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Leberbeteiligung
bei viralen Atemwegsinfektionen von Kindern

Liver involvement
in viral respiratory infections of children

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This dissertation is dedicated to my lovely husband and my son as they are my strength in everything I do.

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List of abbreviations

ALAT	Alanine aminotransferase
ALP	Alkaline phosphatase
ASAT	Aspartate aminotransferase
AV	Adenovirus
BMI	Body mass index
BoV	Bocavirus
CMV	Cytomegalovirus
CoV	Coronavirus
CRP	C-reactive protein
DNA	Deoxyribonucleic acid
EBV	Epstein-Barr virus
EV	Enterovirus
FluV	Influenza virus
γ-GT	Gamma-Glutamyltransferase
HBoV	Human bocavirus
HCoV	Human coronavirus
HHV-4	Human herpesvirus 4
HHV-5	Human herpesvirus 5
HHV-6	Human herpesvirus 6
HIV	Human immunodeficiency viruses
HMPV	Human metapneumovirus
HRV	Human rhinovirus
LRTI	Lower respiratory tract infection
LVE	Liver values elevation
PCR	Polymerase chain reaction

PIV	Parainfluenza virus
RNA	Ribonucleic acid
RSV	Respiratory syncytial virus
RT-PCR	Real-Time-Polymerase chain reaction
RV	Rhinovirus
SARS	Severe acute respiratory syndrome
URTI	Upper respiratory tract infection
VZV	Varicella-zoster virus

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1 Introduction/ Aim of dissertation

Acute respiratory infections are an important factor of morbidity in infants and children (Bharaj et al., 2009). The main causative agent of these infections is in 45-60% of the cases viruses (Marinheiro, Sanalios, Santos, Costa, & Hársi, 2009). Clinical manifestations may involve both the upper respiratory tract with symptomatology of rhinitis, pharyngo-tonsilitis, otitis, sinusitis or lower respiratory tract with bronchiolitis, asthma attacks and pneumonia (R. M. Kliegman, B. M. Stanton, J. S. Geme, & N. F. Schor, 2015).

Acute bronchiolitis is mainly caused by respiratory syncytial virus (RSV). By the age of two, almost 100% of children have been diagnosed with RSV infection (Gern, Rosenthal, Sorkness, & Lemanske Jr, 2005). Other viral agents involved in respiratory infections are parainfluenza viruses (PIV), influenza virus (FluV) A & B, adenovirus (AV), rhinoviruses (RV) and echo viruses. At the beginning of the 21st century, newer respiratory viruses such as coronaviruses (CoV), human metapneumovirus (HMPV) and bocavirus (BoV) have been identified and have been associated with respiratory infections as well (Ruohola et al., 2009).

It is well known that Epstein – Barr virus (EBV) causes hepatitis with elevated liver enzyme levels and hepatomegaly (Schechter & Lamps, 2018). The main symptoms of an EBV infection are tonsillitis, cervical lymphadenopathy and fever. In more than 90% of the cases of EBV-related infection, there is liver involvement, but in the majority of these cases it is subclinical and self-limited or manageable with supportive treatment (Suh, Liapis, Misdraji, Brunt, & Wang, 2007; Vouloumanou, Rafailidis, & Falagas, 2012). EBV infection can also lead to severe or fatal hepatitis, mainly in immunocompromised patients and rarely in immunocompetent (Edoute et al., 1998; J. L. Mellinger et al., 2014). There has also been reported cases where the infection can be recurrent or even chronic, which has been linked to a very poor prognosis (Drebber et al., 2006). Although there are many hepatic findings that vary among patients, the liver lesions can be severe and therefore an EBV infection can also lead to death (Drebber et al., 2006).

Other viruses that cause respiratory tract infection are, also, not restricted to this organ (Papic et al., 2012). In patients with upper or lower respiratory tract infection it has been noticed, not rarely, that they also show elevated liver enzyme levels, which suggest liver involvement. In literature, we have frequently encountered references for the involvement of the liver in viral infections caused by a lot of respiratory viruses, such as AV (B. Han, Son, Yoon, & Lee, 1998), RSV (Michael Eisenhut, 2006), CoV (Farcas et al., 2005) and FluV (Al-Refaee, 2012; Papic et al., 2012; Ru et al., 2011). However, there are not many large scale prospective studies that

primarily investigate the liver involvement in respiratory tract infections in children and adolescents.

The aim of this work was to investigate the relationship between respiratory tract infections and the occurrence of elevated hepatic enzyme values in children and adolescents. More specifically, we wanted to study how frequent is the involvement of the liver in pediatric patients with respiratory infections, which are the most common pathogenic factors in these cases and also which viruses may cause concomitant hepatitis.

For this purpose, we conducted a prospective clinical study according to the state of the art. Prospective studies investigate for results, for example the development of a disease and try to relate this, during the study period, to a number of factors. The reason why we conducted a prospective clinical trial was because we have noticed in clinical practice that during viral infections of the respiratory tract concomitant hepatitis is induced (Al-Refaee, 2012; Michael Eisenhut, 2006; Farcas et al., 2005; B. Han et al., 1998; Papic et al., 2012). However, in viral infections, since antibiotics cannot be used, we tend to treat the symptoms rather than the cause (antipyretics, antitussives, mucolytics, etc.). Therefore, we wanted to investigate whether hepatitis is caused by the virus or the medication administered.

For the above mentioned reasons, we included 6 pediatric practices (outpatients) and one pediatric hospital (inpatients) in Germany in our study. The subjects were aged 1-18 years old suffering from symptoms of respiratory tract infections. During the study the medical history of the patients was documented and also blood was sampled for several laboratory tests. The results were also statistically analyzed.

2 Literature Discussion

2.1 Liver anatomy

The liver is the largest gland of the human body with exocrine and endocrine fates. It is the chemical factory of the body. It is located in the upper abdomen and occupies the right subchondria, the largest part of the epigastric and part of the left hypochondrium. Its weight in adults is from 1200 to 1800 grams and represents about 2% of the total body weight of an adult (Hirata et al., 2004). In the liver we can see two surfaces, the upper or diaphragmatic and the lower or visceral (Abdel-Misih & Bloomston, 2010).

Most of the liver is surrounded by peritoneum except of a part on the back of the lower surface that comes in direct contact with the central tendon of the diaphragm (bare surface). The recurrences of the peritoneal lamella of the peritoneum around the naked surface of the liver constitute the anterior and posterior lamella of the coronary ligament. The two petals meet at both ends and form the right and left triangular ligament of the liver. The anterior petal of the coronary ligament approximately in the midst retracts forward and forms the falciform ligament, dividing the liver into right and left lobes (Figure 1). Falciform ligament has a free end that adheres to the abdominal wall up to the navel and contains the round joint which is the obtuse embryo umbilical vein (Abdel-Misih & Bloomston, 2010).

The liver hilum is located on the visceral surface of the organ and connects two sagittal sulci. The left hosts fetal vessel remnants, in front of which there is the round ligament (occluded umbilical vein), and behind the vein ligament, which is the obstructed venous duct. The right sagittal sulcus in front appears enlarged and forms the cystic fossa that holds the gall bladder. The back of the sulcus receives the inferior vena cava. Between the two sulci in front of the portal is formed the quadrate lobe and behind the portal is the caudate lobe (Figure 2). The hepato-duodenal ligament transports to the liver portal the liver artery and portal vein (Sibulesky, 2013).

Except of the anatomical separation of the liver in lobes there is also the so-called surgical (according to QUINAULD), where the liver is divided into eight (VIII) sections. Specifically, the caudate is Section I, the left lobe consists of Sections II and III whereas the quadrilateral lobe and the remaining right lobe consist of Sections V, VI, VII and VIII (Figures 3 and 4) (Sibulesky, 2013).

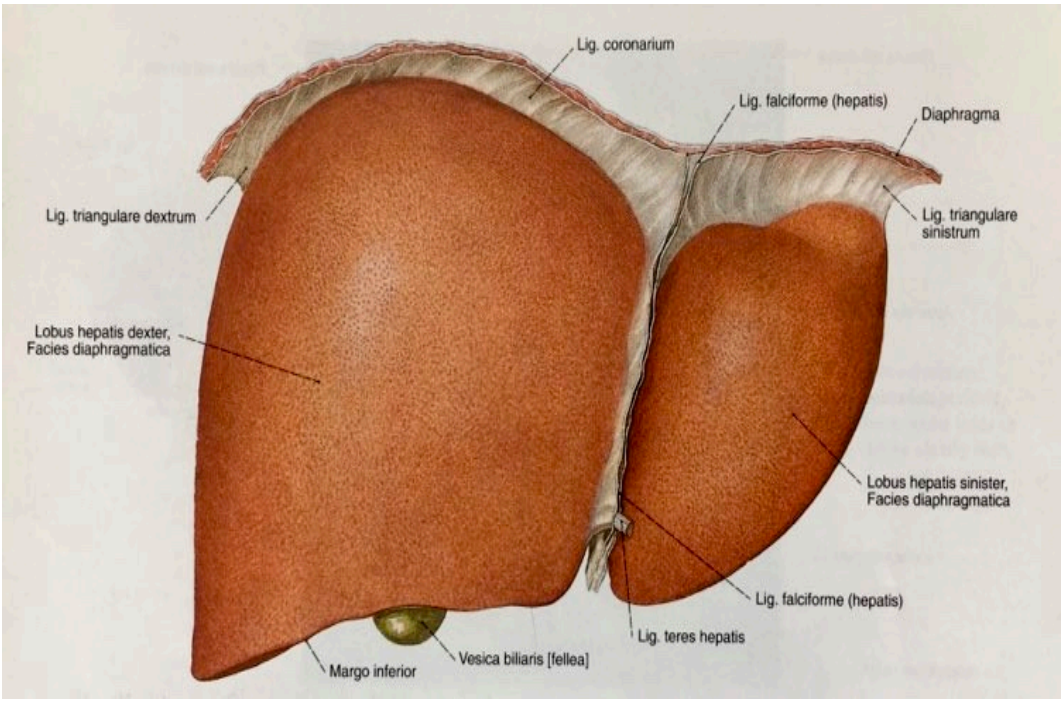


Figure 1: Ventral view of the liver (Sobotta, Atlas of Human Anatomy).

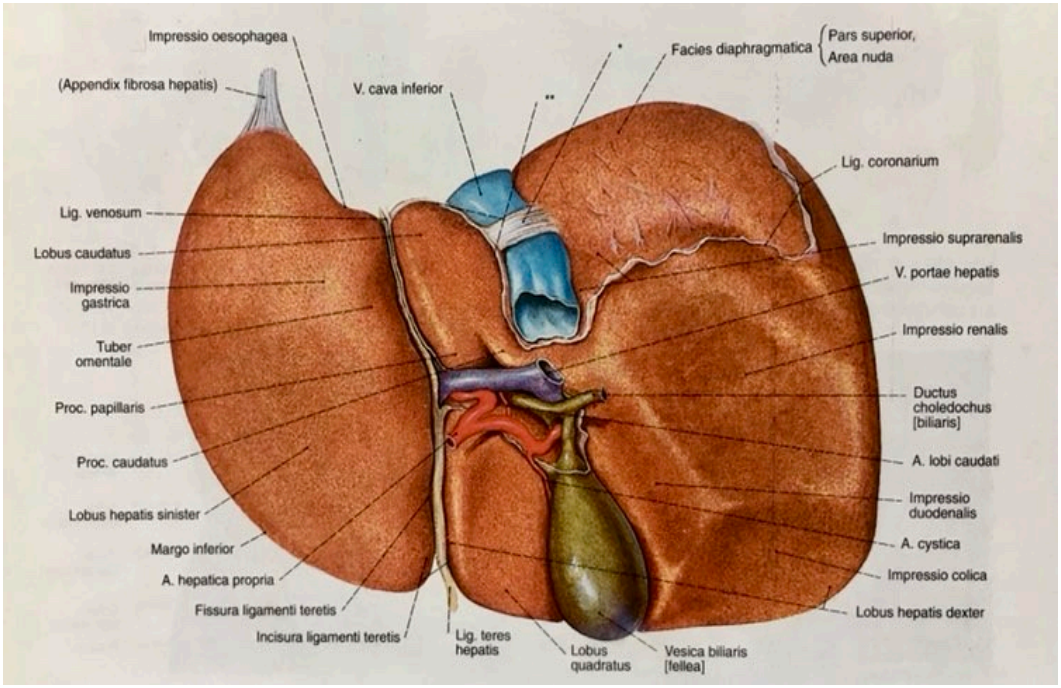
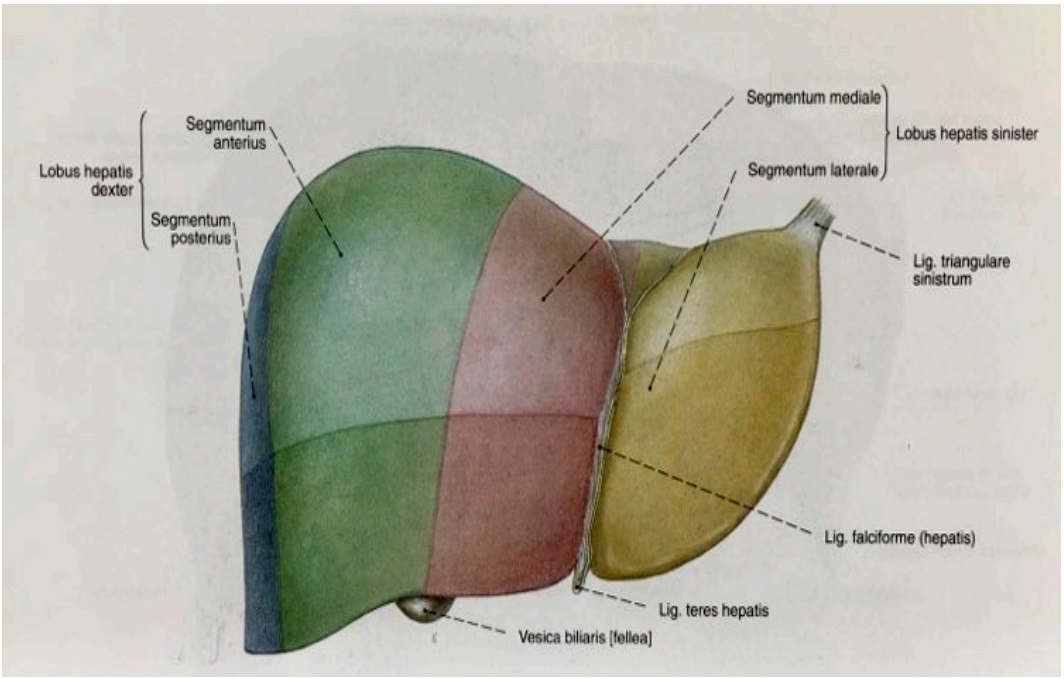
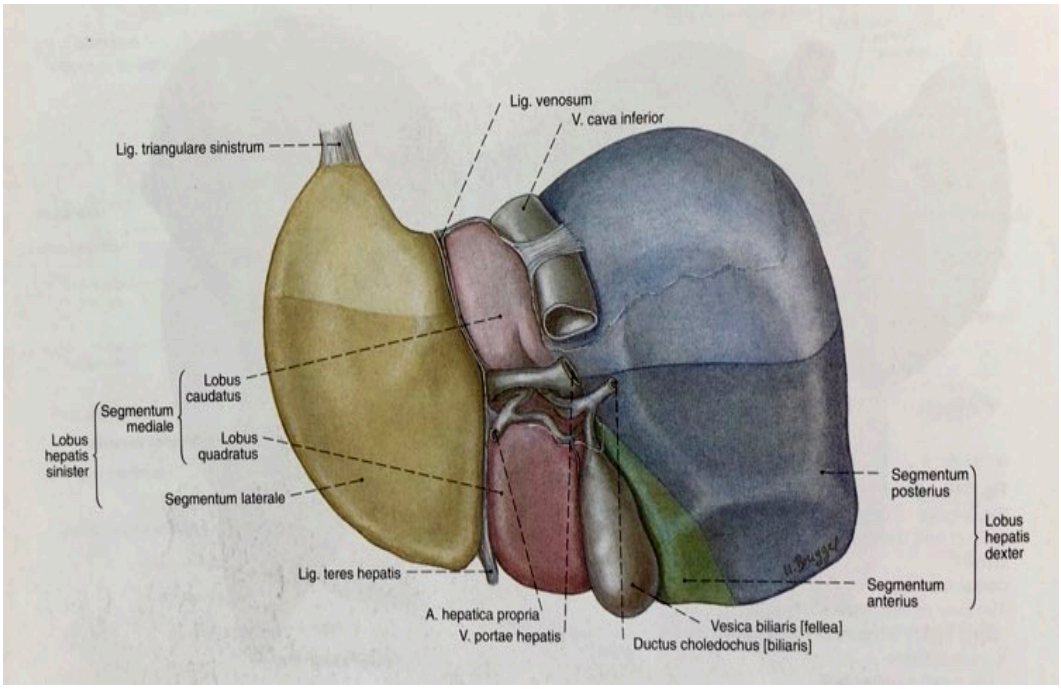


Figure 2: Dorsal view of the liver (Sobotta, Atlas of Human Anatomy).





Figures 3 and 4: Ventral and dorsal view of the liver. Surgically these segments are further divided into an upper (light colours) and lower part (dark colour) (Sobotta, Atlas of Human Anatomy).

2.1.1 Hepatic vasculature

Liver hematopoiesis is double. The liver artery and the portal vein are responsible for this task. The common hepatic artery is a branch of truncus coeliacus which emerges from the abdominal aorta. From the ventricular artery, also appear the left gastric artery for stomach hematopoiesis and the spleen artery for spleen hematopoiesis (Abdel-Misih & Bloomston, 2010).

The common hepatic artery provides the gastroduodenal artery for the duodenal and pancreatic hematopoiesis and continues to the liver as a proper hepatic artery through the hepatodysplastic bundle into the bile duct. At the height of the liver hilum it is divided into right and left branches. From the right branch, the cystic artery develops for the hematopoiesis of the gallbladder (Figure 5) (Abdel-Misih & Bloomston, 2010).

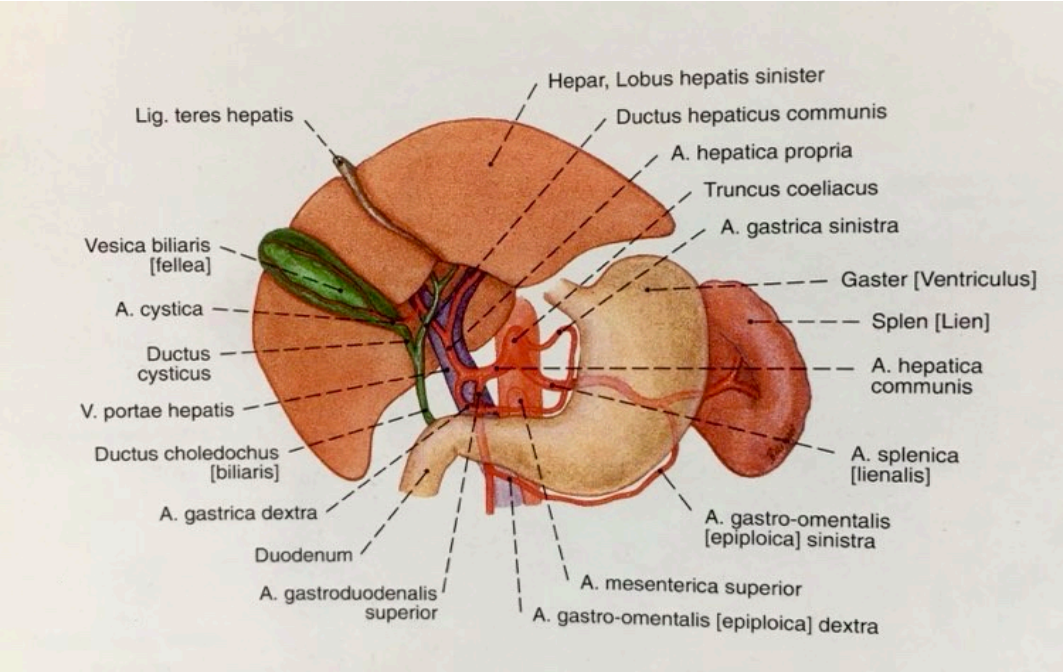


Figure 5: Arterial blood supply of the liver (Sobotta, Atlas of Human Anatomy).

The portal vein is formed by the confluence of the splenic vein with the superior mesenteric vein behind the pancreas head. Venous blood from the unilateral abdominal viscera (lower esophagus, stomach, gastrointestinal tract to upper rectum, spleen, pancreas) is collected in the portal venous system, which is carried by the portal vein to the liver. In the liver the blood passes through the capillary system of the portal vein and finally with the hepatic veins ejects into the inferior vena cava (Sibulesky, 2013).

2.1.2 Histology of the liver

The liver is surrounded by the Glisson capsule, which creates protrusions to the liver parenchyma creating a loose connective tissue network (Glisson sheath). At the intervals of the network there are epithelial liver cells (hepatocytes) that form the liver lobes. The hepatic lobule is the basic unit, has small size (1.5 - 2mm) and a polyhedral shape which is classically described as a hexagon. Corresponding to the angles of the hexagon, the medullabelous spaces are enlarged, containing a branch of the hepatic artery, a branch of the portal vein, a bile duct and lymphatic vessels and constitute the portal triad (Abdel-Misih & Bloomston, 2010).

Hepatocytes form anastomous trabeculae of one or two cells carried radially toward the periphery of the lobe. Between the trabeculae there are the sinusoids capillaries in which the blood flows from the hepatic artery and portal vein. In the center of the lobulum is the central vein in which ends the blood from the sinusoids. Sinusoids constitute the exchange track of the liver hilum (Sibulesky, 2013). Between the endothelium of the sinusoids and the hepatocytes there are the Kupffer asteroid cells which show phagocytic properties (Figures 6 and 7) (Kahle, Leonhart, & Platzer, 1986).

The central veins assembled end up in the liver veins. The classic hepatic lobe as described presents the liver texture morphologically. From a functional point of view, however, it can be described as a botrix where the portal triad is at the center (Kahle et al., 1986).

The concentration of oxygen, nutrients and hormones is greater around the portal triad and decreases gradually as the blood is directed from the sinusoids to the central veins. This functional heterogeneity of the hepatic lobe can be expressed in the form of three concentric zones around the portal triad. Thus zone 1 around the portal triad is the best oxygenated while zone 3 around the central veins is the poorest oxygenated. The intermediate area in the middle of the lobe is zone 2 (Abdel-Misih & Bloomston, 2010).

Bile ducts are formed between two adjacent hepatocytes and do not have their own cell wall. Bile exits from the center of the lobe to the periphery of the mesolobian bile ducts which have an epithelial wall and which are constantly joined by adjacent ones and brought to the liver hilum. At this height, from the joining of the right with the left hepatic duct, the extravascular bile duct begins. From their union, a common liver duct, 4-6cm long, is created, which together with the cystic duct is the bile duct. The cystic duct comes from the gallbladder and is 3-4cm long. The bile duct, 10 cm long, is carried through the hepato-duodenal ligament, behind the first duodenum to reach the back of the inner wall of the duodenal collapsed duct, where in 77% of the cases it expands together with the pancreatic duct at the major duodenal papilla (Vater's papilla) (Kahle et al., 1986).

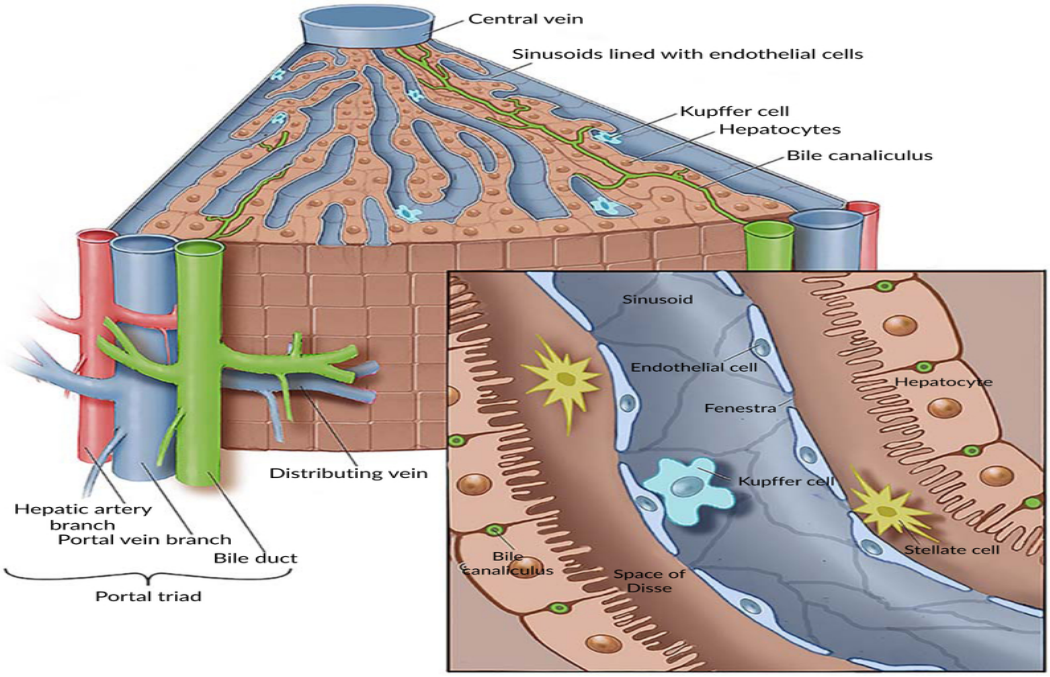


Figure 6: Microscopic anatomy of human liver (Anan Abu Rmilah et al,.).

