Journal of Cystic Fibrosis xxx (xxxx) xxx

Contents lists available at ScienceDirect

Journal of Cystic Fibrosis

journal homepage: www.elsevier.com/locate/jcf

Original Article

Cystic fibrosis newborn screening in Switzerland – evaluation and scenarios for improvement after 11 years of follow-up

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ARTICLE INFO

Keywords:
Cystic fibrosis
Newborn screening
Evaluation
Performance
Screening algorithm
Safety net
Sensitivity
Specificity
Positive predictive value
Simulation

ABSTRACT

Background: Newborn bloodspot screening (NBS) for cystic fibrosis (CF) is important for early diagnosis and treatment. However, screening can lead to false-positive results leading to unnecessary follow-up tests and distress. This study evaluated the 11-year performance of the Swiss CF-NBS programme, estimated optimal cut-offs for immunoreactive trypsinogen (IRT), and examined how simulated algorithms would change performance.
Methods: The Swiss CF-NBS is based on an IRT–DNA algorithm with a second IRT (IRT-2) as safety net. We analysed data from 2011 to 2021, covering 959,006 IRT-1 analyses and 282 children with CF. We studied performance based on European Cystic Fibrosis Society (ECFS) standards including sensitivity, specificity, positive predictive value (PPV), false negative rate, and second heel-prick tests; identified optimal IRT cut-offs using receiver operating characteristics (ROC) curves; and calculated performance for simulated algorithms with different cut-offs for IRT-1, IRT-2, and safety net.
Results: The Swiss CF-NBS showed excellent sensitivity (96 %, 10 false negative cases) but moderate PPV (25 %). Optimal IRT-1 and IRT-2 cut-offs were identified at 2.7 (>99th percentile) and 5.9 (>99.8th percentile) z-scores, respectively. Analysis of simulated algorithms showed that removing the safety net from the current algorithm could increase PPV to 30 % and eliminate >200 second heel-prick tests per year, while keeping sensitivity at 95 %.
Conclusion: The Swiss CF-NBS program performed well over 11 years but did not achieve the ECFS standards for PPV (≥30 %). Modifying or removing the safety net could improve PPV and reduce unnecessary follow-up tests while maintaining the ECFS standards for sensitivity.

1. Introduction

Newborn bloodspot screening (NBS) for cystic fibrosis (CF) has become an essential component of early CF diagnosis in many countries [1,2]. The main goal of NBS programmes is to detect infants with a treatable disease early, to initiate treatment, prevent symptoms, and decrease mortality [3]. While CF-NBS programmes have benefits for children with CF, it is important to be aware of possible harmful effects. Every follow-up test, such as a second IRT measurement or sweat test, due to a false-positive CF-NBS result causes distress for the family and burdens the health-care system [4–6]. Furthermore, screening can result in an unclear diagnosis, currently known as CF transmembrane

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https://doi.org/10.1016/j.jcf.2024.04.008
Received 15 December 2023; Received in revised form 11 April 2024; Accepted 15 April 2024

conductance regulator (*CFTR*)-related metabolic syndrome or cystic fibrosis screening positive inconclusive diagnosis (CFSPID) [7]. Since most children with CFSPID remain healthy throughout their childhood and adolescence, most European programmes try to minimize the detection of children with CFSPID, because no early treatment can be offered to them and there is a risk of over-medicalisation [7–11] . Regular evaluation of screening programmes is essential to ensure high sensitivity while unnecessary testing, stress, and overtreatment of healthy children are kept minimal [12,13].

CF-NBS programmes differ between countries due to regional differences in *CFTR* variants and different health care and economic systems [8]. According to the European CF Society (ECFS), sensitivity of a CF-NBS programme should be at least 95 % and the positive predictive value (PPV) at least 30 % [14]. A European study from 2019 including 22 national and 34 regional CF-NBS programmes found large differences in performance between algorithms with sensitivities ranging from 67 to 100 % and PPVs from 2 to 91 % [9]. In Switzerland, NBS for CF was introduced in 2011 [15,16] based on an immunoreactive trypsinogen (IRT) - deoxyribonucleic acid (DNA) screening algorithm. A second heel-prick test with IRT-measurement was included as a safety net in case no CF-causing *CFTR* variant is detected in children with an elevated initial IRT [17–20]. The Swiss CF-NBS was followed-up prospectively since its implementation, offering a unique opportunity for a long-term evaluation. This study aimed to evaluate the 11-year performance of the Swiss CF-NBS, estimate the optimal cut-offs for the first and second IRT tests, and investigate simulated (hypothetical) scenarios for algorithm optimisation.

2. Materials and methods

We used data from the national NBS laboratory on all newborns screened in Switzerland by heel-prick tests from 01.01.2011 to 31.12.2021 (N = 959,006), and from the national CF-NBS database, which includes all children with a positive CF screening result (N = 1106), and those diagnosed with CF outside of the CF-NBS in the same period (N = 10). Children lost to follow-up or those who died after a positive screening without receiving a final diagnosis were excluded from the analyses (n = 16), resulting in an analysis set of N = 1100 children (1090 screen positive with final diagnosis and 10 false negative children). The Swiss CF-NBS programme consists of two parts [6,15,16,19,21]: screening by the NBS laboratory, and, for positively screened infants, diagnostic evaluation by specialist paediatric CF centres (Supplemental Figure 1).

