Single-centre prospective evaluation of the first 5 years of cystic fibrosis newborn screening in Germany

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Shareable abstract (@ERSpublications)
CF newborn screening in Germany should be adapted to increase its PPV. The tracking system in Bavaria, Germany is very effective and could serve as a template for other tracking models. Macroduct is more accurate than Nanoduct when performed regularly. https://bit.ly/48UqsC5

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Abstract

Background In 2016, nationwide cystic fibrosis newborn screening (CFNS) was newly implemented in Germany, using an immunoreactive trypsin/pancreatitis-associated protein/DNA screening algorithm that differs from most other nationwide screening programmes.

Methods We analysed real-life feasibility of the confirmation process with respect to our pre-specified procedural objectives. These included overall accuracy through false-negative and false-positive results, effectiveness of the Bavarian tracking system, and accuracy of Macroduct and Nanoduct sweat conductivity compared with quantitative chloride determination. All consecutive CFNS-positive newborns assigned to our CF centre and born between 1 September 2016 and 31 August 2021 (n=162) were included.

Results The German CFNS was feasible at our CF centre as all procedural objectives were met. The positive predictive value (PPV) of positive CFNS was low (0.23) and two initially negatively screened children were later diagnosed with CF. The tracking system was highly efficient with a 100% tracking rate. The Macroduct and Nanoduct systems had comparable success rates (93.2% *versus* 95.9%). Importantly, conductivity *via* Macroduct was more accurate than *via* Nanoduct (zero and four false-positive newborns, respectively).

Conclusions CF confirmation diagnostics of neonates in a certified regional CF centre was well managed in daily routine. The PPV of the German CFNS needs to be improved, *e.g.* by extending the DNA analysis within the screening algorithm and by increasing the number of variants tested. The Bavarian tracking system can serve as a successful model for other tracking systems. We preferred the Macroduct system because of its more accurate sweat conductivity readings.

Introduction

The discovery of elevated serum immunoreactive trypsin (IRT) as a diagnostic marker for newborn cystic fibrosis (CF) patients led to the implementation of the first community-wide CF newborn screening (CFNS) programmes in Australia and New Zealand in 1981 [1, 2]. The screening algorithm has since been adapted from the original IRT/IRT pathway to a combined IRT/DNA pathway used in all states of Australia and New Zealand by 2005, improving sensitivity [2]. The benefits of CFNS are well established, and include improvements in patient metrics such as weight, height, lung function, intellectual development and hospitalisation rates of CF patients [3–6]. To date, many countries worldwide have implemented CFNS; in Europe, CFNS was performed in 21 countries by the end of 2015, with 17 countries participating in a nationwide screening programme [7].

In Germany, there was a regional CFNS programme from 1996 to 2000, evaluating an IRT/DNA pathway, and two regional CFNS programmes from 2008 onwards, evaluating an IRT/pancreatitis-associated protein

(PAP) pathway first suggested by Sarles *et al*. [8], further optimising the sensitivity by introducing an IRT-dependent "safety net" [9–12]. Nationwide CFNS was introduced in Germany on 1 September 2016. After lengthy negotiations due to concerns regarding the German Genetic Diagnostics Act, an IRT/PAP/DNA screening algorithm was established [13]. In the first step, IRT is measured. If IRT is <99.0th percentile, CF is unlikely and the CFNS is negative. If IRT is ⩾99.9th percentile, CF is likely and the CFNS is positive (so-called "safety net" or "fail-safe"), directly followed by the confirmation test (sweat test). If IRT is ⩾99.0th and <99.9th percentile, PAP is measured. If PAP is ⩾87.5th percentile, DNA panel analysis is performed for the 31 most common CF transmembrane conductance regulator (*CFTR*) variants in Germany (*CFTR* panel analysis), covering 95.5% of variants in the German population [14]. If no variant is found, the CFNS is negative. If one or two variants are found, the CFNS is positive, followed by the confirmation test (figure 1).

Many countries use a two-tier screening system with IRT/DNA, while PAP is used only in Germany, the Netherlands and Portugal. The German screening algorithm has a positive predictive value (PPV) of only 20% [13], leaving many families worried by a false-positive screening result. Informing parents of a positive screening result has been shown to cause anxiety in the majority, but parental distress can be reduced by minimising the delay between notification and confirmation testing, and by having trained professionals communicate results and facilitate follow-up [15, 16]. Therefore, when national CFNS was implemented in Germany in 2016, the main goal of our confirmation process was to establish the final diagnosis (CF, non-CF or CF screen positive inconclusive diagnosis (CFSPID)) as quickly and reliably as possible, to minimise the psychological burden on families and to initiate specific therapy if indicated. To achieve this goal, we prospectively defined eight specific procedural objectives in topics such as information management, time management and making a diagnosis. We also formulated eight questions to be evaluated by the study, concerning the effectiveness of the Bavarian tracking system, overall accuracy through false-negative and false-positive results, adequacy of the scope of DNA analysis, and accuracy of Macroduct or Nanoduct sweat conductivity compared with quantitative chloride determination (table 1).

