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# Differences in clinical condition and genotype at time of diagnosis of cystic fibrosis by newborn screening or by symptoms

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ABSTRACT

*Background:* Early diagnosis through newborn screening (NBS) and early treatment of cystic fibrosis (CF) do lead to better prognosis. In the Netherlands, the median age for a clinical diagnosis is six months, and after newborn screening this is 30 days. It is unknown if being diagnosed at the age of six months or before two months leads to a clinically relevant difference of the clinical condition at the time of diagnosis.
*Aim:* The aim of this study is to assess the differences in clinical parameters at diagnosis between children with CF identified by newborn screening (NBS) or by clinical diagnosis (CD) in the Netherlands.
*Methods:* From July 1st, 2007 to January 1st, 2012 all newly diagnosed CF patients were reported to the Dutch Paediatric Surveillance Unit (DPSU). All paediatricians received a questionnaire to collect data on mutations and clinical condition at diagnosis. Non-classical CF was excluded from the analysis on clinical condition.
*Results:* 204 new CF diagnoses were reported to the DPSU, 33 were reported twice and three had no CF after further testing. 127 questionnaires were returned (76%); 85 children were diagnosed because of clinical symptoms, 40 after NBS and two because of a positive family history. The median age at diagnosis was 34 weeks for a clinical diagnosis and 3 weeks after NBS. Non-classical CF was more prevalent in the NBS group (6 clinical, 14 NBS), mostly F508del/R117H7T (12). Compared to the NBS group, significantly more patients in the CD group showed failure to thrive, respiratory symptoms, and hospitalizations. 62% of the CD group showed abnormal signs at physical examination compared to 4% of the NBS group.
*Conclusion:* At the time of diagnosis infants detected after NBS are in a significantly better condition than after a clinical diagnosis. Growth retardation is already seen when after NBS the diagnosis is confirmed, but NBS leads to a diagnosis before respiratory symptoms have developed.



## 1. Introduction

Newborn screening for cystic fibrosis (NBSCF) is being implemented in an increasing number of countries globally. The generally accepted opinion is that the benefits of newborn screening (NBS) outweigh the potential harm [1].

Early diagnosis through newborn screening and early institution of treatment of CF does lead to better nutritional status, improved growth, longer preservation of lung function and longer survival in early adulthood [2,3].

For maximum benefit of screening, the diagnosis should be confirmed before the age of two months [4,5]. In the 1990s the median age at diagnosis in the Netherlands was 14 to 18 months [6]. Recent data showed that the median age at diagnosis, excluding infants diagnosed through screening, was about five months in 2008 [7]. At the same time the median age at diagnosis after newborn screening was 30 days, and all patients were diagnosed before the age of two months [8]. It is unknown if being diagnosed at the age of five months or before two months leads to a substantial difference in clinical condition at the time of diagnosis.

In this study we assessed the differences in clinical parameters at diagnosis between children with CF identified by NBS or by clinical diagnosis in the Netherlands.

## 2. Methods

### 2.1. Registration

From July 1, 2007 to January 1, 2012 all newly diagnosed CF patients in the Netherlands were registered by the Dutch Paediatric Surveillance Unit (DPSU). The DPSU was initiated by the Dutch Paediatric Society

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(NVK). The purpose of the surveillance system is to gain insight into the prevalence of rare and new diseases in children (0–18 years) on a population level, and to promote scientific research addressing the background, nature and prognosis, as well as the treatment and prevention, of these diseases [9]. Paediatricians were asked to report all new CF diagnoses monthly. Diagnosis was made by newborn screening, based on clinical symptoms or by family history. Confirmation of the diagnosis and treatment of CF is performed by seven CF centres in the Netherlands.

### 2.2. Newborn screening for CF

From January 1, 2008 until May 1, 2011, a pilot study of newborn screening on CF was performed in four provinces in the southern and middle parts of the Netherlands (Cystic fibrosis Heel prick amOng a newborn Population In the Netherlands; CHOPIN study). In the other areas, there was no newborn screening for CF [8]. From May 1, 2011 newborn screening for CF was added to the routine Dutch heel prick screening programme. The incidence of CF in the Netherlands is 1:4750 [10], and the incidence in the NBS region was 1:6062 [8].

### 2.3. Study population

All children diagnosed with CF, classic as well as non-classic CF in the Netherlands and reported to the DPSU between July 2007 and January 2012 were included in the study.

### 2.4. Classic and non-classic (equivocal) CF

Classic CF was defined as an infant with two cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations with symptoms and/or a positive sweat test result (sweat chloride value ≥60 mmol/l) or a positive family history [11].

We defined non-classic CF as an infant with two CFTR mutations, of which one or two have an unclear phenotypic outcome and a normal or intermediate sweat test result (sweat chloride value between 30 and 60 mmol/l [12].

