CYSTIC FIBROSIS IN ADULTS
Diagnostic, epidemiologic and quality-of-life aspects

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To my great family

and especially to

Thomas, Aron, Arvid, Annika and Ylva

Contents

Abstract

Background: Cystic fibrosis (CF) is a severe hereditary disease. The type of mutation in the Cystic Fibrosis Transmembrane conductance Regulator (*CFTR*) gene will determine the degree of CFTR chloride channel malfunction. Disturbed salt transport leads to production of sticky mucus, blocking exocrine gland ducts and persistent airway infection, starting early in life. Most patients are pancreatic insufficient, and almost all males are infertile due to obstruction of the vas deferens. Diagnostic criteria of CF are identification of two CF causing mutations and/or laboratory evidence of disturbed chloride transport in a patient with symptoms compatible with CF. Survival has increased due to improved care and today most patients are adult.

CFTR mutations and elevated sweat chlorides are common in infertile men with congenital bilateral absence of the vas deferens (CBAVD).

Aim: To address issues of importance for adults with CF; 1) review diagnosis of CF in adult age, 2) to find out if men with CBAVD, CFTR mutations and intermediate or elevated sweat chloride concentrations have evidence of early airway disease, 3) to analyze outcome of pregnancy and 4) to construct a health-related quality of life questionnaire.

Methods: CF patients in Toronto (*Paper I-IV*), Göteborg and Lund (*Paper IV*) were included. Demographic, diagnostic and pregnancy data was extracted from the patient database and completed with chart review and patient interviews. Bronchoscopy with bronchoalveolar lavage (BAL) was performed in men with CBAVD, *CFTR* mutations and intermediate or elevated sweat chloride concentration. CF-related health issues were collected, a provisional questionnaire constructed and translated to Swedish, interviews with 135 patients performed in order to rank the items of importance to quality of life by frequency of occurrence and mean importance. The specific questionnaire was constructed based on the interview result.

Results: In patients diagnosed as adults, pancreatic sufficiency, lung disease, inconclusive sweat test results and a high prevalence of uncommon mutations were common. Nasal potential difference measurement was a diagnostic aid. There was light growth of opportunistic gram-negative bacteria in BAL in 6/8 men with CBAVD. IL-8 and TNF alpha levels were higher in men with CBAVD. Absence of *Burkholderia cenocepacia*, pancreatic sufficiency and pre-pregnancy $FEV_1 > 50\%$ of predicted was associated with better maternal survival. Pregnancy did not affect overall survival or decline in FEV_1 when compared to the whole adult female CF population. The Cystic Fibrosis Quality of life Evaluative Self-administered Test (CF-QUEST) was constructed; CF-related health issues were collected. A provisional 114-item questionnaire was constructed and semi-structured interviews with adult CF patients in Toronto and Sweden were conducted. Items were ranked according to frequency of occurrence and mean rated importance. The final questionnaire was constructed based on the results.

Conclusion: Diagnosis of CF in adults often requires extensive diagnostic methods. Some men presenting with CBAVD in adult age have a mild form of CF. Outcome is good for most pregnant women with CF. CF-QUEST is a new patient-derived HRQL instrument for adults with CF. Field studies to assess repeatability and responsiveness will follow.

Keywords: Cystic fibrosis, cystic fibrosis transmembrane conductance regulator, pregnancy, male infertility, bronchoalveolar lavage , quality of life, questionnaire

List of Publications

1. Gilljam M, Ellis L, Corey M, Zielenski J, Durie P, Tullis DE: Clinical manifestations of cystic fibrosis among patients diagnosed in adulthood. Chest 2004; 126: 1215-1224

2. Gilljam M, Moltyaner Y, Downey G, Devlin R, Durie P, Cantin A, Zielenski J, Tullis E: Airway inflammation and infection in congenital bilateral absence of the vas deferens. Am J Resp Crit Care Med. 2004; 169: 174-179

3. Gilljam M, Antoniou M, Shin J, Dupuis A, Corey M., Tullis E: Pregnancy in cystic fibrosis; Fetal and maternal outcome. Chest 2000; 118:85-91

4. Tullis E*, Gilljam M*, Corey M, Eriksson L, Bergman B: Development of the Cystic Fibrosis Quality of life Evaluative Self-administered Test (CF-QUEST), a new disease specific quality-of-life instrument for adults with cystic fibrosis. Submitted

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Abbreviations

ATP	adenosine triphosphate
ASL	airway surface liquid
BAL	bronchoalveolar lavage
cAMP	cyclic adenosinemonophosphate
CBAVD	congenital bilateral absence of the vas deferens
CF	cystic fibrosis
CFQ	Cystic Fibrosis Questionnaire
CFQoL	Cystic Fibrosis Quality of Life Questionnaire
CF-QUEST	Cystic Fibrosis Quality of Life Evaluative Self-administered Test
CFRD	cystic fibrosis related diabetes
CFTR	cystic fibrosis transmembrane conductance regulator
CRQ	Chronic Respiratory Questionnaire
DIOS	distal intestinal obstruction syndrome
DNA	deoxyribonucleic acid
ENaC	endothelial sodium channel
ER	endoplasmatic reticulum
FEV_1	forced expiratory volume in one second
HRQL	health-related quality of life
IL-6	interleukin 6
IL-8	interleukin 8
IL-10	interleukin 10
IRT	immuno reactive trypsin
LTB4	leukotriene B4
LUL	left upper lobe
MSD	membrane spanning domains
mRNA	messenger ribonucleic acid
Nasal PD	nasal transepithelial potential difference measurement
NBD	nucleotide binding domain
NE	neutrophil elastase
NHP	Nottingham Health Profile
OGTT	oral glucose tolerance test
QOL	quality of life
QWB	Quality of Well-Being Scale

PI	pancreatic insufficiency
PKA	protein kinase A
PS	pancreatic sufficiency
R	regulatory domain
RML	right middle lobe
SGRQ	St George´s Respiratory Questionnaire
SIP	Sickness Impact Profile
TNF-α	tumor necrosis factor-alpha

Introduction

Historical background, genetics and cell biology

Cystic Fibrosis (CF) is a severe autosomal recessive genetic disease that was first described in 1936 by the Swiss pathologist, Guido Fanconi, who reported the autopsy and clinical characteristics of three patients with bronchiectasis and pancreatic insufficiency (1). In 1938, Dr Dorothy Andersen published an autopsy study of 38 infants, described the findings as "cystic fibrosis of the pancreas" and recognised the syndrome as an inherited disease (2).

In the early reports CF was considered a lethal disease with most patients dying in early childhood (3). With improved treatment, survival has steadily increased with an adult population outgrowing the child population. The median survival age is now almost 40 years and for children born in 2000 the predicted median survival probably exceeding 50 years (4-7).

The abnormal salt transport in CF became clear during a heat wave in 1952, when children with CF were admitted to hospital with severe dehydration and salt loss (8). The discovery of a high salt concentration in the sweat of CF patients lead to the development of the sweat test, the main diagnostic tool for CF for over 50 years now (9). The mechanism for the defect chloride transport in the sweat glands was demonstrated in 1983 (10), and in 1985 the gene was localized to the long arm of chromosome 7 (11). In 1989, through a collaborative research project, the gene was finally identified using linkage analysis and chromosome jumping and walking techniques (12-14). This was the first time a disease-causing gene was cloned solely on the basis of its position, without any knowledge of the gene product.

The large gene named cystic fibrosis transmembrane conductance regulator (the *CFTR* gene), consists of 27 coding exons. The encoded gene product, the CFTR glycoprotein, is a cyclic adenosine monophosphate (cAMP)-regulated low voltage chloride channel without preference for direction of ion secretion. It is composed of 1480 amino acids in two membrane-spanning domains (MSD) with six segments each, two nucleotide-binding domains (NBD) and a regulatory domain (R). CFTR belongs to the adenosine triphosphate (ATP)-binding cassette family, and is mainly expressed in the apical membrane of epithelial cells in exocrine glands (15,16) (Figure 1).

Protein is synthesised by a process of transcription of a single stranded DNA to mRNA, which in turn is translated to the final protein. Every amino acid is determined by a codon of 3 sequential nucleotides. Stop codons will terminate the protein synthesis. On the DNA level, there are three mechanisms for mutations; deletions, insertions or substitutions of one or more basepairs, resulting in missense (incorporation of an incorrect amino acid), nonsense (point mutations that convert a codon to a stop codon), frame shift (substitutions or deletions that often cause disruption of the reading frame) and splice site mutations (often at junction of exon-introns). There are today over1500 known *CFTR* mutations (17).

The functional consequences for the CFTR protein are depending on type of mutation and position on the gene. The *CFTR* mutations can be divided into five functional classes; I - absence of synthesis, II - defective protein maturation and premature degradation, III – disordered regulation, such as diminished ATP binding and hydrolysis, IV – defective chloride conductance and V - reduced number of *CFTR* transcripts due to a promoter or splicing abnormality (18,19). An additional classVI, causing accelerated turnover from the cell surface, has been suggested but is not generally agreed upon (20,21) (Figure 2). Class I, II, III and VI, with non-functional, unstable or absent CFTR protein at the apical membrane, are usually associated with a more severe phenotype (pancreatic insufficiency) and the Class IV and V mutations with a milder phenotype (pancreatic sufficiency). The categorization is

not strict and some mutations can belong to more than one functional class. DeltaF508, the first identified and most common mutation, is a class II mutation. It is caused by deletion of three base pairs in exon 10, and as a consequence, deletion of the corresponding amino acid phenylalanine on position 508 in the first nucleotide-binding domain (NBD).

