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Diagnostic Modalities of Cystic Fibrosis among Children

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Abstract: ***Background:*** Cystic fibrosis (CF) remains one of the most prevalent, life-shortening genetic diseases in the Caucasian population. CF has remained under-diagnosed in many developing countries because its clinical characteristics are similar to respiratory and gastrointestinal issues associated with malnourishment, failure to thrive and high infant mortality. The CF phenotype is characterized by complex, multi organ involvement, analyzed in context of its various clinical components such as, pulmonary disease, pancreatic exocrine dysfunction or sweat chloride abnormality among others. Clinical consequences of the CFTR defect are site-specific and range from severe (lungs, pancreas, male reproductive tract) to mild (intestine) to asymptomatic (sweat glands). CF is diagnosed when an individual has both: at least one clinical presentation of the disease, and evidence of CFTR dysfunction. Evidence of CFTR dysfunction must be demonstrated either by: An elevated chloride concentration in sweat (≥ 60 mmol/L) in at least two independent measurements, and/or The demonstration of at least two cystic-fibrosis-causing CFTR mutations intrans, i.e., one mutation on each of the two chromosomes, and/or The demonstration of a characteristic type of CFTR dysfunction with nasal potential difference measurement (NPD) or intestinal short-circuit current measurement (ICM).

Keywords: *Cystic Fibrosis, Children*

Introduction

According to the Cystic Fibrosis Foundation (CFF), there are approximately 30,000 people living with CF in the United States and upwards of 70,000 worldwide, yet the exact prevalence of CF is difficult to determine globally due to variable quality of disease registries and medical reports ***(1).***

CF is most common in European and European-derived populations. With the lowest incidence among Hispanics (1:8,400 births), African Americans (1:15,000 births), and the Asian population of Hawaii (1:89,000 births). CF has remained under-diagnosed in many developing countries because its clinical characteristics are similar to respiratory and gastrointestinal issues associated with malnourishment, failure to thrive and high infant mortality. Without a high level of suspicion and the belief that CF is not present in the population, CF can go undetected. The increasing awareness of CF, as well as the availability of diagnostic tests such as sweat testing and/or DNA testing, often leads to a higher number of people being diagnosed. For many years, the

exact incidence of CF in the Arab world was unknown and thought to be non-existent. The few studies available suggest the presence of many undetected patients and emphasize the suspicion of a higher incidence, especially due to the high incidence of consanguinity. The population of the region has a large family structure, high fertility rate, high maternal and paternal age, and high rate of marriage within the same family ***(2).***

Clinical picture

The CF phenotype is characterized by complex, multi organ involvement, analyzed in context of its various clinical components such as, pulmonary disease, pancreatic exocrine dysfunction or sweat chloride abnormality among others. Clinical consequences of the CFTR defect are site-specific and range from severe (lungs, pancreas, male reproductive tract) to mild (intestine) to asymptomatic (sweat glands). Although cystic fibrosis is a complex disorder affecting many organs, 85% of the mortality is a result of lung disease. There is a strong correlation between clinical phenotype and the degree of CFTR activity. Indeed, patients with residual function mutations generally have a milder course and less organ involvement ***(3).***

1.Respiratory tract involvement:

As the disease progresses, repeated infections associated with inflammatory cell accumulation and release of cell contents damage bronchial walls, leading to loss of bronchial cartilaginous support and muscle tone and eventual bronchiectasis. Disease progression includes acute exacerbations of cough, tachypnea, dyspnea, increased sputum production, malaise, anorexia, and weight loss. These acute events are associated with acute, transient loss of lung function that improves with treatment but that often progresses to permanent loss of lung function over time ***(4).***

2.Pancreatic disease:

Approximately two-thirds of CF patients exhibit insufficiency of the exocrine pancreas from birth, with an additional 20% to 25% developing this condition during the first several years of life, and most exhibiting signs of fat malabsorption by one year of age . Overall, approximately 85% of individuals with CF eventually develop clinically significant pancreatic insufficiency ***(5).***

Common symptoms and signs of pancreatic insufficiency include steatorrhea, characterized by frequent, bulky, foul-smelling stools that may be oily, as well as failure to thrive or poor weight gain resulting from malabsorption of fat and protein. Infants with severe untreated pancreatic insufficiency occasionally present with edema, hypoproteinemia, electrolyte loss, and anemia due to malabsorption of macro-and micronutrients. Some patients also may present with symptoms caused by deficiencies of the fat-soluble vitamins A, D, E, and K. Vitamin K deficiency can present as a coagulopathy and vitamin D deficiency as rickets ***(5).***

3. Sinus disease:

Most CF patients develop sinus disease**.**Sinus disease can present with chronic nasal congestion, headaches, cough caused by chronic postnasal drip, and sleep disturbances. Sinus infections can trigger lower respiratory exacerbations in some patients, although organisms found in sinuses do not always match those recovered from lungs. Meanwhile, some individuals with isolated chronic rhinosinusitis have signs and symptoms suggestive of CFTR dysfunction that do not satisfy CF diagnostic criteria, prompting clinicians to refer to this affliction as CFTR-related disorder. Notably, in one case-control study, the single CFTR mutation rate for a group of chronic rhinosinusitis cases was significantly higher than the corresponding rate for the general population (7% versus 2%) ***(6).***

4. Digestive system diseases:

10% to 20% of newborns with CF present with meconium ileus, particularly those with the ΔF508/ΔF508 genotype, which is a risk factor for poor CF prognosis and sometimes associated with prenatal instestinal perforation and peritonitis**.** Rectal prolapse, which previously was rarely detected in children with CF, has been detected frequently in recent years and appears to be associated with constipation and/or malnutrition. Straining with constipation forces the anterior wall of the upper rectum into the anal canal, and with time, the rectal attachments to the sacrum become loose so that a circumferential prolapse occurs ***(7).***

