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Original Research

A retrospective cohort study of children diagnosed with Cystic Fibrosis after implementation of a newborn screening program in Turkey

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ABSTRACT

Introduction: Newborn screening (NBS) for cystic fibrosis (CF) facilitates early diagnosis and has been shown to significantly improve long-term clinical outcomes. In this study, we aimed to evaluate the 7-year results of the immunoreactive trypsinogen (IRT)/IRT NBS of Turkey.

Methods: The study included all CF patients who were born after NBS implementation, and who were enrolled in the CF Registry of Turkey (CFRT) in 2022. Patients were divided into three groups according to NBS results: Group 1 with positive NBS, Group 2 with negative NBS, and Group 3 with no screening or unknown screening results. All clinical and demographic data were compared between the three groups.

Results: A total of 853 patients were included in the study, 668 (78.3%) patients were in Group 1, 90 (10.5%) in Group 2, and 95 (11.2%) in Group 3. The age at diagnosis was 0.17 (0.08-0.33) years in Group 1, 0.50 (0.25-1.0) in Group 2, and 0.33 (0.17-0.75) in Group 3 ($p<0.001$). The first and second sweat test results and frequency of pancreatic insufficiency were lowest in Group 2 ($p<0.05$). Median FEV1 (%) was 88 (77-103) in Group 1, 90 (71.5-104) in Group 2, 89.5 (81.75-97.5) in Group 3 ($p>0.05$). 49% of the patients had a severe genotype and it was detected most frequently in Group 1 ($p=0.021$).

Conclusions: Patients with pancreatic sufficiency may be missed by IRT/IRT NBS and lower and negative sweat test results may contribute to delays in CF diagnosis. Approximately 22% of patients are not diagnosed through this screening method.

1. Introduction

Early diagnosis and treatment are critical for reducing cystic fibrosis (CF)-related mortality and morbidity. Newborn screening (NBS) is significantly associated with improved quality of life and life expectancy, owing to earlier diagnosis and implementation of CF therapy [1–4].

Worldwide, NBS protocols employ various methods, including the two-stage immunoreactive trypsinogen (IRT)/IRT, IRT/DNA, three-stage IRT/DNA/IRT, IRT/Pancreatitis-Associated Protein (PAP)/IRT, IRT/DNA with extended genetic analysis (EGA), and IRT/PAP/DNA/EGA methods [3–7]. The initial stage of each protocol involves IRT testing, and each method offers unique advantages and disadvantages related to efficacy, cost, and genetic diversity [3,4,8]. In Turkey, the immunoreactive trypsinogen (IRT)/IRT protocol was introduced for CF newborn screening (NBS) in 2015 [5]. In our NBS protocol, a blood sample is collected on Guthrie paper, dried, and analyzed using a standardized fluorometric enzyme immunoassay method within 72 h after birth. If the IRT value in the first blood sample is $\geq$ 90 μg/L, a second IRT sample is collected between the 7th and 14th day after birth. If the second IRT sample value is $\geq$ 70 μg/L, the child is considered positive for IRT/IRT screening and is referred to a CF center for sweat testing [9,10]. While the IRT/IRT protocol for CF NBS has been in place since 2015, its effectiveness in identifying CF cases remains unclear.

CF Registry of Turkey (CFRT) was established in 2007 which systematically records demographic and annual clinical characteristics of CF patients, enabling detailed analyses and regular follow-up [11,9]. In this study, we aimed to assess the 7-year results of the national NBS program using data from the CFRT which is the first to evaluate the long-term outcomes of the IRT/IRT NBS protocol in Turkey. Additionally, it includes the first pulmonary function test (PFT) evaluations of patients diagnosed via NBS.

2. Methods

This was a registry-based retrospective cross-sectional study. All procedures performed in studies involving human participants were prepared in accordance with the ethical standards of the institutional and/or national research committee (University Ethics Board, reference numbers: HEK 07/16–21 and GO 18/473-31) and the Declaration of Helsinki and subsequent amendments or comparable ethical standards.