Figure 1. *Normal CFTR in the cell membrane (a) and the channel function (b) is illustrated below. The CFTR anion channel belongs to the ATP-binding transporter superfamily that is important in trans epithelial fluid and electrolyte transport. It consists of a regulatory domain (R) with numerous phosphorylation sites, two membrane-spanning domains (MSD) and two nucleotide-binding domains (NBD) with ATP-binding sites. Phosphorylation of the R domain by protein kinases A and C stimulates ATP-dependent channel gating. G=glycosylation, C=carboxy terminus, N=amino terminus.*

a)

b)

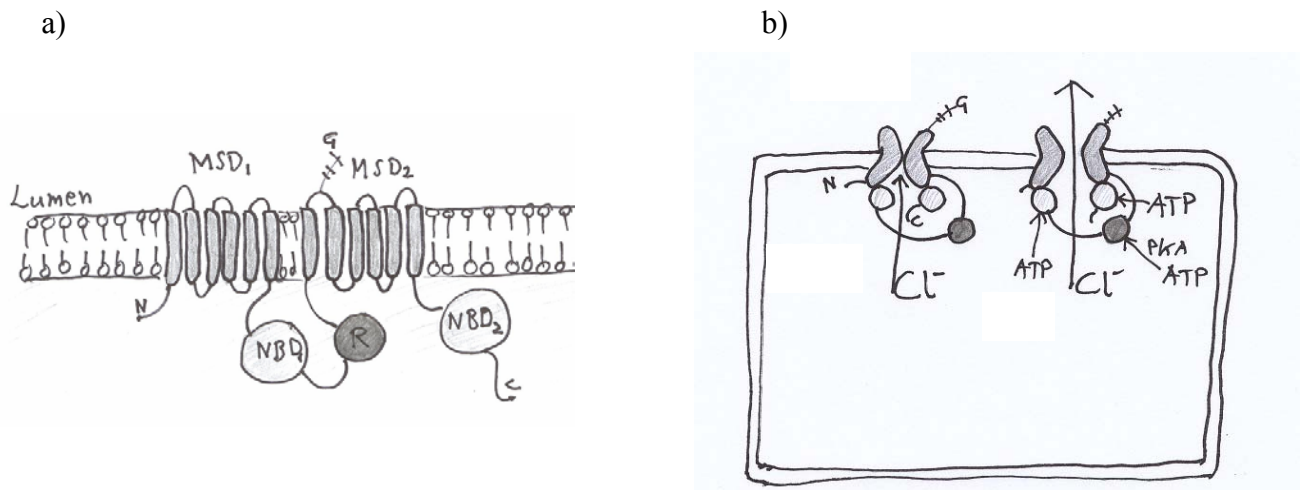*Fig 2*. *The functional classes of the CFTR gene mutations are illustrated; I - absence of synthesis, II - defective protein maturation and premature degradation, III – disordered regulation, such as diminished ATP binding and hydrolysis, IV – defective chloride conductance, V - reduced number of CFTR transcripts due to a promoter or splicing abnormality, VI- accelerated turnover from the cell surface. ER=endoplasmatic reticulum.*
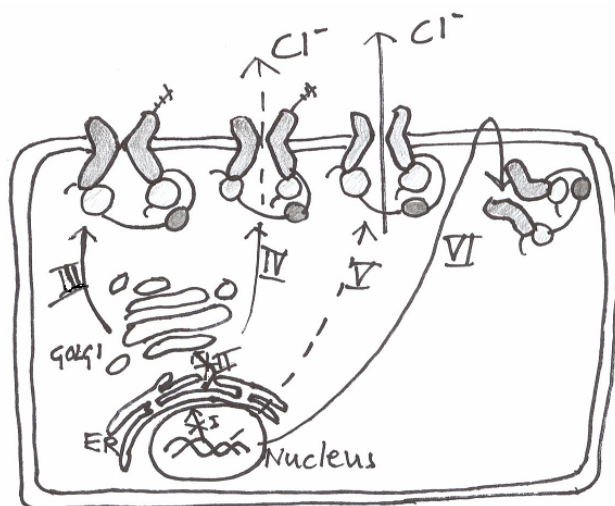
Genetic factors, other than the *CFTR* mutations, may affect the phenotype. Normal polymorphic sequence variations within the *CFTR* gene have the potential to alter protein structure and function (22). One example is how the variation of the polypyrimidine T-tract length in intron 8 will affect splicing of exon 9. The 5T variant results in less efficient splicing than the 7T and 9T and skipping of exon 9 in a high proportion. The consequence is reduced CFTR expression as in a Class V mutation. In addition, the length of the adjacent TG repeats can modulate the effect of the 5T variant (23-25). CF is a monogenic disease but the phenotypic variations seen may also be affected by polymorphisms in other genes, so called modifier genes (22,26).

Except for its role in chloride transport, CFTR also acts through regulation of other ion channels, most important being the amiloride-sensitive, epithelial sodium channel (ENaC). There is growing evidence of other regulatory functions, not related to ion transport, and that CFTR also plays a role in developmental regulation in the fetus (27). CFTR may co-act in metabolic processes in the vicinity of the apical membrane and thus CFTR dysfunction may be linked to inflammation via the fatty acid metabolism (28-31).

CFTR is expressed mainly in the apical membrane of epithelial cells in exocrine gland ducts. Disease manifestation in CF will depend on type of mutation, expression of the CFTR protein at the cellular level in different organs, modifier genes and environmental factors, as well as the role of CFTR in the particular organ. CFTR is expressed in mucus-rich epithelial tissues such as the respiratory, gastrointestinal and reproductive tract, where the altered properties and amount of macromolecules cause ductal obstruction and tissue damage. It is also expressed in non-mucus rich tissues (sweat glands and kidneys), as well as non-epithelial tissues (heart and brain) with no similar pathology (32). In the airways, CFTR is found mainly in the secretory serous cells of the submucosal glands (16).

General phenotype

Most patients are diagnosed during the first year of life. Failure to thrive, fatty stools or pulmonary infections and obstructive symptoms are the most common reasons for investigation. Some infants are diagnosed at birth because of meconium ileus or as a consequence of newborn screening. There is a large variability in organ manifestations and severity of symptoms. Approximately 85-90% of people with CF are pancreatic insufficient (PI) (33) and 98% of males are infertile (34-36). Other ductal complications seen are periportal biliary cirrhosis with portal hypertension, cholelithiasis and pancreatitis (in pancreatic sufficiency (PS) only). CF-related diabetes (CFRD), due to fatty replacement and scarring in the pancreas, is usually described to occur in patients with pancreatic insufficiency and is characterized by decreased insulin production, a gradual onset and an incidence in the population at risk of 8-15%, with a peak age of onset of 20-25 years (37). Distal intestinal obstruction syndrome (DIOS) is caused by impaction of inspissated intestinal contents (mucofeculent material and undigested fat) in the terminal ileum, caecum and proximal colon and may occur at any age. Nasal polyposis and chronic sinusitis are common upper airway manifestations. CF lung disease is the major cause of morbidity and mortality. Other complications are, essential fatty acid deficiency, deficiency of fat-soluble vitamins, hypertrophic pulmonary osteoarthropathy and osteoporosis.

CF lung disease

Lung disease in CF is variable in severity, but in general characterised by progressive inflammation and infection and the cause of a shortened lifespan. The chronic bacterial infection distinguishes airway disease from ductal manifestations in CF. The direct contact of the airways with the environment, in conjunction with several abnormalities in the microenvironment of the lung, cause a viscous cycle of infection, inflammation, mucus

plugging, bronchial obstruction and destruction of the normal airway architecture leading to bronchiectasis and fibrotic changes (19,38-44). This process starts early and bronchoscopy studies in infants have shown elevated levels of inflammatory markers in bronchoalveolar lavage (BAL) fluid, with or without the presence of bacteria, as early as 4 weeks of age (45,46). The common description of persistence of bacteria in the airways as "chronic colonization", implying that bacteria can colonise the airways without doing any harm, has been suggested to be abandoned for "chronic infection" (47).

Fertility

Male infertility is almost universal and is caused by azoospermia due to blockage of the ductal system (the vas deferens, seminal vesicles and epididymis) during fetal development or early childhood (34-36,48). The testes are normal and active spermatogenesis occurs. The 3849 + 10kb C>T mutation is however consistent with male fertility, low sweat chloride concentration and variable lung disease (49). With improved assisted reproductive techniques, using sperm aspiration and in vitro fertilisation, there are now a significant number of men with CF who have fathered children (50-52).

Females usually conceive spontaneously but fertility may be reduced due to thick cervical mucus (53,54), or anovulatory cycles (55). The early reports of pregnancy in women with CF were discouraging, describing deterioration during pregnancy and permanent loss of lung function and early death post partum (56-59), while later reports have been more optimistic, at least for those with good lung function (60-62).

Diagnostic criteria

Diagnostic criteria according to a 1998 North American CF consensus report, states that for a diagnosis of CF, laboratory evidence of CF is required in conjunction with typical clinical symptoms, positive newborn screening or a family history of CF (63) (Figure 3). Sweat chloride concentration >60 mmol/l on 2 or more occasions, identification of two CF-causing mutations and/or evidence of disturbed chloride transport on nasal potential difference measurement are accepted laboratory test results.

The sweat test should be performed with an accepted method in an accredited laboratory (64). *CFTR* mutations have to be considered as CF-causing in order to be diagnostic. Today only 24 of 1500 identified mutations (17) are classified as severe enough to be labelled CF-causing (63). Abnormalities of ion transport can be demonstrated in vivo by change in nasal potential difference (nasal PD) during perfusion with different solutions, but this is a complicated research method and not generally available for diagnostic purposes (18,65-68). European diagnostic guidelines was recently published and will be discussed later (69).

Principles of care

The improved prognosis for patients with CF is due to centralised CF care, focusing on a multidisciplinary approach with frequent clinical visits, early treatment of complications, and nutritional therapy aimed at preventing the typical malnutrition of pancreatic insufficiency (70,71). The development of new therapies (improved antibiotics, mucolytics etc) has further boosted survival. CF therapy is time-consuming and costly due to extensive physiotherapy treatment routines and use of multiple, expensive medications (72). Care has shifted somewhat from hospital-based to patient-administered home care (73). Due to an increasing adult CF population the increased need for treatment of severe complications as well as supportive and hospital-based care has been delayed until adulthood. During the last two decades, lung transplantation has become a realistic option, increasing survival (74,75).

Fig 3. The North American diagnostic guideline was published following a consensus conference. Rosenstein. J Pediatrics 1998.

Diagnostic criteria for Cystic Fibrosis

One of:
Sweat chloride concentration > 60 mmol/l on at least two occasions
Two CF-causing mutations
Abnormal chloride transport (nasal PD)

and
One of:
Clinical characteristics, typical for CF
Sibling with CF
Positive newborn screening

Quality of life

Quality of life (QOL) has become a relevant measure of efficacy in clinical trials. The World Health Organization proposed a broad definition of health in 1948; "Health is a state of complete physical, mental and social well-being and not merely the absence of disease". This definition does not cover most non-health domains of quality of life, which however should be included in any measurement of health-related quality of life (76).

There are several instruments available for measurement of health-related quality of life (HRQL). Generic instruments have been constructed to cover a wide variety of areas and can be used for comparisons between populations and in cost-utility analyses, but may be less responsive for changes in specific conditions. Examples are the Nottingham Health Profile (NHP)(77), SF-36 (78), the Quality of Well-Being Scale (QWB) (79) and the Sickness Impact Profile (SIP) (80).The aim of specific HRQL instrument is to increase the responsiveness by focusing on the aspects of health status that are of primary interest to the specified disease, function or problem. The Chronic Respiratory Questionnaire (CRQ) (81) and St George´s Respiratory Questionnaire (SGRQ) (82) are two disease-specific instrument available for patients with chronic lung disease, mainly used in patients with chronic obstructive pulmonary disease.

