# Neonatal screening for cystic fibrosis

**Gezondheidsraad** **President**
Health Council of the Netherlands

To the Minister of Health, Welfare and Sport

Subject : presentation of advisory report *Neonatal screening for cystic fibrosis*
Your reference : PG/OGZ-2940324
Our reference : I-185/09/ES/db/852-J
Enclosure(s) : 1
Date : March 5, 2010

Dear Minister,

In reaction to your request for advice dated 9 July 2009, I hereby present the *Neonatal screening for cystic fibrosis* advisory report. It has been drafted by a Committee which was established specifically for this purpose. The Committee also consulted external experts. The draft advisory report was reviewed by the Standing Committee on Genetics of the Health Council of the Netherlands.

The Committee has examined the results of a trial of Cystic fibrosis heel prick screening of neonates in the Netherlands (CHOPIN), which was conducted in 2008 in four provinces of the Netherlands, and the results of screening in various other countries.

The results led the Committee to conclude that expanding the neonatal screening programme to include cystic fibrosis would clearly provide additional benefits, that test methods now exist that would enable responsible screening, and that the screening would not involve higher costs but could reduce the cost of health services.

The Committee calls for attention to be paid to the information provided to future parents on screening. This also involves aspects which play a role in neonatal screening for other diseases, such as those concerning the nature of the disease, the importance of early diagnosis and the significance of being a carrier (including the option of whether or not to be provided with information on being a carrier).

I endorse the Committee's conclusions and recommendations.

Yours sincerely,
(signed)
Professor J.A. Knottnerus

P.O.Box 16052
NL-2500 BB The Hague
Telephone +31 (70) 340 71 80
Telefax +31 (70) 340 75 23
E-mail: ej.schoten@gr.nl

Visiting Address
Parnassusplein 5
NL-2511 VX The Hague
The Netherlands
www.healthcouncil.nl

# Neonatal screening for cystic fibrosis

to:

the Minister of Health, Welfare and Sport

No. 2010/01E, The Hague, March 5, 2010

The Health Council of the Netherlands, established in 1902, is an independent scientific advisory body. Its remit is "to advise the government and Parliament on the current level of knowledge with respect to public health issues and health (services) research..." (Section 22, Health Act).

The Health Council receives most requests for advice from the Ministers of Health, Welfare & Sport, Housing, Spatial Planning & the Environment, Social Affairs & Employment, Agriculture, Nature & Food Quality, and Education, Culture & Science. The Council can publish advisory reports on its own initiative. It usually does this in order to ask attention for developments or trends that are thought to be relevant to government policy.

Most Health Council reports are prepared by multidisciplinary committees of Dutch or, sometimes, foreign experts, appointed in a personal capacity. The reports are available to the public.

The Health Council of the Netherlands is a member of the European Science Advisory Network for Health (EuSANH), a network of science advisory bodies in Europe.

The Health Council of the Netherlands is a member of the International Network of Agencies for Health Technology Assessment (INAHTA), an international collaboration of organisations engaged with *health technology assessment*.

This report can be downloaded from www.healthcouncil.nl.

Preferred citation:
Health Council of the Netherlands. *Neonatal screening for cystic fibrosis*. The Hague: Health Council of the Netherlands, 2010; publication no. 2010/01E.



ISBN: 978-90-5549-817-8

# Contents

# Executive summary

The Health Council of the Netherlands has in 2005 provided the Minister for Health, Welfare and Sport with an advisory report on Neonatal Screening. In this advisory report, the Health Council highlights the advantages of neonatal screening for cystic fibrosis (CF), namely a better feeding status, prevention of an often protracted and aggravating diagnostic process and a decrease in the number of incidents of sickness and hospital admissions. Evaluations made of screening programmes performed abroad after 2005 have also demonstrated these advantages. However, the Health Council also underlined the imperfections of the screening methods available at the time, which was the basis for the recommendation to undertake research into better screening methods.

The CHOPIN study (Cystic fibrosis Heel prick screening in a newbOrn Population In the Netherlands) was undertaken in 2008 as a result of this recommendation. Based on the outcome of this study, the Health Council now concludes that an adequate method is available and – in view of the advantages of screening stated above – recommends to include cystic fibrosis in the neonatal screening programme. The recommended protocol comprises four steps, whereby each step is followed by a decision depending on preset criteria whether the next step will be performed or not. The successive steps are the determination of the immunoreactive trypsinogen concentration, the determination of the pancreatitis associated protein concentration, the analysis of 36 mutations in the cystic fibrosis transmembrane regulator gene which occur frequently in patients with cystic

fibrosis, and an extended analysis of mutations. Annex C provides a detailed description of the method. When two mutations associated with cystic fibrosis are found, newborns are referred to one of the centres specialising in cystic fibrosis in children, where teams composed of (paediatric) specialists, nurses, nutritional specialists, physiotherapists, social workers and others will provide optimal care. Parents of a newborn with CF, and parents whose child carries one mutation and therefore is a carrier of CF will be referred to a clinical geneticist for genetic counselling, the latter group unless they have indicated not to want to receive information about carriership.

The CHOPIN study included a limited number of patients, meaning that the probability of false-negative outcomes continues to be a concern (a false-negative result is obtained if the test indicates absence of disease whereas in fact the newborn has the disease). To prevent false-negative outcomes, it is therefore recommended for the time being to utilise a failsafe procedure described by the researchers. This procedure concerns additional mutation analyses if none of the 36 frequently occurring CF mutations is present, but a high concentration of immunoreactive trypsinogen is found.

In view of quality control the mutation analysis should be performed under the supervision of a centre for clinical genetics that is specialised in cystic fibrosis. The analysis should be evaluated systematically, and if necessary amended. As is customary in the current programme, patient registration is required for the valuation of the screening results, which is performed in the centres specialised in cystic fibrosis.

The CHOPIN researchers have estimated that the net annual costs of the full programme using the methods outlined above will be EUR 140,000. The additional annual costs for the failsafe procedure are EUR 39,000. The net costs of the full programme may be lower, however some aspects of the cost calculation are governed by uncertainty. If screening results in a decrease in treatment costs, it may even lead to a cost-saving on health care expenses.

The clinical course of newborns with forms of cystic fibrosis that are considered less severe should be monitored, as it is as yet unclear what treatments are optimal for the patients concerned. Neonatal screening will lead to the identification of a limited number of carriers of mutations, a finding that is relevant to the probability of eventual subsequent children developing cystic fibrosis. In the same way as in neonatal screening for sickle cell anaemia, parents should be given the option whether they wish to receive information about being carriers or

not. The committee takes the view that parents should not be informed about any findings unrelated to disease or that are not relevant in any other way. The importance of good information about the nature of the disease, the importance of an early diagnosis, the meaning of being a carrier (including the choice to either opt in or out of being informed)are emphasized by the committee. The information should also state that screening does not fully rule out the disease and only identifies a small proportion of carriers.

Chapter 1

# Introduction

In 2005 the Health Council of the Netherlands published an advisory report on neonatal screening.[1] The Health Council's advisory report included an assessment and discussion of more than forty disorders on the basis of various criteria. One of the Health Council's recommendations was to include cystic fibrosis (CF) in neonatal heel prick screening as soon as test methods became available with a high specificity (as low specificity leads to a great deal of clinical investigation of unaffected neonates). Following the publication of the results of the CHOPIN study[2] the Minister of Health, Welfare and Sport requested an advisory report from the Health Council on possibly expanding neonatal heel prick screening to include CF (see Annex A for complete request for advisory report). In response, the President of the Health Council set up the Neonatal Screening for Cystic Fibrosis Committee composed of members of the former Neonatal Screening Committee (see Annex B).

The Minister of Health, Welfare and Sport specifically asked the following questions in the request for the advisory report:

1 What is the Health Council's advice on adding CF to the diseases covered by neonatal heel prick screening?
2 Has it been established that early screening for CF would provide significant additional benefits with regard to the health of neonates with CF?

3 If the Health Council advice is affirmative on adding CF to the diseases covered by neonatal heel prick screening, what would be the preferred test method and what are the assessments that form the basis for this preference?

4 What are the Health Council's recommendations on providing information on being a carrier and on detecting mild variants, given the above and taking into account the Health Council's advice on point three regarding the test method?

The above questions are answered in chapter 5 ‘Answers to the questions in the request for an advisory report’. The following matters are discussed to substantiate the answers provided: relevant background information on cystic fibrosis (symptoms; therapy; and new information on screening, Chapter 2); a factual description of the CHOPIN population screening trial study, whereby the focus is on the various screening methods; the cost-effectiveness of screening; and parents’ opinions on screening (Chapter 3); and the significance of the results of the CHOPIN study for addressing the request for an advisory report (Chapter 4).