Episodes of bowel obstruction due to the thickened mucus, intussusception and intestinal volvulus are not rare even later. There is also a possibility of mucous impaction of the appendix with subsequent appendicitis and periappendicular abscess ***(5).***

5. Nutrition and growth disorders:

Children with CF are at increased risk of malnutrition due to impaired absorption of nutrients due to pancreatic insufficiency, increased basal energy requirement, and recurrent pulmonary exacerbations, affecting growth. Other co-morbidities like gastro-esophageal reflux disease, CF-related liver disease, and CF-related diabetes can also contribute to inadequate nutrition. Nutritional status is assessed with common anthropometric parameters including weight, height, and body mass index (BMI), and calculating Z score from local growth standards. Because of the predisposition to pulmonary infection, a relationship has been found with increased breathing, reduced appetite, and increased calories at the expense of inflammatory catabolism ***(8)***.

In developed countries, patients with CF have good age-appropriate nutrition with good nutritional intake, pancreatic enzyme replacement therapy (PERT) and high fat diet, but resource-poor countries persist to have significant undernutrition. Nutritional status has been associated with respiratory health of the patients and nutritional interventions like high fat/ high calorie diet have been shown to be temporally related with improved nutrition and lung functions in studies from United States and Canada ***(9).***

6.acid base and electrolyte imbalance:

Most children with CF presenting as hypoelectrolytaemia and metabolic alkalosis (so-called pseudo-Bartter) were under the age of 6 months.

Defective *CFTR* results in excessive loss of NaCl in the sweat. This results in salt depletion in these infants and the compensatory activation of renin-angiotensin-aldosterone system(RAAS). Compensatory Na^+ reabsorption by aldosterone results in secretion of K^+ and H^+ in the renal collecting ducts, resulting in hypokalemia and metabolic alkalosis. This constellation of abnormalities- hyponatremia, hypochloremia, hypokalemia, and metabolic alkalosis are referred to as Pseudo-Bartter syndrome (PBS).

That occurred mostly in <2.5years old age group and may be subacute or chronic. That may be recurrent even before the clinical diagnosis of CF is considered. Circulating renin and aldosterone levels are almost always elevated. The features of PBS were associated with dehydration, excessive sweating, fever, chest infections, vomiting and failure to thrive ***(10).***

7.Hepatobiliary disease:

With the increasing life span, however, hepatobiliary dysfunction is becoming increasingly prevalent. Focal biliary cirrhosis caused by inspissated bile is present in many patients and may cause elevated serum alkaline phosphatase and lobular hepatomegaly. A minority of CF patients develops periportal fibrosis; cirrhosis, symptomatic portal hypertension, and variceal bleeding that are associated with progressive liver disease ***(11).***

8.Reproductive system diseases:

More than 95% of men with CF are infertile because of defects in sperm transport, although spermatogenesis is not affected. Interestingly, nearly one-half of all men with congenital bilateral absence of the vas deferens (CBAVD) and normal lung function possess two *CFTR* mutations. Clinical diagnosis is based on impalpable vas deferens and ultrasonographic examination of the urogenital tract confirms the absence or atresia of the vas deferens ***(12).*** Semen analysis shows constant azoospermia. Isolated CBAVD represents the most typical form of CFTR-RD.

Females with CF are less fertile than normal healthy women, due to malnutrition and the production of abnormally tenacious cervical mucus. However, females with CF may become pregnant and should be counselled accordingly about contraception and childbearing decision ***(13).***

- CF is diagnosed when an individual has both: at least one clinical presentation of the disease, and evidence of CFTR dysfunction ***(14).***

1) Clinical Presentation of CF includes:

- A positive neonatal screening test.
- A sibling carrying the diagnosis of cystic fibrosis.
- At least one clinical sign of cystic fibrosis.

2) Evidence of CFTR dysfunction must be demonstrated either by:
   - An elevated chloride concentration in sweat (≥ 60 mmol/L) in at least two independent measurements, **and/or**
   - The demonstration of at least two cystic-fibrosis-causing CFTR mutations intrans, i.e., one mutation on each of the two chromosomes, **and/or**
   - The demonstration of a characteristic type of CFTR dysfunction with nasal potential difference measurement (NPD) or intestinal short-circuit current measurement (ICM).