The importance of measuring quality of life in patients with CF was first recognised in an expert meeting in 1986 (83). Several studies of Quality of Life have been performed in CF, using generic instruments (84-92) or the CRQ (93,94). CF is different from most lung diseases as it is usually manifested in childhood, has a large variability in disease severity and may have primary CF manifestations in other organ systems. Generic instruments and instruments designed for chronic lung diseases may thus be less responsive to changes in CF (95).

Congenital bilateral absence of the vas deferens (CBAVD)

Azoospermia, defined as complete absence of sperm from the ejaculate, is present in less than 1% of all men and in 10 to 15 percent of infertile men (96). Epididymal, vasal, or ejaculatory duct pathology is responsible for approximately 40% of cases of azoospermia. Genitourinary infections, iatrogenic injury, and congenital anomalies, such as congenital bilateral absence of the vas deferens (CBAVD), are other causes of obstructive azoospermia. Diagnosis is made

by physical examination and, in doubtful cases, confirmed by rectal ultrasound. Genetic studies have revealed that men with CBAVD carries *CFTR* gene mutations and the 5T variant in a high proportion (97-102). CBAVD is almost universal in CF and thus it has been suggested that CBAVD may represent a very mild form of CF, or a CFTR-related disease (99,103-105).

Prolonged overall survival but still no cure

Despite identification of the *CFTR* gene almost twenty years ago there is still no gene therapy with proven clinical efficacy available (106). Several pharmacological approaches to correct the disturbed CFTR function are under investigation. Correction of the functional defect will require tailored solutions depending on type of disturbance in the CFTR protein (107). In this developmental process it is crucial with a profound knowledge of the basic genetic mistake and its consequence for the CFTR protein, genotype-phenotype relations, as well as to what extent environmental factors contribute to the absence or presence of clinical disease. Until drugs that correct the CFTR malfunction are available, patients with CF will have to balance life choices and expectations for the future with the need to perform daily extensive treatment and frequent clinical visits. Education, work, family planning and financial issues are important for adolescents and adults with CF and have to be addressed in the CF clinic. In the era of lung transplantation, the decision of terminal care has become more complicated (108). In evaluation of new drugs and cost-benefit analysis of the expensive treatments it is important to assess all aspects of effects and side effects, including quality of life.

Aims of the thesis

The overall aim of this thesis was to address aspects of cystic fibrosis of importance for the adult CF population.

Specifically:

1. To define clinical characteristics and diagnostic parameters for patients diagnosed with CF in adulthood at the Toronto CF Program, with focus on those diagnosed between 1990-2001.

2. To investigate if healthy men with CBAVD, and some evidence of CFTR malfunction have evidence of airway disease. We hypothesized that these men may have early, sub-clinical pulmonary disease with inflammation and/or infection, not evident by routine pulmonary function testing or radiographic investigations.

3. To study the effect of pregnancy on pulmonary function, nutritional status and maternal survival and to study the fetal outcome.

4. To construct an evaluative, disease-specific, self-administered quality-of-life questionnaire for adults with cystic fibrosis in English and Swedish.

Methods

Study design

Paper 1: retrospective cohort study

Paper 2: investigational case-control study

Paper 3: retrospective cohort study combined with patient interviews

Paper 4: multi-centre interview study

Study populations

Patients diagnosed with cystic fibrosis at *(Paper I)* or followed at the Toronto Cystic Fibrosis Program *(Paper II-IV)*, or at one of two Swedish CF Centres (the West Swedish or Lund CF Centres) *(Paper IV)*.

Since 1990, the Toronto CF Program has been divided into separate Pediatric and Adult Centres with a common Research Program and patient database. Patients with CF attend the Pediatric CF Program until age sixteen to eighteen years, when they are transferred to the adult CF Program. Patient data are consecutively entered into the database at the Research Program. Patients diagnosed at age ≥18 years, or in the adult clinic, were considered diagnosed in adulthood. Patients diagnosed between 1944 and 1959 (92 children) were excluded due to incomplete data *(Paper I)*.

The West Swedish CF Centre is a combined Adult and Pediatric Centre, while the Lund CF Centre is divided into Adult and Pediatric Clinics with a common Research Program. Patients are referred to the Adult CF Clinic at eighteen years of age. Each program has now a patient population of approximately 145 patients of whom approximately 60% are adult (*Paper IV*).

Eight non-smoking, healthy, infertile men with congenital bilateral absence of the vas deferens (CBAVD), were recruited from a group of men participating in a genotype-phenotype study *(Paper II)*. These men had been referred to the CF clinic due to CBAVD rather than for typical CF symptoms. All had one or two CFTR mutations and intermediate (40-59 mmol/l) or elevated (≥ 60 mmol/l) sweat chloride concentration. All men had been offered a comprehensive clinical evaluation, including information of what possible implication the mutations may have on their future health.

Four age-matched healthy males from the hospital staff were included for the bronchoscopy study (*Paper II)*.

Diagnostic criteria and laboratory tests

The 1998 North American consensus criteria guided the diagnostic decisions (63) *(Paper I-IV)*.

Genetic analyses: Genomic DNA was isolated from lymphocytes (109). Analysis for 31 of the most common CFTR mutations was performed. If two mutations were not identified, the polymerase chain reaction-based multiplex heteroduplex gel shift analysis on MDE0/00 gel matrix (BMA; Rockefelland, ME) was utilized for detection of CFTR mutations (110). The analysis included all the exons, their flanking intron sequences, and the promoter region. Fragments displaying aberrant migration pattern were further characterized by direct-sequencing analysis using the Thermo Sequenase Radiolabeled Terminator Cycle Sequencing Kit (Amersham-Life Science; Cleveland, Ohio). Three variants (9T, 7T and 5T) of the polythymidine tract (T-tract) in intron 8 was also analysed (100) *(Paper I-III)*.

Sweat test was done on at least two occasions by the method described by Gibson and Cooke (9). Until late 1980s, the urecholine method (111) was used in Toronto. Sweat chloride concentration < 40 mmol/l were considered negative, 40-59 mmol/l intermediate and ≥ 60 mmol/l elevated (*Paper I-IV*).

Nasal PD measurement was done according to the protocol by Knowles et al (65) In brief, measurements were performed during perfusion with Ringer lactate (maximum PD) followed by perfusions with amiloride (blocking sodium transport), addition of chloride-free solution (generating a chloride gradient), and isoproterenol (cyclic adenosine monophosphate activation of chloride permeability) (*Paper I-III*).

Demographic and physiological data

The Toronto CF database was used for extraction of demographic, diagnostic and clinical data that were prospectively collected and retrospectively analysed (*Paper I-IV*).

Pancreatic function was determined by 72-h dietary records and faecal fat determination (112,113), and in some cases, confirmed by duodenal intubation and pancreatic stimulation studies (33,114). Serum Trypsinogen was analyzed initially, and then serially in patients with pancreatic sufficiency (PS), in order to detect the onset of pancreatic insufficiency (PI) (115) *(Paper I-III)*.

Oral glucose tolerance test (OGTT) was performed in clinically stable, non-diabetic subjects, with measurement of venous plasma glucose levels in a fasting state and 2 h after ingestion of 75 g of glucose. The results were categorized as described by Moran et al (116) *(Paper I and III)*.

Chest radiographic findings were described as normal, minor changes (sole linear scar or bronchial wall thickening), or bronchiectasis *(Paper I and II)*.

Spirometry was performed according to the American Thoracic Society guidelines (117), and the Forced expiratory volume in one second (FEV_1) was reported as percentage of predicted (118) *(Toronto population, Paper I-IV)* or according to the European Respiratory Society guidelines and reference values (119) *(Swedish population, Paper IV)*.

Bronchoscopy

Flexible bronchoscopy during mild sedation was performed in an area separated from CF care. Bronchoalveolar lavage (BAL) was performed with three 50-ml aliquots of pre-warmed (37°C) sterile, isotonic saline in the right middle lobe (RML). The procedure was repeated in the left upper lobe (LUL) *(Paper II)*.

Gram staining and quantitative bacterial cultures of BAL fluid was performed according to the local standards for CF, using four agar plates (*Burkholderia cepacia* select agar [Oxoid, Basingstoke,UK], MacConkey agar without crystal violet, Fildes medium(120) , and blood agar). Unusual glucose non-fermenting gram-negative bacteria were identified at the Toronto Public Health Laboratory (Toronto, ON, Canada). *B cepacia* strains were typed at the Hospital for Sick Children and at the *B cepacia* reference laboratory(121) *(Paper II)*.

For *virus screening,* BAL fluid was inoculated onto monolayers of MK and MRC-5 cells in shell vials (influenza A and B, parainfluenza, respiratory syncytial virus, herpes simplex virus, cytomegalovirus, and rhinovirus) *(Paper II)*.

After cell/differential count and viability testing, the rest of the BAL fluid was processed and the samples frozen in -70°. Analysis of *cytokines* was later done using a commercially available ELISA kit (Cytoscreen; Biosource International, Camarillo, CA) for Tumor necrosis factor alpha (TNF-α), Interleukin 6, 8 and 10 (IL-6, IL-8, and IL-10) levels. *Neutrophil*

elastase (NE) was quantitated with an EnzChek elastase assay kit (Molecular Probes, Eugene, OR) and the effect of BAL Fluid on NE release was assessed. Peripheral blood neutrophils, isolated from healthy donors, were incubated with BAL fluid from normal control subjects and from men with CBAVD and release of NE was quantified *(Paper II)*.

Interview studies

A short semi-structured interview during clinical visits was used for information on obstetric data and breast-feeding habits, not available in the database *(Paper III)*.

One medical student conducted semi-structured interviews with 75 adult patients in Toronto and one research nurse with 60 adult patients attending either of the two Swedish CF centres, using the same interview instructions *(Paper IV, Item-reduction stage)*.

Statistical methods

Data are presented as mean ± SD or median (range).

The Student´s *t* test, the Mann–Whitney rank-sum test, the χ^2 test with Yates correction for continuity and Fisher´s exact test were used to compare groups *(Paper I- IV)*.

The Paired *t* test and the Wilcoxon signed rank test was used to compare paired data *(Paper II- III)*

The Kaplan-Meier method was used for calculation of survival curves and the Log Rank test for comparison between groups *(Paper III)*

The Cox proportional hazard analysis was used to assess the effect of pregnancy on survival, in a model using all female patients in the clinic, and taking age and clinical variables into the model *(Paper III)*.

