2. Diagnosis in adults

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1. INTRODUCTION

- The median age of CF diagnosis remains at 6 months, but the diagnostic yield in subjects older than 18 years has been increasing, probably due to improved clinical awareness and better access to 2nd-level testing. Switzerland introduced newborn screening for CF in 2011 (IRT/DNA/IRT protocol), which may in time lead to earlier diagnosis, prompt management and better outcomes.
- The diagnostic work-up of adults can be challenging because:
  - some of the classic clinical characteristics of the disease may be absent
  - different cut-off values of diagnostic tests may apply
  - uncommon *CFTR* variants may be observed in those patients presenting with later onset disease, modifying the optimal approach to genetic testing
- Diagnostic terminology is still evolving as our knowledge of the spectrum of CFTR disorders increases.
- Several clinical entities associated with CFTR dysfunction but not fulfilling the diagnostic criteria for CF are called CFTR-related disorders (CFTR-RD). The best-recognized CFTR-RD are:
  - congenital bilateral absence of the vas deferens (CBAVD)
  - acute recurrent or chronic pancreatitis
  - diffuse bronchiectasis
- Terms such as “mild” or “atypical” CF may be misleading and it is best to classify patients where possible as having CF or CFTR-RD.

2. SYMPTOMS AND CLINICAL FEATURES SUGGESTIVE OF CF OR CFTR-RD IN ADULTHOOD.

- The spectrum of CF clinical features **(Table 1)** is broad characterized by varying degrees of organ involvement, disease severity and progression rate.

3. DIAGNOSTIC WORK-UP

In cases in which the clinical picture is suggestive of CF or CFTR-RD in an adult patient, diagnosis should be established by specific laboratory tools such as:

- The sweat chloride test
- *CFTR* gene analysis
- CFTR bioassays

Thoracic imaging, pulmonary function tests, fecal analysis of elastase or a spermogram, may support the initial clinical suspicion and characterize the phenotype and the severity of the disease.

Table 1: Symptoms and clinical features suggestive of CF or CFTR-RD in adulthood (adapted from[1-4])

Chronic sinopulmonary manifestations:

1. Airway colonization/infection with typical CF pathogens, including *Staphylococcus aureus*, *Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia, Burkholderia cepacia* complex*, Achromobacter xylosoxidans*
2. Symptoms: Chronic productive cough, haemoptysis associated with diffuse pulmonary disease in the absence of an alternative diagnosis
3. Persistent radiographic abnormalities (e.g. bronchiectasis, atelectasis, persistent infiltrates, hyperinflation, radiographic evidence of chronic pansinusitis)
4. Allergic bronchopulmonary aspergillosis (ABPA)
5. Obstructive airways disease
6. Pansinusitis, nasal polyps
7. Digital clubbing

Gastrointestinal and nutritional abnormalities

1. Intestinal: distal intestinal obstruction syndrome (DIOS)
2. Pancreatic : exocrine or endocrine pancreatic insufficiency, recurrent acute or chronic pancreatitis, pancreatic abnormalities on imaging
3. Hepatic: chronic hepatic disease manifested by clinical or histological evidence of focal biliary cirrhosis or multilobular cirrhosis, primary sclerosing cholangitis
4. Nutritional: hypoproteinemia and oedema, complications secondary to fat-soluble vitamin deficiencies, osteopenia/osteoporosis diagnosed at <40 years of age

Salt loss syndromes: acute salt depletion, chronic metabolic alkalosis

Infertility in males: obstructive azoospermia due to congenital bilateral absence of the vas deferens (CBAVD)

3.1 Sweat chloride test

- **It remains the gold standard of CF diagnosis and it should be performed in a specialized centre**.
- **Method**: application of pilocarpine nitrate (cholinergic drug) on the skin, preferably the flexor surface of the forearm or alternatively the upper arm, thigh or calf → iontophoresis using two electrodes (one positive and one negative) and a gradually increasing current up to 4 mA for less than 5 min → the positively charged pilocarpine ions move away from the positive electrode and into the skin → intracellular Ca^{+2} concentration increases → opening of the calcium-activated Cl^- channel → production and collection of sweat during 20-30 min → sweat analysis of the concentration of Cl^-.
  - Sweat Cl^- is increased in CF.
  - Osmolality and conductivity have poor discriminatory power for the diagnosis of CF.
- The lower limit of Cl^- detection provided by the laboratory should be ≤ 10mmol/l, whereas the upper end of reportable results should be ≤160 mmol/l, as a higher concentration is not physiologically possible.

