Incidence of viral respiratory pathogens causing exacerbations in cystic fibrosis patients

Thesis

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Abstract

BACKGROUND: Cystic fibrosis is a genetic disease characterized by progressive epithelial secretory gland dysfunction associated with repeated respiratory infections. Although bacteria have historically been considered to play a major role in cystic fibrosis (CF) airway damage, a strong impact of respiratory viral infections is also now recognized. Emerging evidence confirms that respiratory viruses are associated with exacerbation and facilitation of bacterial colonization in CF patients.

METHODS: This is a cross sectional observational study recruiting 60 patients diagnosed as CF. Following in CF clinic, Children's Hospital, Cairo University, throughout a period of 7 months. Their age ranged from 6 months to 13 years. Children had nasal swabs and sputum samples obtained during pulmonary exacerbations. Multiplex PCR technique was used to detect respiratory viruses from nasal swabs.

RESULTS: We detected viruses in 48 patients during exacerbation (80%), the most common virus was Rhinovirus 43.4% present in all seasons, followed by bocavirus 20%, adenovirus 13.3%, enterovirus 10%, HMPV 6.7%. Co infection with double viruses was in 10 patients.while bacteria present in 56.7% of patients and the most common organism found was pseudomonas spp in 20% of patients then staph aeurus, MRSA, Klebsiella and Hemophilus influenza. Podsitive CRP was detected in 53.3% of patients.There was significant correlation between positive bacterial culture and and certain viruses as influenza A virus, Enterovirus, HMPV with P value (<0.01, 0.002, 0.002 respectively).There was significant relation between both influenza A virus and Enterovirus with the need for oxygen with p value (0.004, 0.02 respectively).There was no significant relation between viruses and the need for

ICU admission .There was significant correlation between HMPV and seasonal variation with (p value 0.001).

Conclusion: The present study demonstrated that viruses present in CF patients during exacerbation more than bacteria, also presence of virus co infection in the same episode.With elevated CRP levels. There was significant relation between bacteria and certain viruses (Influenza A, Enterovirus and HMPV).And significant relation between certain viruses with the need for hospital admission and the need for oxygen (Influenza A and Enterovirus).

Keywords: Cystic fibrosis, Exacerbation, Respiratory viruses, Rhinovirus.

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List of abbreviations

ABC	ATP binding cassette
A1C	glycated hemoglobin
ACTH	Adrenocorticotrophic hormone
ADV	Adenovirus
ARIC	Acute Respiratory Illness Checklist
ASL	airway surface liquid
ATP	Adenosine triphosphate
BAL	Bronchoalveolar lavage
BHR	bronchial hyperreactivity
BiPAP	Noninvasive positive pressure ventilation
cAMP	Cyclic Adenosine monophosphate
CF	Cystic fibrosis
CFBD	CF-related bone disease
CFF	Cystic Fibrosis Foundation
CFPE	CF patients experience intermittent pulmonary exacerbations
CFRD	CF related diabetes
CFTR	cystic fibrosis transmembrane conductance regulator gene
cGMP	Cyclic guanosine monophosphate
CI	confidence interval
Cl	Chloride
COPD	chronic obstructive pulmonary disease
CoV	Human coronaviruses
CRP	C-reactive protein
CT	Computed tomography
CXR	Chest x-ray
DCs	dendritic cells
DIOS	distal intestinal obstruction syndrome
DMARDs	Disease –modifying anti-rheumatic drugs
DNA	Deoxynucleic acid
EnaC	epithelial sodium channel
ER	endoplasmic reticulum
EV	Enteroviruses
F	Fosfomycin
FEV1	Forced expiratory volume in one second
FVC	Forcsd vital capacity

FTI	fosfomycin/tobramycin
GA	Golgi apparatus
GER	Gastroesophageal reflux
GI	Gastrointestinal
GSNO	S-nitrosoglutathione
GSNOR	S-nitrosoglutathionereductase
HA	Hemagglutinin
HadVs	Human adenoviruses
HIV	Human immunodeficiency virus
HRCT	high-resolution computed tomography
HRV	Human rhinovirus
HTS	High throughput screening
ICAM	intercellular adhesion molecule
ICU	Intensive care unit
IFN	Interferon
IL	Interluken
IRT	immunoreactive trypsin
IV	Influenza virus
LDLR	low-density lipoprotein receptor
LPS	lipopolysaccharide
LRP	LDLR-related protein
MHC	Major histocomptability complex
MRSA	Methicillin-resistant S. aureus
MPV	Metapneumovirus
NA	Neuraminidase
Na	Sodium
NAD	nasal transepithelial potential difference
NBD1&2	nucleotide binding domains
NBS	Newborn screening
NEP	nuclear export protein
NP	Nucleocapsid protein
NP swab	Nasopharyngeal swab
NPD	nasal potential difference
OGTT	oral glucose tolerance test
P	Phosphorylation
PA	Pseudomonas aeruginosa
PBS	Pseudo-Bartter syndrome
PCD	Primary ciliary dyskinesia
PCR	polymerase chain reaction
PCT	Practical clinical trial
PD	Potential difference

PDE	Phosphodiesterase
PERT	pancreatic enzyme replacement therapy
PFT	Pulmonary function tests
PI	Pancreatic insufficiency
PIV	Parainfluenza virus
PTC	premature termination codon
PTCs	premature termination codons
R	Regulatory domain
RNA	Ribonucleic acid
RNP	Ribonucleoprotein
RSSQ	Respiratory and Systemic Symptoms Questionnaire
RSV	respiratory syncytial virus
SBDS	Shwachman-Bodian-Diamond syndrome
SPSS	Statistical Package for the Social Sciences
SST	short synacthen test
T	Tobramycin
TGF	transforming growth factor
TNF	tumour necrosis factor
US	Ultrasound
USA	United States of America
VFGF	vasoactivepeptides (bradykinin), and growth factors
VP	Viral protein
VTM	viral transport media
W/W	weight-to weight ratio

Introduction

Cystic fibrosis

Cystic fibrosis (CF) is a common life-shortening genetic disorder in the Caucasian population (less common in other ethnic groups) caused by the mutation of a single gene that code for the production of the cystic fibrosis transmembrane conductance regulator protein (CFTR). This protein coordinates the transport of salt (and bicarbonate) across cell surfaces and the mutation most notably affects the airways. In the lungs of people with CF, defective protein results in a dehydrated surface liquid and compromised mucociliary clearance. The resulting thick mucus makes the airway prone to chronic infection and inflammation, which consequently damages the structure of the airways, eventually leading to respiratory failure ***(Aslam et al., 2017).***

CF is a chronic disorder, of autosomal recessive inheritance, which principally affects the respiratory tract, pancreas, gastro-intestinal tract and liver. In the respiratory tract, abnormalities result in mucus plugging of the airways and susceptibility to respiratory tract infection. This leads to neutrophil -dominated airway inflammation with consequent lung damage (bronchiectasis), and eventually respiratory failure and death. Inflammation is also important in the development of the bronchial hyperreactivity (BHR) and airway instability that may be associated with CF. ***(Balfour-Lynn and Welch 2016).***

CF is a genetic disease caused by mutations in the CFTR. Mutations in CFTR result in airway mucus buildup and chronic airway infections. CF patients experience intermittent pulmonary exacerbations (CFPE), events that are poorly defined clinically, but known to lead to lung function decline and accelerated disease progression ***(Goss and Burns, 2007).***

The lung affection is characterized by dehydration of airway surface liquid and impaired mucociliary clearance. As a result, there is difficulty clearing pathogens from the lung, and patients experience chronic pulmonary infections and inflammation. Although CF is a complex disorder affecting many organs, 85% of the mortality is a result of lung disease ***(Flume et al., 2009).***

The median age at death is approximately 25 years. Every year, many children with CF die from respiratory failure ***(Liou et al., 2008).***

Aim of the work

The aim of the study was to assess:

1- The relation between respiratory viruses and exacerbation in cystic fibrosis children.

2- The relation between different bacteria and exacerbation in cystic fibrosis children.

Review of literature

Genetics of cystic fibrosis

About 70 000 people have CF worldwide with prevalence varying by location and ethnic background. It is most common in white people of Northern European descent and therefore its prevalence is highest in Europe, North America, and Australia. The prevalence is much lower in the Middle East, South America, Africa ***(Macneill et al., 2015).*** CF is a multi-organ disease with an autosomal recessive pattern of inheritance.

Molecular basis of CFTR dysfunction

About 2000 mutations have been identified in the CFTR gene since its discovery in 1989, and about 242 mutations have currently been confirmed to cause cystic fibrosis. Despite the allelic diversity in this disease causing gene, 85-90% of white people with cystic fibrosis carry at least one copy of the F508del mutation. Previously, people with cystic fibrosis were genotyped only to confirm the diagnosis or to predict disease severity, but with the recent approval of mutation specific treatments, genotypic information is considered essential ***(Ramsey et al., 2011).***

Normal CFTR structure and function

CFTR is a member of the ATP binding cassette (ABC) family of transporter proteins, characterized by two membrane spanning domains that form the channel pore and two nucleotide binding domains that bind and hydrolyze ATP. Unlike other ABC transporters, CFTR has an additional regulatory domain that regulates channel opening and closing ***(Riordan, 2008).***

Phosphorylation of the regulatory domain by protein kinase A, followed by binding of ATP and its hydrolysis by the nucleotide binding domains leads to dimerization of the nucleotide binding domains and structural realignment of

the membrane spanning domains to allow opening (or gating) of the CFTR channel pore (fig 1).***(Kanelis et al., 2010).***

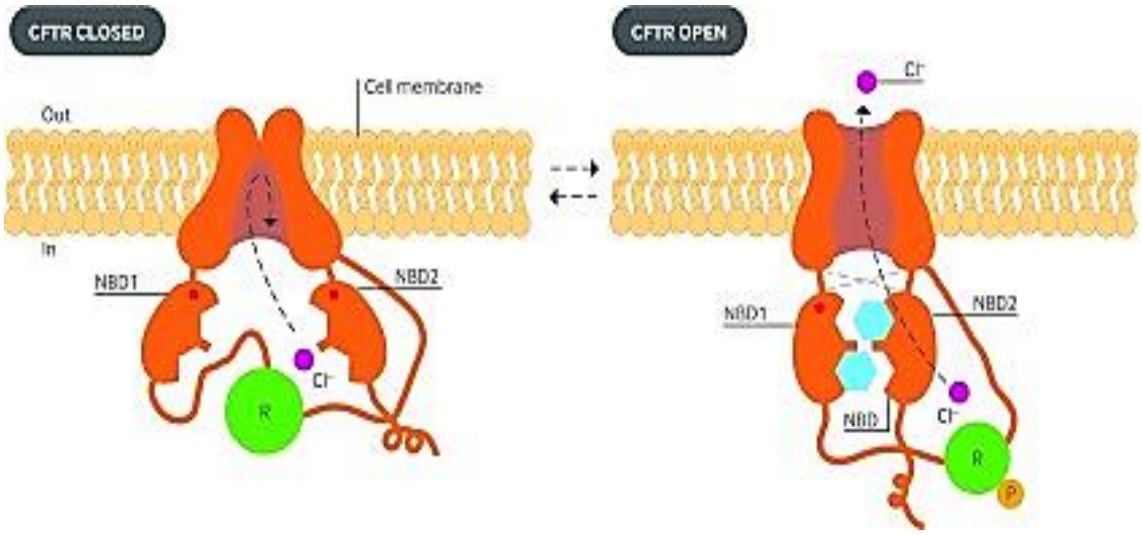


Figure 1: proposed structure of the cystic fibrosis transmembrane conductance regulator (CFTR) in its closed (left) and open (right) configurations.

The two transmembrane spanning domains form the channel pore. Gating of the channel is controlled by the two intracytoplasmic nucleotide binding domains (NBD1 and NBD2) as they bind and hydrolyze ATP, in addition to a regulatory domain (R), which contains numerous sites of phosphorylation (P). Normal activation of the protein requires phosphorylation of the R domain. The NBDs bind and hydrolyze ATP, inducing channel gating by conferring opening of the pore through interfaces with the transmembrane domains via their extracellular loops, which also function to stabilize the protein. Cl^- =chloride ion. **(Hwang and Sheppard, 2009).**

The primary role of the CFTR protein is to transport anions (such as chloride and bicarbonate) through the apical membrane of epithelial cells, thereby creating an osmotic gradient for fluid secretion. The CFTR also has an absorptive role in some epithelial structures, such as the sweat gland. Absence or dysfunction of the CFTR results in dehydrated, thickened secretions that obstruct epithelium lined ducts (such as airways and biliary and pancreatic ducts) resulting in tissue damage.

Specific to the airways, reduced airway surface liquid (ASL) volume causes impaired mucociliary clearance and obstruction of small and medium sized airways with inspissated secretions ***(Cantin et al., 2015).***

Inherent abnormalities of CF mucus that increase its viscosity and adhesion to the epithelial surface also affect the lungs and other organs. This creates a vicious cycle of mucus retention, infection, and inflammation, which further perpetuates the airway damage. In addition to impairments in mucociliary clearance, there is strong evidence that CFTR dysfunction itself leads to innate and adaptive immune defects that result in compromised bacterial clearance and dysregulated inflammation ***(Birket et al., 2014).***

Inheritance of CFTR Gene

CF is an autosomal recessive disorder, when an individual has one copy of the CFTR delta 508 allele and another normal allelic variant, he or she is said to be heterogenous and is a carrier for CF.

In this insistence, there is one functional CFTR allele and one nonfunctional (deleterious) CFTR delta F508. On the other hand, if an individual has 2 copies of the same allele, then that individual is homozygous for the CFTR delta F508 allele means an individual will exhibit the classic CF phenotype. If a female has one deleterious copy of the CFTR gene, were pregnant or considering a pregnancy, her partner should be offered testing so that the partners CF carrier status can be determined ***(Wolpert et al., 2005).***

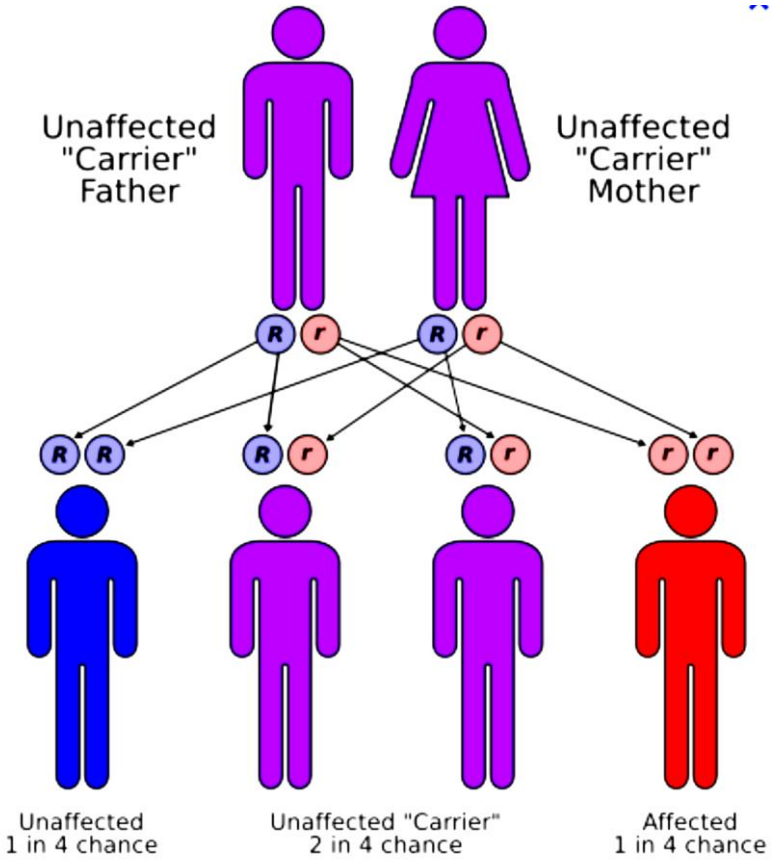


Figure 2: Inheritance of cystic fibrosis.

(Wolpert et al., 2005).

Molecular consequences of variants in CFTR

CFTR mutations can be broadly classified into six categories on the basis of the mechanisms that cause aberrant CFTR synthesis or function (fig 3).

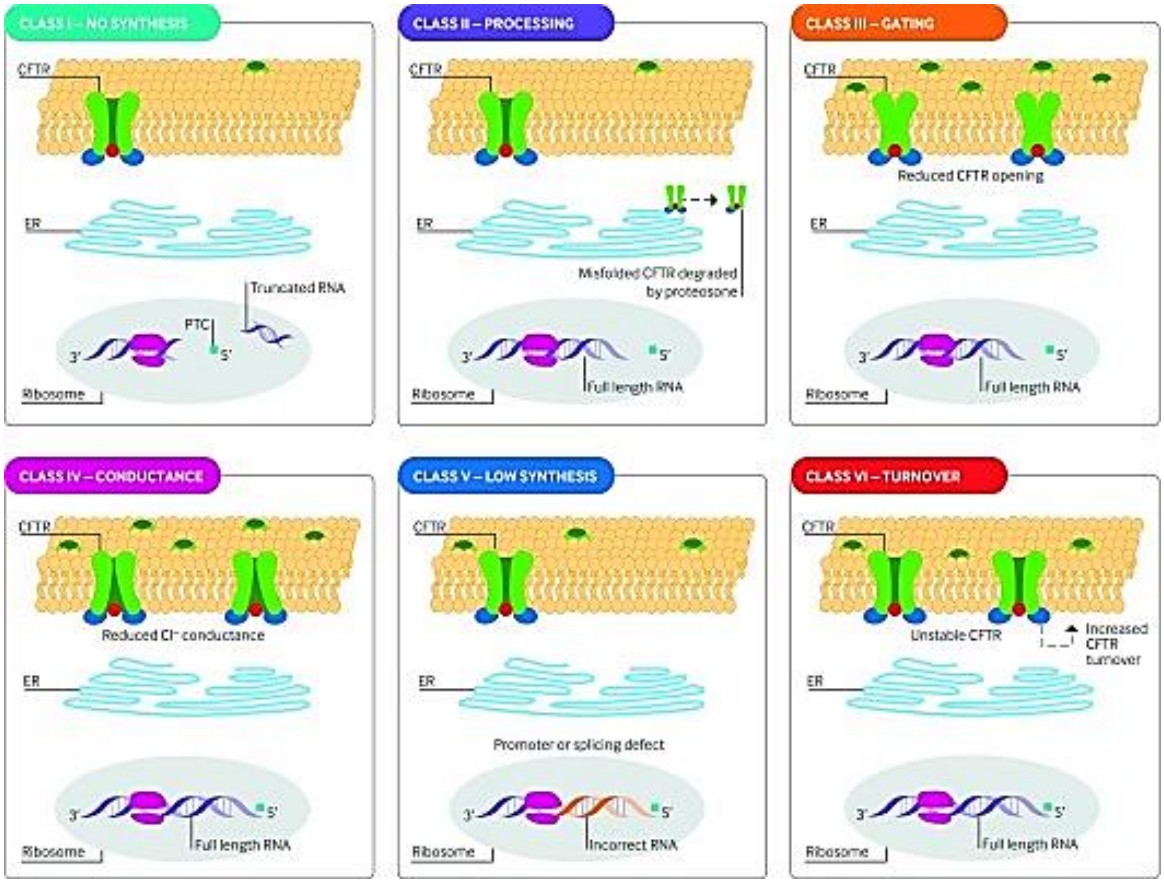


Figure 3: CFTR mutational classes and molecular consequences.

CFTR=cystic fibrosis transmembrane conductance regulator; ER=endoplasmic reticulum; PTC=premature termination codon

Class I mutations include in-frame UAA, UAG, or UGA stop codons in the mRNA preceding the native stop codon at the 3′ end of mRNA, and are thus referred to as premature termination codons (PTCs). The transcribed mRNA is truncated and unstable resulting in absent protein production **(Mendell and Dietz, 2001).** Other major insertions and deletions that disrupt normal translation are also included in this category.

Class II mutations cause abnormal CFTR processing or trafficking, which result in reduced amounts of CFTR at the cell surface. For example, the F508del-CFTR mutation is a three base pair deletion that leads to an amino acid deletion with subsequent misfolding of the CFTR protein. The misfolded protein fails to transport to the cell surface owing to premature degradation by the proteasome ***(Welch, 2004).***

Class III mutations are often referred to as "gating" mutations because they lead to disordered activation of the CFTR channel.

Class IV mutations exhibit normal gating, but changes in conductivity of the channel pore cause abnormal chloride permeability.

Class V mutations are located within promoter or splice sites in the gene; they lead to fewer CFTR transcripts and reduced protein production.

Class VI mutations are the most recently discovered and result in reduced stability of CFTR at the cell surface, leading to increased turnover. Several mutations exhibit features of more than one class. For example, although the primary abnormality of F508del is aberrant cellular processing, it also exhibits defective gating and a reduced surface half life. R117H is often described as a partially active conductance mutation, but it also exhibits partially disrupted gating and is located in cis with mutations that affect its expression ***(Castellani et al., 2008).***

Impact of mutational class on CFTR activity

Each individual patient's disease phenotype is partly determined by overall CFTR activity, which in turn is determined by the net impact of both of the disease causing alleles on the quantity and function of the CFTR. In general, people with two loss of function alleles (classes I-III) have low levels of CFTR

activity (<10% of normal) and more serious lung disease and pancreatic insufficiency consistent with classic cystic fibrosis. By contrast, those with at least one residual functional allele are expected to have residual CFTR activity (>10% of normal) and milder lung disease and pancreatic sufficiency consistent with non-classic or mild cystic fibrosis ***(Quon, 2016) (McKone et al., 2003).***

Moreover, substantial phenotypic variation exists even for patients with the same combination of CFTR mutations owing to environmental influences and additional genetic variation that contributes to the final phenotype (genetic "modifiers" of disease). For example, polymorphisms in the gene encoding transforming growth factor β (TGF-β) modify the severity of lung disease in F508del homozygous patients ***(Drumm et al., 2005).***

Diagnosis of CF

I- Clinical manifestations:

CF is a complex multiorgan disease in which lung disease accounts for nearly 85% of the mortality ***(Flume et al., 2007).*** It is highly variable in onset and intensity ***(Gibson et al., 2003).***

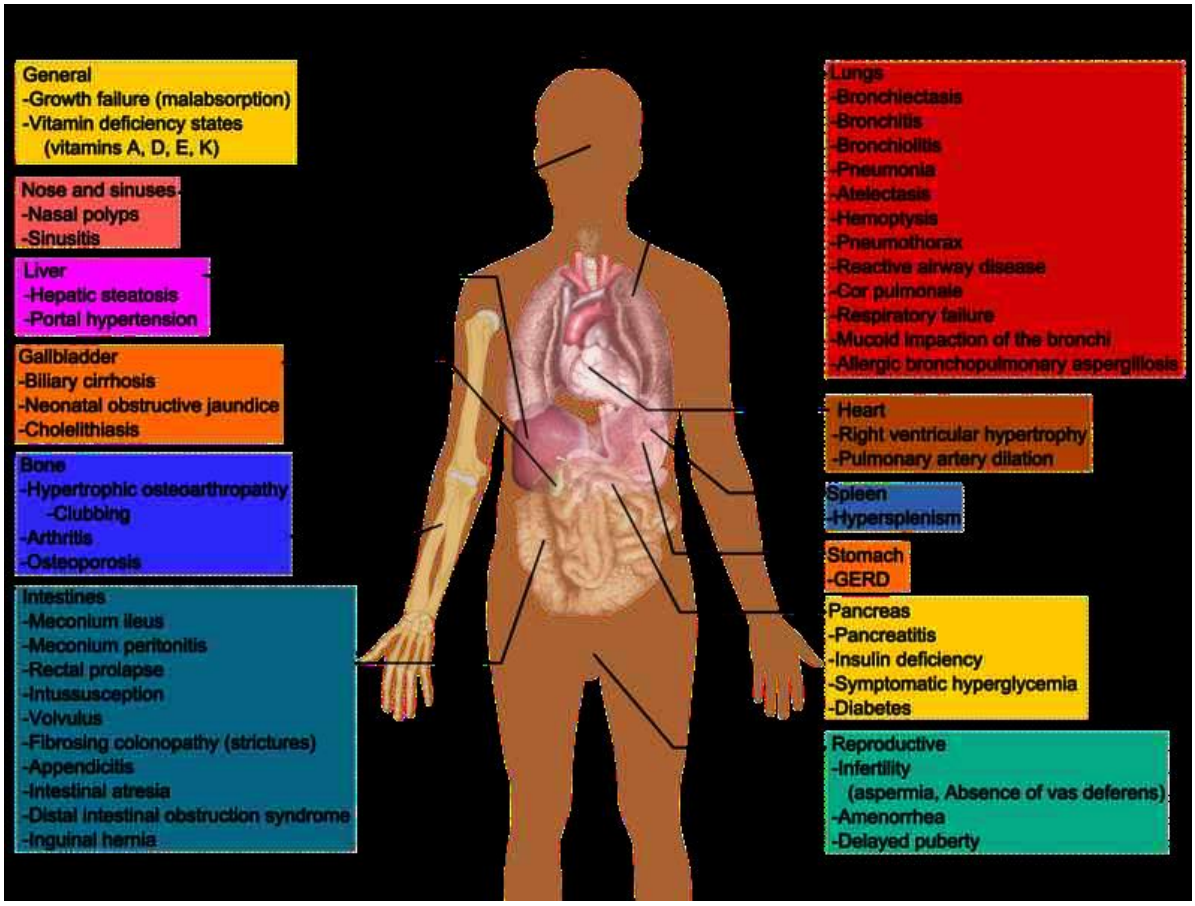


Figure4: Manifestations of CF

(Kliegman et al., 2006).