The Neonatal Screening for Cystic Fibrosis Committee requested additional advice from the following external experts on various aspects of the results of the CHOPIN study and a number of studies conducted abroad: Dr H.G.M. Arets, paediatric pneumonologist, Utrecht University Medical Centre; Dr D.J.J. Halley, clinical molecular geneticist, Erasmus Medical Centre; and Dr M.F. Wildhagen, health care economist, Erasmus Medical Centre.

The Committee has discussed adding CF to the diseases covered by neonatal heel prick screening and the additional benefits of doing so for the health of neonates with CF; the test methods, the costs and benefits of screening; the detection of carriers and non-classical types of CF; the turnaround times; and the provision of information.

Chapter 2

# Cystic fibrosis: symptoms, therapy and new information on screening

## 2.1 Symptoms

Cystic fibrosis is also known as mucoviscidosis. The main symptoms of CF are chronic obstructive pulmonary disease, pancreatic fibrosis and hepatic fibrosis. Pulmonary infections often occur in the first year of life, especially as a result of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Nutritional problems occur in the vast majority of patients: pancreatic enzymes are secreted too slowly, which leads to poor nutrient uptake and damage to the pancreas, which in turn can lead to diabetes mellitus. Almost all males with CF are infertile owing to secondary azoospermia (no passage of spermatozoa). However, spermatozoa can be obtained by testicular or epididymal aspiration, so that men with CF can nevertheless become biological fathers by means of introcytoplasmatic sperm injection (ICSI). The need for medical assistance to become pregnant is higher than average among female sufferers owing to menstrual disorders and the formation of thick secretions.

CF is caused by mutations in the *CFTR gene* (cystic fibrosis transmembrane regulator gene), which is important for transporting chloride ions across membranes. The disease is an autosomal recessive disorder, which means that a CF patient has inherited a mutation from both parents. If both parents are carriers (i.e. have one mutation but no symptoms of the disease), there is a one in four likelihood of each pregnancy resulting in a child with CF.

## 2.2 Therapy

As indicated in the 2005 advisory report, there is a broad, general consensus on the best therapy for patients with CF. As a result of better treatment, the median life expectancy at birth of CF patients has increased considerably, from 25 years in 1985 to 38 years in 2007.[3] Irreparable damage to the lungs remains the main cause of death. A great deal of research is therefore conducted into new types of therapies[4a], such as those which focus on small molecules which cancel the effect of certain mutations (for example PTC124, which causes a premature stop codon to be read through[4b] or which can make a mutated *CFTR protein* more functional (for example by using VX-770, which is being tested in phase III clinical trials, see www.clinicaltrials.gov).

## 2.3 New information on screening

A great deal of new research has been conducted into various aspects of CF since the publication of the 2005 advisory report on Neonatal Screening. Some of the research results are relevant for the screening of neonates for this disorder.

The first important fact is that, besides the immunoreactive trypsinogen (IRT), another protein has been discovered which acts as an indicator in screening, namely pancreatitis associated protein (PAP).[5] Neonates with CF generally have higher concentrations of IRT and PAP. Determining the concentration of both IRT and PAP enables a considerable reduction in the number of follow-up examinations.

Secondly, studies of patients have revealed more than 1,600 different mutations in the *CFTR gene*,[6] details of which have been included in an international database (www.genet.sickkids.on.ca/cftr). Although the exact significance for the disease is still unclear in many cases, more information has been obtained on the relationship between these mutations and the severity of CF.[7] Some of the mutations result in a less severe form, designated as non-classical CF. Some other variants in the *CFTR gene* are known not to cause CF. Although it is plausible that new information on mutations will become available, sufficient knowledge is available to warrant the inclusion of mutation analyses in the protocol. Relatively little information is available on mutations among population groups of Turkish and Moroccan descent (see section 4.1 and Annex E).

Thirdly, studies have been conducted of the birth prevalence of CF in the Netherlands. It was published in 2005 that the estimated prevalence figure for the years 1961-1965 of 1 in 3,600 neonates[8] had decreased to 1 in 4,750 between 1974 and 1994.[9] According to the patient records of the Dutch Cystic Fibrosis Foundation (NCFS), between 20 and 38 patients were found to have CF in the years 2000-2006 (personal communication, Dr V.A.M. Gulmans). On the basis of these new data, an average of 29 CF patients per year can be expected among cohorts of 185,000 neonates, which represents a decrease to 1 in 6,300. A decrease of this kind is partly explained by the increase in the number of parental couples who request genetic counselling, generally following the birth of a CF patient in the immediate or close family. Various choices are available to these parental couples, such as deciding not to have more children, opting for invasive prenatal diagnostics (according to the Annual Reports of the Prenatal Diagnostics Working Party of NVOG and VKGN, the average annual figure since 1995 has been approximately 25, of which an average of six cases a year were followed by pregnancy termination) or opting for pre-implantation genetic diagnostics (the option taken by a total of 21 couples since 1995, according to the 2008 Annual Report on PGD in the Netherlands). A decrease in the birth prevalence of CF was also found in other Western countries or regions.[10,11a,11b] Knowledge of being a carrier appears to have played a role in the decrease. In a region of Italy where screening for carriers was offered as part of routine practice the decline was sharper than in a region where screening was only offered to relatives of patients and carriers, or prior to fertility treatment.[11b] Another explanation for the small decrease is the number of births in populations in which fewer *CFTR mutation*s occur. It is not known whether other factors also play a role.

Chapter 3

# Results of CHOPIN study

In 2006, the Ministry of Health, Welfare and Sport informed the Netherlands Organisation for Health Research and Development (ZonMw) of the position of the Health Council of the Netherlands on the possible expansion of neonatal heel prick screening to include CF[1]. To ascertain amongst other things whether early CF screening would provide additional benefits for the health of neonates, ZonMw funded a population screening trial, CHOPIN (Cystic fibrosis heel prick screening of neonates in the Netherlands). In 2008, during this pilot, blood obtained from the heel pricks of 72,874 neonates in Noord-Brabant, Utrecht, Gelderland and Limburg was analysed to determine whether suitable methods existed for neonatal screening for cystic fibrosis and what the features and costs of the tests were for the various strategies. Moreover, a survey was conducted of the opinions of parents and care providers on the inclusion of CF in the heel prick programme and the level of parental knowledge was assessed.[2]

## 3.1 Methods

Heel prick blood was analysed in two ways: 1) by screening for IRT followed by analysis of PAP; and 2) by screening for IRT followed by mutation analysis. Neonates with CF generally have higher concentrations of IRT and PAP. Concentrations may be normal in the case of meconium ileus (obstruction of the small intestine); the diagnosis of CF is then based on demonstrating that the obstruction exists. The concentrations of IRT and PAP can be determined by using a

fluorimetric method. The mutation analysis – referred to as the DNA method in the CHOPIN report – was carried out using a panel of 36 mutations which are frequently described in CF patients, and was possibly followed by more detailed mutation analysis (referred to as *extended gene analysis* or EGA in the CHOPIN report). EGA consists of determining the sequence of exons and intron-exon boundaries of the *CFTR gene*.

Using the IRT-PAP method, the test was deemed to be positive if the IRT concentration was 50 µg/l or higher and the PAP concentration was 1.8 µg/l or higher, or if the IRT concentration was 100 µg/l or higher and the PAP concentration was 1.0 µg/l or higher. Using the IRT-DNA method, the test was deemed to be positive if the IRT concentration was 50 µg/l or higher and two mutations were found in the first mutation analysis; in the case of one mutation being found EGA was used to find a possible second mutation.

Sweat tests were performed on all neonates with a positive test result from either the IRT-PAP method or the IRT determination in combination with DNA analysis. An increased salt concentration in sweat is considered to be the gold standard in international reference literature, which means it is referred to as the clearest evidence for the CF diagnosis.[12] However, sweat tests in children under the age of three months often are problematic owing to the low level of sweat that is produced. The CHOPIN study provided further confirmation of this: for five of the ten CF neonates identified through screening, the first sweat test was doubtful, had failed or was negative (Table 2.6[2]). Using DNA analysis makes diagnosis at an early age largely independent of the sweat test. The sweat test and other analyses based on defective chloride transport continue to be valuable for clinical diagnosis of CF when molecular analysis fails to provide an answer and clinical symptoms nevertheless exist.

