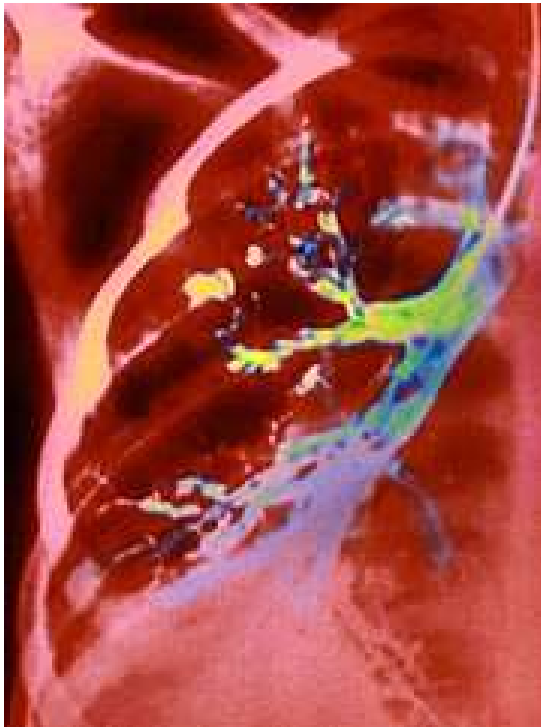




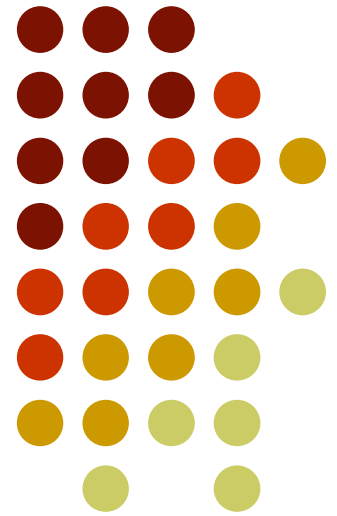
Cystic Fibrosis (Diagnosis)

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Cystic Fibrosis



- Cystic fibrosis (CF) is a multisystem disorder caused by pathogenic mutations of the CFTR gene (CF transmembrane conductance regulator)

Overview



- Epidemiology
- Definitions
- Clinical features
- Newborn screening
- Diagnosis
- Differential Diagnosis

EPIDEMIOLOGY



In the United States, CF occurs in approximately

- 1:3200 White Americans,
- 1:10,000 Hispanic Americans,
- 1:10,500 Native Americans,
- 1:15,000 Black Americans,
- and 1:30,000 Asian Americans

Cont.



- CF is increasingly recognized worldwide
- Prevalence estimates are likely to rise with:
 1. increasing recognition of disease in all populations,
 2. use of newborn screening,
 3. and increasing recognition of individuals with mild disease or disease limited to one organ system.

Asia; Iran



- The statistical analysis of the various continents regarding prevalence of CF showed that Asia has the lowest rate of patients with the disease.
- According to data from the WHO, there is no detailed information on the prevalence of CF in Asia
- The reported rate for Iran was 1 in 100,000 (2014)