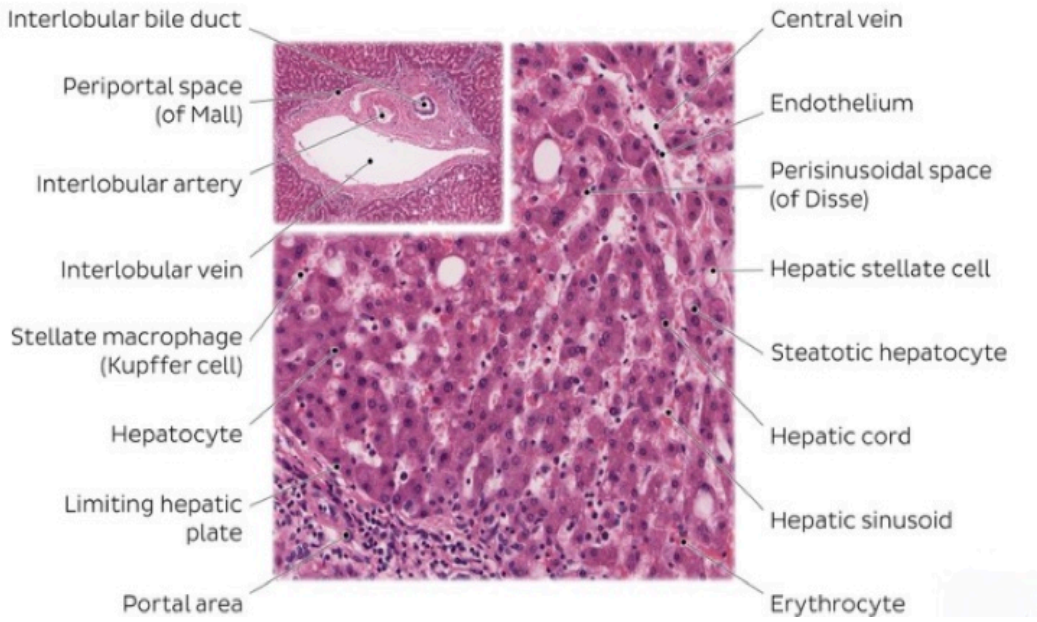


Figure 7: Liver histology (Pawlina & Ross, 2018).

2.2 Hepatitis

2.2.1 Viral hepatitis

Viral hepatitis is defined as the diffuse inflammation of the hepatic parenchyma (Alter, 2003; Cecil, Goldman, & Schafer, 2012; Katsanos et al., 2009; B. Kliegman, J. Stanton, N. Geme, & R. Schor, 2015; Shepard, Finelli, & Alter, 2005; Zumla, 2010) which is usually caused by hepatoproliferative viruses A, B, C, D and E. Viral hepatitis is divided into two major categories, depending on their path: acute and chronic viral hepatitis (Cecil et al., 2012; Kasper & Harrison, 2012; Zumla, 2010). Acute hepatitis may appear abruptly a few weeks after the viral infection and can be self-healed within a few months. A typical example of this is hepatitis A. Chronic hepatitis can be caused by the hepatitis B, D viruses and mainly hepatitis C virus (Cecil et al., 2012; Hatzakis et al., 2011; Papaevangelou, 1992; Zehender et al., 2012). In recent studies, chronic hepatitis may also result from the hepatitis E virus, mainly in immunosuppressed, transplanted and human immunodeficiency viruses (HIV) patients (Fujiwara et al., 2014; Passos-Castilho et al., 2014; Pischke et al., 2012; Riveiro-Barciela et al., 2014). Usually, acute hepatitis is self-healed and resolves without residual hepatic damage or persistence of the virus in the body. However, a proportion of certain forms of hepatitis lead to persistent infection with chronic liver damage. Chronic hepatitis includes conditions characterized by chronic necrotic and inflammatory liver damage. The disease is characterized as chronic if there is evidence of progressive damage for more than six months (Cecil et al., 2012; Kasper & Harrison, 2012; Zumla, 2010).

2.2.2 Viruses causing hepatitis

- Herpesviruses

Herpesviruses (Herpesviridae) are a family of double stranded DNA viruses that cause a wide variety of diseases in humans and animals, including herpes, chickenpox and infectious mononucleosis. Characteristic of these viruses is their ability to remain dormant in host cells for a long time and to operate only in stressful conditions. There are 100 known viruses belonging to this family and divided into three subfamilies. It has been reported that in case of congenital infection the virus may cause severe disease in multiple organs, including hepatitis. Also, in immunocompromised patients infection by herpesviruses represents a major of morbidity and mortality (Stumpf, Laidlaw, & Jansen, 2002).

EBV, human herpesvirus 4 (HHV-4) was first described by M.A. Epstein, G.B. Achung and Y.M. Barr in 1964 (Drebber et al., 2006). It is of the genus Lymphocryptovirus and belongs to the family of Herpesviruses and specifically to the gammaherpesviridae subfamily. The EBV

seems to play an important role in liver pathology, since some studies have suggested EBV triggers for an autoimmune hepatitis (Krawitt, 2006). EBV-related hepatitis is not quite common. However, in these cases there is a dense lymphocytic inflammatory infiltrate in sinusoids as well as in portal tracts. Also, the lymphoid cells seem to be enlarged and atypical. In some patients apoptotic hepatocytes have been observed, but the cellular damage is smaller in comparison to the inflammation that have taken place. Moreover, although the lymphoid infiltrate may be present, that does not destroy cells at the portal interface (Koch et al., 2007; Jessica L Mellinger et al., 2014).

Cytomegalovirus (CMV) or human herpesvirus 5 (HHV-5), is a member of the herpesvirus family and belongs to the beta-herpes subfamily. CMV does not seem to have a specific geographic location and appears globally with a different frequency per region. It has been shown that the virus appears more frequently in developing countries and in societies with lower socio-economic level (Ho, 2008). About 40% of the adults worldwide will be infected with the virus at some stage of their life. CMV is also the most common virus in developing embryos and can cause various abnormalities in their development. In most of the cases of congenital CMV the patient is asymptomatic, although in some cases it has been reported that it can cause prematurity, neurologic dysfunctions, jaundice and hepatosplenomegaly (Taylor, 2003). CMV can cause mononucleosis syndrome similar to that caused by EBV and sometimes liver dysfunction is also a symptom, which occasionally may lead to fulminant hepatic failure (Ho, 2008). Also, in patients with primary CMV infection and mononucleosis, hepatitis may occur. In these cases a transient increase in hepatocellular enzyme levels can be observed as well as, of course, the patient may develop jaundice. Although the disease has a favorable prognosis, deaths have been reported in immunosuppressed patients (Kenneson & Cannon, 2007). Pathological features are penetration of mononuclear cells from the virus entry region and also development of granulomatous infection. Neonatal hepatitis is also common in some cases of congenital CMV, with portal and lobular inflammation, cholestasis, variable degrees of extramedullary hemopoiesis, and giant cell transformation of hepatocytes (Kenneson & Cannon, 2007).

Human herpesvirus 6 (HHV-6) was isolated for the first time from immunocompromised patients in 1986 (Lopez et al., 1988). This virus is the reason for exanthema subitum in children. As is the case with the rest of herpesviruses acute infections of HHV-6 show in most cases no or very few symptoms with a spontaneous favorable outcome. However, in immunosuppressed patients, serious clinical manifestations that affect the central nervous system, liver, gastrointestinal tract, lungs, and bone marrow may be observed (Agut, 2011). In those cases, mainly in allograft recipients, HHV-6 may be the cause of acute hepatitis, which may, therefore, lead to fulminant hepatitis (Asano et al., 1990).

Varicella-zoster virus (VZV) is one of the well-known eight types of herpes virus that severely affects humans and no other mammalian species. It is estimated that, worldwide, it infects 90% of the population until it reaches adulthood (Dworkin et al., 2007; Dwyer & Cunningham, 2002; Gnann Jr, 2007; Heininger & Seward, 2006). The reactivation of the virus causes herpes zoster, which causes a painful cutaneous eruption with dermatomal distribution. In most of the cases herpes zoster can cause postherpetic neuralgia as complication (Saxena, 2017). Herpes zoster virus is almost never observed in immunocompetent patients, however, in patients that have undergone steroid treatment or chemotherapy submassive/massive hepatic necrosis may be seen. In these cases, there are a lot of pathognomonic intranuclear herpetic inclusions (Herrero et al., 2004).

Herpes simplex viruses are important human pathogens causing diseases in a variety of different tissues and animal species. Herpes simplex viruses types 1 and 2 as well as VZV belong to the alphaherpesviruses, which show great host range, a quite short life cycle and establish latent infections preferentially in sensory ganglia (Cote-Daigneault, Carrier, Toledano, Wartelle-Bladu, & Willems, 2014). Herpes simplex viruses does not cause hepatitis often, however it may lead to acute liver failure, liver transplantation, and/or death. Herpes simplex viruses most commonly affects immunosuppressed patients but it may also affects, rarely, immunocompetent individuals as well (Côté - Daigneault, Carrier, Toledano, Wartelle - Bladu, & Willems, 2014; Levitsky et al., 2008). As far as histology is concerned, in herpes simplex viruses hepatitis we observe fulminant hepatic necrosis and serum aminotransferase levels are 100 to 1,000-fold above normal (Goodman, Ishak, & Sesterhenn, 1986). In the case of pregnants or neonates there is wide hepatic necrosis but there are no viral inclusions and no inflammatory response (Jacques & Qureshi, 1992).

- Adenoviruses (AV)

AV, members of the Adenoviridae family, are non-enveloped, double DNA viruses that were isolated by Rowe et al. in 1953 (G. Wadell, Hammarskjöld, Winberg, Varsanyi, & Sundell, 1980). They have a wide range of vertebrate hosts. On 68 serotypes have been found to cause a wide variety of conditions, mainly respiratory tract infections in children and infants. The serotyping is based on their neutralization by specific animal antisera. All human AVs belong to the genus Mastadenovirus. The 68 human serotypes are classified in 7 species / subgroups (A – G) (Berk, 2007; Harrach et al., 2011).

The virus only rarely causes hepatitis in immunocompetent patients but it can cause severe hepatitis in immunicompromised patients that can even lead to acute hepatic failure (Ronan et al., 2014). Hepatitis caused by AV shows scattered necrotic areas all over the parenchyma, and the hepatocytes that are near those areas include nuclear viral inclusions. Also, there are

a lot of cells that are related to inflammation such as macrophages and lymphocytes (Larranaga, Kajon, Villagra, & Avendano, 2000).

- Respiratory syncitial virus (RSV)

RSV is a RNA virus that belongs in the genus of Pneumoviruses and the family of Paramyxoviridae (Jha, Jarvis, Fraser, & Openshaw, 2016). This virus is quite common in children, since together with influenza can cause severe infections of the respiratory track in children and infants (Pilar Orive et al., 1998). There has also been described extrapulmonary manifestation of severe RSV infection (Njoku & Kliegman, 1993). In the literature it has been shown that RSV can also cause hepatitis. Specifically, in almost 50% of children with RSV bronchiolitis, there were elevated hepatic enzymes levels, while infants showed more severe symptoms of the infection (M. Eisenhut & Thorburn, 2002; M. Eisenhut, Thorburn, & Ahmed, 2004). However, RSV associated hepatitis is quite rare in immunocompetent patients (Nadal, Wunderli, Meurmann, Briner, & Hirsig, 1990). Also, individuals with Reye's syndrome caused by RSV infection can exhibit liver involvement as well (Griffin, Keeling, & Tomlinson, 1979).

- Coronaviruses (CoV)

CoV, belong to the family of Coronaviridae (King, Adams, Carstens, & Lefkowitz, 2012) and are the causative agent of respiratory infections, mainly, in young children. However, patients with respiratory tract infections often exhibit dysfunctions in other organs as well, including liver (Chan-Yeung & Yu, 2003; Peiris et al., 2003). Although, in many cases, the hepatic viral load of the patients and the transaminases levels are elevated, there is only minor inflammation in the liver (Farcas et al., 2005). The existing data seem to support the hypothesis that CoVs mediate liver dysfunction (Farcas et al., 2005).

As far as the new CoV (COVID-19) is concerned, there are studies that clearly show the presence of elevated liver enzyme values in patients with COVID-19 disease (Fix et al., 2020; Hao et al., 2020; Moon & Barritt, 2020; Sultan et al., 2020), however there are no specific histopathological findings in these patients (Jothimani, Venugopal, Abedin, Kaliamoorthy, & Rela, 2020). Therefore, more data are needed in this area.

- Influenza viruses (FluV)

FluVs are classified into the family of Orthomyxoviridae (Bouvier & Palese, 2008). FluV is responsible for the largest and most deadly viral epidemics suffered and usually causes respiratory disease with relatively few extrapulmonary manifestations (Neuzil, Mellen, Wright, Mitchel Jr, & Griffin, 2000). It has been reported, however, that in patients with influenza there

is liver damage that can also lead to acute hepatitis, with or without liver failure (Papic et al., 2012). Clinical manifestations of hepatitis caused by FluV include jaundice, elevated liver enzymes and coagulopathy (Whitworth et al., 2006). These findings strongly suggest that FluV, although rarely, may exhibit a hepatotropic action (Al-Refaee, 2012).

2.3 Viral infections of the respiratory system

The respiratory system may be infected by a large group of viruses that affect the respiratory epithelium and cause a variety of syndromes that differ in severity of symptoms, from mild cold to cases of pneumonia and bronchiolitis. Respiratory tract infections are among the most common diseases worldwide, causing a significant amount of morbidity and mortality in humans (Templeton, 2007).

They are distinguished in upper respiratory tract Infection (URTI) and lower respiratory tract infection (LRTI) respiratory system, divided at the vocal cords, depending on the symptomatology and the anatomical area involved. URTIs, though mostly self-serving, often lead to the doctor and unnecessary antibiotic prescribing. LRTIs are accompanied by more severe symptoms and usually a large proportion of patients requires hospitalization and treatment (Dasaraju & Liu, 1996). Influenza-like illness is also commonly used in the world literature to describe viral URTIs with symptoms such as fever higher than 38ºC, cough and / or sore throat, in the absence of known cause (Aguilera et al., 2003; Nichol, 2006).

2.3.1 Infections of the upper respiratory system

- Rhinitis

Rhinitis is the result of a viral or bacterial infection of the nasal major cavity and in the majority of cases is followed by inflammation of the adenoid outgrowth in children or the sinus cavities (in children and adults). The most common causative factors are RV, CoV, FluV and PIV, RSV and Coksackie virus. Some of the symptoms are: productive cough, development of swelling in the mucous membranes of the nasopharynx, swallowing difficulty, nasal cavity pain, vibrant vocal chords, low fever, headache, loss of appetite, drowsiness, irritability, gradual change in nasal secretions, rarely in children younger than three years old, night cough lasting about a week (Dykewicz & Hamilos, 2010). The symptoms last for a few days and in most cases rhinitis is self-healing. Treatment is supportive rather than causal (Dykewicz & Hamilos, 2010).

- Pharyngitis/ Tonsilitis

We refer to pharyngeal inflammation and tonsil edema due to infection of the individual organs of the upper respiratory tract by an infectious agent (Vincent, Celestin, & Hussain, 2004). It is caused by a heterogeneous group of viruses and microorganisms with the most frequent microbiological etiological infectious agent of β-haemolytic Streptococcus. It is estimated that in children aged 7 to 15 years beta-hemolytic Streptococcus is responsible for more than 25% of all cases of pharyngitis or tonsillitis (Ridway, 2001). Viruses that can cause the disease are AV, RV, CoV, EBV, CMV and the herpes virus (Bisno, 1996).

The transmission of the disease usually occurs after frequent contact with the patient. The highest incidence of streptococcus and CoV infections is the period between winter and spring, while RVs are usually caused in autumn and spring (Gerber, 2005). We therefore conclude that acute pharyngitis or sore throat or pharyngitis - tonsilitis are infections of the upper respiratory tract that occur primarily in childhood with seasonal upgrading according to the virus that causes them (Seaton, Seaton, & Leitch, 2000).

Some of the symptoms are: pain located in the pharynx, disruption, fever, headache, nausea, vomiting, abdominal pain, pharyngeal redness, tonsil edema, hyperplasia of lymphatic tissue in the posterior pharynx and tonsils (Gerber, 2005; Selby, 2002).

- Pararhinocolpitis

Sinus infections can cause inflammation, acute, chronic or recurrent (Slavin et al., 2005). More frequent is sinusitis, however, it is possible to infect both the frontal sinuses (Lund & Kennedy, 1995). Sinuses are typically free from microorganisms and contaminated by microbes of the nose and the mouth flora. The main microorganisms involved are Pneumococcus, Haemophilus, Streptococcus and anaerobes (Dykewicz & Hamilos, 2010). Pain in the face that is getting worse in appearance, purulent smelly secretion, headache with or without fever are the main manifestations. The classic symptoms of acute sinusitis in children usually follow a cold that does not improve or one that gets worse after 5 to 7 days of symptoms (Wald, 1992a, 1992b). Findings indicative of the disease are periorbital edema and local pain and / or sinus sensitivity (Wald, 1992a, 1992b).

2.3.2 Infections of the lower respiratory system

- Bronchiolitis

Bronchiolitis is one of the most common infections of the lower respiratory tract, which primarily affects children younger than 2 years old. The most well-known cause of the disease is the

RSV. A specific feature of this virus is its period of exacerbation, which dates back to November until April. Other viruses responsible for the development of bronchiolitis in children is the PIV, FluV and various AVs. The result of the infection is the inflammation of the bronchioles, where there is air obstruction and so the alveoli cannot carry out the gas exchange (Aherne, Bird, Court, Gardner, & McQuillin, 1970; Smyth & Openshaw, 2006).

The symptoms of bronchiolitis usually occur 1-3 days after the onset of common cold symptoms and include the following: nasal congestion, nasal discharge, persistent productive cough, fever ranging from 38.2 - 39.4^{o}C, loss of appetite, rapid breathing (60 to 80 breaths / minute), dyspnoea, wheezing upon inhalation, difficulty eating due to nasal congestion, rarely can cause dehydration of the child due to reduced intake of fluids, apnoea (especially in children of one year, where breathing is interrupted by 10 up to 20 seconds) (Gadomski & Scribani, 2014; Ralston et al., 2014). Treatment is mostly symptomatic (Ralston et al., 2014).

- Pneumonia

Pneumonia is the inflammatory reaction of the alveoli and / or of the medial tissue of the pulmonary parenchyma from infectious causes. Staying indoors during the winter months results in closer contact and easier transmission of infections. Schools and nurseries, in fact, are the ideal environment for the transmission of viral and bacterial infections (Rudan, Boschi-Pinto, Biloglav, Mulholland, & Campbell, 2008; Wardlaw, Salama, Johansson, & Mason, 2006).

Viruses are the most common cause of pneumonia in children aged 1 month - 2 years old. More specifically, in preschoolers the cause is Haemophilus infuenzae and in school-aged children Pneumococcus and Mycoplasma pneumoniae (Rudan et al., 2008; Ruuskanen, Lahti, Jennings, & Murdoch, 2011; Virkki et al., 2002; Wardlaw et al., 2006).

The clinical manifestation of pneumonia includes signs and symptoms such as: catarrh, nasal congestion, fever, productive cough and respiratory distress. In pneumococcal pneumonia the clinical picture is noisy with sudden fever, high fever, shivering, shortness of breath, thoracic or abdominal pain, malaise, pallor and rales (Ruuskanen et al., 2011)

The treatment involves patient support depending on the cause of the disease. Supportive treatment includes good hydration, antipyretics and treatment of respiratory distress by the administration of liquefied oxygen or antibiotics (McCracken, 2000; Rudan et al., 2008).

- Tracheobronchitis

Tracheobronchitis is one of the frequent infections of childhood, mainly in the form of acute respiratory infection due to tracheal and bronchial inflammation (Luxner, 2005; Sherry, Klainer, Wolff, & Gerhard, 1988). FluV (type A and B). PIV, AVs, and RSV are the main virulence factors

affecting children aged 4-6 years that can cause the disease (Chapman, Henderson, Clyde, Collier, & Denny, 1981; Luxner, 2005).

Main symptoms are: cough, which in the early days is characterized as dry while in continuously productive, low fever (ranges from 37.8 - 38.5^{o}C), feeling of chest discomfort, nasal congestion, nasal discharge, headache, pharyngitis, loss of appetite, irritability, physical weakness (Burger, 2012; Luxner, 2005).

The symptomatology may last from 3 to 5 days. However, if the signs deteriorate and the immune defense system of the child is unable to cope with the further clinical progression of tracheobronchitis, a therapeutic adaptation must be made in order to avoid a relapse of the infection within 7 days from the absence of symptoms (Luxner, 2005).

2.3.3 Viral agents in respiratory infections in children

- Respiratory syncytial virus (RSV)

RSV is a RNA virus that belongs in the genus of Pneumoviruses and the family of Paramyxoviridae (Jha et al., 2016). It was first isolated in 1956 from a sick chimpanzee during an epidemic that looked like common cold in laboratory animals. The virus took the name syncytial due to the characteristic lessions that it causes in cell cultures, syncytia (Azevedo et al., 2003; Jha et al., 2016; Thompson et al., 2003).

RSV causes epidemics every year (Hall et al., 2009; Resch, 2012). The impact of primary infection on infants in urban areas is estimated at 50-60% for each epidemic, whereas the incidence of re-infection is 10-20%. The severity of the disease is greater in re-infection, so 1-3% of infants affected by RSV need hospital admission (Hall et al., 2009; Resch, 2012). All children are infected with the virus in the first 2-5 years of their life (Hall, 2001; Hall et al., 2009; Resch, 2012).

RSV only affects humans (B. Kliegman et al., 2015). Infants show more severe symptoms of the disease since they come into contact with the virus for the first time and have reduced ability to produce neutralized antibodies and secreted IgA (Hall et al., 2009; Resch, 2012). The disease is even more severe in neonates, prematures and babies with congenital heart disease, bronchopulmonary dysplasia or immune deficiency. Infection in infancy usually causes lower respiratory tract disease, bronchiolitis, bronchopneumonia (Jha et al., 2016). Disease in older children usually occurs as upper respiratory tract disease, bronchitis and asthma crisis (Pediatrics, 2012).

- Rhinovirus (RV)

RVs are the most important and frequent causative factors for the common cold and URTIs (Dick, Jennings, Mink, Wartgow, & Inhorn, 1987). RVs were first discovered in 1953 in England, by a biologist who had an URTI and who isolated the virus after a nasal wash culture in human lung explants (Andrewes, CHAPRONIERE, GOMPELS, Pereira, & Roden, 1953).

Human Rhinoviruses (HRVs) are the major pathogens that infect humans, since they are responsible for about 50% of the common cold (Takeyama et al., 2012). They are global diasporas and infect patients of all ages. Infections from hRVs can occur throughout the year, but their frequency increases mainly in the spring and autumn. During autumn, these viruses account for 90% of cases of URTI (Peltola et al., 2008). Only 50% of people infected with disease, as well as asymptomatic vectors, disperse the virus in the environment. In a community and at a certain time, there are at the same time 3-4 hRV serotypes, of which, as a rule, one prevails (Takeyama et al., 2012). Then, after about a year, and due to the gradual growing immunity of the population, a new group of serotypes invade the community and so on (Wang et al., 2011). The most vulnerable are the children, who are the main hosts of hRVs and "import" the virus into the family, as well as the elderly people, where these infections can lead to hospitalization or even death (Baillie, Olwagen, & Madhi, 2018; Lemanske et al., 2005).

HRVs are effectively transmitted mainly to families and schools with three mechanisms. The main mode of transmission is with droplets from the patient's respiratory system (Aponte et al., 2015). They are also transmitted, by direct contact with the hands of infected humans, since viral particles can remain even when individuals do not show symptoms, and then by self-immunization in the nose mucosa or the conjunctiva of the eye. Finally, in indirect contact, transmission involves contact with infected objects. HRVs can survive in various objects that contact infected people such as glasses and doors for several days (Wat, 2004).

HRVs are responsible for most episodes of common cold (Hayden, 2004). Pharyngalgia, wheezing, coughing and nasal obstruction are some of the typical symptoms. Sometimes, fever, headache, shivering and malaise are present (Kwon et al., 2014). The recovery time varies and healing occurs about two weeks after (Hayden, 2004; Lemanske et al., 2005).

- Bocavirus (BoV)

The human bocavirus (hBoV) was first discovered in Stockholm in September 2005 by Allander and his colleagues who detected the existence of a previously unknown virus in nasopharyngeal swabs of children (Allander et al., 2005). This new virus seems to be responsible for a significant proportion of the respiratory tract infections, especially in young children (Allander, 2008).

HBoV belongs to the family of Parvoviridae, a large family of viruses divided into two subfamilies of Parvovirinae and Densovirinae. Although Densovirinae members infect only arthropods and insects, Parvoviruses known to cause infection in vertebrates are fused to Parvovirinae that are subdivided into five genera: Parvovirus, Erythrovirus, Amdovirus, Dependovirus and Bocavirus (Allander, 2008; Jartti et al., 2012; Lindner & Modrow, 2008).

Since its discovery, hBoV has been detected globally in nasopharyngeal specimens, serum, stools and urine that are mainly derived from young children (Jartti et al., 2012). In infants with respiratory infection, the incidence of the virus varies between 2.7 - 19%, with most cases involving patients under 2 years of age (Longtin et al., 2008). This difference in the prevalence of hBoV is likely to be due either to seasonal fluctuations in the virus infection or to the different populations included in the various studies. Additionally, viral DNA has been detected in 0.8 - 9.1% faecal samples from patients with acute gastroenteritis as well as in urine samples (Lindner & Modrow, 2008). In contrast to hBoV1, the newer hBoV2, hBoV3 and hBoV4 species appear to be mostly isolated from stool specimens (Chieochansin, Kapoor, Delwart, Poovorawan, & Simmonds, 2009; Chow, Ou, & Esper, 2010) and rarely from respiratory tract samples (T. H. Han, Chung, & Hwang, 2009; Song et al., 2010).

HBoV is also detected to a large extent in mixed infections with other viral and bacterial pathogens, e.g. hRV, AV, hRSV and Streptococcus spp., ranging from 18% to 90% (Allander et al., 2005; Fry et al., 2007). HBoV has been detected in young children up to 2 years of age with acute upper and lower respiratory tract infection, e.g. pneumonia, bronchiolitis and wheezing. Other symptoms observed in hBoV positive patients include cough, fever, rhinitis and rarely conjunctivitis or rashes (Longtin et al., 2008).