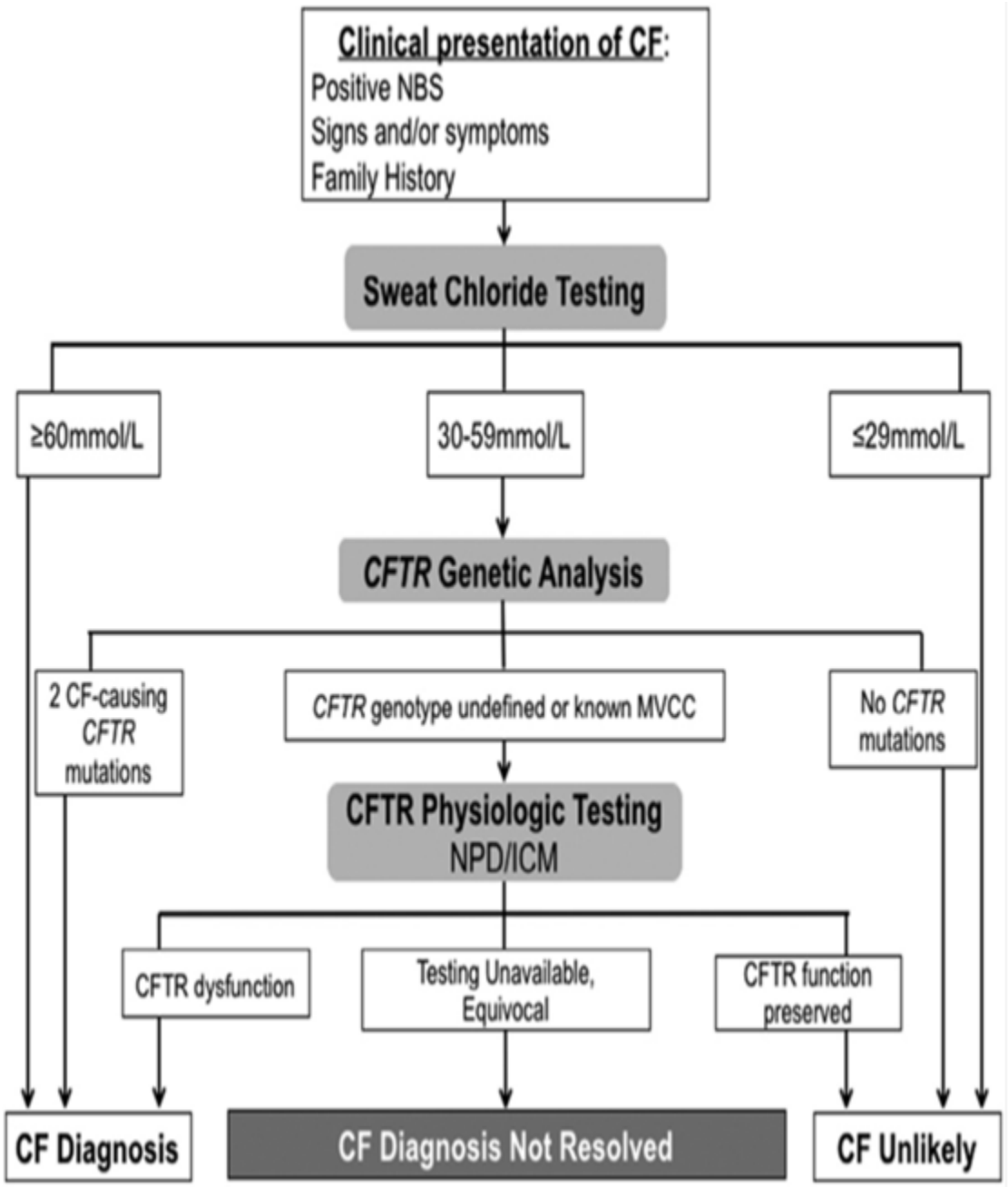

Fig .1: Alghorithm for CF diagnosis (15).

A. **Sweat chloride test (SCT):**

Even with recent advances in molecular diagnostics, the quantitative pilocarpine iontophoresis sweat chloride test remains the gold standard confirmatory test in diagnosing CF which can accurately diagnose more than 98% of cases of CF. Sweat conductivity can be used as a screening test**.** Chloride measurement in sweat is 96.5% sensitive and 99% specific and is the method of first choice. The informativeness of this test depends crucially on quality control in its performance and assessment, which is a requirement for certified cystic fibrosis center It can be carried out from the third day of life onward, and optimally from the 14th day of life onward, in children with a body weight of at least 3000 g and a gestational age of at least 36 weeks. The performance of two separate tests on a single day increases the rate of evaluable test results, especially in neonates ***(17).***

The standard laboratory assays for CF consist in the measurement of chloride in sweat**,** which is often collected by the absorption method of Gibson and Cooke (proposed in 1959), based on the use of pilocarpine iontophoresis for sweat stimulation, that is generally regarded as the reference method for sweat testing. It is recommended to perform ST in an accredited care center by a trained technician**.** The ST is typically performed on the patient's arm or leg. The test starts with iontophoresis of pilocarpine, a parasympathomimetic alkaloid, which acts on the cholinergic receptors by mimicking acetylcholine, to stimulate sweat production by sweat glands. Collection of two simultaneous samples is recommended because of the variability of the test and insufficient sample risk ***(18).***

In the original Gibson and Cooke method, iontophoresis is done by placing two electrodes on the patient's arm or leg and covering one of them with pilocarpine-soaked gauze and the other with deionized water–soaked gauze. An electric current of maximum 1.5 mA is then applied for 5 min to stimulate sweat production. The electrical stimulation is painless and causes no discomfort. Sweat is collected for a period of up to 30 min. For the gauze or filter paper method, the stimulated area must be 2 × 2 inches. The filter paper is then placed in a laboratory dish of known weight so that the quantity of the collected sweat can be calculated. The minimum quantity required for sweat collected from the gauze method is 75 mg ***(18).***

However, conventional procedures, such as those using gauze and filter paper, carry a significant risk of evaporation unless performed by trained and experienced staff. Errors made during sweat stimulation and collection and analysis can cause skin burns and also volumetric, gravimetric, condensate, and evaporation inaccuracies. This is especially significant in young, particularly preterm, infants ***(19).***

Nanoduct® is a new diagnostic system that induces, collects and analyzes sweat in one step while the required electrodes and sensors are attached to the patient**.** Compared to sweat volumes between 75 to 100 µl required by other systems, this system only needs 3 µl of sweat and test results are available within half an hour ***(20).***

If the concentration of chlorine is greater than 60 mmol/L, the diagnosis of CF is confirmed, while a high concentration of 40-60 mmol/L is suspicious, and a concentration <40 mmol/L is normal (excluding adrenal insufficiency). However, new clinical guidelines indicate that a sweat chloride concentration <30mmol/L is the normal threshold for all age groups (excluding adrenal insufficiency). The 2017 CFF Consensus Guidelines for interpretation of sweat chloride concentration are as follows; ≤ 29 mmol/L: normal, CF unlikely (exceptions occur) 30-59 mmol/L: intermediate, possible CF, ≥ 60 mmol/L: abnormal, indicative for diagnosis of CF. Sweat chloride values decrease in the first few weeks of life, which requires that age-specific interpretation ranges be used ***(21).***