All demographic, clinical, and laboratory data were obtained from the CFRT as of 2022. Data of patients diagnosed with CF who were born on or after January 1, 2015, which was the initiation date of the IRT/IRT NBS were included in the study. Patients born before the implementation of the NBS program or with incomplete data were excluded. The diagnosis of CF was made based on the European Cystic Fibrosis Society (ECFS) criteria, and patients meeting these inclusion criteria were enrolled in the CFRT. The inclusion criteria were two sweat tests >59 mmol/L chloride, one sweat test >59 mmol/L chloride, and DNA Analysis/Genotyping - two identified disease-causing CF mutations. If the sweat value is less than or equal to 59 mmol/L, at least two of the listed criteria must be met; DNA Analysis/Genotyping - two identified diseases causing CF mutations and/or clinical presentation - typical features of CF [12,13]. Sweat tests were performed following ECFS standards. Pancreatic insufficiency was recorded if fecal elastase levels were below 200 μg/g, while levels of 200 μg/g or above were recorded as pancreatic sufficiency. Genetic mutations were evaluated, and patients with two mutations in classes 1, 2, or 3 were classified as having a “severe genotype”, whereas those with at least one mutation in classes 4, 5, or 6 were classified as having a “mild genotype” [14]. Variants that are not included in CFTR variant databases and with no accessible variant classifications are defined as "unknown". PFT was conducted following the criteria recommended by the American and European Respiratory Societies [15]. In Turkey, PFTs are usually performed after the age of six. NBS results were interpreted in CFRT as ‘performed--positive’, ‘performed-negative’, and ‘results unknown/not performed’ without IRT levels.

Weight, height, and body mass index (BMI) z-scores were recorded using reference values published by the Centers for Disease Control [16]. Data collected included the patient’s age, body weight, height, BMI z-scores, 1st and 2nd sweat test results, fecal elastase levels, fecal fat levels, genetic mutations, PFT values, sputum culture results, and colonization status (e.g., *Pseudomonas aeruginosa* (PA), chronic Methicillin-sensitive *Staphylococcus aureus* (MSSA), chronic Methicillin-resistant *Staphylococcus aureus* (MRSA), chronic *Burkholderia cepacia* complex, chronic *Haemophilus influenzae*, nontuberculous mycobacteria, *Stenotrophomonas maltophilia*, Achromobacter species, and fungal cultures). The last recorded weight and height are entered into the registry, but if the patient had a PFT in 2023, the best FEV1 value of the year along with the concurrent weight and height are recorded. Sputum culture results from 2023 were included in the study.

Therapeutic data included the use of pancreatic enzyme replacement therapy (PERT), inhaled and oral treatments (e.g., recombinant human deoxyribonuclease [rhDNase], inhaled hypertonic saline [for more than three months], inhaled mannitol [for more than three months], continuous inhaled bronchodilators [for more than three months], inhaled steroids, oral steroids, continuous macrolides [for more than three months], ursodeoxycholic acid [UDCA], proton pump inhibitors [PPI], enteral nutrition, multivitamins, and vitamin A, D, E, and K, calcium supplementation), as well as oxygen therapy and modulator treatments. Noninvasive mechanical ventilation (NIPPV) status, the

presence and number of pulmonary exacerbations in the last year, the number of days in the hospital, and the number of days on IV antibiotics were also recorded.

CF-related complications such as Pseudo-Bartter syndrome, chronic liver disease, CF-related diabetes, distal intestinal obstruction syndrome (DIOS), allergic bronchopulmonary aspergillosis (ABPA), sinusitis, and major hemoptysis were systematically documented in the CFRT [11,12].

Patients were divided into three groups based on their NBS results: Group 1 included those with positive NBS results, Group 2 included those with negative NBS results, and Group 3 included those who were either never screened due to family rejection or technical reasons, or whose NBS results were unknown. In Group 2, patients with an initial IRT value below 90 and, if performed, a second IRT value below 70 were considered IRT screening negative. Clinical and demographic data were compared across the three groups.