Classic and non classic

1-Classic:

The classic or typical form of CF is diagnosed if a patient demonastrates clinical disease in one or more organ systems and has elevated sweat chloride (>60 mmole /L). Most of these patients have disease manifestations in multiple organ systems (pancreas, upper and lower respiratory tract, and male reproductive tract) ***(De Boeck et al., 2006).***

2-Non classic:

About 2 percent of patients fulfill diagnostic criteria for CF, but have anormal or intermediate sweat chloride result. In these patients, the diagnosis of CF depends on DNA analysis (two copies of a disease causing CFTR mutation) or on measurement of nasal potential difference.

This disease pattern is usually termed (non classic) or (atypical) CF ***(Boyle, 2003).***

The term non classic has also been used to describe patient with clinical disease limited only one organ system (eg. Isolated obstructive azoospermia or chronic pancreatitis). However, this definition depends on the stringency of the diagnostic method used to determine clinical disease. Therefore, the sweat-test based definition of non-classic disease can be applied more consistently and is preferred ***(Boyle, 2003 and De Boeck et al., 2006).***

Patients who are first diagnosed with CF as adults are more likely to have non classic features, with normal or intermediate sweat chloride result, milder lung disease, and/or little or no gastrointestinal disease ***(Keating et al., 2010).***

Picture of the classic type:

A-Respiratory tract manifestations

Typical respiratory manifestations of CF include a persistent productive cough, hyperinflation of the lung fields on chest radiograph, and pulmonary function test that are consistent with obstructive airway disease. As the disease progresses, chronic bronchitis with or without bronchiectasis develops and is accompanied by acute exacerbations characterized by increased cough, tachypnea, dyspnea, increased sputum production, malaise, anorexia and weight loss ***(Newton, 2009)***

Digital clubbing is often seen in patients with moderate to advanced disease. Colonization of the airway with pathogenic bacteria often occurs early in life. Staphylococcus aureus and haemophilus influenza are common pathogens during early childhood, but pseudomonas aeruginosa is ultimately isolated from the respiratory secreations of most patients. This predisposition to infection may be in part because of impaired clearance directly induced by a defect in CFTR ***(Cohen and Prince, 2012).***

1-Bronchiectasis

Chronic inflammation causes lung damage that ultimately advances to the stage of irreversible bronchiectasis and progressive respiratory failure .Terminal findings often include severely comgested parenchyma, with grossly purulent secretions in and around dilated airways. The airway epithelium is hyperplastic, often with areas of erosion and squamous metaplasia.Plugs of mucoid material and inflammatory cells are often present in the airway lumen .Submucosal gland hypertrophy and hyperplasia of airway smoothmuscle may also be noted (***Hays et al., 2005).***

2-Pulmonary exacerbations

The clinical course of most patients with CF is punctuated by episodes of acute worsening of respiratory status.These pulmonary exacerbations appear to drive long-term deterioration in lung functions ***(Sanders et al., 2011).***

B-Sinus disease

The majority of CF patients develop sinus disease. Radiographs reveal panopacifications of the paranasal sinuses in 90 to 100 percent of patients older than eight months of age ***(Yung et al., 2002).***

C- Gastrointestinal manifestations

1-Pancreatic disease

Some patients with marginal pancreatic function at birth will develop overt evidence of pancreatic insufficiency in later childhood. It is important to consider CF in a child whose respiratory symptoms suggest the diagnosis even in the absence of malabsorptive symptoms. Most patients with CF develop progressive pancreatic damage as a result of defective ductular and aciner pancreatic secretions. The fast majority of patients with classic CF have exocrine pancreatic insufficiency. Acute or recurrent pancreatitis can be seen, particularly in patients who are pancreatic sufficient, although they may subsequently progress to pancreatic insufficiency **(De Boeck et al., 2006 and Augarten et al., 2008).**

Patients with exocrine pancreatic insufficiency often develop disfunction of the endocrine pancreas, leading to glucose intolerance and CF-related diabetes. Children and adolescence with CF frequently have growth failure, caused by the combination of malabsorption, increased energy needs, and reduced appetite. Nutrient delivery and correction of maldigestion and malabsorption are essential to achieve normal growth to support optimal pulmonary function and to prolong life .The nutritional risks and requirements for a patient with CF also vary along this disease spectrum but do not precisely coincide with the severity of pulmonary disease ***(Flume et al., 2009).***

2-Meconium ileus and distant ileal obstruction

Meconium ileus can occur in patients with a variety of CFTR mutations. A familial reccurence rate of nearly 30 percent suggests that other genetic modifiers predispose to the development of meconium ileus. Episodes of small bowl obstruction may also occur in older children and adults, and are known as distal intestinal obstruction syndrome (DIOS) (or meconium ileus equivalent). DIOS occurs in 10 to 47 per cent of CF patients, depending upon the criteria used to make the diagnosis ***(Lin and Wong, 2012).***

3- Biliary Disease

Focal biliary cirrhosis caused by inspissated bile is present in many patients, and this may cause elevations of serum alkaline phosphatase and lobular hepatomegaly.A symptomatic liver disease is a common finding at autopsy. In a minority of patients, the liver disease is progressive, with periportalfibrosis, cirrhosis, symptomatic portal hypertension, and variceal bleeding. CF is the third leading cause for liver transplantation in late childhood. Several observational studies have suggested that administration of ursodeoxycholic acid may arrest the progression of liver disease related to CF, but the efficaty of this therapy has not been confirmed in randomized trials ***(Cheng et al., 2012).***

D-Reproductive system manifestations

More than 95 per cent of men with CF are infertile because of defects in sperm transport, although spermatogenesis is not affected. Most of these men have incompletely developed Wolffian structures, most commonly absent vas deferens but potentially also by other mechanisms such as causing azoospermia, teratospermia and oligoasthenospermia **(Chen et al., 2012).**

Female CF patients also suffer from decreased fertility due to abnormal cervical mucus (**Abdel-Magid AF, 2016).**

E-Musculoskeletal system manifestations

A CF-associated arthropathy occurs in 2 to 9 percent of patients and is characterized by brief episodes of pain and swelling of joints. These features are occasionally accompanied by painful nodular skin lesions and purpura.although it is generally recognized that cystic fibrosis-related arthritis can be episodic and resolve spontaneously, treatment with analgesic and anti- inflammatory agents may be needed ***(Thornton and Rangarag, 2012).***

F-Recurrent venous thrombosis

CF appears to be a risk factor for recurrent venous thrombosis ***(Takemoto, 2012).***

G- Renal manifestations

Nephrolithiasis and nephrocalcinosis are common in patients with CF ***(Ozcelik et al., 2004).***

Enteric hyeroxaluria (due to fat malabsorptionresuting from decreased secretion of pancreatic enzymes) and hypocitraturia (due to chonic metabolic acidosis) are putative risk factors ***(Hoppe et al., 2005).***

II- Complications

1-CF – related diabetes mellitus

Nearly 50% of adult patients with cystic fibrosis have diabetes .the occurrence of CF related diabetes (CFRD) is preceded and is associated with deterioration of lung function and nutritional status. microvascular complications can occur, but the main cause of death is respiratory failure rather than cardiovascular causes as in type1 or type2 diabetes because other methods such as glycated hemoglobin(A1C) levels are less sensitive in patients with CF the recommended screening test is the oral glucose tolerance test(OGTT) ***(Boudreau et al., 2016).***

2-Bone disease

With the increasing life expectancy of patients with CF, prevelance of late complications such as CF-related bone disease (CFBD) has increased.it was initially described in 24% of the adult population with CF and has also been reported in the pediatric population .CFBD is multifactorial and progresses in different steps. Both decreased bone formation and increased bone resorption (indifferent amounts) are observed. CFBD is likely primitive(directly related to the CFTR defect itself), but it also worsened by acquired secondery factors such as lung infections, chronic inflammation, denutrition, vitamin deficiency,and decreased physical activity.CFBD may be clinically apparent (mainly vertebral and costal fractures), or clinically asymptomatic***.(Braun et al.,2016).***

3-Growth failure

Poor linear growth and inadequate weight gain are very common problems CF children. The most important factor involved in growth failure are undernutrition or malnutrition, chronic inflammation, lung disease, and corticosteroid treatment.nutritional support and pharmacological therapy with recombinant human growth hormone are essential for a good management of

children with CF, although these children are shorter and lighter than healthy children, and despite the catch-up growth observed after diagnosis; deficit in length/height and weight continues to be seen until adulthood. Early diagnosis is essential to ensure better nutritional status and growth, potentially associated with better respiratory functions and prognosis.Growth failure in CF children has a role in the disease morpidity and in clinical outcome, especially in relation to progressive decline of pulmonary function ***(Scaparrotta et al., 2012).***

4-Gas exchange

As bronchiectasis and airway obstruction become more pronounced, ventilation-perfusion mismatch leads to hypoxemia. This may initially occur only during sleep or exercise, but patients with advanced disease often require continuous oxygen supplementation. Hypercapnia occurs relatively late in the course of CF lung disease. Chronic hypoxemia and hypercarbia may lead to muscular hypertrophy of the pulmonary vasculature, right ventricular hypertrophy and eventually corpulmonale with right heart failure. Unfortunately, particularly for those requiring endotracheal intubation and invasive mechanical ventilation for respiratory failure, the outcome continoues to be poor ***(Efrati et al., 2010).***

5-Hemoptysis

Minor hemoptysis is a common occurrence in patients with CF, particularly during pulmonary exacerbations. Other than assuring that vitamin K deficiency is not a contributing factor, it requires no special treatment beyond the usual approach for the exacerbation. However, even minor hemoptysis can be alarming to patients and reassurance as to its usually benign nature is needed.

The guidelines recommended suspension of all chest physiotherapy in the event of massive hemoptysis ***(Flume et al., 2010).***

III- Diagnostic criteria for CF

Newborn screening (NBS) for CF is increasingly being implemented and is soon likely to be in use throughout the United States, because early detection permits access to specialized medical care and improves outcomes. The diagnosis of CF is not always straightforward, however. The sweat chloride test remains the gold standard for CF diagnosis but does not always give a clear answer. Genotype analysis also does not always provide clarity; more than 2000 mutations have been identified in the CF transmembrane conductance regulator (CFTR) gene, not all of which result in CF. Harmful mutations in the gene can present as a spectrum of pathology ranging from sinusitis in adulthood to severe lung, pancreatic, or liver disease in infancy.***(Philip et al., 2008).***

To provide guidance for the diagnosis of both infants with positive NBS results and older patients presenting with an indistinct clinical picture, a combination of clinical presentation, laboratory testing, and genetics is needed to confirm a diagnosis of CF ***(Philip et al., 2008).***

Table 1: Diagnostic Criteria For Cystic Fibrosis (CF)

Presence of typical clinical features (respiratory,gastrointestinal,or genitourinary)
OR
A histroy of CF in a sibling
OR
A positive newborn screening test
PLUS
A laboratory evidence for CFTR dysfunction:
Two elevated sweat chloride concentrations obtained on separate days
OR
Identification of two CF mutations
OR
An abnormal nasal potential difference measurement.

(Kliegman et al., 2011)

Table 2: Phenotypic features consistent with a diagnosis of CF

1. Chronic sinopulmonary disease, manifested by: a. Persistent colonization/infection with typical CF pathogens,includingStaphylococcus aureus, nontypeableHaemophilusinfluenzae, mucoid and nonmucoid Pseudomonas aeruginosa, Stenotrophomonasmaltophilia, and Burkholderiacepacia b. Chronic cough and sputum production c. Persistent chest radiograph abnormalities (eg, bronchiectasis, atelectasis, infiltrates, hyperinflation) d. Airway obstruction, manifested by wheezing and air-trapping e. Nasal polyps; radiographic or CT abnormalities of the paranasal sinuses f. Digital clubbing
2. Gastrointestinal and nutritional abnormalities, including: a. Intestinal: meconium ileus, distal intestinal obstruction syndrome, rectal prolapse b. Pancreatic: PI, recurrent acute pancreatitis, chronic pancreatitis, pancreatic abnormalities on imaging c. Hepatic: prolonged neonatal jaundice, chronic hepatic disease manifested by clinical or histological evidence of focal biliary cirrhosis or multilobular cirrhosis d. Nutritional: failure to thrive (protein-calorie malnutrition), hypoproteinemia and edema, complications secondary to fat-soluble vitamin deficiencies
3. Salt loss syndromes: acute salt depletion, chronic metabolic alkalosis
4. Genital abnormalities in males, resulting in obstructive azoospermia

(Mishra et al., 2005)

IV- Investigations:

1-sweat chloride test:

The confirmatory test, the gold standard, is the quantitative analysis of electrolytes in sweat, with an accuracy >90%. Sweating is stimulated by pilocarpine, performed by iontophoresis; sweat is obtained using the Gibson and Cooke method. The sweat collection system with a capillary micro tube Macro duct has been widely used due to its simplicity and efficiency. The test is positive if the chloride concentration is >60 mmol/L in at least two independent measurements. For infants younger than 6 months, values between 30 mmol/L and 50 mmol/L are considered suspect ***(Mishra et al., 2008).***

The conductivity test also constitutes an alternative and valid method for the laboratory diagnosis of CF. The system measures the capacity of the sweat to conduct electrical current in milliamperes, which depends on the concentration of Na^+ and Cl^-.

The Sweat Check 3120conductivity analyzer specifically designed for use with the Macroduct sweat collector, measures conductivity in a 6–10 μL sample. Values >80 mmol/L and a compatible clinical setting justify the start of treatment, whereas values between 50 mmol/L and 80 mmol/L indicate the need to perform the quantitative test, because conductivity is considered a screening method, as it is not selective to the chloride ion***(Beauchamp and Lands , 2005).***

Interpretation

For infants younger than 6 months of age, a wide intermediate range is used because sweat chloride concentrations in healthy newborns gradually decrease during the first weeks of life ***(Parad et al., 2005).***

Sweat chloride results for this age group are interpreted as follows:

- ≤29mmol/L: Normal (CF very unlikely).
- 30 to 59 mmol/L: Intermediate (possible CF).
- ≥60 mmol/L: Abnormal (Diagnosis of CF).

For infants> 6 months, children, and adults, sweat chloride results are interpreted as follow:

- ≤39mmol/L: Normal (CF very unlikely).
- 40 to 59mmol/L: Intermediate (possible CF).
- ≥60mmol/L: Abnormal (Diagnosis of CF).

(Farrell et al, 2008).

After the newborn period, sweat chloride concentrations in a healthy population rise with age. Median sweat chloride concentration in a healthy

population rises from 13mmol/L in mid-childhood to 23mmol/L in young adults ***(Mishra et al., 2008).***

For patients with intermediate sweat chloride results, sweat chloride teasting should be repeated. For asymptomatic infants, the sweat chloride test should be repeated at 1 to 2 months of age, and then at 6 to 12 month intervals until the diagnosis is clear.in symptomatic infants or children, or for infants who were younger than 2 weeks old when first tested, it may be appropriate to repeat the sweat chloride test sooner. Intermediate results of sweat testing also should be investigated with DNA analysis; using a CFTR multimutation method approximately 20% of children with intermediate sweat chloride results will have DNA evidence of CF on expanded analysis ***(Lebecque et al., 2002).***

A sweat chloride value greater than 60 meq/L is sufficient to confirm the diagnosis of CF in patients with clinical symptoms of CF.This value distinguishes most patients with CF from those with other forms of chronic pulmonary disease. Sweat chloride levels vary slightly throughout childhood. A variety of other clinical conditions may be associated with elevated sweat chloride levels, but none of these are readily confused with CF ***(Mishra et al., 2008).***

Very rarely, apparently healthy individuals have sweat chloride values>60 mmole/L.Therefore, positive results of sweat testing should be confirmed by repeat sweat chloride testing and/or DNA analysis. Another problem is that hypoproteinemic edema and concurrent administration of steroids can lead to a false decrease in the sweat chloride concentration ***(Farrell et al., 2008).***

Causes of false-negative and false-positive sweat test results are summarized in (table3) ***(Wayne, 2009).***

Table 3 Causes of false-negative and false positive sweat test results

False-positive results	False-negative results
Evaporation of the sweat sample Severe malnutrition Anorexia nervosa Atopic dermatitis(eczema) Familial hypoparathyroidism Pseudohypoaldosteronism Adrenal insufficiency Glucose-6-phosphatase Nephrogenic diabetes insipidus Mauriac syndrome Fucosidosis Klinefelter's syndrome Familial cholestatic syndrome	Dilytion of sweat sample Oedema Dehydration Hypoproteinaemia Mineralocorticosteroid treatment Some CFTR mutations,e.g.R117H

(Wayne, 2009)

2-Gene diagnosis:

CF is caused by loss of function mutations in the CFTR gene, which is located on chromosome 7. The CFTR protein is a cAMP/ATP-mediated ion channel that is expressed in a variety of cell types, including secretory and absorptive epithelial cells. Normal CFTR protein channels regulate chloride and bicarbonate anion flux through the cell membrane of epithelial cells to maintain electroneutrality and osmolarity across the epithelial membrane. It also regulates the activity of other ion channels and proteins. (**Abdel-Magid AF, 2016).**

There are about 2000 known mutations in the CFTR gene. The majority of them is extremely rare and does not lead to CF. only about 125 CFTR mutations have been identified as CF disease-causing mutations. The most common of these mutations is F508del mutation (deletion of a phenylalanine on position 508), which causes defective processing of CFTR in the endoplasmic reticulum (ER) (**Abdel-Magid AF, 2016).**

3-Nasal potential difference measurements

The profile of CF patients is characterized by hyperpolarization of basal increased Na+ channel activity, which can be detected by evaluating the magnitude of nasal transepithelial potential difference (NPD) recorded in the presence of a Na+ channel blocker (amiloride), and defective adenosine cAMP-mediated Cl- secretion. Detected by the PD changes in response to B-adrenergic agonists in a low chloride solution ***(Sermet-Gaudelus et al., 2006).***

Abnormalities in epithelial chloride secretion can be demonstrated in most CF patients by evaluating the NPD. The test provides supportive evidence of CFTR dysfunction, and can be helpful in evaluating a patient in whom sweat chloride testing and DNA testing are inconclusive; in these patients nonclassic (atypical) CF may be suspected because of CF-like disease in one or more organ systems***(Sermet-Gaudelus et al., 2010).***

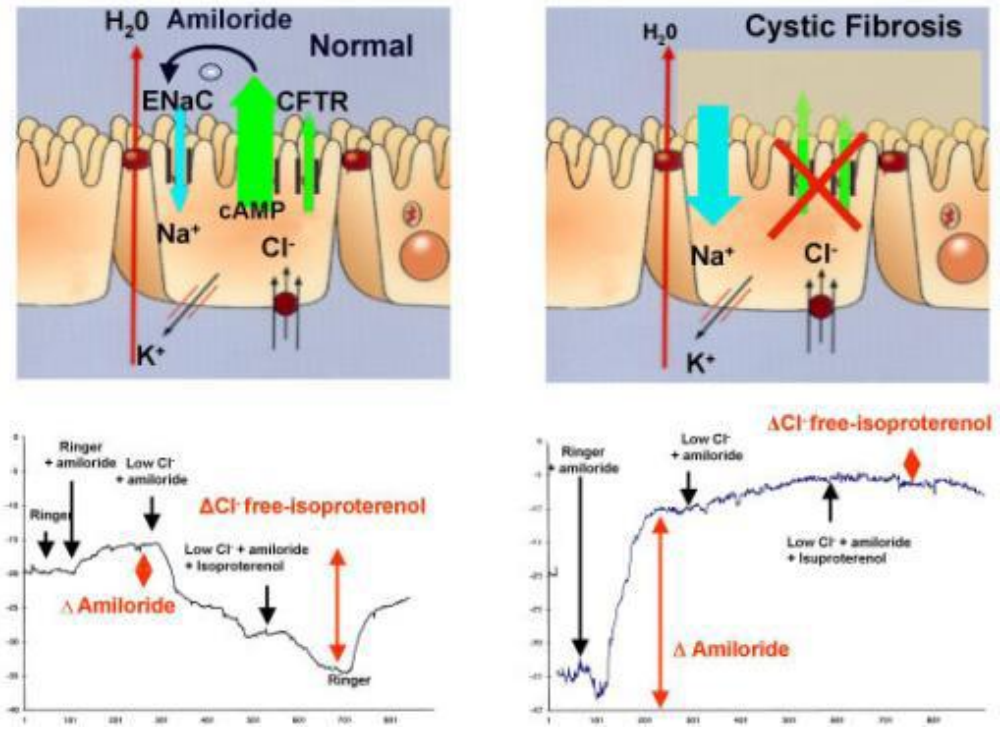


Figure 5: Ion transport in normal and CF nasal epithelium. Nasal PD Measurements in a healthy subject and in a CF patient (Sermet-Gaudelus et al., 2006).