Severe CFTR mutations have been associated with higher sweat chloride concentrations; however, there is wide variation in sweat chloride concentrations within genotypes, and sweat chloride concentrations < 60 mmol/L have even been reported in a patient homozygous for the typically severe F508del CFTR mutation ***(23).***

Importantly, a normal sweat chloride concentration is observed in approximately 1 percent of CF patients with unusual genotypes, such as the c.3717+12191C >T (legacy name: 3849 + 10 kb C-T) or poly-T defects ***(21).***

The most common reason for a false positive ST is technical error during the procedure, such as evaporation of the sweat sample. The incidence of this problem is reduced by correct implementation and adherence to recommended testing procedures and by ensuring that the test is performed in adequately equipped laboratories and by properly trained personnel ***(18).***

Sweat Cl^- levels may also be elevated falsely in other pathologic conditions, including atopic dermatitis, ectodermal dysplasia, pseudohypoaldosteronism, untreated hypothyroidism, glycogen storage disease type I, carbonic anhydrase XII mutations, malnutrition, and anorexia nervosa. Elevated sweat Cl^- concentrations in non-CF patients may also be related to iatrogenic causes, such as mineralocorticoid, $NaCl^-$ perfusion, and topiramate treatment ***(24).***

The underlying mechanism for false positive results in many conditions is unknown. The possible sweat gland function impairment associated with the skin manifestations may be the reason for high levels of sweat Cl^- in patients with atopic dermatitis and ectodermal dysplasia. Hyperchlorhidrosis caused by autosomal recessive inherited Carbonic Anhydrase XII deficiency should be considered in the differential diagnosis of a positive ST, especially with the clinical findings of hyponatremic dehydration during infancy. High sweat Cl^- levels during treatment with topiramate may be the result of the inhibition of carbonic anhydrase isotypes in the sweat gland ducts. There are several potential causes for a falsely low sweat chloride result: Technical problems as failure to dry skin prior to sweat collection and errors in weighing, dilution, elution, or computation, Physiologic as inadequate volume secondary to low sweat rate and edema ***(25).***

In addition, several mutations in CFTR have been associated with either borderline or normal sweat chloride concentrations: 384910 kb C>T, R117H, G551S, A455E, D1152H, IVS8 (5T), L206W,27895 G>A**.** Sweat chloride concentration dosage has also been useful to demonstrate the function of the CFTR protein after the administration of correctors, potentiators, or stabilizers drugs by personalized/precision medicine Therefore, the future role of ST should include the successful monitoring of personalized medicine therapy. ***(25).***

- ***Difficulties of sweat testing***

Insufficient yield of sweat volume is called 'quantity not sufficient' (QNS), and may prolong CF diagnosis and initiation of appropriate therapy. The CFF recommends QNS rates ≤ 10% for infants <3 months of age. As younger infants are now commonly referred for diagnostic testing following identification through abnormal CF Newborn Screening results rather than onset of CF symptoms, this can be a difficult goal to meet ***(25)*** and provides the rationale for CF centers and institutions to assess5their screening and diagnostic programs.

Individuals presenting with a positive newborn screen, symptoms of CF, or a positive family history, and sweat chloride values in the intermediate range on 2 separate occasions may have CF. They should be considered for extended CFTR gene analysis and/ or CFTR functional analysis ***(17).***

There is a small risk of urticaria or burn associated with sweat-testing, as some patients react to pilocarpine or electrical stimulation. Other causes for irritation include an iontophoresis current greater than 4 mA, the skin coming into contact with the bare metal of the electrode, the reagent interface not being moist enough, or a damaged electrode surface. As well, some patients may simply be more sensitive to the electrical current than others. However, with properly trained technicians, these risks rarely manifest ***(25).***

B. <u>Molecular Diagnostic Testing:</u>

The purpose of molecular diagnostic testing is to provide genetic characterization of individuals with clinical or suspected CF. Reasons for doing such diagnostic testing include prenatal diagnosis in a carrier couple, newborn screening follow-up, clinical symptoms consistent with CF phenotypes, and a family history of a relative with CF or with a CF-like condition. The benefits of testing include earlier and definitive diagnosis, improved CF-specific care, clarification of atypical cases, and attainment of the information required for providing counseling regarding recurrence risk and fertility options ***(26).***

Individuals who meet sweat chloride criteria for CF diagnosis still benefit from CFTR genetic testing because of the availability of CFTR-modulating therapies that are specific to certain mutations. Patients with intermediate sweat test results should undergo CFTR genotyping for confirmation of diagnosis. Therefore CFTR genotyping has become an equally important part of CF diagnosis ***(15).***

DNA analysis represents a modern method in the diagnostics of CF. However, most laboratories can detect only most frequent mutations, so that genetic verification of rare variants of the disease are likely to go unnoticed ***(27).***

C. Newborn screening (NBS):

Newborn screening was initially used for detection of rare inborn errors of metabolism, but has expanded to commonly include endocrine, hematologic, and hearing disorders. Discussions surrounding the addition of CF to newborn screening date back to the 1970s, but it was not until the new millennium that the consensus was reached that CFNBS was warranted based on moderate benefit and low risk of harm ***(26).***