3. Statistical

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 x^2 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 data that did not conform to the normal distribution, and the independent sample *t*-test was used in 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 were evaluated by Pearson's correlation test. P-values less than 0.05 were considered statistically significant.

4. Results

In 2022, there were a total of 2088 children with CF, of whom 931 were born after the initiation of the NBS program. A total of 78 patients were excluded due to missing data, leaving 853 patients included in the study. Fig. 1 illustrates the distribution of these patients.

When divided into three groups based on NBS results, there were 668 patients (78.3 %) in Group 1, 90 patients (10.5 %) in Group 2, and 95 patients (11.2 %) in Group 3. Of the total cohort, 410 (48 %) were girls, and 443 (52 %) were boys. The median age at diagnosis was 0.47 years (range: 0–7 years), and the current median age was 4.33 years (range: 2.5–6.16 years).

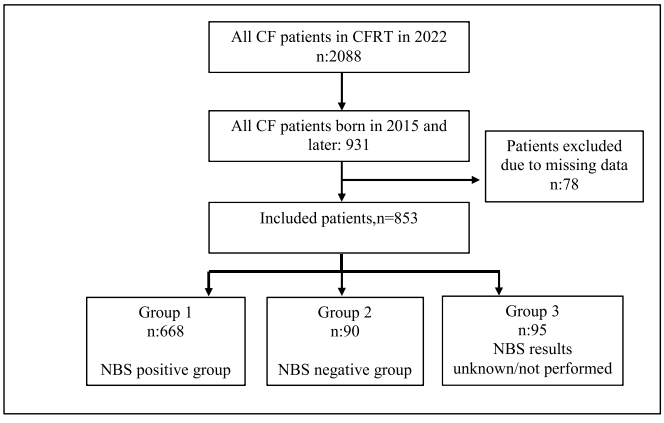

Fig. 1. Flowchart of study population *(CFRT: Cystic Fibrosis Registry of Turkey, NBS: Newborn screening)*.

The median weight z-score was −0.74 (range: 6.37 to 6.20), height z-score was −0.81 (range: 10.97 to 5.27), and BMI z-score was −0.39 (range: 4.31 to 8.57). The median first sweat test value was 70 mmol/L (range: 50–89 mmol/L), and the median second sweat test value was 69 mmol/L (range: 42–87 mmol/L). The median FEV1 was 87 (range: 39–132), and the FVC was 93 (range: 39–145). Among all patients, 49 % had a severe genotype, 18 % had a mild genotype, and 33 % had an unknown genotype.

The demographic and clinical data of the groups were compared. The proportion of females was significantly lower in Group 3 ($p = 0.049$). The median age at diagnosis was 0.17 years (range: 0.08–0.33) in Group 1, 0.50 years (range: 0.25–1.0) in Group 2, and 0.33 years (range: 0.17–0.75) in Group 3 ($p < 0.001$). Patients in Group 1 had the highest current median age (4.5 years compared to 4.08 years in Group 2 and 3.5 years in Group 3; $p = 0.016$).

There were no statistically significant differences in weight, height, or BMI z-scores among the groups ($p = 0.067$, $p = 0.083$, and $p = 0.234$, respectively). Group 2 had the lowest median first sweat test value (63 mmol/L compared to 76 mmol/L in Groups 1 and 3; $p = 0.006$) and the lowest median second sweat test value (59.5 mmol/L compared to 76 mmol/L in Group 1 and 65 mmol/L in Group 3; $p = 0.002$).

Pancreatic insufficiency was observed in 541 patients (81 %) in Group 1, 52 patients (58.4 %) in Group 2, and 77 patients (82.8 %) in Group 3, with the lowest prevalence in Group 2 ($p < 0.001$). No differences were observed among the groups in terms of FEV1 ($p = 0.934$) and FVC ($p = 0.735$).

Severe genotypes were significantly more common across all groups ($p = 0.016$) and were most frequently observed in Group 1, where 344 patients (60.1 %) had severe mutations ($p = 0.021$). A detailed comparison of the demographic and diagnostic findings across the groups is presented in Table 1.