Measurements are taken 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 (***Fajac et al., 2004).***

The presence of nasal polyps or inflammation may result in false negative results. Only a few centers performing this test have adapted the technique for use in children ***(Farrell et al., 2008 and Sermet-Gaudelus et al., 2010).***

The degree of abnormality in nasal transepithelial potential difference is not correlated with the severity of CF lung disease. However, specific abnormalities in nasal transepithelial potential difference are associated with distinct phenotypes of CF. In general, abnormal chloride secretion is associated with pancreatic insufficiency, while sodium hyperabsorption is more common among patients with severe lung disease ***(Fajac et al., 2004).***

4- Newborn screening for CF:

The goal of prepregnancy or prenatal carrier screening is to identify couples at increased risk of having a child with CF so that they can make an informed decision about pregnancy. In 2011, the American college of obstetricians issued an updated committee opinion on CF screening, based in part on the screening experience of the previous 10 years. This opinion reiterated that, ideally, counseling and screening are performed before conception or in the first or early second trimester. It also stated that screening for CF should be offered to all patients contemplating pregnancy. However, because mixed ethnicity is common, it is reasonable to offer CF carrier screening to all patients. Special consideration should be given to screening individuals with a personal or family history of CF ***(American College of Obstetricians, 2011).***

Most infants with CF have, elevated blood levels of immunoreactive trypsin (IRT), which can be quantified by radioimmunoassay or by an enzyme-linked immunoassay. The test can be performed on the dried blood sample obtained for newborn screening purposes, and allows detection of at least 95 percent of newborns with CF ***(Wagener et al., 2004).***

IRT levels fall rapidly during infancy, and a negative result is not informative after eight weeks of age ***(Farrell et al., 2003 and Wagener et al., 2004).***

In addition, the rates of false-positive and false-negative results are relatively high in many series ***(Wagener et al., 2004 and Fritz and Farrell, 2012).***

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. In most US states, DNA analysis for mutations in the CF gene also is used to screen newborns. This may be used 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. An IRT/IRT screening protocol has somewhat lower costs, but more delayed or missed diagnosis as compared with an IRT/DNA screening protocol ***(Wells et al., 2012).***

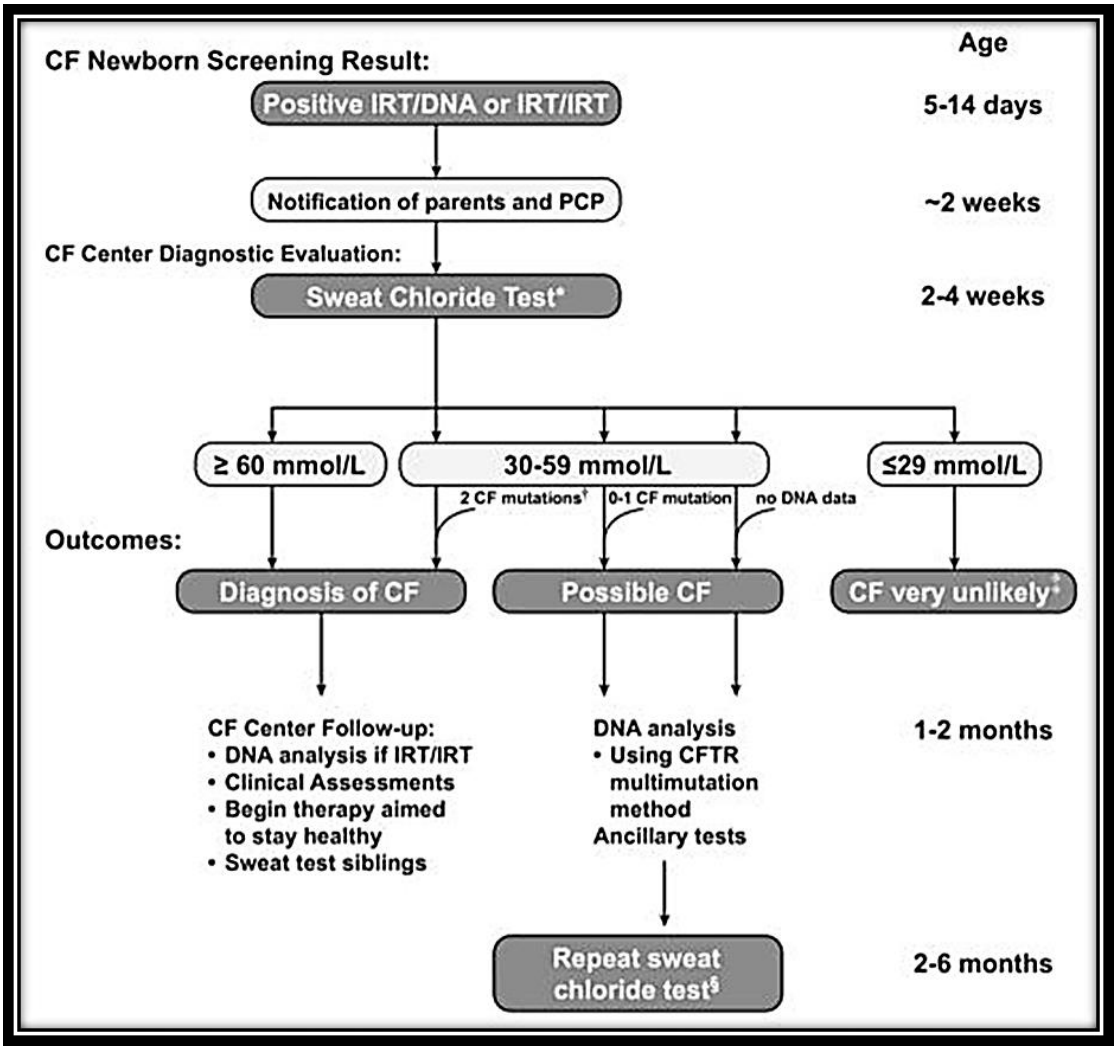


Figure 6: The CF diagnostic process for screened newborns.

(Farrell et al., 2008)

5- Pancreatic function testing

a- Fecal elastase

Measuurements of fecal elastase provide a useful index of exocrine pancreatic function ***(Walkowiak et al., 2004).***

This test should be performed for each patient with CF to help determine the need for pancreatic enzyme replacement therapy (PERT).Because it is not a quantitative test, it is not valuable as a measure to monitor the effectiveness of PERT ***(Borowitz et al.,2004).***

Fecal elastase values less than 200 micrograms/gram indicate pancreatic insufficiency ***(Cystic Fibrosis Foundation, 2009).***

Fecal elastase testing has high sensitivity and specificity in detecting severe pancreatic insufficiency in children with CF. However, the test performs less well for detecting mild or moderate pancreatic insufficiency, and also displays variability with repeat testing in this type of patient. Thus, results of fecal elastase testing should be combined with clinical observations, including nutritional status and symptoms of steatorrhea, to determine the need for PERT ***(Weintraub et al., 2009).***

Determination of fecal elastase can be performed on a single stool sample that requires no special storage, and does not require discontinuation of pancreatic enzymes. Thus, it is more clinically practical than a 72-hour collection of fecal fat which is not ideal. The 72 hour stool collection procedure is onerous for patients, and PERT must be discontinued during the collection period. Furthermore, the test does not distinguish between hepatobiliary, mucosal, and pancreatic causes of fat malabsorption ***(Borowitz et al., 2007).***

Determination of fecal elastase on a single stool sample is also more clinically practical than secretin stimulation tests. The results of fecal elastase testing correlate well with the secretin stimulation test. The sensitivity and

specificity of fecal elastase in detecting severe pancreatic insufficiency in children with CF range from 89 to 100 percent and 86 to 100 percent, respectively, depending upon the cut-off values (<100 versus<200 mcg/g stool) and the gold-standard for diagnosis (eg, secretin stimulation, fecal fat collection, clinical evaluation) ***(Borowitz et al., 2007).***

b- Secretin stimulation tests

Secretin stimulation tests which involve the collection of duodenal aspirates after stimulation of the pancreas with a secretagogue such as secretin. The basis for this test is that secretin causes the secretion of bicarbonate-rich fluid from the pancreas. A peak bicarbonate concentration of less than 80 mEq/L is consistent with pancreatic exocrine insufficiency ***(Borowitz et al., 2004).***

c- C breath tests

Several (13) C-labeledsubstrates that are digested by pancreatic enzymes have been proposed for breath tests, thus assessing the intraluminal activity of pancreatic enzymes and therewith the pancreatic exocrine function. Particularly in pediatrics, (13) C breath tests are suited not only for diagnosis of pancreatic exocrine disorder, but also for therapy control under pancreatic enzyme substitution. However, the costs of substrates, the high time expenditure, and the lack of standardization still limit the clinical use of these breath tests ***(Braden, 2010).***

***D*- CF-related diabetes mellitus**

The CFF and Amercan Diabetes Association recommended annual screening for CFRD beginning at age 10 years, carried out at a tme of clinical stability ***(Moran et al., 2010).***

An oral glucose tolerance test (OGTT) should be used for screening because either fasting plasma glucose or hemoglobinA1c has low sensitivity in this patient group. Continuous glucose monitoring is a new technique that might be more sensitive than OGTT for detecting abnormal hyperglycemia. However, this technique requires a sophisticated device and is probably not practical for routine clinical screening ***(O'Riordan et al., 2009).***

6 -Imaging technique

a- Plain films

In patients with mild lung disease the chest radiograph may appear normal for many years, but in most patients at least mild radiographic findings are evident in the first decade of life. The first discernible change is usually hyperinflation, which may initially be reversible with treatment for acute exacerbations of infection. As the disease progresses, hyperinflation becomes persistent, and the bronchovascular markings become more prominent. For unclear reasons, abnormalities tend to appear in the upper lobes first, progressing to the lower lobes with advancing disease. With time, the bronchovascular markings progress to a pattern of bronchiectasis and cyst formation. Peribronchial cuffing and "tram tracks" (parallel lines caused by thickened bronchial walls in longitudinal section) appear, followed by the rounded shadows of saccular bronchiectasis ***(Farrell et al., 2009).***

Increasing hyperinflation leads to progressive flattening of the diaphragms, a prominent retrosternal space, and kyphosis in late stages of disease. Thin-walled cysts (most common in the upper lobes) may appear increasing frequency in older patients ***(Flume et al., 2005).***

In advanced stages of disease, the chest radiograph may demonstrate little or no correlation with acute clinical changes. With good clinical care and

careful attention to daily airway clearance, the classic radiographic signs of CF may be delayed for many years ***(Quinton and O'Connor, 2007).***

b- Chest computed tomography

Computed tomography (CT) of the chest may be helpful in defining the extent of bronchiectasis in some patients ***(De Jong et al., 2004).***

This is of particular interest in patients who have focal areas of advanced disease, which may sometimes be amenable to surgical resection. CT surveillance may also be useful to monitor the presence and/or progression of disease caused by atypical mycobacteria. The degree of bronchiectasis noted on CT is associated with infection with mucoid strains of Pseudomonas aeruginosa. However, the degree of bronchiectasis is only weakly correlated with measures of pulmonary function, and not well correlated with exercise performance ***(Farrell et al., 2009).***

Serial high resolution CT scans often display progressively severe disease, despite apparently stable pulmonary function ***(De Jong et al., 2007).***

Changes in pulmonary function may be more subtle than or lag behind the CT abnormalities. CT scanning may demonstrate evidence of unsuspected air trapping and inhomogeneities of lung inflation even in very young asymptomatic children, and are thus a sensitive indicator of early disease in chidren too young to perform pulmonary function testing ***(Brody et al., 2005)***

7-Pulmonary function tests

In infants with CF, subtle changes in lung structure and function may be identifiable from a very early age, even before clinical signs of disease are apparent ***(Belessis et al., 2012).***

Pulmonary function tests (PFT) in infant are performed using a technique of forced expiration and are well validated. In infants with CF, the tests

typically are normal at the time of diagnosis by newborn screening, but may deteriorate by six months of age ***(Linnane et al., 2008).***

The decline in infant pulmonary function is associated with signs of pulmonary inflammation and infection ***(Pillarisetti et al., 2011).***

Infant lung function tests have been shown to correlate with results of standard pulmonary function testing performed six years later ***(Harrison et al., 2009).***

V- Differential Diagnosis:

The differential diagnosis of CF includes the following ***(Moskowitz, 2008):***

Dysphagia with chronic descending tracheal aspiration and gastroesophageal reflux (GER) with or without ascending tracheal aspiration.

- Similarities: chronic cough in infancy; may be associated with failure to thrive; either of these conditions may present as secondary complications in individuals with CF lung disease during infancy, or in the case of GER, later in childhood.
- Differences: chronic aspiration is typically associated with focal densities in the lower lobe of the right lung; cough is often temporally associated with feedings; steatorrhea is not associated with primary dysphagia and primary GER.

Immunologic abnormalities (esp. severe combined immunodeficiency)

- Similarities: may present with recurrent respiratory infections and chronic diarrhea in infancy.
- Differences: GI or nutritional mnifestations that are typically present in individuals with CF with respiratory symptoms during infancy are not associated with these anomalies.

Airway anomalies

- Similarities: Chronic cough and wheezing during infancy.
- Differences: GI or nutritional manifestations that are typically present in individuals with CF with respiratory symptoms during infancy are not associated with these anomalies.

Primary ciliary dyskinesia (PCD)

- Similarities: cough and sputum production during infancy; Pseudomonas aeruginosa or other opportunistic bacterial pathogens may be isolated from airway secretions (chronic sinus disease); may progress to chronic bronchiectasis.
- Differences: Situsinversus occurs in about 50% of persons with PCD; steatorrhea and failure to thrive are not associated with PCD; associated with mutations in multiple genes encoding different structural components of cilia.

Shwachman-Diamond syndrome (congenital lipomatosis of pancreas; Shwachman-Bodian syndrome; Switchman-Bodian-Diamond syndrome)

- Similarities: pancreatic insufficiency, steatorrhea, and failure to thrive; respiratory symptoms may occur during infancy as a result of hypoplasia of the chest wall.
- Differences: Chronic neutropenia, anemia, thrompocytopenia, or pancytopenia; additional hematologic abnormalities (myelodysphasia) with increased risk of leukemic transformation; skeletal abnormalities (metaphysealdysostosis type); caused by

autosomal recessive mutation of the (Shwachman-Bodian-Diamond syndrome) SBDS gene.

Biliary atresia

- Similarities: Rarely, individuals with CF may present in infancy with symptoms of biliary obstruction, but without other clinically apparent GI or respiratory manifestations.
- Differences: Serum levels of immunoreactive trypsinogen and stool levels of elastase should be normal in primary biliary atresia, whereas CF liver disease in invariably associated with evidence of pancreatic duct obstruction.

Other diseases:

Non-cystic fibrosis bronchiectasis

Non cystic fibrosis bronchiectasis in children presents as a wide spectrum of disease severity. It is usually due to acute or chronic infection or inflammation, anatomic airway obstruction, or underlying congenital disease that predisposes to chronic infection. The presentation includes recurrent respiratory infections, productive cough, shortness of breath, and occasional hemoptysis. Bronchiectasis is more likely to occur in children born prematurely and with both early onset and increased frequency of lower respiratory infections.Because bronchiectasis is radiographically or anatomically visualizing the typical changes. In patients with suspectedbrochiectasis without characteristic chest radiograph findings, a high-resolution computed tomography (HRCT) scan is the diagnostic procedure of choice ***(Singleton et al., 2013).***

Pseudo-Bartter syndrome (PBS)

PBS is an uncommon cause of metabolic alkalosis that has been seen as a presenting feature of CF as well as a complication in those with known disease. It is accompanied by chronic salt depletion and sometimes failure to thrive without severe dehydration. Principal findings are hypokalaemichypochloraemic metabolic alkalosis, sometimes with hyponatraemia. This may be preceded by anorexia, nausea, vomiting, respiratory exacerbations, fever and weight loss ***(Kose et al., 2008).***

PBS is characterized also by hyperaldosteronism, hyperreninism, normal blood pressure and hyperplasia of the juxtaglomerular apparatus. The most dangerous complication of PBS is hypokalemia. Hypokalemia caused by vomiting, diarrhea, prolongedfasting, abuse of potassium-depleting drugs and may be present in patients with binge purging from of anorexia or bulimia nervosa ***(Gentile, 2012).***

Primary adrenal hyperplasia

Primary adrenal hyperplasia is a destructive disease marked by deficient adrenocortical secretion and characterized by extreme weakness, loss of weight, low blood pressure, gastrointestinal disturbances, and brownish pigmentation of the skin and mucous membranes. However, these findings are relatively unspecific and can be found in the context of several conditions other than adrenal insufficiency. To establish the diagnosis of adrenal insufficiency with confidence, a short synacthen test (SST) needs to be performed. This test is also known as an ACTH stimulation test or a cosyntropin test ***(Arlt and Allolio, 2003).***

Treatment of Cystic Fibrosis

The treatment of CF lung disease is experiencing a period of rapid evolution, supported by well-designed clinical trials and improved understanding of the genetics and pathophysiology of the disease ***(Strausbaugh and Davis, 2007 and Ratjen, 2009).***

While the focus is on pulmonary therapies, it must be kept in mind that management is often suboptimal unless the multisystem nature of the disease is considered. Sinus infection, nutritional status, glucose control, and physiological issues must all be assessed at regular intervals ***(Yankaskas et al., 2004).***

1- TREATMENT OF RESPIRATORY COMPLICATIONS

A-Treatment of infection

Respiratory disease is the major cause of mortality and morbidity in CF. Life expectancy of people with CF has increased dramatically in the last 40 years. One of the major reasons for this increase is the mounting use of antibiotics to treat chest exacerbations caused by bacterial infections. The optimal duration of intravenous antibiotic therapy is not clearly defined. ***(Plummer et al., 2016).***

Individuals usually receive intravenous antibiotics for 14 days, but treatment may range from 10 to 21 days. A shorter duration of antibiotic treatment risks inadequate clearance of infection which could lead to further lung damage. Prolonged courses of intravenous antibiotics are expensive and inconvenient and the incidence of allergic reactions to antibiotics also increases with prolonged courses. The use of aminoglycosides requires frequent monitoring to avoid some of their side effects. However, some organisms which infect people with CF are known to be multi-resistant to antibiotics, and may require a longer course of treatment. ***(Plummer et al., 2016).***

The onset and rate of chronic airway infection varies widely among patients due to differences in environmental influences, genetic effects, and medical interventions. Nonetheless, some patterns of infection can be observed. Clinical microbiology laboratories identify staphylococcus aureus as the most prevalent infecting bacteria in childhood; it continues to be a frequent pathogen throughout adulthood ***(Cystic Fibrosis Foundation, 2010).***

- The portion of S. aureus that is methicillin resistant has been increasing ***(Dasenbrook et al., 2010).***
- Haemophilus influenza is present in 20 to 30 percent of patients in childhood, but it becomes less prevalent in adult.
- Pseudomonas aeruginosa, which can be isolated in about 25 percent of infants, becomes the most frequently isolated bacteria in adult, reaching a prevalence rate of up to 80 percent.

Antibiotic therapy for CF patients is directed at preventing, eradicating, or controlling respiratory infections. The therapy generally starts with oral and inhaled therapies in an outpatient setting and the use of intravenous route for patients with severe exacerbations ***(Sriramulu, 2013).***

The fluoroquinolones (e.g., ciprofloxacin) are the most commonly used oral agents to treat acute exacerbations caused by P. aeruginosa infection. Other agents that have long been used by inhalation in CF patient for the treatment of P.aeruginosa lung infection are tobramycin, aztreonam, or colistin ***(Sriramulu, 2013).***

Current standard care guidelines for antibiotic recommend in CF patients for most commonly bacterial species are described in Table 4.

New antibiotic combinations have been developed **(Anderson et al., 2013).** One example is the combination of fosfomycin/tobramycin (FTI), an

inhaled antibiotic with broad-spectrum antibacterial activity for treatment of bacterial respiratory infections. FTI consists of fosfomycin (F) and tobramycin (T) in a 4:1 weight-to weight ratio (w/w); this combination has promising activity against MRSA and P. aeruginosa with greater activity under aerobic and physiologically relevant anaerobic conditions, compared to F or T alone ***(McCaughey et al.,2013).***

Inhaled antibiotics have been probably the safest and most effective therapy for P.aeruginosa chronic lung infection in CF patients ***(Máiz et al., 2013).*** The use of inhaled antibiotics allows it to be delivered directly to the target area, with a lower dose than more conventional oral or intravenous delivery methods, with reduced systemic absorption and consequently reduced risk of toxic effects ***(Hoppentocht et al., 2014).***

Table 4: Antibiotic therapy use for bacterial species most commonly associated with CF airway disease

Species	Infection phase	Antibiotic therapy
P. aeruginosa	First isolated from patients Chronic infection	Oral ciprofloxacin or Inhaled colistin or tobramycin or aztreonam Two inhaled antibiotics among the following: colistin, tobramycin, aztreonam
H. influenza	—	Oral or intravenous amoxicillin + clavulanic acid depending on the severity.
S. aureus	First isolated from patients Chronic Infection	Oral flucloxacillin or Oral flucloxacillin + oral or intravenous rifampicin or fusidic acid Oral flucloxacillin
MRS A: Methicillin-resistant Staphylococcus aureus	First isolated from patients Chronic infection	Oral rifampicin + fusidic acid Intravenous vancomycin or teicoplanin or linezolid
B. cepacia	—	At least two intravenous antibiotics: Intravenous ticarcillin + clavulanic acid or piperacillin + tazobactam

(Döring et al., 2012).

Chronic treatment with oral antibiotics to control infection is not encouraged because the benefits have not outweighed the problems associated with antibiotic resistance ***(Breen et al., 2012)*** with two exceptions:

- Azithromycin is recommended for many patients with CF; its benefits may be due to its anti-inflammatory and/or antibacterial properties.
- Chronic treatment with nebulized antibiotics directed against pseudomonas aeruginosa (eg, tobramycin and aztreonam) appears to improve lung function and is recommended for many patients ***(Ryan et al., 2003).***

Macrolide antibiotics

Macrolide therapy was found to be beneficial for patients with panbronchiolitis, a non-CF lung disease that is seen predominantly in Japan and is manifested by bronchiectasis and chronic pseudomonas infection. A systematic review concluded that six months of treatment with azithromycin improves respiratory functions in patients with CF and reduces the frequency of pulmonary exacerbations ***(Southern et al., 2012).***

B-Bronchodilators

Airflow obstruction is a central feature of CF lung disease, and is caused by several mechanisms. Impairment to flow is due to bronchial plugging by purulent secretions, bronchial wall thickening due to inflammation, and airway destruction. A subgroup of CF patients also has airflow obstruction from bronchial hyperreactivity, many, but not all, of these patients show typical signs and symptoms of asthma, such as chest tightness, wheezing, and cough following exercise or exposure to allergens or cold air. Some of these patients are colonized with aspergillus species and fulfill diagnostic criteria for allergic bronchopulmonary aspergillosis ***(Stevens, et al, 2003).***

1-Inhaled Beta-2-adrenergic receptor agonists

In general, the recommendations of a guidelines committee of the Cystic Fibrosis Foundation***(Flume et al., 2007)*** advice the regular use of inhaled Beta-2-adrenergic receptor agonists in virtually all patients with CF (albuterol or a similar Beta-2adrenergic receptor agonist with rapid onset of action) for virtually all patients with CF, in the following situations :

- Immediately prior to sessions of chest physiotherapy and exercise to facilitate clearance of airway secretions.
- Immediately prior to inhalation of nebulized hypertonic saline, antibiotics, and/or DNase to limit nonspecific bronchial constriction induced by these agents and to potentially improve penetration and distribution of the drugs within the airways.

2-Other bronchodilators

The anti-cholinergic agents ipratropime bromide can induce bronchodilation following acute administration in patients with CF. numerous studies have established that it is a safe and effective therapy and that it improves a range of important clinical outcomes.for example, compared with placebo, tiotropium is associated with superior bronchodilation, increased inspiratory capacity, increased exercise capacity, reduced dyspnea, improved health status, and reduced exacerbations ***(Avi et al., 2005).***

However, inhaled ipratropium bromide is preferred over beta-2 agonists by many ***(Karner et al., 2012)*** as the bronchodilator of choice in COPD for the following reasons:

- Its minimal cardiac stimulatory effects compared to those of beta agonists.

- Its greater effectiveness than either beta agonist or methylxanthine bronchodilators in most studies of patients with COPD.