The Spearman rank correlation test with correction for ties was used for correlation between FEV_1 percent predicted and item importance scores, and to examine concordance *(Paper IV)*.

Ranking of overall importance, defined as the product of frequency and mean importance for each item (the mean score for those responding) was the main method for item reduction *(Paper IV)*

Factor analysis using the principal components extraction method and varimax rotation was used to guide reduction of emotional items, and to assess how items with unclear domain belonging were grouped *(Paper IV)*.

Statistical significant difference was set at $p<0.05$ *(Paper I-IV)* or $p<0.01$ *(Paper IV)*

Computer software SAS, version 6.12 or StatView version 5.0 (SAS Institute, Cary, NC) was used for all analyses.

Ethical considerations

The local Ethical Committees approved on the studies and informed written consent was obtained from the study participants.

A research nurse contacted and informed men with CBAVD already recruited in the genotype-phenotype study regarding the bronchoscopy study *(Paper II)*. The guideline of the World Medical Association Declaration of Helsinki from 1975 was followed.

Results

Diagnosis of CF in adult age *(Paper I)*

Between 1960-June 2001, the overall proportion of patients diagnosed with CF as adults was 73/1051 (7%). The proportion diagnosed in adulthood increased over time with 3% before 1990 compared to 18% afterwards (11% when patients derived from studies were excluded). The mean sweat chloride value was lower for those diagnosed as adults (75 ± 26 mmol/L), compared to those with a diagnosis as children (100 ± 19 mmol/L) and adults were more likely to be pancreatic sufficient (73%) compared to children (13%). In 46 patients diagnosed since 1990, the reason to investigate for CF was pancreatitis (4%), pulmonary symptoms (39%), pulmonary and gastrointestinal symptoms (22%), infertility (26%) and genetic screening (9%). The diagnosis could be confirmed by sweat test alone in 30 of 46 patients (65%), by mutation analysis alone in 15 patients (33%), and by a combination in 31 patients (67%). Nasal PD measurement confirmed abnormal chloride transport in the remaining 15 patients (33%). The 24 different mutations identified are listed in Table 1. One patient was homozygous for the deltaF508 mutation, and this mutation accounted for 33/74 (44%) of the alleles with mutated *CFTR*.

Table 1. CFTR mutations identified in 44/46 patients, diagnosed with CF in adult age in Toronto between 1990 and 2001

Mutation (Number of alleles affected)			
DeltaF508	(33)	L1077P †	(1)
1717-1G>A	(1)	3489+10kb>C	(2)
G551D	(4)	S549R †	(1)
R334W	(2)	1677delT>A †	(1)
2789+5G>A	(3)	A455E	(1)
711+3A>G	(1)	W1282	(1)
R347P	(1)	4016 insT †	(1)
1898+1G>A	(1)	621+1G>T	(1)
R117, 5T	(1)	G239R †	(1)
R117H,7T	(5)	D1152H †	(2)
R117L,7T	(1)	P67L †	(2)
5T	(6)	I1234V	(1)

† Not included in the 31-mutation screening panel.

Bronchoscopy with BAL in men with CBAVD *(Paper II)*

There was light growth of *Staphylococcus aureus* in one of eight men with CBAVD, and small numbers of opportunistic gram-negative bacteria in six of eight men with CBAVD and in one control subject. BAL cell count, NE, and cytokine levels were within the normal range. IL-8 and TNF-α levels were higher for men with CBAVD than for control subjects. The only

man with CBAVD with apparent purulent airway disease on bronchoscopy had growth of *S aureus* and *B cepacia* and an inflammatory response in BAL.

Pregnancy (*Paper III*)

Between 1963 and 1998, there were 92 pregnancies in 54 women. There were 11 miscarriages and 7 therapeutic abortions. Forty-nine women gave birth to 74 children. The mean follow-up time after pregnancy was 11 ± 8 years. One patient was lost to follow-up shortly after delivery, and one after 12 years. The overall mortality rate was 19% (9/48 patients). Absence of *B cepacia,* pancreatic sufficiency, and pre-pregnancy $FEV_1 > 50\%$ predicted was associated with better survival rates. When adjusted for the same parameters, pregnancy did not affect survival compared to the entire adult female CF population. The decline in FEV_1 was comparable to that in the total CF population. Three women had diabetes mellitus, and seven developed gestational diabetes. There were six preterm infants and one neonatal death. CF was diagnosed in two children.

Health-related Quality of Life, the CF-QUEST (*Paper IV)*

The question "What do you think is important to quality of life in CF" generated ~200 written responses from patients with CF, CF health-care workers in different English-speaking countries. The responses were reviewed and a provisional 114-item-questionnaire, categorized into 6 domains, was constructed. The domains were physical health, emotional issues, social issues, treatment-related issues, vocational and financial issues and activity limitation.

Altogether 135 adult CF patients from Toronto and Sweden participated in an interview study for item ranking. There was no difference between the populations for demographic or clinical parameters, except that a higher proportion of the Toronto patients were infected with *B cepacia*. The most important item for both the Canadian and the Swedish population was "having a supportive family" and a "supportive spouse" followed by "working or being around smoke, dust or fumes". The highest ranked physical items were "cough" and "chest congestion". As expected, higher scoring was seen in severe disease, specifically for negative emotional items and items related to physical symptoms and activity. Treatment-related items were, unexpectedly, given low scores in general with the item, "Therapy being too time-consuming" being highest ranked and the item "Therapy being tedious" being highest ranked within this domain by the subgroup of patients with FEV_1 <30% predicted. The correlation between lung function, given in FEV_1 % predicted, and physical symptoms, as well as with activity limitation was moderate. Correlation between lung function and treatment-related issues was low.

The final instrument; the Cystic Fibrosis Quality of life Evaluative Self-administered Test (CF-QUEST) was constructed, guided by overall importance ranking lists, correlations and factor analysis. In pre-testing the questions were reported to be appropriate and easy to understand.

Discussion

Study population

The whole Toronto CF population, except those diagnosed elsewhere, was included in the diagnostic study (*Paper I*). The Toronto CF Centre was the first CF Centre in Ontario established, and one of the first in Canada. Ontario has a population of approximately 10 million inhabitants and is more densely populated in the south where also all eight CF-centres are located. There are 3500 people with CF in Canada, 1100 of them in Ontario and the calculated incidence is 1/300 newborn in the Caucasian population. A proportion of 47% adults was reported for the whole of Canada, which is slightly lower than the data for Toronto (4). Some patients had previously been investigated for CF at regional hospitals but usually patients were referred to Toronto for diagnostic procedures, either directly or if the first sweat test was abnormal.

We found a decreased number of patients diagnosed in childhood during the last two decades, probably reflecting the increased number of diagnosing centres as well as the possibility of genetic screening and prenatal diagnosis that in some cases would lead to abortion of CF fetuses. During the last decade, there were a high proportion of patients diagnosed as a consequence of genotype/phenotype studies in Toronto, which may cause selection bias when these patients are included in the analyses. Due to the increased awareness of CF in the medical community and the close research collaboration with the CF clinic in Toronto, it is also likely that more patients will be referred for diagnostic investigations. This could cause a selection bias, when comparing Toronto to other diagnostic settings.

Of those diagnosed in adulthood, 87% had pulmonary symptoms and thus likely to primarily consult specialists in internal medicine, pulmonary medicine, infectious diseases or general medicine. Patients may however also first present to gastroenterologists, endocrinologists, ear-nose-and-throat specialist and in fertility clinics as some of the patients in our study (*Paper I*).

To examine the **Swedish** experience a **cross-sectional survey** was performed, including all patients followed at the four Swedish CF centres during 2006 (not previously published). Sweden has a population of 9 million inhabitants and is like Ontario more densely populated in the south. CF is however less common in Sweden, with only 600 patients. The most recent study from 2002 reported a calculated incidence of 1/5600 live births (5). In a study from 1982 a much higher incidence was suggested (122) while the result from the first study from 1962 was comparable to the recent data (123). The proportion of adults with CF in Sweden is now 54%. Of 47 (8%) patients diagnosed in adult age, the majority was pancreatic sufficient and carried other mutations than deltaF508 (Table 2). The experience was thus similar as in Toronto.

The aim of the bronchoscopy study (*Paper II*) was to investigate the relationship between *CFTR* gene mutations, CFTR protein malfunction (sweat chlorides, nasal PD), CBAVD and CF airway disease. The relationship between CBAVD and CF is unclear; in some cases CBAVD is the only clinical symptom in a patient with elevated sweat chloride levels and/or abnormal nasal PD. Our hypothesis was that some men with CBAVD and laboratory evidence of CFTR malfunction may in fact have asymptomatic mild airway inflammation, with or without presence of bacteria, in analogy with the early inflammation and infection seen in infants with CF (45,46).

It is difficult to do a correct power calculation in a pilot study with very limited data available. We based our power calculation on the assumption that neutrophils in BAL for men with CBAVD would be increased to a level between values for the normal population and the

hundred-fold increase seen in infants with CF, with a standard deviation of 50%. We aimed for 10 men with CBAVD and 10 controls in this pilot study but did not reach this number. The bronchoscopy study (*Paper II*) was part of a large genotype study, starting with genetic screening of all infertile men (124,125), followed by sweat test and nasal PD measurement in men with infertility due to CBAVD (126) and clinical assessment (in manuscript). There could be a risk of selection bias as men with some airway symptoms would be more willing to participate in the clinical assessment part of the study, as well as the bronchoscopy. There were however only 2 patients who declined to participate, and this was because of work schedules. We discussed the possibility of also including men with CBAVD without CFTR mutations but abandoned this idea, due to financial and logistic reasons, as this group had not been through the diagnostic and clinical evaluation. All age-matched controls were hospital staff-members but only one had been in recent contact with CF patients. Finally, the unforeseen decision to close the Wellesley Hospital delayed and hampered the project and also forced us to stop the procedures before we had reached the goal of twenty study subjects.

All women, followed at some point in time during or after their pregnancy, were included in the pregnancy study (*Paper III*). We chose to exclude pregnancies with gestational length shorter than 26 weeks in the statistical analysis of survival and effect on pulmonary function. The effect of pregnancy, in relation to CF, would be very limited for women with early miscarriages or abortions. Including women who had undergone early therapeutic abortion due to poor health could erroneously result in interpreting pregnancy as a risk factor for deteriorating health and early death.

In the quality-of-life study *(Paper IV),* approximately 40 % of all adult CF patients attending the CF centres in Lund or Göteborg, and 30 % of all adult CF patients in Toronto at the time of the study were included. The study group were representative of the adult populations in each country, except that there were only 37% females in the Canadian population.