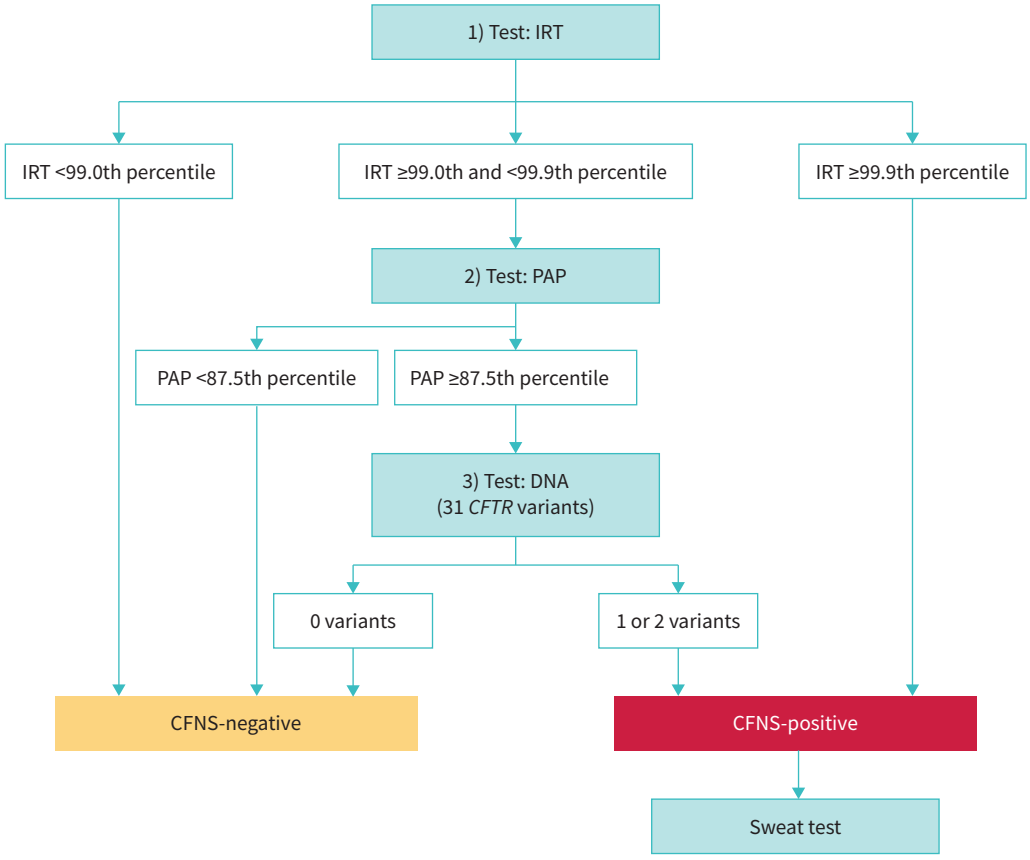

FIGURE 1 German cystic fibrosis newborn screening (CFNS). IRT: immunoreactive trypsinogen; PAP: pancreatitis-associated protein; *CFTR*: CF transmembrane conductance regulator. See main text for a detailed description.

TABLE 1 Procedural objectives and practical questions defined before implementation of cystic fibrosis newborn screening (CFNS) at our CF centre for prospective evaluation

Topic	
Objectives	
Information management	Initial information should be given to parents by a CF-experienced medical doctor
	A CF-experienced medical doctor should give information about the likely test result and proceedings after the sweat test
	A CF-experienced medical doctor should talk to the families and explain the final diagnosis when established
Time management	Informing the family should take place within the first month of life
	A sweat test should be offered for the same or next day to reduce psychological burden
	A final diagnosis should be communicated within 1 week of the sweat test taking place
Final diagnosis	The final diagnosis should be established by quantitative chloride measurement
	Final CF or CFSPID diagnosis should be supported by genetic testing
Questions	
Lost to follow-up	Is the Bavarian tracking system effective?
False-positive CFNS	What is the false-positive rate for CFNS?
False-negative CFNS	Is the CF diagnosis made in patients who are primarily negative for CFNS and if so, when?
Genetic testing	Are the 31 *CFTR* variants currently used in the German CFNS adequate?
Sweat test	Is it possible to identify factors influencing the sweat test success rate?
	Does the sweat test success rate improve over time?
Sweat conductivity	Is Macroduct sweat conductivity feasible and valid?
	Is Nanoduct sweat conductivity feasible and valid?