CF NBS leads to early diagnosis, which improves clinical outcomes. By 2010, all US states had approved CF NBS**.** Most patients (59% in 2011, as opposed to 9.4% in 2001) are now identified by CF NBS for immunoreactive trypsinogen (IRT), a protein marker of pancreatic inflammation/disease, with clinical and molecular follow-up when elevated. CF NBS samples are collected via heel stick puncture, typically within 48 hours of birth. Laboratory tests for CF NBS include IRT enzyme testing, pancreatitis-associated protein (PAP) testing, DNA mutation analysis, and sweat chloride testing**.** Infants with CF have elevated IRT levels, hypothesized to be because of pancreatic duct dysfunction in both pancreatic-sufficient and pancreatic-insufficient infants. False negatives are occasionally seen with meconium ileus, and therefore IRT is not used as a diagnostic test. False positives are more commonly seen, particularly in cases of prematurity, perinatal stress, low Apgar scores, and African ethnic origin ***(28).***

A wide variety of physiological or medical conditions have been associated with hypertrypsinaemia in the neonatal period. Increased IRT has been noted in trisomies 13 and 18. Perinatal stress has also been reported to be a significant factor in hypertrypsinaemia, Elevated IRT levels have been found in association with congenital infections, renal failure, and bowel atresia and in a case of nephrogenic diabetes insipidus ***(16).***

The population distribution of blood IRT concentrations in the newborn period is slightly higher in babies of North African parentage and in African Americans than in babies of North European origin**.** Pancreatitis-associated protein is a non-specific stress protein elevated in the blood of newborns with CF, and the combination of IRT with PAP is being examined in several populations in Europe ***(28).***

The single IRT and IRT + IRT repeat testing algorithms are reported to have a sensitivity of 85% to 90%, with many false positives (less specificity) ***(21).***

The increased sensitivity is potentially associated with more referrals of individuals who have an initial elevated IRT and happen to carry a single CFTR mutation but do not have CF. Last, another CFNBS algorithm is based on IRT + PAP screening, which has been evaluated in conjunction with IRT + DNA testing in several European countries. This approach provides easy testing of both samples simultaneously, may save cost, and is less likely to identify unaffected carriers, but conversely also misses milder cases**.** Which ever algorithm is followed, infants with a positive screening result are referred for sweat chloride testing, further clinical evaluation, and additional molecular testing if clinically indicated. Despite mandated CF NBS in 2010 across the United States, in 2018 only 61.5% of newly diagnosed CF patients were identified through NBS ***(21).***

The European Cystic Fibrosis Society Patient Registry Report in 2018 similarly stated that 74% of CF patients aged 5 years had undergone CF NBS at birth. Taken together, these data support additional quality improvement in CF NBS to better understand the current screening practices in place identify factors contributing to delays in diagnostic testing and identify barriers to receiving specialized CF multidisciplinary, clinical, and psychosocial care.

D. **Nasal Potential Difference*(NPD)* and Intestinal Current Measurement*(ICM):***

Measurements of CFTR (and the epithelial sodium channel) activity in nasal epithelium readily distinguish the healthy young infant from one with CF. In fact, NPD, when attempted in babies with intermediate sweat chloride levels by very experienced, skilled operators, can provide reliable results ***(21).***

The assay includes the perfusion of different solutions in standardized concentrations across the nasal mucosa and monitoring the transepithelial potential difference by a probe electrode.The solutions used include Ringers, Ringers plus amiloride (to block epithelial sodium channel and sodium resorption), zero chloride solution and amiloride (with gluconate replacing chloride to produce a chloride secretory gradient), the addition of isoproterenol (to stimulate CFTR) and adenosine triphosphate (to activate CFTR-independent chloride transport and serve as a positive control of epithelial integrity). ***(29).***

However, NPD is not possible or reliable in every situation, and analysis of CFTR function in the intestine (ICM) may be considered, aided by the fact that CFTR is highly expressed in intestinal epithelia, offering high specificity and sensitivity for the test. Like NPD, ICM measurements must be conducted in specific high quality reference centers with experienced, very skilled personnel. ICM can be used to confirm a diagnosis of CF in the context of intermediate sweat chloride levels ***(29).***

Ion transport in the intestine is a very sensitive measure of CFTR function: only 10% of wild-type CFTR is necessary to prevent intestinal pathology in CF, and a very small gain in *CFTR* expression (from 1% to 5% of wildtype) results in large gains in chloride secretion (from 5% to 25% of wild-type levels) ***(29).***

Because of this sensitivity, ICM can be used to better characterize variants of unknown disease liability. Combining results from ICM and NPD, when available, can provide an even clearer picture of the spectrum of CFTR function, from CF-causing to healthy levels ***(21).***

Additional investigations:

1. ***Fecal Elastase (FE):***

The fecal elastase-1 test is highly sensitive (using a monoclonal rather than polyclonal antibody) and involves an enzyme linked immunosorbent assay to determine levels of this human pancreas-specific enzyme in a small specimen of well-formed feces; thus, it is simply a diagnostic tool of pancreatic function ***(30).***

Demonstration of low FE levels <200 mg/g (in the absence of diarrhea) has been proposed as an indicator of pancreatic insufficiency and a diagnostic marker for CF. FE values fluctuate through the first 12 months of life. ***(30).***

FE may be useful as an interim measure in those infants with pancreatic insufficiency who have "quantity not sufficient" sweat test results, permitting appropriate treatment until repeat sweat testing is successful. This strategy has been used in Switzerland ***(20).***

However, despite the early interest for this biomarker, FE is of limited value in diagnosing CF definitively, as many individuals with CF retain normal levels of FE ***(21).***

The advantages of FE determination are that the test is simple, inexpensive, results are not affected using PERT and stool specimens can be assayed as late as 5 days from the time of collection. There can be false positive results if the test is performed on a watery stool specimen; therefore, all FE testing should be performed on a formed or semi-formed stool specimen. Individuals with severe malnutrition may also have false positive FE***,*** although in the case of a patient with CF the PI is the likely cause of the malnutrition. In non-CF patients, testing should be repeated after nutritional repletion. ***(21).*** An Egyptian study concluded that fecal-elastase-1 measurement in cystic fibrosis children can diagnose the exocrine pancreatic insufficiency even without clinical manifestations of steatorrhea. FE-1 level can predict pathologic changes of pancreas detected by transabdominal ultrasound ***(22).***