- For an individual patient, results should always be interpreted within the clinical context and all positive tests must be confirmed by a second sweat Cl^- test or by *CFTR* genetic testing.
- The diagnosis of CF is **supported** by sweat Cl^- levels ≥60 mmol/l.
- Diagnosis of CF is **not supported** when the sweat Cl^- level is <60 mmol/l
  - Some *CFTR* variants, including some CF-causing variants, such as 3849+10kbC>T, 2789+5G>A, R117H, R117L, A455E, A309D, G551S, IVS8 (5T), L206W, L997F, D1152H may result in a sweat Cl^- <60 mmol/l when they are associated with another *CFTR* variant.
- Current guidelines suggest that sweat Cl^-<30 mmol/l is considered normal.
- Values of sweat Cl^- between 30-59 mmol/l are called "borderline" or "intermediate" values and indicate some degree of CFTR dysfunction.
- Causes, other than CF, that may lead to a false positive or false negative sweat test are presented in **Tables 2 and 3** respectively.

Table 2: Causes other than CF which may be associated with a false positive sweat Cl- test - in alphabetical order (Adapted from[5-9])*

Likely
*Anorexia nervosa**
Arsenic toxicity
*Atopic dermatitis**
Carbonic anhydrase XII mutations
Malnutrition (if severe)
*Munchausen syndrome by proxy**
*Technical issues**
– Evaporation of sweat sample
– Application of amethocaine at the site of testing
Topiramate therapy
Possible but further validation is required
Ectodermal dysplasia
Familial cholestasis
Fucosidosis type I
Glycogen storage disease type I
Hypogammaglobulinemia
Hypoparathyroidism
Hypothyroidism (untreated)
Pseudohypoaldosteronism
Psychosocial failure to thrive

(continued)

Unlikely**
Autonomic dysfunction
Celiac disease
Glucose 6 phosphatase (G6PD) deficiency
KID syndrome
Klinefelter syndrome
Mauriac syndrome
Mucopolysaccharidosis type I
Nephrogenic diabetes insipidus
Prostaglandin E1 infusion
Pyelonephritis
Shwachman-Diamond syndrome
Systemic lupus erythematosus
Triosephosphate isomerase deficiency
Trisomy 21
Untreated Addison's disease

*Conditions that should always be considered.
** Some reports but very limited or insufficient/contradictory evidence.

Table 3: Causes which may be associated with a false negative sweat Cl- test (Adapted from [5,7])*

Likely
*Technical factors**
– Sweat sample dilution
– Failure to dry skin before sweat collection
Possible but further validation is required
Mineralocorticoid treatment
Unlikely**
Young age
*Edema**
*Hypoproteinemia**
Penicillin therapy

*Conditions that should always be considered.
** Some reports but very limited or insufficient/contradictory evidence

3.2 CFTR *genetic testing*

- DNA analysis should be performed in a specialized genetic laboratory. **All CF/CFTR-RD patients should be genotyped.** *CFTR* genetic testing is used for diagnostic confirmation, for accurate genetic counselling and for personalized treatment decisions (e.g. ivacaftor for gating variants such as G551D, G1244E, G1349D, G178R, G551S, S1251N, S1255P, S549N, S549R, ivacaftor for the class IV variant R117H, ivacaftor/lumacaftor for F508del/F508del) **(See also Chapter "*CFTR modifiers*")**.

- The diagnosis of CF is supported by the identification of two CF-causing genetic variants.
- The laboratory should report on relevant identified CFTR variants and make clear whether they are: a) CF-causing and predicted to be associated with CFTR residual function or not, b) of varying clinical consequences (VVCC) or c) of unknown significance (VUS). Variants which are likely to have no clinical relevance are generally not reported. However, it must be kept in mind that interpretation of clinically relevant genomic variation can be challenging **(see also Chapter "*Pathogenesis of CF*")**.
- The frequency of CF-causing variants in patients varies considerably according to ethnic origin. To adapt the genetic analysis and/or interpretation appropriately, **it is important that the clinician asks the ethnicity of each parent and transmits the information to the laboratory**.
- Most commercially-available **first-level screening assays** test 30-50 CF-causing variants. In Switzerland, their sensitivity to detect CF alleles is 85-90% based on ethnic background. Such tests allow detection of:
  - both (2/2) disease-causing variants in 70-80% of CF patients
  - 1/2 in 20-30% of patients
  - 0/2 in 1-2% of patients (false-negative result)
- In some clinical situations (e.g. when only one variant is identified by first-level screening), **second-level testing with high-sensitivity analysis** of the entire *CFTR* gene may be necessary.
  - **Gene sequencing** has a sensitivity of ≥98%, but is more expensive than first-level screening.
  - Some common variants are not routinely detected by sequencing analysis and should be specifically tested when appropriate (e.g. the Eastern European dele2,3 deletion and the Hispanic 1811+1.6kbA>G intronic variant).
  - Gene deletions and duplications (1-5% of CF alleles) are not detected by sequencing; further testing by MLPA or other techniques may be necessary.
  - Second-level testing can identify variants of unknown significance; the laboratory should provide the most accurate possible interpretation.
- Whenever two potentially disease-causing variants are detected, *cis* or *trans* phase **(see Chapter "*Pathogenesis of CF*", section "*Complex alleles*")** should be determined. This is especially true for variants of varying clinical consequences (VVCC), whose roles are often unclear. Ideally, phase is determined by family testing (e.g. parents) whereas in some cases it can be probabilistic (e.g. F508del and R117H are essentially certain to be in *trans*).
- In case of a strong clinical suspicion of CF but without confirmation by the presence of two disease-causing *CFTR* variants, firstly the genetic testing should be re-evaluated ("was it sufficiently sensitive?"). If this was the case, genetic heterogeneity could be considered: although such cases are extremely rare, other genes than CF could be responsible for a clinical phenotype of CF. However, at present no reliable testing exists for such forms.
- Genetic testing laboratories should provide results within a defined and appropriate **turnaround time** (TAT). TAT for routine testing should be <1 week and <2 weeks for 1st- and 2nd- level testing respectively. Urgent requests should generally be reported in ≤3 working days (both levels).

