positive patients and in 40% of patients diagnosed without NBS. While there was mild involvement in the chest x-rays of patients with positive NBS, severe findings such as atelectasis, multiple infiltration, and bronchiectasis were found in patients diagnosed with clinical findings. Early diagnosis and treatment initiation with NBS may prevent chronic *S. aureus* infection and early lung damage.

In our study, there were 20 patients without NBS. Refusal of NBS can be prevented by giving families detailed information about NBS and the diseases being screened. Problems of IRT tests due to technical reasons should be decreased to a minimum. There were 40 patients with normal NBS in our study. It is known that the IRT/IRT protocol has lower sensitivity than other protocols such as IRT/DNA or

IRT/IRT/DNA[37]. In our country, DNA analysis is not performed in NBS due to the large variety of mutations[5]. In many countries, as in our country, DNA analysis cannot be made economically.

Our study included the results of a large number of patients across the country. There are few NBS studies evaluating the registry data of countries using the IRT/IRT protocol. Although the results of our study are informative, there are some limitations. IRT levels appear to be able to stratify risk of CF disease even amongst those who have an indeterminate diagnosis of CF at time of initial NBS[38]. In prospective studies, children with CRMS/CFSPID have been shown to have significantly lower IRT values compared to children with CF[39]. IRT results could not be evaluated because there is no data in CFRT about IRT. In our study, 40 of the patients had false-negative NBS. This was the limitation of the screening test. Although ECFS was not recommended to confirm the diagnosis of CF, sweat conductivity test was used in 40.9% of our patients[7]. In this study, the registry data of the patients at only three years were evaluated. Long-term follow-up results of patients with CF who were born during the NBS period may reflect the effects of NBS on patients over the years.

Conclusions

There is a wide spectrum of manifestations and that NBS is merely a screening test. Normal NBS and sweat chloride test results are not sufficient to exclude the diagnosis of CF. It should be kept in mind that CF can also be seen in patients with normal NBS. Normal NBS and sweat chloride tests can also be detected in patients with severe mutations. NBS allows an earlier diagnosis of CF which is associated with improved clinical outcomes.

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Author contribution

T.R.G., A.T.A., P.A., T.S.E., E.C., N.C., S.P., D.U.A., G.K.O., A.B., N.S., E.C., G.D.T., A.O. and K.H. designed the study; M.K., N.E., Z.T., H.Y., G.O., E.T., D.C., P.K.E., G.C., M.K., S.O., G.C., D.D., U.D., E.Y., V.S., O.E., A.A.K. and H.Y. performed experiments; D.U.A., G.K.O., A.B., N.S., E.C., G.D.T., A.O., K.H., M.K., N.E., Z.T., H.Y., G.O., E.T., D.C., P.K.E., G.C., M.K., S.O., T.R.G., A.T.A., P.A., T.S.E.,

E.C., N.C. and S.P. collected and analyzed data; M.K., N.E., Z.T., H.Y., G.O, E.T., D.C., P.K.E., G.C., M.K., S.O., G.C., D.D., U.D., E.Y., V.S., O.E., A.A.K. and H.Y. provided reagents and mice; T.R.G., A.T.A., P.A., T.S.E., E.C., N.C. and S.P. the manuscript; G.C., D.D., U.D., E.Y., V.S., O.E., A.A.K., H.Y., D.U.A., G.K.O., A.B., N.S., E.C., G.D.T., A.O. and K.H. gave technical support and conceptual advice. All authors read and approved the final manuscript.