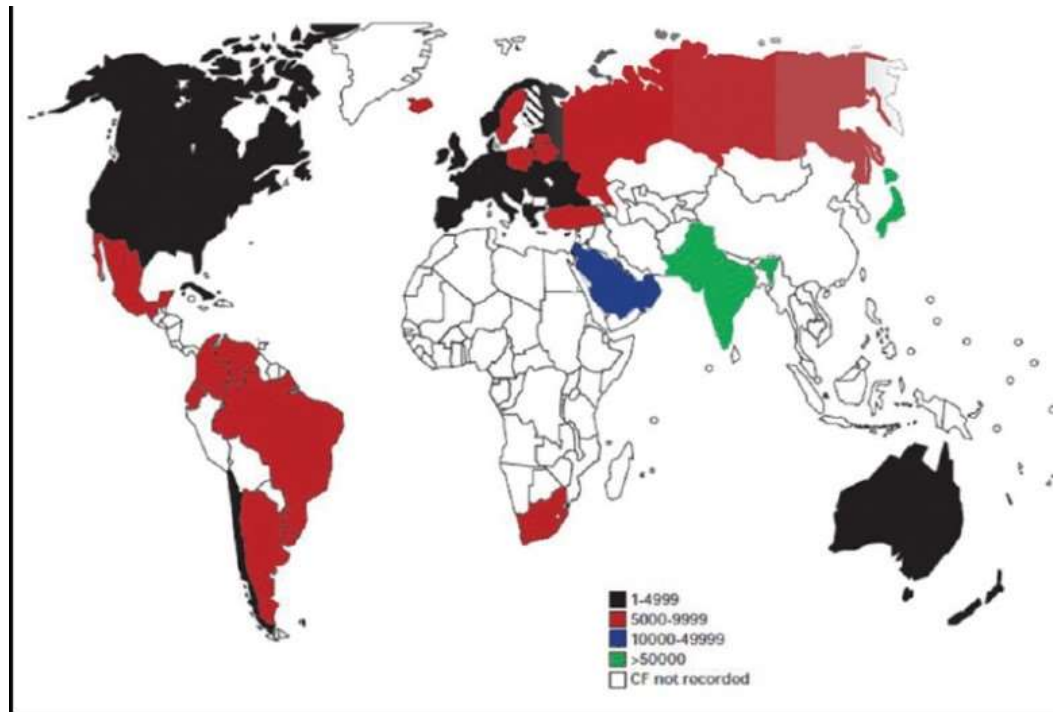


Figure 5

The incidence of cystic fibrosis on the map according to the World Health Organization

Source

Geographical Distribution of Cystic Fibrosis;
The past 70 Years of Data Analysis

Biomedical and Biotechnology Research
Journal (BBRJ)1(2):105-112, Oct-Dec 2017.

DEFINITIONS



- Cystic fibrosis — The classic or typical form of CF is diagnosed if a patient demonstrates clinical disease in one or more organ systems and has elevated sweat chloride (≥ 60 mmol/L).
- normal or intermediate sweat chloride result;
- ❑ two copies of a disease-causing mutation in the CFTR gene on each parental allele (ie, in trans) or
- ❑ abnormal nasal potential difference (NPD).
- These patients are more likely to present later in childhood or adulthood and to have unusual CFTR mutations
- In the past, these phenotypes were called "non-classic" or "atypical" CF

Cont.



- **A CFTR-related disorder**

is defined as clinical disease limited to only one organ system associated with some evidence of CFTR dysfunction that does not meet full genetic or functional criteria for a CF diagnosis.

- **Clinical manifestations may include**

isolated obstructive azoospermia,
chronic sinusitis,
chronic pancreatitis,
or pulmonary disease that presents in adulthood.

Cont.



- In such patients, testing may identify only one disease-causing mutation in CFTR and intermediate sweat chloride and NPD measurements.
- These patients should have complete gene sequencing, to confirm the absence of two disease-causing mutations.

Cont.

CRMS/CFSPID



- CFTR-related metabolic syndrome (CRMS) is a term that describes infants and children with an equivocal diagnosis following newborn screening for CF and is found in 3 to 4 percent of infants with a positive newborn screen.
- The term "CF screen positive, inconclusive diagnosis" (CFSPID) is equivalent and is used in Europe
- CRMS/CFSPID describes an asymptomatic infant with positive newborn screening results and
 - Intermediate sweat chloride results (30 to 59 mmol/L) on two separate occasions and fewer than two CF-causing mutations
- OR
- Normal sweat chloride results (≤ 29 mmol/L) on two separate occasions and two CFTR mutations, at least one of which is not clearly categorized as CF-causing

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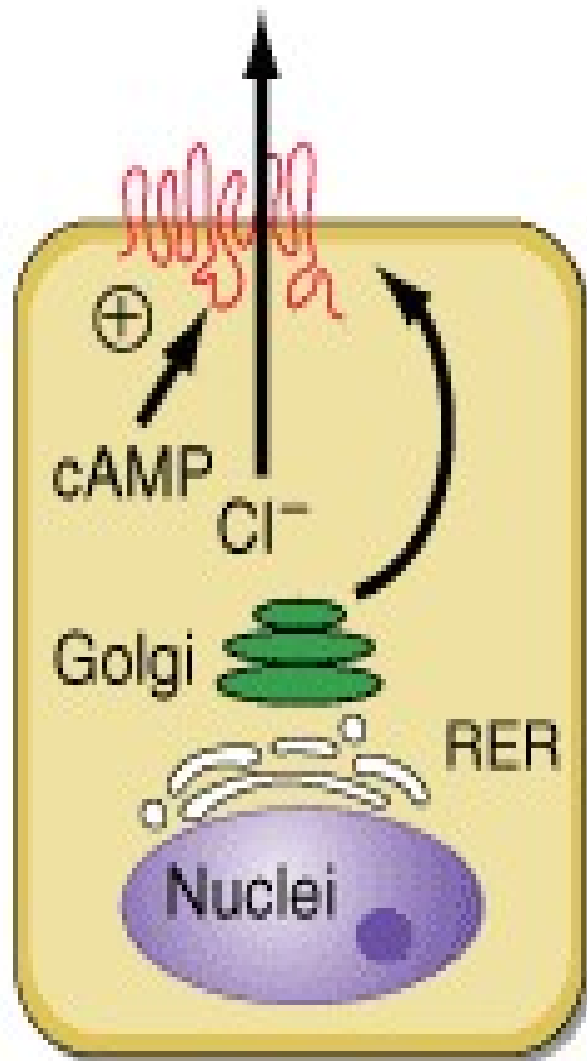
- Further evaluation and monitoring should include repeat sweat chloride testing at six months of age and annually until at least eight years of age, or until a diagnosis of CF is confirmed or excluded
- Additional monitoring should include measurement of fecal elastase on at least one occasion and tracking growth parameters.