When the treatments of the three groups were compared, bronchodilator use was highest in Group 2, with 9 patients (10.1 %) using it ($p = 0.003$), while PERT use was lowest in Group 2, with only 52 patients (58.4 %) receiving it ($p < 0.001$). No statistically significant differences were observed among the groups in terms of the use of rhDNase, mannitol, hypertonic saline, inhaled antibiotics, azithromycin prophylaxis, oxygen, inhaled steroids, UDCA, or PPI ($p > 0.05$). When evaluating the use of modulator therapies, it was observed that the use of ivacaftor was statistically significantly higher in Group 2 ($p < 0.001$). A detailed comparison of treatments across the groups is provided in Table 2.

No statistically significant differences were found among the groups in terms of microbiological agents ($p > 0.05$). A comparison of chronic infection status and respiratory tract cultures across the groups is presented in Table 3.

There were no statistically significant differences among the groups in terms of complications such as liver disease, meconium ileus, ABPA, DIOS, Pseudo-Bartter syndrome, pneumothorax, hemoptysis, diabetes, presence of pulmonary exacerbations in the last year, number of pulmonary exacerbations, number of days of hospitalization, or days of IV antibiotic use ($p > 0.05$). A comparison of complications across the groups is shown in Table 4.

There was a statistically significant negative correlation between age at diagnosis and first sweat test results in Group 1 ($p = 0.001$, $r = -0.173$) and Group 3 ($p = 0.025$, $r = -0.354$) (see Table 5). There was a statistically significant negative correlation between age at diagnosis and second sweat test results and in Group 1 ($p = 0.001$, $r = -0.211$) and Group 2 ($p = 0.018$, $r = -0.365$).

5. Discussion

This study showed that patients with pancreatic sufficiency may be missed by IRT/IRT NBS. Lower or negative sweat test results may contribute to delays in CF diagnosis. Further evaluation should be

Table 1
Comparison of the demographic characteristics and diagnostic findings of the groups.

	Group 1 [median(min-max)]	Group 2 [median (min-max)]	Group 3 [median(min-max)]	P
Female n (%)	334 (50)	41 (45.6)	35 (36.8)	**0.049**
Male n (%)	334 (50)	49 (54.4)	60 (63.2)	
Age of diagnosis (year)	0.17 (0.08–0.33)	0.50 (0.25–1.0)	0.33 (0.17–0.75)	**<0.001**
Current age (year)	4.5 (2.5–6.16)	4.08 (2.16–6.28)	3.5 (2.08–5.5)	**0.016**
Weight z-score	−0.93 (−1.60–−0.21)	−0,45 (−1.46-0.22)	−0.98 (−1.48–−0.14)	0.067
Height z-score	−0.67 (−1.91-0.35)	−0.36 (−1.63-0.78)	−0.87 (−1.95–−0.13)	0.083
BMI z-score	−0.61 (−1.32-0.19)	−0.51 (−1.59-0.44)	−0.39 (−1.23-0.54)	0.234
Pancreatic insufficiency n (%)	541 (81)	52 (58.4)	77 (82.8)	**<0.001**
1st ST (mmol/L)	76 (10–160)	63.0 (11–118)	76 (17–115)	**0.006**
2nd ST (mmol/L)	76 (10–140)	59.50 (14–112)	65 (21–105)	**0.002**
PFT	n:65	n: 9	n: 16	
FEV1 (%)	88 (77–103)	90 (71–104)	89.5 (81–97)	0.934
FVC (%)	93 (80–107)	100 (80–105)	93 (82–111)	0.735
Genotype[a]				
Mild	111 (16.6)	23 (25.5)	20 (21)	
Severe	344 (51.5)	33 (36.7)	41 (43.2)	**0.021**
Unknown	213 (31.9)	34 (37.8)	34 (35.8)	

BMI: Body mass index, FEV1: Forced Expiratory Volume in 1 s, FVC: Forced Vital Capacity, PFT: Pulmonary Function Test, ST: Sweat test.
[a] Column percentage.