C-Agents to promote airway secretion clearance

Difficulty clearing purulent secretions from the airways is a universal complaint from CF patients who have moderate to severe lung diease. Chemical analysis of CF sputum has shown that its high viscosity is caused by the interaction of several macromolecules, including mucus glycoproteins, denatured DNA, and protein polymers such as actein filaments ***(Flume et al., 2007).***

1- Inhaled DNase I

The endonuclease DNase I can decrease the viscosity or purulent CF sputum by cleaving long strands of denatured DNA that are released by degenerating neutrophils. The human DNase I gene has been cloned, and the protein that it encodes can help liquefy CF sputum. A small but statistically significant reduction in the number of hospital days for exacerbations of respiratory disease was also seen in patients receiving the drug ***(Flume et al., 2007).***

A metaanalysis of randomized trials concluded that DNase treatment improves lung function and is well tolerated ***(Jones et al., 2010).***

A guideline committee of the CF Foundation recommends the chronic useof DNase for all children with CF older than 6 years of age, regardless of symptoms or pulmonary function tests ***(Flume et al., 2007).***

2- Inhaled hypertonic saline

Hypertonic saline has been administered by inhalation to hydrate inspissated mucus that is present in the airway of patients with CF. It is presumed that the high osmolality of the solution draws water from the airway

to re-establish the aqueous surface layer that is deficient in CF***(Donaldson et al., 2006).***

Because the mechanisms of action of DNase and hyertonic saline are different, their benefits may well be complementary. The guidelines committee of the CF foundation addressed each treatment separately and recommended both for the majority of patients with CF without assigning priority of one over the other ***(Flume et al., 2007).***

3-Inhaled N-acetylecystine

N-acetylecystine, a free sulfhydryl reagent that cleaves disulfide bond within mucus glycoproteins, can liquefy CF sputum in vitro. Although originally developed as an inhaled mucolytic agent, there are no well-designed studies that demonstrate its clinical utility ***(Flume et al., 2007 and Nash et al., 2009).***

D- Chest physiotherapy

Retained purulent secretions are an importatnt cause of airflow obstruction and air way injury in CF. In 1950, chest physiotherapy in the form of postural drainage and percussion was introduced to CF care and became the standard method to promote secretion clearance. Increasingly, methods that can be performed without the aid of another person are replacing the traditional technique in older children and adults. These alternatives include a variety of breathing and coughing techniques such as “autogenic drainage”, “active cycle of breathing”, and “huffing” ***(Hardy et al., 1996).***

Medical devices of varying cost and complexity have been developed to assist with airway clearance. These include airway oscillating devices, external

percussion vests, and intrapulmonary percussive ventilation ***(Bradley et al., 2006).***

Patients who produce sputum should be instructed in chest physiotherapy for secretion clearance. Adherence to chest physiotherapy is often poor, particularly among patients with mild disease ***(Arias et al., 2008).***

E- Exercise

Many patients with CF report that they mobilize secretions during aerobic exercise ***(Bradley et al., 2006).***

These with moderate or advanced disease should participate in organized pulmonary rehabilitation programs ***(Schnciderman et al., 2000).***

F- Anti-inflammatory therapy

Intense neutrophilic inflammation is a dominant pathological feature of the airways of patients with CF. although the inflammatory response was formerly viewed as being necessary to prevent the spread of infection, increasing information indicates that the amount of inflammation developed is probably excessive and harmful ***(Chmiel et al.,2007).***

1- Ibuprofen

Based on the recognition that anti-inflammatory glucocorticoids reduce the rate of FEV1 decline in CF, ibuprofen was studied to determine if similar benefits could be obtained without the prohibitive side effects of glucocorticoids. The clinical value of high-dose ibuprofen was demonstrated in patients with mild CF lung disease ***(lands et al., 2007).***

The guidelines committee of the CF foundation suggests the use of high-dose ibuprofen in children older than 6 years of age who have good lung function (FEV1>60 percent predicted) ***(Flume et al., 2007).***

2-Glucocorticoids

- Systemic glucocorticoids

The guidelines committee of the CF Foundation recommends against the routine chronic use of oral corticosteroids for children with CF aged 6 to 18 years, in the absence of asthma or allergic bronchopulmonary aspergillosis, because of the associated adverse effects. The committee found insufficient data on which to judge the value of chronic glucocorticoids in adults (***Cystic Fibrosis Foundation, 2009).***

- Inhaled glucocorticoids

Inhaled glucocorticoids have been prescribed in an effort to obtain the benefits that were demonstrated in the oral glucocorticoids trials while reducing the adverse effects of oral therapy although it seems reasonable to continue prescribing aerosolized glucocorticoids to CF patients who have definite signs and symptoms of asthma or allergic bronchopulmonary aspergillosis, there is insufficient evidence to warrant broader use ***(Balfour and Welch, 2012).***

One of the reasons for caution is that inhaled glucocorticoids may modestly impair linear growth in children with CF or asthma.these effects are dose related and less severe than those seen in children treated with systemic glucocorticoids (***Allen et al., 2006 and DeBoeck et al., 2007).***

G-Disease –modifying anti-rheumatic drugs (DMARDs):

Randomized controlled trials to review the effectiveness and safety of disease –modifying anti-rheumatic drugs (DMARDs) for the management of arthritis related to CF in adults and children suggested, that when episodic symptoms progress to persistent disease, disease-modifing anti-rheumatic drugs may be needed to limit the course of the disease ***(Thornton and Rangaraj,2012).***

H-Supplemental oxygen

Supplemental oxygen for patients with CF is recommended to treat intermittent or chronic hypoxemia. It is appropriate to assume that supplemental oxygen will delay or ameliorate the complications of chronic hypoxemia in CF, as it does in chronic obstructive pulmonary disease ***(Yankaskas et al., 2004).***

I-Noninvasive positive pressure ventilation

Noninvasive positive pressure ventilation (BiPAP) has been used for patients with advanced CF lung disease and hypercapnia ***(Young et al., 2008).***

In a randomized trial in adults with daytime hypercapnia, nocturnal use of BiPAP was compared to supplemental oxygen or placebo (air). Six weeks of BiPAP improved chest symptoms, exertional dyspnea, nocturnal hypoventilation, and peak exercise capacity, without measurable improvement in lung function. Based on these studies, it would be appropriate to offer nocturnal noninvasive BiPAP to patients whose arterial carbon dioxide level remains elevated (eg, $\geq$ 50 mmhg) despite maximizing other treatments ***(Granton et al., 2002).***

I-Intensive care unit treatment

Outcomes for CF patients requiring treatment in an intensive care unit was previously reported to be uniformly poor, but has fortunately improved. In modern series, survival was dependent upon the severity of respiratory failure, with the best outcomes for those who could be managed by noninvasive ventilation and the worst for those requiring endotracheal intubation-ventilation ***(Sood et al., 2001 and Texereau et al., 2006).***

J- Recent lines of treatment of CF

1- Gene therapy

Basic principle of gene therapy is deleviring a new correct copy of the gene to the cell, so that the cell can produce the correct protein and thus function normally **(Tim, 2005).**

Molecular basis of new and emerging treatments

CFTR modulators

CFTR modulators are designed to treat the underlying cause of cystic fibrosis by targeting the CFTR protein defect. Small molecule pharmacologic agents that target defects in CFTR gating, processing, and synthesis have undergone rigorous preclinical evaluation over the past decade and include CFTR potentiators, correctors, and translational read-through agents, respectively (fig 4).

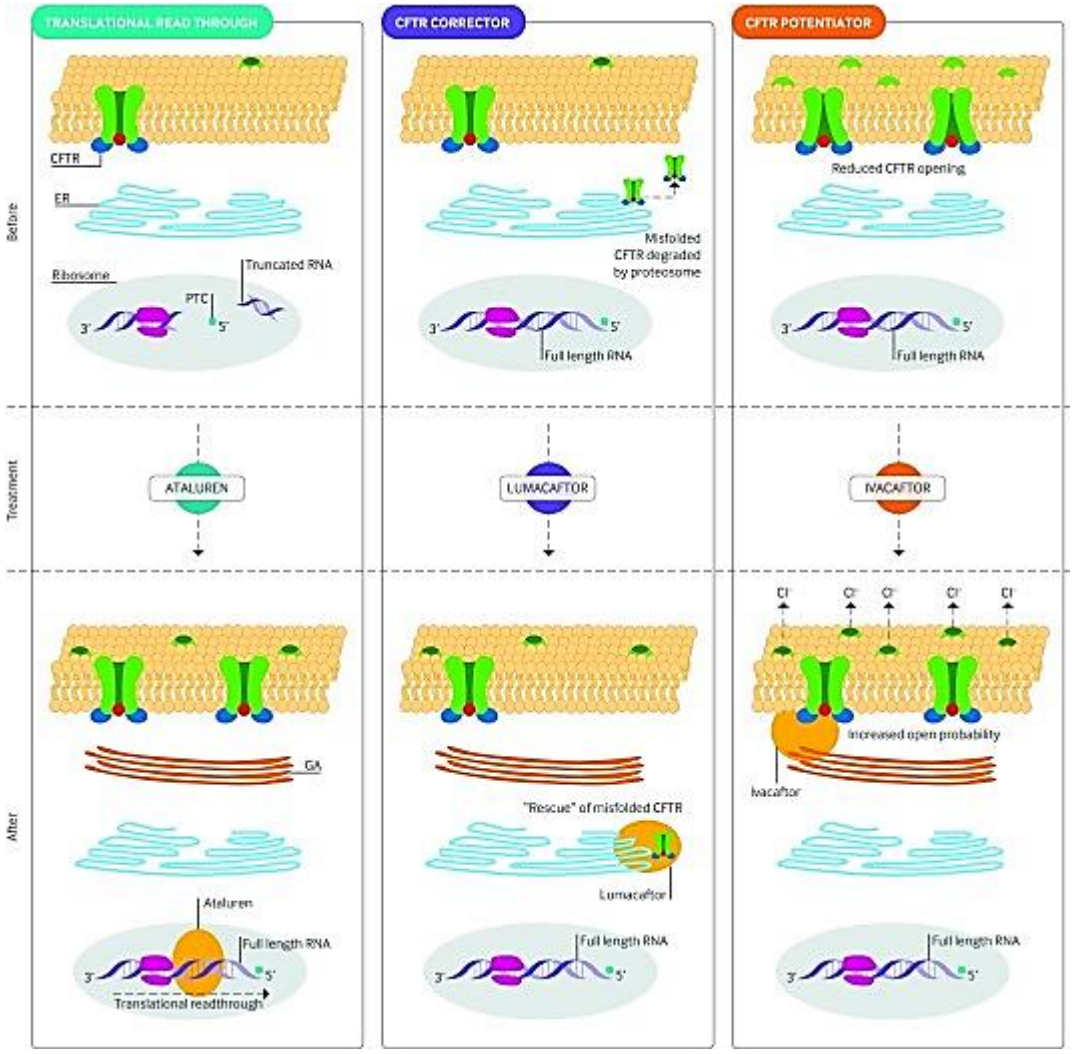


Figure 7: Molecular basis of CFTR modulators: fate of CFTR before and after CFTR modulator treatment (Quon, 2016).

Ataluren permits selective ribosomal read-through of the premature termination codon allowing the production of full length transcript and CFTR protein. Lumacaftor is capable of interacting directly with the CFTR to facilitate its correct folding or by modulating components of the cellular quality control machinery to allow proper trafficking of CFTR to the cell surface. Ivacaftor stabilizes the open state of the CFTR, thus increasing channel opening time. Areas shaded in red are the presumed targeted sites of action. The size of the inner dark circle of the CFTR channel at the apical surface reflects the extent of channel opening before and after CFTR potentiator therapy. CFTR=cystic fibrosis transmembrane conductance regulator; GA=Golgi apparatus; ER=endoplasmic reticulum; PTC=premature termination codon

CFTR potentiators

CFTR potentiators increase the flow of ions through surface localized, activated CFTR channels **(Van Goor et al., 2009).** People with class III mutations such as G551D (c.1652G>A) have normal amounts of CFTR protein at the cell surface but have primary defects in CFTR channel gating, making them ideal targets for potentiator therapy.

In preclinical studies, ivacaftor (formerly VX-770) was identified as a promising CFTR potentiator after HTS of more than 200 000 chemically diverse compounds and medicinal chemistry optimization. Using G551D recombinant cell lines, ivacaftor increased CFTR channel opening time sufficiently to increase CFTR activity from about 5% to about 50% of wild-type levels as assessed by electrophysiologic measurements ***(Van Goor et al., 2009).***

Ivacaftor also approximately doubled wild-type CFTR chloride transport. Although the precise mechanism of its action remains incompletely understood, evidence suggests that ivacaftor stabilizes the open state of CFTR, thus increasing channel opening time. The discovery of ivacaftor has provided proof of the concept that CFTR related chloride secretion can be potentiated **(Jih and Hwang, 2013).**

CFTR correctors

CFTR correctors repair defective CFTR processing by facilitating proper maturation and delivery of protein to the plasma membrane. Correctors act by interacting directly with CFTR to facilitate its correct folding or by modulating components of the cellular quality control machinery (***Rowe and Verkman, 2013).***

Patients with class II mutations (such as F508del) are primary targets for CFTR corrector therapy because the misfolded protein is retained within the endoplasmic reticulum and prematurely degraded. The potential impact of

developing efficacious CFTR corrector therapy is profound, because 85-90% of patients with cystic fibrosis have at least one copy of the F508del allele.

Alongside the discovery of ivacaftor, HTS identified small molecules capable of augmenting F508del-CFTR activity in recombinant cell based assays ***(Van Goor et al., 2011).***

Using human bronchial epithelial cell lines from the lungs of F508del homozygotes, lumacaftor (formerly known as VX-809) improved CFTR maturation eightfold and enhanced F508del-CFTR mediated chloride transport fourfold. However, lumacaftor only partially rescued the F508del-CFTR processing defect, because the maximum chloride transport achieved was estimated to be about 15% of wild-type levels. Mechanistic studies have shown that lumacaftor probably increases the conformational stability of F508del-CFTR, thus reducing cellular misprocessing and allowing at least some of the CFTR to move from the endoplasmic reticulum to the cell surface ***(Mendoza et al., 2012).***

CFTR read-through agents

CFTR read-through agents promote the ribosomal "read-through" of PTCs in CFTR mRNA. The first read-through agents examined in cystic fibrosis were aminoglycoside antibiotics, which are commonly used in cystic fibrosis to combat Gram negative bacteria such as Pseudomonas aeruginosa. Aminoglycoside antibiotics such as gentamicin are capable of inhibiting ribosomal "proofreading" by binding to the decoding site of rRNA. This reduces the fidelity of the codon-anticodon pairing and permits the erroneous addition of an amino acid to the polypeptide chain at the site of the PTC, allowing translation to continue to the end of the gene **(Quon, 2016).**

Read-through seems to be selective to PTCs because in vitro studies have found no detectable elongation beyond the native stop codon located at the 3′ end of mRNA owing to mechanistic differences between premature and normal termination codons.55 Unfortunately, high systemic levels of gentamicin, which can cause serious renal toxicity and ototoxicity, are needed to induce translational read-through ***(Welch, et al., 2007).***

Efforts have been made to modify the aminoglycoside chemical structure to provide higher read-through activity with less toxicity ***(Xue et al., 2014).***

For example, NB124 is a novel aminoglycoside derivative that was rationally designed to provide 2.5-fold greater read-through activity than gentamicin, restoring CFTR function to roughly 7% of wild-type levels. This compound was also less cytotoxic than gentamicin when evaluated in a tissue based model of ototoxicity ***(Xue et al., 2014).***

Ataluren (formerly PTC124) was identified as a lead candidate after medicinal chemistry optimization. The compound has no structural similarity to aminoglycosides or other clinically developed drugs.Subsequent in vivo experiments found that PTC124 could suppress the G542X nonsense mutation in a cystic fibrosis mouse model expressing the human CFTR-G542X transgene, restoring CFTR expression and function ***(Du et al., 2008).***

CFTR combination therapies

It has been estimated that 10-35% of normal CFTR function can result in a milder cystic fibrosis phenotype, offering promise that CFTR function does not have to be completely restored to have therapeutic benefit. In vitro and early clinical studies have shown that neither correctors nor potentiators alone are sufficiently active to provide clinical benefit in patients who are homozygous for the F508del mutation ***(Boyle et al., 2014).***

A pre-clinical study of lumacaftor and ivacaftor combined found that F508del-CFTR mediated chloride transport nearly doubled compared with lumacaftor alone, increasing chloride transport to about 25% of wild-type levels ***(VanGoor et al., 2011).***

Similar to the strategy used to treat HIV, a therapeutic cocktail consisting of multiple CFTR correctors, potentiators, and stabilizers might be needed to enhance CFTR function in challenging multidimensional genotypes such as F508del ***(Okiyoneda et al., 2013).***

Because many CFTR channels also exhibit abnormal gating, and even those with normal gating can be activated further, CFTR potentiators may also be useful to augment function when less than complete rescue has occurred with other agents.

There is evidence that wild-type CFTR counteracts the absorptive function of the epithelial sodium channel (ENaC), thus preventing excessive sodium and water intake.66 Unopposed ENaC function therefore exacerbates ASL desiccation, which is also caused by a lack of CFTR mediated fluid secretion. An investigational ENaC inhibitor to restore ASL known as P-1037 is being evaluated in a phase II placebo controlled trial of patients (aged ≥12 years) with mild to moderate lung disease **(Boucher, 2007).**

Other CFTR modulators

Other CFTR modulators currently in clinical development include agents that can directly and indirectly modulate the nitric oxide pathway, including phosphodiesterase-5 inhibitors (PDE-5; sildenafil) and guanylatecyclase stimulators (riociguat), and a new class of small molecule inhibitors of S-nitrosoglutathionereductase (GSNOR) (see fig 5).

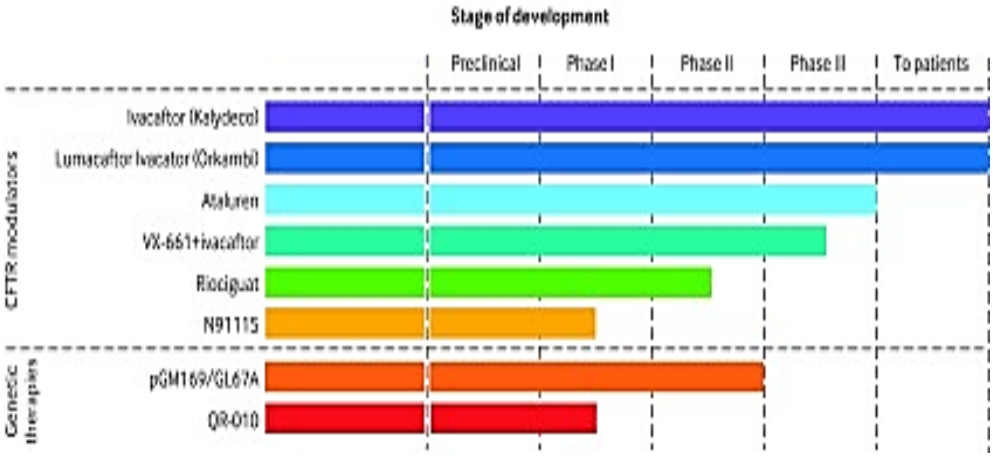


Figure 8: Cystic fibrosis transmembrane conductance regulator (CFTR) modulators and genetic therapies in development. (Quon, 2016).

Preclinical studies have shown that PDE-5 inhibitors can act as both potentiators and correctors of mutant CFTR through cGMP dependent and independent mechanisms, respectively **(Leier et al., 2012).**

Preclinical studies have also shown that soluble guanylate cyclase stimulators have CFTR corrector action and an ongoing phase II study is evaluating riociguat in F508del homozygous CF adults.

N91115 is an orally bioavailable inhibitor of GSNOR. In preclinical studies, increased levels of S-nitrosoglutathione (GSNO) achieved through GSNOR inhibition modified CFTR chaperone proteins, stabilizing the CFTR both inside the cell and at the cell surface ***(Zaman et al., 2014).***

N91115 thus represents a unique CFTR stabilizer under investigation. A phase Ib PCT is currently evaluating the safety and pharmacokinetics of N91115 as a sole CFTR modulator in F508del homozygous subjects. Subsequent clinical trials will focus on combining N91115 with ivacaftor or lumacaftor (or both) as a combined CFTR potentiator-corrector-stabilizer treatment for F508del homozygotes **(Quon, 2016).**

2- Vaccinations and palivizumab

- **Influenza vaccine**

Viral respiratory infections have been implicated as a frequent cause of exacerbations of CF lung disease ***(Cystic Fibrosis Foundation, and Jain et al., 2009).***

Based on efficacy in other populations, annual vaccination against viral influenza is recommended for all patients with CF older than 6 months of age, using an inactivated vaccine delivered by injection, but not the live attenuated vaccine delivered by intranasal spray ***(Jain et al., 2009).***

- **Pneumococcal vaccine**

The pneumococcal vaccine is recommended for all patients with CF because of a favorable risk-benefit profile, although Streptococcus pneumonia is not a major cause of pulmonary exacerbations in CF ***(Jain et al., 2009).***

- **Palivizumab**

Retrospective studies of palivizumab, a humanized monoclonal antibody against respiratory syncytial virus for children younger than 24 months of age, have suggested possible efficacy in CF, but definitive studies have not been performed, preventing firm recommendations ***(Robinson et al., 2012).***

3- Lung transplantation

Virtually all lung transplants for patients with CF require replacing both lungs, because leaving a native lung in place would present a huge source of infected secretions that would threaten the transplanted lung ***(Christie et al., 2010).***

Based on a consensus report from the International Society for Heart and Lung Transplantation ***(Orens et al., 2006)***, recommending that a patient should

be refered to a transplant center when any of the following indications are present:

- FEV1 below 30 percent predicted or arapid decline in FEV1, particularly in young female patients.
- Increasing frequency of exacerpations requiring antibiotic therapy.
- Refractory and/or recurrent pneumothorax.
- Recurrent hemoptysis not controlled by empolization.

Patients with CF who undergo lung transplantation have better survival rates compared with those of patients who are transplanted for other disease indications ***(Christie et al., 2010).***

K-treatment of pancreatic insufficiency:

The mainstay of treatment for pancreatic insufficiency in CF is pancreatic enzyme replacement therapy. Multiple formulations of pancreatic enzymes exist with different combinations of lipase, protease and amylase. ***(Cystic Fibrosis Foundation et al., 2009 and Stallings et al., 2008).***

Pancreatic enzymes are extracts of porcine pancreas. Most enzyme preparations are in the form of granules or microspheres that are coated with a ph-sensitive material that protects the enzyme from destruction by acid in the stomach. The coating dissolves in the alkaline medium of the duodenum, releasing the enzyme **(food and drug administration 2012).**

Dosing considerations

Most pancreatic enzyme preparations consist of capsules containing microspheres. Older children and adults generally swallow the whole capsule. For younger children and infants, enzymes are administrated by opening the capsule and sprinkling the microspheres on food. The food should be soft so that it does not require chewing and should be relatively acidic to avoid dissolving the enteric coating. Dosing of pancreatic enzymes is based upon the

units of lipase determined as a function of patient weight or dietary fat intake (***Pillarisetti et al., 2011).***

The weight based method can generally be used at any age. The starting dose for children less than four years of age is 1000 lipase units/kg body weight per meal, and for children older than four years of age is 500 lipase units/kg body weight per meal ***(Stallings et al., 2008).***

The fat based method is useful for infants who take a known amount of formula or in patients who receive tube feedings. The dose starts at approximately 2000 lipase units/120ml of formula or per breast feeding (about 1600 lipase units/gram of fat ingested per day). The dose can be adjusted up to no more than 2500 lipase units per kg body weight per feeding, with a maximum daily dose of 10000 lipase units per kg ***(Cystic Fibrosis Foundation, 2009).***

Pulmonary exacerbation in cystic fibrosis

The natural history of cystic fibrosis (CF) lung disease is characterized by a progressive decline in lung function with episodes of acute worsening of respiratory symptoms called "pulmonary exacerbations." ***(Flume et al., 2009).*** Although there is no universally accepted definition of a pulmonary exacerbation, features include symptoms such as increased cough or sputum production, fever, weight loss, decreased exercise tolerance, and absenteeism from school or work due to illness as well as new clinical findings, including tachypnea, new crackles, decreased pulmonary function tests, reduced oxyhemoglobin saturation ***(Rosenfeld et al .,2001).***

Although milder events are commonly treated with oral antibiotics on an outpatient basis, hospitalization or intravenous antibiotics are often used in severe pulmonary exacerbations ***(Goss and Burns, 2007).***

The annual number of acute pulmonary exacerbations was identified as a characteristic of the disease that predicted survival. Despite extensive knowledge regarding the effects of severe pulmonary exacerbations on outcomes in CF, little is known about the effects of milder exacerbations treated with oral antibiotics in individuals with CF. A single-center, retrospective study suggested that oral antibiotic treatment circumvented the need for intravenous therapy in approximately 74% of cases, although the associated impact on lung function was not described ***(Briggs, et al., 2012).***

Definitions of Exacerbation in Children with CF

Similar to other airway diseases, defining pulmonary exacerbations is a challenge in CF. Due to the variability in presenting symptoms, many definitions are linked to interventions, such as the administration of intravenous antibiotics, and none has been validated a priori. The Fuchs criteria for a pulmonary exacerbation, which are commonly used in clinical trials, rely on

treatment with intravenous antibiotics for 4 of 12 predefined respiratory signs or symptoms to identify an exacerbation state (Table 5) ***(Waters and Ratjen., 2015).***

Definitions have been used in major clinical trials evaluating new treatments in CF.They have combined patient symptomatology, laboratory data and clinician evaluation. These definitions of a pulmonary exacerbation have revolved around the clinician's decision to treat a constellation of symptoms, but a treatment decision-defined outcome is, by its nature, problematic. **(Goss and Burns, 2007).**

Two additional scoring systems were developed to diagnose pulmonary exacerbations and were used in two recent phase 2 CF clinical trials. The first score used was the Acute Respiratory Illness Checklist (ARIC). **(Piedra PA et al., 2003).** It was used as a symptom score to identify patients with lower respiratory tract infections, with the goal of capturing a wider spectrum of CF exacerbations in study participants. The second diagnostic score was the Respiratory and Systemic Symptoms Questionnaire (RSSQ; M W Konstan, personal communication); this score was created to have a uniform approach to identifying CF-related pulmonary exacerbations including mild events not necessitating intravenous antibiotics. Table 6 compares the signs and symptoms assessed in these four different instruments to diagnose a CF pulmonary exacerbation. **(Goss and Burns, 2007).**

Components of these definitions have been examined to see which clinical characteristics best predict a pulmonary exacerbation. The signs and symptoms that were most predictive of a pulmonary exacerbation in all of these studies were increased cough, change in sputum (volume or consistency), decreased appetite or decreased weight, change in respiratory examination and respiratory rate.**(Ferkol et al., 2006).**

Table 5: Definitions of pulmonary exacerbations in cystic fibrosis 1:

- Fuchs et al - Pulmozyme®: (25)
  "Exacerbation of respiratory symptoms": a patient treated with parenteral antibiotics for any 4 of the following 12 signs or symptoms:
  - Change in sputum
  - New or increased hemoptysis;
  - Increased cough;
  - Increased dyspnea;
  - Malaise, fatigue of lethargy;
  - Temperature above 38°C;
  - Anorexia or weight loss;
  - Sinus pain or tenderness;
  - Change in sinus discharge;
  - Change in physical examination of the chest;
  - Decrease in pulmonary function by 10 percent or more from a previously recorded value;
  - Radiographic changes indicative of pulmonary infection

- Ramsey et al - inhaled tobramycin: (17)
  Pulmonary exacerbation indicated by at least 2 of the following seven symptoms during the study:
  - Fever (oral temperature >38°C);
  - More frequent coughing (increase of 50%);
  - Increased sputum volume (increase of 50%);
  - Loss of appetite;
  - Weight loss of at least 1 kg;
  - Absence from school or work (at least 3 or preceding 7 days) due to illess;
  - Symptoms of upper RTI.