Table 2. *Patients diagnosed with CF in adult age in Sweden*

Number, No (%)	47 (8%)
Gender, No (%)	
Male	32 (68)
Female	15 (32)
Age at diagnosis,	35 ± 13
Mean sweat chloride*	81 ± 21
Genotype, No (%)	
Del F508/Del F508	4 (9)
Del F508/Other	17 (36)
Other/Other	26 (55)
Pancreatic function, No (%)	
Pancreatic sufficiency	33 (70)
Pancreatic insufficiency	14 (30)

A cross-sectional survey, including patients alive, and followed during 2006. * Data missing for 8 patients

Method consideration

The sweat test, using the method of pilocarpine iontophoresis and quantitative analysis of sweat chlorides according to Gibson and Cooke method, has been the gold standard for almost a half century(9) but has in many centres been replaced by the Macroduct® sweat collection system (Wescor,Inc.) (64,127,128). Analysis of conductivity is a quick method that may be used for screening only, while for diagnosis of CF, measurement of chloride concentration has to be performed. The same time is required for sweat collection regardless of method of analysis. Some laboratories do sequential analyses on the sample while other always determinate chloride concentration directly. A negative test in a patient with typical symptoms should always be repeated. Validation studies of the Macroduct system have mainly been based on healthy controls with normal (>40 mmol/l) sweat chloride concentrations or "classic" pancreatic insufficient patients with high (often >80 mmol/l) sweat chloride concentrations. According to recent data, there seems to be acceptable agreement between the methods also for the intermediate 40-60 mmol/l range (personal communication P. Durie, Jan 2007) which is important given the diagnostic difficulties in the group of patients with borderline sweat chloride concentration and atypical presentation.

The sweat test requires a certain amount of training. The unreliability of these methods, performed and evaluated in the smaller settings, is exemplified by several patients (*Paper I*) diagnosed with CF with abnormal chloride levels in adult age, despite having had previous sweat testing performed, with normal results, due to CF in a sibling. In some cases the previous test was performed recently in a symptomatic patients but in other cases we cannot exclude that the changed chloride concentration was a manifestation of disease progress. Elevated sweat chloride levels in the absence of CF can be seen in malnutrition and in some rare metabolic and endocrine disorders (129).

Nasal PD is a complicated, time-consuming method of measuring ion transport *in vivo*, which requires a skilled technician who performs frequent tests for reliable results. The function of the sodium and chloride channels are assessed using a voltage meter attached to two electrodes: one reference electrode (subcutaneous needle) corresponding to the intracellular space, and one exploring electrode (double lumen catheter) under the inferior turbinate in the nose, corresponding to the airway lumen. The results are unreliable if there is acute upper airway infection/inflammation or if major sinus surgery has been performed. Protocols for standardized measurements have been published (67) but there are still no consensus on diagnostic criteria.

Alternative methods for assessing ion transport are *in vitro* studies of intestinal or rectal biopsies (130-133).There are two different protocols for rectal biopsies and no standardised diagnostic criteria. These methods also have limitations as they are invasive and the biopsy procedure has to be performed close to the laboratory to allow immediate analysis. Pancreatic stimulation test, with analysis of duodenal secretions, will also reveal the disturbed fluid and ion transport but is an invasive method and not useful for diagnosis as a routine (134).

Mutation analysis

Mutation panels have to be targeted for the specific ethnic population (135). The Toronto population is multicultural with patients originating from all over the world. For some minority groups, screening for certain mutations, in addition to deltaF508, such as the W1282 in Ashkenazi Jews (136) or the A455 and 621+1G->T mutations in Saguenay-Lac-Saint-Jean region in Quebec, Canada would detect the majority of carriers (137). If the screening panel does not identify two mutations, further more expensive analysis with gene sequencing has to be performed. The benefit compared to both sweat test and nasal PD is that complementary

mutation analysis can be done without the patient coming to clinic again. Patents diagnosed late in life often carry uncommon mutations and thus extensive analyses are often required.

In the 1998 consensus document only 24 mutations were listed as fulfilling the specific requirements for a CF-causing mutation; 1) causes a change in the amino acid sequence that severely affects CFTR synthesis and/or function, 2) introduces a premature termination signal, 3) alters the "invariant" nucleotides of spliced sites, or 4) causes a change in the amino acid sequence that does not occur in the normal genes from at least 100 carriers of CF mutations from the same ethnic group (63). Obviously there must be several other mutations fulfilling these criteria but not yet listed as CF-causing. For definitive evidence of the physiologic cellular disturbance caused by a mutation, it has to be proved *in vitro* that by correcting the specific *CFTR* gene defect, the CFTR protein function is also corrected, and such experiments have only been performed for a few mutations (138).

Bacteriology

When BAL cultures from the first patient *(Paper II)* revealed light growth of *B cepacia complex*, we were concerned of possible bacterial acquisition in the hospital or contamination in the laboratory. There was however almost no possibility for spread in the clinical setting; these men were seen infrequently and on days separate from CF clinics, bronchoscope equipment was not used for CF patients and the cleaning procedures had been followed. Furthermore, none of the *B cepacia* strains identified in the study belonged to the Toronto *B cenocepacia* ET 12 cable-pili positive strain, infecting over 40% of all CF patients at the time. This Toronto strain was, and still is, a major cause of death, often with a very dramatic course, the Cepacia syndrome (*Paper I and III*) (139).

The *B cepacia* species are classified into Genomovars; I-VIII belonging to the B cepacia complex. *B cepacia, B multivorans, B cenocepacia, B stabilis, B vietnamensis* are the genomovars most often cultured from patients with CF. The most virulent of these strains is without doubt *B cenocepacia* which is highly transmissible, multi-resistant, can survive intra-cellularily and is able to cause severe, rapidly progressive lung infection, as well as extra-pulmonary infection and septicaemia in patients with CF (140,141) and chronic granulomatous disease (142,143). It has also caused outbreak of bacteraemia in hemodialysis patients from contaminated material.

Ralstonia pickettii, formerly *Pseudomonas (Burkholderia) pickettii* are isolated from plants and soil, may be part of the commensal flora but usually of low virulence. It has been associated with nosocomial outbreaks caused by contaminated solutions used for patient care. It has also been recovered from sputum cultures of CF patients and described to cause airway or bloodstream infection (144,145). The fact that one healthy control had light growth of *R pickettii* raised the question if health care workers and family members transiently or persistently can harbour CF bacteria. Spread of an epidemic strain of *P aeruginosa* from a CF patient to both parents, causing significant pulmonary disease, has been reported (146) but cross infection from CF patients to CF health care workers, or non-CF family members is in general not a clinical problem. Parents of CF patients are expected to have 50% normal CFTR function, which should be sufficient for a normal phenotype. *Brevundimonas diminuta*, previously assigned to the genus Pseudomonas, has caused infection in cancer patients but has to my knowledge not been reported in patients with CF (147). *Burkholderia gladioli* has caused abscesses in a patient with CF (148) and also infections in lung transplant recipients(149).

These bacteria, identified in the bronchoscopy study, are environmental strains, but usually not described as part of the normal airway flora. We cannot exclude the possibility that the normal airway flora has changed, or that these bacteria previously have been missed but now

identified in the specialised CF bacteriological laboratory in the study situation. The bacteria were however detected by routine methods for CF sputum. Improved laboratory methods and the changed and complicated taxonomy make historical comparisons difficult. Bronchoscopy with BAL in health care workers, relatives and healthy controls with quantitative cultures analyzed in the CF bacteriological laboratory would target some of these questions.

CF airway inflammation and infection; relation to CFTR function

The pathophysiology of CF lung disease is complicated and not fully understood. Absent or dysfunctional CFTR is leading to altered ion fluxes across the epithelial membrane but the type of change and resultant consequence has been debated. CFTR is a chloride channel, but maybe more important is the interaction with other ion channels and metabolic processes in the cell. The high salt hypothesis suggests that, in analogy with the sweat glands, the chloride concentration in the airway surface liquid (ASL) is higher than in normal individuals. The high chloride concentration would inactivate defensins (small antimicrobial peptides) with impaired killing of bacteria and chronic airway infection as a consequence (150-152). In the low-volume hypothesis ASL is suggested to be isotonic, as in healthy individuals, but water absorption is increased and ASL depleted, leading to deficient mucociliary clearance and entrapment of bacteria in hypoxic mucus and thus causing chronic infection (43,153). The increase in arachidonic acid and prostanoids and altered metabolism of nitric oxide in CF may also contribute to the exaggerated inflammation (29,30,154,155). CFTR is highly expressed in the serous epithelial cells of the submucosal glands (156) and loss of CFTR function may alter the secretion of lysozyme, lipids, mucins and other components, and thereby changing the viscosity, gel hydration and anti-bactericidal properties (157).

Early in childhood bacterial infection with *S aureus* and *Hemophilus influenzae* is common, while later a large proportion of the patients become chronically infected with *Pseudomonas aeruginosa* or other opportunistic gram-negative bacteria. *P aeruginosa* often convert to a mucoid highly resistant phenotype, able to form biofilms. An exaggerated inflammatory response is seen in BAL from patients with CF, with increased number of neutrophils and the pro-inflammatory cytokines IL-6, IL-8, TNF-α and LTB-4, but decreased levels of the anti-inflammatory cytokine IL-10.

Altered epithelial surface glycosylation and the possibility that CFTR may serve as a receptor for *P aeruginosa* or is a transporter for peptides involved in the inflammatory defense may also contribute to the persistent airway infection (158,159). Glutathione, an important antioxidant in the lung, is reduced in CF ASL. The fact that glutathione is able to permeate through CFTR suggests a direct link between CFTR and antioxidative defense in the lung (160).

It is unclear if the severe lung inflammation and infection in CF is a direct consequence of dysfunctional CFTR, or if there are other primary disturbances of the innate immunity. CF mouse model studies have indicated that inflammation may arise at least partly from a primary defect in the regulation of neutrophil recruitment, independently of infection and not related to CFTR. When human fetal CF trachea was grafted into severe combined immune deficiency mice, progressive intraluminal inflammation leading to destruction of the lung parenchyma was seen in the graft in the absence of infection (161,162).

B cepacia can survive and replicate in human CF airway cells (140) and can also cause severe infections in patients with chronic granulomatous disease, a disorder with disturbances in phagocytic function (141-143,163). However, so far there is no evidence of a specific chemotactic or phagocytic defect in CF.