2.1. Procedure in the newborn screening laboratory

The Swiss CF-NBS measures IRT (IRT-1) using blood from a heel-prick test (Guthrie card) on the 4th day of life (72–96 h) for all newborns in Switzerland (Supplemental Figure 1). If IRT-1 is above the specified cut-off (99.2nd percentile (P) since May 2011, currently >95 ng/mL [20]) or the child has meconium ileus, the blood sample is tested for the most common CF-causing *CFTR* variants in Switzerland: initially, only 7 *CFTR* variants covering 98 % of CF cases were tested (F508del, 3905insT, G542X, R553X, W1282X, 1717–1G>*A*, N1303K) [15], and since 2013 additional 11, as the components of the original self-developed inhouse kit were no longer available and it was necessary to switch to a commercially available test kit (Test strip A, Vienna Lab Diagnostics GmbH, Vienna, Austria)(Supplemental Table 1). If at least one variant is found, the newborn is designated a positive screening result and referred for further evaluation to a specialist paediatric CF centre. These children are considered direct referrals. Due to the strict genetic law in Switzerland, the NBS laboratory is not allowed to communicate the specific *CFTR* variant detected in the genetic screening to the CF centre, as we do not have written informed consent from the parents. Therefore, the NBS laboratory only reports whether one or two of the 18 *CFTR* variants screened were detected. If no variant is found and IRT-1 was >99.6th P (currently >110 ng/ml), the safety net is initiated. The midwife or the family physician calls the family back within 2–3 weeks for a second heel-prick test with IRT measurement (IRT-2). If IRT-2 is again above the same cut-off as for IRT-1 (99.2nd P) the infant is also designated a positive screening result and referred to a specialist paediatric CF centre. These children are considered indirect referrals via safety net. The screening (IRT-1, *CFTR* variants, IRT-2, blood taking institution) and basic demographic information (date of birth, sex, gestational age, birth weight, and age at blood sampling) for all children referred to a specialist paediatric CF centre are entered by the staff at the national newborn screening lab into a central CF-NBS database hosted at the Institute of Social and Preventive Medicine (ISPM), University of Bern.

2.2. Diagnostic evaluation in the specialist paediatric CF centres

All infants designated as screening positive are invited to the nearest specialist paediatric CF centre for a diagnostic sweat test. In all CF centres, two different sweat tests (Macroduct and Nanoduct) are used simultaneously [21]. If both sweat tests are negative, the infant is considered a healthy carrier. If the sweat tests are positive or intermediate, genetic analysis (50 most common CF-causing *CFTR* variants) is performed. If only one *CFTR* variant is found, a complete *CFTR* gene sequencing is performed. If too little sweat is available (minimum 15 µl required for the Macroduct sweat test) to determine chloride content, a faecal elastase test is performed. If the faecal elastase is normal, the sweat test is repeated when the child weighs at least 4000 g. If the faecal elastase result is pathological or two known CF-causing *CFTR* variants were identified in the screening, the child is also referred for genetic analysis (Supplemental Figure 1) [17]. Clinical data, diagnostic test results, and *CFTR* variants of children who undergo genetic analysis are recorded by the CF physicians in an assessment sheet, which is sent to and entered into the central CF-NBS database by staff at the ISPM. Children born after 2011 and diagnosed with CF based on clinical symptoms (false negative NBS cases) are reported to the central CF-NBS database by the clinicians.

2.3. Changes to the Swiss CF-NBS algorithm

Since 2011, there have been small changes to the Swiss CF-NBS algorithm including changes to IRT cut-offs, the number of *CFTR* variants screened, laboratory tools used, and procedures implemented for repeating the sweat test (Supplemental Table 1).

2.4. Statistical analysis

Descriptive statistics are presented as means with standard deviations (SDs) for continuous variables and numbers with percentages for categorical variables. The national newborn screening laboratory changed the analysis method from *PerkinElmer* to *Labsystems* in the beginning of February 2019, which led to a shift in absolute IRT levels. We therefore transformed IRT-values into z-scores, to make them comparable across the whole analysis period. We used a reference dataset from the Swiss NBS laboratory including all IRT tests done between 2011 and 2022 including data on IRT-values, age at blood sampling (days), and presence of ≥1 *CFTR* variants (yes/no). We calculated z-scores based on the mean and SDs of all blood samples taken at day 4 and 5 of this reference dataset for *PerkinElmer* (N = 694,192) and *Labsystems* (N = 262,923) (Supplemental Table 2) [22].

First, we described the 11-year performance of the screening programme by presenting the overall number of children at each stage in the programme and calculated the sensitivity, specificity, PPV, and false negative rate.

Second, we identified the optimal IRT cut-offs by plotting a receiver operating characteristics (ROC) curve for IRT-1 and IRT-2 z-scores to discriminate between children diagnosed with and without CF. The ROC

curve for IRT-1 was based on all IRT assessments performed at the national newborn screening laboratory between 2011 and 2021 (n = 959,006; from the NBS reference dataset); the IRT-2 ROC curve was based on data from all children in the central CF-NBS database who had performed a second IRT test (n = 239). We used the Youden index to estimate the optimal cut-off where sensitivity and specificity are maximised [23].

Third, we evaluated the performance of different simulated screening algorithms using data from 2013 to 2021 as several changes to the algorithm were implemented during 2011 and 2012. We calculated the number of referred children (total, direct, and via safety net), number of detected CF cases, number of second heel-prick tests (NBS reference dataset was used to calculate this parameter), sensitivity, PPV, false negative rate, and ratio of CF to CFSPID for each of the simulated algorithms. Sensitivity and PPV are presented with a 95 % exact binomial confidence interval (CI). We compared all simulated scenarios to the current algorithm as it was implemented in clinical practice (true algorithm/numbers A1). In a first scenario (A2), we simulated performance if the current algorithm was strictly followed (referral exactly according to IRT z-score cut-offs), and in a second scenario, we simulated performance if the safety net was removed from the current algorithm (A3). Next, we simulated scenarios keeping the IRT-1 cut-off at the 99.2nd P but with stepwise increases in the IRT-1 cut-off for performing IRT-2 and stepwise increases in the IRT-2 cut-off (B1 – B3). Afterwards, we simulated scenarios with stepwise increases in the IRT-1 cut-off and changes in the safety net: IRT-1 at 99.3rd P (C1-C2), IRT-1 at 99.4th P (D1-D2), and IRT-1 at 99.5th P (E1-E2). In a sensitivity analysis we provide all results for the simulated scenarios for the whole period from 2011 to 2021. The simulated scenarios were based on fixed IRT cut-offs in contrast to floating cut-offs, as the Swiss NBS laboratory during the 11-year evaluation period used fixed cut-offs. The Swiss NBS has too small daily batch sizes, which would lead to uncertain estimates and estimates sensitive to outliners in the calculation of floating IRT cut-offs [23].