Table 2
Comparison of the groups in terms of treatments.

	Group 1 n (%)	Group 2 n (%)	Group 3 n (%)	P
Pulmonary Treatments				
RhDNase	516 (77.5)	69 (74.2)	69 (77.5)	0.776
Mannitol	4 (3.1)	0	0	0.578
Hypertonic saline	49 (7.3)	7 (7.9)	6 (6.5)	0.931
Inhaled antibiotic	61 (9.1)	8 (9)	7 (7.5)	0.879
Azithromycin	33 (4.9)	3 (3.4)	2 (2.2)	0.413
Bronchodilator	14 (2.1)	9 (10.1)	3 (3.2)	**0.003**
Oxygen	6 (0.9)	0	3 (3.2)	0.071
NIPPV	5	0	0	0.504
Inhaled steroid	73 (10.9)	13 (14.6)	11 (11.8)	0.586
Oral steroid	5 (0.7)	0	0	0.504
Other Treatments				
PERT	541 (81)	52 (58.4)	77 (82.8)	**<0.001**
UCDA	82 (12.3)	9 (10.1)	8 (8.6)	0.523
PPI	54 (8.1)	2 (2.2)	4 (4.3)	0.071
Modulator therapies				
Ivacaftor	6 (0.9)	6 (6.7)	0	**<0.001**
Lumacaftor/Ivacaftor	8 (1.2)	0	1 (1.1)	–
Tezacaftor/Ivacaftor	0	0	0	–
Elexacaftor/Tezacaftor/Ivacaftor/Ivacaftor	7 (1)	1 (1.1)	1 (1.1)	0.664
Total	21 (3.1)	7 (7.7)	2 (2.2)	

RhDNase: Recombinant human DNAase, NIPPV: Non-invasive positive pressure ventilation, PERT: Pancreatic enzyme replacement therapy, PPI: Proton Pomp Inhibitor, UCDA: Ursodeoxycholic asid

Table 3
Comparison of chronic infection status and respiratory tract cultures among the groups.

	Group 1	Group 2	Group 3	P
MSSA	78 (11.8)	12 (13.6)	9 (9.6)	0.661
PA	44 (6.7)	3 (3.4)	8 (8.6)	0.587
MRSA	18 (2.7)	1 (1.1)	4 (4.3)	0.677
H. influenzae	3 (0.5)	2 (2.3)	1 (1.1)	0.241
B. cepacia complex	1 (0.2)	0	0	–
S. maltophilia	2 (0.3)	0	0	–
Achromobacter	2	0	0	–
Non-TB mycobacteria	3	0	0	0.548
Fungal culture positivity (at least 1+/year)	42 (6.4)	6 (6.7)	7 (7.4)	0.959
A. fumigatus	3 (0.5)	0	1 (1.2)	–

MSSA: Methicillin-sensitive *Staphylococcus aureus*, PA: *Pseudomonas aeruginosa*, MRSA: Methicillin-resistant *Staphylococcus aureus*

Table 4
Comparison of CF-releated complications of the groups.

	Group 1	Group 2	Group 3	P
Liver disease n (%)	64 (9.5)	6 (6.7)	8 (8.4)	0.155
Meconium ileus n (%)	47 (7.2)	5 (5.7)	8 (8.5)	0.929
ABPA n (%)	3 (0.5)	1 (1.1)	0	–
DIOS n(%)	2 (0.3)	0	0	–
Salt loss n (%) (Pseudo-Bartter Syndrome)	32 (4.8)	4 (4.4)	4 (4.3)	0.966
Pneumothorax n (%)	1	0	0	–
Major hemoptysis n (%)	1	0	0	–
CFRD n (%)	4 (0.6)	1 (1.1)	2 (2.1)	0.676
Presence of pulmonary exacerbation last year n (%)	127 (19.2)	15 (16.7)	14 (14.9)	0.549
Number of pulmonary exacerbations (mean ± SD)	0.32 (±0.03)	0.26 (±0.07)	0.29 (±0.09)	0.579
Number of days of hospitalization (mean ± SD)	4.55 (±0.45)	5.25 (±2.04)	3.90 (±1.27)	0.189
Days of IV antibiotic use (mean ± SD)	3.64 (±0.37)	2.44 (±0.74)	3.53 (±1.17)	0.526
Patient died n (%)	0	3	0	–

ABPA:Allergic Bronchopulmoner Aspergillosis, CFRD: Cystic fibrosis-related diabetes, DIOS: Distal Intestinal Obstruction Syndrome, IV: intravenous.