CLINICAL FEATURES



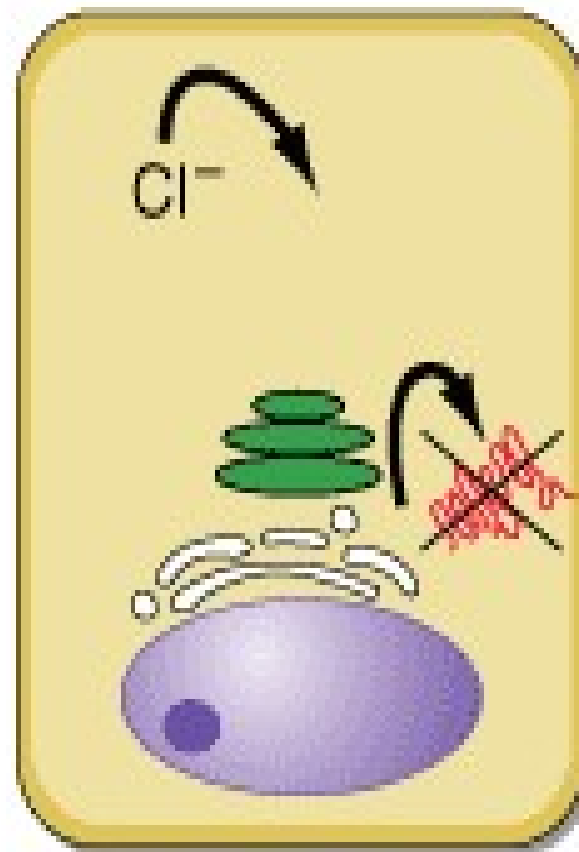
- CF is caused by mutations in the CFTR protein, a complex chloride channel and regulatory protein found in all exocrine tissues.
- Deranged transport of chloride and/or other CFTR-affected ions, such as sodium and bicarbonate, leads to thick, viscous secretions in the lungs, pancreas, liver, intestine, and reproductive tract and to increased salt content in sweat gland secretions

Functional in apical plasma membrane

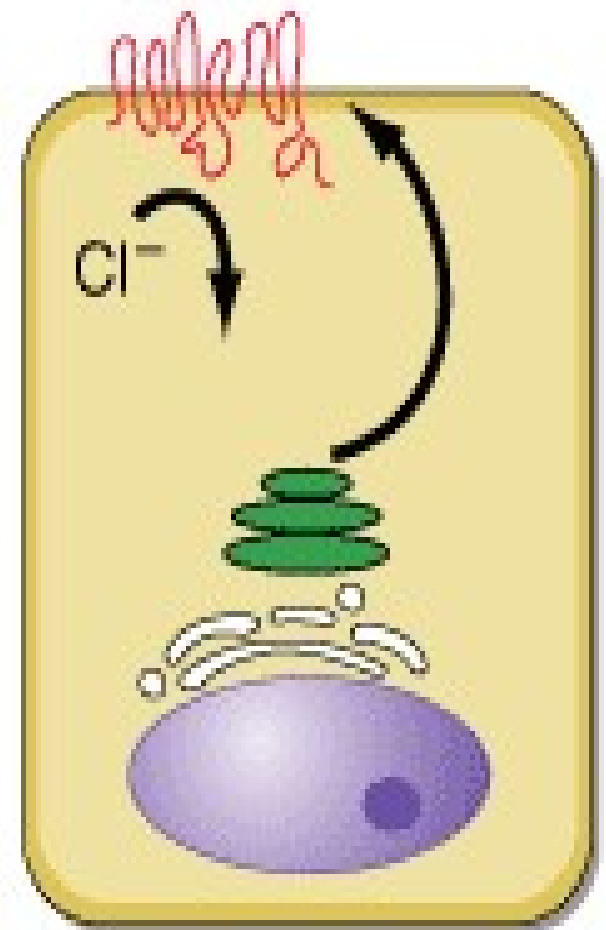


Normal cell

1. Does not reach apical plasma membrane



2. Does not function normally at apical plasma membrane



Cystic fibrosis cell

Prenatal findings



- Some cases of CF present with abnormal findings on routine prenatal ultrasonography, including hyperechogenic bowel.
- The risk of CF is highest if there is evidence of meconium peritonitis (scattered calcifications are seen throughout the fetal peritoneum), bowel dilatation, or absent gallbladder
- If these findings are present on fetal ultrasonography, we suggest offering the parents prenatal CF carrier screening.