- Coronavirus (CoV)

CoVs belong to the genus CoV and together with the genus Torovirus make up the family of Coronaviridae (King, Adams, Carstens, & Lefkowitz, 2012). The first reference in the literature on CoVs was made in 1931 by Schalk and Hawn (Schalk, 1931), who observed a respiratory infection in chickens. The factor they observed was later characterized and named avian influenza virusbronchitis virus (Beaudette, 1937). In 1965, Hamre and Procknow, using pharyngeal washes from medical students with clinical features of superior respiratory infection, managed to isolate, after culturing in human embryonic kidney monolayers, a factor, the original strain of which was defined as 229E (Hamre & Procknow, 1966). Almeida and Tyrrell (Almeida & Tyrrell, 1967), observed that the morphology of this new strain was very similar to that of influenza virusbronchitis virus, which a year later was given the name corona, from the characteristic "krona" in its shell, as revealed by negative staining, electron microscopy (Tyrrell et al., 1975). Finally, McIntosh and his colleagues (McIntosh, Dees, Becker, Kapikian, &

Chanock, 1967), identified six strains of viruses called "OC" (organ culture). One of these strains, named OC43, is now referred to as a common human CoV.

CoVs 229E and OC43 were the only ones that were identified in humans until 2003, when a new strain that caused severe acute respiratory syndrome (SARS) and was called SARS-CoV (Chan et al., 2003; Drosten et al., 2003; Rota et al., 2003) appeared. In 2004, researchers from the Netherlands isolated a new Human CoV (hCoV), named NL63, from a 7 month old child suffering from fever, bronchiolitis and conjunctivitis (van der Hoek et al., 2004).

Except for SARS-CoV, which appeared in November 2002 and disappeared in April 2004, the remaining hCoVs cause a large number of infections each year, mainly in children (Esposito et al., 2006; Monto & Lim, 1974). "Older" hCoVs (229E and OC43) have a worldwide spread and circulate mainly during the winter months and the beginning of spring, causing small exactions every 2-4 years (Gaunt, Hardie, Claas, Simmonds, & Templeton, 2010).

“Older” coronaviruses are responsible for 5-30% of respiratory infections in young children worldwide. "New" hCoVs (NL63 and HKU1) have also been detected globally during the winter, with NL63 being responsible for 1-9.3% of respiratory infections, mainly in children and HKU1 occupying 1-6%, although, according to sero-epidemiological studies, the majority of cases involve asymptomatic patients (Principi, Bosis, & Esposito, 2010).

HCoVs are often detected in cases of mixed infections with other respiratory viruses such as hRSV and FluV. Immunity against the virus does not last for a long time after the infection and re-contaminations may occur even during the same year (Gaunt et al., 2010). HCoV is usually transmitted by inhalation of infectious droplets but also through the hands to the mucosa of the nose or eyes (Gaunt et al., 2010; Mailles et al., 2013).

The hCoVs invade the respiratory system mainly through the nose and after a three days incubation period on average, cause common cold symptoms, including nasal obstruction, sneezing, nasal discharge and sometimes coughing (Gaunt et al., 2010). Symptoms resolve after a few days, during which the virus is excreted with respiratory secretions (Gerna et al., 2006). HCoVs are still associated with asthma attacks and chronic bronchitis and are less often the cause of severe LRTI. Cases of pneumonia due to hCoVs have been reported in infants and elderly people but also immunocompromised patients. There are also reports of the high incidence of NL63 in patients with acute laryngotracheobronchitis (croup) (van der Hoek et al., 2005).

The new CoV (COVID-19), which appeared in December 2019, is responsible for the most recent coronavirus disease (Zhu et al., 2020). COVID-19 is a beta-CoV that has an incubation period of 2-5 days and is transmitted between people through respiratory droplets and contact routes. It has a low mortality rate generally (2-3%) and only two cases of mortality in children have been reported so far. Although, children are just as likely as adults to become infected,

most of them are asymptomatic or have milder symptoms (Dong et al., 2020). Symptoms that have been observed in children are mostly fever (59%) and cough (46%), and also nasal congestion, runny nose, conjunctivitis, wheezing, myalgia and expectoration. Rarely symptoms such as nausea, vomiting, diarrhea, have been reported. Dyspnea, cyanosis, poor feeding, irritability, decreased response, respiratory distress and multiorgan failure are even more rare symptoms of the disease. As far as biochemical results are concerned elevation of transaminases, myoglobin, muscle enzymes, and D-dimers might be seen in severe cases only (Cai et al., 2020; Cao, Chen, Chen, & Chiu, 2020; Chen et al., 2020; Dong et al., 2020; Mazzotta, Troccoli, & Bonifazi, 2020; Wei et al., 2020; Yang, Liu, Li, & Zhao, 2020). The clinical examination is mostly negative for pulmonary signs but it has been reported the existence of rales and thoracic retractions. In the majority of the cases children recover one to two weeks after the onset of the disease (Lassandro et al., 2020).

- Human metapneumovirus (HMPV)

HMPV is one of the most recently recognized viruses. It was discovered in 2001 in the Netherlands by the team of van den Hoogen et al who monitored and examined 28 hospitalized children and infants with acute respiratory infection who had signs and symptoms of RSV infection (Shahda, Carlos, Kiel, Khan, & Hage, 2011). It belongs to the family Paramyxoviridae, Paramyxovirinae, genus Metapneumovirus (M. Hamelin et al., 2008).

The hMPV is the cause of LRTI in 20% of infections which are negative for common viruses (Huck et al., 2006; Williams et al., 2005). It has been isolated in 5-10% of children with acute respiratory infection (Esper, Boucher, Weibel, Martinello, & Kahn, 2003; Longtin et al., 2008; Peret et al., 2002) (Esper et al., 2004). It presents a seasonal distribution with winter and spring excursions and annual fluctuations (Noyola et al., 2005). The outbreaks of infections with the virus appear in the months of the outbreak of infections with the RSV (Van Den Hoogen, Osterhaus, & Fouchier, 2004). They are a local phenomenon in contrast to the FluV where 2 or 3 strains are spread on the planet each year (Broor, Bharaj, & Chahar, 2008). The hMPV infection is transmitted through the community, although in-hospital transmissions have been described (Esper et al., 2003). Based on serological studies, it has been found that by the age of 5 years 77.3% of children have been exposed to the virus (Peltola et al., 2008).

HMPV has been isolated from children with the upper or lower respiratory tract infections, with asthma or laryngotracheobronchitis. The mean age of children infected with the lower respiratory tract virus is 11 months, while the upper respiratory tract infection is more common in older children with an average of 18 months of age (Williams et al., 2005).

- Influenza viruses (FluVs)

FluVs are classified into the family of Orthomyxoviridae. These viruses are divided into three types: A, B and C. Types A and B, are divided into serotype different strains, are mainly pathogenic and cause epidemic disease. Type C is a sporadic cause of infections mainly of the upper respiratory tract (Bouvier & Palese, 2008; R. M. Kliegman et al., 2015)

FluV A have a complex epidemiology involving animals that act as reservoirs for different strains, which can also infect humans. Migratory birds may also be responsible for the spread of the disease. The A type virus according to the type of hemagglutinin (H1, H2, H3) and / or the neuraminidase antigen (N1, N2) is distinguished in subtypes. FluV B has a lower antigenic capacity and no animal virus reservoir has been found (Bouvier & Palese, 2008; R. M. Kliegman et al., 2015).

Influenza is a disease of the cold months of the year in temperate climates. Transmission takes place through the air with tiny droplets and rarely by infected objects (Tellier, 2006). The disease is transmitted 1-2 days before up to one week after the onset of the symptoms. The incubation time varies from 1-3 days. Dispersion is rapid with a maximum frequency in the first 2-3 weeks. Spread of the virus in hospital can cause complications and hospitalization is considered necessary in younger age groups (R. M. Kliegman et al., 2015; O'Brien et al., 2004; Tellier, 2006).

Epidemics are caused by types A and B. FluV is responsible for the largest and most deadly viral epidemics suffered by man. During the epidemic, up to 40% of preschool children and 30% of school-age children can be affected by the epidemic (Neuzil, Mellen, Wright, Mitchel Jr, & Griffin, 2000).

At the pathological level, the influenza virus causes lytic inflammation of the respiratory epithelium, decreased mucus production and apoptosis of the epithelial layer cells. These changes allow the bacterial infection to be secondary suprainfection (Pediatrics, 2012).

FluV A and B usually cause respiratory disease. The incidence of lower respiratory disease with pneumonia, wheezing, croup, bronchiolitis as a complication of influenza ranges from 0.2 to 25% (Pediatrics, 2012). The onset of the disease is sudden and is characterized by rhinitis, conjunctivitis, pharyngitis and dry cough. More often than any other respiratory virus, influenza is accompanied by high fever, myalgia, fatigue and headaches. The usual duration of fever is 2-4 days, but coughing may persist for a longer period of time, and signs of malfunction of the small airways are found for weeks after healing (Glezen, Taber, Frank, Gruber, & Piedra, 1997; R. M. Kliegman et al., 2015).

- Parainfluenza (PIV)

PIVs are RNA viruses and belong to the family of Paramyxoviridae. They were isolated during the period from 1956 to 1960 from cell cultures. Their name results from the fact that they cause infections similar to those of influenza and also exhibit hemagglutinin and neuraminidase activity (Henrickson, 2003).

The incubation period ranges from 2 to 6 days. The type and severity of the clinical picture depends on the type of virus, the existence of an earlier infection from the same or another type of PIV, the age and the biological background of the patient. Generally, the disease is more severe when it is caused by type 3 or when it happens to infants or babies. PIVs cause nasopharyngitis, laryngotracheobronchitis, bronchiolitis, bronchitis and bronchopneumonia (Iwane et al., 2004). They have also been implicated in asthma exacerbation in children (Azevedo et al., 2003).

Infection by one type of virus causes cross-immunity for other types. Reinfection is possible but symptoms are milder and confined to the upper respiratory tract. The virus is transmitted from person to person with droplets and infected objects (Pediatrics, 2012).

- Adenovirus (AV)

AVs belong to the family Adenoviridae, they are moderate-sized DNAs, which are classified as subgenus A to G. They were isolated in 1953 from the human and animal cell cultures. 52 serotypes have been isolated, of which children are mainly infected by 1, 2, 3, 5, 6, 7, 40 and 41 (G. Wadell, 1984; G. Wadell et al., 1980).

AV infections are spread worldwide. They appear all year round but their impact is higher in spring, early summer and midwinter in temperate climates. Certain types occur in epidemics, types 4 and 7 in respiratory tract epidemics, 3, 7 and 21 in severe pneumonia, type 3 in upper respiratory disease with fever, type 11 in cystitis, and types 8, 19, 37 in keratoconjunctivitis epidemics (Gray et al., 2007).

More than 60% of school-age children have antibodies against common respiratory types of the virus. The disease is transmitted from person to person and rarely by contact with infected objects. The incubation period of the disease ranges from 2-18 days. Many AVs, especially those that affect children, 1, 2 and 5, are excreted for a prolonged period of time from the respiratory and gastrointestinal tract (R. M. Kliegman et al., 2015; Pediatrics, 2012).

AVs cause a wide range of syndromes. They are the only ones among respiratory viruses that can cause events in almost all systems (G. Wadell, 1984). In particular, they cause upper respiratory tract disease, which is the most common infection caused by AV. In these they are included: Bronchiolitis or bronchopneumonia mainly in infants (Chang et al., 2008; Hong et al.,

2001; Carballal, Videla, Misirlian, Requeijo, & Aguilar Mdel, 2002), pertussis-like syndrome (Jones et al., 2007; Pediatrics, 2012), pharyngoconjuctival fever (Jones et al., 2007), conjunctivitis and keratoconjunctivitis (Jones et al., 2007), infections of the gastrointestinal tract (Chen et al., 2004; Jones et al., 2007), haemorrhagic cystitis (Jones et al., 2007), systemic disease (R. M. Kliegman et al., 2015), Reye syndrome and syndromes similar to Reye (R. M. Kliegman et al., 2015), meningoencephalitis (Jones et al., 2007) and hepatitis (Jones et al., 2007).

AV infections account for 5-10% of respiratory infections in children (Chen et al., 2004). Clinical manifestations range from simple cold to croup, bronchitis and pneumonia (Cheng et al., 2008). The youngest age of adenovirus infection is between 6 months and 5 years (Walls, Shankar, & Shingadia, 2003). Serious respiratory infections are caused by serotypes belonging to genera B and C. Very often adenovirus infection resembles a severe microbial infection (Larranaga et al., 2000; Straube et al., 1983).

- Enterovirus (EV)

EVs belong to the family of Picornaviridae and include polio viruses, Echo virus and Coksackie virus and 68-71 EVs (Blomqvist, Paananen, Savolainen-Kopra, Hovi, & Roivainen, 2008).

Human is their only host. They usually entry from the respiratory and digestive tract. The incubation period for nonpolio EVs ranges from 2-12 days and the symptoms usually start 3 days after the initial entry of the virus in an organ (R. M. Kliegman et al., 2015).

Infection by nonpolio EVs causes a clinical picture that varies in severity from asymptomatic to fatal depending on the type of the virus, the age, and the immunological status of the child. Infections by Coksackie virus A and B, Echo virus, enteroviruses 68-71 include: non-specific febrile illness (Rotbart et al., 1999), severe systemic disease in infants and babies mainly from Coxsackie B viruses, infections of the upper respiratory tract (common cold, acute nasopharyngitis, lymphatic pharyngitis, herpangina, acute laryngitis), infection of the lower respiratory tract (bronchiolitis, bronchitis and bronchopneumonia caused by Coxsackie A and B and Echo viruses), Bornholm's disease, acute gastroenteritis, hand, foot and mouth disease (Xu et al., 2012), myocarditis – pericarditis, aseptic meningitis (Cunningham et al., 1999), encephalitis (Cunningham et al., 1999), paralysis of cerebral conjugates and inferior neurons, hemorrhagic conjunctivitis (Wright, Strauss, & Langford, 1992).

3 Materials and methods

3.1 Ethics statement

The study was approved and supervised by the Ethics Committee of the Westfalen-Lippe Medical Association and the University of Westfalen-Wilhelms Medical School. All legal guardians were informed prior to inclusion of the subject into the study and also signed informed consent.

3.2 Study design

A prospective clinical study in a cohort of children and adolescents in 6 pediatric practices (outpatients) and one pediatric hospital (inpatients) in Germany was conducted. This study was performed between January 2014 and December 2016. The study consisted of two visits per subject. More specifically, at the time point of the first presentation (Visit 1) and after 3 to 7 days (Visit 2).

3.3 Patients and sample collection

3.3.1 Inclusion criteria

All patients must meet the following criteria:

- all children and adolescents must be between 1 -18 years old
- they should have a diagnosis of non-complicated respiratory infection (Rhinitis, sinusitis, pharyngitis, bronchitis, pneumonia)
- the patients and their guardians should approve their participation after detailed explanation / clarification of the purpose of the study
- all blood and nasal fluid samples should be taken within 24 hours after the onset of hospitalization or primary visit

3.3.2 Exclusion criteria

Patients that show at least one of the following are excluded from the study:

- Patients with severe infections who require mechanical support or medication with drugs that support circulatory disease are excluded

- Patients with known inherited / inborn diseases or metabolic diseases that will affect the outcome of the study
- Patients with bronchopneumonia, pleuropneumonia, lobar / partial pneumonia
- Patients that show symptoms of the infections for more than 5 days already
- Patients that have jaundice at the first physician contact

3.3.3 Tests

For all the patients demographic data, medical history and pre-medication data were documented at visit 1.

Blood samples

Two blood samples were taken for each subject. One at visit 1 and the second at visit 2. For each patient we tested:

- Complete blood count: hemoglobin, hematocrit, leucocytes, platelets, differential blood picture: (leucocytes, lymphocytes, neutrophils, eosinophilic granulocytes, basophils, monocytes), C-reactive Protein (CRP), electrolytes (Na +, K +, Ca ++, Cl-), venous blood gas analysis (pH, CO_2, bicarbonate, base excess).
- Hepatic markers: aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), gamma-Glutamyltransferase (γ-GT), alkaline phosphatase (ALP), total bilirubin, direct (conjugated) bilirubin, indirect (unconjugated) bilirubin and serum albumin.

For the above mentioned tests we used the analysis system Integra 800 Roche and later on the analysis System Cobas 6000 from the same company.

Other tests

Oxygen saturation was measured via portable oximeter Philips SureSigns VS2+. Also, blood pressure and pulse were measured on visit 1 to the clinic.

Nasopharyngeal samples

Nasopharyngeal samples were obtained using nasal lavage, in order to identify the virus using multiplex-Polymerase chain reaction (PCR) according to the protocol previously described by Bonzel et al. 2008. Table 17 (see Appendix) shows the reference strains, cells as well as the Gen-Bank Accession-numbers used for the establishment of the real-time-TaqMan PCR for the following viruses:

- Influenza A (incl. H1N1sw) und B
- Parainfluenza 1, 2 und 3

- Respiratory syncytial virus A und B
- Human Metapneumovirus A und B
- Coronavirus 229E, OC43 und NL63
- Rhinoviruses
- Enteroviruses
- Adenoviruses
- Bocaviruses

3.4 Extraction of Viral Nucleic Acids

In order to obtain the viral nucleic acids (DNA, RNA) from the clinical samples and the cell culture supernatants we used the EZ 1 Virus Mini Kit (Qiagen, Hilden, Germany). For this purpose, we used 200 µL from each sample, which resulted in 60 µL eluates of viral nucleic acid in buffer solution (Bonzel et al., 2008).

3.5 Selection of Primers and Probes

The primers and probes that were selected for each assay are shown in Table 18 (see appendix) (available online only). For the selection we followed the protocol previously described by Bonzel and colleagues (Bonzel et al., 2008).

3.6 Real-Time Polymerase Chain Reaction (RT-PCR)

For the real time RT-PCR we used the Qiagen's Quantitect Multiplex RT-PCR kit. In more detail, the assay was carried out in a reaction mixture of 25 µL. The reaction mixture contained 12.5 µL of 2x QuantiTect Multiplex RT-PCR master mix which included Hot-StarTaq DNA Polymerase, Quantitect Multiplex RT-PCR buffer, dNTP Mix, Rox passive reference dye, 11 mM $MgCl_2$, and 1 µL of the Quantitect Multiplex RT mix, which included Omniscript Reverse Transcriptase and Sensiscript Reverse Transcriptase. The final concentration of all the primers and the probes was 400 nM. Following that, we distributed the reaction mixture onto an 8-tube reaction strip (MicroAmp optical 8-tube strips, ABI) before adding primers and probes in specific positions on the reaction strip (Table 2, available online only). Subsequently, we stored reaction strips at -20°C, in order to use them. For the PCR thermal protocol we did the following steps: cDNA synthesis for 20 minutes at 50°C, initial denaturation at 95°C for 15 minutes, 45

cycles of denaturation at 94°C for 45 seconds, and combined annealing and extension at 60°C for 1 minute and 15 seconds. Confirmation of all the positive results was obtained using a monoplex assay with the same primer and probe sets described in Table 18 (Appendix) (available online only).

3.7 Statistical analysis

Statistical significance was determined using SAS (version 9.4).

4 Results

4.1 Demographics

In our study we included 992 patients, 572 (57.7%) of which were male and 420 (42.3%) female (Diagram 1). The vast majority of our patients (945 patients, 95.3%) were of Caucasian/White ethnicity while only 47 patients (4.7%) reported other ethnicities (Diagram 2). As far as the age was concerned there were three age group: i) children with ages between 1 and 5 years old, ii) children with ages between 6 and 12 years old and iii) adolescents (13-17 years old). At the first age group there were 576 patients (58.1%), at the second 307 cases (31.0%) and the third 109 patients (11.0%) (Diagram 3). The age of the patients ranged from 1 to 17,9 years, with an average age of 6,2 years and a median age of 4,83 years.

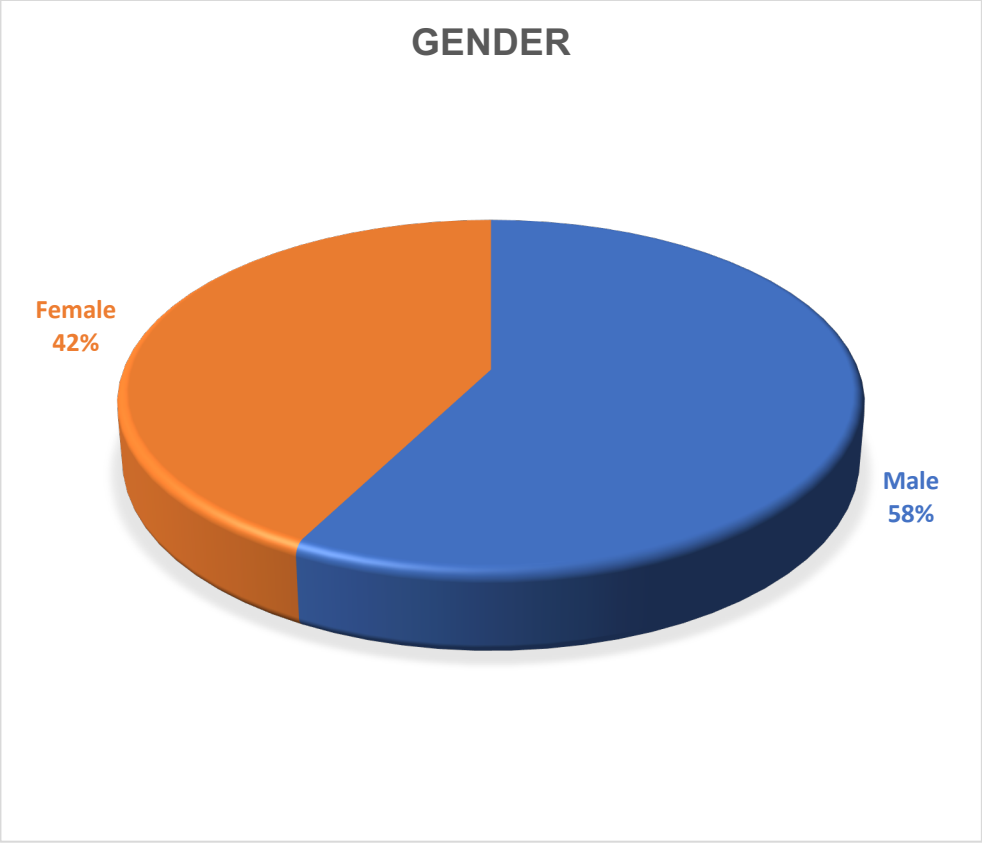


Diagram 1: Demographic data - gender distribution of patients analyzed.

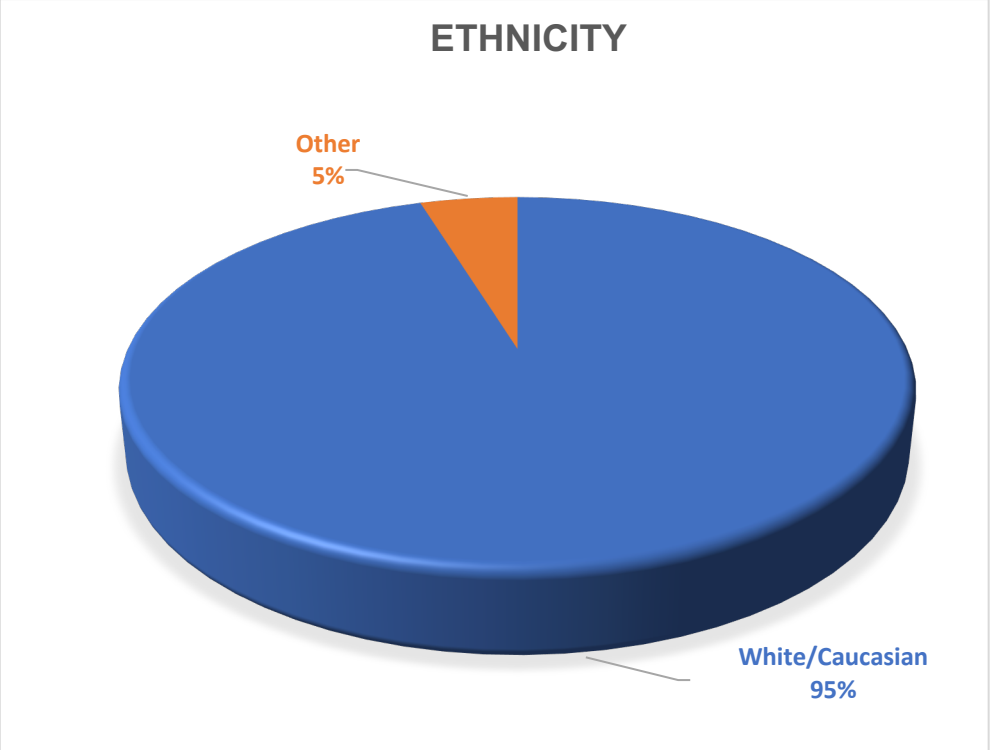


Diagram 2: Demographic data - Ethnicity distribution of patients analyzed.