  These symptoms had to have been associated with at least one of the following 3 additional criteria:
  - Decrease in FVC of at least 10%;
  - An increase in respiratoty rate of at least 10 breaths per minute;
  - a peripheral blood neutrophil count of 15 000 per cubic millimeter or more.

(Goss and Burns, 2007)

Table 6: Definitions of pulmonary exacerbations in cystic fibrosis 2:

	Fuchs	Ramsey	ARIC	RSSQ
Signs and symptoms (new or increased)				
Pulmonary signs and symptoms				
Increased dypsnoea with exertion	×			×
Decreased exercise tolerance				×
Increased work of breath			×	
Cough	×	×		×
Day cough			×	
Night cough			×	
Wet or congested cough			×	
Chest congestion	×			×
Frequency of cough				×
Cough up mucus	×			
Wheezing			×	
Haemoptysis/coughing up blood	×		×	×
Sputum volume	×	×	×	×
Change in sputum appearance		×		×
Change in sputum colour			×	×
Change in sputum consistency			×	×
Increased respiratory rate		×		
Decreased lung function	×	×		
Upper respiratory tract symptoms				
Sore throat/runny nose		×	×	
Sinus pain/tenderness	×			×
Change in sinus discharge	×			×
Constitutional and GI signs and symptoms				
Malaise/fatigue/lethargy	×			×
Abdominal pain				
Fever	×	×		×
Decreased appetite/anorexia	×	×		×
Weight loss		×		×
Work/school absenteeism		×		×

ARIC, Acute Respiratory Illness Checklist; RSSQ, Respiratory and Systemic Symptoms Questionnaire (© Boehringer Ingelheim).

(Goss and Burns, 2007).

Causes of exacerbation

Exacerbations of respiratory symptoms in individuals with CF are associated with multiple factors, including shifts in the microbial composition, host characteristics, and environmental exposures ***(Goss andBurns, 2007).***

Increasing concentrations of nitrogen dioxide on the day before an exacerbation, for example, have been associated with an increased risk of antibiotic use ***(Goeminne et al., 2013).***

In addition, proton pump inhibitor use has been suggested to be associated with an increased frequency of CF pulmonary exacerbation, which

parallels their effect in the non-CF population in which they have been linked to a higher risk of pneumonia ***(Dimango et al., 2014).***

Regarding microbial factors, studies have demonstrated that, unlike other diseases such as chronic obstructive pulmonary disease, CF exacerbations are not associated with the acquisition of new strains of preexisting bacteria such as Pseudomonas aeruginosa ***(Aaron et al., 2004).***

Although sputum bacterial density of dominant pathogens such as P. aeruginosa is known to decrease with antimicrobial treatment of pulmonary exacerbations, there is little evidence to support the notion that exacerbations are due to transient increases in bacterial numbers within the CF lung ***(Stressmann et al., 2011).***

Although bacterial composition does not change in a consistent fashion, markers of pulmonary and systemic inflammation decrease with antimicrobial treatment of exacerbations, and failure to adequately decrease inflammation during pulmonary exacerbations, especially sputum neutrophil elastase, is associated with a lack of lung function recovery and an earlier time to subsequent exacerbation ***(Waters et al., 2015)***

Systematic reviews have demonstrated that significant increases in blood C reactive protein levels occur from stable to exacerbation states in patients with CF, but these markers of inflammation are nonspecific as to the cause of the exacerbation ***(Shoki et al., 2013)***

Concurrent viral infections are a possible explanation, especially in young children, and are discussed further below

Role of bacterial infection in exacerbation

Pulmonary exacerbations are usually caused by bacteria which are typically associated with CF (Staphylococcus aureus, Haemophilus influenzae,

P. aeruginosa, Burkholderiacepacia complex and other emerging CF pathogens). However, in clinical practice, it is often found that the respiratory tract cultures obtained at the time of exacerbation do not grow the typical bacteria. In a retrospective study looking at 672 admissions over 5 years, 17% overall were negative for the typical CF bacteria but had signs and symptoms of a pulmonary exacerbation and responded to treatment ***(Zemanick et al., 2010).***

S. aureus, H. influenzae (nontypeable) and occasionally S. pneumoniae are the bacteria most often encountered in pulmonary exacerbations in infancy and early childhood. CF subjects chronically colonized with Methicillin-resistant S. aureus (MRSA) can experience pulmonary exacerbations due to MRSA ***(Bhatt, 2013).***

Initial infection with P. aeruginosa is often environmentally acquired and can be cleared with eradication treatment. Subsequent intermittent isolates can also be eradicated but it eventually becomes an established chronic infection with biofilm formation in most patients. It has been proposed that biofilms play a role in infection persistence and planktonic forms play a role in pulmonary exacerbations. The worsening of symptoms during a pulmonary exacerbation, the proven clinical efficacy of b-lactam antibiotics in treating exacerbations and the selection for b-lactam resistance in vivo, suggest that the release of more sensitive planktonic organisms from the biofilm layer play a significant role in pulmonary exacerbations in CF ***(Van Devanter et al., 2005).***

Pulmonary exacerbations can be associated with the acquisition of new organisms. however, the majority of pulmonary exacerbations are not due to acquisition of new strains of Pseudomonas, but are instead due to clonal expansion of existing strains, especially in adult patients with CF ***(Aaron et al., 2004).***

A change in the bacterial density of colonizing flora has also been proposed as another potential mechanism of pulmonary exacerbation, as bacterial concentrations of Pseudomonas were shown to be high during an exacerbation and decreased with treatment with antimicrobial agents (with an associated reduction in symptoms and improvement in lung function). ***(Stressmann et al., 2011).***

Table7: Bacterial species most commonly associated with CF airway Disease

Species	*Clinical significance*
Pseudomonas aeruginosa	Arguably the most important pathogen; presents a prevalence of 80 % at ages ≥18 years; ability to develop biofilms that protect from host responses and numerous antibiotics
Haemophilus influenzae	Most frequently isolated during infancy and/or early childhood; ability to form biofilms
Staphylococcus aureus	Infects young patients, but can also be cultured from adolescents and adult patients; ability to cause chronic infection
Burkholderia cepacia complex	Important opportunistic pathogens Ability to cause a progressive, invasive and fatal pulmonary disease known as "cepacia syndrome"

Adapted from Huang et al (Huang and Lynch, 2011).

Role of viral infection in exacerbation

Respiratory viruses are responsible for more ill health across the globe than any other cause. Included in this spectrum of pathogens are human rhinovirus, the principal cause of the common cold, and influenza, while viruses such as respiratory syncytial virus (RSV) and human parainfluenza viruses lead to a huge burden of disease in children as the cause of bronchiolitis and croup ***(Cherry, 2008).***

Viral respiratory infections are universal and will typically affect children five to eight times per year while the average adult will experience up to four such illnesses annually ***(Flight and Jones, 2017).***

Infants and the elderly are disproportionately affected by these infections and are at greater risk of serious morbidity and mortality. A further group at risk is those with chronic lung disease. Respiratory viruses are responsible for up to 80% of acute exacerbations of asthma ***(Khetsuriani et al., 2007)*** as well as a third of exacerbations of chronic obstructive pulmonary disease (COPD).

Respiratory virus infection has been shown to play a role in a variety of other patient groups including those with bronchiectasis, pulmonary fibrosis and lung transplant recipients ***(Gao et al., 2015).***

Anecdotally, however, CF patients will frequently report that colds and other upper respiratory tract infections are the predominant cause of flares of their respiratory symptoms. Despite this, there is a relative paucity of literature on the role of respiratory viruses in CF lung disease and a matching lack of therapeutic options for these pathogens. ***(Flight and Jones, 2017).***

Respiratory viruses have been detected in 13 to 60% of patients with CF with increased respiratory symptoms ***(Asner S et al., 2012).***

Rates of detection are highest in children, although a significant number of adult patients with CF also have viral infections, most commonly rhinovirus ***(Goffard et al., 2014).***

In an analysis of over 400 pulmonary exacerbations requiring intravenous antibiotic therapy in adults with CF, exacerbations associated with a positive viral polymerase chain reaction (PCR) had a greater fall in lung function at presentation with higher serum inflammatory markers (including C reactive protein) ***(Etherington et al., 2014).***

In addition, these patients received more days of intravenous antibiotics, showed less response to treatment (in terms of change in FEV1 and FVC1%predicted), and had a shorter time to subsequent pulmonary exacerbation compared with matched control subjects. These findings have also been replicated in the pediatric population with CF in which patients with exacerbations and a positive viral PCR were less likely to recover lung function back to baseline compared with those with a negative viral PCR (***Esther et al., 2014).***

Although children with CF and healthy control subjects have similar frequencies of viral respiratory infections, there is evidence that there is greater virus-related morbidity in patients with CF ***(van Ewijk et al., 2008).***

The exact mechanism of how respiratory viruses affect lung disease in CF is not completely understood. Several studies have noted that new bacterial infections, such as P. aeruginosa infections, are often preceded by viral infections in children with CF and in otherwise healthy children; children with CF, however, are less able to clear P.aeruginosa colonization ***(Van Ewijk et al., 2006).***

Respiratory viruses can also directly affect the innate host defense system. Bronchoalveolar lavage studies have demonstrated a similar level of neutrophilic inflammation in patients infected with viruses or bacteria.

The viral immune response in patients with CF appears to be virus specific, with influenza and rhinovirus inducing distinct antiviral gene expression ***(Ramirez et al., 2014).***

In summary, although the exact mechanism by which viral infections may trigger a pulmonary exacerbation in children with CF has not yet been defined, viruses likely play a key role and need to be considered when studying exacerbations in this population. Yearly influenza A immunization is recommended for individuals with CF ***(Grohskopf et al., 2014)*** and early detection and treatment of influenza infections with oseltamivir may improve the outcomes of associated pulmonary exacerbations.

However, with the exception of oseltamivir, there are currently no effective antiviral therapies for the treatment of respiratory viruses. Despite this, understanding how these viruses alter the host inflammatory response and disrupt the pulmonary microbiome may allow us to target antimicrobial and adjunctive treatments more effectively during CF pulmonary exacerbations.

Respiratory viruses:

1-Human rhinoviruses (HRVs) belong to Picornaviridae family, genus Enterovirus, and are divided in three species (HRV-A, HRV-B, and HRV-C) with about 100 serotypes within these species ***(Palmenberg et al., 2010).***

The development of highly-sensitive molecular techniques for characterization of the HRV genome has recently allowed recognition of the HRV-C species. There is already evidence that this new species may be more virulent and more strongly associated with lower respiratory tract infections than HRV-A and HRV-B ***(Jacobs et al., 2013).***

HRV is the most common cause of upper respiratory tract infections, being responsible for at least 50% of cases of the common cold. This leads to considerable economic burden in terms of medical visits and both school and work absenteeism. HRVs have also been linked to lower airway effects that result in significant morbidity and mortality,such as exacerbations of chronic pulmonary disease, severe bronchiolitis in infants and children, as well as fatal pneumonia in elderly and immunocompromised adults ***(Jacobs, et al ,2013).***

In a study of 100 adult patients with CF followed prospectively over the course of a year, viral infections (predominantly rhinoviral) were associated with an increased risk of pulmonary exacerbation but not with a greater decline in lung function over the subsequent 12 months ***(Flight et al., 2014).***

In vitro studies have shown that rhinovirus can impair LPS-induced TNF-a and IL-8 secretion by alveolar macrophages, the major immune cells in the airways. Using bronchial epithelial cells from young children with CF, demonstrated a combination of decreased apoptosis, increased IL-8 response, and increased viral replication compared with non-CF cells after in vitro rhino viral infection, suggesting a dysregulated inflammatory response in CF epithelium ***(Sutanto et al., 2011).***

Interestingly, an independent study found that the proinflammatory cytokine response was similar in CF and non-CF human bronchial epithelial cells with increased cell death in CF cells in response to rhinovirus infection ***(Kieninger et al., 2012).***

In general, HRV infections occur during spring and autumn **(Litwin CM, Bosley JG, 2014)** and manifest differently depending on whether the lower or upper respiratory tract is infected. Infections of the upper respiratory tract ordinarily include symptoms of the common cold, but can present as acute otitis

media or rhino sinusitis. On the other hand, infections of the lower respiratory tract can cause severe symptoms and result in bronchiolitis and pneumonia.

HRV receptors, entry, and replication

HRVs are non-enveloped with a ss (+) RNA genome that is protected by an icosahedral protein capsid built of 60 copies each of the four viral proteins VP1–VP4 ***(Jacobs et al., 2013).***

Based on phylogeny, more than 150 HRV types are classified as species A, B, and C. Twelve HRV-A (the minor group) bind members of the low-density lipoprotein receptor (LDLR) family whereas the remaining A and B types (the major group) bind intercellular adhesion molecule-1 (ICAM-1) ; for HRV-C, the recently identified CDHR3 might serve as a receptor ***(Bochkov et al .,2015).***

For infection, the cognate receptor must be accessible to the virus, i.e., at the apical surface of ciliated epithelial cells. While reports on the location of ICAM-1 in the healthy nasal mucosa are contradictory, it is generally agreed that this receptor is upregulated upon inflammation ***(Blaas and Fuchs, 2016).***

Reinvestigating this issue, we detected ICAM-1 at the ciliated surface of all nasal epithelial cells in the nasal tissue from healthy individuals. As expected from its "normal" physiologic function, LDLR is located at the basolateral plasma membrane of the polarized airway, intestinal, renal, and hepatic cell lines. We were thus surprised to find that LDLR and LDLR-related protein 1 (LRP-1) are present at the apical side of the nasal epithelial cells and thus available for uptake of virus at its main port of entry ***(Blaas and Fuchs, 2016).***

HRVs of species A and B investigated so far enter cells by receptor-mediated endocytosis. In the endosomal lumen, they convert into subviral A

(altered) particles devoid of the innermost capsid protein VP4 but still containing the RNA genome. After the release of the RNA (uncoating) into the cytoplasm, empty capsids remain (Fig. 1). Minor group HRVs exclusively depend on the low endosomal pH for this conformational modification and uncoating occurs even at 20 °C ***(Jurgeit et al., 2010).***

Although uncoatingof HRV-A2 is receptor-independent, the ß-propeller of LDLR and LRP plays a role in releasing the virus in early endosomes thus enabling its transport to late endosomes (pH≤ 5.6), a station most suitable for RNA transfer into the cytosol ***(Fuchs and Blaas, 2010).***

On the other hand, it is generally accepted that conversion of ICAM-1 binding HRVs into A particles is facilitated by the receptor above 26 °C; in addition, depending on the serotype, the process also requires low endosomal pH.

However, major group HRV-A89 can also convert at 20 °C in a low pH-dependent but presumably receptor-independent manner and, even more importantly, it follows a route different from the one taken by HRV-B14 .

While HRV-A89 productively uncoats in the perinuclear recyclingcompartment, HRV-B14 penetrates into the cytoplasm byrupturing endosomes en route to the lysosomes in atemperature- (≥20 °C), low-pH, and ICAM-1-dependentmanner (Fig. 1). Whether this is related to different affinityof ICAM-1 for the respective virus—and associated differencesin dissociation of the virus-receptor complex—is currentlyunder investigation.

Once the RNA has arrived in the cytoplasm, it is translated into a polyprotein. After autocatalytic cleavage intothe structural (capsid) and non-structural proteins, the RNA is replicated by the viral polymerase. Finally, infectiousprogeny is assembled and released into the nasal cavity (***Jacobs et al., 2013).***

In contrast to HRV infection in tissue culture cells, airway epithelial cells of patients are not lysed for virus release; as shown for other enteroviruses, it is thus possible that cell-to-cell spread might occur via virus-carrying microvesicles ***(Inal and Jorfi, 2013).***

Host response to HRV infectionsAlthough initially believed that HRV infection was limited to the upper airways, replicating virus was found in ciliated epithelial cells of the lower respiratory tract. Infected cells appear in patches, and only 10 % of the ciliated cellsproduce viral proteins and RNA. Similar results hadbeen obtained with in situ hybridization in nasal biopsies, again indicating that only a small proportion of cells were infected. Nevertheless, basal cells are more susceptible to infection as compared to fully differentiated ciliated cells ***(Jakiela et al., 2008).***

This might be related to the higher expression level of ICAM-1 in basal cells versus ciliated cells. The absence of visible cytopathic alterationsin the airway epithelium led to the hypothesis thatthe symptoms are rather due to the immune response of the host ***(Kennedy et al., 2012).***

Upon HRV entry into and replication in ciliated epithelial cells, signalling pathways are activated leading to the release of various cytokines (IL-1ß, TNF, IL-8, IL-6, IL-11), chemokines (Rantes, MCP-1, MP-10), vasoactivepeptides (bradykinin), and growth factors (VFGF) ***(Kennedy et al., 2012).***

Consequently, inflammatory cells (leukocytes, granulocytes and monocytes) become activated and invade the submucosa. This results in amplification of the inflammatory processand the typical symptoms of the common cold.

Conversely, HRV infections are controlled by innate and adaptive immuneresponses. Type-I interferons are the early mediatorsof the innate immune system, while neutralizing IgA and IgG in serum and secretions are

observed 1–2 weeks after infection as a consequence of the adaptive immune response.

Nasal epithelial cells express the pIgA receptor (pIgR) and the neonatal Fc-receptor (FcRn) that transportthe respective immunoglobulins into nasal secretions ***(Heidl et al., 2015).***

We presented evidence for HRV-A2 transferring its genome into the cytosol via apore in the membrane and the remaining empty capsidbeing directed towards lysosomes where it is degraded. Onthe other hand, HRV-B14 breaks the endosomal membraneresulting in arrival of viral proteins in the cytoplasm ***(Fuchs and Blaas, 2010).***

As a consequence, one might hypothesize that the proteins of incoming virus are presented to the immune system either as products of proteasomal (HRV-B14) or lysosomal (HRV-A2) processing. Degradation products of the formerwould thus be mainly presented by the MHC-I system andthe latter mainly by the MHC-II system. Furthermore, pattern recognition receptors in the endosome are different from those in the cytosol ***(Jacobs et al., 2013).***

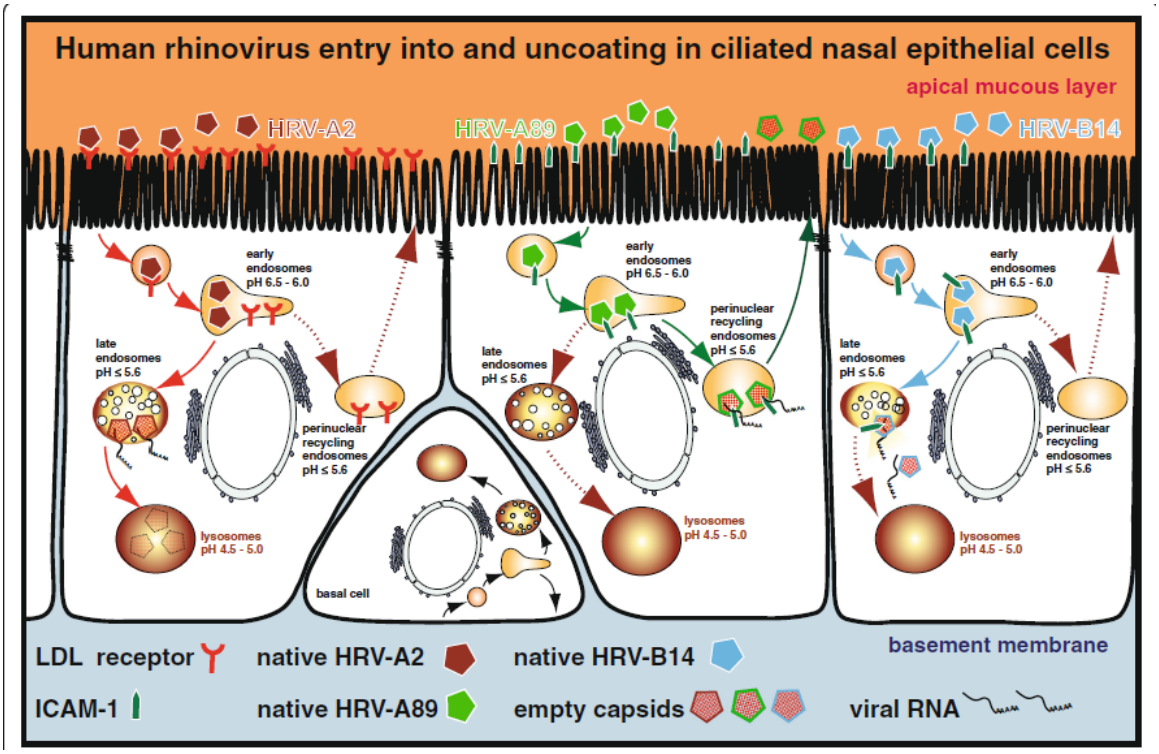


Figure 9: Human rhinovirus entry into and uncoating in ciliated nasal epithelial cells

2-Respiratory syncytial virus (RSV) is a major cause of pneumonia and bronchiolitis among infants and children globally **(Nair H et al., 2010).** Although immune responses develop in those who have had RSV infection during childhood, these persons remain susceptible to RSV upper respiratory tract reinfection throughout life **(Agoti et al., 2012).**

Respiratory syncytial virus (RSV) is the major cause of lower respiratory tract disease in infants and young children and a major viral agent responsible for respiratory tract disease in immunosuppressed individuals and the elderly **(Malloy et al., 2013).**In 2009, the World Health Organization estimated that globally there are 64 million annual RSV cases leading to 160,000 deaths . Antiviral drugs and vaccines that effectively target RSV infections are currently unavailable, representing a significant challenge in regards to RSV disease prevention and treatment **(Turner et al., 2014).**

In infants with CF, research has primarily focused on respiratory syncytial virus (RSV)-induced respiratory exacerbations. Small studies have suggested that infants with CF who are infected with RSV have not only a higher rate of respiratory exacerbations but also prolonged hospitalizations and prolonged symptoms over the ensuing 2 years.

RSV has been shown to enhance P. aeruginosa adherence to CF epithelial cells **(Van Ewijk BE et al., 2007)** and to cause more severe acute P. aeruginosa lung infection in a mouse model ***(Vrankrijker et al., 2009).***

Virus infection is initiated via inoculation of the nose or eyes which can occur through direct contact or inhalation of airborne infectious particles.This results in viral replication in the nasopharynx over 4–5 days, the virus occasionally spreads to the lower respiratory tract over the next few days ***(Collins and Graham, 2008).***

RSV is a member of the Pneumoviridae family, which consists of two genera, Metapneumovirius andOrthopneumovirus, with RSV belonging to the latter ***(Afonso et al., 2016).***

3-Adenovirus (ADV) is one of the most common respiratory pathogens in childhood, and 13-17% of children hospitalized with a viral respiratory tract infection are diagnosed with an ADV infection ***(Kwon et al., 2013).***

Although most cases are self-limiting, ADV infection can lead to more severe complications and death. Specific serotypes of the ADV (serotypes 1, 3, 5, 7, 8, 21, and 55), a younger age (less than one year), and immunocompromised hosts have been reported as risk factors for severe ADV infection ***(Cao et al., 2014).***

Adenoviruses are frequently detected in CF patients but at low rates (not more than 11.8% **(Asner et al., 2012)** and were reported to be associated with impaired lung function, decreased FVC and FEV1 and increased airway obstruction beyond the acute phase of the illness **(Billard et al., 2017).**

Human adenoviruses (HAdVs) are non-enveloped, double-strandedDNA viruses of the genus Mastadenovirus in the Adenoviridae family. There are seven different species of HAdV (HAdV-A to -G), with over 50 serotypes and 70 distinct genotypes. Different clinical manifestations of infection are, in part, due to the tissue tropism of each species ***(Ghebremedhin, 2014).***

The existence of over 70 distinct genotypes among the more than 50 serotypes of HAdV suggests ongoing changes in the viral genome as one mechanism of evolutionary diversity. HAdV serotypes are differentiated based on antibody neutralization assays ***(Cook and Radke, 2017).***

In addition to random mutations, new adenoviral genotypes also result from homologous recombination. On clinical isolates of HAdV-C showed that recombination events are common among circulating adenoviruses2. Recombination requires co-infection of the same cell with different viral types. These recombination events usually occur between members of the same species (e.g. HAdV-B with another HAdV-B strain). Interspecies HAdV recombination increases the rate of molecular evolution and results in novel HAdVs that could theoretically have increased viral fitness, altered cell tropism, and increased virulence ***(Cook and Radke, 2017).***

However, HAdV recombination usually goes unnoticed because of the self-limited nature of the infections. It is only when mutations or recombinations result in a more pathogenic HAdV genotype that causes

outbreaks of more severe disease that the effects of these genetic changes are realized ***(Cook and Radke, 2017).***

4-Influenza

Influenza virus is a major pathogen that represents an ongoing health threat to several species as diverse as poultry, swine, and mammals including humans, generally via respiratory morbidity and mortality ***(Eyer and Hruska, 2013).***

A study followed 103 children with CF over 1 year and collected nasopharyngeal aspirates during routine visits and during respiratory exacerbations for viral studies (by PCR testing for 12 respiratory viruses including rhinovirus) ***(de Almeida et al., 2010).*** Although the presence of respiratory viruses was overall more common during respiratory exacerbations, only the presence of influenza A was associated with an increased risk of hospital admission.