Table 1 shows the CHOPIN results, including the theoretical calculation supplied by the researchers of the figures for the IRT-PAP-DNA-EGA combination. A few cases involved non-classical CF; in these cases two mutations were found in neonates, one being a known classical CF mutation but its combination with a second mutation pointed to a less severe form. Setting the limit for the IRT concentration at 60 µg/l would mean that the number of patients with classical CF would be the same but that approximately half the number of cases of non-classical CF (and the number of carriers) would be detected. Likewise, using the IRT-PAP-DNA-EGA combination would have detected the same number of classical CF patients, and fewer non-classical CF patients and carriers in comparison

*Table 1* Results heel prick screening (CHOPIN study) of 72 874 newborns.

| | IRT ≥50 and PAP ≥1,8 of IRT ≥100 and PAP ≥1,0 | IRT ≥50 followed by DNA-EGA | IRT-PAP-DNA-EGA (calculated result) |
|---|---|---|---|
| Abnormal results | 119 | 20 | 12 |
| Classical CF | 10 | 10 | 10 |
| Non-classical CF | 0 | 9 | 2 |
| Carriers | 0 | 89 | 5 |

with using the IRT-DNA-EGA combination. This formed the basis for the researchers' recommendation that this combination should be applied in the screening protocol. The researchers also recommended that a CF centre should monitor the course of non-classical CF in children to enable a better assessment over the years of whether these children need care and, if they do, the type of care that is needed (see Annex D for CF centres).

## 3.2 Cost-effectiveness

Based on the figures stated in the CHOPIN report (Tables 3.2, 3.4 and 3.5[2]), the total cost of laboratory analysis for screening 185,000 neonates (Statistics Netherlands has recorded a birth rate of over 11 per 1,000 in the population) would come to the amounts shown in Table 2.

On the basis of the similar costs after expansion of neonatal screening to include adrenogenital syndrome in 2000, the annual cost of implementation would be EUR 154,000.

The costs of the various methods at an IRT cut-off value at 60 µg/l differ relatively little and the same therefore applies to the cost-effectiveness of the three methods. The CHOPIN researchers therefore concluded that other factors, such as the stress and the number of carriers detected, are more important for the choice of the method.

*Table 2* Annual costs of laboratory assays.

| | IRT cut-off value at 60 µg/l | IRT cut-off value at 50 µg/l |
|---|---|---|
| IRT-PAP-method | € 1,007,000 | € 1,007,000 |
| IRT-DNA-EGA-method | € 1,530,000 | € 1,087,000 |
| IRT-PAP-DNA-EGA-method | € 1,087,000 | € 1,061,000 |

The costs of screening methods were compared with those of diagnostics costs without screening. The latter were estimated to be EUR 9986 per patient, namely the indexed costs of analysis and admissions prior to diagnosis[13] and EUR 858,888 for more than 3000 sweat tests at EUR 274, for diagnostic analysis of patients who ultimately prove not to have CF. These costs (EUR 1,172,220, Table 3.2-5[2]) were deducted from the screening costs in the calculations.

The lifetime treatment costs (EUR 25.6 million) of a cohort year of CF patients could also possibly decrease, as screening could lead to savings, for which estimates vary from 0 to 5% (Table 3.1[2]).

## 3.3 Opinions of parents

Many parents were able to give proper answers to simple questions they were asked to ascertain their knowledge (Figure 6.1[2]). Parents from ethnic minority groups experienced many more problems with the information on heel prick screening. Heredity appears to be a particularly difficult subject for them but the same applied to some indigenous parents and care providers. Little use was made of the heel prick information provided on the website of the National Institute for Public Health and Environmental Protection (RIVM).

It was decided when the CHOPIN study was set up that parents would not be informed if they were carriers. CHOPIN researchers concluded on the basis of the questionnaires and group discussions that parents would like to be informed if they are carriers. According to CHOPIN researchers, the importance of proper information on being a carrier cannot be overemphasised.

Parents who receive positive test results are understandably extremely shocked and worried. After false-positive results (test incorrectly indicates that a person has the disease when this is not actually the case), many parents continue to be concerned even after a follow-up analysis has confirmed that the child does not have CF. Most parents feel that they have to wait too long (an average of four days) for the appointment for the follow-up analysis. The average period between carrying out the sweat test and hearing the results was 2-3 days, in a range of 0-28 days.

Chapter 4

# Conclusions on screening for cystic fibrosis

In 2005 the Health Council of the Netherlands drew attention to the additional benefits of screening for CF. It also pointed out that the screening methods used at the time had a relatively poor specificity and sensitivity (parameters for the number of false-positive or false-negative results: low specificity leads to a great deal of clinical investigation of unaffected neonates and insufficiently sensitive methods result in a failure to identify CF patients). It was also pointed out that the screening costs would probably be low.[1] These aspects are discussed below in relation to the results of the CHOPIN study and the turnaround times, detection of carriers, and provision of information are also discussed.

## 4.1 Additional benefits of screening

Various countries have introduced neonatal screening for CF.[12] Screening for an increase in IRT is always the first step in screening and the second step is generally mutation analysis. Evaluations of the screening results confirm the Health Council's previous conclusions on the additional benefits of screening.[1] Patients who were identified by neonatal screening proved to have a better nutritional status in their childhood years than those who had not been screened. Screening enables children and parents to avoid the aggravating uncertainty of a protracted diagnostics process. Screening also results in a slight decrease in morbidity[14] and is followed by fewer hospital admissions. Nutritional status also appears to be important for lung function.[15,16] Many experts deem the favourable effects to be

sufficiently great to recommend screening, as in the case of the European consensus meeting on screening, for example.[12]

The Health Council's 2005 advisory report divided possible neonatal screening checks into categories, namely disorders whereby considerable irreparable damage can be prevented (category 1), disorders in cases where the latter is less possible or has not been satisfactorily proved (category 2), and disorders whereby no health damage is prevented by neonatal screening (category 3). Screening for CF provides health benefits but they are less substantial than for the disorders in category 1, such as phenylketonuria and congenital hypothyroidism. CF is therefore in category 2, with the qualification that it is a borderline case.[1] The evaluations of screening results abroad[4,17,18] confirm that screening provides benefits but that they are not sufficient to warrant a revision of CF's category 2 classification. As mentioned in section 2.2, a great deal of research is being conducted into new types of therapies. Future developments in treatment may mean that CF ought to be placed in category 1.

Achieving the additional benefits of screening is dependent on the availability of proper medical care. Guidelines defined in Europe on screening for CF (*European best practice guidelines*)[12] and those in the United States (*Cystic Fibrosis Foundation workshop report*)[19] stress that the availability of this care is a precondition. CF expertise centres have been established in the Netherlands for the provision of the care (see Annex D) and they are obliged to meet set quality requirements. The minimum conditions according to the guidelines of the Institute for quality in care (CBO) on CF diagnostics and treatment (*Diagnostiek en behandeling van CF*)[20] are: at least 50 patients who are provided with continuous/chronic disease care; a CF team composed of a CF specialist (paediatrician/pneumonologist), physiotherapist, dietician, CF nurse, social worker, psychologist, clinical pharmacologist, microbiologist, secretary and database manager; the CF team and the patient files are available 24 hours a day for CF patients. The Ministry of Health, Welfare and Sport has provided the Dutch Cystic Fibrosis Foundation (NCFS) with a project subsidy to enable quality inspections for the CF centres. At the international level there is consultation on the optimum treatment of neonates when screening points to CF, and guidelines have been established on counselling their parents.[12] It would also be advisable to draft protocols/guidelines in the Netherlands on further diagnostics and treatment of neonates identified by screening as having CF and on following up.

Besides the aforementioned benefits, another advantage of screening is that the number of sweat tests and other aggravating examinations of non-CF patients can be reduced. CHOPIN researchers estimate the current number of sweat tests performed on non-CF patients to be 100 per diagnosis made.[2] This amounts to around 3,000 tests in a cohort year of 185,000 children. The tests are time-consuming for parents, laboratory staff and other care providers. This burden would be considerably reduced in the event of introducing neonatal screening for CF. Also in case the sweat test were to be replaced by DNA analysis to exclude CF as a diagnosis for children with long-term pulmonary problems and/or retarded growth, the reduction in the number of examinations would be beneficial. A temporary advantage of introducing screening would be that in families in which neonates are found to have CF, any older siblings who are affected but who have not yet had a diagnosis could be provided with a diagnosis quickly.

Treatment should be monitored to enable an assessment of the additional benefits of screening. Access to new treatment options is important for reducing the mortality rate but most definitely also for improving the quality of life.[4] Besides the aforementioned quality requirements for the CF expertise centres, it is therefore also important for the centres to participate in scientific research, in the form of clinical trials, for example.

The Committee's conclusion on the grounds of the above is that neonatal screening for CF clearly provides additional benefits and that the benefits have been confirmed by the results of research conducted since 2005.