Table 5
Corelation of the groups, sweat test and diagnose age.

	Group 1		Group 2		Group 3	
	Age at diagnose		Age at diagnose		Age at diagnose	
	p	r	p	r	p	r
1st Sweat test	0.001	−0.173	0.114	−0.217	0.025	−0.354
2nd Sweat test	0.001	−0.211	0.018	−0.365	0.141	−0.291

conducted in patients with clinical suspicion, even if the NBS result is negative. A positive IRT/IRT NBS for CF allows for earlier diagnosis, and severe mutations were more commonly observed in these patients. Although IRT/IRT NBS for CF has been implemented in Turkey since 2015, approximately 22 % of patients with CF were not diagnosed through national NBS.

One of the key findings of this study is the presence of 90 patients (10.5 %) who were initially negative for NBS but were diagnosed later based on clinical suspicion, history, and clinical findings. In clinical practice, CF should be considered in the differential diagnosis when there is clinical suspicion, even if the NBS result is negative. A negative NBS result and normal or near-normal sweat test results should not rule out the diagnosis of CF. In CF patients with negative NBS results, genotypic characteristics tend to reflect a phenotype with less pancreatic insufficiency, near-normal sweat test results, and a milder disease course

compared to those with positive NBS results. In a study by Ramaslı et al., using similar methodology during the third year of the CF IRT/IRT NBS in Turkey with CFRT data, patients with negative NBS results were diagnosed later, had less pancreatic insufficiency, and their sweat test results were near normal [17]. Based on these findings, it can be concluded that IRT levels are usually higher in patients with pancreatic insufficiency than in pancreatic sufficient patients, which may lead to pancreatic sufficient patients being missed by the IRT/IRT NBS protocol. Therefore, it is not always possible to diagnose CF through NBS in this group.

The goal of NBS programs for CF is to identify as many CF cases as possible while minimizing false positives and keeping costs as low as possible. It is recommended that the appropriate screening program be selected based on the genetic diversity of the population and current economic conditions. According to the ECFS Best Practice Guidelines, the IRT/IRT method currently used in our country is recognized as a viable approach for CF NBS [18]. When comparing the 7th-year data with the 3rd-year data, in the study by Ramaslı et al., it was observed that the percentage of patients not diagnosed through CF NBS was 16.7 % in the 3rd year, but this rate increased to approximately 22 % in the 7th year. Additionally, one in five patients was not diagnosed through NBS [17]. Maybe reducing the cut-off points of IRT-1 and IRT-2 may avoid a late diagnosis in severe genotypes, and would be more accurate cut-off points without increasing very much the false positives and the final costs.

Various methods are employed for CF NBS worldwide, with all protocols starting with the IRT test. A study conducted in Poland over 20 years evaluated different CF NBS methods, including IRT/IRT, IRT/IRT/DNA, IRT/IRT/DNA-IRT/DNA, and IRT/DNA/EGA. The study found that 11 patients were not diagnosed through CF NBS, 10 of whom were missed because their screening was terminated at the first stage due to normal IRT test results. This finding underscores that if the first-stage IRT test is normal, these patients cannot be detected, regardless of the complexity or accuracy of subsequent methods [19]. Similarly, our study highlights the critical importance of the NBS test results in determining the outcome of the entire screening process. A normal NBS result means the patient is not flagged for further testing, which can lead to undiagnosed CF cases even when the disease is present. For this group of patients, clinical suspicion remains vital, and CF should still be considered in cases where symptoms or history warrant further investigation.