2. ***coefficient of fat absorption:***

Assessment of the coefficient of fat absorption (CFA) involves 72-hour stool collection, recording of dietary fat during the stool collection period, and calculation of the percentage CFA. This test, which is very weighty for

the patient, is the most valuable tool for assessing fat maldigestion in PERTsupplemented patients with poor nutritional status or inadequately controlled gastrointestinal symptoms, or clinical trials to evaluate PERT efficacy. The gold standard for diagnosing steatorrhea is a 72h fecal fat estimation using the van de Kamer method ***(31).***

A positive test is defined as >7 g of fat over a 24 h period. This is only indicative of the presence and not the cause of fat malabsorption. It remains, however, the most common test performed for research, especially to assess the effectiveness of pancreatic enzyme replacement therapy. As this test involves consuming a high fat diet (100 g fat diet each day for adults) for 5 days with the collection of all stool output over the last 3 days, it is not commonly performed due to its inconvenient and burdensome nature. The test measures steatorrhea, which only occurs once pancreatic lipase output has fallen to 5–10% of normal*.* It does not measure other elements of pancreatic exocrine function and cannot identify mild or moderate insufficiency. It should be noted that infants have a normal coefficient of fat absorption of ≥85%, compared to adult normal valued of ≥93; thus, when this test is used in infants a different standard must be applied. Qualitative fecal fat ("spot fecal fat") is not recommended due to its lack of specificity since a high fat intake in a normal patient can lead to false positive result and diets rich in calcium can lead to increased fecal fat excretion ***(31).***

3. Trypsinogen:

Trypsinogen levels are already used to identify infants at high risk for CF and may be used to better advantage ***(31).*** Serum trypsinogen levels were serially examined over the first 36 months of life in 82 infants categorized as CFSPID and 80 infants diagnosed with CFOverall, infants with CFSPID had significantly lower NBS IRT than did infants with CF. Furthermore, nine of the 82 (11%) infants with CFSPID were subsequently diagnosed with CF, and these patients had significantly higher serial serum trypsinogen levels than did those infants who remained in the group with CFSPID. Thus, serial trypsinogen levels may contribute useful information. ***(31).***

\Oral glucose tolerance test:

According to recent guidelines released by the CFF, the American Diabetes Association, and the Pediatric Endocrine Society, the oral glucose tolerance test (OGTT) is recommended yearly in patients with CF over 10 years of age*.* Some authors recommend annual OGTT after the age of 6 years in CF patients with pancreatic insufficiency ***(32).***

4. Imaging:

a. Pulmonary:

Originally, chest X-ray was employed to depict morphological changes in the CF lung. It has often been replaced by computed tomography (CT) at specialized centers, because of its higher sensitivity for early and subtle changes in the CF lung ***(33).***

Affection of the small airways, which are usually not visualized by CXR, may lead to visibility of grouped mottled shadows. CXR has the lowest sensitivity for early changes in the CF lung, whereas CT is considered the reference standard because of its high isotropic resolution*.* However, the use of CT for short-term follow-up in infants and preschool children as well as lifelong longitudinal monitoring are accompanied by an accumulation of radiation dose*.* Most recently, magnetic resonance imaging (MRI) has emerged as a radiation-free technique for assessing the CF lung ***(34).***

Morphological changes of the CF lung

1. Airways:

Characteristic airway abnormalities in CF are mucus plugging together with inflammatory airway wall thickening and progressive bronchiectasis that usually appear in heterogeneous combinations of different severity ***(35).***

Recent CT and MRI studies in infants and young children with CF also demonstrated high variability and regional heterogeneity of early lesions throughout the lung without predilection for a specific region that, especially in early disease, cannot be captured by global measures, such as spirometry, due to functional compensation by structurally normal areas ***(34).***

Bronchiectasis is considered one of the earliest irreversible structural abnormalities detected by morphologic imaging even in asymptomatic infants identified by newborn screening, and also correlates with disease severity and exacerbation rate***(35).***

Recent CT studies reported bronchiectasis in approx. 30 % at the age of 3 months, and progression to approx. 60 % at the age of 3 years ***(35).***

Bronchiectasis may appear as superimposed line shadows and ring shadows on CXR, depending on the course of the airway in relation to the image planeMultiplanar reformats help to identify central to . peripheral bronchiectasis and the visualization of small airways is precluded by the system-inherent resolution of 200 – 300µm ***(36).***

If small airways (by convention smaller than 1mm in diameter) are affected by wall thickening, mucus plugging or bronchiectasis (usually a combination of all three), they may increase in size over the resolution threshold and become visible as centrilobular nodules, often grouped with a tree-in-bud appearance. In more advanced disease, sacculations, or cystic bronchiectasis, may be observed, which ultimately may lead to the destruction of a whole lung lobe. ***(36).***

2. <u>Parenchyma:</u>

Consolidations are typical signs of infection and are found in pulmonary exacerbations in CF. In many cases, an atelectasis with reduced volume and displacement of the pulmonary fissures occurs in exacerbation, unlike typical lobar pneumonia in otherwise healthy patients. CXR usually has the lowest sensitivity, while CT and MRI performe equally well. On MRI, consolidations stand out brightly on T2-weighted sequences. In case of a destroyed segment or lobe, bronchiectasis embedded in persistent consolidation and volume loss are evident ***(37).***

Peripheral consolidations may lead to pleural thickening and enhancement of the adjacent pleura ***(34).*** Recent work using quantitative CT has confirmed earlier histopathological descriptions that adolescent and adult CF patients develop emphysema (age range: 7 – 66 years, median age: 20.1 years)***.*** This is also supported by a mouse model showing that emphysema formation in advanced CF is pathophysiologically linked to emphysema in COPD ***(37).***

b. <u>Pancrease:</u>

Imaging in most CF patients reveals fatty replacement, calcification, and atrophy of the pancreas consistent with chronic injury and consequent insufficiency. Fat deposition results in high-intensity signal T1-weighted magnetic resonance imaging, and fibrosis appears as low intensity T1- and T2-weighted images ***(38).***

c. <u>GIT:</u>

(Littlewood, 39) characterized Fibrosing Colonopathy as:

• "Severe submucosal thickening by mature fibrous connective tissue

• Intraluminal fusiform narrowing but with little change in the external bowel diameter, mainly in distal caecum and ascending colon.