Symptomatic presentation in infants and children



- Prior to the implementation of widespread newborn screening in the United States and many other countries, infants and children were typically diagnosed with CF after presenting with one or more of the following symptoms:
 - Meconium ileus – 20 percent of patients
 - Respiratory symptoms – 45 percent of patients
 - Failure to thrive – 28 percent of patients

Cont.



Infants with severe untreated pancreatic insufficiency occasionally present with a syndrome of:

- edema with hypoproteinemia,
- electrolyte loss,
- anemia,
- alopecia, dermatitis, and
- failure to thrive

due to malabsorption and malnutrition, including deficiencies in essential fatty acids, zinc, vitamins, and proteins.

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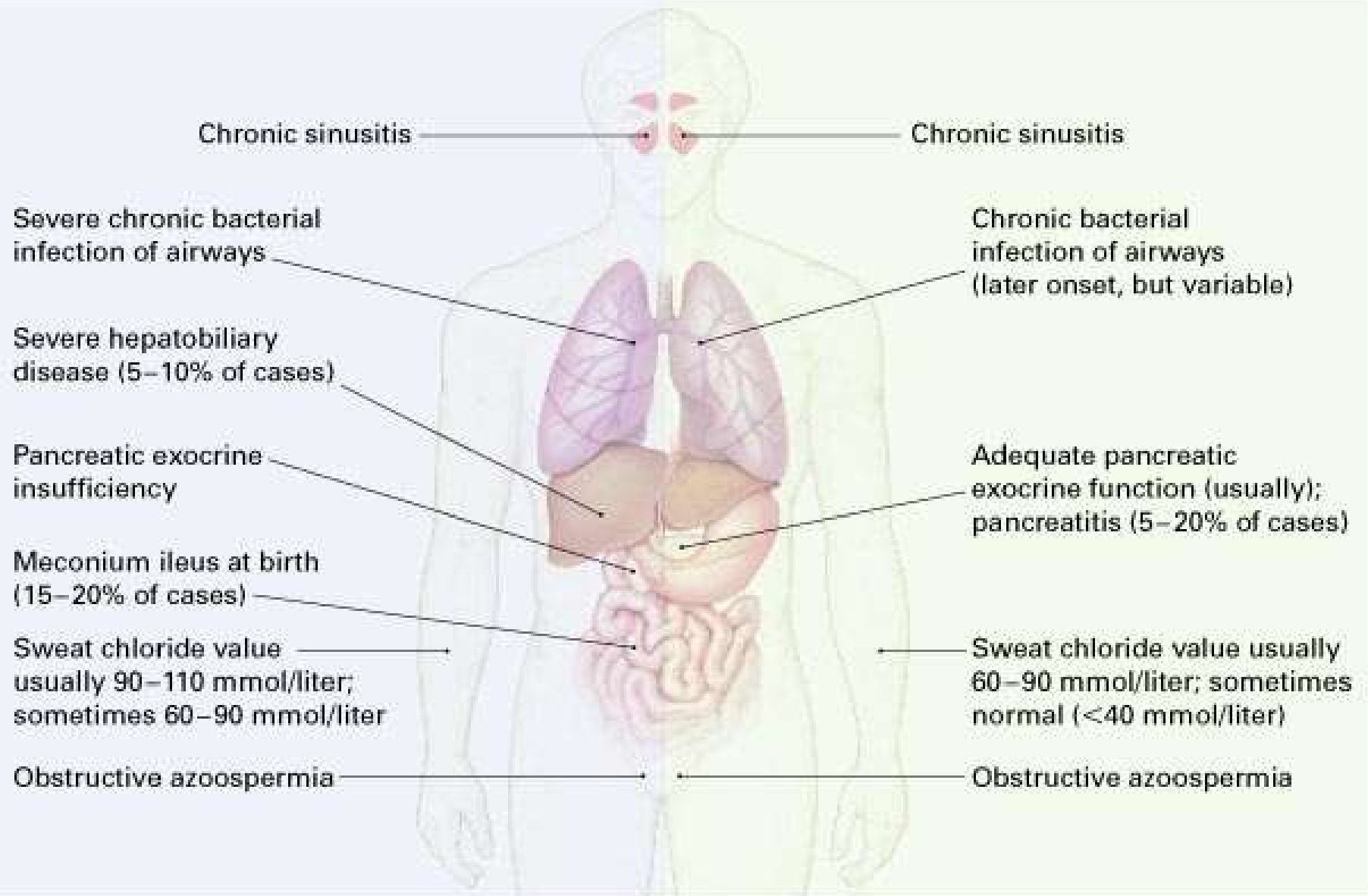