A study from the Netherlands evaluated the performance of the IRT/PAP/DNA/EGA method for CF NBS by narrowing the cut-off values for the initial IRT and PAP tests and increasing the number of analyses through two different protocols. The study demonstrated that increasing the number of analyses and stages improved the true positive rate while reducing the false negative rate, with only a minimal increase in costs [20]. Given that some patients, particularly those with negative NBS results, are not diagnosed through the current screening method, reassessing and strengthening the screening approach is crucial. In this regard, increasing the number of analyses and screening stages could enhance the true positive rate and reduce false negatives; however, the associated costs make implementing more advanced screening protocols currently unfeasible in our country.

In Denmark, the IRT/DNA(F508del)/Next Generation Sequencing (NGS) method is used for CF NBS. Over two years, 126,338 newborns were screened, and 22 were diagnosed with CF through NBS. However, 2 patients were missed and did not receive a CF diagnosis via NBS, resulting in a false negative rate of 8.33 % among diagnosed CF patients. According to genetic analyses conducted in Europe, the Danish population has the highest proportion of the F508del mutation among CF patients [21]. Although this method is well-suited to the genetic profile of the Danish population, it does not apply to our country due to the high genetic diversity of CF mutations reported in our population [22].

A study conducted in Brazil evaluated 840,976 newborns screened for CF using the IRT/IRT NBS protocol. During the study period, 49 children were diagnosed with CF, of whom 39 were identified through NBS, while 10 (20.4 %) were diagnosed based on clinical suspicion (false-negative NBS). In our study, the proportion of patients with negative NBS results was 10.5 %, and those with unknown NBS results were 11.2 %, indicating that 21.7 % of CF patients were not diagnosed through the IRT/IRT NBS method. This finding aligns with studies from Brazil, suggesting that the IRT/IRT method may fail to diagnose CF in approximately 10 % of cases [23].

Early diagnosis of CF has been shown to improve prognosis, increase survival rates reduce complications, and enhance the overall quality of life and long-term health outcomes in affected individuals. NBS programs facilitate early diagnosis of CF and play a crucial role in the more effective management of respiratory and gastrointestinal complications [24,25]. A study by Leung et al. [26]. demonstrated that children diagnosed with NBS showed better weight gain in the first year of life compared to previous cohorts. In our study, when evaluating growth parameters, chronic colonization status, and CF-related complications, no significant differences were found among the three groups. This was attributed to the possibility of closer monitoring, as all patients were diagnosed within the first year of life.

In CF, chronic pathogen colonization and pulmonary exacerbations are well-known as leading contributors to morbidity and mortality [27,28]. In the 3rd-year data by Ramaslı et al., there was no information available on the use of modulator therapies. However, in the 7th-year data, 30 patients (3.5 %) were documented as having received modulator therapy. The reduction in pathogen colonization observed between the 3rd and 7th years may be attributed to the longer follow-up periods and the increased use of modulator treatments during this time [23,29]. Also, a greater proportion of the children in Group 2 were receiving bronchodilators, which may be related to the increased asthma phenotype in relatively milder CF patients.

The use of CFTR-specific modulator therapies has revolutionized the treatment of CF, particularly in managing pulmonary complications [30,31]. Ivacaftor is a single-agent modulator that binds to the CFTR protein, prolonging the duration of the channel's open state. It is highly effective in patients with gating mutations due to this mechanism [32]. Studies by Ramsey et al. [33] and Gould et al. [34] have shown that CFTR modulators improve exocrine pancreatic function and help alleviate pancreatic insufficiency. In our study, children in the NBS-negative group used less pancreatin and more Ivacaftor. These findings suggest that the mutations in this group are more likely associated with gating mutations in CF. Personalized treatments should be prioritized in CF management, as selecting appropriate modulator therapies based on a patient's specific mutations can significantly improve prognosis.