- **Reimbursement and insurance issues**
  - Swiss obligatory health insurance reimburses the cost of laboratory tests which "serve to **diagnose or treat a disease and its consequences**" (LAMal Art 25. Al.1) when the disease is included in the Analysis List, as is the case for both cystic fibrosis ("fibrose kystique", "Cystische Fibrose") and CAVD ("Aplasie congénitale du canal deferent", "Congenitale Aplasie des Vas Deferens").
  - Carrier testing, for example in relatives of CF patients or partners of CF patients or known carriers, is not reimbursed by obligatory health insurance. However, this is not a reason to avoid discussing carrier testing when medically recommended.
  - Patients should be aware that obtaining private insurance can be difficult or expensive after the diagnosis of CF.
  - The costs of 1st- and 2nd-level testing vary according to the exact techniques used but are approximately 550 CHF and 1'700-3'000 CHF at the time of writing; carrier testing typically costs between 350-550 CHF. The laboratory can provide accurate costs for individual situations.
- **Post-test counselling and cascade testing**
  - **All individuals who test positive for one or two CF-causing variants must be offered genetic counselling** to explain the eventual reproductive and/or familial consequences of the result. Counselling should be timely, taking into account the fact that relatives as well as the patient are potentially at increased risk of having offspring with CF.
  - Genetic counselling and "cascade" carrier testing should be offered to all relatives of individuals with CF-causing variants. As stated above, the fact that carrier testing is not obligatorily reimbursed by health insurance is not a justification for not offering counselling/testing.
  - Carrier testing should not be automatically offered for variants of varying clinical consequences (VVCC), because of their reduced penetrance and clinical variability. However, genetic counselling should always be an option, and clinical judgement should be used to decide when testing is appropriate.

  Note: prenatal diagnosis for CFTR-RD genotypes is not generally considered appropriate and should not be routinely offered.
- **Informed consent (Table 4)**.

Table 4: Facts and recommendations concerning informed consent before genetic testing for CF or CFTR-RD

Informed consent is required before all genetic testing (Federal Law on Human Genetic Analysis)
It is the responsibility of the physician requesting the test to ensure that appropriate counselling is provided and that consent is given
Oral consent is sufficient for diagnostic testing; the doctor should make a **written note**, for example "the patient was counselled and consent was given"
Written consent is obligatory before prenatal, pre-symptomatic and carrier testing
Parents or guardians must consent for persons unable to give informed consent (e.g. children).

3.3 CFTR bioassays

- Bioassays provide direct evidence of CFTR dysfunction. At the time of writing these tests are not available in Switzerland.
- **Nasal potential difference (NPD) measurement (in vivo):**
  - It is based on the observation that bioelectric potential differences across respiratory epithelia are abnormal in CF patients.
  - Voltage differences are measured between two electrodes: the exploring electrode which rests on the top of the nasal mucosa and the reference electrode which is placed under the forearm skin.
  - Sequential application of 5 different solutions on the nasal mucosa: 1) Ringer's solution, 2) Ringer's solution and amiloride (ENaC inhibition), 3)chloride-free solution and amiloride (Cl^- secretion mediated by the activation of CFTR, Ca^{+2} dependent Cl^- channels and ORCC), 4) chloride-free solution, amiloride and isoproterenol (further increase of CFTR mediated Cl^- transport), 5) chloride-free solution, amiloride and ATP (activation of Cl^- secretion through calcium-activated chloride channels).
  - Compared to normal subjects, the typical pattern observed in CF consists of a more negative basal NPD, an exaggerated response to amiloride and no response to the Cl^- free+amiloride+ isoproterenol solution. **Figures S1-S3 of the Supplement** show examples of NPD measurements in a healthy control and a CF patient.
  - Standard operating procedures (SOPs) are available for NPD. However, the test has several technical challenges and it is important to be performed by an experienced operator. Patient-related factors that can affect CFTR response should also be considered (e.g. smoking, acute rhinitis within 2 weeks prior to the study, allergic rhinitis within a month prior to the study, polyp removal, certain drugs such as amiloride, hypertonic saline, inhaled antibiotics, DNAse).
  - Interpretation of NPD: The parameter used the most is the change in potential difference between the application of solution 3 (chloride-free solution + amiloride) and 4 (chloride-free solution + amiloride + isoproterenol). This difference is called $\Delta PD_{0Cl/Iso}$ (Iso referring to isoproterenol). Although there is no wide consensus regarding the cut-off points, the following categories have been used in the literature for $\Delta PD_{0Cl/Iso}$
    - i) normal <-12 mv,
    - ii) abnormal >-7.7 mV and
    - iii) intermediate between -12 and -7.7mV.