Finally, environmental factors may have a direct effect on CFTR function, influencing the disease expression (164,165). The importance of environmental factors, treatment or socioeconomic factors is also suggested by preliminary report from an ongoing twin study in the United States; monozygous twins had a more discordant phenotype than dizygous twins or siblings, but only as long as they lived together (166). This is contrasted by the European study demonstrating a significantly higher concordance in severity of lung disease for monozygous than for dizygous twins, suggesting a strong genetic contribution to variability in severity of lung disease (167). Based on the US data the heritable genetic contribution to lung disease in CF was calculated to approximately 0.6-0.7 (166). Sample size is however a great concern in both these studies (22).

Genotype-phenotype aspects

The relationship between genotype and phenotype in CF is schematically illustrated in Figure 4. Some patients with classic CF, homozygous for deltaF508, may retain a supra-normal lung function and no radiographic evidence of CF airway changes on high resolution computer tomography in their thirties, while others develop a severe infection already in childhood despite early diagnosis and adequate treatment. It is very likely that normal variations or modifier genes account for at least part of such variation. The combination of mutations, as well as the possibility of a third modulating mutation has also to be taken into consideration (168,169). A large number of possible modifier genes have been suggested, but due to the variability of the disease and the necessity of large sample sizes, and thus including multiple centres with different treatment policies, it is difficult to draw any definitive conclusions from studies performed (22,170,171).

The common class IV mutation R117H ís listed as CF-causing, but only when combined with the 5T, and not the 7T or 9T variants on the same allele. It is consistent with pancreatic sufficiency. Some patients with the R117H mutation are diagnosed on neonatal screening with elevated immunoreactive trypsin (IRT) but equivocal sweat chloride concentrations. Pancreatic stimulation test may however reveal secretory disturbances, typical for CF, but not severe enough to cause pancreatic insufficiency (134). This mutation is also, in combination with the 7T or 9T variant, frequently detected in men with CBAVD. We don not know the long-term prognosis for these patients. It is possible that some will develop typical CF disease but with a late onset due to the "mild" characteristics of this genotype, as seen in two of our patients (*Paper I*) and also reported by others (172-174). There are several reasons to follow these patients in the CF clinic; they may develop clinical manifestations of CF and should also be offered information of male infertility as well as genetic counselling. Genotype is correlated to pancreatic function but there are exceptions and patients with class III and IV mutations may occasionally develop PI in adult age. We and others have experience of adult siblings with discordant pancreatic function (175). The pancreatic function status is usually categorised as "either or not", but disturbances are seen on pancreatic stimulation tests in patients with pancreatic sufficiency and some have had recurrent pancreatitis before evident pancreatic insufficiency.

The dilemma in diagnostic decision and classification.

The starting points for investigation for CF are listed in Table 3.

The diagnostic criteria established at the North American consensus conference (63) are easier to remember than the recent European diagnostic guidelines (69), but have some limitations. Infants with elevated IRT on screening may have *CFTR* mutations that are not classified as CF-causing and/or normal sweat chloride levels, and do thereby not meet the diagnostic criteria for CF. Nevertheless, some of these infants will eventually develop typical CF disease and should be followed. The same applies to patients identified to have two *CFTR*

mutations on screening due to family history or prior to pregnancy. CBAVD is a clinical manifestation of CF in males but there is no equivalent finding in females so it is more likely for a man to meet the diagnostic clinical criteria. Nasal PD was the laboratory test confirming disturbed chloride transport in CFTR protein function in 33% of our patients (*Paper I*). This test is not generally available at CF centres and specific criteria of what constitutes an abnormal response has not been determined.

Fig 5. Schematic genotype-phenotype relationship in CF. There is a 25% chance for the offspring of two healthy carriers to inherit one mutation from each parent in this autosomal recessive disorder. The type and combination of mutations will decide the genotype. Additional genetic factors, environmental factors, disease modulation and age will influence the phenotype. Adapted after Zielenski, Respiration, 2000.
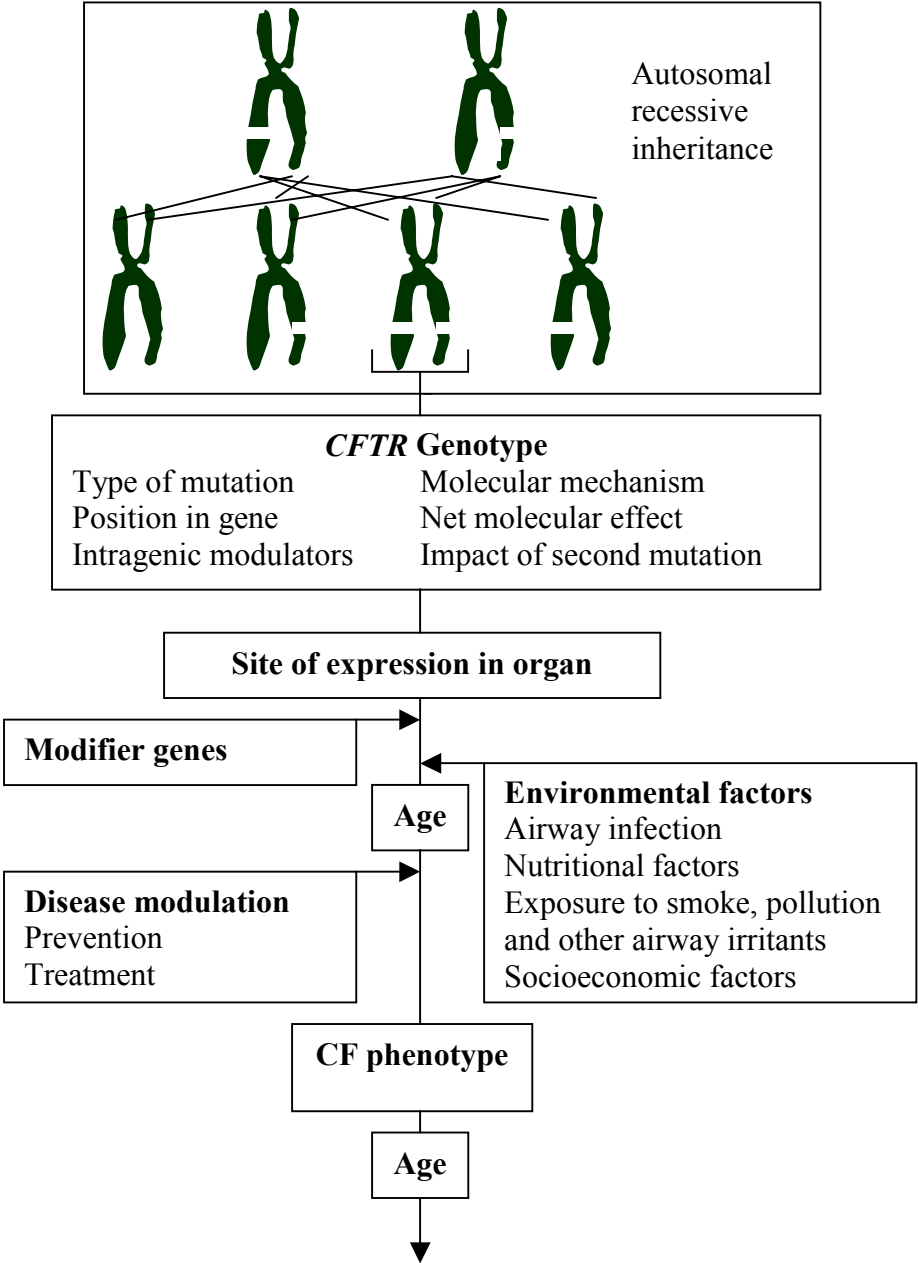
Table 3. *Starting point for diagnostic investigation for CF*

1. Positive newborn screening

2. Positive screening in a patient with a family history of CF or prior to pregnancy

3. Typical symptoms of malabsorption, with or without pulmonary symptoms

4. Pulmonary disease but no symptoms of malabsorption

5. Single organ manifestations, including CBAVD*, pancreatitis, salt-loss syndrome

*CBAVD= congenital bilateral absence of the vas deferens

Patients with "typical" disease have CF if sweat chloride concentrations are abnormal or if two CF-causing mutations are identified. If not, and if nasal PD is not available, CF cannot be excluded. The decision to label someone as having CF or not, for most patients diagnosed as a consequence of screening or because of single-organ manifestations and *CFTR* mutations, will have to be a clinical decision. Depending on facilities the patient may be followed in the CF clinic or at the local pulmonary physician or gastroenterologist.

Ideally, the decision of a diagnosis of CF should not be influenced by socioeconomic and legal factors. A patient with health problem requiring medications and clinical visits may be more likely to "appreciate" being diagnosed with a severe, potentially life-threatening disease than the healthy person diagnosed on screening and in the middle of a career. Ability to obtain life insurance is another important issue that must be addressed.

The terminology for classification of CF is confusing. Mutations are called "mild" or "severe" depending on the functional consequence for the CFTR protein but CF disease can also be clinically "mild" or "severe", regardless of type of mutation. CF has been divided into "classic CF" (sweat chlorides often 90-100, PI, often severe lung-disease, may have meconium ileus and biliary cirrhosis) and "non-classic CF" (sweat chlorides >60 but <80, often milder lung disease, PS and no meconium ileus or biliary cirrhosis) (176) or synonymously "typical" and "atypical CF". The terms "non-classic" and "atypical" are however also used to describe patients not fulfilling the diagnostic criteria, with borderline sweat chloride concentrations, 1 or 2 *CFTR* (but not 2 CF-causing) mutations and/or single organ disease manifestations (177,178). In the European Diagnostic Consensus Guidelines, first published online Dec 2005, two diagnostic algorithms are presented (69). One algorithm begins from the mutation analysis and one from the sweat test, in an attempt to target the different starting points for diagnostic evaluation. In order to identify patients with borderline sweat chloride concentration, the lower normal limit was decreased from 40 to 30 mmol/l. Patients with intermediate sweat chloride concentrations are here classified as non-classic CF, or according to the WHO diagnostic list for single organ disease phenotypes associated with *CFTR* mutations (179,180). The nasal PD is incorporated in the algorithm as a standard test, albeit not generally available. The algorithms are complicated in comparison with the diagnostic guidelines from 1998 and the different reference values for sweat chloride interpretation in the two documents is confusing. The European guideline has no list of CF-causing mutations and the North American consensus mutation list is apparently not comprehensive. Diagnostic guidelines will need to be revised, hopefully as a worldwide project.

For now, the diagnosis will at most CF centres have to be based on clinical presentation, the sweat test and mutation analysis. Specialized core centres should provide nasal PD and other complicated methods for diagnosis, as well as for studies, evaluating the correctional effect of new drugs. Nasal PD measure the CFTR function in respiratory epithelium *in vivo* and it is thus not surprising that this method is correlated to the clinical expression of CF lung disease (181). Hopefully new and better diagnostic tools will be available in the future. Some modified sweat test methods for examination of CFTR function *in vitro,* to assess drug safety and efficacy are currently under investigation (182).