There are some limitations in this study. It was a retrospective study, 78 patients were excluded due to missing data, and PFT could not be performed by all patients. Our study focuses on the IRT/IRT protocol results and patients diagnosed with CF through NBS, as well as those who did not receive a CF diagnosis through NBS. However, it does not include newborns who underwent screening but did not receive a CF diagnosis. Therefore, the sensitivity, specificity, and predictive values of the test could not be directly calculated. The classification of CFTR mutations as "severe" (class 1, 2, and 3) and "mild" (class 4, 5, and 6) is based on general functional effects; however, there are some exceptions. For example, L206W may not always result in a severe phenotype, while certain class 4 mutations, such as R347P and R334W, can lead to a more severe disease course. Additionally, some class 5 and 6 mutations, such as 1811 + 1,6kbA > G, may not be considered mild. Therefore, the classification of CFTR mutations should be interpreted with caution, and this represents another limitation of our study. The lack of genotype stratification in Group 2 patients is a limitation of our study, as it may affect the interpretation of disease severity, treatment burden, and respiratory status. While further analyses incorporating genotypes, such as regression models, could provide a more comprehensive evaluation, these were beyond the scope of our current study.

In conclusion, patients with less commonly detected pancreatic

insufficiency may be missed by IRT/IRT-based NBS. Lower or negative sweat test results may contribute to delays in CF diagnosis. In cases of clinical suspicion, CF should be investigated in patients even if their NBS result is negative. It should be considered that CF patients may have lower or intermediate sweat test results due to milder mutations. The absence of differences in growth parameters among patients may be attributed to the fact that all patients were diagnosed within the first year of life. Although IRT/IRT NBS has been implemented for CF in Turkey, approximately 22 % of children with CF remain undiagnosed through this screening method. Based on these findings, especially for patients not diagnosed through NBS, it would be valuable to review and potentially revise the method and implementation of the NBS program in Turkey. Longer follow-ups may provide clearer results.

CRediT authorship contribution statement

Handan Kekeç: Writing – original draft. **Tuğba Şişmanlar Eyüboğlu:** Project administration. **Ayşe Tana Aslan:** Supervision. **Zeynep İlkşen Hocoğlu:** Data curation. **Ebru Yalçın:** Data curation. **Birce Sunman:** Data curation. **Burcu Çapraz Yavuz:** Data curation. **Velat Şen:** Data curation. **Suat Savaş:** Data curation. **Ayşe Ayzıt Kılınç:** Data curation. **Azer Kılıç Başkan:** Data curation. **Hakan Yazan:** Data curation. **Gökçen Ünal:** Data curation. **Yakup Canıtez:** Data curation. **Nihat Sapan:** Data curation. **Figen Gülen:** Data curation. **Gökçen Kartal Öztürk:** Data curation. **Özlem Keskin:** Data curation. **Elif Arık:** Data curation. **Mehmet Köse:** Data curation. **Ali Ersoy:** Data curation. **Derya Ufuk Altıntaş:** Data curation. **Mahir Serbes:** Data curation. **Abdurrahman Erdem Başaran:** Data curation. **Ayşen Bingöl:** Data curation. **Ali Özdemir:** Data curation. **Meral Barlık:** Data curation. **Gökşen Dilşa Tuğcu:** Data curation. **Işıl Bilgiç:** Data curation. **Hülya Anıl:** Data curation. **Beste Özsezen:** Data curation. **Merve Nur Tekin:** Data curation. **Hasan Yüksel:** Data curation. **Gönül Çaltepe:** Data curation. **Melih Hangül:** Data curation. **Zeynep Gökçe Gayretli Aydın:** Data curation. **Mehmet Kılıç:** Data curation. **Mina Hızal:** Data curation. **Nilay Baş İkizoğlu:** Data curation. **Gizem Özcan:** Data curation. **Nagehan Emiralioğlu:** Data curation. **Güzin Cinel:** Data curation. **Sevgi Pekcan:** Data curation. **Erkan Çakır:** Data curation. **Uğur Özçelik:** Data curation. **Deniz Doğru:** Project administration.

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Declaration of competing interest

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