Influenza virus is a member of the Orthomyxoviridae family with an enveloped, negative sense-single stranded RNA ***(Zhang et al., 2013).***

They can be classified into three types: A, B, and C. The influenza A virion genome consists of eight RNA segments that are varying in sizes, with coding ability of 11 proteins, including Hemagglutinin (HA), Neuraminidase (NA), Matrix proteins (M1 and M2), Polymerase basic protein (PB1, PB2 and PA), Nucleocapsid protein (NP), PB1-F2 and non-structural proteins ***(NS1 and NS2; Oh and Hurt, 2014).***

HA functions as a mediator for virus entry into the cell by membrane fusion activity and receptor binding. Meanwhile, NA mediates the progeny virions release by viral receptor enzymatic cleavage. Integral membrane protein, M2, is a multi-functional, proton-selective, ion channel which has roles in both virus entry as well as in virus assembly and budding. The matrix protein (M1)

plays an important role in the virion structure and also as a mediator for the ribonucleoprotein (RNP) core and the viral lipid membrane.PA, PB1, PB2 and NP make up the RNP core which plays a critical role in mediating the packaging and binding of the viral genome.

NS1, NS2, nuclear export protein (NEP) and PB1-F2are the Three other proteins witch are expressed during replication of The virus and are not merged to the mature virion ***(Zhang et al., 2013; Coleman,2007).***

It has been investigated that NS1 protein Acts as a immunosuppressor by inhibiting type I IFN release and Attenuates the capacity of dendritic cells(DCs) to induce Tcell Responses and maturation resulting ininhibition of innate and Adaptive immunity, respectively ***(Fernandez-Sesma et al., 2006).***

Other human respiratory viruses

Coronavirus, Parainfluenza virus (PIV), Adenovirus, Enterovirus (excluding RV), Bocavirus and Metapneumovirus are detected at low rates in CF patients, and sometimes in coinfection with other viruses, making it difficult to specify their clinical impact **(Burns et al., 2012; da Silva Filho et al., 2012).**

Human coronaviruses (CoV) are known to cause upper and lower airway infection in the general population.The prevalence of these viruses generally did not exceed 11% in CF patients **(Emerson et al., 2013; Goffard et al., 2014; Keravec et al., 2015),** except in one 6-month pediatric cohort study which reported higher prevalence for each of the four CoV species (OC43, NL63, HKU1 and 229E) **(van Ewijk et al., 2008).**

In another CF pediatric cohort, these four CoV species were identified during exacerbation, but no significant correlation was found between exacerbation and any given CoV species **(da Silva Filho et al., 2012).**

In CF patients, prevalence of parainfluenza viruses was reported to range between 0 and 17.7% **(Asner et al., 2012; da Silva Filho et al., 2012)** but

reached 28.6% during fall in a 2-month cohort of 14 CF children in exacerbation **(Stelzer-Braid et al., 2012).**

Bocavirus has seldom been screened in CF; prevalence was about 5% **(Keravec et al., 2015).** As in the general population, it was detected along with other viral or bacterial pathogens, and its specific pathogenicity remains to be clarified. Besides Rhinoviruses, other Enteroviruses (EV) can be detected in the airways, causing respiratory symptoms similar to RV. In CF children, EV prevalence mostly ranged between 3.2 and 7.75% **(de Almeida et al., 2010; Esposito et al., 2014)**, but can reach 29.4% (Coxsackievirus/Echovirus) or 35%, as reported in two 5-month studies **(Asner et al., 2012; van Ewijk et al., 2008**, respectively).

Bacteria–virus coinfection

The respiratory tract is a complex environment, with viral, bacterial and fungal populations evolving under selection pressures exerted by both host and microbiome **(Lopes et al., 2014).** Various studies have shed light on virus–bacteria interactions in respiratory tract infection and coinfection, which may be synergistic or antagonistic, with severe health impact. Mechanisms vary widely according to strain. Viruses may be involved in bacterial infection even without viral symptoms, and vice-versa.

Viruses influence bacterial biofilm: e.g., RV releases planktonic bacteria, which are more pro-inflammatory, from biofilms, concomitantly with increased chemokine production, aggravating exacerbation **(Chattoraj et al., 2011b).**

Moreover, in vivo and in vitro, apical release of the iron binding protein transferrin is increased in RSV infection, increasing formation of P. aeruginosa biofilm **(Hendricks et al., 2016).** RSV may also be a coupling agent, increasing P. aeruginosa adherence to host epithelium cells **(van Ewijk et al., 2007).** Conversely, mucoid P. aeruginosa infection suppresses IFN response to RV

infection in CF bronchial epithelial cells, enhancing viral spread **(Chattoraj et al., 2011a).**

Recent studies focused on changes in the microbiome, with higher rates of Haemophilus species, Moraxella species and Streptococcus pneumonia **(Esther et al., 2014)** and S. aureus **(Coscia et al., 2016)** after viral infection.

Seasonality

Virus seasonality is the same in CF and non-CF patients **(van Ewijk et al., 2008)**. Some respiratory viruses (e.g., Adenovirus) cause infection all year round but others, such as RSV, IV or Parainfluenza virus 3, mainly in winter, while RV peaks more often in winter, and spring. RSV and Metapneumovirus (MPV) infection occurs in the same proportions in CF as in non-CF children, mostly from October to January **(Garcia et al., 2007).** Higher frequency was reported for CF exacerbation during winter, with a highly significant association with the IV season **(Ortiz et al., 2010).**

Diagnosis of respiratory virus infection

The field of virology was transformed with the development of molecular microbiological techniques; principally polymerase chain reaction (PCR) assays **(Mackay IM, 2004).**Prior to this technology, the diagnosis of viral infection relied on time-consuming, inefficient methods including cell culture and serology.

Studies from this era are likely to have underrepresented the incidence of confirmed viral infection. PCR-based viral diagnostics rely on the identification of viral nucleic acids in clinical samples and have proven exquisitely sensitive. Increasingly automated processes for the extraction of nucleic acids and running of PCR assays have been adopted in many clinical virology laboratories to

optimize the efficiency of these tests. PCR has also allowed the identification and classification of many new viral species including human metapneumovirus and human bocavirus ***(Allander et al., 2005).***

Prevention of respiratory virus infection

Preventing the spread of respiratory viruses is a priority both at a public health level and within the clinical or domestic setting. Good respiratory and hand hygiene can help limit transmission of viral infections. Segregation or co horting of infected individuals within hospital environments can also be effective, as shown in efforts to halt nosocomial outbreaks of SARS and MERS coronavirus infection ***(Seto et al., 2003).***

Rigorous infectioncontrol measures are recommended as part of standardCF care with the aim of preventing cross-infection withtransmissible pathogens such as P. aeruginosa. As a result, CF services in the developed world are well placedto control the spread of respiratory viruses but in our experienceit are still possible for outbreaks of influenza to affect CFunits. Part of the explanation may be that staff caring for CFpatients are prone to viral respiratory infection and may beinvolved in nosocomial transmission. It remains to be seen ifmeasures such as the wearing of face-masks by CF patients inhospital, as recommended in US infection control guidance ***(Saiman et al., 2014)*** help reduce the spread of respiratory viruses in thispopulation.

1. Pharmacological prophylaxis for respiratory viruses

Pharmacological interventions to help prevent viral infection are available. Palivizumab, for instance, is a monoclonal antibodythat is effective in the prevention of RSV infection ininfants with bronchopulmonary dysplasia. Small studies ininfants with CF have failed to show a clear benefit from palivizumab and its use is further limited by its significantcost and the

requirement for repeat treatment during the RSV season ***(Robinson et al., 2016).***

2. Influenza vaccination in CF

Vaccination is a more attractive preventative strategy butcurrently is only available against influenza A and B amongviral respiratory pathogens. Influenza vaccines are formulatedeach year with the aim of providing coverage against thestrains of influenza predicted to be circulating in the forthcominginfluenza season. Influenza vaccines are currentlyavailable in an inactivated intramuscular formulation and alive-attenuated nasal preparation ***(Treanor, 2016).***

Influenza vaccines containeither three or four strains of influenza and will typicallyinclude both influenza A and B. Influenza vaccine effectivenessin case-control studies varies from 10% to 60% although thevaccine efficacy in a meta-analysis was found to be 59% (95% confidence interval (CI): 51–97%) ***(Treanor, 2016).***

Success of theinfluenza vaccine will depend on the match with each year'smajor circulating strains as well as the ability to achieve sufficientcoverage within the wider population. Emergence of anovel pandemic strain, through the process of antigenic shift, leads to influenza viruses for which there is minimal protectionfrom existing influenza vaccines. Although international guidelines recommend annual influenzavaccination for people with CF, there are no randomizedcontrolled trials in this population. It has been shown previously, however, that people with CF mount a satisfactoryimmune response to intra-muscular influenza vaccination ***(William Flight and Andrew Jones, 2017).***

Observational data have suggested that an influenza vaccination in child with CF significantly reduces the rate of clinical illness with CF **(Wat et al, 2008).** In contrast, a small study among a cohort if adults wih CF failed to

detect a beneficial effect of influenzavaccination over two winter seasons ***(Flight et al., 2011).***

Given the potentiallyserious effects of influenza to patients with CF, however, it isappropriate that all patients with CF and healthcare workers involved in their care receive annual influenza vaccination.

Treatment of respiratory virus infection

1. Neuraminidase inhibitors for influenza in CF

Treatment options for viral respiratory tract infections are severely limited. Influenza is the only respiratory virus for which there are licensed anti-viral therapies available.

Amantadine has been shown to have modest efficacy for the prevention of influenza A, but this has not been translated into widespread clinical use due to a poor side effect profile and ready development of viral resistance. Two neuraminidase inhibitors, oseltamivir and zanamivir, are commercially available and have been shown to havesome benefit for the treatment and prevention of influenza ***(Jefferson et al., 2009).***

A long-acting neuraminidase inhibitor, laninamivir, has shown comparable efficacy to oseltamivir but is not currently widely available ***(Watanabe et al., 2010).***

An intravenous formulation as peramivir has also been studied in seasonal influenza although further data are required to assess its effectiveness ***(Yoshino et al., 2016).***

Neuraminidase inhibitors are at their most effective when taken within 48 h of onset of symptoms, although a case series of patients with severe influenza A/H1N1 infection suggested that benefit may still be obtained with delayed initiation of these therapies ***(Viasus et al., 2011).***

Of concern in the CF context, a meta-analysis by Cooper and colleagues has found evidence of reduced benefit of neuraminidase inhibitors in people with chronic lung disease compared with previously healthy persons ***(Cooper et al., 2003).***

Zanamivir is currently only licensed in an inhaled preparation which further hinders its use in conditions such as CF with underlying structural lung damage. Another issue of significant concern for the future is the development of viral resistance to neuraminidase inhibitors. Cases of oseltamivir-resistant influenza infection in patients with CF have been reported

2. Potential antiviral therapeutic strategies

Beyond neuramnidase inhibitors, there are a number of promising avenues of antiviral research. From a low-tech approach, zinc supplementation has been found to have a modest effect in reducing the duration of the common cold ***(Hemilä, 2011).***

Given the risk of zinc deficiency in people with CF, there is a rationale for a future trial of high-dose zinc supplements in this patient group. Prophylactic oral macrolides, principally azithromycin, are already widely used in CF with its strongest evidence base in patients with chronic P. aeruginosa infection.

In vitro data suggests that azithromycin has a degree of antiviral action, which would support its wider use among people with CF who are not infected with Pseudomonas ***(Gielen et al., 2010).***

Inhaled interferon-β has been studied as a potential intervention in asthma and if proven to be effective in this setting then it may also prove to have a role in CF and other suppurative lung diseases ***(Djukanović et al., 2014).***

Vaccination against human rhinoviruses has long been an aspiration but given the huge diversity of these organisms little progress has been made to date ***(Papi and Contoli, 2010).***

However, this is a very attractive strategy in both CF and asthma given the predominance of rhinoviruses as the cause of acute exacerbations in both conditions and May well see developments over the coming years **(Rohde GGU, 2010).**

The great variability in the incidence of CF is not only influenced by the ethnic makeup but also by rate of consanguinity, geographical origin, certain tribal descent and religious background prevalent in a certain population. Therefore, CF incidence and specific mutations have to be assessed specifically for any population ***(Kambouris et al., 2000).***

Patients and methods

Patients

This is a cross sectional observational study recruiting 60 patients diagnosed as CF, based on clinical manifestations and confirmed by a positive sweat chloride test, coming in acute exacerbation to the cystic fibrosis clinic in children's hospital, Cairo University.Their age ranged from 6 months to 13 years.

Study Design:

Type: cross sectional observational study.

Inclusion criteria:

1- Age range from (6months-13 years old).

2- Both genders were included.

Ethical aspect of the study:

Informed consent was optained from the parents after explanation of the aim of the study, its benefits for their children.

Methodology:

The patients were subjected to the following:

1-Full medical history and through clinical evaluation with special stress on respiratory system:

Respiratory manifestations: Fever, exaggerated cough, frequency of exacerbation, need for oxygen, need for hospital admission, and ICU admission, type of breathing, oxygen saturation.

2- Laboratory investigations:

a) CRP:

Blood was collected by venipuncture, allowed to clot and serum was separated by centrifugation at room temperature and was frozen at -20.the analysis of all samples was carried out at the laboratory of the department of clinical pathology, Kasr el eini Hospital, Cairo University.

b) Sputum culture:

The sputum was collected in sterile ice - cream cups after instructing the patient to rinse his/her mouth thoroughly with water and cough forcefully to bring out the material from the tracheobronchial tree.

Suction can also be used to collect a sputum sample. A soft, flexible tube (called a nasotracheal catheter) is put through the nose and down the throat. Suction is applied for up to 15 seconds to collect the sample. This method is often used for people who are unable to cough.

The sputum sample was placed in a container with a growth medium or culture medium. Any bacteria that grow will be found under a microscope or by chemical tests.

c) Nasopharyngeal swab:

- Sample Collection:

Samples were in the form of nasopharyngeal swabs taken on viral transport media (VTM).

- *Measuring*

The distance between the ear lobule and the ala nasi was measured by the NP swab and divided by 2, and the swab marked at this distance to ensure the insertion of the swab in the proper site. This flexible, sterile tip flocked with nylon fiber swab applicator was inserted into the nostril and back to the

nasopharynx and left in place for a few seconds. It was then slowly withdrawn with a rotating motion. The swab was placed in a 15 mL centrifuge tube labeled with the patient unique ID and containing 2mL viral transport media (VTM: consisting of sterile solution of bovine albumin fraction V, HEPES buffer, penicillin and streptomycin in HANK's balanced salt solution). The applicator stick was then cut off.

- *Sample processing:*

The received swabs inside the 15 ml tube was agitated vigorously for 10 seconds using a vortex mixer to free cells from the swab tip, and then swab was removed from the tube and discarded using a forceps. The VTM was immediately placed in a freezer (-70°C) until tested with PCR for presence of respiratory viruses.

- PCR testing for Respiratory viruses

  - Viral Nucleic Acid Extraction: Viral Nucleic acid extraction was done after centrifugation of the sample for 10-20 min and 200ul from the sediment taking as starting material using the Biospin Virus RNA Extraction kit (cat number BSC62M1 from Bioflux), according the manufacturer's instructions.
  - CDNE was done manually by cDNA Synthesis Premix (SGRT801) from Seegene.
  - PCR was done for the following viruses: adenovirus, influenza A virus, influenza B virus, parainfluenza viruses 1–4, rhinovirus, respiratory syncytial virus, bocavirus, metapneumovirus, coronavirus 229E, coronavirus NL63, coronavirus OC43, and enterovirus. PCR was done by real-time multiplex PCR using AnyplexTM II RV16 Detection (v1.1)

(cat. no. RV7G01Y) supplied by Seegene, operated on a CFX96TM Real-Time PCR Detection System (Bio-Rad).

- Interpretation of the results was done according to the manufacturer's instructions, in addition to automatic analysis using the Seegene viewer software after exporting the run data to it **(Kim et al., 2013).**

3-Radiological imaging:

CXR done for all patients during exacerbation of cystic fibrosis.

Statistical analysis:

Data were coded and entered using the statistical package SPSS (Statistical Package for the Social Sciences) version 24. Data was summarized using mean, standard deviation, median, minimum and maximum in quantitative data and using frequency (count) and relative frequency (percentage) for categorical data. Comparisons between quantitative variables were done using the non-parametric Kruskal-Wallis and Mann-Whitney tests ***(Chan, 2003a)***. For comparing categorical data, Chi square ($\chi 2$) test was performed. Exact test was used instead when the expected frequency is less than 5 ***(Chan, 2003b)***. P-values less than 0.05 were considered as statistically significant.

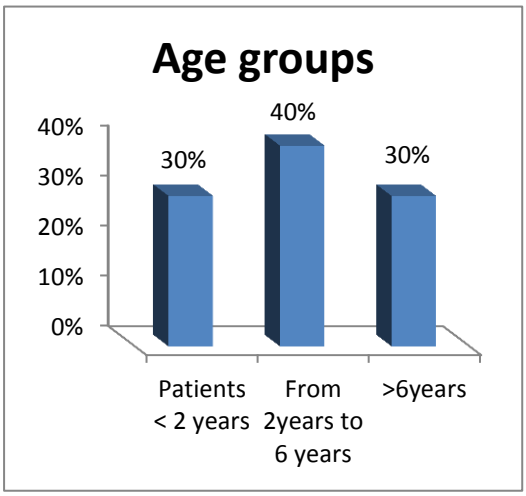
Results

This is a cross sectional observational study recruiting 60 CF patients during exacerbation, from allergy and Pulmonology Unit, Children's hospital, Cairo University. Both genders were included.

Our study included 38 male (63.3%)and 22 female(36.7%) with male to female ratio 1.7:1 and a median age of 4 years and range from 6 months to 13 years. The majority of patients were diagnosed as CF below the age of 1 year.

Table 8: Demographic data of the study population:

Characteristic	N=60	%
Age groups		
Patients < 2 years	18	30%
From 2 years to 6 years	24	40%
>6years	18	30%
Gender		
Male	38	63.3%
Female	22	36.7%



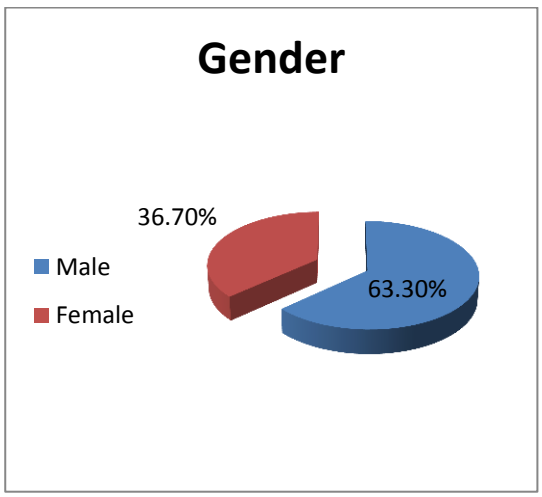


Figure 10: Age group **Figure 11: Patient's gender**

In our study 73.3% of patients were below the 5th centile for weight while 53.3% were below the 5th centile for height.

Table 9: Percentiles of weight, height and BMI:

Percentile of weight		
Below 5th centile	44	73.3%
From 5th to 95th centile	16	26.7%
Percentile of height		
Below 5th centile	32	53.3%
From 5th to 95th centile	28	46.7%
Percentile of BMI		
Below 5th centile	20	47.6%
From 5th to 95th centile	22	52.4%

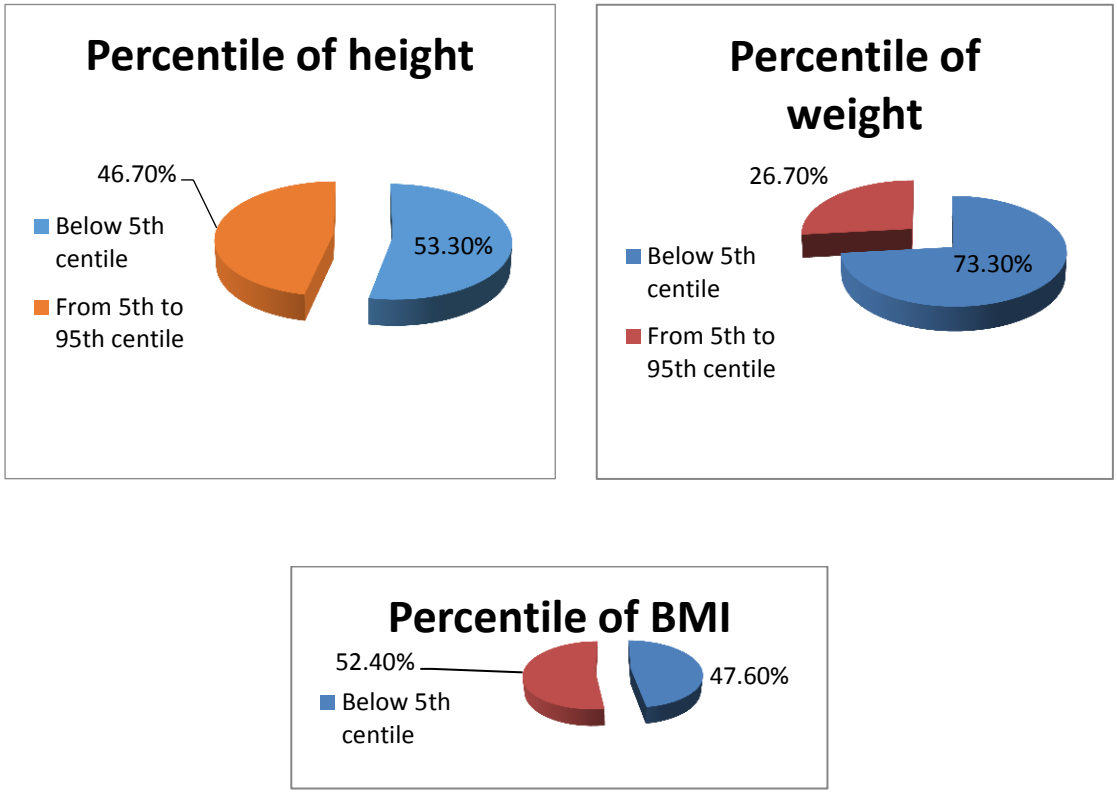


Figure 12: Percentiles of weight, height and BMI.

Table 10: Demographic data of our patients:

	Mean ± standard deviation(SD)	Median	Range
Age(months)	58.70 ± 41.11	49.50	6.00 ─ 156.00
Weight(Kg)	12.73 ± 6.84	11.52	3.00─ 28.00
Height(cm)	93.03 ± 23.50	89.5	55.00─ 140.00
Age at diagnosis	30.70 ± 39.34	10.00	2.00─ 132.00
BMI	13.81 ± 1.30	14.00	11.20─ 15.60

Nearlly all patients presented with fever and exaggerated cough, the most common sign was increased wheezes and crepitations in 36% of patients.