    Measurements are performed in both nostrils. Currently the mean $\Delta PD_{0Cl/Iso}$ is used for diagnostic purposes but this approach may underestimate CFTR function. A recent study suggested that the best $\Delta PD_{0Cl/Iso}$ value should also be taken into consideration. **Figure S1b of the Supplement** shows an example of how $\Delta PD_{0Cl/Iso}$ is measured on an NPD curve.
- **Intestinal current measurement (ICM) (ex vivo):**
  - It is based on the observation that bioelectric potential differences across intestinal epithelia are abnormal in CF patients.
  - Freshly obtained suction rectal biopsies (minimally invasive procedure) → intestinal tissue specimen is placed in mini-Ussing chambers → application of amiloride (to block ENaC) and stimulation with CFTR activatory agents → ion transport → measurement

of the transepithelial short-circuit current or potential difference → evaluation of CFTR function.
    - It has been mainly used in research, however increasing evidence suggests that it may be a useful tool in clinical practice and that it may be superior to NPD.
    - Currently there are 2 different SOPs available (not standardized across laboratories).
- **β-adrenergic sweat secretion test (in vivo):**
    - Assessment of sweat gland CFTR activity using an evaporimeter, to measure the rate of CFTR-mediated sweat secretion.
    - Sequential intracutaneous administration in the forearm of the following solutions: carbachol (induction of cholinergic secretion) → atropine (inhibition of cholinergic secretion) → solution containing atropine, isoproterenol, aminophylline (pure β-adrenergic secretion).
    - Measurement of the mean maximal β-adrenergically stimulated sweat rate is a) absent in CF, b) reduced or absent in CFTR-related disorder, c) half the rate of healthy controls in cases of heterozygotes and d) equal to cholinergic sweat rate in healthy controls **(examples in Figure S4 of the Supplement)**.
- **Intestinal organoids (ex vivo):**
    - This technology was first described in 2013. It is a quantitative assay for CFTR function and, and although it is currently a research tool, it is considered to be very promising not only for diagnosis but also for CFTR-specific drug development **(see also Chapter "*CFTR modifiers*")**.
    - Suction rectal biopsies (minimally-invasive procedure) → Primary intestinal culture, intestinal stem cells proliferate under the effect of growth factors → they expand into closed organoids which are similar to in vivo tissue structures (crypt-like structures and internal lumen lined by differentiated cells, CFTR expressed at the apical membrane of the crypt cells) → When forskolin is applied to organoids derived from healthy controls, it induces rapid organoid swelling but its effect is strongly reduced in CFTR-deficient organoids → drug-induced restoration of CFTR-function can be assessed with this technique.

4. DIAGNOSTIC TERMINOLOGY

- The diagnostic terminology is still evolving as our knowledge of the spectrum of CFTR dysfunction increases.
- **Established CF diagnosis**: 1) clinical features of CF in at least one organ **and** 2) CFTR dysfunction demonstrated through **any** of the following
    - Sweat chloride ≥60 mmol/l (repeated twice) or
    - Two CF-causing variants in trans (i.e. in each *CFTR* gene) or
    - An abnormal functional testing (NPD or ICM) characteristic of CF
- **CFTR-RD: clinical entities that are associated with CFTR dysfunction but do not fulfil the diagnostic criteria of CF;** they are characterized by clinical features and findings of CFTR dysfunction and none or one CF causing variant. The best-recognized CFTR-RD are:
    - congenital bilateral absence of the vas deferens (CBAVD)
    - idiopathic chronic pancreatitis

- diffuse bronchiectasis

Note: According to the 2001 WHO classification, CFTR-RD includes isolated obstructive azoospermia, chronic pancreatitis, allergic bronchopulmonary aspergillosis, disseminated bronchiectasis, diffuse panbronchiolitis, sclerosing cholangitis, neonatal hypertrypsinogenaemia.