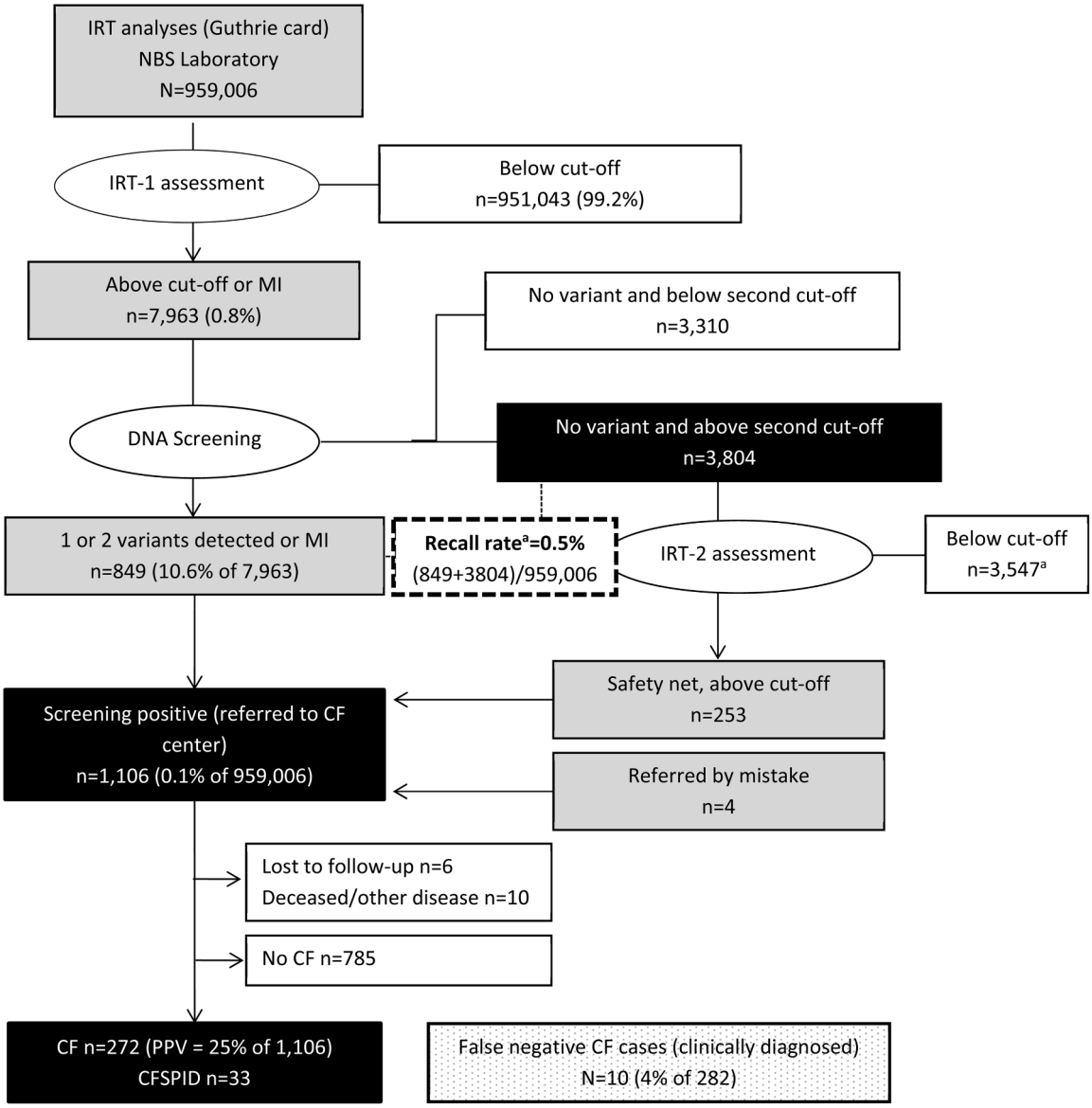

Fig. 1. Flow diagram of the Swiss CF-NBS from 2011 to 2021.
Figure 1 shows the number and proportion of children who underwent screening at each stage of the CF-NBS screening from January 2011 to December 2021.
[a]The n = 3547 include 1–3 children per year who did not get a 2nd heel-prick test because they moved abroad or deceased. Abbreviations: CF, cystic fibrosis; CF-NBS, cystic fibrosis newborn bloodspot screening; CFSPID, cystic fibrosis screening positive inconclusive diagnosis; DNA, deoxyribonucleic acid; IRT, immunoreactive trypsinogen; MI, meconium ileus; n, number; PPV, positive predictive value.

All analyses were performed in STATA, version 17 (StataCorp LP, College Station, Texas, USA).

3. Results

3.1. Description of the Swiss CF-NBS 2011–2021

In Switzerland, 959,006 IRT-1 analyses were performed between 2011 and 2021 of which 951,043 (99.2 %) were below the cut-off. For these children no further analyses were done (Fig. 1). In 7963 children, IRT-1 was above the specified cut-off (99.2nd P), or meconium ileus (MI; $n = 59$) was reported to the NBS laboratory, and DNA screening was performed. Among those, 849 children (11 %) had one or two CF-causing *CFTR* variants and were directly referred to a CF centre. A total of 257 infants were referred to a CF centre without one or two CF-causing *CFTR* variants: 253 after 2 elevated IRT levels (safety net) and 4 by mistake; the median age at IRT-2 was 19 days (IQR 17–22). Among the 1106 children referred, 272 were diagnosed with CF, 33 were designated CFSPID, and 16 were deceased or lost to follow-up. Ten cases were diagnosed outside the NBS (false negative cases) among whom median time to diagnosis was 6 months (range 1–30). Of these 10 children, 8 had an IRT-1 below the cut-off, and 2 had a *CFTR* variant which was not screened for as part of the Swiss NBS algorithm at the time.