• Loss of haustral pattern sometimes with a 'cobblestone' appearance of the intestinal epithelium, although the epithelium is generally intact but with some localized defects. Altered architecture suggests there has been repair of previous damage.

• Little or no evidence of inflammation or other lesions suggesting Crohn's disease. Some slight inflammation and fat around blood vessels.

• The small bowel is not involved.

• A few patients have chylous ascites.

DIOS Ultrasound and computed tomography may be used to confirm the diagnosis. On imaging, dilated bowel loops with bubble laden intraluminal material with or without air fluid levels are key features. Intussusception: Classic imaging signs include ''coiled spring'' on barium enema and, on ultrasound, ''donut sign'' (transverse) and ''pseudokidney'' (longitudinal). These imaging findings may also be present on asymptomatic patients, but 25 % of patientswith intussusception present with bowel obstruction ***(40).***

5. Pulmonary function tests and arterial blood gas (ABG):

Pulmonary function tests and arterial blood gas analysis also can be used to quantify CF progression. Pulmonary function tests are used to determine the severity of pulmonary exacerbations as well as disease progression of disease; ABG analysis can be useful in early diagnosis as well as determining the severity of exacerbations. Patients with declining lung function may exhibit hypoxemia and respiratory acidosis on ABG analysis ***(41).***

References:

1. Jackson, A.D.& Goss, C.H. (2018):Epidemiology of CF: how registries can be used to advance our understanding of the CF population. J. Cyst. Fibros.; 17: 297–305.
2. El-Seedy, A.S., Shafiek, H., Kitzis, A., Ladevèze,V. (2017): CFTR Gene Mutations in the Egyptian Population: Current and Future Insights for Genetic Screening Strategy. Front. Genet; 8, 37.
3. De Gracia, J., Mata, F., Alvarez, A., Casals, T., Gatner, S., Vendrell, M., de la Rosa, D., Guarner, L., Hermosilla, E. (2005):Genotype-phenotype correlation for pulmonary function in cystic fibrosis.Thorax60:558–563
4. Chen, Q., Shen, Y., Zheng, J. (2021): A review of cystic fibrosis: Basic and clinical aspects. Animal Models and Experimental Medicine, 4(3), 220-232
5. Gaskin, K., Allen, J. (2008): Exocrine pancreatic disease including cystic fibrosis. In: Duggan C, Watkins JB, Walkler AW, editors. Nutrition in Pediatrics. Hamilton: BC Decker Inc;.577-588.
6. Wang, X., Moylan, B., Leopold, D.A., et al.(2000): Mutation in the gene responsible for cystic fibrosis and predisposition to chronic rhinosinusitis in the general population. JAMA.; 284(14):1814-1819.
7. El-Chammas, K. I., Rumman, N., Goh, V. L., Quintero, D., & Goday, P. S. (2015): Rectal prolapse and cystic fibrosis. Journal of pediatric gastroenterology and nutrition, 60(1):110-112.
8. Sabharwal S. (2016): Gastrointestinal manifestations of cystic fibrosis. Gastroenterol Hepatol.; 12:43-7.
9. Goss, C.H., Sykes, J., Stanojevic, S., Marshall, B., Petren, K., Ostrenga, J., et al. (2018): Comparison of nutrition and lung function outcomes in patients with cystic fibrosis living in Canada and the United States. Am J Respir Crit Care Med.; 197:768-775.
10. Scurati-Manzoni E, Fossali EF, Agostoni C, et al. (2014): Electrolyte abnormalities in cystic fibrosis: systematic review of the literature. Pediatr Nephrol;29: 1015–1023.
11. Ledder, O., Haller, W., Couper, R.T., Lewindon, P., Oliver, M., et al. (2014): Cystic fibrosis: an update for clinicians. Part 2: hepatobiliary and pancreatic manifestations. J Gastroenterol Hepatol.;29(12):1954-1962.
12. Daudin, M., Bieth, E., Bujan, L., et al. (2000): Congenital bilateral absence of the vas deferens: clinical characteristics, biological parameters, cystic fibrosis transmembrane conductance regulator gene mutations, and implications for genetic counseling. Fertil Steril;74(6):1164-1174.
13. Sueblinvong, V., Whittaker, L.A. (2007): Fertility and pregnancy: common concerns of the aging cystic fibrosis population. Clin Chest Med.;28(2):433-443
14. Naehrig, S., Chao, C. M., and Naehrlich, L. (2017):Cystic fibrosis: diagnosisand treatment. Deutsches Ärzteblatt International, 114(33-34), 564.
15. Sosnay, P.R., Salinas, D.B., White, T.B., Ren, C.L., Farrell, P.M., Raraigh, K.S., et al. (2017):Applying cystic fibrosis transmembrane conductance regulator genetics and CFTR2 data to facilitate diagnoses. J Pediatr.;181:S27–32 e1.
16. Castellani, C., Duff, A.J.A., Bell,S.C., et al. (2018): ECFS best practice guidelines: the 2018 revision. J Cyst Fibros; 17: 153–178.
17. Naehrlich, L., Ballmann, M., Davies, J., Derichs, N., Gonska, T., Hjelte, L., et al. (2014): Nasal potential difference measurements in diagnosis of cystic fibrosis: an international survey. J Cyst Fibros;13:24-28.
18. CLSI. Sweat testing (2019): specimen collection and quantitative chloride analysis. CLSI Guideline C34. 4th ed. Wayne, PA: Clinical and Laboratory Standards Institute.
19. McColley, S.A., Elbert, A., Wu, R., Ren, C.L., Sontag, M.S., LeGrys, V.A. (2020): Quantity not sufficient rates and delays in sweat testing in US infants with cystic fibrosis. Pediatr Pulmonol. 55:3053–3056.
20. Barben, J., Ammann, R.A., Metlagel, A., Schöni, M.H. (2005): Conductivity determined by a new sweat analyzer compared with chloride concentrations for the diagnosis of cystic fibrosis. J Pediatr 146:183–188.
21. Farrell, P.M., White, T.B., Howenstine, M.S., Munck, A., Parad, R.B., Rosenfeld, M.,& McColley, S.A.(2017):Diagnosis ofcystic fibrosis in screened populations. The Journal of pediatrics;181:33-44.
22. Sarhan, D. T., Sameer, H., Hegab, S. S., Abd-Allah, A. M., & Salah, K. M. (2024): Fecal elastase-1 and ultrasonography for assessment of exocrine pancreatic function in children with cystic fibrosis. Zagazig University Medical Journal.
23. Desmarquest, P., Feldmann, D., Tamalat, A., Boule, M., Fauroux, B., Tournier, G., et al. (2000):Genotypic analysis and phenotypic manifestations of children with intermediate sweat chloride test results. Chest;118:1591–1597.
24. Dayasiri, K., Hull, J., Rao, S. (2021): NICE guidance on diagnosis and management of cystic fibrosis. Arch Dis Child Educ Pract Ed. 106:31–34.