CFSPID: CF screen positive inconclusive diagnosis; CFTR: CF transmembrane conductance regulator.

Methods

Subjects and study design

When we prepared the implementation of CFNS in our CF centre, we defined objectives and practical questions as shown in table 1. Data concerning CFNS-positive newborns were collected in a standardised and prospective manner for each consecutive CFNS-positive subject using a separate printed screening form. Our objectives regarding the process of CFNS were included in our standard operating procedures. All staff members involved in the CFNS process received specific procedural training.

We included all consecutive CFNS-positive newborns born from 1 September 2016 to 31 August 2021 assigned to our CF centre for confirmation diagnostics.

The analysis of the collected data was pseudonymised. The study was approved by the Institutional Review Board for Human Studies of Dr von Hauner Children's Hospital, LMU University Hospital, LMU Munich, Munich, Germany, on 17 March 2014.

Information and tracking

In the state of Bavaria in Germany, the screening laboratory notifies the certified regional CF centre (total number of certified CF centres in Germany: 30, including seven in Bavaria) closest to the newborn's place of residence of a positive CFNS finding. The centre contacts the person with custody, informs them about the suspected diagnosis and arranges an appointment for a sweat test on the same or following day during this telephone conversation [17].

In addition, a tracking mechanism is in place through the Bavarian State Office for Health and Food Safety (Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit (LGL)). Each positive CFNS is reported to the LGL and followed up by them until the case is resolved (CF, non-CF or CFSPID).

Medical equipment

Macroduct

Sweat testing was performed with the Macroduct 3700 iontophoresis and sweat analysis system (ELITech Group, Logan, UT, USA) using Pilogel discs (ELITech Group). Macroduct sweat conductivity was measured using the Wescor Sweat-Chek 3120 sweat conductivity analyser (ELITech Group). All procedures were performed according to the respective manuals.

Nanoduct

Sweat collection and sweat conductivity measurements were performed using the Nanoduct 1030 neonatal sweat analysis system (ELITech Group) and Pilogel discs (ELITech Group) according to the manual.

Chloride analyser

Quantitative sweat chloride concentration was measured coulometrically in our clinical laboratory using the Corning 925 chloride analyser (Bayer Diagnostics, Leverkusen Germany). To assure the accuracy of the chloride measurement, our clinical laboratory participates in ring trials several times per year.

Diagnostic evaluation at our CF centre

Using the Macroduct system we collected sweat (a minimum of 30 µL to allow for possible re-testing in the laboratory) to perform confirmation diagnostics by quantitative chloride measurement according to the German guideline [18]. Thresholds of <30 mmol·L^{-1} for healthy children, 30–59 mmol·L^{-1} for CFSPID and ⩾60 mmol·L^{-1} for CF were used according to the German guideline and the European Cystic Fibrosis Society (ECFS) best practice guidelines [18, 19]. At the same time, we measured sweat conductivity through Nanoduct and Macroduct simultaneously to use as a reference for informing parents about the likely test result. For this, thresholds of <60 mmol·L^{-1} for healthy children, 60–79 mmol·L^{-1} for CFSPID and ⩾80 mmol·L^{-1} for CF were used according to previous publications [20–23]. If sweat collection failed or yielded <30 µL, the sweat test was repeated ∼2 weeks later. If Nanoduct sweat conductivity failed, it was not repeated. The final diagnosis was established based on the quantitative chloride measurement result only. If the sweat test was positive (chloride ⩾30 mmol·L^{-1}), a stepwise approach of genetic analysis followed to complete the diagnosis. First, the 31 most frequent *CFTR* variants in Germany according to the national guideline were analysed [18]. If these were not found, the entire *CFTR* gene was sequenced. If the findings were again inconspicuous, an analysis of potential deletions or duplications within the *CFTR* locus followed.

Statistical analysis

Cohort data were collected in pseudonymised form. Statistical analyses were performed using Excel 365 (Microsoft, Redmond, WA, USA) and SPSS version 29.0 for Windows (IBM, Armonk, NY, USA). Data are given as median (range). For comparison of categorical variables, the Chi-squared test and Fisher's exact test were applied as appropriate. Percentages for Macroduct and Nanoduct sweat conductivity were based on the respective total number of valid tests (Macroduct n=161 and Nanoduct n=143).

Correlation analyses were performed using Pearson's correlation coefficient. Comparison of Pearson's correlation coefficient was calculated according to the Hittner–May–Silver modification [24] of the Dunn–Clark z [25] using a back-transformed mean Fisher's z procedure. We calculated Bland–Altman plots [26], which allow the identification of proportional bias, *i.e.* whether the difference between the two tests (Macroduct and Nanoduct sweat conductivity, respectively, *versus* quantitative chloride measurement) is equal throughout the range of sweat test measurements.