5. DIAGNOSTIC ALGORITHMS

- The algorithms intended for the diagnosis of CF recommend specific reasoning pathways for the establishment of the diagnosis in clinically suspected cases of CF.
- **The algorithm of the Swiss adult CF centres is an adaptation of the European, and the 2008 and 2017 CF Foundation algorithms. It presents the pathway of investigations performed in patients referred to Swiss Adult CF centres based on available diagnostic tests (Figure 1).** Both sweat test and DNA analysis are proposed in suspected cases to allow further cascade screening of the patients' relatives and personalized treatment decisions. Functional testing is proposed in selected cases at the last step, as these tests are not currently available in Switzerland.
- The major differences between these algorithms are presented in **Table 5.**

6. EXAMPLES OF CASES

- **Example 1:** 1) absence of phenotypic characteristics of CF or CFTR-RD, 2) normal sweat chloride test and 2) one CF-causing variant. *Diagnosis:* healthy carrier.
- **Example 2:** 1) at least one phenotypic characteristic of CF, 2) 2 CF-causing variants (one on each *CFTR* gene) and 3) a sweat Cl^- concentration > 60 mmol/l. *Diagnosis:* CF.
- **Example 3:** 1) at least one phenotypic characteristic of CF, 2) detection of two CF-causing variants (one on each *CFTR* gene) *and* 3) a normal (<30 mmol/l) or borderline (30-59 mmol/l) sweat Cl^- concentration. *Diagnosis:* CF.
- **Example 4:** 1) At least one phenotypic characteristics of CF, 2) one CF-causing and one variant of varying clinical consequences (such as VVCC associated with CFTR-RD) in *trans* and 3) a borderline (30-59 mmol/l) sweat chloride concentration. *Diagnosis:* CFTR-RD.
- **Example 5:** 1) at least one phenotypic characteristic of CF, 2) a borderline sweat Cl^- concentration (30-59 mmol/l) and 3) one CF-causing variant in the 1st level genetic analysis → Subsequent confirmation of only one CF-causing variant with 2nd level genetic analysis (gene sequencing). *Diagnosis:* CFTR-RD. Consider performing NPD or ICM (if available).
- **Example 6:** 1) Phenotypic characteristics highly suggestive of CF, 2) no detection of CF-causing variants after 1st level and 2nd level genetic analysis and 3) sweat chloride test normal (<30 mmol/l)
  - DNA sequencing is recommended, with testing for intronic variants and/or deletions and insertions of one or more exons of the *CFTR* gene.
  - NPD or ICM is recommended to document CFTR dysfunction.
  - Although very rare, genetic heterogeneity cannot be excluded since the CF phenotype could conceivably be caused by a genetic factor other than CFTR (if, as mentioned above, the sweat Cl^- test is normal).

Table 5: Major differences among different algorithms for the diagnosis of CF and CFTR-RD in adults[1-3,10-13]

	European guideline (2006)[1]	CF Foundation guideline (2008)[3]	CF Foundation guideline (2017)[2]	Swiss adult CF centres recommendations (2017)
Diagnostic categories	• Classic CF • CFTR dysfunction • Inconclusive • CF unlikely	• CF • CFTR-related disorder • Inconclusive • CF unlikely	• CF • CFTR-related disorder (not in the algorithm) • Not resolved • CF unlikely	• CF • CFTR-related disorder • Inconclusive • CF unlikely
Definition of CFTR-RD	The term CFTR-dysfunction is used. Non-classic CF or single organ phenotype associated with CFTR mutations (WHO diagnostic list)*[1]	0-1 CF-causing mutations and clinical signs (possibly multiple-organ) suggestive of CF	A symptomatic entity that does not meet diagnostic criteria for CF	Clinical entities that are associated with CFTR dysfunction but do not fulfil the diagnostic criteria of CF; clinical findings of CFTR dysfunction and 0-1 CF causing variant
Initial testing (algorithm entry)	Sweat chloride or CFTR genotyping	Sweat chloride	Sweat chloride	Sweat chloride and 1st level CFTR genotyping
Sweat chloride upper limit of normal for no disease	30 mmol/l	30 mmol/l at an age up to 6 months and 40mmol/l thereafter	30 mmol/l	30 mmol/l