## 4.2 Sensitivity and specificity

A conversion of the figures on the basis of the average number of newborns in recent years (185,000) shows that the screening methods used in the CHOPIN study would identify 25 classical CF patients per year. This figure is in line with the anticipated average number of 29, whereby the diagnosis for 4 of these patients (an estimated 17%) would be based on a meconium ileus. As indicated in the CHOPIN report, this figure of 25 is too low to allow final conclusions to be drawn on sensitivity. The follow-up period is also too short for this. Reference literature on screening using IRT, PAP and DNA analysis also lacks sufficient specific data on sensitivity.[12] Important to the sensitivity that can be expected is the actual birth prevalence. Adding CF to the neonatal screening programme would provide more clarity about this.

In various programmes, the limit for screening using IRT was set at the highest 1%, or even a lower percentage. A limit of 50 µg/l (the 2.43% highest concentrations) was adopted in the CHOPIN study and the calculation for an IRT concentration of 60 µg/l concerns the highest 1.03%. Relatively low IRT concentrations were found in CF patients with a meconium ileus.[19] Increasing the limit to 60 µg/l produces a sharp drop in the number of non-classical CF patients identified by screening.

The sensitivity of the PAP determination is not high. Of the 10 patients identified in the CHOPIN study, five had a concentration below the limit of 1.8 mg/l (CHOPIN report, page 27). However, all patients identified by IRT and DNA screening would also be identified by the combination of IRT ≥60 µg/l *and* PAP ≥1.8 µg/l, or IRT ≥100 µg/l *and* PAP ≥1.0 µg/l.

The percentage of clinically relevant mutations that occur in the DNA panel is an important factor in the sensitivity of the mutation analysis based on the initial test for the 36 frequently occurring mutations. In the case of the panel used for the CHOPIN study, this percentage is estimated for the indigenous population to be approximately 94%; for the F508delta-mutation – the most frequently occurring mutation – the percentage is approximately 76%. On the basis of this, the likelihood of failing to identify a patient with two mutations is 0.36%. Sensitivity is lower for screening immigrant population groups because the 36 mutations used in the DNA analysis panel occur less often in these CF patients. It appears from a survey of CF mutations among Turkish and North African immigrants in Europe21 that F508del is the most frequently occurring mutation in their case too, but that it occurs considerably less frequently than in the indigenous Dutch population (Annex E).

The survey results were compared with the DNA panel to determine how many neonates with CF in immigrant populations would be identified using IRT-PAP-DNA-EGA screening. In the case of Turkish immigrants 44% of the identified mutations were present in the panel and the figure for North African immigrants was 69% (appendix E). The number of CF patients expected in the Turkish and Moroccan population groups can be approximated on the basis of the number of residents, the prevalence of carriers, and the birth rates. The estimated annual figure for the two groups is 0.69 and 0.62 respectively, which corresponds with the NCFS data (11 Turkish and 11 Moroccan patients among 650 patients from the age of 0 to 19 years). A calculation based on combination with the detection percentage shows that using the IRT-PAP-DNA-EGA method would lead to a fail-

ure to detect one patient every three years in the Turkish population group and one patient every five years in the Moroccan population group (Annex E). Various relevant mutations in immigrant groups are expected to be added to the mutation panel during the coming years.

As it is not possible to draw a final conclusion on sensitivity – especially with regard to mutations among immigrant population groups – the Committee's recommendation for the time being is that a *failsafe* procedure should be taken into account, as was also mentioned by the CHOPIN researchers. In such a procedure, in the case of an IRT concentration of ≥100 µg/l, an EGA should also be conducted if no mutation is identified using the DNA panel. This would involve an average of 94 determinations annually (Annex C). Within the same framework, it is important that CF patients be reported to the Centre for Population Screening's Neonatal Screening Advisory Committee on CF, which is due to be established.

The CHOPIN researchers calculated specificity on the basis of the numbers of classical CF patients, while stating that they could also have opted for classical *and* non-classical. In the latter case, specificity is 100% for the protocols using DNA-EGA. An impression could be created that there are two clearly distinguishable groups of CF patients but there is actually a continuum. Severe and less severe types of the disease can be expected with certain combinations of mutations, and less severe types and healthy ones with other combinations, depending on other factors (modulator genes and living conditions). Choices need to be made for screening with regard to the limits set for concentrations of IRT and PAP as well as with regard to which mutations may be deemed to be positive. For example, discussions are underway about the importance for screening of the relatively frequently occurring R117H mutation.[22,23] These choices can be adjusted on the basis of growing knowledge, as has been the case in the past with various other cut-off values in neonatal screening. In making these choices it is important that the detection of less severe forms is not a primary objective of screening, as was also pointed out by the CHOPIN researchers. Evaluation of the results may also lead to the conclusion that the *failsafe* procedure is superfluous or should be replaced by another procedure.

The results of the CHOPIN study shown in Table 1 indicate that the IRT-PAP method failed to detect any non-classical patients and carriers, but that numerous deviant results make follow-up diagnostics necessary. It is pointed out here that the follow-up diagnostics conducted in connection with the deviant results will

lead to more non-classical patients and carriers being identified than would be the case with a screening protocol involving IRT-PAP-DNA-EGA.

More than 1,600 mutations are known in the *CFTR gene*[6] but the use of EGA could nevertheless lead to more previously undescribed mutations being identified. It is clear in some cases that a mutation causes classical CF, such as when the mutation prevents the formation of *CFTR protein*, for example. When there is no clarity about this the term *unknown variants* is used. These mutations present a problem in large-scale mutation analyses conducted in populations with a low risk, but in the small group of neonates in which the risk of CF is considered high on the grounds of the concentrations of IRT and PAP, such a result should be assumed positive for the neonates concerned.

Sensitivity and specificity are furthermore dependent on the reliability of the test methods and an accurate interpretation of the results. The IRT, PAP and DNA determinations were carried out using standard methods which have been thoroughly tested in practice and lead to relatively few interpretation problems. More expertise and experience are required for EGA, owing to the wide range of mutations/variants in the *CFTR gene*. Knowledge must also be kept up-to-date for the evaluation of the DNA panel used for simple mutation analysis. In the Netherlands, the expertise is available in the molecular diagnostics laboratories which are attached to the clinical genetics departments of Erasmus MC, UMCG and VUMC. The *European best practice guidelines* mentioned in section 4.1 state that accreditation (ISO 15189 or equivalent) is required for laboratories that conduct mutation analyses. DNA panels have to be validated and must participate annually in international quality controls.[12] The Committee is in favour of these quality requirements and recommends that both the simple and extended mutation analysis be carried out in close cooperation with a clinical genetics centre which specialises in cystic fibrosis. To this end, clear arrangements should be made in advance on the responsibilities, tasks, method of working and protocol (see also (in Dutch) www.st-ab.nl, Wet op bijzondere medische verrichtingen [Special Medical Procedures Act], Besluit aanwijzing bijzondere medische verrichtingen 2007 [Special Medical Procedures Designation Decree 2007]).

Given the limitations of the sweat test for neonates, as also confirmed by the CHOPIN study, the test should not be included in the screening protocol. Sensitivity and specificity are inadequate. The role of the sweat test and other functional tests in supplementary diagnostics will be left to the paediatrician/ pneumonologist who the patient is referred to for treatment.

The CHOPIN report uses the term ‘DNA analysis’ for the analysis of 36 mutations and ‘EGA’ for the sequence determination of exons and exon-intron boundaries. As ‘DNA analysis’ can also be interpreted as the analysis of the entire genome, it would be preferable to use the term ‘limited mutation analysis’ in the information provided for patients. The term *extended gene analysis* may also lead to more being expected than the aforementioned sequence determination and it would therefore be better to replace it with the term ‘extended mutation analysis’.

The Committee concludes that high specificity screening for CF can be performed using the IRT-PAP-DNA-EGA protocol. Bearing sensitivity considerations in mind, the Committee recommends that screening should be carried out using the IRT-PAP-DNA-EGA protocol, with the aforementioned *failsafe* procedure for the time being. Evaluations of mutation analysis may indicate that changes in the mutation panel and/or the *failsafe* procedure are advisable.

## 4.3 Costs and savings

Early detection of CF appears to cost less than the clinical diagnostics used at present, and the treatment costs of screened patients who are therefore identified at an early stage are usually lower. Calculations based on data from the Netherlands[13] and the United Kingdom[24] indicate cost savings. However, long-term results are difficult to predict, also because the effects and costs of new treatments may play an important role in this.

The CHOPIN researchers estimated the net cost of screening using the IRT-PAP-DNA-EGA method to be approximately EUR 140,000[2] and concluded that the costs of the examined methods with an IRT cut-off value at 60 µg/l differed relatively little from those with an IRT cut-off value at 50 µg/l, and this therefore also applies to cost-effectiveness. It emerged from the explanation of laboratory costs that a major cost saving could be achieved by adjusting the deployment of resources for PAP screening into line with the actual numbers.