Results

General characteristics of subjects

Of 162 newborns tested by quantitative chloride measurement, 33 (20.4%) were diagnosed with CF, four (2.4%) were diagnosed with CFSPID and 125 (77.2%) were non-CF (healthy) (figure 2); the PPV of positive CFNS was 0.23. The median (range) age of the subjects at the time of sweat testing was 24 (14–103) days and they weighed 3985 (2698–7300) g. 101 subjects (62%) were female and 61 (38%) subjects were male.

Objectives

Information management

Of 162 families, 137 families (84%) were first contacted by a CF-experienced medical doctor from our CF centre. 22 families (13%) were first contacted by the maternity unit/midwife. In three cases, the documentation of the first contact was missing.

All families were seen by a doctor from our CF centre during the sweat test, who discussed the likely test result (healthy, CF or CFSPID) with the parents and informed them of the following quantitative chloride measurement.

After 2 (0–23) days, all families were contacted by a CF-experienced medical doctor to confirm the final diagnosis and to explain further management.

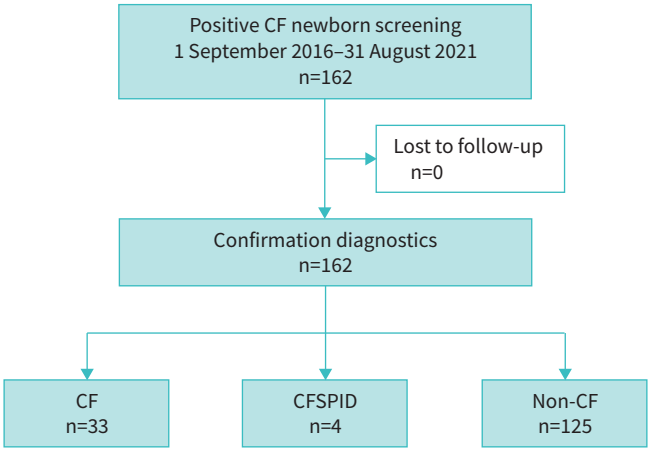

FIGURE 2 Flow diagram of study cohort. 162 subjects were enrolled in the study: 33 (20.4%) were classified as cystic fibrosis (CF), four (2.4%) as CF screen positive inconclusive diagnosis (CFSPID) and 125 (77.2%) as non-CF (healthy).

Time management

Our CF centre was informed by the screening laboratory about a positive CFNS 18 (5–39) days after birth. We informed the family 22 (7–48) days after birth. We informed the family preferably on Monday and always offered a sweat test on the same day or alternatively the next day. 124 subjects (76%) had a sweat test within 1 day (49 (30%) the same day and 75 (46%) the next day). 18 subjects (11%) had a sweat test within 7 days and 20 subjects (12%) had a sweat test more than 7 (8–55) days after being informed.

A telephone call informing the parents of the quantitative chloride measurement result was made 2 (0–23) days after the sweat test was performed.

Final diagnosis

We performed parallel testing with Nanoduct and Macroduct whenever possible, collecting a minimum of 30 µL of sweat with the Macroduct system for (possibly repeated) quantitative chloride measurement. In 11 subjects, there was not enough sweat for chloride measurement at the first visit and the sweat test was repeated. In all subjects, the final diagnosis was made by quantitative chloride measurement. In all CF patients, the disease-causing *CFTR* variants were genetically confirmed. Of the four patients with CFSPID, genetic testing was performed in two patients. The other two patients supposedly left the country shortly after the positive screening result and could not be contacted anymore by us or their respective paediatricians.

Questions

Is the Bavarian tracking system effective?

Of 162 CFNS-positive newborns referred to our CF centre, we performed a sweat test in all subjects, resulting in a 100% tracking rate. We contacted the families up to 16 times (median 2 times) by calling the phone number provided by the parents when giving consent to the CFNS before contacting the LGL and asking for assistance in providing other contact information. The LGL was contacted in 15 cases (9%).

What is the false-positive rate for CFNS?

In our CFNS study cohort, there were 125 healthy newborns, resulting in a false-positive CFNS rate of 77%.

Is the CF diagnosis made in patients who are primarily negative for CFNS and if so, when?

Two infants born in 2018 and 2019 who participated in the CFNS were primarily negative in the CFNS, but were later diagnosed with CF. One infant had recurrent pulmonary infections and was diagnosed by sweat test at 6 months of age. His IRT was ⩾99.0th and <99.9th percentile and his PAP was <87.5th percentile, therefore the CFNS was negative. The other infant was also diagnosed at 6 months of age by sweat testing due to poor growth. Her IRT was ⩾99.0th and <99.9th percentile and her PAP was >87.5th percentile, but the specific *CFTR* variants were not detected as she was of Indian descent and had two

variants not included in the panel of 31 *CFTR* variants, rendering the CFNS negative. Both infants' disease-causing *CFTR* variants were later found with *CFTR* sequencing after the diagnosis was made with a positive sweat test.