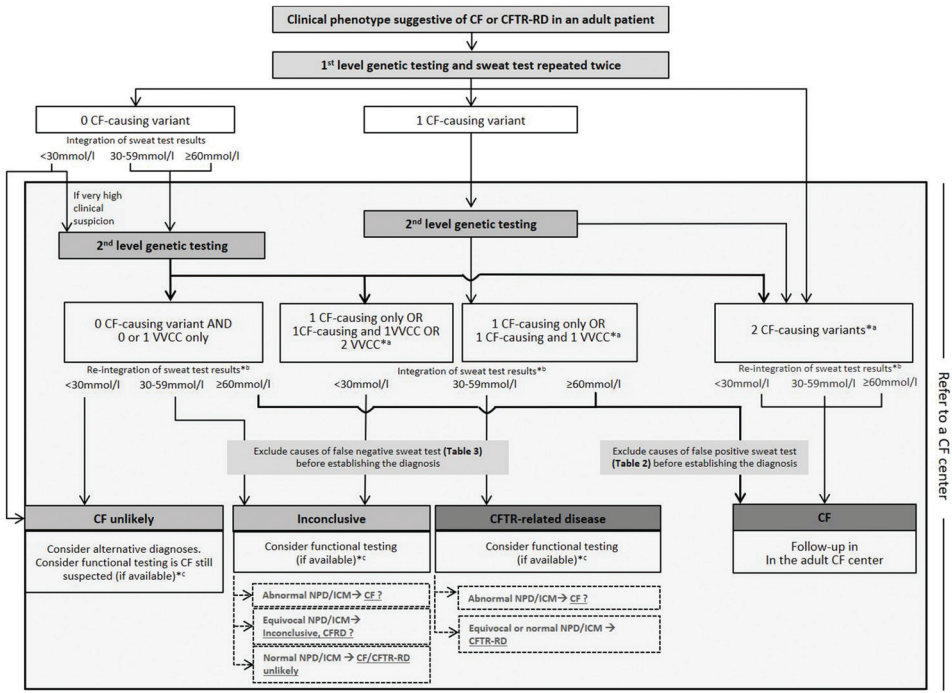
(continued)

CFTR variant panel	Variants that reflect the distribution and frequency in the appropriate population	23 variants established by the American College of Medical Genetics	Use CFTR2 mutation list, with guidelines given for mutations not included in CFTR2*[2]	First-level screening assays test 30-50 CF-causing variants. Adaptation of variant testing according to ethnicity.
CFTR variant categories	• CF-causing • Involved in CFTR related disease • Not disease causing • Unknown functional consequences	• CF-causing • No resulting clinical effect • Unknown functional consequences	• CF-causing • Mutation of varying clinical consequences (MVCC) • Non-CF-causing • Unknown functional consequences	• CF-causing • Variant of varying clinical consequences (VVCC) • No clinical consequences • Variant of unknown significance (VUS)
CFTR functional testing in the algorithm	NPD	NPD	NPD/ICM	NPD/ICM - Currently not available in Switzerland

*[1] Refers to the 2001 WHO classification that includes: isolated obstructive azoospermia, chronic pancreatitis, allergic bronchopulmonary aspergillosis, disseminated bronchiectasis, diffuse panbronchiolitis, sclerosing cholangitis, neonatal hypertrypsinogenaemia

*[2] Clinical and Functional Translation of CFTR project accessible in **http://www.cftr2.org/index.php**

Figure 1: Algorithm for the diagnosis of CF and CFTR-related disorders in adults, in Switzerland



CFTR genetic analysis should be performed in specialized laboratories and interpretation of the results should be done by an experienced geneticist.

First level genetic testing refers to commercially-available assays testing for 30 to 50 CF-causing variants.

Second level genetic testing refers to high-sensitivity analysis of the entire *CFTR* gene, notably gene sequencing or additional techniques whenever necessary (e.g. MLPA, specific research of some suspected variants that are not identified with gene sequencing – see text).

The term **VVCC** refers to variants of varying clinical consequences.

The terms **"CF?" and "CFTR-RD?"** are used to characterize cases that do not fulfil the diagnostic criteria for CF or CFTR-RD respectively, but present evidence of CFTR dysfunction. For these patients a close follow-up is recommended to timely identify the development of clinical features compatible with CF or CFTR-RD.

*a Whenever two potentially disease-causing variants are detected, phase (*cis* or *trans*) should be determined. This is especially true for variants of varying clinical consequences, whose roles are often unclear. Ideally, phase is determined by family testing (e.g. parents) whereas in some cases it can be probabilistic (e.g. F508del and R117H are essentially certain to be in *trans*).

*b *CFTR* variants, such as 3849+10kbC>T, 2789+5G>A, R117H, R117L, A455E, A309D, G551S, IVS8 (5T), L206W, L997F, D1152H, that result in sweat Cl^- levels <60 mmol/l when they are associated with another *CFTR* variant, should be considered when interpreting sweat test results.

*c CFTR bioassays are not available in Switzerland at the moment of writing.