Risk factors for deterioration and death

The diagnosis of adults will contribute to the increased life expectancy, but still the proportion diagnosed in adulthood is small and improved centralised care with focus on preventive measures and early treatment of complications are the main reasons for this improvement (3,5,7). Poor survival in countries without established CF centres and the difference in outcome depending on access to healthcare also stress the importance of modern specialised care (183). Because of the variability of the disease and the close monitoring and rigorous treatment, we sometimes meet patients diagnosed in adult age with "mild" mutations" who in fact have a more severe lung disease than patients of the same age, followed since childhood with "classic" CF.

We chose to divide patients in two FEV_1 groups below and above 50% predicted, instead of the more common categorization into mild, moderate or severe disease (FEV_1 >70%, 40-70% or <70% predicted) (*Paper III*). According to our clinical experience, we felt that an FEV_1 of below 50% correlated to when we would be hesitant to recommend pregnancy. This cut-off has been used by others (184,185). Division into two, instead of three groups was also based on the limited patient population. In one large study from the US registry, no effect of pregnancy on survival was seen, even for subgroups with poor lung function (FEV_1 <40% predicted) insulin-dependent diabetes, pancreatic insufficiency or chronic infection with *P aeruginosa* (186). These positive results are consistent in several reports as well as the overrepresentation of women with pancreatic sufficiency, FEV_1 >50% and mutations other than deltaF508 with completed pregnancies (52,61,184,185,187-189). It is however difficult to predict the outcome in the individual case. Two women with poor lung function were stable 8 and 18 years after their first pregnancy while one woman with pre-pregnancy FEV_1 of 95% predicted died from *B cepacia* syndrome 3 years post partum. She was healthy until shortly before her quick deterioration and death. The demands of the care of infants and toddlers are risk factors for deterioration post pregnancy. A comprehensive discussion regarding these issues as well as the possibility of early death of the mother should be taken prior to pregnancy (190).

The major impact of *B cenocepacia* for the CF population in Toronto has to be stressed and is a confounding factor in survival analysis if not adjusted for (139,191).

Quality of life in CF

Generic Instruments: The results from earlier QOL studies in CF, using generic measures, have been variable. The QWB is a utility measure that takes 10-15 minutes to complete by interview. It has been shown to correlate to changes in pulmonary function in patients with CF(84) but in a recent review of quality of life outcome in randomised controlled trials, no change in quality of life could be detected for 3 of 4 studies using the QWB scale as a secondary outcome (192).

The NHP is a frequently used, self-administered health profile that takes 10-15 minutes to complete and is divided in two parts; 1) subjective assessment of health status and 2) a brief

indicator of handicap. When the NHP was used in a cross sectional survey in CF patients 16 years of age or older, different patterns of perceived quality of life were seen in men and women, with an age-related trend of more distress/disability for some dimension for older men. For the whole group the scores in Part 1 was similar to patients with minor non-acute conditions and the authors warned against use of the measure in health economics planning (86). Poor correlation was seen between change in NHP scores and physiological change over time in the 2-year follow-up report. The CF patients´ scores appeared to be skewed and the instrument of limited use in CF (87). The instrument has also been suggested to be most suitable for an older patient population with substantial disability (193).

The SF-36 health survey is self-administered and takes 10 minutes to complete and has been described to identify minor levels of discomfort in relatively well people, missed by the NHP (193). The instrument has been used in cross-sectional studies in adolescent and adults with CF. However in one study, where the SF-36 was compared with two generic German instruments, a lot of ceiling effects were seen for SF-36. It was proposed that the different validity of instruments has to be considered in choosing a questionnaire appropriate to the purpose of measuring QOL (90). For reasons of sample size, multi-centre studies are often required in medical research. Including QOL as a secondary outcome will require access to translated and validated QOL questionnaires. When using SF-36 in a study comparing German and English adolescents and adults with CF, differences in quality of life was seen that could not be explained by different disease status or treatment, but rather culturally determined or due to translational problems (89). It is possible that by developing an instrument simultaneously in different languages some of these queries may be overcome, a method previously used by the European Organization for Research and Treatment of Cancer in the development of the Quality of Life Questionnaire (194).

The *Chronic respiratory questionnaire (CRQ)* was constructed for patients with chronic airflow limitation (81) evaluating four dimensions: dyspnoea, fatigue, emotional function, and the patient's feeling of control over the disease (mastery). The initial version was interview-administered and took up to 30 minutes to complete but one self-administered and one version with a standardized activity domain are also available (195,196). The instrument has been used in follow-up studies in patients with CF (192,197,198) and also adapted for CF (199). The instrument was however initially constructed for an older group than the majority of patients with CF.

Construction of the CF-QUEST: When our project started there were no quality of life measures for CF available and the instruments used seemed to have several limitations. We believe that by developing the questionnaire in English and Swedish simultaneously, rather than to translate the final instrument, this will increase the cross-cultural validity of the final questionnaire. We found that the positive emotional items were higher scored for Swedish patients and believe that this was caused by a linguistic problem. The same interview instructions were used but there was unfortunately no direct communication between the two interviewers. In the provisional questionnaire every question was asked: "In relation to CF, have you experienced the following items during the last year?" and this expression caused problem for the positive emotional questions. These questions were therefore asked more in general terms and it may have been a difference in the way the question were asked in the two countries. We will keep this problem in mind in the planned field study for validation of the CF-QUEST. We decided to include positively phrased questions, as a "very negative" questionnaire may not be applicable, or even offensive, for a patient with mild disease.

Factor analysis was used as a complement for assessment of domain belonging when constructing the final questionnaire. This is a statistical method aimed to reduce a large number of interrelated variables to smaller number of common dimensions or factors. A

factor is a group of variables with high correlation within the group but weak correlation between variables within the group and those outside of the group. In an exploratory factor analysis no prior knowledge of the structure of postulated function of the questionnaire is necessary. The correlation matrix is the starting point for analysis. In the primary correlation matrix it is often difficult to interpret the pattern of how the variables are loading on different factors. There is often more than one mathematical solution, and the computer program can perform different transformations (rotations) that simplify the overall structure. The Eigen value is a measure of how much variation in the data is accounted for by each factor. A rough rule is to keep the number of factors with an Eigen value greater than one (i.e. that the factor extracts at least as much as the equivalent of one original variable), and/or to look at the graphic scree plot and keep those above the point where the smooth decrease of Eigen values appears to level off. The factors on the right side of this point probably have little additional to contribute. Based on the results we moved "difficulty sleeping" to the emotional domain but decided to keep "feeling tired" in the physical domain, despite high loading with the emotional domain. Fatigue is a well-known symptom of infectious exacerbation and in the pre-testing study of CF-QUEST in patients starting intravenous antibiotic therapy due to infection this was also the physical item with the highest score.

English is spoken in different cultures and parts of the world and we therefore avoided slang or expressions that we felt could be interpreted in different ways, or that might cause difficulties in future translations.

There are currently a few disease-specific instruments for CF available; among them two instruments in English. *The Cystic Fibrosis Questionnaire (CFQ)* was originally developed in France by Henry and colleagues (200) in three versions; one version for children 6 to 13 years, one for parents of school-age children and one for adolescents and adults. The CFQ has been translated into English, Dutch and Spanish recently, and validation of the translated versions are in progress (201,202). The developmental process started with qualitative interviews with 44 children and adults with CF and their parents. Three initial questionnaires were constructed and pilot testing with 30 participants was performed. After adjustments there were 534 questionnaires completed to assist in developing the final French questionnaire, which in part was financed by a pharmaceutical company.

The *Cystic Fibrosis Quality of Life Questionnaire (CFQoL)* was developed in the UK by Gee and colleagues (89,203). It is a self-administered questionnaire for adolescents and adults including 52 questions –or rather statements- across nine domains, asked "in relation to CF" and to be responded on a six-point scale. Items were generated from literature review, examination of existing instruments, discussion with CF-staff, and interviews with patients. The instrument has been reported to be appropriate for use in clinical trials, for cross sectional comparison between groups of CF patient with different levels of disease, as well as for longitudinal follow up of patients.

Characteristics of the three CF-specific instruments are listed in Table 4. Some features of the new instrument, CF-QUEST, are the individualized activity questions, a simple layout and a wide response range of 1-7 with only the extreme alternatives stated in text. The purpose is to increase the applicability in a wide range of functional level, to make it easy to complete and to increase responsiveness. A field study is now planned for validation of the instrument. Some important issues are the clinical importance of a measured difference in QOL scores and ceiling and floor effects. Correlation between for example FEV_1 and QOL scores is often interpreted as an indicator of validity but HRQL measures are constructed to provide additional information not captured by these tests and a very high correlation should not be expected.

Table 4. *Disease-specific Quality of Life Instruments in English for adults with CF*

Instrument	Layout Domains (number of questions)	Language
CFQ 14+	48 questions divided in 3 sections. A demographic section is included. Self-administered. Takes 15 min to complete. Items are rated in terms of frequency, difficulty, true-false categories or weighted statements with 4 alternatives. Three versions available, 1) ≥14 years, 2) children <14 years and 3) their parents. Quality of life, 9 domains (33) Physical functioning, Energy and well-being, Emotional state, Social limitation, Role limitation, Body image, Eating disturbance, Treatment constraints, Embarrassment School, work, or daily activities (3) Symptom difficulties (12)	French English German Dutch Italian Portuguese Spanish

CFQoL	52 questions with 9 domains, divided in 8 sections. Self-administered. Ten minutes to complete. Scale 1-6, defined response alternative, Transformation of scores to 0-100.
	    Physical functioning (10)
	    Social functioning (4)
	    Treatment issues and Chest symptoms (7)
	    Emotional functioning (8)
	    Interpersonal relationships (10)
	    Career issues (4)
	    Body image (3)
	    Concerns for the future (6)
		English

CF-QUEST	47 questions, divided in a main module, separate GI module and 2 global questions. Self-administered. Ten minutes to complete. Semi-linear scale 1-7 with the extremes defined as "not at all" and "very much". Transformation of scores to 0-100.
	    Main module (40)
	        Physical and Treatment-related issues, Emotional issues, Social and vocational issues, Activities (individualized)
	    Gastrointestinal complaints (5)
	    General Health and Quality of Life (2)
		English Swedish

CFQ=Cystic Fibrosis Questionnaire
CFQoL= Cystic Fibrosis Quality of Life Questionnaire
CF-QUEST= Cystic Fibrosis Quality of Life Self-administered Tests

Future

There is lot of hope for the future. The understanding of genotype-phenotype relations, CFTR function and interactive events in the airway microenvironment leading to infection and inflammation is increasing. The goal is to find a method for definitive correction of the CFTR function with gene therapy (204,205), stem cell therapy (206) or by pharmacologic correction (207). New drugs aimed to restore the microenvironment in the airways are under investigation. Meanwhile, patients will have to adhere to the cumbersome daily treatment, with the CF health care team supporting them throughout different phases of their life and stages of disease. For patients diagnosed in adult age, the diagnostic procedure has often been prolonged and there is a need for directed information and psychosocial intervention during the first time following diagnosis. Diagnosis of atypical cases in adult age, family screening and prenatal diagnosis of *CFTR* mutations will influence the future epidemiology of CF, as well as legal and religious aspects of prenatal diagnosis, and pre-implantation diagnosis for in-vitro fertilization techniques.