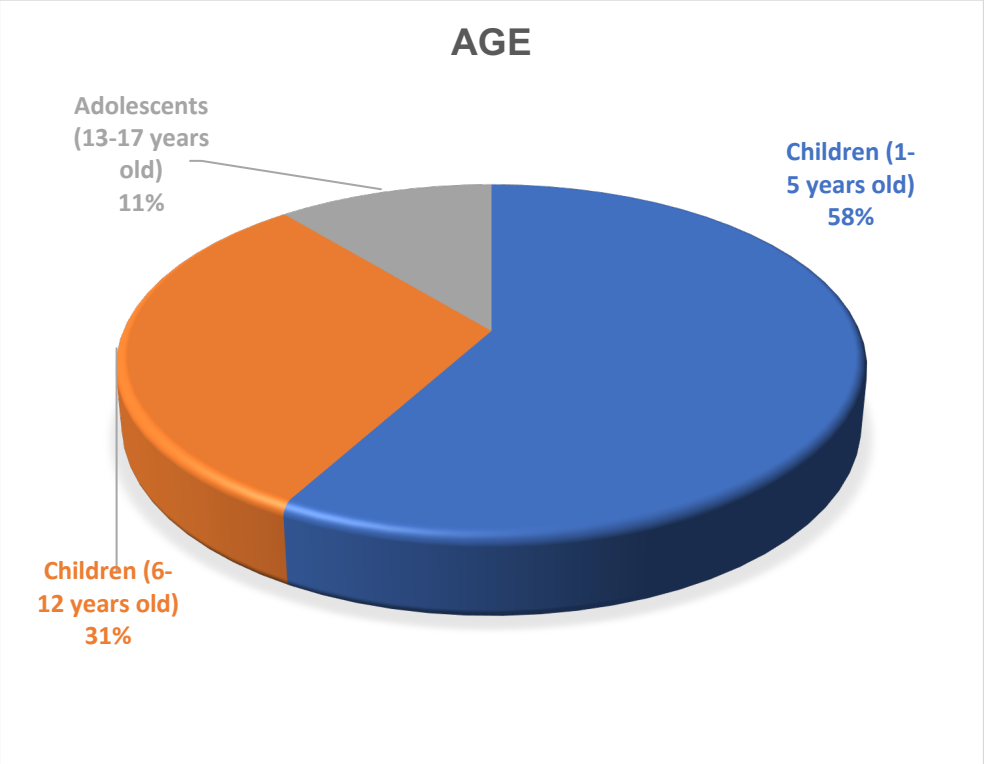


Diagram 3: Demographic data - Age distribution of patients analyzed.

The viruses that were searched in our sample included FluV A (H1N1, H3N2) and B, PIV 1, 2 and 3, RSV A and B, hMPV A and B, CoV (229E, OC43 and NL63), RV, EV, AV and hBoV. From our patients, 617 cases had a positive PCR for virus detection (63.0%) while 363 cases showed a negative PCR (37.0%) as shown in Diagram 4. In the age groups it was shown that 420 of the 569 (73.8%) children (1 – 5 years old) had a positive PCR for virus detection in contrast to 150 out of 304 (49.3%) children (6 – 12 years old) and 47 of the 107 (43.9%) adolescents (Diagram 5). The statistical analysis using Fisher's Exact Test showed a significant difference between the age-groups meaning that younger children are more prone to have positive PCR for virus detection ($p<0.0001$). As far as the gender of the patient was concerned it was shown that 370 of the 564 (65.6%) male patients had a positive PCR for virus detection contrary to 247 of the 416 (59.4%) female patients (Diagram 6). The statistical analysis concerning the patient's gender and the PCR result did not demonstrate a statistically significant difference between females and males ($p<0.0523$).

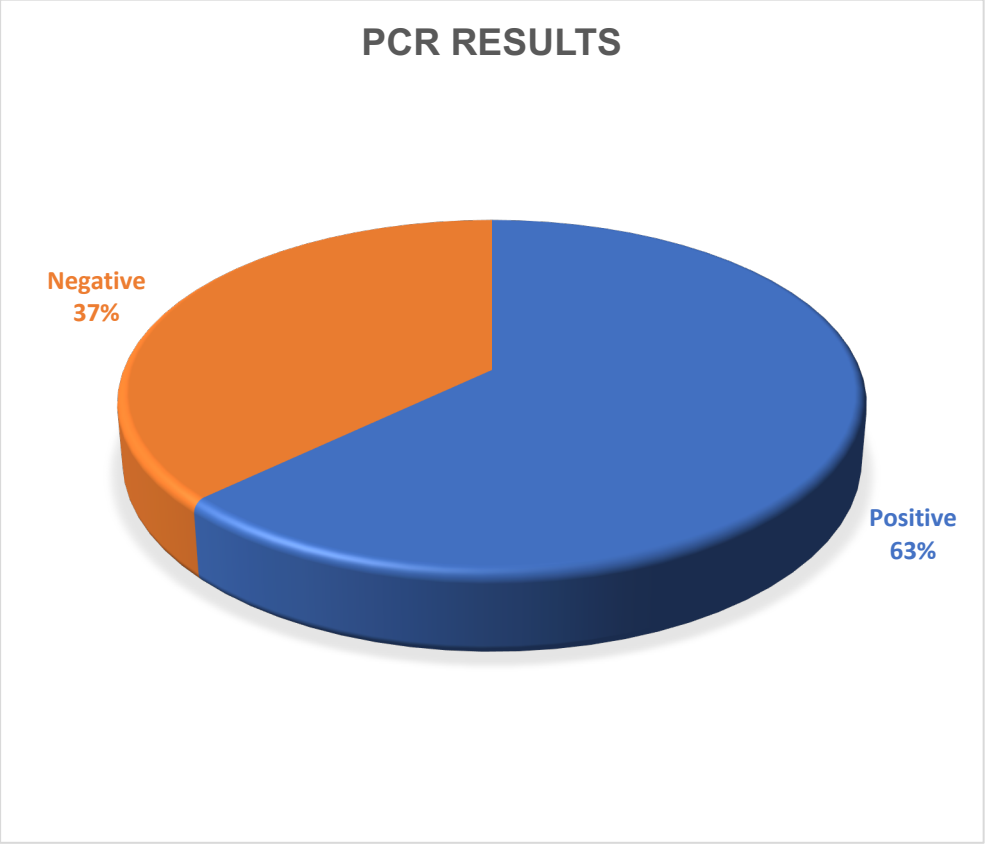


Diagram 4: Patients with positive / negative PCR virus detection in total.

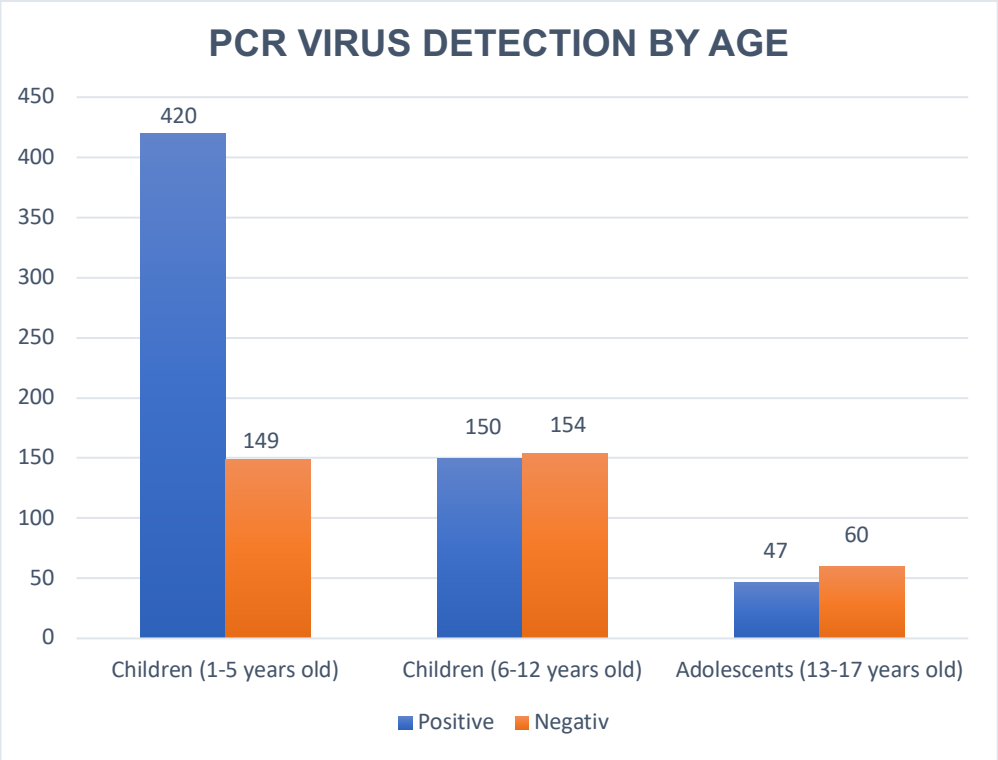


Diagram 5: Patients with positive / negative PCR virus detection by age.

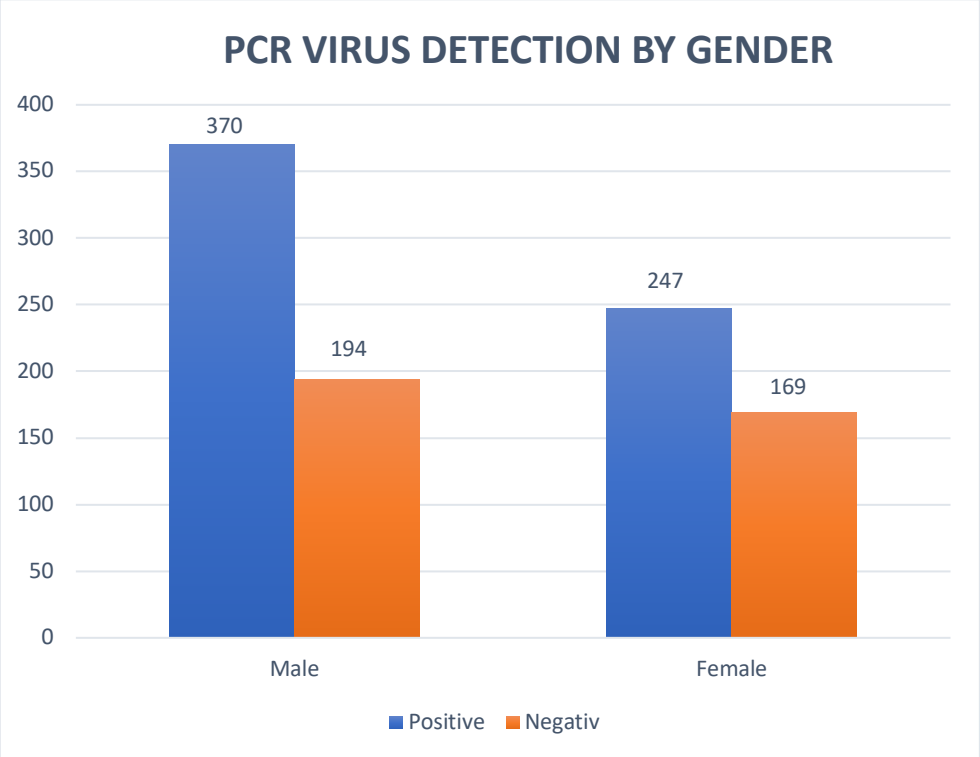


Diagram 6: Patients with positive / negative PCR virus detection by gender.

Regarding the spectrum of the detected viruses it was shown RV was detected in 179 cases (18.0%), RSV (A, B) in 105 cases (10.6%), FluV B in 92 cases (9.3%), PIV (1,2,3) and CoV (229E, OC43, NL63) in 69 (7.0%) and 63 cases (6.4%) respectively, AV in 62 cases (6.3%), hBoV and hMPV (A, B) in 42 (4.2%) and 41 cases (4.1%) respectively, FluV A (H1N1, H3N2) in 39 cases (4.0%) and EV in 26 cases (2.6%). These results are demonstrated in Diagram 7.

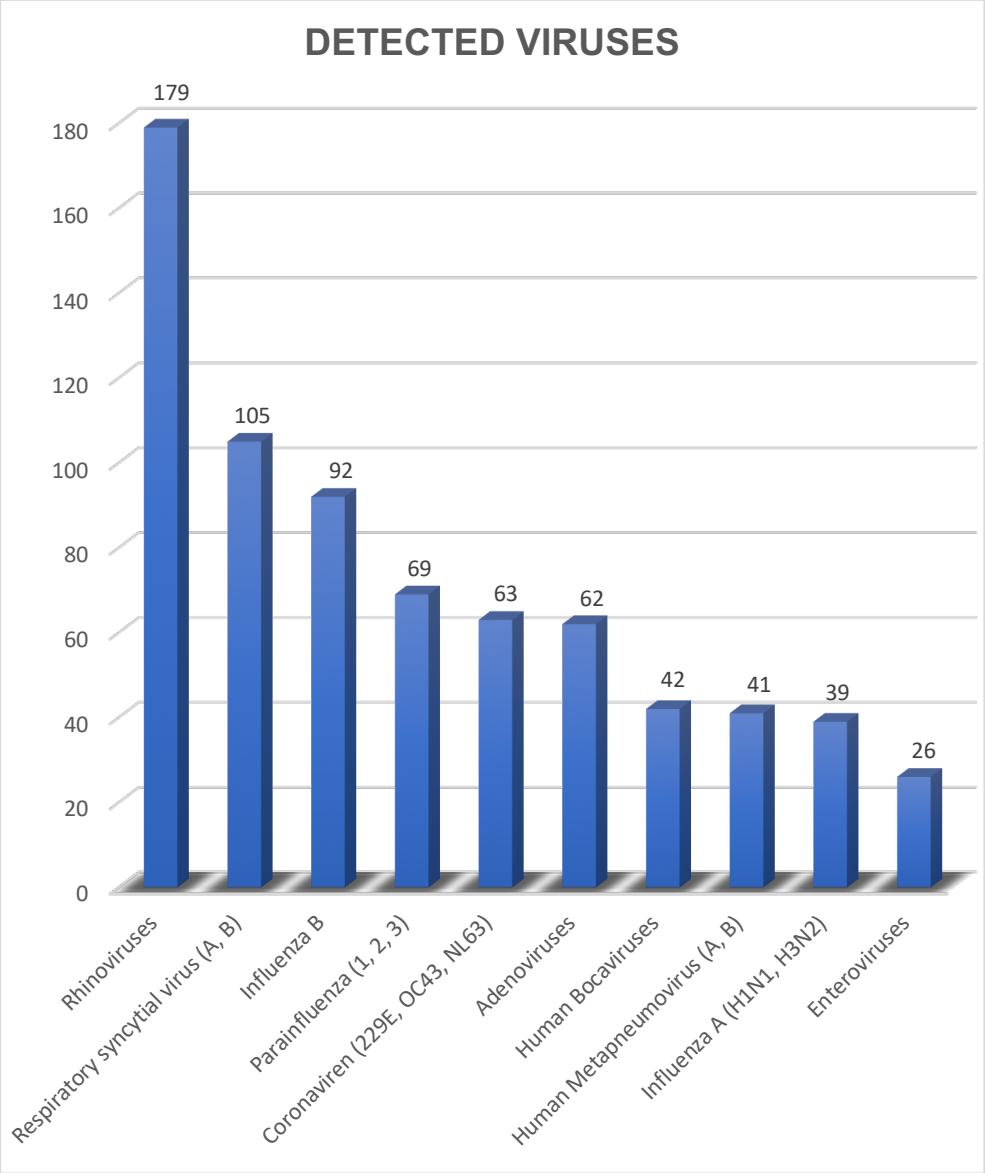


Diagram 7: Spectrum of detected viruses (percentage has been evaluated using N=992 patients).

Moreover, the analysis of the detected viruses' spectrum with regards to the season of the detection reported that the vast majority of the virus detection was conducted during winter season (RV, RSV, FluV A and B, CoV, AV, PIV, hMPV and hBoV) while only RV, PIV, AV and EV were detected mostly in summer season (Diagram 8).

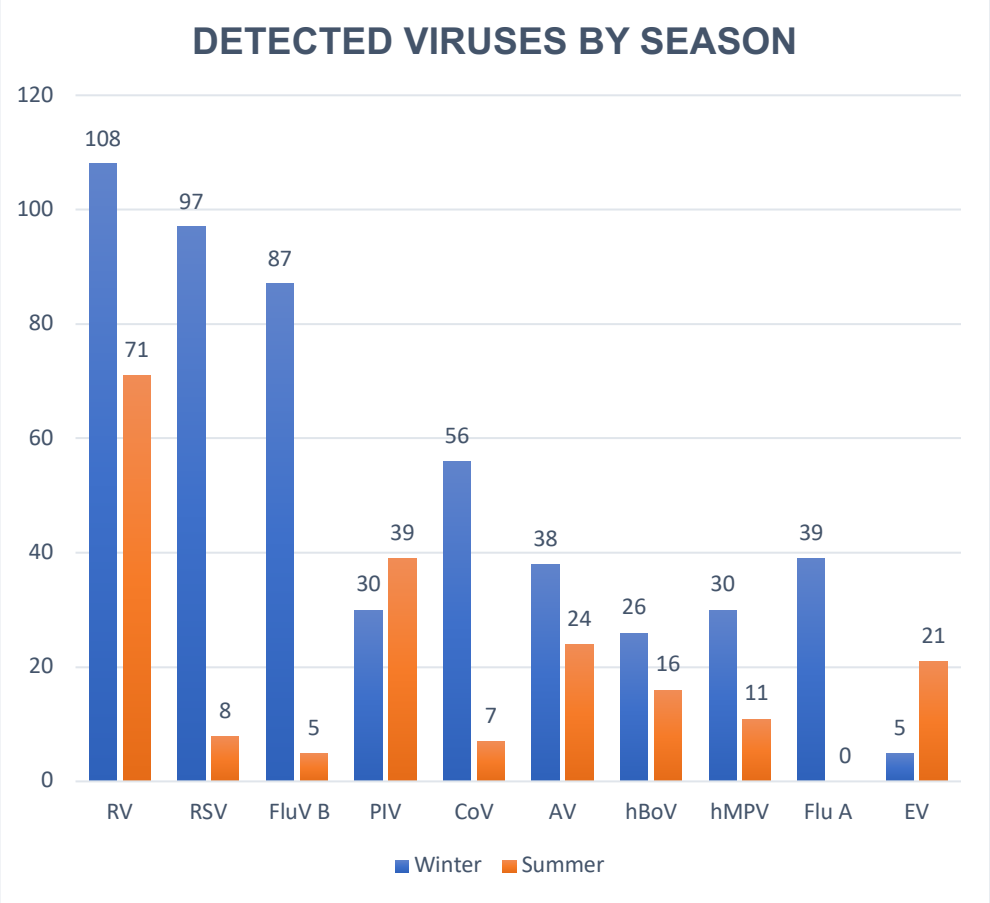


Diagram 8: Spectrum of detected viruses in winter (October until April) and summer (May until September).

Also, we studied the Body mass index (BMI) of all our subjects. BMI ranged from 11.4 to 45.25. Mean BMI was 17.5 while median was 16.49. For the BMI analysis, using the BMI reference percentiles of Robert Koch Institute, second extended edition, 2013, children were considered to be of normal weight as far as their BMI was at or below the 90th percentile of the applicable age group, overweight when their BMI ranged above the 90th percentile and at or below the 97th percentile, and obese when their BMI was above the 97th percentile (Neuhauser H., et al., 2013). Diagrams 9 and 10 show BMI categories and gender as well as age.

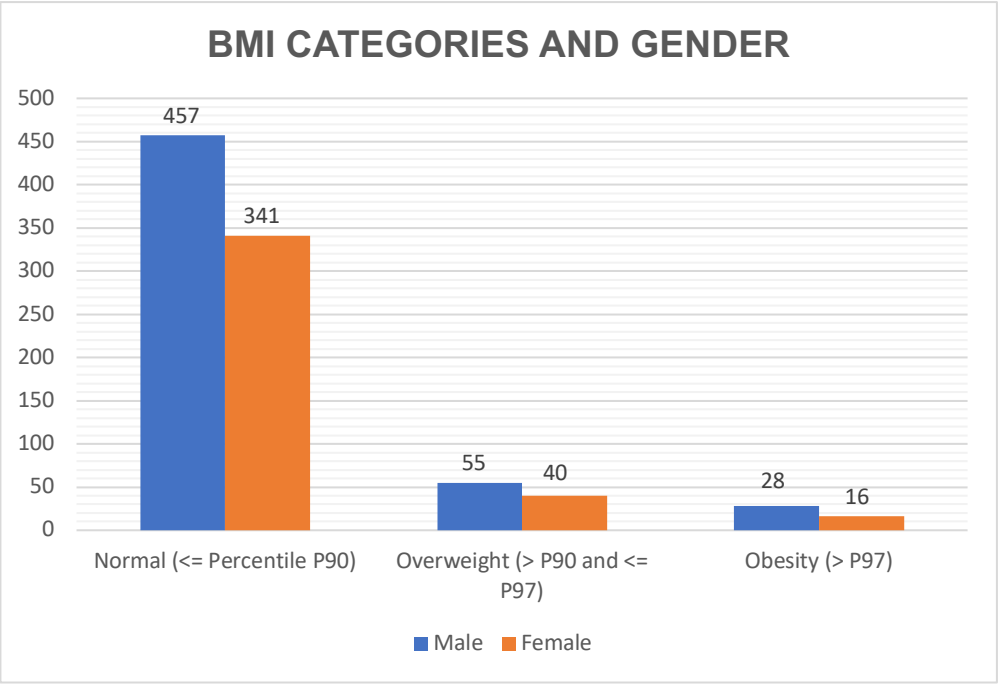


Diagram 9: BMI categories and gender of subjects (BMI categories were calculated according to the reference percentiles of Robert Koch Institute, second extended edition, 2013).

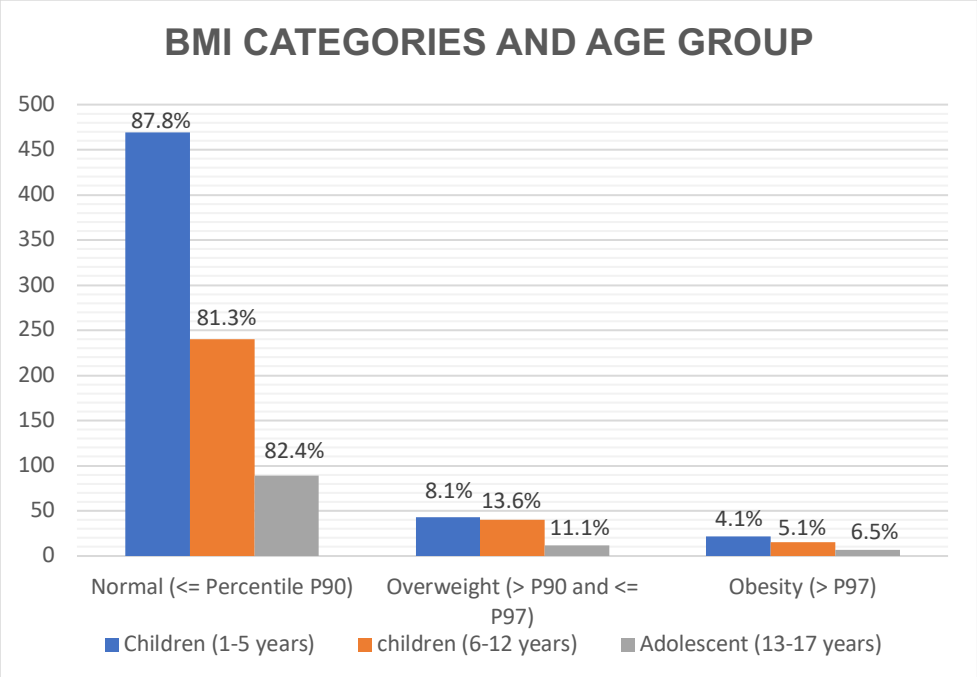


Diagram 10: BMI categories and age group of subjects (BMI categories were calculated according to the reference percentiles of Robert Koch Institute, second extended edition, 2013).

4.2 Liver enzymes and liver values elevation (LVE)

There are many tissues where alkaline phosphatase (ALP) can be found, such as the canalicular membrane of hepatocytes, bone osteoblasts, small intestine enterocytes, proximal tubules of kidneys, the placenta and white blood cells. However, its role is still unknown. The level of ALP in the serum varies significantly with age. In more detail, it has been reported that children manifest high serum ALP levels of bone origin. This is known as transient hyperphosphatasemia of infancy, which shows increased levels of ALP in an infant or child younger than 5 years old without any liver disease or bone problem (Posen et al., 1977; Krause et al., 1985). In this case, the rise in ALP values is sometimes ten times higher the upper limit of the laboratory reference values. They return to normal after some weeks or months. Thus, an increased ALP value does not indicate liver or biliary disease on its own, when the other liver biochemical tests are normal. Increased ALP serum levels in the absence of liver disease can be due to pregnancy, familial inheritance, chronic renal failure, blood groups B or O, and transient hyperphosphatasemia in infancy (Haghighat, M. 2014). Also, a study that was conducted by Ruiz et al., (2014) wanted to investigate the connection between physical activity and liver enzyme values in adolescents from 9 European countries. The results from this study showed that subjects who had 60 minutes per day of moderate to vigorous intensity physical activity were more prone to have higher levels of AST and AST/ALT no matter of the time spent sedentary.

For these reasons, we also investigated the values of the three primary liver parameters (ASAT, ALAT and γ-GT) for each patient after each visit (Visit 1 and Visit 2) in order to detect any increase (LVE) in these parameters. A patient has a liver value increase if and only if at least one of the three above mentioned primary liver parameters lies above the normal range (calculation: liver value / upper standard value> 1) (Table 1).

Parameter	Unit	Age	Gender	Range
Serum chemistry				
ASAT (SGOT)	U/L	1-18 yr	M	10-50
			F	10-35
ALAT (SGPT)	U/L	1-18 yr	M	10-50
			F	10-35
Gamma-GT	U/L	1-18 yr	M	8-61
			F	5-36

Table 1: Normal range values for ASAT, ALAT, GGT (given by our laboratory; analysis system Integra 800 Roche and Cobas 6000 Roche).