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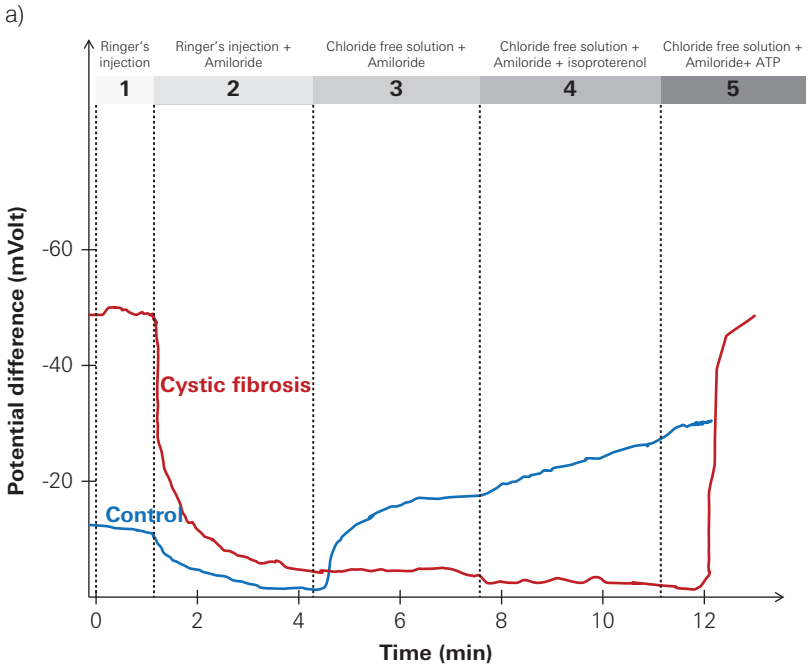
S2. Diagnosis in adults

Figure S1: **a)** Nasal potential difference measurement (NPD) in a control (blue line) and a CF patient (red line), measured during sequential application of 5 different solutions on the nasal mucosal. Note that the y axis has negative values. Compared to normal subjects, the typical pattern observed in CF consists of a more negative basal NPD, an exaggerated response to amiloride and no response to the isoproterenol solution. Solution 2 causes ENaC-inhibition, solution 3 activates CFTR, Ca^{+2} dependent Cl^- channels and ORCC, solution 4 increases further CFTR-mediated Cl^- transport and solution 5 activates calcium-activated chloride channels. **b)** The parameter used the most is the $\Delta PD_{0Cl/Iso}$ (i.e. change in potential difference between the mucosal perfusion with solution 3 and solution 4). Figure S1b shows an example of how $\Delta PD_{0Cl/Iso}$ is measured on an NPD curve. Although there is no wide consensus regarding the cut-off points, <-12 mV is considered normal, >-7.7 mV abnormal and values between -12 and -7.7mV intermediate.



b)

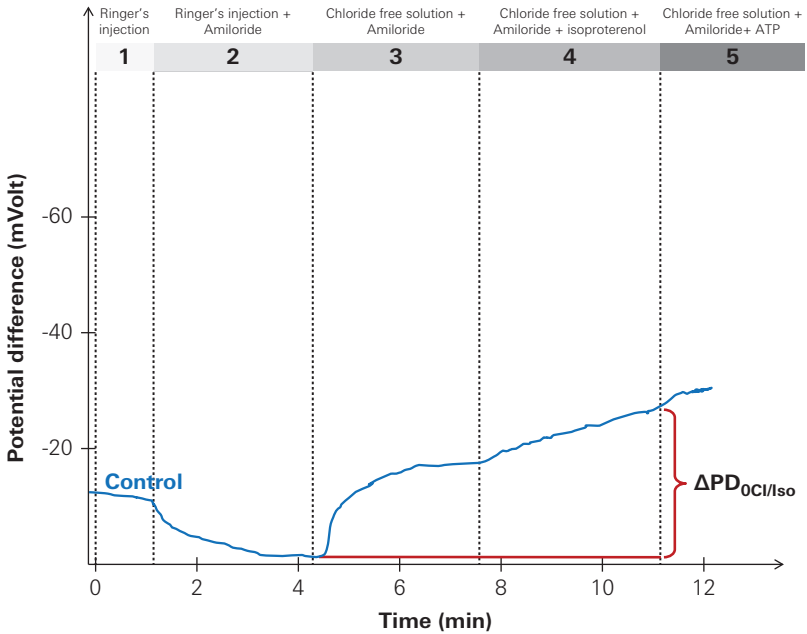
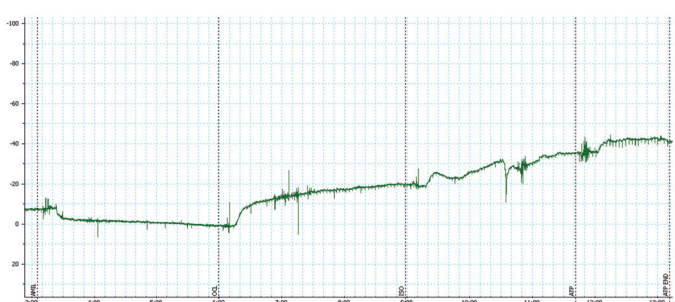