23% of our patients were in need for hospital admission, 26% needed supplemental oxygen therapy and 6.7% needed ICU admission

Table 11: clinical presentations of our patients during exacerbation:

Clinical presentation	N=60	%
Fever	50	83.3
Exaggerated cough	60	100
Hospital admission	14	23.3
Need for Nasal oxygen	16	26.7
Need for ICU	4	6.7
⇈Wheezes in chest	58	96.7
⇈Crepitations in chest	22	36.7

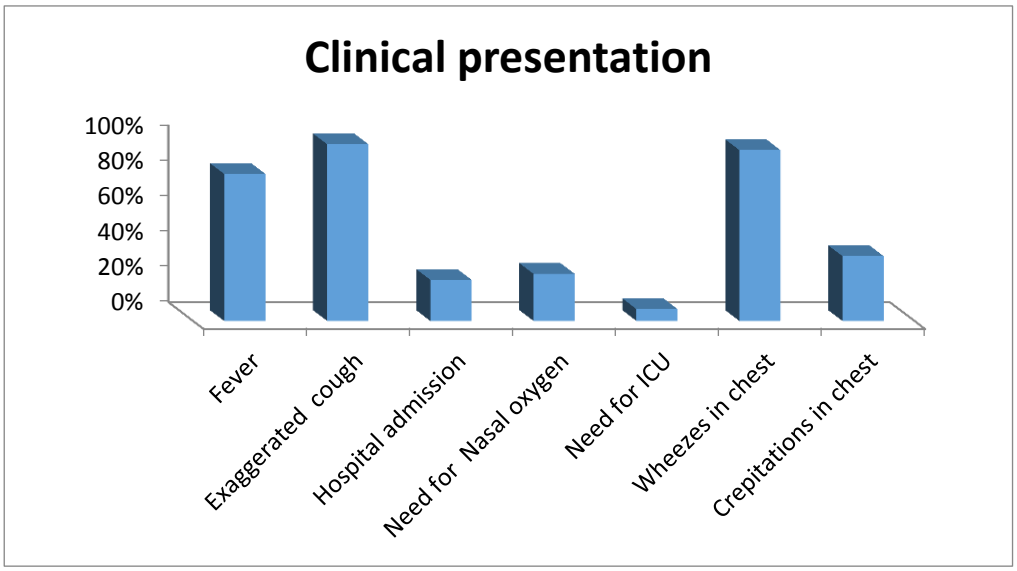


Figure 13: clinical presentations of our patients during exacerbation.

Most patients had a history of exacerbation more than once per month 70%, and only 13.3% had associated liver affection.

Table 12: pulmonary exacerbations in patients with CF:

Frequency of exacerbation	**N=60**	**%**
Twice per year	6	10%
≥3 times per year	12	20%
≥Once per month	42	70%

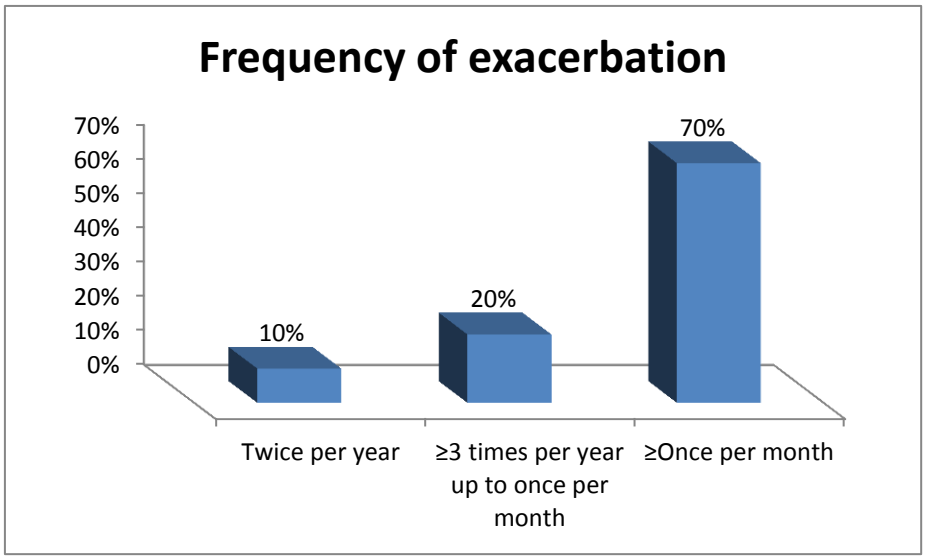


Figure 14 : Pulmonary exacerbations in patients with CF.

In our study 53.3% of CF patients had positive CRP during exacerbation, and the most common organism found in sputum culture was pseudomonas in 20% of patients, Klebsiella in13.3%, staph aureus in 10% and Hemophilus influenza in 6.7%.

Table 13: Sputum culture and CRP results at time of exacerbation:

		Count	Percent
Sputum culture	Staph aureus	6	10%
	Pseudomonas spp	12	20%
	MRSA	2	3.3%
	Hemophilus influenza	4	6.7%
	Klebsiella	8	13.3%
	E coli	2	3.3%
	Mixed flora	26	43.3%
CRP	Positive	32	53.3%
	Negative	28	46.7%

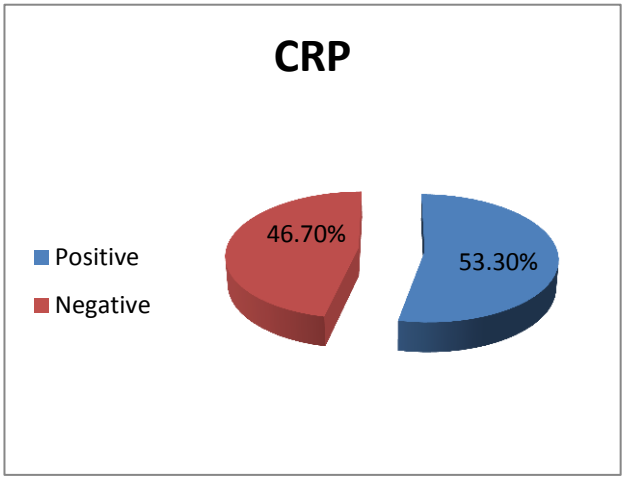


Figure 15: CRP results at time of exacerbation.

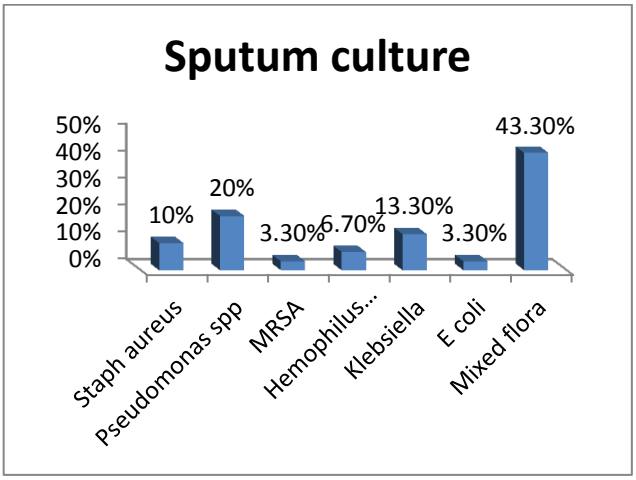


Figure 16: Sputum culture results at time of exacerbation.

Abnormal CXR was detected during exacerbation in 80% of patients in the form of consolidation in 20%, hyperinflation in 23.3%, increase bronchovascular markings in 26.7% and air bronchogram in 10%, while 20% of patients had normal CXR.

Table 14: Chest X- ray findings during exacerbation:

		Count	%
X ray	Air bronchogram	6	10%
	Consolidation	12	20%
	Hyperinflation	14	23.3%
	Increase bronchovascular markings	16	26.7%
	Normal	12	20%

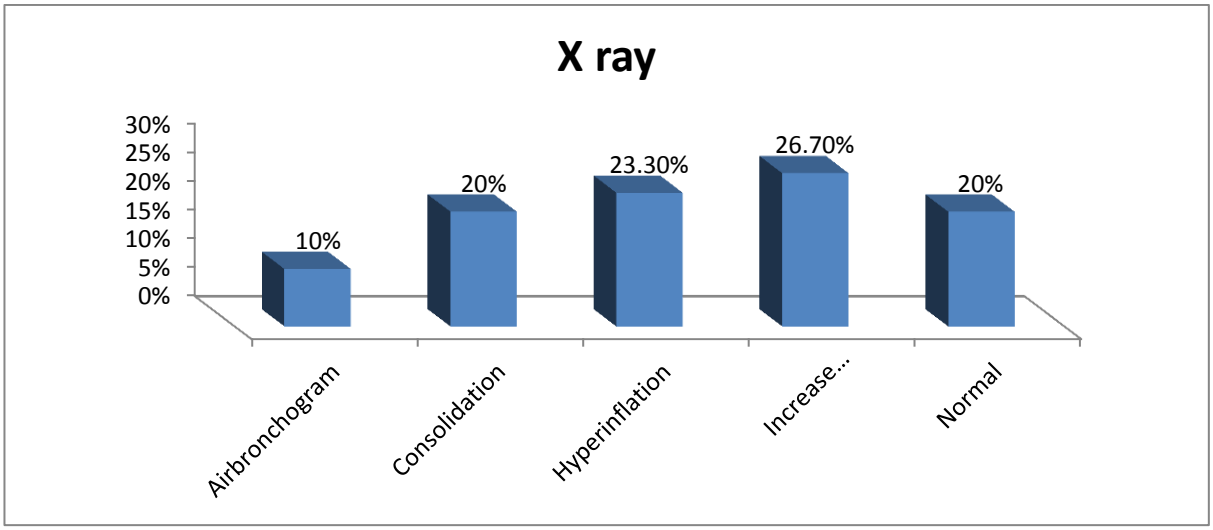


Figure 17: Chest X- ray findings during exacerbation.

In our study 23.3% of patients were in need for hospital admission during exacerbation and with mean duration of 11.14 ± 6.62 day and hospital stay ranged from 3 to 20 days.

Table 15: Duration of hospitalization of CF patients during exacerbation.

	Mean ± standard deviation	Median	(Range) Minimum –maximum
Duration of hospitalization.(days)	11.14 ± 6.62	8	3 - 20

Table16: Percentage of virus detection during exacerbation.

	Count	%
Virus positive	48	80
Virus negative	12	20

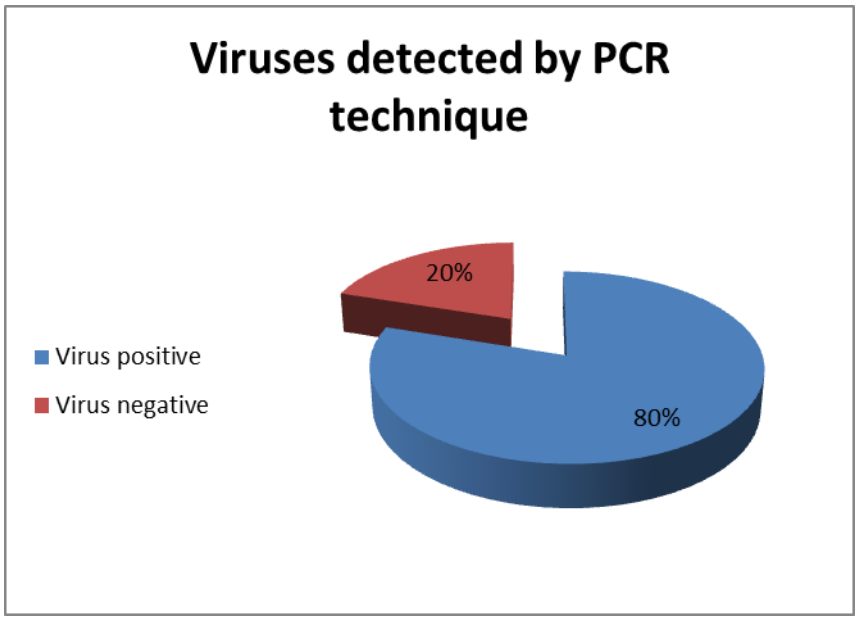


Figure 18: Percentage of virus detection during exacerbation.

Most common virus detected was Rhinovirus in 43.4% of patients, followed by bocavirus in 20%, adenovirus in 13.3%, enterovirus in 10% and HMPV in 6.7%. Detection of viruses used a semi quantitative method to assess the viral load in the sample (+, ++, +++).

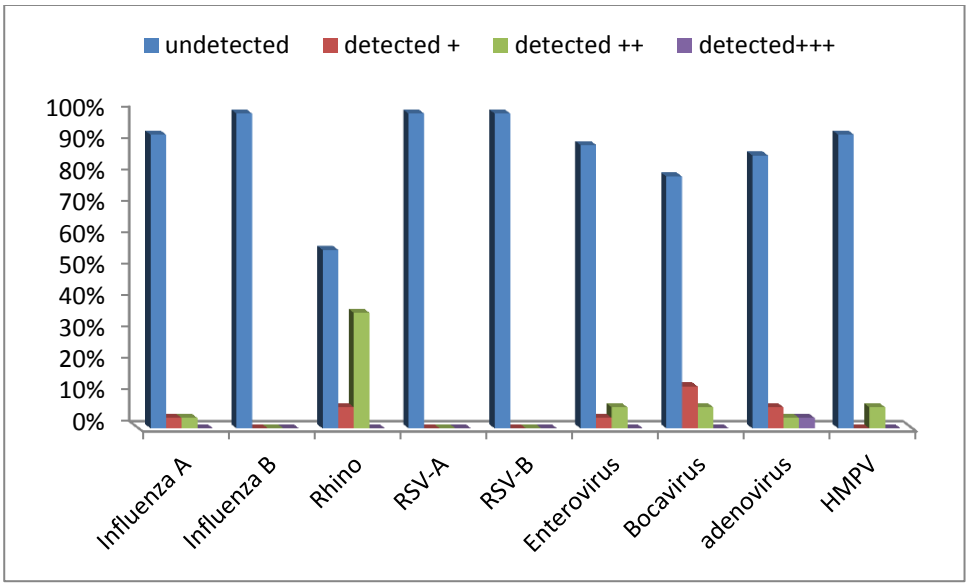


Figure 19: viruses detected.

Table 17: Viral load of CF patients during exacerbation:

		Count	%
Influenza A	**Undetected**	***56***	***93.3%***
	detected +	***2***	***3.3%***
	detected ++	***2***	***3.3%***
	detected+++	***0***	***.0%***
Influenza B	**Undetected**	***60***	***100.0%***
Rhino	**Undetected**	***34***	***56.7%***
	detected +	***4***	***6.7%***
	detected ++	***22***	***36.7%***
	detected+++	***0***	***.0%***
RSV-A	**Undetected**	***60***	***100.0%***
RSV-B	**Undetected**	***60***	***100.0%***
Enterovirus	**Undetected**	***54***	***90.0%***
	detected +	***2***	***3.3%***
	detected ++	***4***	***6.7%***
	detected+++	***0***	***.0%***
Bocavirus	**Undetected**	***48***	***80.0%***
	detected +	***8***	***13.3%***
	detected ++	***4***	***6.7%***
	detected+++	***0***	***.0%***
Adenovirus	**Undetected**	***52***	***86.7%***
	detected +	***4***	***6.7%***
	detected ++	***2***	***3.3%***
	detected+++	***2***	***3.3%***
HMPV	**Undetected**	***56***	***93.3%***
	detected +	***0***	***.0%***
	detected ++	***4***	***6.7%***
	detected+++	***0***	***.0%***

Correlation between bacteria in sputum culture and viruses:

Significant correlation was detected between positive bacterial culture and certain viruses as influenza A virus, Enterovirus and HMPV (with p value <0.001, 0.002, 0.002 respectively).

Table 18: Correlation between bacteria and viruses in CF patients during exacerbation:

	Sputum culture														
	Staph. Aureus		Pseudomonas spp		MRSA		Mixed flora		Klebsiella		Hemophilus influenza		E coli		P value
	Count	%	Count	%	Count	%	Count	%	Count	%	Count	%	Count	%	
Influenza A	2	33.3%	0	.0%	0	.0%	0	.0%	0	.0%	0	.0%	2	.100%	< 0.001
Rhino	2	33.3%	4	33.3%	0	.0%	12	46.2%	4	50%	2	50.0%	2	100.0%	0.169
Enterovirus	0	.0%	4	33.3%%	0	.0%	0	.0%	0	.0%	0	.0%	2	100.0%	0.002
Bocavirus	0	.0%	0	.0%	0	.0%	10	38.5%	2	25.0%	0	.0%	0	.0%	0.427
Adenovirus	0	.0%	4	33.4%	0	.0%	4	15.4%	0	.0%	0	.0%	0	.0%	0.721
HMPV	0	.0%	2	16.7%	2	100.0%	0	.0%	0	.0%	0	.0%	0	.0%	0.002

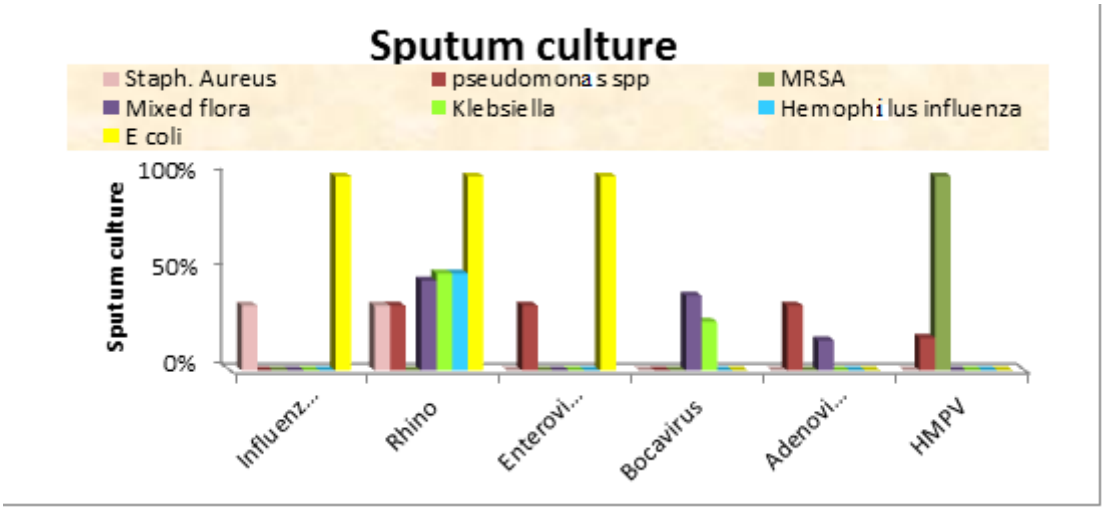


Figure 20: Correlation between bacteria and viruses in CF patients during exacerbation.

Relation of viruses with hospital admission:

Many viruses were detected in CF patients during exacerbation necessitating hospital admission as: Rhinovirus, influenza A, Enterovirus, Bocavirus, Adenovirus. But significant relation was found only between hospital admission and (Influenza A and Enterovirus).

Table 19: Relation of viruses with hospital admission:

	Hospital admission				
	Yes		**No**		**P value**
	Count	**%**	**Count**	**%**	
Influenza A	***4***	***28.6%%***	***0***	***.0%***	***0.002***
Rhino	***8***	***57.1%***	***18***	***39.1%***	***0.248***
Enterovirus	***4***	***28.6%***	***2***	***4.3%***	***0.012***
Bocavirus	***2***	***14.3%***	***10***	***21.7%***	***0.726***
Adenovirus	***2***	***14.3%***	***6***	***13%***	***0.093***
HMPV	***0***	***.0%***	***4***	***8.7%***	***0.564***

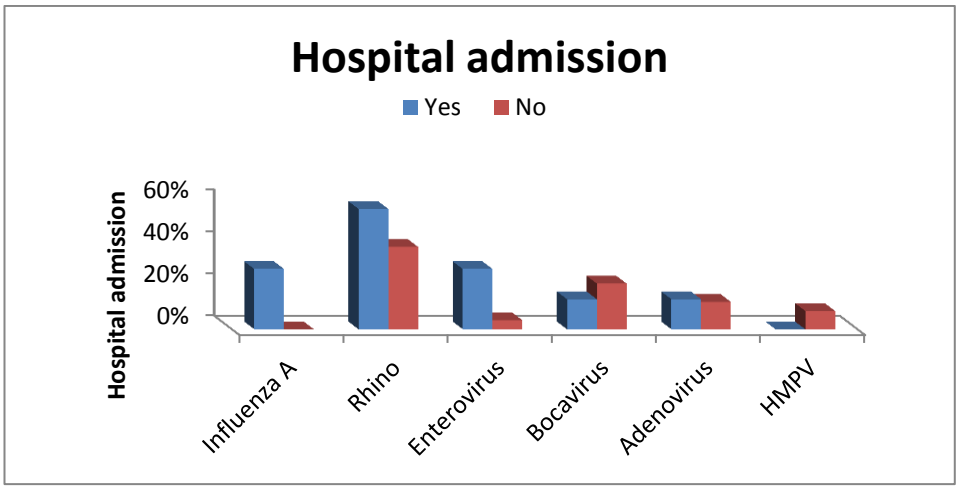


Figure 21: Relation of viruses with hospital admission.

Correlation between the age group and viruses:

Statistically significant relation was noted between different age group and Influenza A, Rhinovirus, Enterovirus and Adenovirus (with P value 0.036, 0.011, 0.002, 0.018 respectively).

Table 20: Correlation of age group with viruses:

	Age groups						
	infant up to 2years		from2y to6y		>6years		P value
	Count	%	Count	%	Count	%	
Influenza A	*0*	*.0%*	*0*	*.0%*	*4*	*20.0%*	*0.036*
Rhino	*6*	*33.3%*	*6*	*27.3%*	*14*	*70.0%*	*0.011*
Enterovirus	*0*	*.0%*	*0*	*.0%*	*6*	*30.0%*	*0.002*
Bocavirus	*4*	*22.2%*	*6*	*27.3%*	*2*	*10.0%*	*0.117*
Adenovirus	*6*	*33.3%*	*2*	*9.1%*	*0*	*.0%*	*0.018*
HMPV	*0*	*.0%*	*2*	*9.1%*	*2*	*10.0%*	*0.537*

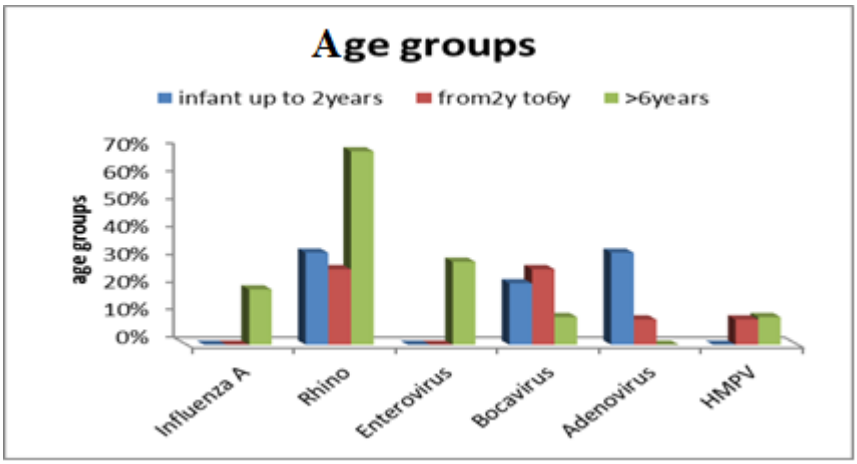


Figure 22: Correlation of age group with viruses.

Relation of viruses with oxygen:

Significant relation was detected between the presence of Influenza A and Enterovirus and the need for oxygen (with p value 0.004 and 0.02 respectively).