Therefore, our results showed that 877 (88.9%) of the patients showed no LVE in visit 1 compared to 110 (11.1%) that showed increase and 796 (88.8%) patients showed no LVE at visit 2 compared to 100 (11.2%) that had an increase (Diagram 11). Out of 110 children and adolescents with elevated liver enzymes values at visit 1, 93 (84.5%) showed elevation below an index value of 2, 10 (9.1%) with an index value $\geq$2 and <3, and 7 showed at least one elevation with an index value $\geq$ 3. Moreover, out of 892 patients, whose laboratory assessments at both visits were available, 63 (7.1%) had elevated liver enzymes values both at visit 1 and visit 2, 41 (4.6%) only at visit 1, and 37 (4.1%) only at visit 2. Thus, 141 patients (15.8%) had at least one elevated liver enzyme value during one visit, at least. From the subjects that a LVE was detected, 89 had one-time higher liver values, 16 two times higher and 5 three times higher liver values in visit 1 while 76 had one time higher, 16 had two times higher and 8 had three times higher liver values in visit 2. The mean for the three hepatic enzymes ALAT, ASAT and γ-GT were 18.55, 34.80, 12.87 respectively in visit 1 and 18.99, 32.69, 13.69 in visit 2 (Table 19, Appendix).

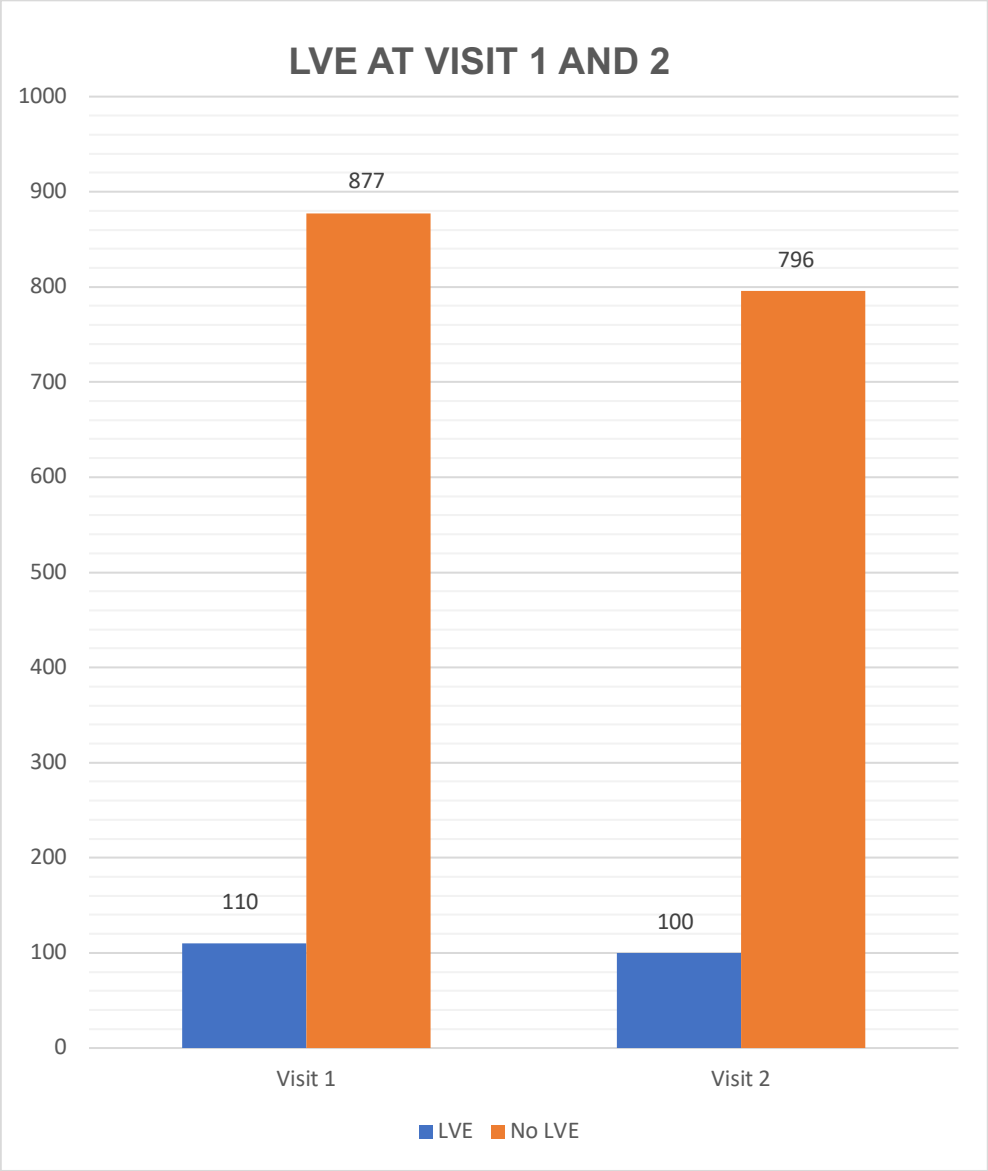


Diagram 11: Liver value elevation at visit 1 and visit 2.

As far as the values of the three hepatic enzymes ASAT, ALAT and γ-GT are concerned our results showed that elevated values of at least one of the three hepatic parameters according to the judgment of the investigator were found in 11,1% of the subjects at visit 1 and 11,2% at visit 2. The elevation of hepatic enzyme values according to the age groups 1-5 years, 6-12 years and 12-17 years and gender is manifested in Table 2.

		Elevated liver enzyme values			
	Gender	**1-5 years old**	**6-12 years old**	**12-17 years old**	**Total**
Visit 1	Boys	9.5%	15.1%	25%	12.8%
	Girls	7.6%	12.1%	6.1%	8.9%
	Total	8.7%	13.8%	16.5%	11.1%
Visit 2	Boys	7.6%	13.1%	29.3%	11.7%
	Girls	8.8%	12.1%	12.7%	10.4%
	Total	8.1%	12.7%	22%	11.2%

Table 2: Overall rates of liver value elevation (elevated values of at least one of the three relevant liver enzymes (ASAT, ALAT, γ-GT) as assessed by the treating physician) according to age groups.

The following table presents the number and rate of children with elevated liver enzyme values at visit 1, 2 or both. For all three cases the rate of children who persistently showed elevated enzyme values in both visits, was at least as high as that of children with normal values at visit 1 but elevated values at visit 2. Moreover, the proportion of children whose values were elevated during treatment was not appreciably higher or even lower than that of children whose values returned to normal during the period of observation (Table 3).

Laboratory measure	N	Value normal at V1 elevated at V2	Value elevated at V1 normal at V2	Value elevated both at V1 and V2
ALAT	886	17 (1.9%)	11 (1.2%)	18 (2.0%)
ASAT	886	22 (2.5%)	37 (4.2%)	24 (2.7%)
γ-GT	862	20 (2.3.%)	8 (0.9%)	29 (3.4%)

Table 3: Children with normal and elevated liver enzyme test results at visit 1 and 2 based on patients with valid data for both visits.

Regarding the virus detection and the LVE it was shown that in 75 patients (12.2%) that were positive for virus also showed an increase in at least one liver value (Diagram 12). According to the Fisher's exact test there is no statistical significance between the detected viral agent and the elevation of hepatic enzyme values in these subjects (p=0.2496).

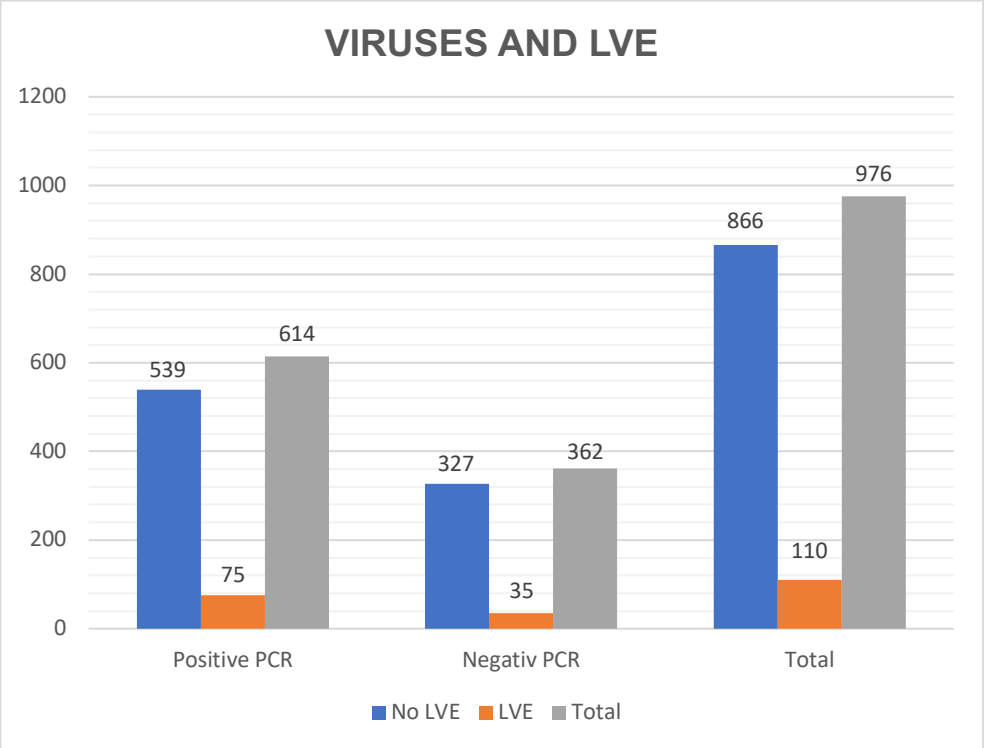


Diagram 12: Viruses and liver value enzymes elevation.

A considerable proportion of subjects with an acute respiratory tract infection, had a RV, RSV, FluV B, PIV, CoV and AV infection (Diagram 13), while elevated liver enzyme levels were observed in patients with FluV B (24.4%), hMPV (14.6%), RSV (13.3%), FluV A (12.8%), CoV (12.7%) and PIV (11.6%) infection (Table 4).

Virus	**Liver value elevation (LVE)**		
	Total	LVE	No LVE
Total	715	83 (11.6%)	632
Influenza A (H1N1, H3N2)	39 (5.5%)	5 (12.8%)	34
Influenza B	90 (12.6%)	22 (24.4%)	68
Parainfluenza (1, 2, 3)	69 (9.7%)	8 (11.6%)	61
Respiratory syncytial virus (A, B)	105 (14.7%)	14 (13.3%)	91
Human Metapneumovirus (A, B)	41 (5.7%)	6 (14.6%)	35
Coronaviruses (229E, OC43, NL63)	63 (8.8%)	8 (12.7%)	55
Rhinovirus	178 (24.9%)	12 (6.7%)	166
Enterovirus	26 (3.6%)	2 (7.7%)	24
Adenovirus	62 (8.7%)	4 (6.5%)	58
Human Bocavirus	42 (5.9%)	2 (4.8%)	40

Table 4: Detected viruses and liver value elevation at visit 1 (the first number is the absolut number followed by % of detected patients).

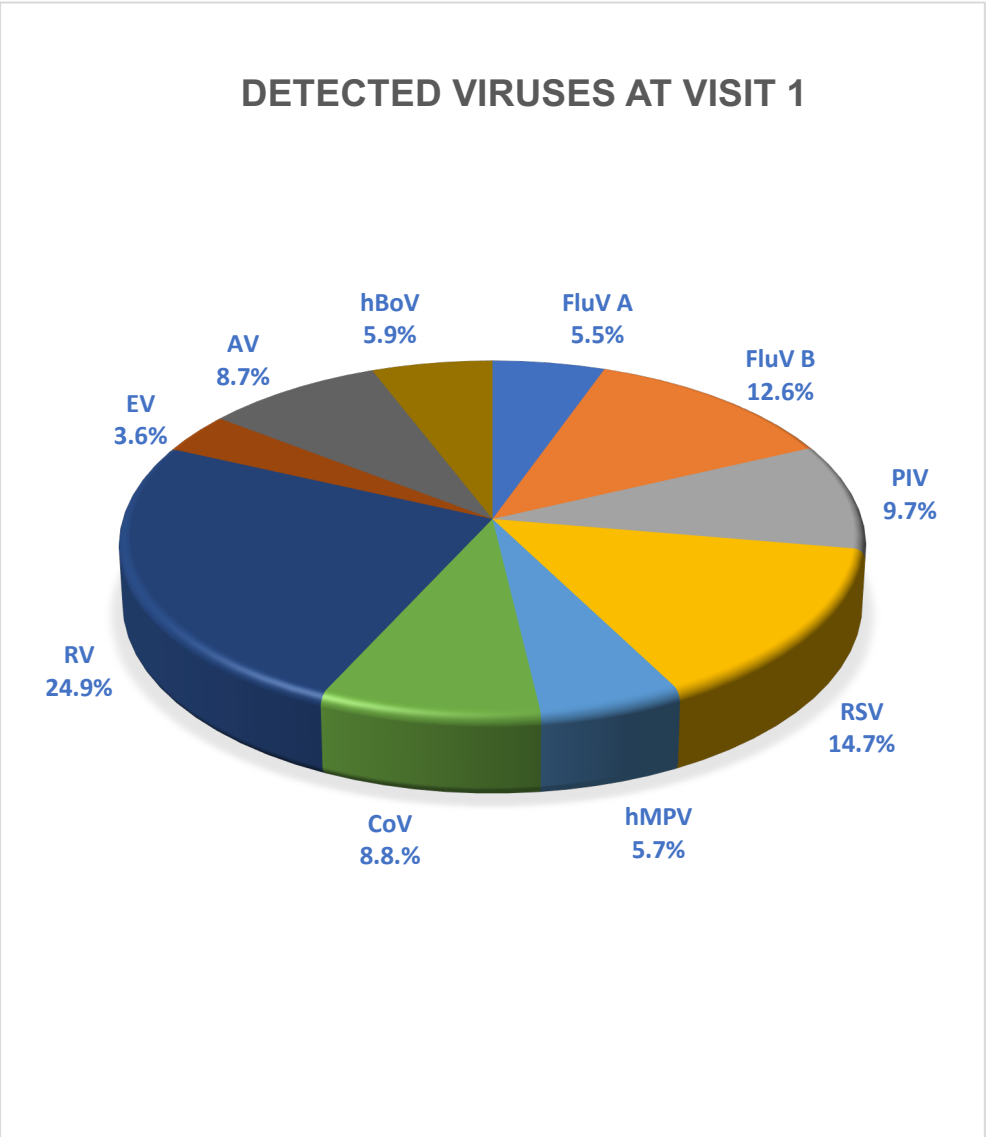


Diagram 13: Detected viruses at visit 1.

As far as BMI is concerned, we found out that as children grow older BMI is increased and also that there is an increase in liver enzyme values in older children and adolescents. It was shown that in 136 overweight/obese patients at Visit 1, 32 (23.5%) had LVE while in 125 overweight/obese patients at Visit 2, 30 patients (24%) manifested LVE. Table 5 shows LVE at visit 1 and visit 2 by age group and BMI category.

LVE at visit 1

BMI	Age group		
	1-5 years old	6-12 years old	>12 years
Normal weight	38/468 (8.1%)	27/239 (11.3%)	8/89 (9.0%)
Overweight / obese	8/64 (12.5%)	14/53 (26.4%)	10/19 (52.6%)

LVE at visit 2

BMI	Age group		
	1-5 years old	6-12 years old	>12 years
Normal weight	30/415 (7.2%)	21/224 (9.4%)	14/88 (15.9%)
Overweight / obese	7/58 (12.1%)	14/50 (28%)	9/17 (52.9%)

Table 5: Liver value elevation at visit 1 and 2, by age group and BMI.

In our study we also investigated whether the patients had any premedication and if they had, what kind of premedication they had. Our results showed that 619 (62.4%) of the patients did not have any premedication while the remaining 373 (37.6%) had. Specifically, regarding our age groups it was shown that 249 (43.2%) of children aged 1-5 years, 91 (29.6%) of children aged 6-12 years as well as 33 (30.3%) of adolescents had premedication (Diagram 14).

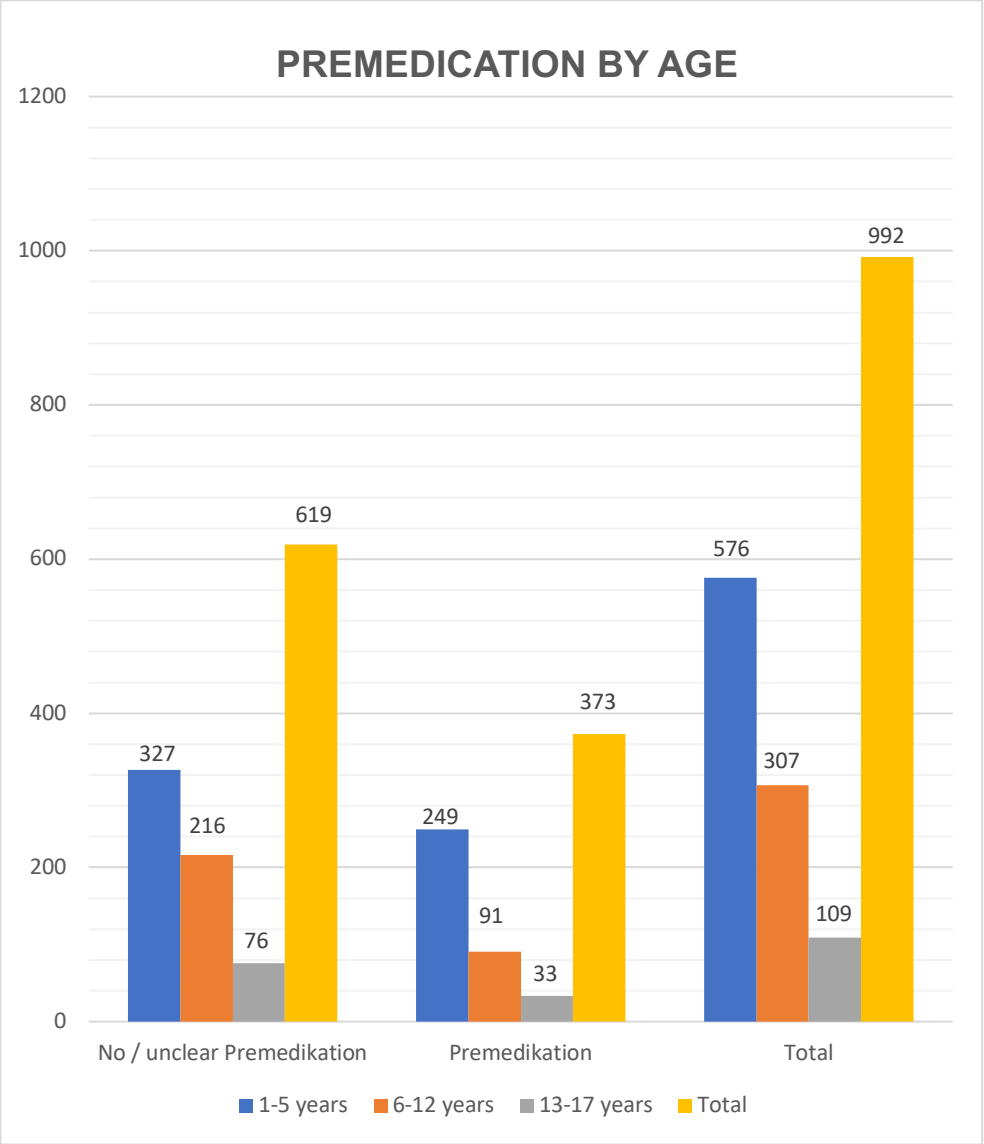


Diagram 14: Premedication by age.

As far as premedication and liver values elevation are concerned, we found that 48 out of 987 patients (4.9% in total) in Visit 1 that had premedication also showed elevated values in at least one of the main liver enzymes and 42 out of 896 patients (4.7% in total) in Visit 2 (Table 6). Fisher's Exact Test also showed no statistical significance with respect to LVE between the patients with premedication compared with the patients without premedication on Visit 1 (p=0.1762) as well as on Visit 2 (p=0.3810).

Premedication	Liver value elevation at visit 1		
	Total	LVE	No LVE
Total	987	110 (11.1%)	877 (88.9%)
Yes	372 (37.7%)	48 (4.9%)	324 (32.8%)
No/ Unclear	615 (62.3%)	62 (6.3%)	553 (56%)

Premedication	Liver value elevation at visit 2		
	Total	LVE	No LVE
Total	896	100 (11.2%)	796 (88.8%)
Yes	337 (37.6%)	42 (4.7%)	295 (32.9%)
No/ Unclear	559 (62.4%)	58 (6.5%)	501 (55.9%)

Table 6: Premedication and liver value elevation on visits 1 and 2 (the first number is the absolut number followed by % of detected patients).

Statistical analysis showed that 17% of the patients in visit 1 and 16.6% of the patients in visit 2 that answered positively for premedication (Table 7), had taken analgesics/ anti-inflammatory drugs, notably ibuprofen (134 patients, 13.5%) and paracetamol (55 patients, 5.5%) (Table 9). Specifically, for those patients it was demonstrated that 23 out of 168 patients, who had taken analgesics/ anti-inflammatory drugs in Visit 1 and 25 out of 149 in visit 2 manifested elevated liver enzymes values (Table 7). Fisher's exact test showed no statistical significance between analgesics and LVE in both visits ($p=0.3412$ and $p=0.0589$ respectively).

Analgesics - Anti-inflammatory	**Liver value elevation at visit 1**		
	Total	LVE	No LVE
Total	987	110 (11.1%)	877 (88.9%)
Analgesic/ Anti-inflammatory	168 (17%)	23 (2.3%)	145 (14.7%)
No / unclear premedication	615 (62.3%)	62 (6.3%)	553 (56%)
No / unclear analgesic / anti-inflammatory	204 (20.7%)	25 (2.5%)	179 (18.1%)

Analgesics - Anti-inflammatory	**Liver value elevation at visit 2**		
	Total	LVE	No LVE
Total	896	100 (11.2%)	796 (88.8%)
Analgesic / Anti-inflammatory	149 (16.6%)	25 (2.8%)	124 (13.8%)
No / unclear premedication	559 (62.4%)	58 (6.5%)	501 (55.9%)
No / unclear analgesic/ anti-inflammatory	188 (21%)	17 (1.9%)	171 (19.1%)

Table 7: Analgesic / anti-inflammatory premedication and liver value elevation during visit 1 and 2 (the first number is the absolut number followed by % of detected patients).

Antibiotics were taken from 6.5% of our subjects at visit 1 and 6.4% at visit 2 (table 8). In more detail, the antibiotics used were cefaclor (20 patients, 2.0%), amoxicillin (18 patients, 1.8%), erythromycin (6 patients, 0.6%), clarithromycin (4 patients, 0.4%), azithromycin, gentamycin and penicillin (3 patients for each antibiotic, 0.3%) and ampicillin (2 patients, 0.2%). Also, mucolytics were taken from 75 patients (7.6%), beta 2 and alpha-1 agonist from 47 (4.7%) and 41 patients (4.1%) respectively, antitussives by 36 patients (3.6%), glucocorticoids by 34 (3.4%), alpha and beta agonists by 15 patients (1.5%) while 8 patients (0.8%) took beta-2 agonists and glucocorticoids, 6 (0.6%) patients took leukotriene receptor antagonists, 3 (0.3%) took anticholinergics and 3 (0.3%) unknown premedication (Table 9). Also, only 9 patients in Visit 1 and Visit 2 were found to have elevated liver enzymes (Table 8). The statistical analysis showed no connection between the antibiotic premedication and LVE in visit 1 (p=0.3361) and visit 2 (p=0.3933).

Antibiotics	**Liver value elevation at visit 1**		
	Total	LVE	No LVE
Total	987	110 (11.1%)	877 (88.9%)
Antibiotic	64 (6.5%)	9 (0.9%)	55 (5.6%)
No/unclear premedication	615 (62.3%)	62 (6.3%)	553 (56%)
No/unclear antibiotic	308 (31.2%)	39 (4%)	269 (27.3%)

Antibiotics	Liver value elevation at visit 2		
	Total	LVE	No LVE
Total	896	100 (11.2%)	796 (88.8%)
Antibiotic	57 (6.4%)	9 (1%)	48 (5.4%)
No/unclear premedication	559 (62.4%)	58 (6.5%)	501 (55.9%)
No/unclear antibiotic	280 (31.3%)	33 (3.7%)	247 (27.6%)

Table 8: Antibiotic premedication and liver value elevation during visit 1 and 2 (the first number is the absolut number followed by % of detected patients).

Drug group			
Analgesics / anti-inflammatory drugs:			
	Ibuprofen	134	13.5%
	Paracetamol	55	5.5%
	Aspirin	1	0.1%
Antibiotics:			
	Cefaclor	20	2.0%
	Amoxicillin	18	1.8%
	Erythromycin	6	0.6%
	Clarithromycin	4	0.4%

	Azithromycin	3	0.3%
	Gentamycin	3	0.3%
	Penicillin	3	0.3%
	Ampicillin	2	0.2%
	Cefotaxim	1	0.1%
	Cefuroxim	1	0.1%
	Ciprofloxacin	1	0.1%
	Sulfonamide	1	0.1%
	Floxal	1	0.1%
Others		109	11%
Mucolytics		75	7.6%
Beta-2 agonists. sympathomimetic		47	4.7%
Alpha-1 agonists. sympathomimetics		41	4.1%
Antitussives		36	3.6%
Glucocorticoids		34	3.4%
Alpha and beta agonists. sympathomimetic		15	1.5%
Beta-2 agonists. Sympaticomimetica / glucocorticoids		8	0.8%
Leukotriene receptor antagonists		6	0.6%
Anticholinergics		3	0.3%
Unknown		3	0.3%

Table 9: List of premedication by drug group.

23.6% of our patients in visit 1 (233 subjects) and 23.2% in visit 2 (208 subjects) had taken premedication with known hepatic influence. 12.9% of those patients (30 out of 233 subjects) showed elevation in at least one liver enzyme value in visit 1 while the corresponding rate in visit 2 was 14,9% (31 out of 208 subjects) (Table 10). According to Fisher's exact test there was no correlation between the premedication with hepatic influence and the LVE in visit 1 ($p=0.3744$) and visit 2 ($p=0.1302$).