Figure S2: Example of NPD measurement in a healthy control a) right nostril, b) left nostril.
(Courtesy of Julie Avolio RN and Dr Tanja Gonska, Program of Physiology and Experimental Medicine, Research Institute, the Hospital for Sick Children, Toronto, ON, Canada)

a)



b)

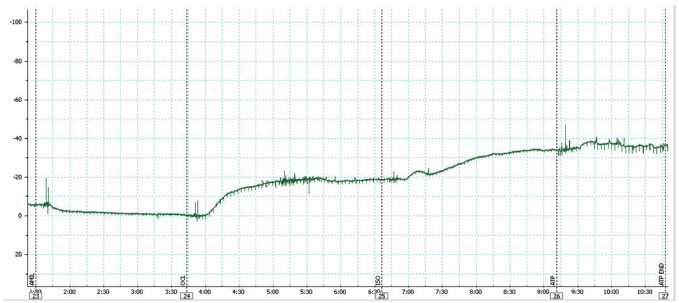
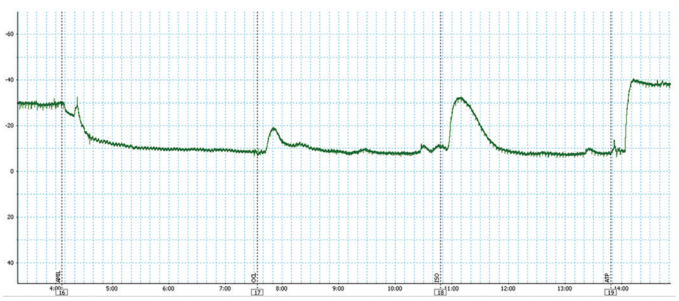


Figure S3: Example of NPD measurement in a CF patient with a G551D/G551D genotype a) right nostril, b) left nostril
(Courtesy of Julie Avolio RN and Dr Tanja Gonska, Program of Physiology and Experimental Medicine, Research Institute, the Hospital for Sick Children, Toronto, ON, Canada)

a)



b)

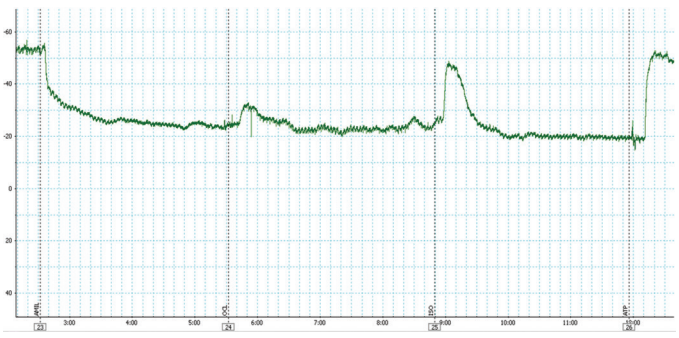
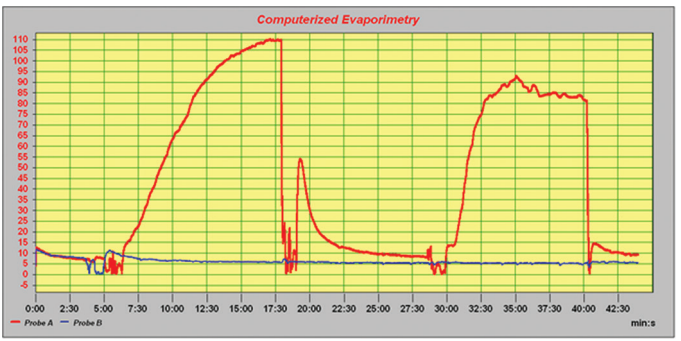
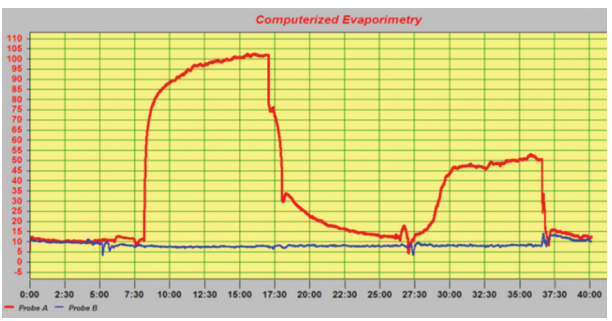


Figure S4: Example of a β-adrenergic sweat test in a) a healthy control, b) a heterozygote and c) a CF patient. The rate of CFTR-mediated sweat secretion is measured with an evaporimeter. The mean maximal β-adrenergically stimulated sweat rate is absent in CF and half the rate of healthy controls in cases of heterozygotes. *(Courtesy of Julie Avolio RN and Dr Tanja Gonska, Program of Physiology and Experimental Medicine, Research Institute, the Hospital for Sick Children, Toronto, ON, Canada)*

a)



b)



c)