The 1100 children included in the analysis (1090 screen positive children + 10 false negative cases) had an average birth weight of 3242 g (SD=601) and gestational age of 38.9 weeks (SD=2.5; Table 1). Most children (71 %) had been referred directly to a CF centre because of a high IRT-1 and detection of ≥1 CF-causing *CFTR* variants, 22 % had been referred via the safety net, 5 % due to MI, and 1 % ($n = 10$) due to clinical symptoms (false negatives). Most were F508del heterozygous ($n = 595$, 55 %) and 11 % ($n = 119$) were F508del homozygous.

The sensitivity of the Swiss CF-NBS over all 11 years was 96 % (272/282, 95 % CI 93.6–98.3) and the PPV was 25 % (272/1090, 95 % CI 22.4–27.6). The CF:CFSPID ratio was 8:1 (272 CF cases to 33 CFSPID cases; Supplemental Table 3). There was some variability of the screening parameters over the years (Supplemental Table 1). Sensitivity ranged from 91 % to 100 %, and PPV from 18 % to 33 %.

3.2. Calculation of the optimal IRT-1 and IRT-2 cut-offs

The optimal cut-off of the IRT-1 calculated by the Youden index was 2.7 z-scores (>99th P) with a sensitivity of 95 % and specificity of 98 % at the cut-off point. The area under the curve (AUC) was 0.97 (Fig. 2). The optimal cut-off point for IRT-2 was 5.9 z-scores (>99.8th P) with an AUC=0.92.

3.3. Comparison of screening parameters for different simulated algorithms

We explored different simulated algorithms using data from 2013 to 2021 after the initial pilot phase (between 2011 and 2013). These resulted in large variability in numbers of children referred and performance parameters (Table 2, Fig. 3). Compared to the current algorithm as it was followed in real-life clinical practice (scenario A1), the current algorithm strictly followed (simulation A2) would have rendered fewer referrals ($N = 771$) and a higher PPV (29 %) during the 9-year period. Removing the safety net from the current algorithm (simulation A3) would lead to 59 fewer referrals and a higher PPV (30 %) than the current strictly followed algorithm (A2). Eliminating the safety net would have prevented more than 2000 children from taking a second unnecessary heel-prick test but increased the number of false negative cases from 6 to 11 between 2013 and 2021 (Table 2). In algorithm B1-B3, we simulated scenarios with a stable IRT-1 cut-off and varying cut-offs for IRT-2. An IRT-2 cut-off at the 99.7th percentile (B1) would reduce referrals further (down to 694 children from 771) with no increase in false negative cases and a PPV of 32 %, while higher IRT-2 cut-offs would render more false negative cases (B2-B3). In algorithm simulations C1-E2, we gradually increased cut-offs for IRT-1. In algorithm C1, IRT-1 was set at the 99.3rd percentile, IRT-2 at the 99.8th percentile, and the cut-off for performing IRT-2 at the 99.7th percentile. This algorithm resulted in $n = 609$ referrals with a PPV of 36 % and only one extra false negative case since 2013. Further simulations in which IRT-1 and IRT-2 cut-offs were increased and the safety net was removed rendered fewer referrals, but with the expense of more false negative cases (E1, E2). Results for the whole period from 2011 to 2021 are provided in Supplemental Table 3.

Table 1
Characteristics of newborns referred to a CF diagnostic centre in Switzerland between 2011 and 2021 ($N = 1100$; 1090 screen positive with final diagnosis and 10 false negative cases[a]).

	Total ($N = 1100$)[a]
Birth weight (g, mean (SD))	3242 (601)
Gestational age (weeks, mean (SD))	38.9 (2.5)
IRT-1 (ng/mL, mean (SD))	
Perkin Elmer ($n = 750$)	88 (56)
Labsystems ($n = 348$)	144 (88)
IRT-1 (z-score, mean (SD))[b]	6.7 (5.3)
IRT-2 (ng/mL, mean (SD))	
Perkin Elmer ($n = 159$)	71 (35)
Labsystems ($n = 80$)	106 (38)
IRT-2 (z-score, mean (SD))[b]	4.9 (3.1)
Institution taking the NBS bloodspot	
Hospital	816 (74 %)
Midwife (home visit)	155 (14 %)
Birthing centre	29 (3 %)
Other	95 (9 %)
CF-causing variants in the NBS[c]	
Homozygous F508del	119 (11 %)
Heterozygous F508del	595 (55 %)
Two *CFTR* variants but not F508del	11 (1 %)
Carrier of only one *CFTR* variant	119 (11 %)
No *CFTR* variants found	243 (22 %)
Mode of referral to the CF centre	
Direct with elevated IRT-1 and ≥1 *CFTR* variants	784 (71 %)
Indirect with two elevated IRTs (safety net)	243 (22 %)
With MI and ≥1 *CFTR* variants (independent of IRT-1)	59 (5 %)
Unclear / error in the NBS testing	4 (0 %)
False negative (due to clinical symptoms)	10 (1 %)
Final diagnosis	
No CF	785 (71 %)
CRMS/CFSPID	33 (3 %)
CF	282 (26 %)
Reported meconium ileus among those with CF[d]	*44 (16 %)*
False negative cases among those with CF	*10 (4 %)*

Numbers are given as frequency with proportions (%) if not stated otherwise. Abbreviations: CF, cystic fibrosis; CRMS/CFSPID, cystic fibrosis transmembrane conductance regulator (*CFTR*)-related metabolic syndrome / cystic fibrosis screening positive inconclusive diagnosis; DNA, deoxyribonucleic acid; IRT, immunoreactive trypsinogen; MI, meconium ileus; NBS, newborn bloodspot screening; n, number. Missings: we had the following missing information in the continuous variables: birth weight ($n = 56$), gestational age ($n = 73$), IRT-1 ($n = 2$), IRT-2 ($n = 4$ out of 243 who were referred based on two elevated IRT values).