25. Legrys, V.A. (1996):Sweat-testing for the diagnosis of cystic fibrosis: practical considerations. J Pediatr;129: 892–897
26. Sly,P. D., , Brennan , S.C., Gangell de Klerk, N. , Murray, C., Mott, L.,Stick,S.M.,Robinson,P.J.Robertson,C.F.,Ranganathan, S.C. (2009):AustralianRespiratory Early Surveillance Team for Cystic Fibrosis (AREST-CF), Lung disease at diagnosis in infants with cystic fibrosis detected by newborn screening. Am. J. Respir. Crit. Care Med. 180, 146–152
27. Boat, T.F., Acton, J.D. (2007): Cystic fibrosis. In: Kliegman RM, Behrman RE, Jenson HB, Stanton BF, editors. Nelson Textbook of Pediatrics. Philadelhia: Saunders;1803-1817.
28. Rock, M.J., Mischler, E.H., Farrell, P.M., Bruns, W.T., Hassemer, D.J., Laessig, R.H.(1989): Immunoreactive trypsinogen screening for cystic fibrosis: characterization of infants with a false-positive screening test. Pediatr Pulmonol, 6:42-48
29. Rowe SM, Clancy JP, Wilschanski M. (2011): Nasal potential difference measurements to assess CFTR ion channel activity. Methods Mol Biol;741: 69–86.
30. Schneider, A., Funk, B., Caspary, W., Stein J. (2005):Monoclonal versus polyclonal ELISA for assessment of fecal elastase concentration: pitfalls of a new assay. Clin Chem.;51:1052-1054.
31. Singh M, Prasad R, Kumar L. (2002): Cystic fibrosis in North Indian children. Indian J Pediatr.; 69:622 – 629.
32. Ode, K.L., Frohnert, B., Laguna, T., et al. (2010): Oral glucose tolerance testing in children with cystic fibrosis. Pediatr Diabetes ;11: 487–492
33. Davis, S.D., Fordham, L.A., Brody, A.S., et al. (2007): Computed tomography reflects lower airway inflammation and tracks changes in early cystic fibrosis. Am J Respir Crit Care Med; 175: 943–950
34. Wielpütz, M.O., Puderbach, M., Kopp-Schneider, A. et al. (2014):Magnetic Resonance Imaging Detects Changes in Structure and Perfusion, and Response to Therapy in Early Cystic Fibrosis Lung Disease. Am J Respir Crit Care Med; 189: 956–965
35. Sly, P.D., Brennan, S., Gangell, C., et al. (2009): Lung disease at diagnosis in infants with cystic fibrosis detected by newborn screening. Am J Respir Crit Care Med; 180: 146–152
36. Kauczor, H.U., Wielpütz, M.O., Owsijewitsch,M.,et al. (2011): Computed tomographic imaging of the airways in COPD and asthma. J Thorac Imaging; 26: 290–300
37. Wielpuetz ,M.O., Eichinger, M., Zhou, Z., et al. (2011): In vivo monitoring of cystic fibrosis- like lung disease in mice by volumetric computed tomography. Eur Respir J; 38: 1060–1070
38. King, L.J., Scurr, E.D., Murugan, N., et al. (2000): Hepatobiliary and pancreatic manifestations of cystic fibrosis: MR imaging appearances. Radiographics.;20:767–777.
39. Littlewood, J.M. (1999): Update on intestinal strictures. J R Soc Med.;92 Suppl 37:41-49.
40. Rowe SM, Clancy JP, Wilschanski M. (2011): Nasal potential difference measurements to assess CFTR ion channel activity. Methods Mol Biol;741: 69–86.
41. Martiniano, S.L., Hoppe, J.E., Sagel, S.D., Zemanick, E.T. (2014): Advances in the diagnosis and treatment of cystic fi brosis. Adv Pediatr.; 61(1):225-243.