- Respiratory tract involvement
- Sinus and nasopharyngeal disease
- Pancreatic disease
- Rectal prolapse
- Hepatobiliary disease
- Infertility
- Musculoskeletal disorders
- Recurrent venous thrombosis

Classic and Nonclassic Cystic Fibrosis

Classic cystic fibrosis
(no functional CFTR protein)

Nonclassic cystic fibrosis
(some functional CFTR protein,
providing survival advantage)



Meconium ileus and distal ileal obstruction



- Meconium ileus is characterized by obstruction of the bowel by meconium in a newborn infant.
- It is the presenting problem in 10 to 20 percent of newborns with CF
- Conversely, 80 to 90 percent of infants with meconium ileus have CF
- ❖ premature infants may be more likely to have meconium ileus without CF

Aquagenic wrinkling



- After seven minutes of immersion, all of the children with CF had wrinkling and many had papules (94 percent), edema (56 percent), pruritus (14 percent), and pain (3 percent). Thus, aquagenic wrinkling may be sufficiently sensitive to serve as a screening tool for CF in low-resource settings.

Symptomatic presentation in adulthood



- Patients presenting with CF later in life are more likely to have atypical symptoms.

CF in Childhood vs. adulthood:

- Patients diagnosed in adulthood were more likely than children to present with gastrointestinal symptoms, diabetes mellitus, and infertility.
- In addition, adults presenting with CF were more likely than children to have unusual genetic mutations, normal pancreatic function, and equivocal results on sweat chloride tests.

NEWBORN SCREENING



- In the past, most patients were diagnosed with CF after presenting with symptoms.
- newborn screening programs during the past 20 years
- In 2001, fewer than 10 percent of CF cases in the United States were diagnosed on the basis of newborn screening programs.
- By 2021, 64.4 percent of total new CF diagnoses and 93.8 percent of diagnoses among infants under six months old were detected by newborn screening.
- There is evidence that individuals diagnosed prior to the onset of symptoms have better lung function, neurocognitive testing scores, and nutritional outcomes later in life and less utilization of health care resources



Cont.

- Techniques: Newborn screening typically employs two serial assays; infants with abnormal results for the first assay are retested with a second assay.
- The two assays used for newborn screening are serum immunoreactive trypsinogen (IRT) and DNA analysis for mutations in the CFTR gene.

IRT assay



- IRT is a precursor to trypsin.
- In infants with CF and pancreatic dysfunction, release of pancreatic enzymes is impaired and IRT is not readily removed from the blood stream for conversion to its active form.
- Therefore, most infants with CF (regardless of whether they are pancreatic insufficient or pancreatic sufficient) have elevated blood levels of IRT, which can be quantified by radioimmunoassay or by an ELISA.

Cont.



- IRT levels fall rapidly during infancy.
- After eight weeks of age, a negative result is not informative, although a positive result still strongly supports a diagnosis of CF
- The test is primarily used for neonatal screening but also may be useful for small or malnourished infants, in whom the sweat chloride test cannot be successfully performed.

DNA assay



- DNA analysis as a secondary screen to confirm the diagnosis in patients with abnormal initial IRT assays (IRT/DNA protocol) or it may be used as a primary method of screening.
- The DNA assays use panels to test for the most common CFTR mutations in the local population (23 to full gene sequencing).
- Infants with one or more mutations are referred for **sweat testing**, which further helps to identify children with true CF. The remainder may be diagnosed as carriers or given the provisional diagnosis of CFTR-related metabolic syndrome (CRMS).



- Infants with positive CF newborn screening results should undergo sweat chloride testing to determine whether they have CF.
- For optimal accuracy, sweat testing should be performed when the infant is at least two weeks of age and weighs >2 kg, using laboratory techniques stipulated by the Cystic Fibrosis Foundation

Diagnosis



- The diagnosis of CF is based upon compatible clinical findings with biochemical or genetic confirmation
- The **sweat chloride test is the mainstay of laboratory confirmation**, although tests for specific mutations, nasal potential difference (NPD), immunoreactive trypsinogen (IRT), stool fecal fat, or pancreatic enzyme secretion may also be useful in some cases.