[a] N=1100 includes all children with positive screening result who have a final diagnosis ($n = 1090$, excluding 16 children lost to follow-up/deceased) plus false negative cases ($n = 10$) referred to a CF centre between 2011 and 2021.

[b] Z-scores were calculated based on all IRT results of the most frequent days of blood sampling (most normal samples, day 4 and 5) for PerkinElmer ($N = 694,192$) and Labsystems ($N = 262,923$) from 2011 to 2022.

[c] N=1087 (1100 minus 10 false negatives minus 3 missings), percentages are based on available data in this variable.

[d] Includes only meconium ileus diagnoses reported to the National Newborn Screening Lab and considered in the screening algorithm.

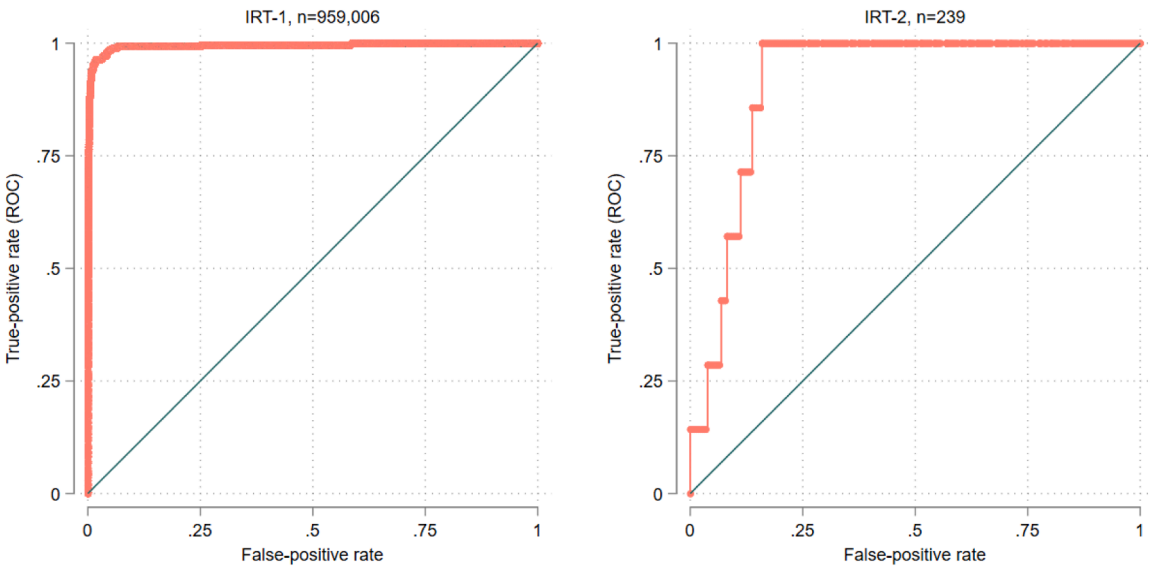

Fig. 2. Receiver operating characteristics (ROC) curves for IRT-1 and IRT-2 z-scores.
Figure 2 shows the receiver operating characteristics (ROC) curves for the IRT-1 z-score and IRT-2 z-score demonstrating its performance to discriminate between children diagnosed with and without CF. Children designated with CFSPID were combined with children without CF. Optimal cut-off point for IRT-1 = 2.7 z-scores; sensitivity = 96 %, specificity = 98 %, AUC = 0.97. Optimal cut-off point for IRT-2 = 5.9 z-scores; sensitivity = 100 %, specificity = 84 %, AUC = 0.92.
Abbreviations: AUC, area under the curve; CF, cystic fibrosis; CFSPID, cystic fibrosis screening positive inconclusive diagnosis; IRT, immunoreactive trypsinogen; n, number; ROC, receiver operating characteristic.

4. Discussion

This is the first study to evaluate 11-years of follow-up and data collection after implementation of a national CF-NBS programme and compared different simulated scenarios to optimise the programme. The overall performance of the Swiss CF-NBS programme was good with an excellent sensitivity of 96 % and an adequate PPV of 25 %. In total, over the 11-year period, 282 children were diagnosed with CF, and only 10 children had a false negative screening result. Simulations suggest that adaptions made only to the safety net, without changing the IRT-1 cut-off, could increase PPV and decrease the number of second heel-prick tests, with minimal effect on sensitivity (simulations A3 and B1-B3).

According to the ECFS recommendations, the outcomes and performance of every NBS programme should be evaluated annually and strategies for collecting accurate and long-term data on ECFS key outcome parameters should be implemented to achieve ECFS standards [9,24–26]. The last ECFS survey showed that many NBS programmes do not reach the ECFS standard of sensitivity of ≥95 % and a PPV of ≥30 % [9]. It can however be difficult to compare the different programmes because of different screening algorithms, laws, health care systems, and ways of processing positive NBS results. For example, in most NBS programmes, an infant with only one *CFTR* variant detected is referred for sweat testing (which lowers PPV and increases sensitivity), whereas in other NBS programmes, these infants are considered as carriers with a negative NBS result (which increases PPV but might decrease sensitivity depending on how many *CFTR* variants are screened). The type and number of *CFTR* variants screened (often regulated by law) also affects the number of CFSPID detected, which as per ECFS standards, should be as low as possible [8,9].

Our evaluation showed that the low PPV of 25 % in Switzerland can be primarily attributed to the safety net, which had led to 243 referrals but only 7 additional CF cases detected over the 11-year study period (PPV of safety net = 3 %). In addition, the safety net led to 3804 second heel-prick tests, most of which were unnecessary, stressful for families, and a burden to the health care system [4,6]. The focus of our simulations was thus to investigate how the PPV could be increased and the number of second heel-prick tests reduced, without affecting sensitivity and the ratio of CF to CFSPID.