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Table 1. Comparison of the demographic characteristics of the groups

	Group 1	**Group 2**	**Group 3**	**p**
Number of patients n (%)	299 (83.3)	40 (11.1)	20 (5.6)	
Gender n (%)				
Female	155 (51.8)	20 (50)	16 (80)	**0.022** [a]

Male	144 (48.1)	20 (50)	4 (20)	
Current age (year) (median–range)	2.0 (0.08–3.5)	1.7 (0.08–3.5)	3.2 (1.17–3.5)	0.163[b]
Age at diagnosis (year) (median–range)	0.17 (0.08–2)	0.5 (0.08–3)	0.21 (0.08–2)	**0.001**[b*#]
Weight z-score (aged < 2 years)	-0.89±1.34	-0.47±1.51	-1.15±1.27	**0.017**[c*+]
Height z-score (aged < 2 years)	-0.91±1.56	-0.76±1.65	-0.69±1.51	0.138[c]
Body mass index z-score (aged > 2 years)	-0.53±1.42	-0.64±1.24	-0.76±1.22	0.911[c]

[a] Chi-square test

[b] Kruskal-Wallis H test

[c] One-way variance analysis

P-values less than 0.05 were considered statistically significant and marked in bold.

*When differences were significant ($p<0.05$), a post hoc test was performed to identify the source of the difference.

#Level of significance: Group 1>Group 3

+Level of significance: Group 2>Group 1

Table 2. Comparison of the diagnostic findings of the groups

	Group 1	**Group 2**	**Group 3**	**p**
History of meconium ileus n (%)	17 (5.6)	2 (5)	5 (25)	**0.018** [a]
First sweat chloride test (mean ± SD) (mmol/L)	77.1±19.5	62.2±27.0	74.2±19.5	**0.009**[b*#]

Second sweat chloride test (mean ± SD) (mmol/L)	78.3±18.6	46.9±21.5	69.6±9.6	**0.001**[b*+]
The number of patients with low fecal elastase levels n (%)	69 (23.8)	2 (5.4)	5 (25)	**0.033**[a]

[a] Chi-square test.

[b] One-way variance analysis.

P-values less than 0.05 were considered statistically significant and marked in bold.

*When differences were significant ($p<0.05$), a post hoc test was performed to identify the source of the difference.

[#] Level of significance: Group 2>Group 3

[+]Level of significance: Group 2>Group 3

Table 3. Comparison of the groups in terms of their treatments

	Group 1 n (%)	**Group 2 n (%)**	**Group 3 n (%)**	**p**
Antibiotic (> 3 months)	19 (6.3)	1 (2.5)	0	
RhDNase	245 (81.9)	25 (62.5)	17 (85.0)	**0.024**[a]
Inhaler bronchodilator	23 (7.6)	2 (5.0)	2 (65.0)	0.768[a]

Azithromycin	10 (3.3)	0	0	
Ursodeoxycholic acid	34 (11.3)	3 (7.5)	1 (5.0)	
Proton pump inhibitor	24 (8.0)	2 (5.0)	1 (5.0)	
Pancreatic enzyme replacement therapy	266 (88.9)	26 (65.0)	18 (90.0)	**0.001**[a]
Oxygen therapy	4 (1.3)	0	0	
Noninvasive mechanical ventilation	2 (0.6)	0	0	

[a] Chi-square test.

P-values less than 0.05 were considered statistically significant and marked in bold.

Table 4. Comparison of chronic infection status and respiratory tract cultures of the groups

	Group 1 n (%)	**Group 2 n (%)**	**Group 3 n (%)**	**p**
Chronic *Pseudomonas aeruginosa*	29 (9.6)	3 (7.5)	1 (5.0)	
Chronic *Staphylococcus aureus*	48 (16.0)	3 (7.5)	8 (40.0)	**0.004**[a]
Haemophilus influenzae	7 (2.3)	2 (5.0)	1 (5.0)	
Non-tuberculous mycobacteria	1 (0.3)	0	0	
Stenotrophomonas maltophilia	14 (4.6)	0	0	

***Achromobacter* species**	2 (0.6)	0	1 (5.0)	
Methicillin-resistant *Staphylococcus aureus*	11 (3.6)	2 (5.0)	3 (15.0)	0.105[a]

[a] Chi-square test.

P-values less than 0.05 were considered statistically significant and marked in bold.