Diagnostic criteria



- Both of the following criteria must be met to diagnose CF:

● Clinical symptoms consistent with CF in at least one organ system, or positive newborn screen or having a sibling with CF

AND

- Evidence of CFTR dysfunction (any of the following):

- Elevated sweat chloride ≥ 60 mmol/L
- Presence of two disease-causing mutations in the CFTR gene, one from each parental allele
- Abnormal NPD



- The accuracy of sweat chloride and NPD measurements are operator-dependent, so it is critical that testing be performed in experienced centers

Sweat chloride



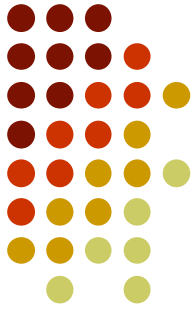
- The sweat chloride test remains the primary test for the diagnosis of CF;
- sweat testing is performed by the collection of sweat with pilocarpine iontophoresis and by chemical determination of the chloride concentration

Indications



Patients with the following characteristics should undergo sweat testing to clarify a diagnosis of CF:

- Infants with positive CF newborn screening results (perform after two weeks of age and >2 kg if asymptomatic)
- Infants with symptoms suggestive of CF (eg, meconium ileus)
- Older children and adults with symptoms suggestive of CF (eg, male infertility, chronic respiratory infections, or chronic sinusitis)
- Siblings of a patient with confirmed CF, if the diagnosis cannot be established based on genetic testing



Interpretation of sweat chloride results is as follows:

Median sweat chloride concentrations in a healthy population rise from 13 mmol/L in mid-childhood to 23 mmol/L in young adults

Interpretation of sweat chloride test results for diagnosis of cystic fibrosis

Interpretation of sweat chloride test results for diagnosis of cystic fibrosis

Sweat chloride concentration	Result	Interpretation
≤29 mmol/L	Normal	CF very unlikely
30 to 59 mmol/L	Intermediate	Possible CF, additional testing recommended*
≥60 mmol/L	Abnormal	Diagnosis of CF, if confirmed by a second test and if clinical symptoms consistent with CF are present†

Asymptomatic infants with equivocal results of the diagnostic process (sweat chloride test and DNA analysis) are given a provisional diagnosis of CRMS, also known as CFSPID.⁽¹⁾

CF: cystic fibrosis; CRMS: cystic fibrosis-related metabolic syndrome; CFSPID: cystic fibrosis screen positive, inconclusive diagnosis.

* Additional testing usually consists of molecular diagnosis (DNA analysis) and a second sweat test.

† Clinical symptoms of CF are not required among newborns identified through a screening program, or among siblings of patients with CF who are diagnosed by shared genotype.

Normal (Cont.)



- Normal – Sweat chloride ≤ 29 mmol/L is normal
- normal sweat chloride concentration is observed in approximately 1 percent of patients with CF, who have unusual genotypes such as the 3849 + 10kb C-T or poly-T defects
- Occasionally, infants with two disease-causing CFTR gene mutations on the newborn screen have normal sweat chloride results.
 1. The first step is to repeat the genetic testing by CFTR sequencing to ensure accuracy of the screening result.
 2. The next step is to perform DNA analysis of both parents. If both mutations were inherited from the same parent, the infant does not have CF.

Intermediate (Cont.)



- Sweat chloride 30 to 59 mmol/L is intermediate.
- This result suggests possible CF and calls for further evaluation by repeating sweat chloride testing and also CFTR sequencing
- For asymptomatic infants with intermediate results, the sweat chloride test should be repeated at one to two months of age and then at 6- to 12-month intervals until the diagnosis is clear.

Abnormal (Cont.)



- Sweat chloride ≥ 60 mmol/L is abnormal.
- If confirmed on a second occasion, this is sufficient to confirm the diagnosis of CF in patients with clinical symptoms of CF.
- Clinical symptoms are not required for infants who were identified by newborn screening.
- Very rarely, apparently healthy individuals have sweat chloride values ≥ 60 mmol/L .
- Therefore, positive results of sweat testing should be further evaluated by CFTR sequencing and repeat sweat chloride testing

Technique



- Sweat testing is performed by the collection of sweat with pilocarpine iontophoresis and by chemical determination of the chloride concentration
- Collection of insufficient quantities of sweat is a common problem, particularly in young infants; at least 75 mg of sweat (or 15 microliters using a Macroduct system) must be collected within a 30-minute period for the test to be valid.
- Hypoproteinemia edema and concurrent administration of steroids can decrease the sweat chloride concentration (false-negative test result) , while topiramate can increase sweat chloride (false-positive test result)

Molecular diagnosis



- If two CF-causing mutations are detected and the sweat test is intermediate or positive, the diagnosis of CF is confirmed.
- If two CF-causing mutations are not identified, the sweat test should be repeated.
- If CF cannot be definitively diagnosed or excluded, the infant is given the provisional diagnosis of CRMS, and further genetic evaluation should be undertaken.

Cont.



- Genetic screening panels – Screening of newborn infants is typically performed with panels of CFTR gene mutations.

