Clinical Chemistry Trainee Council
Pearls of Laboratory Medicine
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TITLE: Cystic Fibrosis

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Slide 1:
Cystic Fibrosis (CF) is the most common life-threatening autosomal recessive disorder in Caucasians. The incidence is 1 in 3500 newborns in the United States, with a disease prevalence of 30,000 adults and children. It is estimated that 2-4% of non-Jewish Caucasians are heterozygous carriers of a CF disease-causing mutation. Fortunately for the affected population, this disease has become better characterized leading to major changes in diagnosis and therapy in the past few decades.

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CF is a disorder of epithelial transport which affects fluid secretion in the epithelial lining of multiple organ systems including the respiratory tract, gastrointestinal, and reproductive systems, as well as various exocrine glands. The responsible gene has been identified as the Cystic Fibrosis Transmembrane Regulator (CFTR) on Chromosome 7q31.2. CFTR protein is responsible for the flow of electrolytes, particularly chloride, across epithelial surfaces. Depending on the functional consequences of the mutation, the disease can have varied penetrance and phenotypic presentation across individuals and organ systems.

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Clinically, CF has a wide variety of abnormalities which can be associated with the defective CFTR protein. Severe lung disease is the most characteristic and pervasive clinical manifestation of CF. The various lung abnormalities relate to the development of thickened mucus within the pulmonary parenchyma. When chloride is unable to be effectively transported across membranes, there is a general decrease in water content of the extra-cellular spaces, leading to thickening of the mucus in various tissues resulting in clinical disease. Aside from lung abnormalities, the GI system, specifically the exocrine pancreas, is next most affected, followed by reproductive abnormalities.

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Pulmonary disease severity is the primary predictor of survival in CF patients. Advances in therapy over the past decades have aimed in delaying and preventing the sequelae of CF. Bacterial colonization and infection are major problems. CF patients develop infection and colonization with aggressive and resistant bacteria such as Pseudomonas Aeruginosa, Staphylococccus Aureus, Hemophilus Influenzae, and Burkholderia cepacia. Recurrent infections can lead to the development of bronchiectasis, atelectasis, and hyperinflation with resulting compromise in lung function. Additionally, 85% of patients will have pancreatic insufficiency, requiring pancreatic enzyme supplementation throughout their life to maintain adequate nutrition. CF can be suspected in the prenatal or neonatal period with the presence of echogenic bowel on ultrasound or meconium ileus at birth (which is seen in 10-20% of affected neonates). Later in life, reproductive abnormalities in males can become apparent as men may be affected with obstructive azoospermia or congenital absence of the vas deferens. CF can present later in life in patients with milder phenotypes. With aggressive treatment targeting disruption of lung mucus, prevention of bacterial infection, and nutritional supplementation, life expectancy has increased to over 30 years of age.

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Diagnosis of CF incorporates a number of dissimilar techniques, each serving a unique role. Since the mid-1990s, newborn screening has been a key factor in early identification of patients with CF. In 1996, newborn screening for CF was routinely performed in only 2 states. By 2000, 40 states had incorporated this testing and by 2008, CF newborn screening was mandatory in all 50 states. The newborn screening algorithms vary by state. First-line testing is commonly performed by immunoreactive trypsinogen (IRT). Trypsinogen is a pancreatic enzyme that is increased in the blood in patients with CF, particularly those with exocrine pancreatic dysfunction. The test has a high sensitivity and algorithms for confirmation allow for improvements in diagnostic specificity. Depending on the state-specific algorithm, elevated results may trigger a repeat IRT test, with the predictive value of 2 high IRTs greater than that of a single result. Other states may reflex an elevated IRT to genetic mutational analysis. Regardless, IRT is a screening, not diagnostic test. Any positive (suspicious) result must then have sweat chloride testing or mutational analysis performed.

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Sweat chloride testing has been the mainstay of diagnostic testing for CF since it was noted that newborns with CF were found to be “salty.” Dysfunctional CFTR leads to high concentrations of chloride in sweat, which can be measured in the clinical laboratory. Sweat chloride testing techniques are remarkably well-characterized with standards developed for the collection and analysis techniques through CLSI. Collection and testing are challenging and it is strongly recommended that testing be performed only at Cystic Fibrosis Foundation (CFF) accredited centers that must show high quality collection and performance. The test is performed by stimulating the child to sweat over an area of specific dimensions. Pilocarpine (a parasympathetic stimulant) is applied to the arm or leg. Electrodes are then placed on the site and a weak current is briefly applied (approximately 5 minutes) which allows for migration of the pilocarpine across the skin and stimulation of the sweat glands. The electrodes are then removed and the sweat is collected over 30 minutes onto pre-weighed gauze or filter paper, or directly into a Macroduct coil while the area is covered to prevent evaporation.

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As mentioned previously, sweat testing should only be performed at CFF-accredited centers in which the laboratories adhere to the established guidelines and maintain appropriate quality assurance of the total collection and testing process. The weight or volume of the sweat is used to gauge adequacy of collection and is used in the determination of the chloride concentration. 75 mg or 15 uL of sweat is considered a sufficient collection. CFF centers are expected to have an insufficient collection rate of <5% for infants above 3 months old. Testing should not be performed on infants less than 48 hours old as there is a transient increase in sweat chloride concentration in the immediate neonatal period. Chloride concentration measured using coulometric titration with a chloridometer is the accepted analytic method. Factors leading to successful collection include appropriate staff training, observation of age guidelines, and bilateral collection. When performed along with the guidelines, the procedure is quite safe with minimal risk of burns, skin irritation, or other adverse effects. There are a number of causes of inaccurate results including intercurrent illness, edema, poor collection technique, evaporation, and multiple medications. Awareness of these factors will aid in establishing a successful CF sweat testing program. CFF-accredited centers focus on the safety and quality to minimize risk and need for repeat testing.