Conclusions

Different aspects of CF in adults were studied in this thesis. Paper I-III was based on the Toronto CF population. For Paper IV, patients from two of four Swedish centres were included as well.

Survival in CF has improved and the adult population is now larger than the child population. There is a large variability of the disease, both in severity and clinical manifestations. During the last decades an increasing number of patients have been diagnosed in adult age but the diagnosis can sometimes be difficult to confirm in standard diagnostic tests and CF is still a clinical diagnosis. The sweat test results may be inconclusive and extensive mutation analysis is often required. A high suspicion is necessary and patients with possible CF, with or without a positive sweat test, should be referred to a CF centre for diagnostic evaluation.

Almost all men with CF are infertile because of absence of the vas deferens. A large proportion of men with infertility due to CBAVD, carry CFTR mutations and may have borderline or elevated sweat chloride concentration. Bronchoscopy with BAL in some of these men revealed presence of opportunistic bacteria. BAL fluid should normally be sterile and these bacteria are not usually described as belonging to the normal airway flora but are sometimes detected in patients with CF. This suggests that there is a relation between CBAVD and CF and that some of these men may in fact have a very mild airway infection, but without the inflammatory response usually seen in CF.

Pregnancy was well tolerated in the population studied. The risk factors for deterioration and death were the same as for the whole adult female CF population; the most important risk factor was chronic airway infection with the Toronto strain of *B cenocepacia*. The experience of pregnancy in women with FEV_1 less than 50% is however limited, as the majority had an FEV_1 more than 50%. Obstetric data and fetal outcome was comparable to the general population.

A self-administered health-related quality-of-life instrument for evaluation of treatment in adults with CF was constructed in English and Swedish in parallel. With some few exceptions the ranking of items of importance for quality of life was similar in Toronto and Sweden and pre-testing of the final questionnaire suggested it to be easy to understand and with appropriate questions.

Populärvetenskaplig sammanfattning på svenska

Bakgrund: Cystisk fibros (CF) är en ärftlig sjukdom som orsakas av mutationer i *CFTR* genen (Cystic Fibrosis Transmembrane conductance Regulator). Denna gen styr bildningen av äggviteämnet CFTR som fungerar som en kloridkanal i cellväggen. Sjukdomen ärvs autosomalt recessivt vilket innebär att föräldrarna är friska bärare av sjukdomsanlaget och att barnet med CF ärvt det sjuka anlaget i dubbel uppsättning.

På grund av den förändrade kloridtransporten över cellväggen så utsöndrar personer med CF ett ovanligt salt svett. Vid CF bildas bildar kroppens körtlar ett segt, förändrat slem. De flesta personer med CF har bristfällig bukspottkörtelfunktion, nästan alla män är infertila på grund av blockerade och tillbakabildade sädesledare och ibland kan en allvarlig leversjukdom utvecklas. Det är emellertid den kroniska lungsjukdomen som ger de mest påtagliga symtomen och är vanligen orsaken till förkortad livslängd. Det sega förändrade slemmet täpper till luftvägarna och är en grogrund för bakterier. Följden blir en kronisk infektion som kan ge svåra ärrbildningar och nedsatt lungfunktion. Lungsjukdomen börjar tidigt vid CF. Vid bronkoskopi på spädbarn med CF har man funnit bakterier och förhöjda nivåer av inflammatoriska celler och markörer i lungsköljvätska.

För diagnos av CF krävs påvisande av två CF-orsakande *CFTR* mutationer och/eller påvisande av störd kloridtransport samt symtom som är typiska för CF. Det är inte alla av hittills mer än 1500 kända mutationer som bedöms vara tillräckligt allvarliga för att klassas som CF-orsakande. Störd kloridtransport påvisas vanligen med ett så kallat svettest. Efter stimulering till svett sekretion kan svett samlas upp och den förhöjda klorid koncentrationen analyseras. Undersökning av spänningsskillnader över slemhinnor, t.ex. näs-slemhinnan vid samtidig genomsköljning med olika lösningar är en annan komplicerad metod att påvisa den störda salttransporten. Typ av mutation och dess plats i genen, men även normala variationer i genen, andra genetiska faktorer och miljöfaktorer är av betydelse för sjukdomsbilden. De flesta får sin diagnos under första levnadsåret men det finns en stor variation i både symtom och allvarlighetsgrad och ibland ställs diagnosen först i vuxen ålder. Eftersom nästan alla män med CF är infertila på grund av blockerade och tillbakabildade sädesledare så har man undersökt om *CFTR* mutationer är vanligt hos infertila män i allmänhet. Manlig infertilitet orsakas i ett fåtal fall av avsaknad av, eller blockerade, sädesledare (congenital bilateral absence of the vas deferens, (CBAVD)). Man har funnit att en stor andel av dessa män med CBAVD bär på *CFTR* mutationer och att en variant i *CFTR* genen som kallas 5T och innebär att det bildas betydligt mindre mängd av normalt CFTR protein är vanlig. Man har även funnit att många av dessa män har ett onormalt svett test och på grund av dessa fynd har man föreslagit att CBAVD kan vara en lindrig variant av CF. För kvinnor med CF är fertiliteten nedsatt på grund av segt slem i livmoderhalsen men många kvinnor med CF blir spontant gravida och klarar av graviditeten utan större problem. I tidiga rapporter fann man dock en risk för försämring under graviditeten samt risk för prematur förlossning. Med centraliserad vård, nya mediciner och ett multidisciplinärt omhändertagande med fokus på förebyggande åtgärder och tidig behandling av komplikationer har överlevnaden förbättrats dramatiskt. Över hälften av patienterna är nu vuxna i vissa populationer (Sverige och Kanada till exempel) medan ett fåtal nådde vuxen ålder för endast ett par decennier sedan. Fortfarande är dock överlevnaden starkt förkortad i vissa länder. Den dagliga behandlingen är arbets- och tidskrävande och det kan vara mycket svårt för patienten att hitta en bra balans mellan daglig CF-behandling och att leva ett "normalt" liv. Vid utvärdering av behandlingsmetoder bör något mått på livskvalitet ingå eftersom traditionella mått på hälsa, såsom lungfunktion, vikt och sänkereaktion, inte kan fånga alla aspekter av sjukdomens betydelse för individen.

Målet med våra studier var att undersöka områden av intresse för vuxna med CF.

I studie 1-4 ingick patienter från vuxen CF centrat i Toronto och i studie 4 ingick även vuxna patienter från CF mottagningarna i Göteborg och Lund.

Studier: 1) Data från patienter som fått diagnosen CF i vuxen ålder i Toronto mellan 1960 och juni 2001 analyserades avseende debutsymtom och diagnostiska undersökningar. Vi fann att personer som fick diagnosen CF i vuxen ålder ofta hade lungsjukdom, normal bukspottkörtelfunktion, ovanliga *CFTR* mutationer och ibland normala eller intermediära svettklorid nivåer. Påvisande av störd salttransport över nässlemhinnan var ett diagnostiskt hjälpmedel. 2) Vid bronkoskopi med sköljprov från lungan på infertila män med CBAVD, intermediära eller förhöjda svett kloridnivåer och en eller två *CFTR* mutationer fann man växt av ovanliga bakterier, som normalt inte ska finnas i lungan men som förekommer vid CF, hos 6/8 män men endast hos 1/4 friska kontroller. Vissa inflammatoriska markörer (IL8 och TNF-alpha) var högre hos män med CBAVD än hos friska kontroller. 3) Graviditeter hos kvinnor med CF mellan 1961 och 1998 analyserades. Det var 49 kvinnor som fullföljde 74 graviditeter. Överlevnaden var bättre hos de kvinnor som inte var infekterade med den speciella svårbehandlade Toronto stammen av bakterien *Burkholderia cenocepacia* , som hade fungerande bukspottkörtel och som hade god lungfunktion. Graviditeten i sig försämrade inte överlevnaden och påverkade inte heller lungfunktionen i jämförelse med hela vuxna kvinnliga CF populationen i Toronto. Få kvinnor som fullföljde en graviditet hade dock kraftigt nedsatt lungfunktion och resultaten måste därför tolkas försiktigt för denna grupp. 4) Ett nytt frågeformulär för att mäta hälsorelaterad livskvalitet vid CF konstruerades. Ett översiktsformulär med 114 frågor relaterade till hälsa och CF konstruerades på basen av 200 svar från patienter och sjukvårdspersonal. Vid en intervju besvarade sedan vuxna patienter i Toronto och Sverige formuläret och angav för varje fråga a) om denna fråga var viktig (ja eller nej) och b) om ja, hur viktig (skala 1-5). Resultatet av intervjustudien vägledde sedan konstruktionen av det slutgiltiga frågeformuläret, CF-QUEST (Cystic Fibrosis QUality of life Evaluative Self-administered Test) som gjordes i en engelsk och en svensk version.

Sammanfattning: Patienter som får diagnosen CF i vuxen ålder har ofta fungerande bukspottkörtel, svårbedömda resultat vid svettest och ovanliga mutationer. Extensiv utredning kan krävas och i enstaka fall kan en klinisk diagnos inte konfirmeras med laboratorietest. En del män med CBAVD har en lindrig form av CF med förekomst av bakterier lungan men utan det inflammatoriska svar som vanligen ses vid CF. De flesta kvinnor med CF klarar av en graviditet bra, utan ökad risk för lungfunktionsnedsättning eller ökad risk för barnet. Kunskapen om kvinnor med nedsatt lungfunktion är dock begränsad. Ett nytt frågeformulär avseende livskvalitet hos vuxna med CF, CF-QUEST, konstruerades på engelska och svenska, för att användas vid uppföljning av behandling.

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