Are the 31 CFTR variants currently used in the German CFNS adequate?

All but one of the positively screened CF patients and both CFSPID patients with genetic *CFTR* analysis carried at least one *CFTR* variant included in the panel of the German CFNS. The one CF patient whose two *CFTR* variants were not included in the panel was of Caucasian descent (Irish father, German mother) and was detected by the screening algorithm's "safety net" (IRT ⩾99.9th percentile).

The CF patient of Indian descent was initially missed because the disease-causing *CFTR* variants were not included in the panel of 31 *CFTR* variants (see earlier).

Sweat test success rate

In 11 of the 162 newborns (6.7%), the sweat test failed due to insufficient sweat (<30 µL) and had to be repeated; one infant required three attempts. The sweat test success rate correlated strongly with the weight of the newborn at the time of testing (figure 3). There was also a learning curve for sweat testing. While 10% of sweat tests had to be repeated in the first 3 years, only 3% had to be repeated in the fourth year and 5% in the fifth year.

Is Macroduct sweat conductivity feasible and valid?

Macroduct sweat conductivity was performed in 161 out of 162 newborns (99.4%). It provided a valid result at the first attempt in 150 out of 161 newborns (93.2%) and after repetition in 11 cases in 100% (see earlier). The scatter plot comparing Macroduct sweat conductivity and chloride concentration showed a significant and robust correlation with $p<0.001$ and $R^2_{linear}=0.965$ (figure 4a). The Bland–Altman plot showed that the conductivity was on average 18 $mmol \cdot L^{-1}$ higher than the chloride concentration with a standard deviation of 5; the bias plot showed that the difference between the two tests was equal over the range of sweat test measurements (figure 4b). All but one CF/CFSPID patient (97.3%) and all healthy newborns (100%) were correctly classified (table 2).

Is Nanoduct sweat conductivity feasible and valid?

Nanoduct sweat conductivity was performed in 149 out of 162 newborns (91.9%). It provided a valid result in 143 out of 149 newborns (95.9%). The scatter plot comparing Nanoduct sweat conductivity and chloride concentration showed a significant correlation with $p<0.001$, although weaker than Macroduct sweat conductivity with $R^2_{linear}=0.797$ (figure 4c). The Bland–Altman plot showed that the conductivity was on average 27 $mmol \cdot L^{-1}$ higher than the chloride concentration with a standard deviation of 14; the bias plot showed that the difference between the two tests was equal over the range of sweat test measurements (figure 4d). 93.4% of CF/CFSPID patients and 97.3% of healthy children were correctly classified with Nanoduct (table 2).

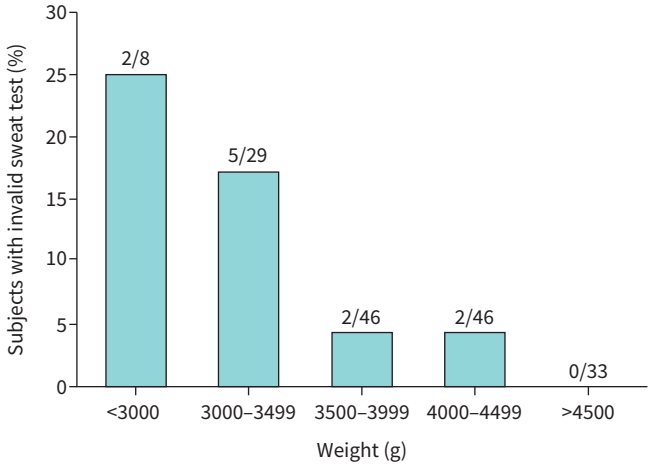

FIGURE 3 Number of invalid sweat tests due to insufficient sweat (<30 µL) as a percentage of the total number of subjects in each weight group. Insufficient sweat correlates with the weight of the newborn.