Further cost savings would result from the reduction in diagnostics costs after the neonatal period, which can be divided into those of CF patients and those of non-CF patients who have been examined because they had similar symptoms or pulmonary or nutritional problems which led to CF being suspected.

The aforementioned diagnostics costs without screening were estimated to come to EUR 1,172,220 (see section 3.2) and were deducted in full from the screening costs. Subject to screening sensitivity proving to be high, the number of diagnostic analyses (sweat tests and DNA tests) can be expected to fall in due course. However, the rate and extent to which this will take place are not yet clear. The introduction of a *failsafe* procedure will increase sensitivity and confidence in screening and is therefore an important factor in the decrease in diagnostics costs. The average estimated costs of this procedure amount to EUR 39,000 per year (94 x EUR 417[2]).

The cost-effectiveness calculation does not include possible savings on the cost of DNA diagnostics. A total of 1261 postnatal DNA analyses for CF were conducted in 2008 at a cost of EUR 740 each (in UMCG, VUMC and Erasmus MC). The Institute for quality in care (CBO) recommends that a mutation analysis should be the first step when there is a clinical suspicion of CF.[20] In due course, once heel prick screening for CF has been introduced, the number of DNA analyses conducted in connection with a clinical suspicion of CF is expected to fall. However, there will be no change in the percentage of postnatal DNA analyses conducted on account of fertility problems or echography findings that indicate CF in an unborn child.

The CHOPIN researchers included only the costs of genetic counselling in case methods involving mutation analysis were used. However, following a positive test result from screening using the IRT-PAP method, mutation analysis and genetic counselling will also usually take place in practice (whereby an average of 220 positives per year can be expected for an IRT concentration limit of 60 µg/l); consequently the IRT-PAP method also involves counselling costs. On the other hand, genetic counselling may also lead to savings, which the CHOPIN researchers did not take into account in the cost-effectiveness analysis.

There is uncertainty about the savings on treatment costs anticipated by various researchers. Studies of these costs conducted in the United Kingdom among patients in the age group up to 10 years concluded that considerable cost savings (averaging 80%) are made in the first three years of life in the group identified by screening, but that there is no significant difference after that between screened and non-screened patients.[14] The British researchers point out that the amounts largely depend on the protocols; for example, a reduction in the number of daily intravenous administrations of antibiotics can make a large difference in the costs. These differences have a major effect on cost-effectiveness because treat-

ment costs are high. For example, the annual costs for children with CF in the Netherlands are estimated to be around EUR 35,000 per child (excluding complications and home care[2]).

Screening costs were estimated by the Committee on the basis of the numbers in the CHOPIN study. The year 2008 was taken as the starting point for the cost of DNA analysis.

On the basis of those estimates, the laboratory costs of the IRT-PAP-DNA-EGA method would be EUR 1,096,000 per year (the IRT determination 185,000 x EUR 3.85, the PAP determination EUR 294,000, the mutation analysis 220 x EUR 234), and the amount for the failsafe procedure would be EUR 39,000. The cost of performing the entire neonatal heel prick screening programme would increase by EUR 154,000 per year with the addition of screening for CF (by way of analogy with the cost increase at the time of adding AGS to the screening programme). The estimated total cost of screening a cohort year would then come to EUR 1,250,000.

With the present policy, the estimated diagnostics costs following clinical suspicion of CF come to EUR 1,754,000, namely EUR 822,000 for 3000 sweat tests and EUR 932,000 for DNA analysis. In the first three years of life, the estimated treatment costs for a cohort year amount to EUR 3,045,000 (29 x 3 x EUR 35,000).

This means that the costs of adding neonatal screening for CF would amount to an estimated 71% of the cost of the present policy. However, the savings on diagnostics and treatment costs were calculated on the basis of data based on relatively little research. Moreover, little is known about how these costs might develop in the future. Nevertheless, the order of magnitude of the stated amounts is plausible and the introduction of screening would probably lead to cost savings.

The Committee concludes that using these methods for neonatal screening does not appear to involve any exceptionally high costs and that doing so could lead to savings. There is little difference between the costs of the screening methods tested and the choice therefore has to be based on other more important factors, such as the stress screening causes for neonates and their parents.

## 4.4 Turnaround times and period of uncertainty for parents

Completing screening within four weeks appears to be possible as a rule. Screening using IRT requires a few days, and PAP determination and the limited mutation analysis jointly take a week, provided they are performed in screening laboratories (as in that case no time is required for sending the cards to another laboratory). This would mean that the vast majority of heel prick samples (>99.9%) would be conclusively analysed. The extended mutation analysis could also be performed along with the analysis of the heel prick sample but would take longer. Given a well-organised programme, it would be possible to conduct the extended analysis within a few weeks. Screening can be expected to identify an average of 25 patients and 12 carriers (Annex C). The parents of 23 patients could be given an appointment for a follow-up examination after a little over a week, and the parents of 2 patients and 12 carriers would have to wait approximately a month. Clear information at the time of arranging this appointment would have to be provided about the analysis to ensure transparency for parents about the follow-up procedure, and an opportunity would have to be created soon for an advisory consult and a follow-up examination. If notice of the result can only be provided after several weeks, it is important to indicate that the delay does not generally involve a major risk for a neonate with CF.

## 4.5 Carriers

It follows that if the neonate is a carrier, one or both parents and possibly other children and family members will be carriers. In the event of both parents being carriers, CF could occur in any subsequent children. Knowledge of being a carrier is therefore relevant for some parents in connection with future family planning choices. The Committe considers the identification of carriers within the scope of neonatal screening a secondary finding rather than the objective. However, the severity of the disorder means that, if requested, it would be necessary to provide genetic advice and treatment options. Certainly, parents must be able to make an informed and conscious choice and the consent of parents is required for the provision of information on being a carrier.[1]

The CHOPIN report states that no carriers are found when the IRT-PAP method is used, at least not by the screening process. However, because parents who receive a positive test result are given a referral, further analysis may nevertheless reveal that a person is a carrier. It emerged from the CHOPIN study that par-

ents are generally of the opinion that they should be able to choose whether information is provided on whether or not their child is a carrier. It is plausible that parents who wish to receive this information would have preferred to have known at an earlier stage about being a carrier, within the scope of preconception care for example.

Identification of carriers in neonatal screening for CF only occurs for a small number of neonates, the annual average being 12 neonates in the case of the IRT-PAP-DNA-EGA strategy referred to in Annex C. This differs sharply from the results in the case of neonatal screening for hemoglobinopathy, for which a method is used that leads to practically all carriers being identified. Parents of a neonate whose screening shows a carrier or CF should be informed of the importance of genetic counselling and the possibility of referral to a clinical geneticist, unless they have indicated that they do not wish to receive information indicating that a person is a carrier. General practitioners should enable parents to make an appointment for genetic counselling and should ensure that the information is properly conveyed to the clinical geneticist. Any follow-up analysis also differs from that conducted in the case of a hemoglobinopathy carrier, as mutation analysis is necessary to determine whether a person is a CF carrier.

## 4.6 Information

A screening programme requires the provision of proper information. Parents should receive information on the nature, prevalence and severity of the disorders being investigated as well as on the importance of early diagnosis; the treatment options and/or other benefits available for the person concerned; the fact that the test results often need to be confirmed and may involve false alarms; the possibility of a failure to identify cases of the disease and that not all diseases are identifiable; the use of residual material from the heel prick for scientific research; the protection of privacy; the possibility that a carrier may be identified and of the option of whether or not to be informed of this.[1] The European and American guidelines[12,19] referred to in section 4.1 also state that providing parents with proper information must be one of the set requirements for the screening programme. The general practitioners of parents whose child is diagnosed as having CF should arrange for their referral to the clinical geneticist and for the information to be conveyed properly. Parents should also be informed of the consequences for other children and family members. In the CHOPIN study parents and health care workers were asked questions to ascertain their knowledge of CF screening. Researchers concluded that parents are well informed about screening

and that they are positive about the information they receive on CF screening. However, in contrast to the positive assessment of the information provided, 15% of obstetricians and screeners 'sometimes' provide information and 13% never do so. Half of the people who provided information did not point out that participation in CF research is voluntary. Moreover, 35% of those who provided the information never provided the brochure and 19% 'seldom' did so. Heredity and being a carrier appear to be problem areas in the information, especially in the case of immigrant parents and those with a low level of education.

The question may arise as to whether parents should be informed if mutations/variants are identified that are not associated with the disease for which screening is performed. In the clinical context, the duty to inform applies as laid down in the Medical Treatment Contracts Act. According to the Act, the care provider who provides the information should be guided by "what the patient reasonably ought to know". Therefore, care providers may limit themselves to providing the information that is of clinical significance or otherwise constitutes relevant and useful knowledge for the patient. The Committee deems a similar approach to be appropriate for population screening and recommends only providing information on mutations if the information is clinically or otherwise useful.