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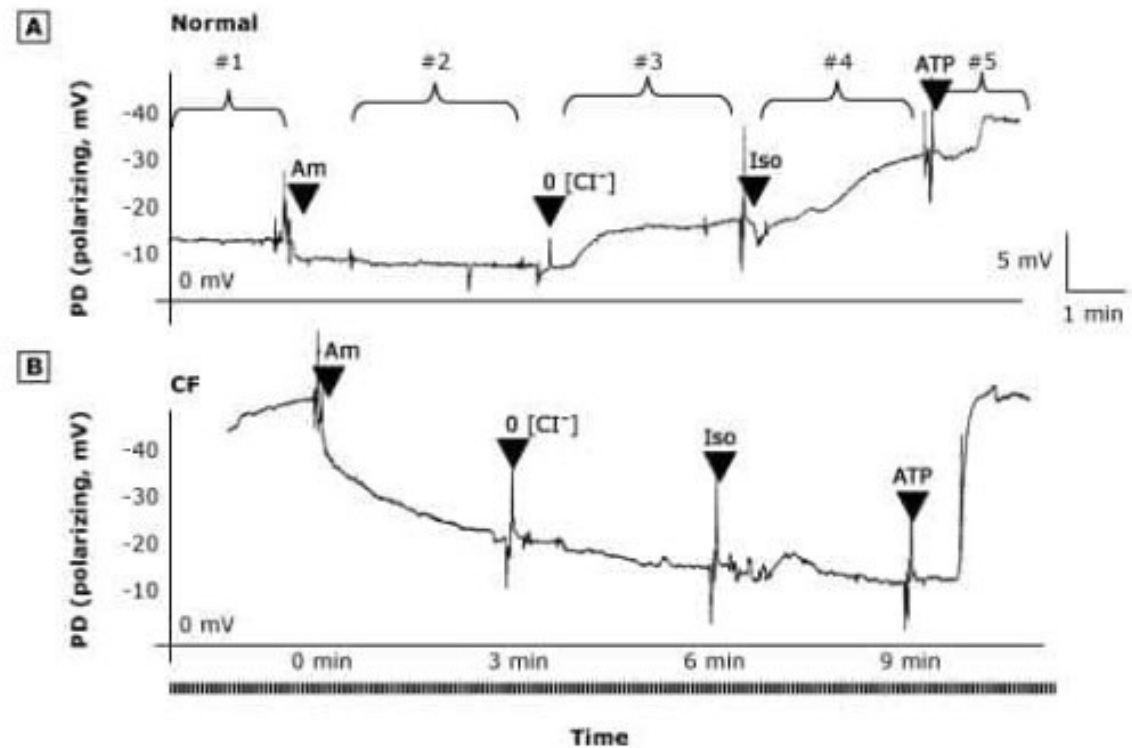
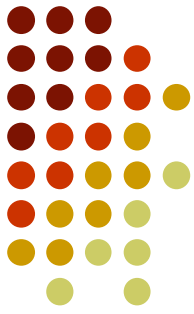
- CFTR sequencing should be performed in individuals with any uncertainty in the diagnosis, including:
 - Patients with intermediate sweat chloride results (in addition to repeat sweat chloride testing)
 - Patients with confirmed or suspected CF, if the genotype is not yet known. In these patients, the gene sequencing confirms the diagnosis and knowledge of the specific CFTR mutation also has important implications for treatment and prognosis
 - Patients with normal sweat chloride results if there is a strong clinical suspicion of CF.

Nasal potential difference measurements



- For patients with inconclusive results of sweat chloride and DNA testing, measurement of NPD can be used to further evaluate for CFTR dysfunction
- In patients with clinical symptoms, clearly abnormal NPD is sufficient to diagnose CF.
- NPD measurements are performed by placing electrodes in the nasal cavity and measuring voltage in the basal state, after nasal perfusion with amiloride to block sodium transport (the major component of NPD) and after nasal perfusion with a chloride-free solution containing a cAMP agonist, such as isoproterenol, to stimulate CFTR-dependent chloride transport.

- Patients with CFTR dysfunction have a high potential difference in the basal state, a greater decline than controls following amiloride, and minimal response to low chloride-isoproterenol perfusion



- The presence of nasal polyps or inflammation may result in false-negative results.
- The degree of abnormality in NPD is not correlated with the severity of CF lung disease

Pancreatic exocrine function



- This may be evaluated indirectly by measurement of **fecal elastase**, which is clinically practical but has limited accuracy.
- Low levels of fecal elastase suggest pancreatic insufficiency and support a diagnosis of CF.
- Normal levels of fecal elastase do not exclude the diagnosis, because pancreatic function is sufficient in a substantial fraction of individuals with CF.