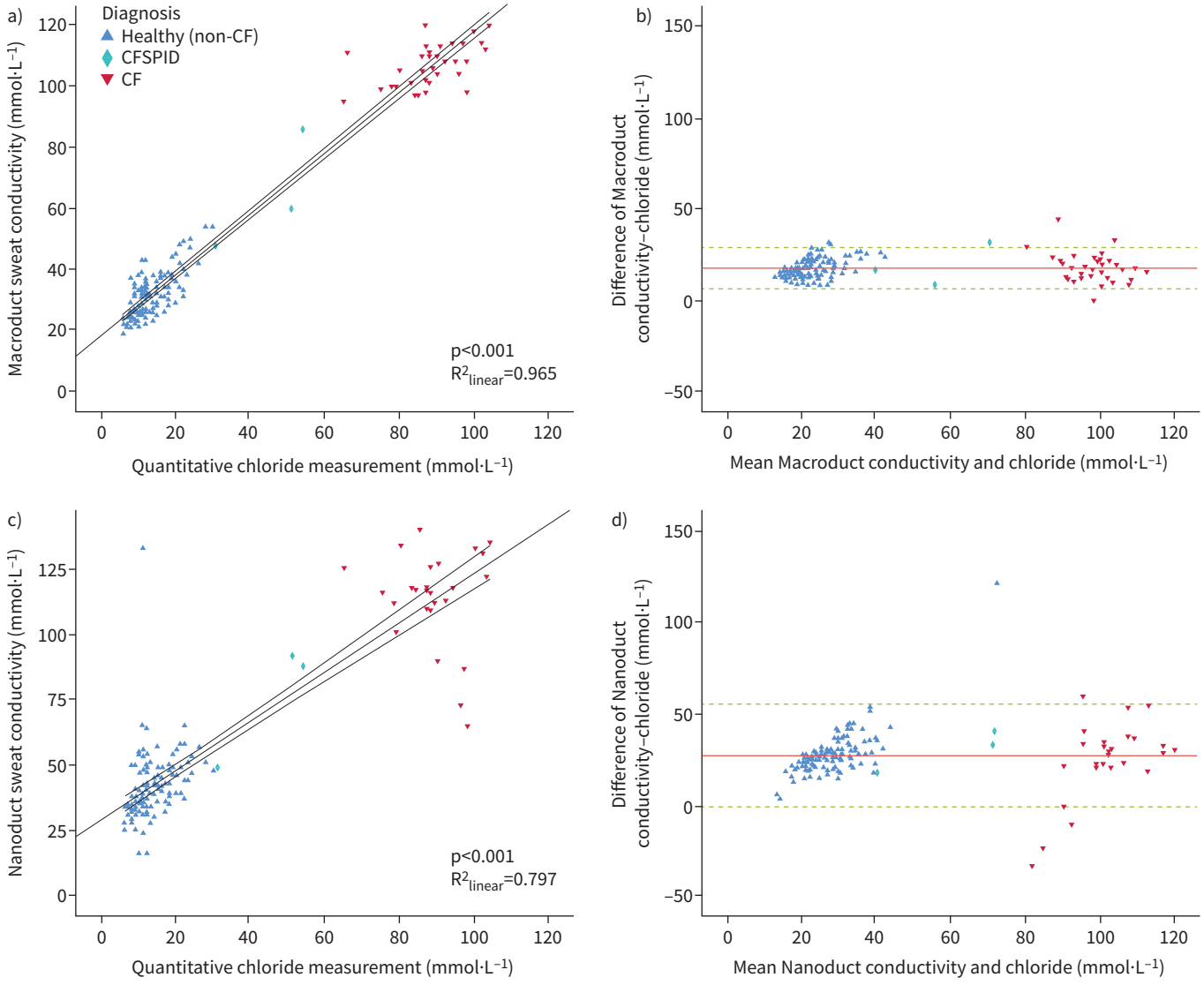

FIGURE 4 a) Scatter plot comparing Macroduct sweat conductivity (*y*-axis) with quantitative chloride measurement (*x*-axis) as of final diagnosis (n=161). The correlation between these two measurements is significant at p<0.001 using Pearson's correlation coefficient. Black lines: estimated linear regression line with 25th and 75th percentiles. R^2=0.96. b) Bland–Altman plot of differences between Macroduct sweat test conductivity and quantitative chloride measurement *versus* their mean by final diagnosis (n=161). The Bland–Altman plot shows the difference in sweat conductivity minus sweat chloride on the *y*-axis plotted against the mean of sweat conductivity and quantitative sweat chloride on the *x*-axis. This allows to identify proportional bias. Red line: mean difference; green dashed lines: ±1.96SD of the mean difference. c) Scatter plot comparing Nanoduct sweat conductivity (*y*-axis) with quantitative chloride measurement (*x*-axis) as of final diagnosis (n=143). The correlation between these two measurements is significant at p<0.001 using Pearson's correlation coefficient. Black lines: estimated linear regression line with 25th and 75th percentiles. Note that the percentiles of the R lines stray further from the centre than in a) with R^2=0.79 (*versus* R^2=0.96 in a)). d) Bland–Altman plot of differences between Nanoduct sweat test conductivity and quantitative chloride measurement *versus* their mean by final diagnosis (n=143). Note that the standard deviation is much wider than for Macroduct in b). Red line: mean difference; green dashed lines: ±1.96SD of the mean difference. CF: cystic fibrosis; CFSPID: CF screen positive inconclusive diagnosis.