In the case of identified mutations which are known to lead to less severe types of CF (non-classical mutations), it is advisable to monitor the course of the disease, which is also what the CHOPIN researchers recommended. The Committee stresses that once mutations of this kind have been identified, parents must be provided with proper information and guidance to avoid lasting concerns.

The Committee concludes that there were gaps in the information provided in the CHOPIN study. In the event of the Minister of Health, Welfare and Sport deciding to introduce neonatal screening for CF, the information should be an important point for attention, especially with regard to the possibility of being a carrier. Improvements are also required in the field of refresher training courses for care providers.

The Centre for Population Screening (CVB) of the National Institute for Public Health and Environmental Protection (RIVM) is responsible for the implementation of the national neonatal heel prick screening programme. CVB has developed information material for parents and professionals and updates it regularly. At every level, care providers and those carrying out heel pricks in the Nether-

lands receive training in the provision of information. This information should be expanded if CF is added to the diseases covered by neonatal screening.

Chapter 5

# Questions and answers addressed by the advisory report

In his request for an advisory report (Annex A), the Minister of Health, Welfare and Sport requested that particular attention be paid to the following four questions. In this final chapter, the Neonatal Screening Committee makes one or more recommendations per question.

### What is the Health Council's advice on adding CF to the diseases covered by neonatal heel prick screening?

In view of the additional benefits of screening and the availability of screening methods with sufficiently high specificity and sensitivity, the Committee recommends including CF in the neonatal screening programme.

### Has it been established that early screening for CF would provide significant additional benefits with regard to the health of neonates with CF?

Studies of screening results conducted abroad confirm the Committee's opinion that screening provides additional benefits, as also described in the Health Council's 2005 advisory report *Neonatal Screening*.

## What is the preferred screening protocol?

The Committee recommends conducting screening in four stages, starting with a determination of the IRT concentration in heel prick blood, with an upper limit of 60 µg/l, followed by a determination of the PAP concentration with an upper limit of 1.8 µg/l. If the IRT concentration is 100 µg/l or higher, the upper limit for the PAP concentration will be 1 µg/l. In the events of these upper limits being exceeded, the Committee recommends conducting a limited mutation analysis using a panel that includes the 36 most frequently occurring CF mutations. If one CF mutation is identified or the IRT concentration is 100 µg/l or higher, an extended mutation analysis should be conducted to ascertain whether there are two CF mutations (Annex C).

It may become clear in practice that a different upper limit should be used for optimal screening, or that alterations are required in mutation analysis.

The costs of various screening methods are not widely different. The costs involved in CF screening are relatively low and can be expected to be entirely or largely offset by the reduction in the cost of diagnosis (in the case of a clinical suspicion of CF in non-CF patients) and the reduction in the cost of treatment, especially in the early years of life (owing to the decrease in the number of hospital admissions of CF patients).

Although not the aim of CF screening, identifying carriers should not be seen as a disadvantage either. The screening method recommended by the Committee would only lead to a small number of carriers being identified.

To avoid damaging confidence in heel prick screening and to maximise utilisation of the possibilities early diagnosis offers, the Committee recommends that the analysis should be completed within four weeks of the heel prick. Approximately one week is required to determine the concentrations of IRT and PAP and to conduct the limited mutation analysis using panels; the anticipated average number of patients who will require this is 23 per year. The extended mutation analysis should be organised so that parents can be informed of a positive result within four weeks; this concerns an average of 2 patients and 12 carriers per year).

The number of less severe forms that will be identified by using the recommended screening protocol is highly restricted by the combination of IRT and PAP determinations using mutation analysis. To enable assessment of the screening protocol and the provision of the best treatment, it would be advisable to monitor the clinical course of the less severe forms of CF identified by screening.

### What are the Health Council's recommendations on providing information on being a carrier and on detecting mild variants?

The Committee notes that information still requires a great deal of attention. Amongst other subjects, it should cover the disorder, the importance of screening, information on the possibility of being a carrier, and the provision of consent. The refresher training courses for the care providers concerned should also be improved. Separate information on less severe forms of CF is not required. After all, variations in severity also occur in other disorders covered by the heel prick programme.

# References


1 Health Council of the Netherlands. Neonatal Screening. The Hague: Health Council of the Netherlands, 2005; publication no. 2005/11.

2 Vernooij-van Langen AMM, Dankert-Roelse JE, namens de CHOPIN-projectgroep. Eindrapport CHOPIN studie (Cystic fibrosis Hielprik Onderzoek bij Pasgeborenen in Nederland), Bilthoven, mei 2009.CHOPIN studie eindrapport mei 2009.

3 Cystic Fibrosis Foundation. Annual Data Report 2007, p3. CFF, Bethesda, VS 2007.

4a O'Sullivan BP, Freedman SD. Cystic Fibrosis. Lancet 2009; 373 (9678): 1891-904.

4b Kerem E, Hirawat S, Armoni S, *et al*. Effectiveness of PTC124 treatment of cystic fibrosis caused by nonsense mutations: a prospective phase II trial. Lancet 2008; 372(9640): 719-27.

5 Sarles J, Berthézène P, Le Louarn C, Somma C, Perini JM, Catheline M, *et al*. Combining immunoreactive trypsinogen and pancreatitis-associated protein assays, a method of newborn screening for cystic fibrosis that avoids DNA analysis. J Pediatr. 2005; 147(3): 302-5

6 Castellani C, Cuppens H, Macek M Jr, Cassiman JJ, Kerem E, Durie P, *et al*. Consensus on the use and interpretation of cystic fibrosis mutation analysis in clinical practice. J Cyst Fibros. 2008; 7: 179-196.

7 Dequeker E, Stuhrmann M, Morris MA, Casals T, Castellani C, Claustres M, *et al*. Best practice guidelines for molecular genetic diagnosis of cystic fibrosis and CFTR-related disorders--updated European recommendations. Eur J Hum Genet. 2009; 17(1): 51-65.

8 Kate LP ten. Cystic fibrosis in the Netherlands. Int J Epidemiol. 1977; 6(1): 23-34.

9 Slieker MG, Uiterwaal CS, Sinaasappel M, Heijerman HG, van der Laag J, van der Ent CK. Birth prevalence and survival in cystic fibrosis: a national cohort study in the Netherlands. Chest. 2005; 128(4): 2309-15.

10 Scotet V, Audrezet MP, Roussey M *et al*. Impact of public health strategies on the birth prevalence of cystic fibrosis in Brittany, France. Hum Genet 2003; 113: 280–5.

11a Hale JE, Parad RB, Comeau AM. Newborn screening showing decreasing incidence of cystic fibrosis. N Engl J Med 2008; 358: 973–4.

11b Castellani C, Picci L, Tamanini A, *et al*. Association between carrier screening and incidence of cystic fibrosis. JAMA 2009; 302(23): 2573-9.

12 Castellani C, Southern KW, Brownlee K, Dankert Roelse J, Duff A, Farrell M, *et al*. European best practice guidelines for cystic fibrosis neonatal screening. J Cyst Fibros. 2009; 8: 153-173.

13 Akker-van Marle ME van den, Dankert HM, Verkerk PH, Dankert-Roelse JE. Cost-effectiveness of 4 neonatal screening strategies for cystic fibrosis. Pediatrics. 2006; 118(3): 896-905.

14 Sims EJ, Clark A, McCormick J, Mehta G, Connett G, Mehta A. Cystic fibrosis diagnosed after 2 months of age leads to worse outcomes and requires more therapy. Pediatrics. 2007; 119(1): 19-2813.

15 McKay KO, Waters DL, Gaskin KJ. The influence of newborn screening for cystic fibrosis on pulmonary outcomes in new South Wales. J Pediatr. 2005; 147(3 Suppl): S47-50.

16 Padman R, McColley SA, Miller DP, Konstan MW, Morgan WJ, Schechter MS, *et al*. Infant care patterns at epidemiologic study of cystic fibrosis sites that achieve superior childhood lung function. Pediatrics. 2007; 119(3): e531-7.

17 Sharp JK, Rock MJ. Newborn screening for cystic fibrosis. Clin Rev Allergy Immunol. 2008; 35(3): 107-15.

18 Southern KW, Mérelle MM, Dankert-Roelse JE, Nagelkerke AD. Newborn screening for cystic fibrosis. Cochrane Database Syst Rev. 2009 Jan 21;(1): CD001402. Update of: Cochrane Database Syst Rev. 2001;(3): CD001402.