Increasing the IRT-1 cut-off runs the risk that more children with CF remain undetected, especially because IRT levels can have high variability [27]. Our study showed that, with a conservative approach as in simulation B1, the PPV would increase to 32 % without affecting sensitivity. However, in this simulation, there would still be >200 unnecessary second heel-prick tests per year. Dropping the safety net (Algorithm A3) would decrease the sensitivity from 97 % to 95 % (5 more false negative cases over 9 years), increase the PPV to 30 %, and eliminate the second heel-prick tests. The ratio of CF:CFSPID improved across all simulations because fewer children would be referred to full genetic analysis. The current CF:CFSPID ratio of 8:1 in Switzerland is above the ECFS target of 10:1. Overall, our results suggest that modifying or even removing the safety net could improve PPV and the CF:CFSPID ratio and drop unnecessary and stressful second heel-prick tests, with only a small risk of increasing false negative cases.

The best algorithm for Switzerland depends on the perspective, which is different for clinicians working with CF patients, parents, experts at the national newborn screening laboratory, public health authorities, and health insurance companies. Based on our calculations, we would keep the existing IRT-1 cut-off at the 99.2nd percentile (scenarios A3, B1–3) due to the uncertainties in the estimations and IRT variability. From the clinicians' point of view, variant B1 would be sufficient to achieve the ECFS standards for PPV without increasing the number of false-negative results. From an economic or parental point of view, it would make sense to completely abolish the safety net with the second heel prick test (scenario A3), which would still fulfil the ECFS standards, but would have produced five more false-negative results. How and whether the Swiss algorithm will be adapted based on the results of the current study, will be discussed, and decided by the Swiss CF Task Force in May 2024. A new algorithm could be implemented from the beginning of 2025.

Another way to optimize NBS for CF would be to introduce next-generation sequencing (NGS) [28]. Sensitivity would be increased at best by using extended genome analysis (EGA) as a second tier, but this would be at the expense of the PPV. This expense is reduced if EGA is applied after testing a variant panel [29]. The increased detection of infants with an inconclusive diagnosis has proven to be a major drawback in programs using EGA. Of the more than 2100 *CFTR* variants

Table 2
Screening parameters for different CF-NBS simulated scenarios for children screened 2013–2021 ($N = 926$[a]; n with CF = 227).

Algorithm			Total number referred to CF center (direct referrals[b]/ safety net)	Total number CF cases detected (among direct referrals[b]/ safety net)	Number 2nd heel-prick tests	Sensitivity (95 % CI)	PPV (95 % CI)	False negatives	Ratio CF:CFSPID
A1[c]	**Current algorithm (true numbers) (IRT-1 > 99.2nd P, IRT-2 > 99.2nd P if IRT-1 > 99.6th P)**		920 (712/208)	221 (216/5)	2993	97 % (94.3 – 99.0)	24 % (21.3 – 26.9)	$n = 6$ (3 %)	221:21 = 11:1
A2[d]	**Current algorithm strictly followed (based on programmed algorithm)**		771 (640/131)	221 (216/5)	2189	97 % (94.3 – 99.0)	29 % (25.5 – 32.0)	$n = 6$ (3 %)	221:20 = 11:1
A3[e]	**Current algorithm without safety net**		712 (n.a.)	216 (n.a.)	0	95 % (91.5 – 97.6)	30 % (27.0 – 33.9)	$n = 11$ (5 %)	216:19 = 11:1
	IRT-1 cut-off	***Safety net***							
B1[f]	**IRT-1 > 99.2nd P**	**IRT-2 > 99.7th P if IRT-1 > 99.6th P**	694 (640/54)	221 (216/5)	2189	97 % (94.3 – 99.0)	32 % (28.4 – 35.5)	$n = 6$ (3 %)	221:19 = 12:1
B2[g]	**IRT-1 > 99.2nd P**	**IRT-2 > 99.8th P if IRT-1 > 99.7th P**	676 (640/36)	220 (216/4)	1632	97 % (93.7 – 98.8)	33 % (29.0 – 36.2)	$n = 7$ (3 %)	220:18 = 12:1
B3[h]	**IRT-1 > 99.2nd P**	**IRT-2 > 99.9th P if IRT-1 > 99.7th P**	654 (640/14)	217 (216/1)	1632	96 % (92.0 – 97.9)	33 % (29.6 – 36.9)	$n = 10$ (4 %)	217:18 = 12:1
C1[i]	**IRT-1 > 99.3rd P**	**IRT-2 > 99.8th P if IRT-1 > 99.7th P**	609 (573/36)	220 (216/4)	1632	97 % (93.7 – 98.8)	36 % (32.3 – 40.1)	$n = 7$ (3 %)	220:15 = 15:1
C2[j]	**IRT-1 > 99.3rd P**	**No safety net**	573 (n.a.)	216 (n.a.)	0	95 % (91.5 – 97.6)	38 % (33.7 – 41.8)	$n = 11$ (5 %)	216:15 = 14:1
D1[k]	**IRT-1 > 99.4th P**	**IRT-2 > 99.8th P if IRT-1 > 99.7th P**	568 (532/36)	216 (212/4)	1632	95 % (91.5 – 97.6)	38 % (34.0 – 42.2)	$n = 11$ (5 %)	216:15 = 14:1
D2[l]	**IRT-1 > 99.4th P**	**No safety net**	532 (n.a.)	212 (n.a.)	0	93 % (89.3 – 96.3)	40 % (35.7 – 44.2)	$n = 15$ (7 %)	212:15 = 14:1
E1[m]	**IRT-1 > 99.5th P**	**IRT-2 > 99.8th P if IRT-1 > 99.7th P**	518 (482/36)	214 (210/4)	1632	94 % (90.4 – 96.9)	41 % (37.0 – 45.7)	$n = 13$ (6 %)	214:11 = 19:1
E2[n]	**IRT-1 > 99.5**th **P**	**No safety net**	482 (n.a.)	210 (n.a.)	0	93 % (88.3 – 95.6)	44 % (39.1 – 97.8)	$n = 17$ (7 %)	210:11 = 19:1

Abbreviations: CF, cystic fibrosis; CI, confidence interval; CFSPID, cystic fibrosis screening positive inconclusive diagnosis; CFTR, cystic fibrosis transmembrane conductance regulator; IRT-1, first IRT measurement in the NBS, usually day 4; IRT-2, second IRT measurement in the NBS if applicable; NBS, newborn bloodspot screening; MI, meconium ileus; n, number; n.a., not applicable; P, percentile; PPV, positive predictive value.