Discussion

After 5 years of CFNS in Germany, we are reporting on the real-life experience of the German CFNS programme from our single-centre perspective.

Analysing our workflow, we found that the work-up of CFNS-positive children was efficient, fast and achieved the set goals. At the beginning of the CFNS in the state of Bavaria, Germany, the instructions sent from the screening laboratory to the maternity hospital or midwife were apparently not clear enough in

TABLE 2 Macroduct and Nanoduct sweat conductivity compared

	Successful tests at first attempt	Successful tests after repetition[#]	Correct classification	False positive	False negative	PPV	NPV
Macroduct sweat conductivity	150/161 (93.2)	161/161 (100)	160 (99.3)	0 (0)	1 (2.7)[¶]	1	0.99
Nanoduct sweat conductivity	143/149 (95.9)		137 (95.8)	4 (2.7)[+]	2 (6.6)[§]	0.87	0.98

Data are presented as n/N (%) or n (%), unless otherwise stated. PPV: positive predictive value; NPV: negative predictive value; CFSPID: cystic fibrosis screen positive inconclusive diagnosis. [#]: Macroduct sweat conductivity was repeated until successful (*i.e.* enough sweat for quantitative chloride measurement), Nanoduct sweat conductivity was performed only once per child; in one CFSPID patient Macroduct conductivity was not performed; in 13 subjects, seven of whom were CF patients, Nanoduct sweat conductivity was not performed. [¶]: one CFSPID patient was classified as healthy. [+]: three healthy newborns were classified as CFSPID, one healthy newborn was classified as CF. [§]: two CFSPID patients were classified as healthy.

that the certified regional CF centre was supposed to initiate contact with the newborn's family. In the first year after introduction, 50% of cases occurred when the maternity unit or midwife informed the family directly. After the information flyer with the instructions was adapted, the frequency of wrong information flow decreased to 2–4 cases per year. Carroll *et al*. [27] showed that 77% of primary care physicians would rather not provide all well-baby care for infants with a confirmed diagnosis of CF. Accordingly, we suggest that the entire process of informing parents, making the diagnosis and, if necessary, continuing treatment should be conducted in a centralised way by the regional CF centre.

Time management goals were mostly met. It is known that anxiety of parents caused by the positive screening information can be reduced by a short latency to confirmation testing and by a CF-experienced doctor informing the parents [16, 28]. Considering that we were informed at a median of 18 days after birth, our goal of informing the family within 1 month was achieved if the families could be reached. When a CF-experienced physician from our CF centre informed the families, we offered a test on the same or next day in all cases, as sweat tests were routinely performed on Mondays and Tuesdays. In cases where the families were informed by their maternity hospitals or midwifes on other days or even on Friday afternoons, this caused obvious anxiety in parents, who often called our CF centre immediately and asked for confirmation testing the same day.

With the Bavarian tracking model and a 100% tracking success rate, we achieved very good results compared with other studies reporting 80% or 95% success rates [29, 30], indicating that a centralised tracking system may be essential to confirm all positive CFNS and should be included in all nationwide CFNS programmes. The two CFSPID patients who could not be reached after the diagnosis of CFSPID was established probably moved abroad. This illustrates the unresolved problem of how to track families who leave the country early after the child's birth.

In our CF centre, the German CFNS algorithm yields a PPV of 0.23, which is below the ECFS best practice guidelines target of a minimum PPV of 0.3 [13, 31]. A PPV of only 0.23 places an unnecessary psychological burden on many families of healthy children that could be avoided. By extending DNA analysis (31 variant *CFTR* panel analysis) to all screenings with an IRT ⩾99.9th percentile (the so-called "fail-safe"), as proposed by Sommerburg *et al*. [13], the PPV could be increased to 0.69.

32 of our 33 CF patients had at least one variant included in the German CFNS genetic panel. The one remaining patient of Caucasian descent would, however, be classified as CFNS-negative in the alternative screening model proposed by Sommerburg *et al*. [13] because their variants are not included in the current German CFNS panel and the "fail-safe" mechanism (IRT ⩾99.9th percentile) would thus not result in a positive CFNS in this case. The two patients who were initially negative in the CFNS but were later diagnosed with CF would also have been missed (one because of rare *CFTR* variants, the other because they would be initially excluded with a PAP <87.5th percentile).