19 Comeau AM, Accurso FJ, White TB, Campbell PW 3rd, Hoffman G, Parad RB, *et al*. Guidelines for implementation of cystic fibrosis newborn screening programs: Cystic Fibrosis Foundation workshop report. Pediatrics. 2007; 119(2): 495-518.

20 CBO. Richtlijn Diagnostiek en Behandeling Cystic Fibrosis. Kwaliteitsinstituut voor de Gezondheidszorg CBO, Utrecht 2007.

21 Lakeman P, Gille JJ, Dankert-Roelse JE, *et al*. CFTR mutations in Turkish and North African cystic fibrosis patients in Europe: implications for screening. Genet Test. 2008; 2(1): 25-35.

22 Scotet V, Audrézet MP, Roussey M, Rault G, Dirou-Prigent A, Journel H, *et al*. Immunoreactive trypsin/DNA newborn screening for cystic fibrosis: should the R117H variant be included in CFTR mutation panels? Pediatrics. 2006; 118(5): 1523-9.

23 Thauvin-Robinet C, Munck A, Huet F, Génin E, Bells G. Gautier E, *et al*. The very low penetrance of cystic fibrosis for the R117H mutation: a reappraisal for genetic counseling and newborn screening. J Med Genet 46(11) : 752-8, online 29 June 2009.

24 Sims EJ, Mugford M, Clark A, Aitken D, McCormick J, Mehta G, Mehta A. Economic implications of newborn screening for cystic fibrosis: a cost of illness retrospective cohort study. Lancet. 2007; 369(9568): 1187-95.

25 Kilinç MO, Ninis VN, Daðli E, Demirkol M, Ozkinay F, Arikan Z, *et al*. Highest heterogeneity for cystic fibrosis: 36 mutations account for 75% of all CF chromosomes in Turkish patients. Am J Med Genet. 2002;113(3):250-7.

# Annexes

Annex **A**

# Request for advice

Letter of 9 July 2009 (reference PG/OGZ-2940324) from the Minister of Health, Welfare and Sport to the President of the Health Council of the Netherlands.

**Background**

In view of recent research and scientific developments a further advisory report is required from the Health Council of the Netherlands on the possible expansion of neonatal heel prick screening to include cystic fibrosis (CF).

On 22 August 2005, at the request of the then State Secretary for Health, Welfare and Sport, the Health Council published an advisory report on neonatal heel prick screening. The advisory report concerned two central topics: should the screening package be expanded and are the criteria for neonatal screening still up-to-date.

The Health Council assessed and discussed 30 disorders and divided them into three categories on the basis of a number of criteria. The Health Council took the view that disorders whereby considerable irreparable damage could be prevented belonged in category 1 and that disorders in cases where the latter was less possible or had not been satisfactorily proven belonged in category 2. The disorders which the Health Council's advisory report recommended should be included in the screening programme were all in category 1.

The Health Council put CF in category 2, with the qualification that it is a borderline case between categories 1 and 2. The Health Council recommended including CF in neonatal heel prick screening as soon as test methods became available with a higher specificity and recommended research into better screening methods.

In November 2005, on the basis of the aforementioned advisory report, the Ministry of Health, Welfare and Sport decided to expand screening with a total of 14 disorders, including metabolic disorders and sickle cell anaemia. In accordance with the Health Council's recommendations, CF was not included in the expansion. Expanded heel prick screening was introduced on 1 January 2007.

### CHOPIN pilot study

On 24 February 2006, the Ministry of Health, Welfare and Sport informed the Netherlands Organisation for Health Research and Development of the advisory report and view of the Health Council on the possible expansion of neonatal heel prick screening to include CF. It was pointed out that the intention was to expand neonatal screening to include CF once it had been conclusively proven that early screening for CF provided additional benefits for the health of neonates, was cost-effective and could be performed using adequate test methods.

The Netherlands Organisation for Health Research and Development subsequently funded the implementation of a CF population screening trial, CHOPIN (Cystic fibrosis heel prick screening of neonates in the Netherlands) in Gelderland, Limburg, Noord-Brabant and Utrecht in 2008, including an extension until June 2009. I received the final report on the CHOPIN study on 22 June 2009 and hereby present it to you. You have already been provided with the digital version.

### Information on being a carrier

Experience has now been gained with the expanded neonatal heel prick screening programme in general and with the information and ‘informed consent’ in particular. Experiences relating to sickle cell anaemia are particularly important for this request for an advisory report. The test used in screening for sickle cell anaemia provides information on whether a person is a carrier, while this is not the primary intention of screening. The same applies to CF, although the number of carriers identified depends on the test strategy chosen.

Parents are informed of the possible outcomes of screening before the child’s birth and therefore prior to screening; this information includes details of whether the child is a carrier and the possible consequences of that information in relation to whether the parents and their other children are carriers. The ‘informed consent’ of parents is required prior to screening, before they may be provided with information on being a carrier after screening has taken place. Parents are free to determine beforehand whether they wish to receive the information. Owing to the complicated message that has to be conveyed, difficulties have emerged in practice with regard to the information as well as the required ‘informed consent’. It has also emerged in practice that a great deal of misunderstanding arises in parents about the child’s health when they are informed of screening results indicating that the child is a carrier (not sick but is a carrier).

An additional problem is anticipated in screening for CF because the disease involves several mutations, some of which are as yet unknown. A need for guidelines on how to handle information on coincidental findings and on being a carrier has arisen in the current practice of performing certain population screening programmes, such as neonatal heel prick screening and prenatal screening. This

need will increase owing to technological developments, such as those which enable various types of genetic screening. Guidelines of this kind are required on providing advice about CF in aid of the possible implementation of screening for CF as part of neonatal heel prick screening.

### CHOPIN project group recommendations

The project group concerned with neonatal screening for CF formulated various recommendations. To summarise, the project group calls for adoption of a screening strategy (combination of tests) with the lowest possible number of false-positive starting points, the lowest possible number of children with mild CF variants and the lowest possible number of identified carriers.

### Specific advice requested

Please pay particular attention to the following requests for advice:

1 What is the Health Council's current advice on adding CF to the diseases covered by neonatal heel prick screening.

2 Has it been established that early screening for CF would provide significant additional benefits with regard to the health of neonates with CF.

3 If the Health Council's advice is affirmative on adding CF to the diseases covered by neonatal heel prick screening, what would be the preferred test method and what are the assessments that form the basis for this preference? Please take the following points into account in making your assessments:
   - Cost aspects and cost-effectiveness;
   - The choice of test method has consequences for the number of CF carriers identified, whereas this is not the primary objective of screening, partly given the project group's recommendations;
   - The consequences for parents with regard to the differences in turnaround times of the screening strategies, whereby in exceptional cases the turnaround time can be up to 87 days from screening to result;
   - Identification of coincidental findings or 'mild variants' in CF screening, also in view of the project group's recommendations.

4 What are the Health Council's recommendations on providing information on being a carrier and on detecting mild variants, given the above and taking into account the Health Council's advice on point three regarding the test method.

I would also ask you to take into account the relevant social, ethical and legal aspects when forming your opinion.

If possible, please present your advisory report before 15 September 2009.

Kind regards,
the Minister of Health,
Welfare and Sport,
(signed)
Dr A. Klink

Annex **B**

# The Committee

- Dr G.C.M.L. Page-Christiaens, *President*
  gynaecologist, Utrecht University Medical Centre (UMC)
- Professor M.F. Niermeijer, *Vice-President* (until 18 November 2009)
  professor of clinical genetics, University Medical Centre St Radboud, Nijmegen
- Professor M.C. Cornel
  professor of community genetics and public health genomics, VU University Medical Centre, Amsterdam
- Dr J.C.J. Dute
  health lawyer, University of Amsterdam
- P.C. Groeneveld, *observer*
  Ministry of Health, Welfare and Sport
- Professor H.S.A. Heymans
  professor of paediatrics, Amsterdam University Medical Centre
- Dr J.G. Loeber
  biochemist, National Institute for Public Health and Environmental Protection, Bilthoven
- Professor G.P.A. Smit
  paediatrician, Groningen University Medical Centre
- Dr M.F. Verweij
  ethicist, University of Utrecht