### 2.5. Study design

We compared the prevalence and distribution of the registered CFTR mutations in both groups. We also compared the age and clinical condition at the time of diagnosis of children detected by NBS with children diagnosed clinically (CD).

### 2.6. Screening methods

During the pilot study two screening strategies were compared [8]. When newborn screening for CF was added to the Dutch routine newborn screening programme, a four step approach, the IRT-PAP-DNA-sequencing strategy was used. In this strategy, all screening samples with high concentrations of immunoreactive trypsinogen (IRT) as well as pancreatitis-associated protein (PAP) (IRT ≥60 and PAP ≥3.0 μg/l, or IRT ≥100 and PAP ≥1.6 μg/l), get a DNA-mutation analysis followed by sequencing of the CFTR-gene when only one mutation is found. As a fail-safe procedure sequencing of the whole CFTR gene was also performed in all samples with an IRT ≥100 μg/l without identified mutations.

Mutation analysis was performed using the commercial kits INNO-LiPA CFTR19 and INNO-LiPA CFTR17 + Tn. CFTR-mutation I148T was ignored because this mutation does not cause CF.

During the pilot study, infants with a positive screening test were referred to one of the four CF centres participating in the study and after the introduction of newborn screening for CF in the routine heel prick programme to one of the seven CF centres in the Netherlands.

### 2.7. Questionnaires

The clinical condition was assessed by questionnaires sent to the attending paediatricians of the reported children.

In the questionnaires doctors were asked to record the following symptoms: meconium ileus, failure to thrive (growth below the 5th percentile or a change in growth that crossed two major growth percentiles in a short time), growth retardation and/or weight loss (weight for height or weight below 50th percentile, physical growth significantly less than peers, or loss of weight), malabsorption, steatorrhoea, recurrent airway infections (infection of the upper (cold) or lower airways (pneumonia) caused by viral or bacterial pathogens), chronic coughing (cough for more than 8 weeks), mucus production, dyspnoea, and ear–nose and throat (ENT) problems (nasal polyps, sinusitis, otitis).

The age at diagnosis, number of hospital admissions, the sweat test results, and the CFTR mutations were also recorded. If available, results of blood coagulation tests, vitamin A, D and E levels, chest X-ray, faecal fat (grams/24 h), faecal elastase and sputum cultures (throat or cough swab) were also recorded.

### 2.8. Statistical methods

Results were considered significant if $p < 0.05$. We used the Pearson chi-square test and Fischer's exact test to compare the differences of symptoms, clinical signs at physical examination, and additional testing in the two groups. Non-parametric tests were used to compare median age at diagnosis and growth parameters (Mann–Whitney-*U* test).

## 3. Results

From July 2007 to January 2012, 204 children with CF were reported to the DPSU (17 children were registered in 2007, 45 in 2008, 65 in 2009, 38 in 2010 and 39 children in 2011). However, 33 children were reported twice and three children did not appear to have CF after further diagnostic testing. Therefore, a total number of 168 CF patients were recorded during the study period. The response rate of the questionnaires was 76% (127/168). The reported 24% patients of whom no questionnaire was returned consisted of 23 CD and 18 NBS patients ($Chi^2$ $p = 0.273$).

85 patients were found because of clinical symptoms (CD), 40 by newborn screening (NBS), and two were found by family history (see Fig. 1). Two infants with a diagnosis after a positive family history

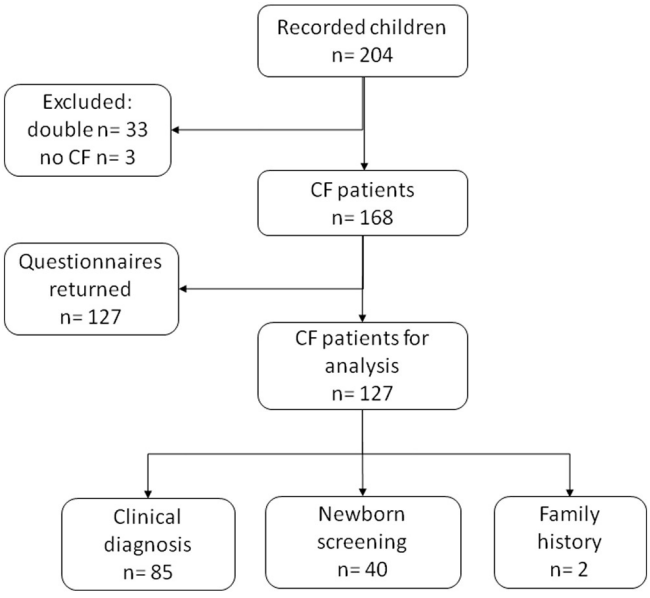


**Fig. 1.** Study population.