We propose that given the increasing number of children born in Germany of other than Caucasian ethnicity, the number of *CFTR* variants screened should be expanded. In order to miss as few CF patients as possible, *CFTR* panel analysis would have to be extended to all cases with an IRT ⩾99.0th percentile regardless of PAP measurement, as is done in many other countries (*e.g.* Ireland, Great Britain, France and Switzerland). IRT/DNA pathways, as indicated earlier, result in a higher number of detected CFSPID, causing long-term psychosocial, medical and financial impacts. Therefore, a recent study in Florence, Italy

was conducted to reduce the number of positive CFNS findings by including PAP in their two-tier IRT/DNA pathway [32]. Since 2005 several studies have already shown the benefits of including PAP in the screening pathway, resulting in fewer detections of healthy carriers and CFSPID as well as lower CFNS costs [12, 32–34]. The inclusion of DNA testing in the screening pathway is improving sensitivity but is also increasing the cost of CFNS significantly. The current German CFNS costs EUR 9.77 on average. If *CFTR* panel analysis were expanded to all CFNS with an IRT ⩾99.0th percentile this would lead to much higher costs to the public healthcare system.

In Germany in 2020, 8270 out of 761 651 CFNS had an IRT ⩾99.0th percentile, of which 551 CFNS were "fail-safe" (IRT ⩾99.9th percentile), resulting in a sweat test. 7507 CFNS had an IRT ⩾99.0th and <99.9th percentile leading to PAP analysis and of these, 1805 CFNS (21.8%) had *CFTR* panel analysis performed [35].

Taking into consideration the need to improve the PPV of the German CFNS and to increase the number of variants tested while keeping the CFSPID detection rate low, the CFNS pathway proposed by Sommerburg *et al*. [13] could be further adapted by performing *CFTR* sequencing instead of *CFTR* panel analysis in all newborns classified as "fail-safe", as well as all newborns with IRT ⩾99.0th and <99.9th percentile and PAP ⩾87.5th percentile. This would increase the number of DNA analyses modestly (to 28.4%), while improving the PPV and including potentially all *CFTR* variants in the analysis. Using this adapted pathway, two of our three CF patients initially CFNS-negative would have been CFNS-positive.

As described in previous studies, the success rate of sweat testing was highly dependent on the weight of the newborn at the time of testing and was >95% at >3500 g body weight. Nevertheless, the overall sweat test failure rate in our CF centre was reduced over time, suggesting that regular practice of sweat testing in newborns improves technical quality and reduces failure rates [36, 37].

Macroduct sweat conductivity had a better success rate and Nanoduct sweat conductivity had a similar success rate compared with other studies [36, 38]. Macroduct conductivity had a significantly better correlation with chloride measurement and a PPV of 1, while Nanoduct conductivity had a lower correlation and a PPV of 0.87. The negative predictive values were similar at 0.99 and 0.98, respectively. Of note, one healthy newborn would have been classified as CF using Nanoduct sweat conductivity, potentially causing unnecessary parental anxiety until corrected by chloride measurement. An argument often made is that Nanoduct offers better practicality because it can provide a result from as little as 3 µL of sweat. In our cohort success rates were similar, with 150 out of 161 (93.2%) successful sweat tests on the first attempt with Macroduct sweat conductivity *versus* 143 out of 149 (95.9%) with Nanoduct sweat conductivity. Overall, Macroduct sweat conductivity was practical and gave more accurate results, given regular practice of using the device with newborns, which, if lacking, seemed to be a reason for lower success rates in other studies [36]. This was one of the reasons why our CF centre stopped using the Nanoduct sweat conductivity system in CFNS evaluation at the end of the 5-year study period. A recent study by Dolce *et al*. [39] compared Macroduct with the Gibson–Cooke method and found no statistically significant differences between the percentages of valid tests with both methods.

Limitations of this study include the single-centre design with a relatively small number of cases. However, our CF centre represents 5.6% of all nationwide CFNS as of 2020 [35] and the statistical data on the 162 subjects, identifying 33 CF patients, were nevertheless robust. On the other hand, the advantages of this single-centre study were the real-life experience reported and the fact that all procedures and routines were rigorously standardised, unlike in multicentre studies.

Another shortcoming is the fact that we did not conduct a written survey on parents' anxiety after being contacted and after performing the sweat test.

Conclusions

We have shown that the German CFNS programme is feasible and has very few false-negative screening results, but according to the ECFS guidelines and in international comparison it generates too many false-positive screening results and should therefore be adapted to increase its PPV. The Bavarian tracking system was very effective and could serve as a template for other tracking models; informing the families by a CF-experienced medical doctor and minimising delays by offering a sweat test on the same day is feasible. Quantitative chloride measurement is practical and safe in diagnosing CF; Macroduct sweat conductivity is just as practical as and more accurate than Nanoduct sweat conductivity when performed by trained and experienced staff who use it regularly on newborns.

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