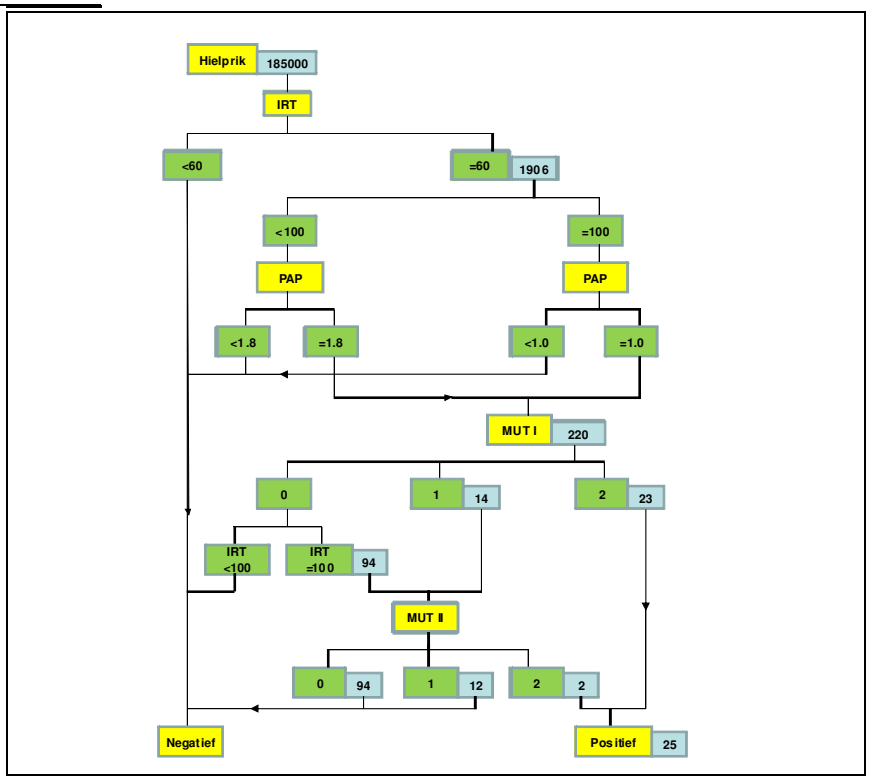
- Dr P.A. Bolhuis, *scientific secretary*
  Health Council of the Netherlands, The Hague

## The Health Council and interests

Members of Health Council Committees – which also include the members of the Advisory Council on Health Research (RGO) since 1 February 2008 – are appointed in a personal capacity because of their special expertise in the matters to be addressed. Nonetheless, it is precisely because of this expertise that they may also have interests. This in itself does not necessarily present an obstacle for membership of a Health Council Committee. Transparency regarding possible conflicts of interest is nonetheless important, both for the President and members of a Committee and for the President of the Health Council. On being invited to join a Committee, members are asked to submit a form detailing the functions they hold and any other material and immaterial interests which could be relevant for the Committee's work. It is the responsibility of the President of the Health Council to assess whether the interests indicated constitute grounds for non-appointment. An advisorship will then sometimes make it possible to exploit the expertise of the specialist involved. During the establishment meeting the declarations issued are discussed, so that all members of the Committee are aware of each other's possible interests.

Annex **C**

# Screening protocol diagram



The average anticipated result for 185,000 neonates (the average of the past five years) was calculated on the basis of the results of the CHOPIN study. This concerns the following determinations: IRT determination for which the result was IRT ≥60 µg/l for 1,906 neonates (1.03%); PAP determination in the case of IRT ≥60 µg/l and PAP ≥1.8 µg/l, or IRT ≥100 µg/l and PAP ≥1.0 µg/l, positive for 220 neonates (p. 55 CHOPIN report: 214 in 180,000); the limited mutation analysis (MUT I) using a panel of 36 frequently occurring CF mutations, positive in 37 neonates (23 with 2 mutations and 14 with 1 mutation); and the extensive mutation analysis (MUT II) for the 14 neonates with one of the mutations in the panel and for 94 neonates with IRT ≥100 µg/l without any of the mutations in the panel (*failsafe* procedure), of these 108 neonates, 2 had a second CF mutation. The total average result that can be expected is 25 positives with 2 mutations, and 12 carriers.

Annex **D**

# Overview of CF centres in the Netherlands

- Amsterdam
  children: VU University Medical Centre, Academic Medical Centre
  adults: Academic Medical Centre
- The Hague
  children: Haga Hospital, location Juliana Children's Hospital
  adults: Haga Hospital, location Leyweg
- Groningen
  children and adults: Groningen University Medical Centre
- Maastricht
  children and adults: Maastricht University Medical Centre
- Nijmegen
  children and adults: Cystic Fibrosis Centre, East Netherlands (location Dekkerswald University Medical Centre, department of pulmonary diseases)
- Rotterdam
  children: Erasmus Medical Centre - Sophia
  adults: Erasmus Medical Centre - Dijkzigt
- Utrecht
  children: Wilhelmina Children's Hospital
  adults: Utrecht University Medical Centre

Annex **E**

# CF among neonates of Turkish or Moroccan descent

The number of CF patients that can be anticipated in Turkish and Moroccan population groups can be approximated on the basis of the number of residents, prevalence of carriers, and birth rates. According to Statistics Netherlands, the figures for the number of residents are 381,000 and 345,000 respectively. Estimates of the number of carriers can only be roughly approximated but are lower than average in the Netherlands and may work out at around 1 in 50 for the Turkish population (LIT 25) and even lower for the North African population. An estimate of 1 in 50 of the population was adopted for both population groups. The birth rate is higher than the average of 1.1% but probably not in excess of 1.8%. It follows from these figures that the average annual number of neonates with CF in these population groups will be 0.69 and 0.62 respectively. This expectation is in line with the fact that of the CF patients (<19 years of age) in the patient records of the Dutch Cystic Fibrosis Foundation, 1.7% are of Turkish descent and 1.7% are of Moroccan descent. With an average likelihood of detection according to the IRT-PAP-DNA-EGA protocol of 0.44 and 0.69 respectively (see Table 3), the annual likelihood of CF patients not being identified is 0.39 and 0.19 respectively, if the data on North African immigrants do not differ significantly from data on people of Moroccan descent. This therefore concerns one patient every three years and one patient every five years respectively.

*Table 3* CF-mutations present in the mutation panel *and* detected among Turkish and North African immigrants in Europe.

| Mutation | Turkey | North Africa | Mutation | Turkey | North Africa | Mutation | Turkye | North Africa |
|---|---|---|---|---|---|---|---|---|
| F508del | 33 | 40 | A455E | 0 | 0 | G542X | 6 | 8 |
| 1717-1G>A | 0 | 0 | S1251N | 0 | 0 | R553X | 0 | 1 |
| R1162X | 0 | 11 | 3272-26A>G | 0 | 0 | W1282X | 3 | 4 |
| 2789+5G>A | 3 | 3 | 711+1G>T | 0 | 14 | E60X | 0 | 0 |
| N1303K | 12 | 10 | 1078delT | 0 | 0 | 3659delC | 0 | 0 |
| 2183 AA>G | 9 | 0 | 3905insT | 0 | 0 | R347P | 0 | 0 |
| 1898+1G>A | 0 | 0 | 2143delT | 0 | 0 | 2184delA | 3 | 0 |
| 3120+1G>A | 2 | 0 | 3199del6 | 0 | 0 | 3489+10kbC>T | 0 | 2 |
| 394delTT | 0 | 0 | 621+1G>T | 0 | 0 | 711+5G>A | 0 | 0 |
| CFTRdel2,3 | 1 | 0 | 1507del | 0 | 0 | G551D | 0 | 0 |
| G85E | 3 | 1 | I148T | 0 | 0 | Q552X | 0 | 0 |
| R117H | 0 | 1 | R334W | 4 | 0 | R560T | 0 | 0 |
| 5T, 7T, 9T | 1 | 0 | | | | | | |

Using INNO-LiPA CFTR 19 and INNO-LiPA 17+Tn, the CF mutations identified in European immigrants of Turkish and North African descent[25] were examined to determine which were included in the mutation panel. The figure was 78 of the 176 mutations (44%) for the population group of Turkish descent and 93 of the 135 mutations (69%) for the population group of North African descent.

Annex **F**

# Abbreviations

| | |
|---|---|
| *CBO* | Institute for quality in care |
| *CBS* | Statistics Netherlands |
| *CVB* | Centre for population screening |
| *CF* | cystic fibrosis |
| *CFTR* | cystic fibrosis transmembrane regulator |
| *CHOPIN* | Cystic fibrosis heel prick screening of neonates in the Netherlands |
| *EGA* | Extended gene analysis |
| *ICSI* | Intracytoplasmic sperm injection |
| *IRT* | Immunoreactive trypsinogen |
| *NCFS* | Dutch Cystic Fibrosis Foundation |
| *NVOG* | Dutch Society for Obstetrics and Gynaecology |
| *PAP* | Pancreatitis associated protein |
| *PGD* | Pre-implantation genetic diagnosis |
| *RIVM* | National Institute for Public Health and Environmental Protection |
| *VKGN* | Dutch Society for Clinical Genetics |
| *VWS* | Ministry of Health, Welfare and Sport |