Table 21: Relation of viruses with the need for oxygen:

	Need for oxygen				
	Yes		No		P value
	Count	%	Count	%	
Influenza A	***4***	***25%***	***0***	***.0%***	***0.004***
Rhinovirus	***8***	***50%***	***18***	***40.9%***	***0.484***
Enterovirus	***4***	***25%***	***2***	***4.5%***	***0.020***
Bocavirus	***2***	***12.5%***	***10***	***22.7%***	***0.648***
Adenovirus	***2***	***12.5%***	***6***	***13.6%***	***0.130***
HMPV	***2***	***12.5%***	***2***	***4.5%***	***0.287***

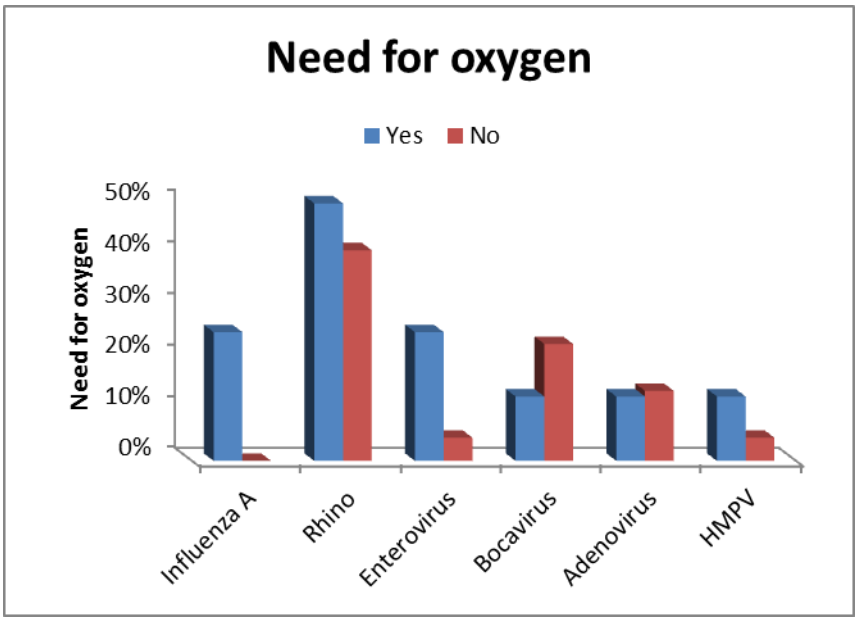


Figure 23: Relation of viruses with the need for oxygen.

Relation of viruses with the need for ICU:

The need for ICU admission in CF patients during exacerbation was detected only in patients with Rhinovirus and Bocavirus. No significant relation was detected between the need for ICU admission and the 2 viruses detected (Rhinovirus and Bocavirus) with p value 0.730 & 0.101 respectively.

Table 22: Relation of viruses with the need for ICU admission:

	Need for ICU				
	Yes		No		P value
	Count	%	Count	%	
Influenza A	***0***	***.0%***	***4***	***7.1%***	***1***
Rhinovirus	***2***	***50.0%***	***24***	***42.8%***	***0.730***
Enterovirus	***0***	***.0%***	***6***	***10.7%***	***1***
Bocavirus	***2***	***50.0%***	***10***	***17.9%***	***0.101***
Adenovirus	***0***	***.0%***	***8***	***14.3%***	***1***
HMPV	***0***	***.0%***	***4***	***7.1%***	***1***

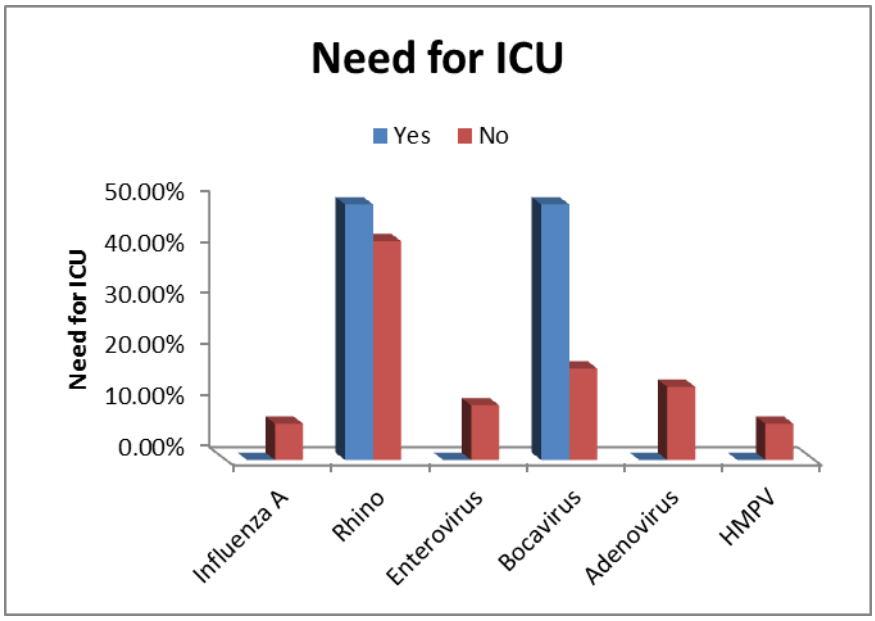


Figure 24: Relation of viruses with the need for ICU admission.

Relation of season with viruses:

There was no significant relation between viruses and season apart from HMPV with P value 0.001.

Table 23: Relation of season with viruses:

	Season								
	Winter(February)		Summer		Spring		Autumn		P value
	Count	%	Count	%	Count	%	Count	%	
Influenza A	*0*	*.0%*	*4*	*16.6%*	*0*	*.0%*	*0*	*.0%*	*0.587*
Rhinovirus	*4*	*40.0%*	*12*	*50%*	*0*	*.0%*	*10*	*45.5%*	*0.253*
Enterovirus	*0*	*.0%*	*6*	*25%*	*0*	*.0%*	*0*	*.0%*	*0.163*
Bocavirus	*0*	*.0%*	*4*	*16.7%*	*0*	*.0%*	*8*	*36.4%*	*0.156*
Adenovirus	*0*	*.0%*	*0*	*.0%*	*0*	*.0%*	*8*	*36.4%*	*0.054*
HMPV	*4*	*40.0%*	*0*	*.0%*	*0*	*.0%*	*0*	*.0%*	*0.001*

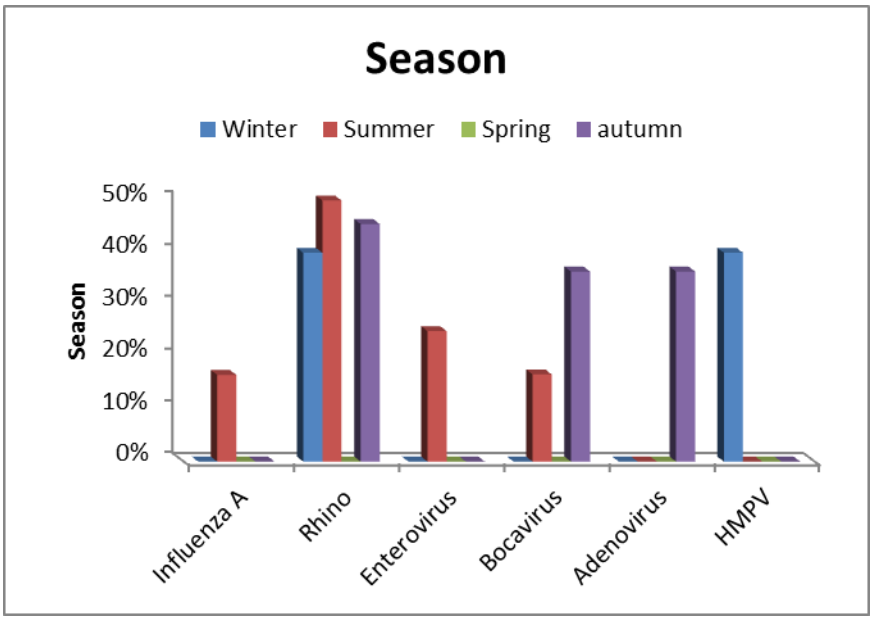


Figure 25: Relation of season with viruses.

Correlation of double viruses and bacteria:

There was no significant correlation between double viruses and bacteria with P value 0.08.

Table 24: Detection of double viruses:

		Count	%
Detection of double viruses.	**Yes**	***10***	***16.7%***
	No	***50***	***83.3%***

Table 25: Correlation of double viruses and bacteria:

		more than 1 virus				
		Yes		No		P value
		Count	%	Count	%	
Sputum culture	**Staph. Aureus**	***0***	***.0%***	***6***	***12.0%***	***0.080***
	pseudomonas spp	***2***	***20.0%***	***10***	***20.0%***	
	MRSA	***0***	***.0%***	***2***	***4.0%***	
	Mixed flora	***6***	***60.0%***	***20***	***40.0%***	
	Klebsiella	***0***	***.0%***	***8***	***16.0%***	
	Hemophilus influenza	***0***	***.0%***	***4***	***8.0%***	
	E coli	***2***	***20.0%***	***0***	***.0%***	

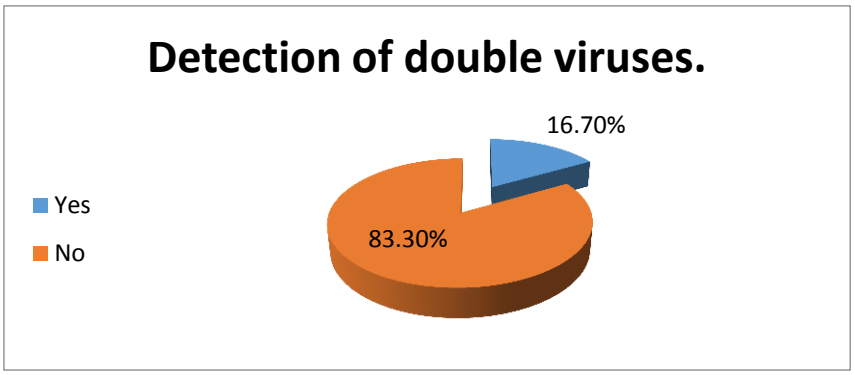


Figure 26: Detection of double viruses.

Discussion

CF is the most common lethal genetic disease in Caucasian populations with 70,000 persons affected worldwide. This autosomal recessive illness is caused by more than1800 known mutations of the gene coding for cystic fibrosis transmembrane regulator protein (CFTR), leading to defective chloride and sodium transport through the epithelium cell membrane **(Elborn, 2016).**

Mutations on this ion channel affect several organs: pancreas, liver, intestine, kidneys and, mostly, lungs. Patients show impaired immune defense, viscous respiratory secretion and reduced mucociliary clearance, fostering chronic microbial infections and progressive deterioration of lung function, the main cause of morbidity, and mortality in this disease. The lungs provide an especially favorable environment. Throughout life, CF patients' airways are sequentially infected by an evolving spectrum of bacteria, strongly impacting disease course **(Billard et al., 2017).**

Pseudomonas aeruginosa (Pa) is a key pathogen. Pa acquisition is a turning point in chronicization, deteriorating health, and is strongly associated with increased hospital admission and decreased survival **(Ong and Ramsey, 2015).** Independently from conventional bacterial pathogens, CF lungs are also colonized by a more complex polymicrobial community **(Surette, 2014).**

Viruses are now considered a major part of the CF microbiota. CF patients, especially during exacerbation, have higher nasopharyngeal and BAL viral loads than non-CF controls **(Kieninger et al., 2013, Esposito et al., 2014),** and children in general have much higher titers **(Krause et al., 2014).**

In our study we detected viruses in 80% of patients with CF during exacerbation. Prevalence of virus-positive CF samples varies from 17.6 to 29% between recent studies in stable CF children **(Esposito et al., 2014; Wat et al., 2008b).**

In concordance with our results, Wat et al conducted a study in the UK , and found that 46% of CF children during exacerbation were positive for respiratory viruses **(Wat et al., 2008b).**

Also a study done in the University of Washington, and Seattle Children's Hospital. including Cystic fibrosis patients aged 6–18 years with new respiratory illness revealed that (66.4%) of CF patients had 1 or more viruses detected **(Emerson et al., 2013).**

In line with our study**,** a pilot french study from January 2009 until March 2013. Longtudinally evaluating children with a diagnosis of cystic fibrosis three times: at baseline (Visit 1), at the diagnosis of pulmonary exacerbation (Visit 2), and after exacerbation treatment (Visit 3) and detected that the proportion of viral respiratory infection was 72% at the diagnosis of PEx (V2), 22% at baseline (V1), and 28% at V3 (**Cousin et al., 2016).**

Also, a spanish study recruting 33 children (aged 10 months to 17 years) following in Cystic Fibrosis Unit in Parc Taulí University Hospital, detected viruses in 154 (41.8%) nasopharyngeal–swab specimens; the most common were rhinovirus (24.4%), adenovirus (5.2%), Enterovirus (4.1%), and parainfluenza (3%) **(Sílvia Miró-Ca˜nísa et al., 2017).**

In contrast to an Italian study conducted for 1 year involving patients aged below 25 years who were regularly following up at the Cystic Fibrosis Centre of the University of Milan, detected viruses in 29% of CF patients in stable condition and in 29.8% in patients during exacerbation **(Esposito et al., 2014).**

The proportion of virus-associated exacerbations documented in our study was significantly higher than those found in prior studies using conventional diagnostic methods (<15%) **(Wang et al., 1984) (Armstrong et al., 1998).** These discrepancies can be attributed to the higher sensitivity and broader range of virus detection afforded by molecular-based methods leading to a better estimation of the true prevalence of respiratory viruses in CF exacerbations.

Unsurprisingly, the principal respiratory viruses involved are RNA viruses: RV, RSV and IV. RSV and IV have long been the most commonly implicated viruses**. (Wat et al., 2008b**

Recently, however, influenza vaccination and RSV prophylaxis have decreased their involvement, and RV is now the predominant viral agent in CF exacerbation. Nevertheless, RSV and IV still induce the most severe clinical impact **(Frickmann et al., 2012).**

In our study we detected Rhinovirus in 43.4% of patients. In line with this, **Stelzer-Braid et al., 2012** which is an Australian study consisting of 37 CF participants (aged between 4.2 and 19.2 years) detected HRV in (50%) of patients during exacerbation and in (26%) of patients not in exacerbation..In contrast to an English study detecting RV in 15.9% of CF patients during exacerbation (**Wat et al., 2008a).**

In our study we detected influenza A virus in 6.7% of CF patients. Similarly a Canadian study of children with CF seen in the setting of a pulmonary exacerbation documented a low proportion of pH1N1 (5.3%) (**Asner et al., 2012**).

In contrast to our study Wat et al., 2008 study done during a typical influenza season reporting higher influenza A and B detection rates (25%) despite a high influenza vaccination uptake of 70 %.

In our study adenovirus was detected in 13.3% of patients, similar rates was detected in Asner et al 2012 study, they found adenovirus in (11.8%) of CF patients.

Bocavirus which has seldom been screened in CF was detected in our study in 20% of CF patients. In contrast to a Brazilian study conducted in a period of 1 year, detecting Bocavirus in (5.2%) of CF patients not in exacerbation and in (6.3%) of CF patients during exacerbation **(de Almeida et al., 2010).**

In our study we detected Metapneumovirus in 6.7% of CF patients. In CF children, it usually varied between 1.1 and 15%. Studies done on an adult CF patients showed that the prevalence of Metapneumovirus ranged from 1.9 to 13.2% **(Flight et al., 2013 and Jones et al., 2011).** On the contrarly to a cohort study done in Texas of older CF children hospitalized for exacerbation, during the RSV season and showed that 20 of 42 (47.6%) had an hMPV infection **(Garcia et al., 2007).**

Enteroviruses (EV) can be detected in the airways, causing respiratory symptoms similar to RV. In our study we detected enteroviruses in 10% of CF patients, similarly de Almeida et al., 2010 study reported EV prevalence was 7.75% , while it can reach 29.4% and 35%, as reported in other studies **(Asner et al., 2012; van Ewijk et al., 2008, respectively).** Prevalence reached 12.5% in an adult CF cohort **(Hoek et al., 2012).**

Viral coinfection is quite frequent in the CF respiratory tract, involving at least two viruses, and sometimes more **(Keravec et al., 2015).** Coinfection detection rates, which had greatly improved by multiplex assay, vary according

to technique. Coinfection increases pathogenicity **(Stelzer- Braid et al., 2012),** and admission rates, especially for children **(Kouni et al., 2013).**

In our study we detected viral coinfection in 16.7% of patients. All of them were double viruses only. (Rhinovirus with enterovirus in 6.6%, Rhinovirus with Bocavirus in 3.3%, Rhinovirus with Adenovirus in 3.3% and Adenovirus with Bocavirus in 3.3%)

Other studies showed viral coinfection rates range up to 34.6% **(Asner et al., 2012 and Goffard et al., 2014).**

Also, an American study included CF Children between 6 and 18 years of age who followed prospectively for up to 2 years. Study visits occurred during pulmonary exacerbations.Two or more respiratory viruses detected in (1.6%) of samples.Mostly rhinovirus with another virus **(Burns et al., 2012).**

The **Cousin et al., 2016** french study detected a viral co-infection in one patient (rhinovirus and metapneumovirus).While in **Olesen et al., 2012** study one sample was positive for both rhinovirus and influenza A.

Concomitant HMPV and RSV was reported in children with respiratory symptoms **(Garcia et al., 2007),** and IV coinfection with Parainfluenza virus 1 **(Ramirez et al., 2014).**

A Spanish study detected multiple viruses in 20samples (5.4%) of CF patients the most common virus–virus coinfection were rhinovirus plus adenovirus in 30% of coinfection samples and rhinovirus plus enterovirus in 20% of coinfection samples **(Sílvia Miró-Ca˜nísa et al., 2017).**

In a study done on CF adults with exacerbation, RV was detected in association with Adenovirus **(Etherington et al., 2014 and Jones et al., 2011).** Other viral coinfections were more rarely described.

Studies consider respiratory viruses as predisposing factors for secondary bacterial infection in CF **(Hendaus et al., 2015).**

In our study we detected significant statistical relation between bacteria and each of influenza A virus, Enterovirus and HMNV (p value < 0.001, 0.002, and 0.002 respectively). On the contrary to our study **(Olesen et al., 2006; Punch et al., 2005)** showed no significant correlation between viruses and bacteria.

Also in **wat et al., 2008** study, there was no statistical difference between the viral and non-viral groups in terms of bacteria isolation (p=0.909). There was also no difference in Pseudomonas isolation between the viral and non-viral groups.

In **Charles Esther et al 2014** study, they did not detect a relationship between positive respiratory viruses and the frequency of typical CF pathogens such as S. aureus or P. aeruginosa, and they considered the possibility that respiratory virus infection might increase the severity of infection manifested as an increased quantity of organisms in the culture.

In our study, significant relation was noted between seasonal variations and HMPV (p value 0.001) .

In **Garcia et al., 2007 study,** Human metapneumovirus (HMPV) and RSV infection occured in the same proportions mostly from October to January.

In our study, we detected significant relation between the presence of influenza A virus or enterovirus and the need for hospital admission (p value 0.002 and 0.012 respectively), as well as the need for oxygen (p value 0.004 and 0.02 respectively).

Infection with respiratory viruses is strongly associated with respiratory morbidity in patients with cystic fibrosis. PCR-based respiratory viral panels

(RVPs)—have suggested that respiratory viruses contribute significantly to CF pulmonary exacerbations. Some studies suggest that CF airway epithelia exhibit an exaggerated inflammatory response to viral infection.

Conclusion

Respiratory viruses are a major concern and frequently act as the harbinger of a pulmonary exacerbation in patients with chronic lung disease as CF.These exacerbations are associated with accelerated lung function decline, the need for intravenous antibiotic therapy at great inconvenience to patients and even an increased risk of death.

Our results provide evidence to support respiratory viruses are commonly found during respiratory exacerbations of CF, particularly Rhinovirus, enterovirus, Bocavirus and Adenovirus along with bacteria such as pseudomonas spp, staph aureus, Hemophilus influenza, Klebsiella spp.

The present study showed significant correlation between positive bacterial culture and certain viruses as influenza A virus, Enterovirus and HMPV.Many viruses were detected in CF patients during exacerbation necessitating hospital admission as: Rhinovirus, influenza A, Enterovirus, Bocavirus, Adenovirus. But significant relation was found only between both Influenza A and Enterovirus and the need for hospital admission. Statistically significant relation was noted between different age group and Influenza A, Rhinovirus, Enterovirus and Adenovirus.

Also, the present study did not show significant relation between the need for ICU admission and viral infection and no significant relation between viruses and season apart from HMPV.

Recommendations

1- Further studies should focus on viral kinetics especially those which has not been thoroughly investigated in CF and its importance for appropriate medical care.

2- Further longitudinal studies on the relation between viral infection and pulmonary exacerbation in CF, aiming to make viral screening part of routine work-up for respiratory infections.

3- Routine vaccination of Egyptian CF children against influenza virus.

Summary

Cystic fibrosis is the most common inherited disorder in childhood being caused by mutations of the cystic fibrosis transmembrane conductance regulator (CFTR gene). It is a chronic condition involving several organs systems that results in life-long morbidity and premature mortality. Lung disease in CF is the major cause of death through a complex process involving impairment of mucociliary clearance, infection, inflammation, and structural injury.

Respiratory viruses are an inescapable part of human existence. For people with CF, respiratory viruses are a major concern and frequently act as the harbinger of a pulmonary exacerbation.

In this cross sectional observational study, we did sputum culture for bacteria and nasopharyngeal swabs for respiratory viruses for 60 patients diagnosed as CF, based on clinical manifestations, examination and confirmed by a positive sweat chloride test. Coming in acute exacerbation to the cystic fibrosis clinic in children's hospital, Cairo University throughout a period of 7 months.Their age ranged from 6 months to 13 years.

The present study detected the presence of viruses in 48 patients during exacerbation (80%), the most common virus was Rhinovirus in 43.4% of patients present allover the year, followed by bocavirus in 20%, adenovirus in 13.3%, enterovirus in 10%, HMPV in 6.7%. Coinfection with double viruses in 16.6% of patients.while bacteria present in 56.7% of patients and the most common organism found was pseudomonas spp in 20% of patients then staph-aeurus, MRSA, Klebsiella and Hemophilus influenza.CRP positive in 53.3%.

There is significant correlation between positive bacterial culture and certain viruses as influenza A virus, Enterovirus and HMPV with P value (<0.01, 0.002, 0.002 respectively).There was significant relation between influenza A virus and Enterovirus with the need for oxygen with p value (

0.004, 0.02 respectively). Significant correlation was noted between HMPV and season with (p value 0.001). There was no significant relation between the need for ICU admission and viral infection.

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الملخص العربى

التليف الكيسى للرئة هو المرض الوراثى الأكثر شيوعا في مرحلة الطفولة الذى يسببه طفرات في الجين المسمى (CFTR).

وهو حالة مزمنة تشمل العديد من الأجهزة والأعضاء التي تؤدي إلى الاعتلال مدى الحياة والوفيات المبكرة. مرض الرئة في التليف الكيسى هو السبب الرئيسي للوفاة من خلال عملية معقدة تنطوي على ضعف في إزالة المخاط ، والعدوى، والالتهاب، والإصابة الهيكلية .

ففيروسات الجهاز التنفسي جزء لا مفر منه من الوجود الإنساني. بالنسبة للأشخاص الذين يعانون من التليف الكيسى، هذه الفيروسات هي مصدر قلق كبير وغالبا ما تكون بمثابة نذير للتفاقم الرئوي .

في هذه الدراسة الرصدية المقطعية، قمنا نحن بزراعة البلغم للبكتيريا ومسحات البلعوم الأنفي لفيروسات الجهاز التنفسي لـ 60 مريضا تم مسبقا تشخيصهم على أنهم مرضى التليف الحويصلى، استنادا إلى المظاهر السريرية والفحص وتأكيد التشخيص من قبل اختبار كلوريد العرق الإيجابي. أتوا فى حالة تفاقم حاد إلى عيادة التليف الكيسى في مستشفى الأطفال، جامعة القاهرة.وقد تراوحت أعمارهم من 6 أشهر إلى 13 عاما .

كشفت هذه الدراسة أن الفيروسات كانت موجودة في 48 مريضا خلال التفاقم الرئوى (80٪)، وكان الفيروس الأكثر شيوعا Rhinovirus 43.4٪ موجود في جميع الفصول، يليه Bocavirus 20٪، . Adenovirus 13.3%، Enterovirus 10٪،HMPV 6.7٪، العدوى الفيروسية المزدوجة 10 مرضى. بينما كانت البكتيريا موجودة فى 56.7% من المرضى وكان الأكثر شيوعا Pseudomonas فى 20% من المرضى ثم Staph وMRSA وKlebsiella وHemophilus influenza. وكان CRP إيجابى فى 53.3%. كانت هناك علاقة قوية بين البكتيريا وفيروس Influenza A وEnterovirus وHMPV وبقيمة بى (< 0.01 ،0.002,0.002) على التوالى. كانت هناك علاقة قوية بين فيروس Influenza A وEnterovirus والحاجة إلى الأوكسجين وبقيمة بى(0.004، 0.02 على التوالى). لم تكن هناك علاقة بين الفيروسات ودخول العناية المركزة. كانت هناك علاقة قوية بين فيروسHMPV والموسم وبقيمة بى (0.001).

نسبة تواجد الفيروسات التنفسية فى مرضى التليف الكيسى اثناء تفاقم المرض

رسالة توطئة للحصول على درجة الماجستير

فى طب الأطفال

مقدمة من

الطبيبة / علا سليمان إمام يونس

بكالوريوس الطب والجراحة

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