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The diagnostic criteria for CF have been established for many years with some minor modifications to allow for the influence of age. All results above 60 mmol/L are considered abnormal and highly suggestive of CF. However, a single abnormal test is not considered sufficient for diagnosis; the elevation must be confirmed by repeat testing or by identification of a mutation in CFTR. There is a "gray" area between 40-59 or 30-59 mmol/L, depending on whether the child is older or younger than 6 months. This area is not clearly diagnostic, but it is not uncommon for affected CF patients to have sweat chloride levels in this range. Patients with levels below 40 or 30 mmol/L, depending on the age group, are considered unlikely to have CF. However, a small percentage (<1%) of all children with CF will not have abnormal sweat chloride results. Therefore, there are some clinical situations where mutation analysis may help resolve a borderline or normal sweat chloride concentration when clinical suspicion is high.

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Genetic testing for CF remains a complex diagnostic process. Over 1000 potentially causative mutations have been identified in the CFTR gene. These mutations are generally categorized based on their functional abnormality resulting from the mutation and grouped by expected disease severity. The low frequency of many of these mutations makes it difficult to judge the expected severity with certainty. Mutations can be classified into 6 types based on the effects on protein expression and function. They are further classified based on severity with Class I-III being more severe and Class IV- V being phenotypically milder. Consequently, the ACMG has determined there are 23 mutations that have been definitively determined to cause CF disease and thus can be considered diagnostic mutations. These mutations account for both disease-causing mutations in the majority (85%) of individuals affected with CF.

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Specifically, the "delta F508" or the deletion of phenylalanine at position 508, is by far the most common CF mutation, accounting for 72% of mutational alleles in non-Hispanic Caucasians (66% in a pan-ethnic group). The methods for mutational analysis are nearly as varied with newer methods taking advantage of multiplexing technologies. The higher prevalence disease-causing mutations are often tested simultaneously in multiplexed panels which may be determined based on ACMG recommended mutations or population-specific panels. Sequencing and evaluation for deletions and duplications of the CFTR gene identify mutations that have a lower prevalence in the population and remain second-line diagnostic methods.

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The genotype-phenotype correlations for CF are not clearly defined. Classic CF patients will have 2 severe mutations and present with severe lung disease and pancreatic insufficiency. In compound heterozygous patients inheriting 1 mild and 1 severe or 2 mild mutations, the phenotype is often less severe with pancreatic sufficiency. When testing is performed, interpretation must take into account the clinical situation that led to testing and the ethnic identity of the patient being tested. These two factors are critical in making clinically appropriate conclusions about the presence, or even more so, the absence of disease-causing mutations. Genetic counseling is commonly recommended to discuss the challenges of test interpretation. There are a number of additional related phenotypes seen with CFTR mutations such as idiopathic chronic pancreatitis, late onset bronchopulmonary disease, and idiopathic bronchiectasis. Additionally, there have been identified some related disease-modifying factors that contribute to the complexities of genotype-phenotype correlation and disease severity.

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As an example of the complexity of CF mutational analysis, I will use the R117H mutation. The R117H mutation can be found alone or in conjunction with another CFTR disease-causing mutation. In and of itself, it is not associated with disease, but another factor must be examined to determine the likely clinical phenotype. In patients with the R117H mutation, the length of the polythymidine tract in intron 8 must be examined. If the tract is 5T in cis with the R117H mutation, this is associated with CF disease. However, if the tract is 7T in cis, the R117H can be associated with male infertility or congenital bilateral absence of the vas deferens. There are other examples of unique physiologic or methodologic abnormalities which one must be aware of when interpreting reports.

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In 1997, an NIH Consensus Panel recommended offering carrier screening to certain ethnic groups with a high carrier frequency for CF disease-causing mutations, particularly non-Jewish Caucasians and Ashkenazi Jews. A 23 mutation panel includes all mutations associated with disease which are found at an allele frequency of >0.1% in the US population. The percent of disease-causing mutations this panel will detect varies with ethnic group, again making the need for discussion of the meaning of a negative test in the context of an individual patient critical.

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Putting all of these diagnostic and screening techniques together, the diagnostic criteria for CF have been defined as follows: the patient must have one or more characteristic clinical feature, a history of a sibling with CF or a positive newborn screen (with either duplicate IRT testing or IRT followed by DNA testing), paired with laboratory evidence of a CFTR abnormality, most commonly sweat chloride testing or identification of two CF disease-causing mutations.

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Points to remember about cystic fibrosis:

1. CF is the most common lethal autosomal recessive disorder in Caucasians, with a high carrier frequency.
2. Newborn screening has been universally implemented in the United States.
3. IRT testing is commonly used; however, alternate tests and diagnostic algorithms may vary by state.
4. Sweat chloride testing remains the gold standard in identifying functional abnormalities in CFTR and clinically affected patients.
5. Lastly, the mutation spectrum is complex and interpretation must be performed in the context of the ethnic background and indications for testing an individual patient.

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