[a] $N = 926$ includes all children with a positive screening result from 2013 to 2021 who have a final diagnosis ($n = 920$) plus 6 false negative cases.

[b] Direct referral: if IRT-1 above cut-off or meconium ileus AND at least one *CFTR* mutation.

[c] Algorithm A1: true numbers from the Swiss CF-NBS algorithm 2013–2021.

[d] Algorithm A2: current algorithm followed strictly from the start and based on IRT z-scores: IRT-1 > 99.2nd P (z-score >3.2), IRT-2 > 99.2nd P if IRT-1 > 99.6th P (z-score >4.3).

[e] Algorithm A3: current algorithm without the safety net.

[f] Algorithm B1: as A2 but IRT-2 cut-off at 99.7th P (z-score >4.8) if IRT-1 > 99.6th P (z-score >4.3).

[g] Algorithm B2: as A2 but IRT-2 cut-off at 99.8th P (z-score >5.6) if IRT-1 > 99.7th P (z-score >4.8).

[h] Algorithm B3: as A2 but IRT-2 cut-off at 99.9th P (z-score >7.5) if IRT-1 > 99.7th P (z-score >4.8).

[i] Algorithm C1: as A2 but IRT-1 cut-off at 99.3rd P (z-score >3.5) and IRT-2 cut-off at 99.8th P (z-score >5.6) if IRT-1 > 99.7th P (z-score >4.8).

[j] Algorithm C2: as C1 but no safety net.

[k] Algorithm D1: as A2 but IRT-1 cut-off at 99.4th P (z-score >3.7) and IRT-2 cut-off at 99.8th P (z-score >5.6) if IRT-1 > 99.7th P (z-score >4.8).

[l] Algorithm D2: as D1 but no safety net.

[m] Algorithm E1: as A2 but IRT-1 cut-off at 99.5th P (z-score >3.9) and IRT-2 cut-off at 99.8th P (z-score >5.6) if IRT-1 > 99.7th P (z-score >4.8).

[n] Algorithm E2: as E1 but no safety net.

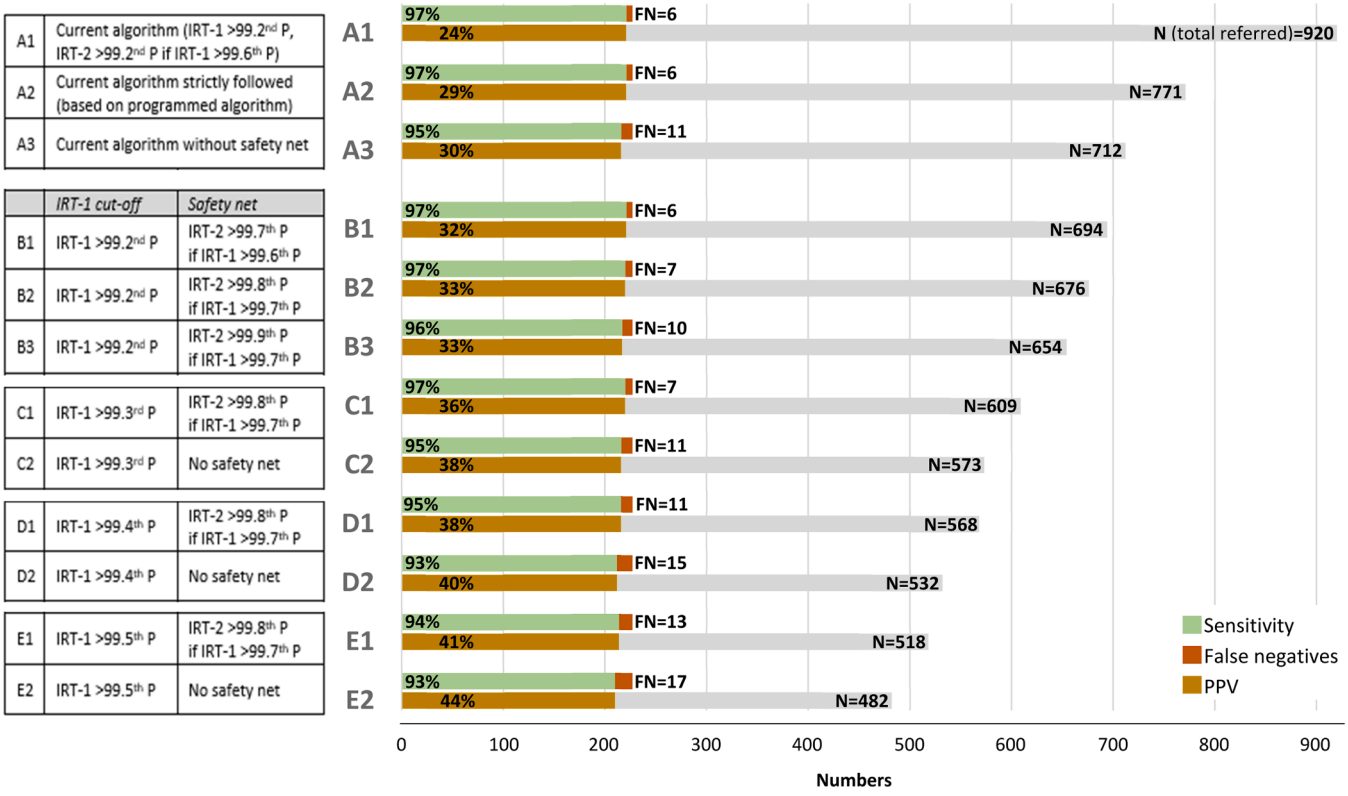

Fig. 3. Graphical display of the key performance parameters of the different simulated algorithms for children screened 2013-2021 ($N = 926$[a]; n with CF = 227). Figure 3 displays the key screening performance parameters of the different simulated algorithms (exact numbers are provided in Table 2). For each algorithm, the lower bar represents the total number of children referred to the CF centre and the proportion diagnosed with CF (PPV) in yellow; the upper bar represents the number of true positive CF cases (always $n = 227$) and the sensitivity of the algorithm in identifying them in green. The red part represents the number of false negatives among all CF cases. The different scenarios are defined as followed:

A1: true numbers from the Swiss CF-NBS algorithm 2013–2021.