Premedication with known hepatic influence	Liver value elevation at visit 1		
	Total	LVE	No LVE
Total	987	110 (11.1%)	877 (88.9%)
Premedication with hepatic influence	233 (23.6%)	30 (3%)	203 (20.6%)
No / unclear premedication	615 (62.3%)	62 (6.3%)	553 (56%)
No / unclear premedication with hepatic influence	139 (14.1%)	18 (1.8%)	121 (12.3%)

Premedication with known hepatic influence	Liver value elevation at visit 2		
	Total	LVE	No LVE
Total	896	100 (11.2%)	796 (88.8%)
Premedication with hepatic influence	208 (23.2%)	31 (3.5%)	177 (19.8%)
No / unclear premedication	559 (62.4%)	58 (6.5%)	501 (55.9%)
No / unclear premedication with hepatic influence	129 (14.4%)	11 (1.2%)	118 (13.2%)

Table 10: Premedication with hepatic influence and liver value elevation during visit 1 and 2 (the first number is the absolut number followed by % of detected patients).

4.3 Patients with elevated liver enzymes values

Our study showed that 13 of the patients showed **three times higher** the upper limit in liver enzyme values. Specifically, when examined the three basic liver parameters (ASAT, ALAT, γ-GT) at least one of them was three times higher than the upper limit either in Visit 1 or Visit 2. All of the aforementioned subjects were of white/caucasian ethnicity. Also, 7 were male and 6 females. The demographics of the patients are demonstrated in Table 11.

	Patient	Age	Gender	Ethnicity	Height	Weight	BMI
1	10021	1.0	male	White/Caucasian	84	12.0	17.0
2	10069	1.5	male	White/Caucasian	-	12.0	-
3	10218	1.5	male	White/Caucasian	85	11.5	15.9
4	10370	17.2	male	White/Caucasian	174	137.0	45.3
5	30005	16.8	female	White/Caucasian	168	84.0	29.8
6	30009	2.3	female	White/Caucasian	90	14.6	18.0
7	40068	12.5	female	White/Caucasian	156	78.0	32.1
8	60054	1.4	male	White/Caucasian	84	13.0	18.4
9	60066	10.1	male	White/Caucasian	142	31.4	15.6
10	60145	13.9	female	White/Caucasian	158	47.5	19.0
11	60204	5.1	male	White/Caucasian	117	26.5	19.4
12	60205	6.8	female	White/Caucasian	130	31.4	18.6
13	70089	4.8	female	White/Caucasian	118	18.8	13.5

Table 11: Demographics of patients with elevated liver enzyme values (at least one of the basic parameters was three times the upper limit of Visit 1 or Visit 2).

From our results it was shown that 10 patients had in both visits three times the upper limit of the enzyme values, 2 patients showed elevation of their values during the visit 2 and in 1 patient it was found that the enzyme values had decreased in visit 2. Moreover, in 6 of these patients were detected viruses. In more detail, the viruses detected were RV, RSV A and B, CoV OC43 and NL63 (Table 12).

Subject number	LVE at Visit 1	LVE at Visit 2	Virus detected
10021	Yes	Yes	RV
10069	Yes	Yes	RSV A
10218	Yes	Yes	RSV B
10370	Yes	Yes	
30005	Yes	Yes	CoV OC43
30009	Yes	Yes	
40068	Yes	Yes	CoV NL63
60054	Yes	No	
60066	Yes	Yes	CoV NL63
60145	No	Yes	
60204	Yes	Yes	
60205	Yes	Yes	
70089	No	Yes	

Table 12: Patients with elevated liver enzyme values and detected viruses (at least one of the basic parameters was three times the upper limit of Visit 1 or Visit 2).

As far as premedication we discovered that out of these 13 patients that showed three times higher the upper limit in liver enzyme values, 1 of them did not had any medication and 5 of them had medication that did not cause increase in liver enzyme values. From these last 5 patients 2 were detected positive for virus. Antibiotics were administered to one patient who was also detected positive for viral infection. Moreover, out of 4 patients that had analgesics, one was virus positive whereas 2 patients had simultaneously antibiotics and analgesics and both of these patients were detected positive for virus. According to the literature, we consider that antibiotics as well as analgesics are medication that increase liver enzyme values (Manov, Motanis, Frumin, & Iancu, 2006; Navarro & Senior, 2006; Stine & Lewis, 2013). The medication that these patients received during their visits as well as the premedication are documented in Table 13.

	Patient	No. of medication	Group of medication	Indication	Start	End
1	10021	1	Beta-2 agonists. Sympathomimitics	Bronchiolitis	07/02/14	-
		2	Anticholinergics	Bronchiolitis	07/02/14	-
		3	Leukotrien receptor-Antagonists	Bronchiolitis	07/02/14	-
		4	Alpha-1 agonists. Sympathomimitics	Stuffed nose	08/02/14	-
		5	Glucocorticoids	Bronchiolitis	07/02/14	07/02/14
2	10069	1	Glucocorticoids	Bronchitis/Dyspnoe	05/05/14	05/05/14
		2	Glucocorticoids	Bronchitis	05/05/14	-
		3	Antibiotics	Bronchitis	05/05/14	-
		4	Other	Bronchitis	05/05/14	05/05/14
		5	Analgetics/ Anti-inflammatory	Fever	05/05/14	05/05/14
		6	Analgetics/ Anti-inflammatory	Fever	06/05/14	06/05/14
		7	Other	Bronchitis	05/05/14	-
		8	Glucocorticoids	Bronchitis	05/05/14	-
3	10218	1	Antibiotics	Bronchitis	18/03/15	21/03/15
		2	Beta-2 agonists. Sympathomimitics	Dyspnoe	18/03/15	25/03/15
		3	Antibiotics	Pneumonia/Bronchitis	22/03/15	-
		4	Alpha-1 agonists. Sympathomimitics	Stuffed nose	21/03/15	25/03/15
		5	Analgetics/ Anti-inflammatory	Fever	21/03/15	21/03/15
		6	Analgetics/ Anti-inflammatory	Fever	18/03/15	21/03/15
		7	Antibiotics	Pneumonia/Bronchitis	21/03/15	22/03/15
4	10370	-	-	-	-	-
5	30005	1	Mucolytics	Bronchitis	18/03/15	20/03/15
		2	Alpha-1 agonists. Sympathomimitics	Rhinitis	18/03/15	20/03/15
6	30009	1	Mucolytics	Rhinitis	27/05/15	-

		2	Mucolytics	Bronchitis	27/05/15	02/06/15
		3	Other	Rhinitis	27/05/15	-
7	40068	1	Other	Vaccination	05/02/16	05/02/16
		2	Other	Vaccination	05/02/16	05/02/16
		3	Analgetics/ Anti-inflammatory	Bronchitis	18/02/16	20/02/16
		4	Mucolytics	Bronchitis	18/02/16	25/02/16
8	60054	1	Other	Rachitisprophylaxis	27/04/15	-
		2	Mucolytics	URTI	21/09/15	28/09/15
9	60066	1	Antitussives	Dry cough	22/02/16	-
		2	Mucolytics	URTI	22/02/16	-
		3	Antibiotics	Otitis externa	24/02/16	-
10	60145	1	Mucolytics	URTI	19/06/16	23/06/16
		2	Antitussives	Dry cough	23/06/16	-
11	60204	1	Mucolytics	URTI	05/11/16	07/11/16
		2	Antitussives	Dry cough	05/11/16	16/11/16
		3	Mucolytics	URTI	07/11/16	09/11/16
		4	Analgetics/ Anti-inflammatory	Fever	07/11/16	09/11/16
		5	Other	Vomiting	09/11/16	10/11/16
		6	Mucolytics	URTI	09/11/16	16/11/16
12	60205	1	Mucolytics	URTI	07/10/16	13/10/16
		2	Alpha-1 agonists, Sympathomimitics	URTI	07/10/16	13/10/16
		3	Antitussives	Dry cough	05/11/16	-
		4	Mucolytics	URTI	07/11/16	16/11/16
		5	Analgetics/ Anti-inflammatory	Oral	07/11/16	09/11/16
		6	Other	Vomiting	09/11/16	11/11/16
13	70089	1	Analgetics/ Anti-inflammatory	Fever and pain	-	-

Table 13: Medication of patients with elevated liver enzyme values (at least one of the basic parameters was three times the upper limit of Visit 1 or Visit 2).

Also, 31 of our subjects showed elevated liver enzyme values with at least one of the three parameters **two times higher** the upper limit either in Visit 1 or Visit 2. 19 of these patients were male and 12 female. As far as the ethnicity is concerned 30 were of white/caucasian ethnicity and 1 of other. The demographics of the patients are demonstrated in Table 14.

	Patient	Age	Gender	Ethnicity	Height	Weight	BMI
1	10021	1.0	male	White/Caucasian	84	12.0	17.0
2	10028	10.6	female	White/Caucasian	140	32.0	16.3
3	10069	1.5	male	White/Caucasian	-	12.0	-
4	10174	1.0	male	White/Caucasian	76	10.4	18.0
5	10179	9.5	female	White/Caucasian	138	40.0	21.0
6	10196	4.6	female	White/Caucasian	98	12.3	12.8
7	10208	1.5	male	White/Caucasian	86	12.3	16.6
8	10218	1.5	male	White/Caucasian	85	11.5	15.9
9	10370	17.2	male	White/Caucasian	174	137.0	45.3
10	10398	4.4	female	White/Caucasian	105	19.7	17.9
11	10401	1.2	male	White/Caucasian	-	-	-
12	30005	16.8	female	White/Caucasian	168	84.0	29.8
13	30009	2.3	female	White/Caucasian	90	14.6	18.0
14	40068	12.5	female	White/Caucasian	156	78.0	32.1
15	40070	17.8	male	White/Caucasian	192	83.7	22.7
16	40073	15.5	male	White/Caucasian	171	88.7	30.3
17	40074	17.8	male	White/Caucasian	183	102.9	30.7
18	40079	17.4	male	White/Caucasian	179	91.0	28.4
19	40096	7.2	female	White/Caucasian	118	20.4	14.7
20	40100	16.9	male	White/Caucasian	164	93.9	34.9
21	60027	1.2	male	White/Caucasian	76	9.5	16.4
22	60054	1.4	male	White/Caucasian	84	13.0	18.4

23	60066	10.1	male	White/Caucasian	142	31.4	15.6
24	60145	13.9	female	White/Caucasian	158	47.5	19.0
25	60155	9.0	male	White/Caucasian	143	32.9	16.1
26	60204	5.1	male	White/Caucasian	117	26.5	19.4
27	60205	6.8	female	White/Caucasian	130	31.4	18.6
28	60250	5.8	male	White/Caucasian	116	16.7	12.4
29	60253	14.9	female	White/Caucasian	160	70.0	27.3
30	60254	17.8	male	Other	172	51.0	17.2
31	70089	4.8	female	White/Caucasian	118	18.8	13.5

Table 14: Demographics of patients with elevated liver enzyme values (at least one of the basic parameters was two times the upper limit of Visit 1 or Visit 2).

It was shown that 16 of these patients were also positive for viral detection. More specifically, the viruses identified were FluV B, CoV OC43 and NL63, RV, RSV A and B, hMPV A, and AV (Table 15).

	Subject Number	**Virus**
1	10021	Rhinovirus
2	10069	Respiratory syncytial virus A
3	10174	Respiratory syncytial virus B
	10174	Adenovirus
4	10179	Coronavirus OC43
5	10208	Human Metapneumovirus hMPV A
6	10218	Respiratory syncytial virus B
7	10401	Respiratory syncytial virus A
8	30005	Coronavirus OC43
9	40068	Coronavirus NL63
10	40070	Influenza B
11	40074	Influenza B

12	40096	Influenza B
13	40100	Rhinoviren
14	60027	Rhinoviren
15	60066	Coronavirus NL63
16	60250	Respiratory syncytial virus A

Table 15: Detected viruses in patients with elevated liver enzyme values (at least one of the basic parameters was two times the upper limit of Visit 1 or Visit 2).

Concerning medication our study showed that out of 31 patients that demonstrated two times higher the upper limit in liver enzyme values, 2 did not have any medication while 1 of them was virus positive. 9 subjects were administrated medication that did not have any effect on the liver enzyme values and 4 out of these 9 patients were detected positive for viral infection. Out of 4 patients that had antibiotics, 2 were virus positive. Also, 3 out of 9 patients that had analgesics were detected positive for virus, whereas 6 out of 7 patients that had an antibiotics – analgesics combination were virus positive. Analgesics and antibiotics are considered to increase liver enzyme values. The medication that these patients received during their visits as well as the premedication are documented in Table 16.

	Patient	No.of medication	Group of medication	Indication	Start	End
1	10021	1	Beta-2 agonists. Sympathomimitics	Bronchiolitis	07/02/14	-
		2	Anticholinergics	Bronchiolitis	07/02/14	-
		3	Leukotrien receptor-Antagonists	Bronchiolitis	07/02/14	-
		4	Alpha-1 agonists. Sympathomimitics	Stuffy nose	08/02/14	-
		5	Glucocorticoids	Bronchiolitis	07/02/14	07/02/14
2	10028	1	Analgetics/ Anti-inflammatory	Fever	27/02/14	-
		2	Analgetics/ Anti-inflammatory	Fever	27/02/14	-

		3	Antibiotics	Pharyngitis	07/03/14	-
		4	Antibiotics	Pharyngitis	07/03/14	-
3	10069	1	Glucocorticoids	Bronchitis/ Dyspnoe	05/05/14	05/05/14
		2	Glucocorticoids	Bronchitis	05/05/14	-
		3	Antibiotics	Bronchitis	05/05/14	-
		4	Other	Bronchitis	05/05/14	05/05/14
		5	Analgetics/ Anti-inflammatory	Fever	05/05/14	05/05/14
		6	Analgetics/ Anti-inflammatory	Fever	06/05/14	06/05/14
		7	Other	Bronchitis	05/05/14	-
		8	Glucocorticoids	Bronchitis	05/05/14	-
4	10174	1	Other	Rachitis prophylaxis	21/12/13	-
		2	Other	Vaccination	07/01/15	07/01/15
		3	Antibiotics	Viral infection/ Flu/ Otitis media	12/01/15	-
		4	Analgetics/ Anti-inflammatory	Fever	10/01/15	13/01/15
		5	Analgetics/ Anti-inflammatory	Fever	12/01/15	12/01/15
		6	Alpha-1 agonists. Sympathomimitics	Otitis	12/01/15	15/01/15
		7	Analgetics/ Anti-inflammatory	Fever	10/01/15	14/01/15
5	10179	1	Analgetics/ Anti-inflammatory	Fever	23/01/15	27/01/15
		2	Antibiotics	Suspicion of Mycoplasma infection	24/01/15	26/01/15
		3	Analgetics/ Anti-inflammatory	Fever	25/01/15	25/01/15

		4	Other	Unc. Kawasaki Syndrom, IG-G was administered	25/01/15	27/01/15
		5	Other	IG-Gabe	27/01/15	27/01/15
6	10196	1	Other	Fever cramps	19/02/15	19/02/15
		2	Other	Oral cavity fungal infection	22/02/15	-
		3	Analgetics/ Anti-inflammatory	Fever	19/02/15	-
		4	Analgetics/ Anti-inflammatory	Fever	19/02/15	20/02/15
		5	Alpha-1 agonists. Sympathomimitics	Otitis media	19/02/15	-
7	10208	1	Mucolytics	Secretolysis	05/03/15	09/03/15
		2	Antitussives	Cough	05/03/15	09/03/15
		3	Antibiotics	Ventilator associated Pneumonia	08/03/15	11/03/15
		4	Other	Obstipation	10/03/15	14/03/15
		5	Analgetics/ Anti-inflammatory	Fever	04/03/15	09/03/15
		6	Analgetics/ Anti-inflammatory	Fever	04/03/15	10/03/15
		7	Antibiotics	Pneumonia	11/03/15	-
		8	Mucolytics	Secretolysis	11/03/15	-
8	10218	1	Antibiotics	Bronchitis	18/03/15	21/03/15
		2	Beta-2 agonists. Sympathomimitics	Dyspnoe	18/03/15	25/03/15
		3	Antibiotics	Pneumonia/ Bronchitis	22/03/15	-
		4	Alpha-1 agonists. Sympathomimitics	Stuffed nose	21/03/15	25/03/15
		5	Analgetics/ Anti-inflammatory	Fever	21/03/15	21/03/15

		6	Analgetics/ Anti-inflammatory	Fever	18/03/15	21/03/15
		7	Antibiotics	Pneumonia/ Bronchitis	21/03/15	22/03/15
9	10370	-	-	-	-	-
10	10398	1	Antibiotics	Infection of the respiratory tract	15/11/16	18/11/16
		2	Alpha and Beta agonists. Sympathomimitics	Pseudo croup/Dyspnoe	20/11/16	21/11/16
11	10401	1	Antibiotics	Pneumonia	14/12/16	15/12/16
		2	Beta-2 agonists. Sympathomimitics	Obstruktive Bronchitis, Dyspnoe	10/12/16	-
12	30005	1	Mucolytics	Bronchitis	18/03/15	20/03/15
		2	Alpha-1 agonists. Sympathomimitics	Rhinitis	18/03/15	20/03/15
13	30009	1	Mucolytics	Rhinitis	27/05/15	-
		2	Mucolytics	Bronchitis	27/05/15	02/06/15
		3	Other	Rhinitis	27/05/15	-
14	40068	1	Other	Vaccination	05/02/16	05/02/16
		2	Other	Vaccination	05/02/16	05/02/16
		3	Analgetics/ Anti-inflammatory	Bronchitis	18/02/16	20/02/16
		4	Mucolytics	Bronchitis	18/02/16	25/02/16
15	40070	1	Other	Infection of the respiratory tract	19/02/16	26/02/16
		2	Antitussives	Infection of the respiratory tract	19/02/16	21/02/16
16	40073	1	Mucolytics	Infection of the respiratory tract	22/02/16	27/02/16

17	40074	1	Mucolytics	Bronchitis	22/02/16	26/02/16
18	40079	1	Analgetics/ Anti-inflammatory	Infection of the respiratory tract	23/02/16	25/02/16
19	40096	1	Analgetics/ Anti-inflammatory	Flu	01/03/16	02/03/16
		2	Beta-2 agonists. Sympathomimitics	Influenza	01/03/16	08/03/16
		3	Glucocorticoids	Flu	01/03/16	08/03/16
20	40100	-	-	-	-	-
21	60027	1	Mucolytics	URTI	05/03/15	12/03/15
		2	Mucolytics	URTI	26/03/15	-
		3	Alpha-1 agonists. Sympathomimitics	URTI	26/03/15	-
		4	Analgetics/ Anti-inflammatory	Fever	25/03/15	28/03/15
22	60054	1	Other	Rachitis prophylaxis	27/04/15	-
		2	Mucolytics	URTI	21/09/15	28/09/15
23	60066	1	Antitussives	Dry cough	22/02/16	-
		2	Mucolytics	URTI	22/02/16	-
		3	Antibiotics	Otitis externa	24/02/16	-
24	60145	1	Mucolytics	URTI	19/06/16	23/06/16
		2	Antitussives	Dry cough	23/06/16	-
25	60155	1	Antitussives	Dry cough	07/07/16	14/07/16
26	60204	1	Mucolytics	URTI	05/11/16	07/11/16
		2	Antitussives	Dry cough	05/11/16	16/11/16
		3	Mucolytics	URTI	07/11/16	09/11/16
		4	Analgetics/ Anti-inflammatory	Fever	07/11/16	09/11/16
		5	Other	Vomiting	09/11/16	10/11/16
		6	Mucolytics	URTI	09/11/16	16/11/16
27	60205	1	Mucolytics	URTI	07/10/16	13/10/16

		2	Alpha-1 agonists. Sympathomimitics	URTI	07/10/16	13/10/16
		3	Antitussives	Dry cough	05/11/16	-
		4	Mucolytics	URTI	07/11/16	16/11/16
		5	Analgetics/ Anti-inflammatory	Oral	07/11/16	09/11/16
		6	Other	Vomiting	09/11/16	11/11/16
28	60250	1	Analgetics/ Anti-inflammatory	Fever	20/12/16	24/12/16
		2	Antitussives	Dry cough	12/12/16	16/12/16
		3	Mucolytics	Bronchitis	20/12/16	-
		4	Antibiotics	Bronchitis	20/12/16	-
29	60253	1	Mucolytics	URTI	25/12/16	03/01/17
		2	Analgetics/ Anti-inflammatory	Fever	26/12/16	29/12/16
		3	Antitussives	Dry cough	27/12/16	-
30	60254	1	Mucolytics	Bronchitis	27/12/16	03/01/17
		2	Antibiotics	Bronchitis	27/12/15	-
31	70089	1	Analgetics/ Anti-inflammatory	Fever and pain	-	-

Table 16: Medication of patients with elevated liver enzyme values (at least one of the basic parameters was three times the upper limit of Visit 1 or Visit 2).

5 Discussion

Viruses are the simplest forms of life. They have been used extensively to investigate the fundamental mechanisms of life and have greatly helped to understand the function of complex organisms at the molecular level. Their study is also of particular interest, because it contributes significantly to the development of methods for the treatment of the diseases they cause (Carter, Saunders, & Saunders, 2007). Viruses were discovered in 1883 by Mayer, but were thought to be the mysterious agents of the "tobacco mosaic" disease. They were initially identified as very small infectious bacteria, which are not visible under a microscope. Ten years later the Russian naturalist Ivanowski found that these pathogens passed through very fine filters, which could hold all the bacteria. They have also been found to be able to multiply only in living cells and not in nutrients, as is the case with bacteria. Finally, in 1935, the tobacco mosaic virus was isolated and studied by Stanley (Creager, Scholthof, Citovsky, & Scholthof, 1999). It was found then, that virus was a reproducible particle, much simpler than bacteria, and composed of nucleic acid and proteins. Unlike the cell, which is the basic unit of life, the virus is nothing more than nucleic acid and protein. Only when it is inside a host cell does the virus begins to replicate (Harrison & Wilson, 1999).

Now, it is well known that viruses are intracellular parasites and necessarily need a host cell to perform all the biological functions necessary for their replication. They are smaller than the smallest bacteria and are visible only with the help of an electron microscope. In addition, they have only one type of nucleic acid, either DNA or RNA, but never both. When a virus infects an organism at first it attaches to a specific host cell via cell’s receptors. The virus then enters the cell and begins to replicate its nucleic acid in order to produce many copies of the viral nucleic acid. At the same time, the components of the viral protein shell are synthesized, which, subsequently, self-assemble together into new viruses. The new viruses are then finally released from the host cell in order to re-infect other cells. Viruses exploit cellular mechanisms and direct host metabolic functions to support their genome proliferation and the synthesis of new viruses (Carter et al., 2007; Norkin, 2010). Moreover, these pathogens are specific both to the organism they infect as well as to the host cells of each organism. In particular, through the receptors they have, they infect host cells of a tissue after they are adhered to them. However, viruses do not just infect one organ but can infect others, causing in most of the cases a number of symptoms. This fact explains why in the case of viral infections not only one organ is infected and why patients manifest several symptoms (Carter et al., 2007; Norkin, 2010).

PCR is a very simple technique but it is a basic tool of Molecular Biology, it has contributed greatly to the development of genetic and wider biological research and has found a plethora

of applications (Berg, Tymoczko, & Stryer, 2008; Primrose, Twyman, & Old, 2001). PCR mimics the process of DNA replication and thus achieves the in vitro production of numerous thousands or millions of copies of a particular DNA region, e.g. of a gene, in a short period of time and even from an initial amount of DNA that can be as little as a single DNA molecule (Mullis, 1990). Any region of any DNA fragment can be selected for amplification by this technique, provided that the nucleotide sequences at both ends of the region of interest are known (Berg et al., 2008; Primrose et al., 2001).

The PCR technique was developed by Kary Mullis in 1983, who in 1993 was awarded the Nobel Prize in Chemistry (Mullis, 1990). It is a simple, inexpensive, easy, reliable, specialized and sensitive technique, which has found a variety of applications in many scientific fields, such as amplification and isolation of specific DNA fragments, gene cloning, directed mutagenesis of genes, genetic fingerprinting with wide application in criminology for the identification of culprits, in the analysis of DNA polymorphisms, in the detection of genetic mutations, in the development of medical genetic tests (e.g. the detection of HIV), in prenatal testing, in paternity testing, in paleontology applications from DNA traces that can be found from fossils, etc. Indicative of the widespread use of the method is the fact that more than 30 different variants of PCR have been developed (Lodish et al., 2008).

PCR, as well as the Real-Time PCR, are widely used in virology as it offers many applications regarding this field (Mackay, Arden, & Nitsche, 2002; Niesters, 2004). Specifically, using these methods we can carry out qualitative and quantitative detection of a large range of viral nucleic acids (Hodinka, 1998). Therefore, this has led to great progress as far as diagnosis is concerned for many viruses (Damen et al., 1996; Espy et al., 2000). Also, these molecular methods have the advantage that there is no need of viral culture, thus are more quick and less expensive (Speers, 2006). Viral detection and diagnosis can be performed with many methods such as electron microscopy, enzyme immunoassays, traditional antigen test, virus cultures and PCR (Jartti, Söderlund-Venermo, Hedman, Ruuskanen, & Mäkelä, 2013; Speers, 2006). However, PCR is the most sensitive and rapid method both for detection and diagnosis (Clark & McKendrick, 2004). Many studies confirm the above mentioned conclusion showing the superiority of this method over other methods used in viral detection and diagnosis (Elden et al., 2002; Lin et al., 2020; Martins Júnior et al., 2014; Steininger, Kundi, Aberle, Aberle, & Popow-Kraupp, 2002; Tsai et al., 2014). Moreover, respiratory viruses that are significant, such as CoV and FluVs, have been detected using PCR methodology with sensitivity that varies from 50-80% in the early stages and goes up to 100% in some cases (Emery et al., 2000; Ho et al., 2005; Lakeman, Whitley, Allergy, & Group, 1995; Long et al., 1998; Ng et al., 2003; Speers, 2006).