Pancreatic exocrine function also can be measured directly by:

1. collecting duodenal fluid after stimulation with secretin and cholecystokinin.
2. calculating the percentage of ingested fat in a 72-hour stool collection

DIFFERENTIAL DIAGNOSIS



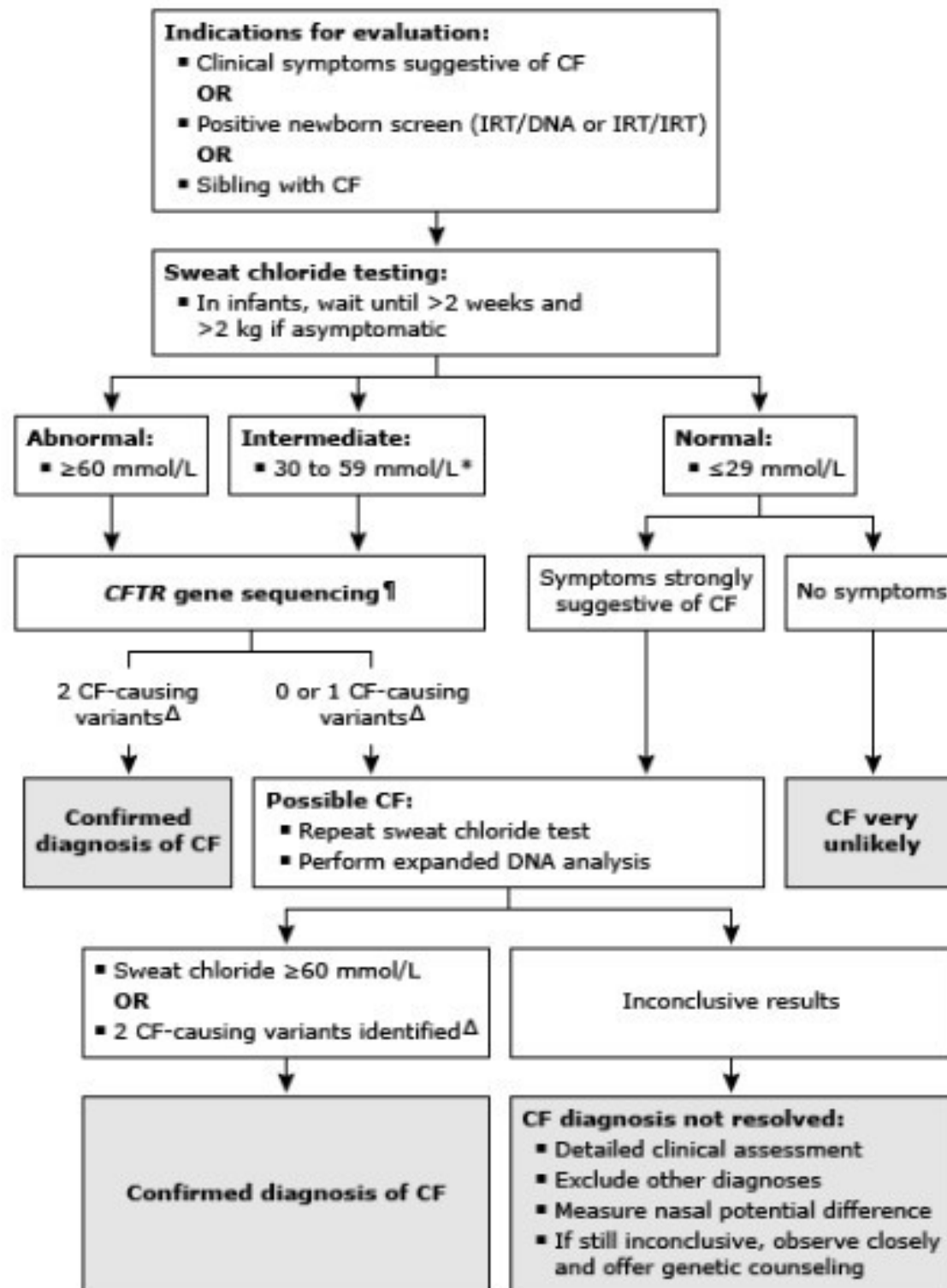
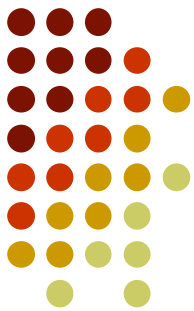
Symptoms of the following disorders may mimic symptoms of CF:

- Primary immunologic abnormalities, (including SCID and CVID)
- Primary ciliary dyskinesia
- Schwachman-Diamond syndrome
- Alpha-1 antitrypsin deficiency
- Plasminogen deficiency

summary



- Clinicians should consider CF in individuals with suggestive symptoms, even when results of the newborn screen are negative or equivocal.
- Evidence of CFTR dysfunction can be provided by sweat chloride testing, molecular testing for CFTR gene mutations, or measurements of NPD.
- In most cases, sweat chloride testing is the first and most important test.
- DNA testing is used for confirmation or for further investigation of patients with intermediate sweat chloride results and for prognostic and epidemiologic purposes in individuals with positive results of sweat chloride testing.



Any Question?



Cystic Fibrosis Care 