A2: current algorithm followed strictly from the start and based on IRT z-scores: IRT-1 > 99.2nd P (z-score >3.2), IRT-2 > 99.2nd P if IRT-1 > 99.6th P (z-score >4.3).

A3: current algorithm without the safety net.

B1: as A2 but IRT-2 cut-off at 99.7th P (z-score >4.8) if IRT-1 > 99.6th P (z-score >4.3).

B2: as A2 but IRT-2 cut-off at 99.8th P (z-score >5.6) if IRT-1 > 99.7th P (z-score >4.8).

B3: as A2 but IRT-2 cut-off at 99.9th P (z-score >7.5) if IRT-1 > 99.7th P (z-score >4.8).

C1: as A2 but IRT-1 cut-off at 99.3rd P (z-score >3.5) and IRT-2 cut-off at 99.8th P (z-score >5.6) if IRT-1 > 99.7th P (z-score >4.8).

C2: as C1 but no safety net.

D1: as A2 but IRT-1 cut-off at 99.4th P (z-score >3.7) and IRT-2 cut-off at 99.8th P (z-score >5.6) if IRT-1 > 99.7th P (z-score >4.8).

D2: as D1 but no safety net.

E1: as A2 but IRT-1 cut-off at 99.5th P (z-score >3.9) and IRT-2 cut-off at 99.8th P (z-score >5.6) if IRT-1 > 99.7th P (z-score >4.8).

E2: as E1 but no safety net.

[a]N = 926 includes all children with a positive screening result from 2013 to 2021 who have a final diagnosis ($n = 920$) plus 6 false negative cases.

known to date (www.genet.sickkids.on.ca), less than 40 % have been characterised as CF-causing in the CFTR2 (www.cftr2.org) and in the French database (https://cftr.iurc.montp.inserm.fr/cgi-bin/home.cgi). Most of the other variants are classified as variants with varying clinical consequences or uncertain (or unknown) significance, and a few are non-pathogenic.

Strengths of this study include the nationwide collaboration between birth institutions, screening laboratories, CF physicians, and databases to carefully document all referred cases since the start of the Swiss CF-NBS program in 2011. Yearly evaluations and meetings have ensured excellent communication between parties involved and high data quality. This has been the basis for adaptations to the screening algorithm over the years which have subsequently been implemented effectively [6,15,17,20,21]. Rigorous follow up of each referred child has led to few children lost to follow-up. The detailed and central data collection has made it possible to evaluate each step of the algorithm over time.

A limitation of the study is the use of two different IRT laboratory systems during the study period (*Perkin Elmer* until January 2019 and *Labsystems* since February 2019). To account for this we transformed the IRT values into z-scores. In addition, when evaluating the performance of different simulated CF-NBS algorithms, we estimated performance assuming that children were referred exactly according to the percentile cut-offs based on the IRT z-scores. This leads to some uncertainty and deviation from what we truly would have observed if the different algorithms had been in place. As such, predictions for future algorithm changes based on IRT cut-offs need to be done carefully. Additionally, collecting data on false negative cases is challenging and additional cases may be detected in the future which will retrospectively affect sensitivity parameters. Our study showed good performance for the Swiss CF-NBS for the study period 2011–2021, but we do not yet have data on the performance of the Swiss CF-NBS after *CFTR* modulator treatment has been approved for use in Switzerland (first approved on 10.12.2020). Several studies have shown that children with 2 CF-causing *CFTR* variants born to mothers on *CFTR* modulator therapy have a low IRT-1 despite having CF (i.e., false negatives) [30,31]. The

careful collection and evaluation of Swiss NBS data remains particularly pertinent in this case, as modifications to the CF-NBS may have to be undertaken, e.g. direct referral of these children to a CF diagnostic centre, independent of their screening result.

4.1. Conclusion

The Swiss CF-NBS demonstrated good performance during the past 11 years with an excellent sensitivity, adequate PPV, and only few CFSPID cases. For future optimisation of the screening algorithm and to achieve the ECFS standards, changing the safety net procedure could improve PPV and reduce unnecessary second heel-prick tests, thus leading to less stress for healthy children and their families. Prospective data collection and evaluation remain crucial in maximising the performance of CF newborn screening programmes.

Declaration of competing interest

No conflict of interest to declare for any of the authors.

Funding

This work is supported by a grant from Cystic Fibrosis Switzerland.

Authorship contributions

ESLP, SHMS, CEK, JB, and CSR made substantial contributions to the study concept and design and interpretation of the data. ESLP, DB, CCMdeJ, MJ, and CSR collected and entered data. CSR analysed the data. ESLP, JB, and CSR drafted the manuscript. ESLP, CCMdeJ, MJ, DB, JS, SHMS, MRB, CEK, JB, and CSR critically revised and approved the manuscript.

Acknowledgements

For their assistance with our study, we thank the lab technicians of the Swiss Newborn Screening Laboratory at the University Children's Hospital of Zurich, the lab technicians at the University Hospital Bern, and all lab technicians who did the sweat tests in the 8 paediatric CF centres in Switzerland.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcf.2024.04.008.

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