Multiplex PCR has, also, become an important procedure in molecular detection and diagnosis (Elnifro, Ashshi, Cooper, & Klapper, 2000). This PCR variant reduces the cost while at the same time increases the volume of samples that can be analyzed (Elnifro et al., 2000). It has many application areas such as nucleic acid diagnostics (Chamberlain, 1990), mutation and polymorphism analysis (Rithidech, Dunn, & Gordon, 1997), quantitative analysis (Sherlock, Cirigliano, Petrou, Tutschek, & Adinolfi, 1998) as well as RNA detection (Zou, Stansfield, & Bridge, 1998). Moreover, it has been shown that multiplex PCR can be used for the identification of many infectious factors such as viruses, bacteria, fungi, and/or parasites (Elnifro et al., 2000). Many studies have confirmed the diagnostic value of this method in upper and lower respiratory viral infections (Echevarría, Erdman, Swierkosz, Holloway, & Anderson, 1998; Ellis, Fleming, & Zambon, 1997; Fan, Henrickson, & Savatski, 1998; Gröndahl et al., 1999; Osiowy, 1998; Stockton, Ellis, Saville, Clewley, & Zambon, 1998). Respiratory viruses such as RSV, influenza viruses, parainfluenza and adenoviruses can cause aRTIs in infants and children, therefore a rapid and sensitive identification method is needed. A study conducted in England showed that multiplex PCR had a better detection rate for influenza viruses in comparison to viral cultures (39.7% versus 32.3%). Also, this method showed great correlation (100%) in the detection of different viral subtypes (Ellis et al., 1997). It has been demonstrated that multiplex PCR can be up to 100% sensitive and 98% specific in comparison to viral culture and antigenic tests (Boivin, Côté, Déry, De Serres, & Bergeron, 2004; Fan et al., 1998; Lee et al., 2007).

The purpose of this dissertation was to analyze as well as investigate the relationship between acute respiratory tract infections and the occurrence of elevated hepatic enzyme values in children and adolescents. More specifically, our aim was to study how frequent is the involvement of the liver in pediatric patients with acute respiratory infections, which consist the most common pathogenic factors in these cases and also which viruses may cause concomitant hepatitis. Moreover, we wanted to examine if other factors such as previous medication, BMI, gender, age etc could also lead to elevated hepatic enzyme values in these patients.

For this purpose, we conducted a prospective clinical study according to the state of the art, which included 6 pediatric practices (outpatients) and one pediatric hospital (inpatients) in Germany. Our patients were aged 1-18 years old suffering from symptoms of acute respiratory tract infections. During the study the medical history of the patients was documented and also blood was sampled for several laboratory test. The results were also statistically analyzed.

5.1 Results

For this prospective study we included 992 children and adolescents suffering from symptoms of acute respiratory tract infections. This study was performed between January 2014 and December 2016. From our patients, 57.7% were male and 42.3% female.

The viruses that were analyzed included FluV A (H1N1, H3N2) and B, PIV 1, 2 and 3, RSV A and B, hMPV A and B, CoV (229E, OC43 and NL63), RV, EV, AV and hBoV. From our subjects, 617 cases had a positive multiplex-PCR for virus detection (63%) while 363 cases showed a negative PCR (37%). Regarding the spectrum of the detected viruses it was shown that RV was detected in 179 cases (18.0%), RSV (A, B) in 105 cases (10.6%), FluV B in 92 cases (9.3%), PIV (1,2,3) and CoV (229E, OC43, NL63) in 69 (7.0%) and 63 cases (6.4%) respectively, AVs in 62 cases (6.3%), hBoVs and hMPV (A, B) in 42 (4.2%) and 41 cases (4.1%) respectively, FluV A (H1N1, H3N2) in 39 cases (4.0%) and EV in 26 cases (2.6%).

Acute respiratory tract infections due to viruses are quite common in children and adolescents causing morbidity (Brealey, Sly, Young, & Chappell, 2015; Monto, 2002). Epidemiological studies that were started almost 80 years ago, have shown that children are at particular risk for viral acute respiratory tract infections (BADGER & DINGLE, 1953; Frost & Gover, 1932; Monto, 1994; Sydenstricker, 1926; Van Volkenburgh & Frost, 1933). These early findings have been verified by the results of currents epidemiological studies. Specifically, it was demonstrated that RVs were by far the most frequent cause of acute respiratory tract infections in the overall population (35%), while FluV (30%), PIV (12%), RSV (11%), AV (8%) and other (4%) followed (Monto, Bryan, & Ohmit, 1987; Monto & Sullivan, 1993). A retrospective study that investigated the etiology and clinical features of severe acute viral LRTI in children showed that 32.1% of the patients were positive for FluV, 29.9% for PIV, 16.0% for RSV, 11.0% for AV, and 11.0% for mixed viruses (Jeon, Kim, Kim, & Hong, 2000). A study conducted in Brazil showed that the main viruses identified in children were RSV (26.2%), AV (6%), FluVs (1.7%), and PIVs (1.5%) (Straliotto et al., 2002). However, in that study subjects were not examined for RV. In another cohort of children in Brazil there was conducted a prospective study in order to investigate the prevalence of viral respiratory infections as well as to identify the viruses involved (Souza et al., 2003). 42.8% of the subjects were positive for viral infection. The viruses identified were: RVs (52%), EVs (15%), AVs (12%), PIVs (11%), FluVs (6%), and RSV (4%). Also, a study in children with asthma had also shown that RV was by far the most prevalent virus (60% among case patients vs 18.2% among control subjects) and the only virus significantly associated with asthma exacerbations (Khetsuriani et al., 2007). Moreover, Singleton and colleagues, in a study conducted in Alaska with hospitalized children, demonstrated that 90% of hospitalized children were detected virus positive. The viruses detected included: RV (44%), AV (30%), RSV (23%), PIV (18%), hMPV (15%), FluV (5%), and

CoV (6%). Also, 52% of the children from the control group were virus positive, mainly with RV (33%) and AV (16%) (Singleton et al., 2010). However, RSV, PIV, hMPV, and FluV were significantly more common in hospitalized children than the control group, while RV, AV, and CoV were not. Also, a longitudinal study with young Andean children, showed that 43% of the subjects were positive for at least one respiratory virus (Howard et al., 2015). The viruses detected were human RV (32%), AV (22%), RSV (10%), and PIV (3%). In a systematic review concerning the respiratory viruses in acute lower respiratory infections in children under five years, the statistical analysis demonstrated a strong connection of the infection and RSV, FluV, PIV and hMPV, and less strong connection with RV in young children presenting with acute lower respiratory infection compared to those without respiratory symptoms (asymptomatic) or healthy children (Shi, McLean, Campbell, & Nair, 2015). In another prospective study, the researchers showed that 24.4% of children were found positive for viral infection, with human RV being the most common virus (17.3%) (Self et al., 2016).

In another study investigating the prevalence of respiratory track viruses in hospitalized children in China, the researchers found that the most common viruses detected were RSV, 68.11 %), AV (ADV, 16.01 %) and PIV-3 (11.0 %) (Wang et al., 2016). Similar results were reported by another study that investigated the incidence or respiratory viral infections in children in Korea (S. J. Lee et al., 2017). In more detail, it was demonstrated that 41.1% of the subjects were positive for virus and the viruses detected included: RSV (18.9%), human RV, (14.8%), AV (9.5%), and hBoV (7.4%). A previous single center study in Korea also confirmed the abovementioned results (Kwon et al., 2014). In a more recent single-center study that wanted to investigate the viral respiratory tract infections in hospitalized children in Iraq it was found that 75.5% of the patients were found positive for viral infections (Hassan, Rachid, & Ziebuhr, 2018). Once again, the most common virus detected was RV (32.7%) and RSV (20.4%), and hMPV (13.4%) followed.

Additionally, in a recent longitudinal, community-based, birth cohort study that included 158 infants, it was demonstrated that 32.7% of the subjects were virus positive (Sarna et al., 2018). Human RV was the most common detected virus (77.8% of all positive detections), followed by hBoV-1 and human polyomavirus KI. Also, in the same study statistical analysis showed that RSV and hMPV were strongly associated with higher risk of lower respiratory symptoms in young children. From the results of the above mentioned studies it is obvious that they are in accordance with the results reported in our study concerning the viruses' prevalence. This fact highlights the accuracy of this study.

5.2 Liver Values Elevation and viruses

In clinical practice it is known that viruses that cause respiratory tract infection are, also, not restricted to this organ and in some occasions they cause liver lesions (Papic et al., 2012). Specifically, it has been reported that in patients with upper or lower respiratory tract infection scientists have noticed, not rarely, that they also show elevated liver enzyme levels, which suggest liver involvement. Therefore, we wanted to investigate the values of the three primary liver parameters (AST, ALT and γ-GT) for each patient after each visit (Visit 1 and Visit 2) in order to detect any increase (LVE) in these parameters. Our results showed that elevated values for at least one of the three hepatic parameters according to the judgment of the investigator were found in 11.1% of the subjects at visit 1 and 11.2% at visit 2. Also, regarding the virus detection and the LVE it was shown that 75 patients (12.2%) who were positive for virus also showed an increase in at least one liver value. According to the Fisher's exact test there was no statistical significance between the detected viral agent and the elevation of hepatic enzyme values in these subjects (p=0.2496).

A considerable proportion of subjects with an acute respiratory tract infection, had a RV, RSV, FluV B, PI, CoV and AV infection, while elevated liver enzyme levels were observed in patients with FluV B, hMPV, RSV, FluV A, CoVs and PIV infection. Specifically, in 715 patients of our study with acute respiratory tract infection that had a positive PCR result it was found that 178 (24.9%) had RV infection, 105 (14.7%) RSV, 90 (12.6%) FluV, 69 (9.7%) PI, 63 (8.8%) CoV and 62 (8.7%) AV. Moreover, 83 (11.6%) of these patients manifested LVE. In more detail, 22 out of 90 patients with FluV B had LVE (24.4%), 6 out of 41 patients with hMPV (14.6%), 14 out of 105 patients with RSV (13.3%), 5 out of 39 patients with FluV A (12.8%), 8 out of 63 patients with CoVs (12.7%) and 8 out of 69 patients with PIV (11.6%).

Although it is well known that a large proportion of children and adolescents with acute respiratory infections may manifest elevated liver enzyme markers without any clinical symptoms, data obtained from prospective, large-scale clinical studies primarily investigating the acute respiratory tract infection-associated hepatic involvement in children and adolescents are as yet lacking. In a study that investigated the distribution of the diseases associated with nonspecific reactive hepatitis in children, as well as the change in the level of AST and ALT (Kim et al., 2008) it was demonstrated that elevated liver enzymes were mostly observed in respiratory and gastrointestinal infections. Between these two infections, the highest levels of liver enzymes were found in the respiratory infections. A review investigated the connection between the infection with respiratory viruses and aminotransferases levels in children (J. S. Oh et al., 2016). The patients were scanned for the following viruses: AV, FluV A and B, PIV, (1,2,3,4), RV (A/B/C), RSV (A and B), BoV (1/2/3/4), metapneumovirus, CoV (229E, NL63, OC43) and EV. It was shown that 13.3% of the patients had elevated liver

enzyme levels. Also, 24.6% of the patients had an RSV A infection, 24.1% RV infection, and 10.9% metapneumovirus infection. The scientists concluded that different types of viruses that cause LRTIs did not significantly affect ALT and AST levels. However, elevated liver enzyme values were more common observed among patients with FluV, RSV, and AV infection as well as among patients who were younger at the time of infection. A more recent study aimed to investigate the prevalence of liver involvement in acute respiratory tract infections in pediatric patients (Baghdasaryan et al., 2019). For this reason, a prospective study including 84 children from Armenia was conducted. Their results showed that almost 40% of the subjects had elevated liver enzymes.

Although there are not many clinical studies that investigate the viral acute respiratory tract infections and the elevated enzyme levels in children and adolescents, there are indeed some smaller studies that investigate liver enzyme involvement and some specific viruses. In more detail, a lot of studies have shown that severe influenza infection can also cause abnormalities in the liver (Adams & Hubscher, 2006). However, these cases are considered rare (Control & Prevention, 2009). Many publications also suggest that infection from FluV can lead to liver damage (Duchini, Viernes, Nyberg, Hendry, & Pockros, 2000; Sellers, Hagan, Hayden, & Fischer, 2017; Whitworth et al., 2006). Analysis of human H5N1 infections had showed that 61-83% of the cases manifest abnormal liver function (A/H5, 2005). In a review investigating the influenza A (H5N1) it was reported that in some cases patients with influenza A showed some liver dysfunction which resolved after the successful clearance of the virus (Yuen & Wong, 2005). Also, Polakos et al., reported that four out of 15 subjects infected with H1N1 developed elevated serum transaminases, suggesting clinically significant hepatitis (Polakos et al., 2006). Later studies, also showed that patients with FluV had moderate liver enzymes elevations (Hien et al., 2009; Liem et al., 2009; Papic et al., 2012; Shinde et al., 2011; Yu et al., 2008). However, a study investigating the pandemic 2009 influenza A reported that this infection can also lead to hepatocellular injury (Papic et al., 2012). In the case of pediatric patients, the studies again are few. Specifically, it has been reported a case of a 10-month-old child with acute myocarditis and fulminant hepatic failure associated with H1N1 (El-Shabrawi, Bazaraa, Zekri, & Rady, 2011). Also, a study conducted in Kuwait, described the case of a 9-year-old boy with H1N1 infection that developed acute hepatic failure (Al-Refaee, 2012).

The present study showed that from the total of 62 (8.7%) patients that were positive for AV infection, only 4 (6.5%) had elevated liver enzyme values. In the case of AV there are some more studies focusing in pediatric patients. A retrospective review of adenoviral infection in pediatric liver transplant recipients showed that out of 484 liver transplant recipients, there were 53 episodes (10.1%) of infection due to AV (Michaels, Green, Wald, & Starzl, 1992). The AV had infected, in most cases, the liver, lung, and the gastrointestinal tract. Another study

investigating 29 pediatric cases of acute respiratory tract infection associated with AV showed that in 37.9% of these cases manifested liver abnormalities among other extrapulmonary findings (Murtagh, Cerqueiro, Halac, Avila, & Kajon, 1993). Also, in a double liver transplant child there was reported a hepatitis due to adenoviral infection (Varki, Bhuta, Drake, & Porter, 1990). Other viruses identified after liver transplant were CMV and EBV (Cen, Breinig, Atchison, Ho, & McKnight, 1991; Chou, 1986; Marcelin, Beam, & Razonable, 2014). A study investigating epidemic adenoviral lower infection in pediatric patients showed that more than half of the patients demonstrated mild elevation of liver enzyme values (B. Han, Son, Yoon, & Lee, 1998). A study conducted in Korea, that investigated lower respiratory tract infections due to AV in children showed that almost half of the infected patients demonstrated elevated liver enzyme values (Hong et al., 2001). Similar previous studies have confirmed these results (Markin, Langnas, Donovan, Zetterman, & Stratta, 1991; Shields, Hackman, Fife, Corey, & Meyers, 1985; Wasserman, August, & Plotkin, 1988; Yagisawa et al., 1989).

We demonstrated that 8 out of 63 subjects (12.7%) that were positive for CoV infection, had an elevation in liver enzyme values. CoVs have been reported that can lead to elevated liver enzyme values. In more detail, SARS that is caused by a novel coronavirus (SARS-CoV) has also shown to cause elevated ALT levels showing that this virus is not mainly affecting the respiratory track (Chan et al., 2005). In a study of 138 cases of SARS, there was reported elevation of liver enzyme values in 23.4% of the patients (N. Lee et al., 2003). A lot of other studies had demonstrated that liver lesions are connected with SARS (Booth et al., 2003; Chau et al., 2004; Choi et al., 2003; Farcas et al., 2005; N. Lee et al., 2003; J. Peiris et al., 2003; Poutanen et al., 2003; Wong et al., 2003). However, the study of N. Lee et al., (2003) was the first study that associated SARS-CoV infection with liver abnormalities. Also, a retrospective study that aimed to evaluate the hepatic function in patients with SARS showed that the majority of patients (70%) had an abnormal liver enzyme activity, which can indicate the replication of the virus in the liver (Cui et al., 2004). These percentages are higher than those reported in other studies (Booth et al., 2003; Cui et al., 2004; Huo et al., 2003), probably because the patients included in the study had severe symptoms. In a more recent retrospective study conducted in Saudi Arabi, it was demonstrated that most patients that were positive for CoV also manifested an elevation in enzyme liver values (Sherbini et al., 2017). Also, the same study showed that deaths among patients with CoV were significantly associated with worse liver enzymes ($p<0.05$).

Liver abnormalities have, as well, been reported for the most recent CoV strain (SARS-CoV-2) that causes COVID-19 disease (Feng et al., 2020; Hao et al., 2020; Hundt, Deng, Ciarleglio, Nathanson, & Lim, 2020; Kulkarni et al., 2020; Mao et al., 2020). However, there are not many studies that investigate these abnormalities in children/adolescents. According to the data so

far, it seems that children positive for this new CoV demonstrate low or zero liver enzyme values elevation (Garrido, Liberal, & Macedo, 2020). In a systematic review on this topic showed that children positive for COVID-19 were less likely to have elevated liver enzyme values in comparison to adult patients, whereas they had the same risk of developing liver lesions (Mao et al., 2020). Another review, also, confirmed these results (Feng et al., 2020). Moreover, in a systematic review that investigated the liver manifestations of COVID-19, it was reported that 17.8% of the pediatric patients of the studies included, had elevated liver enzyme values during the initial stages of the disease while in adults was 24.1% (Kulkarni et al., 2020). The same review reported that 18.4% of children had elevated AST and 12.6% ALT values.

One of the most common viral infections that leads children and infants in the pediatric intensive care unit (PICU) due to respiratory failure is RSV infection (Pilar et al., 1998). RSV infection can also cause non-pulmonary manifestations that among other organs include as well the liver (Gkentzi, Dimitriou, & Karatza, 2018). In our cohort it was shown that 14/105 (13.3%) patients with RSV infection had elevated liver enzymes. An early study with pediatric liver transplant recipients, showed that almost 3% of the subjects were positive for the virus (Pohl, Green, Wald, & Ledesma-Medina, 1992). An earlier case study reported two immunosuppressed children cases after liver transplantation, who developed severe morbidity associated with RSV infection post-transplant (Blanchard, Gerrek, Siegel, & Czinn, 2006). In studies investigating RSV in pediatric patients, the scientists showed that 46 to 49% of ventilated children with RSV bronchiolitis had elevated enzyme liver values. Also, it was reported that patients with elevated enzyme values also had a more severe infection (Eisenhut & Thorburn, 2002; Eisenhut, 2006; Eisenhut, Thorburn, & Ahmed, 2004; Thorburn et al., 2018). A case report investigating hepatitis after RSV infection in a 13-month boy with signs and symptoms of respiratory failure showed a remarkable increase of the liver enzyme values (Kristić Kirin, Zrinski Topić, & Dodig, 2013). Other case studies also linked RSV bronchiolitis in children with elevated liver enzyme values (Giordano et al., 2018).

HMPV is another respiratory pathogen that has been isolated from adults and children with acute respiratory tract infections (Van den Hoogen et al., 2001). HMPV infections have also been associated with altered liver findings (Principi, Bosis, & Esposito, 2006). The present study showed that 14.6% (6/41) of the patients included in our investigation that were positive for hMPV infection, had elevated liver enzymes values. In a study that investigated 587 children hospitalized with respiratory infection, 32 (5.5%) were found positive for hMPV (J. M. Peiris et al., 2003). Additionally, some of these patients that were positive also had altered liver tests. In a study that investigated the prevalence on hMPV in immunosuppressed children it was shown that almost half of the patients (49%) manifested moderate elevation of liver enzyme values (Scheuerman et al., 2016).

RV is, as well, a quite common pathogen in viral acute respiratory tract infections in children, both in upper and lower respiratory tract (Cheuk et al., 2007; Souza et al., 2003; Turner, 2007). Also, it has been linked with asthma, especially in these age groups (Friedlander & Busse, 2005; Johnston et al., 1995). There are not many studies that describe the connection between rhinovirus infection and liver enzyme alterations. We showed that 6.7% (12/178) of patients with RV infection demonstrated liver enzymes elevation. Moreover, in a prospective study that was conducted including hospitalized children in Hong Kong it was demonstrated that 35.4% of the patients were positive for RV but liver findings were normal in the subjects tested (24%) (Cheuk et al., 2007). Moreover, one study that investigated the role of common respiratory viruses in nonspecific hepatitis in pediatric patients showed that, although 24.1% of the subjects were infected with rhinovirus, no significant difference was found between this viral infection and the aminotransferase concentrations (Oh et al., 2016). In another study that examined the complications of RV in critically ill patients, only 1 out of 13 (7.7%) patients had liver lesions (To et al., 2016). Although rhinoviral infections are quite common in children, unfortunately there are not many studies that investigate the correlation between rhinovirus and liver alterations, neither in adults nor in children. This may be due to the fact that RV is thought to cause only mild symptoms (Atmar, 2005). However, this is not true, as RV can lead to severe complications, as well (To et al., 2016). More research is required in this direction.

5.3 Liver Values Elevation and premedication

Another factor that we investigated in our study was if our patients had previous medication and also what kind of previous medication they had. Specifically, we discovered that out of 13 patients that showed three times higher the upper limit in liver enzyme values, 1 of them did not had any medication and 5 of them had medication that did not cause increase in liver enzyme values. From these last 5 patients 2 were detected positive for virus. Antibiotics were administered to one patient who was also detected positive for viral infection. Moreover, out of 4 patients that had analgesics, one was virus positive whereas 2 patients had simultaneously antibiotics and analgesics and both of these patients were detected positive for virus. We consider that antibiotics as well as analgesics are medication that increase liver enzyme values.

Also, for the 31 patients that demonstrated two times higher the upper limit in liver enzyme values we found out that 2 of them did not have any medication while 1 of them was virus positive. 9 subjects were administrated medication that did not have any effect on the liver enzyme values, and 4 out of these 9 patients were detected positive for viral infection. Out of 4 patients that had antibiotics, 2 were virus positive. Also, 3 out of 9 patients that had analgesics were detected positive for virus, whereas 6 out of 7 patients that had an antibiotics

– analgesics combination were virus positive. Analgesics and antibiotics are considered to increase liver enzyme values.

From the above mentioned results, we can see that the proportion of the patients that had premedication (analgesics and antibiotics) and also showed elevated liver enzyme values are similar to the one observed for patients that had other medication. Also, we noticed that most of these patients (with previous or concomitant medication) were also positive for a virus showing that there is a mild correlation between the previous medication and the elevated liver enzyme values. Since in the case of viral infection we don't usually give antibiotics we believe that patients that were given antibiotics had a more severe infection with greater symptoms therefore that can also explain the elevated liver enzyme values in these patients.

The statistical analysis showed no statistical significance between the previous medication and LVE both at visit 1 ($p=0.1722$) and at visit 2 ($p=0.3810$). Moreover, it was found that analgesics and antibiotics did not show a correlation with liver enzyme values in both visits ($p=0.3414$, $p=0.0589$, $p=0.3361$ and $p=0.3933$ respectively). This may be due to the unclear information regarding the previous or concomitant medication of our patients. Therefore, conclusions for this subject should be drawn with caution.

It is well known that analgesic drugs and antibiotics are a major group of drugs that affect liver, causing in some cases liver damage or hepatotoxicity (Manov, Motanis, Frumin, & Iancu, 2006; Navarro & Senior, 2006; Stine & Lewis, 2013). Although drug-related hepatotoxicity is quite rare, there are some drugs, that the reported incidence is between 1 in 10,000 and 1 in 100,000 patients (Larrey, 2002), and therefore the true incidence of this phenomenon is quite difficult to determine (R. Oh & Hustead, 2011). In most of the cases liver injury is indicated by elevations in serum liver enzyme levels, but increases of far more than three times the upper limit of normal may not lead to clinically liver damage. This is due to the ability of the liver to heal injury, while also develop tolerance, as frequently seen with initial exposure to drugs such as isoniazid (Nolan, Goldberg, & Buskin, 1999) and tacrine (Watkins, Zimmerman, Knapp, Gracon, & Lewis, 1994).

Also, a lot of anti-inflammatory and analgesic drugs can cause toxic effects effecting the liver and causing elevated liver enzyme values, but these are reported only after a drug has been used for a significant period of time. Anti-inflammatory drugs and analgesics can be responsible for various clinical, biochemical and structural changes, regardless of their correct or not administration. The most frequently observed and extensively studied are the effects of nonsteroidal anti-inflammatory drugs (NSAIDs) as well as other analgesics on the liver (Manov et al., 2006).

Antibiotics remain the most common drugs that cause hepatotoxicity and acute drug-induced liver injury (Andrade & Tulkens, 2011; Grant & Rockey, 2012; Serranti et al., 2013; Stine &

Lewis, 2013), but there are, also, many other drugs as well as xenobiotics associated with liver damage, although their occurrence is not common enough and most of the time depends on the user and his temperament (Reuben, Koch, & Lee, 2010; Senior, 2008; Zimmerman, 2000). Antibiotics are considered responsible for about 45% of drug hepatotoxicity cases (Serranti et al., 2013). However, these cases are quite rare and moreover their incidence in children are not well characterized since there are few studies that investigate it (Ferrajolo et al., 2010; Molleston et al., 2011; Serranti et al., 2013).

Drug-induced toxicity can mimic all forms of acute as well as chronic hepatic insufficiency, while many of these drugs tend to have a characteristic pattern based on clinical and pathological symptoms and latency (Kaplowitz, 2001). The mechanism by which this is manifested is not always understood, but may be the result of the patients "delayed" immune response to the drug, in combination with its prolonged retention in the body (Kaplowitz, 2004). Undesirable hepatic side effects caused by drugs can be considered either unexpected (high frequency) or unpredictable (low frequency) (Leise, Poterucha, & Talwalkar, 2014). Medications that cause expected liver damage, such as paracetamol, usually show symptoms for a short time (some days) and are the result of the immediate hepatotoxicity caused by the original drug or its metabolites. On the other hand, drugs that cause unforeseen side effects, such as isoniazid, manifest severe or symptomatic diseases but only after a longer period of time, ranging from 1 week to 1 year. The majority of drug induced liver injuries are unpredictable and are either immune hypersensitivity or idiosyncratic (Kaplowitz, 2004; Zimmerman, 2000).

Moreover, it has been reported that antibiotics are more frequently administered to children than to adults. However, little is known about the liver injury caused to children due to antibiotics. Although there are antibiotics that are very studied, such as amoxicillin (Rodríguez, Stricker, & Zimmerman, 1996), amoxicillin/clavulanic acid (M. I. Lucena et al., 2006), penicillinase resistant penicillins (Maraqa, Gomez, Rathore, & Alvarez, 2002), erythromycin (Carson et al., 1993), ciprofloxacin (I. M. Lucena, Andrade, Sanchez-Martinez, Perez-Serrano, & Gomez-Outes, 1998), levofloxacin (Figueira-Coelho et al., 2010), trimethoprim/sulfamethoxazol (Jick & Derby, 1995) and tetracyclines (Björnsson, Lindberg, & Olsson, 1997), there are other antibiotics including cephalosporins or clarithromycin, that little is known. A lot of pediatric case reports have been published, suggesting that liver injury due to antibiotics is the same as in adults (Alberti-Flor, Hernandez, Ferrer, Howell, & Jeffers, 1989; Altraif, Lilly, Wanless, & Heathcote, 1994; Andrade, Guilarte, Salmerón, Lucena, & Bellot, 2001; Bell, Foster, & Townsend, 2010; GORDIN, SIMON, WOFSY, & Mills, 1984; Madronero, Porcel, & Bielsa, 2007; Rodríguez et al., 1996; Schwarze, Schmitz, Fischer, Sauerbruch, & Spengler, 2002; Yao, Behling, Saab, Li, & Hart, 1997). From studies conducted specifically in children it

was shown that antibiotics can cause liver damage in children (Ferrajolo et al., 2010; Molleston et al., 2011; Serranti et al., 2013). The aforementioned antibiotics were the same with those reported for adults. Nevertheless, there have been reported findings that are seen only in children, such as the fact that jaundice is more common in children as in adults (Molleston et al., 2011).

According to the literature, specific medication such as antibiotics and anti-inflammatory medication can lead to altered liver enzyme values in adults as well as children. Our research showed a mild elevation on these values and although no statistical significance was detected, our results are in accordance with the results of other studies showing that premedication in children and adolescents can mildly elevate the liver enzymes values. However, further research must be conducted in this field in order to draw more specific conclusions.

5.4 Liver Values Elevation and BMI

Obesity among children and adolescents has become one of the most serious public health problems in the current century. It is well known that the worldwide prevalence of childhood obesity has increased greatly over the past three decades (J. C. Han, Lawlor, & Kimm, 2010), but also, we now know that obesity can also lead to several pathologies (J. C. Han et al., 2010; LUSTIG & WEISS, 2008).

This is the reason why we also investigated the BMI of our patients and the connection between BMI and liver enzyme values elevation. Our results showed that 139 (14.8%) of our subjects were overweight/obese. More analytically, according to the age groups it was shown that 12,1% of children aged 1-5 years is overweight/obese, in comparison to 18.6% in children aged 6-12 years and 17.6% in adolescents.

It has been reported in various studies conducted, that the prevalence of childhood obesity worldwide demonstrates a great increase (Fraser et al., 2007; Tominaga et al., 1995; Troiano, Flegal, Kuczmarski, Campbell, & Johnson, 1995). Additionally, in the third National Health and Nutrition Examination Survey (NHANES III) study, that included 2450 children and adolescents aged 12-18, it was shown that 16% of adolescents were overweight while 10% were obese (Fraser, Longnecker, & Lawlor, 2007; Strauss, Barlow, & Dietz, 2000). A study conducted by Han et al., (2010) validated the aforementioned results, as it was shown that the prevalence of childhood obesity worldwide had increased over the past three decades (J. C. Han et al., 2010). Specifically, in the United States (US) only, the pediatric obesity incidence has increased from less than 5% to approximately 20% in that period of time (Ogden, Carroll, Curtin, Lamb, & Flegal, 2010). Furthermore, in 2007-2008, 16.8% of children and adolescents in USA according to the BMI were considered obese (Güngör, 2014). Also, a more recent worldwide study showed that from 1975 to 2016, the BMI of children and adolescents increased globally

and therefore the global prevalence of obesity in children and adolescents increased from 0.7% in 1975 to 5.6% in 2016 for females and from 0.9% in 1975 to 7.8% in 2016 for males (Abarca-Gómez et al., 2017). These results are in accordance with the results found in our study. However, another study that investigated BMI in 1107 students aged 12-18 years old showed that 34% of these subjects were overweight and 26.6% were obese (Kelishadi et al., 2009).

Moreover, as far as BMI and the elevated liver enzyme levels concerned, we found out that patients that belonged to the overweight/obese category were more likely to show elevated liver enzyme values both in visit 1 and 2 compared with patients that had normal weight. In more detail, our research demonstrated that in 136 overweight/obese patients, 32 (23.5%) of our subjects showed LVE in visit 1 and in 125 overweight/obese patients, 30 patients (24%) in visit 2. Also, when we studied BMI and LVE according to age group it was shown that adolescents (13-17 years old) that were obese/overweight were more prone to have elevated liver enzyme values in both visits (52,6% at visit 1 and 52,9% at visit 2) in comparison to the other age groups.

Several studies conducted both in general population as well as in obese/overweight subjects have indicated elevated liver enzyme values with increasing BMI category. According to the results of the NHANES III as well as other studies has been shown that overweight and obese children/adolescents are much more likely to have elevated liver enzymes values than normal weight children/adolescents (Alavian, Mohammad - Alizadeh, Esna - Ashari, Ardalan, & Hajarizadeh, 2009; Kelishadi et al., 2009; Strauss et al., 2000). Specifically, in NHANES III study it was demonstrated that 6% of overweight and 10% of obese adolescents had elevated ALT values while more than 60% of the subjects with elevated liver values without hepatitis C or B were either overweight or obese (Strauss et al., 2000). Also, these researchers showed that even without the alcohol consumption obese/overweight adolescents had four times greater risk for elevated liver enzymes values (Strauss et al., 2000). Also, a retrospective review that investigated fatty liver in 742 children and adolescents showed that 16% of overweight children and 38% of obese children had fatty liver (Schwimmer et al., 2006). Moreover, in an Australian study with adolescents the researchers found that adverse concentrations of ALT, GGT and AST increased across BMI categories (Booth et al., 2008). In more details, it was demonstrated a marked increase in the prevalence of liver injury with increasing BMI, which was over 40% in obese boys and nearly 20% in overweight girls. Two studies that investigated liver enzyme levels in adolescents in USA, during the periods 1988–1994 and 2007–2010, showed similar results with the NHANES III study (Welsh, Karpen, & Vos, 2013).

Other studies have also shown greater liver enzymes values (24%-25%) than those reported by the NHANES III study. However, these studies were conducted including only overweight

and/ or obese children/adolescents and did not include a normal BMI children group (Bedogni et al., 2012; Saviano et al., 1997; Tazawa, Noguchi, Nishinomiya, & Takada, 1997; Tominaga et al., 1995; Wiegand et al., 2010). Also, a study conducted in Mexico showed that 42% of obese and overweight children manifested elevated aminotransferases (Flores-Calderón, Gómez-Díaz, Rodríguez-Gómez, & Morán-Villota, 2005).

As we can see, our results are higher than those reported by the NHANES III study but in accordance with other studies conducted. Since there is not another largescale clinical trial to compare our results to, we believe that these results can be interpreted by the manner of our study.

6 Summary

Acute respiratory track infections play an important role in morbidity in infants and children (Bharaj et al., 2009). In the majority of these infections the causative agent in 45-60% of the cases are viruses (Marinheiro, Sanalios, Santos, Costa, & Hársi, 2009). The clinical manifestations of these infections may involve the upper respiratory tract with symptomatology of rhinitis, pharyngo-tonsilitis, otitis, sinusitis or lower respiratory tract with bronchiolitis, asthma attacks and pneumonia (Kliegman, Stanton, Geme, & Schor, 2015).

In clinical practice it is known that a large proportion of children and adolescents with acute respiratory infections may manifest elevated liver enzyme markers without any clinical symptoms. Although there are some studies that investigate the effect of viral acute respiratory tract infections in hepatic enzyme values in adults, there are not many studies that investigate the same subject in children and adolescents. The studies that deal with the role of acute respiratory tract infections in liver function in most of the cases, concern children with immunodeficiency or other severe health issues. Therefore, there is not much information about the role of these infections in the liver function in children and adolescents without an underlying disease.

The aim of this dissertation was to study the relationship between respiratory tract infections and the occurrence of elevated hepatic enzyme values in children and adolescents. More specifically, we wanted to study how frequent is the involvement of the liver in pediatric patients with viral respiratory infections, which are the most common pathogenic factors in these cases and also which viruses may cause concomitant hepatitis. For this reason, we included 992 children and adolescents suffering from symptoms of acute respiratory tract infections. Our results demonstrated that in children and adolescents with acute viral respiratory infection, there were observed clinically significant increases in hepatic markers in a large proportion of patients with little association with previous medication due to limitations of our research. More specifically, it was shown that hepatic enzyme values (AST, ALT, γ-GT) are elevated in a considerable proportion of children and adolescents suffering from acute respiratory tract infection. However, statistical analysis did not show any correlation between the viral causative agent and the liver enzyme values elevation, which is not in accordance with the results of similar studies. We believe that, although, our study did not unveil a statistical significance between the viral infection and the elevated liver enzyme values of our subjects, there was shown a clinical significance, since it was demonstrated that patients with viral acute respiratory tract infections had greater probability of manifesting elevated liver enzymes values as well. These findings also represent a reliable basis for the interpretation of abnormal hepatic enzyme values in the course of clinical trials as well as for the assessment of spontaneous

adverse event reports. We must also point that, to our knowledge, so far, this study is one of the largest prospective studies in that subject and therefore, there are no specific rates concerning pediatric patients on the matter.

Moreover, from this study, it is also obvious that other factors such as BMI can also play an important role, since we discovered that overweight/obese children and adolescents were more prone to show elevated liver enzyme values in comparison to normal weight patients. Other studies also highlight the fact that overweight/obese children and adolescents show a variety of health problems. This fact shows that physiological characteristics such as BMI play an important part in the elevation of liver enzyme values and therefore can lead to liver damage.

As far as limitations are concerned one important limitation of our study was the fact that there were patients who did not provide complete information about previous and/or concomitant medication. For this reason, we cannot extract any safe conclusions consider the effect of premedication in the levels of the liver enzyme markers investigated.

In conclusion, we believe that our study provides important information regarding the viral respiratory tract infections and liver enzyme values elevation in pediatric patients without other underlying health conditions. In addition, there is need for more prospective large-scale clinical studies to be conducted in order to have a more complete view on the matter.

Zusammenfassung

Akute Atemwegsinfektionen spielen eine wichtige Rolle bei der Morbidität von Säuglingen und Kindern (Bharaj et al., 2009). Bei diesen Infektionen handelt es sich in 45-60% der Fälle um Viren (Marinheiro, Sanalios, Santos, Costa & Hársi, 2009). Die klinischen Manifestationen dieser Infektionen können entweder die oberen Atemwege mit Symptomen wie Rhinitis, Pharyngo-Tonsilitis, Otitis, Sinusitis oder die unteren Atemwege mit Bronchiolitis, Asthmaanfällen und Lungenentzündung betreffen (Kliegman, Stanton, Geme, & Schor, 2015).

In der klinischen Praxis ist bekannt, dass ein großer Teil der Kinder und Jugendlichen mit akuten Atemwegsinfektionen, erhöhte Leberenzymmarker ohne klinische Symptome aufweisen kann. Obwohl einige Studien die Wirkung von viralen Infektionen der akuten Atemwege auf die Leberenzymwerte bei Erwachsenen untersuchen, gibt es wenig Studien, die das gleiche Thema bei Kindern und Jugendlichen untersuchen. Die Studien, die sich in den meisten Fällen mit der Auswirkung akuter Atemwegsinfektionen bei der Leberfunktion befassen, betreffen Kinder mit Immunschwäche oder anderen schwerwiegenden Vorerkrankungen. Daher gibt es nicht viele Informationen über die Rolle solcher Infektionen bei der Leberfunktion bei Kindern und Jugendlichen ohne Grunderkrankung.

Ziel dieser Dissertation war es, den Zusammenhang zwischen Atemwegsinfektionen und dem Auftreten erhöhter Leberenzymwerte bei Kindern und Jugendlichen zu untersuchen. Insbesondere wollten wir untersuchen, wie häufig eine Leberbeteiligung bei pädiatrischen Patienten mit viralen Atemwegsinfektionen auftritt, welche in diesen Fällen die häufigsten pathogenen Faktoren sind und welche Viren eine begleitete Hepatitis verursachen können. Aus diesem Grund haben wir in unsere Studien 992 Kinder und Jugendliche eingeschlossen, die an Symptomen akuter Atemwegsinfektionen litten. Unsere Ergebnisse zeigten eine klinisch signifikante Erhöhung der Lebermarker in einem großen Teil der Kinder und Jugendlichen mit akuter Virusinfektion der Atemwege, dies allerdings mit geringer Assoziation mit früheren Medikamenten aufgrund von Einschränkungen unserer Forschung. Insbesondere wurde ein Anstieg der Leberwerte (AST, ALT, γ-GT) bei einem beträchtlichen Anteil der Kinder und Jugendlichen gezeigt, die an einer akuten Infektion der Atemwege litten. Die statistische Analyse zeigte jedoch keine Korrelation zwischen dem viralen Erreger und der Erhöhung der Leberenzymwerte, was nicht mit den Ergebnissen ähnlicher Studien übereinstimmt. Wir glauben, dass, obwohl unsere Studie keine statistische Signifikanz zwischen einer Virusinfektion und den erhöhten Leberenzymwerten unserer Probanden enthüllte, es bei den Patienten mit viralen akuten Atemwegsinfektionen eine größere Wahrscheinlichkeit auch erhöhte Leberenzymwerte nachzuweisen besteht, sodass eine klinische Signifikanz gezeigt wurde. Diese Ergebnisse stellen auch eine verlässliche Grundlage für die Interpretation

abnormaler Leberenzymwerte im Verlauf klinischen Studien sowie zur Auswertung von Berichten über unerwünschte Ereignisse oder Zwischenfälle dar. Wir müssen auch darauf hinweisen, dass diese Studie unseres Wissens bislang, eine der größten prospektiven Studien in diesem Bereich ist und daher gibt es keine spezifischen Raten für pädiatrische Patienten in dieser Thematik.

Darüber hinaus, ist aus dieser Studie ersichtlich, dass auch andere Faktoren wie der BMI eine wichtige Rolle spielen können, da wir festgestellt haben, dass übergewichtige / adipöse Kinder und Jugendliche im Vergleich zu normalgewichtigen Patienten anfälliger für erhöhte Leberenzymwerte sind. Andere Studien betonen auch die Tatsache, dass übergewichtige / adipöse Kinder und Jugendliche eine Vielzahl von Gesundheitsproblemen aufweisen. Diese Tatsache zeigt, dass physiologische Eigenschaften wie der BMI eine wichtige Rolle bei der Erhöhung der Leberenzymwerte spielen und daher zu Leberschäden führen können.

Als wichtige Einschränkung unserer Studie weisen wir auf die unvollständigen Informationen einiger Patienten über frühere und / oder begleitende Medikamente hin. Aus diesem Grund, können wir keine sicheren Schlussfolgerungen ziehen, die die Auswirkung der Prämedikation auf die Spiegel der untersuchten Leberenzymmarker berücksichtigen.

Zusammenfassend glauben wir, dass unsere Studie wichtige Informationen zu viralen Atemweginfektionen und Erhöhung der Leberenzymwerte bei pädiatrischen Patienten ohne andere zugrunde liegende Vorerkrankungen liefert. Darüber hinaus besteht der Bedarf für die Durchführung von weiteren prospektiven klinischen Studien in größerem Umfang, um eine umfassendere Sicht zu diesem Thema zu erhalten.

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8 Appendix

Virus	Reference strain	Cells	Accession-number
Influenza A	A/South Australia/ 51/2005(H1N1))	MDCK	CY021760
Influenza B	(B/HongKong/ 28/2001)	MDCK	CY019598
Parainfluenza 1	Washington/1964	CV1	NC_003461
Parainfluenza 2	1990 Düsseldorf	CV1	NC_003443
Parainfluenza 3	1999 Düsseldorf	CV1	NC_001796
HMPV A	B09 (Bonn)	-	NC_004148
HMPV B	B09 (Bonn)	-	NC_004148
Adenovirus	Adeno 5 (Düsseldorf 1981)	A549	J01966
Coronavirus 229 E	2001 Düsseldorf	MRC-5	AF304460
Coronavirus OC43	2005 (Düsseldorf)	-	NC_005147
Coronavirus NL63	2004 (Bonn)	-	NC_005831
RSV A	ATCC VR-26 (Long-strain)	Hep2	AY911262
RSV B	Virus 9320 (B)	Hep2	AY353550
Rhinovirus	Human Rhinovirus 2	MRC-5	X02316
Enterovirus	Human Enterovirus 68 isolate 37-99	MRC-5	EF107098
Human Bocavirus	Isolate st2 (Bonn)	-	DQ000496

Table 17: Reference strains, cells and Gen-Bank Accession-numbers used for the establishment of the real-time-TaqMan PCR.

Primer and probes	Sequence (5’ → 3’)	Gene and position	Amplicon
Inf A-F	AGCTGCMCARAGRGCAATGG	NP (719-738)	
Inf A-R	RAADATGAGATCTTCRATYTCAGCRT	NP (772-797)	79 bp
Inf A-pr	FAM-ATCAAGTGAGAGAAAGYCGGAACCCAGG-BHQ	NP (742-769)	
Inf B-F	CCCTGCTTGCTCGWAGYATGG	NP (987-1007)	
Inf B-R	TGCTTATGGGAAGMACYACTTTG	NP (1033-1055)	69 bp
Inf B-pr	HEX-CGTTGTTAGGCCCTCTGTGGCGA-BHQ	NP (1009-1031)	
Para1-F	TGATTTAAACCCGGTAATTTCTCA	HN (7619-7642)	
Para1-R	GGCAAGGAGCATAACTGATAACC	HN (7701-7723)	
Para1-pr	FAM-ACAGGARATCATGTTCTGTAATAGCTGCAGGA-BHQ	HN (7663-7694)	105 bp
Para2-F	CTGGAGTCATGCCATGCAAT	HN (8159-8178)	
Para2-R	GGCCACACATCTGCGTACAC	HN (8215-8234)	
Para2-pr	HEX-CAACAAGTTTTTGCCCTGCTA ATTGCATC-BHQ	HN (8180-8208)	76 bp
Para3-F	AAAGGCAAAATAATATTTCTCGGG TAT	HN (7790-7816)	
Para3-R	CCCGGGACACCCAGTTGT	HN (7859-7876)	
Para3-pr	Cy-5-TGAACATCCAATAAATGAGAATGYAATCTG-BHQ	HN (7825-7854)	87 bp
RSVA-F	TCTTAAATCTRTAGCACAAATCACATTATC	G (4788-4817)	
RSVA-R	TGATGGCTGCAATTATAAGTGAAGTT	G (4839-4864)	
RSVA-pr	FAM-TGGCAATGATAATCTC-MGB	G (4823-4838)	77 bp
RSVB-F	TTTAAAATCTATAGCACAAATAGCACTATC	G (4789-4818)	
RSVB-R	TTATGGCTGCAATTATGAGAGAGGTT	G (4840-4865)	
RSVB-pr	FAM-TGGCAATGATAATCTC-MGB	G (4824-4839)	77 bp
HMA-F	GGGTCAGAGAGAGTACAGCAGATTC	N (277-301)	
HMA-R	CCCCAAAGAGTACGTTCTGGTT	N (336-357)	
HMA-pr	HEX-AACTCAGGCAGTGAAGTCCAAGYGGTYT-BHQ	N (307-334)	81 bp
HMB-F	AAACAATGGTGACTTTGCTAA AGGA	N (452-476)	
HMB-R	TTGGTGTGTCTGGTGCTGAAG	N (509-529)	

HMB-pr	FAM-TCATCAGGTAACATCCCACAAAACCAGAGG-BHQ	N (478-507)	78 bp
Ade-F	GCCACGGTGGGGTTTCTAAACTT	Hexon (157-181)	
Ade-R	GCCCCAGTGGTCTTACAT GCACATC	Hexon (267-281)	
Ade-pr	HEX-TGCACCAGACCCGGGCTCAGGTACTCCGA-BHQ	Hexon (197-225)	111 bp
HCoE-F	CAGTCA AATGGGCTGATGCA	N (25693-25712)	
HCoE-R	AAAGGGCTATAAAGAGAATAAGGTAT TCT	N (25740-25768)	
HCoE-pr	FAM-CCCTGACGACCACGTTGTGGTTCA-BHQ	N (25715-25738)	76 bp
HCoO-F	CGATGAGGCTATTCCGACTAGGT	N (29607-29629)	
HCoO-R	CCTTCCTGAGCCTTCAATATAGTA ACC	N (29656-29682)	
HCoO-pr	HEX-TCCGCCTGGCACGGTACTCCCT-BHQ	N (29631-29652)	76 bp
HCoN-F	ACGTACTTCTATTATGAAGCATGATATTAA	R (6055-6084)	
HCoN-R	AGCAGATCTAATGTTATACTTAAA ACTACG	R (6128-6157)	
HCoN-pr	Cy-5-ATTGCCAAGGCTCCTAAACGTACAGGTGTT-BHQ	R (6089-6118)	103 bp
Ent-F	GACATGGTGYGAAGAGTCTAT TGA	5'NCR (409-432)	
Ent-R	GCTCCGCAGTTAGGATTA GCC	5'NCR (464-484)	
Ent-pr	HEX-GTAGTCCTCCGGCCCCTGAATGC-BHQ	5'NCR (441-463)	76 bp
Rhin-F	TGGACAGGGTGTGAAGAGC	5'NCR (396-414)	
Rhin-R	CAAAGTAGTCGGTCCCATCC	5'NCR (520-539)	
Rhin-pr	FAM-TCCTCCGGCCCCTGA ATG-BHQ	5'NCR (434-451)	144 bp
Boca-F	TCGGGCTCATATCATCAGGAA	NP-1 (2554-2574)	
Boca-R	CACTTGGTCTGAGGTCTTCGAA	NP-1 (2607-2628)	
Boca-pr	Cy-5-CCCAATCAGCCACCTATCGTCTTGC-BHQ	NP-1 (2577-2601)	75 bp

Table 18: Properties of primers and probes for Influenza A (Inf A), Influenza B (Inf B), Parainfluenza 1-3 (Para 1-3), HMPV A, B (HM), Adenoviruses (Ade), Human Coronavirus 229 E, OC43, NL63(HCo), Respiratory Syncytial Virus (RSV), Rhinoviruses (Rhin), Enterovirus (Ent) and Bocaviruses (Boca). The primers that were included into the same reaction kit were: a) Influenza A and Influenza B b) Parainfluenza 1-3 c) RSVA, RSVB and HMPV A, d) HMPV B and adenoviruses, e) Human Coronavirus 229 E, OC43, NL63, f) Enterovirus and g) Rhinoviruses and Bocaviruses.

Visit	N	Variable	Label	N	Mean	Std Dev	Min.	Median	Max.
Visit 1	992	ALAT_val	ALAT [U/l]	982	18.55	15.92	5.00	16.00	285.00
		ASAT_val	ASAT [U/l]	983	34.80	15.70	8.00	33.00	335.00
		GGT_val	GGT [U/l]	959	12.87	6.40	3.00	12.00	79.00
Visit 2	992	ALAT_val	ALAT [U/l]	894	18.99	16.55	4.00	16.00	266.00
		ASAT_val	ASAT [U/l]	894	32.69	15.99	13.00	31.00	265.00
		GGT_val	GGT [U/l]	890	13.69	10.85	4.00	12.00	249.00

Table 19: Liver values and visits.

Elevated liver enzyme values	Premedication	1-5 years old	6-12 years old	12-17 years old	Total
Visit 1	No/Unclear	6.1%	14.0%	15.8%	10%
	Yes	12%	13.3%	18.1%	14%
	Total	8.7%	13.8%	16.5%	11.5%
Visit 2	No/Unclear	7%	11%	21.3%	10.4%
	Yes	9.5%	16.5%	23.3%	12.5%

Table 20: Overall rates of liver value elevation (elevated values of at least one of the three relevant liver enzymes (ASAT, ALAT, γ-GT) as assessed by the treating physician) and premedication according to age groups.

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10 Curriculum vitae
Lebenslauf

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01.02.2011-29.03.2016	Master of Science (M.Sc.) an der Universität Thessalia, Masterarbeit: „Adipositas und moderner Lebensstil. Vergleichende Studie von Kindern in zwei europäischen Ländern“. Abschlussnote: 9,19/10, „ausgezeichnet“

05.03.2012	Deutsche Approbation vom Hessischen Landesprüfung- und Untersuchungsamt
30.06.2011-30.03.2012	Besuch eines Sprachenzentrum für deutsche Sprache in Frankfurt
07.03.2006	Griechische Approbation
2004-2005	Prüfungen zur Erlangung der Anerkennung des Medizinischen Diploms (DOATAP), Abschlussnote: 7,12/10, „sehr gut“.
14.11.2003	Diplom des Medizinstudiums, Abschlussnote: 4,92/6, „sehr gut“
1997-2003	Studium der Humanmedizin an der Universität Plovdiv, Bulgarien
1996-1997	Teilnahme an Einführungs- Sprachkursen vor der Immatrikulation an der Medizinischen Fakultät Plovdiv, Bulgarien
1995-1996	Studium in der Computerschule LCPC in Thessaloniki
1987-1996	Besuch eines Sprachenzentrum für Englische Sprache in Thessaloniki, Griechenland

Sprachkenntisse

Griechisch	Muttersprache
Deutsch	Sehr gut in Wort und Schrift (Niveau C1)
Englisch	Perfekt in Wort und Schrift (Certificate of Proficiency in English. University of Cambridge. Niveau C2)
Bulgarisch	Perfekt in Wort und Schrift (Niveau C2)