Universitat Autònoma de Barcelona

THE ROLE OF THE POLYMERIZATION OF THE MUTATED ALPHA- 1 ANTITRYPSIN IN THE PATHOGENESIS OF LUNG AND LIVER DISEASE IN PATIENTS WITH ALPHA1-ANTITRYPSIN DEFICIENCY

Alexa Gabriela Núñez Dubon

Universitat Autònoma de Barcelona

Doctorate Program in Medicine

Department of Medicine

DOCTORAL THESIS

THE ROLE OF THE POLYMERIZATION OF THE MUTATED ALPHA- 1 ANTITRYPSIN IN THE PATHOGENESIS OF LUNG AND LIVER DISEASE IN PATIENTS WITH ALPHA1-ANTITRYPSIN DEFICIENCY

Author: Alexa Gabriela Núñez Dubon

Supervisors: Cristina Esquinas López and Marc Miravitlles Fernández

Tutor: Jaume Joan Ferrer Sancho

Barcelona, 2022

Dedicatoria

A mis padres, que siempre me han apoyado en todas mis decisiones. Gracias por su paciencia y dedicación para darme la mejor educación posible.

A mi hermano, por creer en mí y ser mi medio de transporte durante la carrera.

A Luis, quién ha sido el pilar fundamental en mis años de formación como especialista y ahora durante el doctorado.

A mis hijos, espero siempre darles el mejor ejemplo como persona y profesional.

A Marc, quién ha sido el mejor mentor con quién la vida me pudo haber llevado. Gracias por siempre estar, por tu ayuda y por siempre enseñarme algo nuevo.

A Cristina, quién ha sido el mejor apoyo tanto como persona como profesional. Te admiro mucho en todo lo que haces.

A todo el equipo DAAT, por formar parte de mi proceso de aprendizaje como investigadora pre-doctoral y por los buenos recuerdos a lo largo de estos años.

ABBREVIATIONS

AAT Alpha-1 antitrypsin

AATD Alpha-1 antitrypsin deficiency

ALP alkaline phosphatise

ALT alanine-aminotransferase

ANOVA One- way analysis of variance

APRI AST-to-platelet ratio index

AST aspartate-aminotransferase

BMI body mass index

CAP controlled attenuation parameter

COPD Chronic Obstructive Pulmonary Disease

CP circulating polymers

CRP C- reactive protein

CT computed tomography

EASL European Association for Study of the Liver

ELF enhanced liver fibrosis test

ER endoplasmic reticulum

FEV_1 Forced Expiratory Volume in the first second

FEV_1/FVC Forced Expiratory Volume in the first second/ Forced Vital Capacity

FIB-4 fibrosis-4 score

FVC Forced Vital Capacity

GGT gamma-glutamyl transferase

INR international normalized ratio

IQR interquartile range

KCO carbon monoxide transfer coefficient

kPa Kilopascals

LSM liver stiffness measurement

mAb monoclonal antibody

NAFLD non-alcoholic fatty liver disease

Pi protease inhibitor system

REDAAT Spanish AATD registry: Registro español de Déficit de alfa-1 antitripsina

SD standard deviation

TMB 3,3´, 5,5´- Tetramethylbenzidin

WHO World Health Organization

INDEX

SUMMARY

Alpha-1 antitrypsin deficiency is a genetic condition that is characterized by low circulating levels of the alpha-1 antitrypsin (AAT) protein and by the misfolding and polymerisation of the protein within hepatocytes. The polymerization of the mutated protein may be in relation with the pathogenesis of the liver and lung disease, therefore this thesis focuses in the polymerization of alpha1-antitripsin. Moreover, since in the past years there has been increasing interest in the screening of liver disease in patients with alpha-1 antitrypsin deficiency, this thesis also focuses in demonstrating the utility of transient liver elastography for the diagnosis of liver impairment.

RESUMEN

El deficit de alfa-1 antitripsina es una enfermedad genética rara que se caracteriza por presentar concentraciones séricas bajas de alfa-1 antitripsina y por el pliegue y polimerización de la proteína en los hepatocitos. La polimerización de la proteína mutada podría estar en relación tanto en la enfermedad hepática como pulmonar que causa la enfermedad. Por lo tanto, esta tesis se enfoca en el estudio del papel de la polimerización del alfa-1 antitripsina en la patogénesis de la enfermedad. Además en los últimos años, ha habido un incremento en el interés sobre el cribado de la enfermedad hepática causada por el déficit, por lo que parte de la tesis se enfocará en demostrar la utilidad de la elastografía hepática para el diagnóstico de la afectación hepática.

1. INTRODUCTION

1.1 Alpha- 1 antitrypsin deficiency

1.1.1 Definition

Alpha-1 antitrypsin deficiency (AATD) is a genetic condition that is characterised by low circulating levels of the alpha-1 antitrypsin (AAT) protein. AATD is an inherited codominant recessive condition and the gene is located in chromosome 14. The SERPINA1 gene is highly polymorphic with at least 120 mutations described, out of them 60 are deficient variants (1, 2). The group of variants is known as the Pi (protease inhibitor system). The normal allele present in 95% of healthy individuals is defined as Pi*M and the most common deficient variants are Pi*S and Pi*Z. Individuals with heterozygosis and homozygosis for the Z allele can present plasma levels of AAT of 50% and 10 to 15% of normal, respectively (3, 4).

In normal condition, the AAT is mostly produced and secreted by the hepatocytes, and in least amount by macrophages and monocytes. The main function of AAT is to protect lung tissue from damage caused by proteolytic enzymes such as neutrophil elastase.

In the presence of the Z allele, most of the AAT synthesized accumulates as inclusion bodies in the endoplasmic reticulum (ER) lumen of liver cells (5). The accumulation of this protein leads to apoptosis of the hepatocytes and a compensatory hepatocyte proliferation that progressively produces liver fibrosis evolving into cirrhosis or hepatocellular carcinoma. On the other hand, low concentrations of circulating AAT predispose to early onset panlobular emphysema, especially in individuals with a history of smoke exposure (6-8).

Therefore, clinically, AATD can be mainly manifested as emphysema and liver diseases: neonatal cholestasis, juvenile hepatitis, cirrhosis and carcinoma in children and adults, and less frequently as neutrophilic panniculitis and systemic vasculitis.

Emphysema secondary to AATD is the most common congenital life-threatening disease in adult life (figure 1).

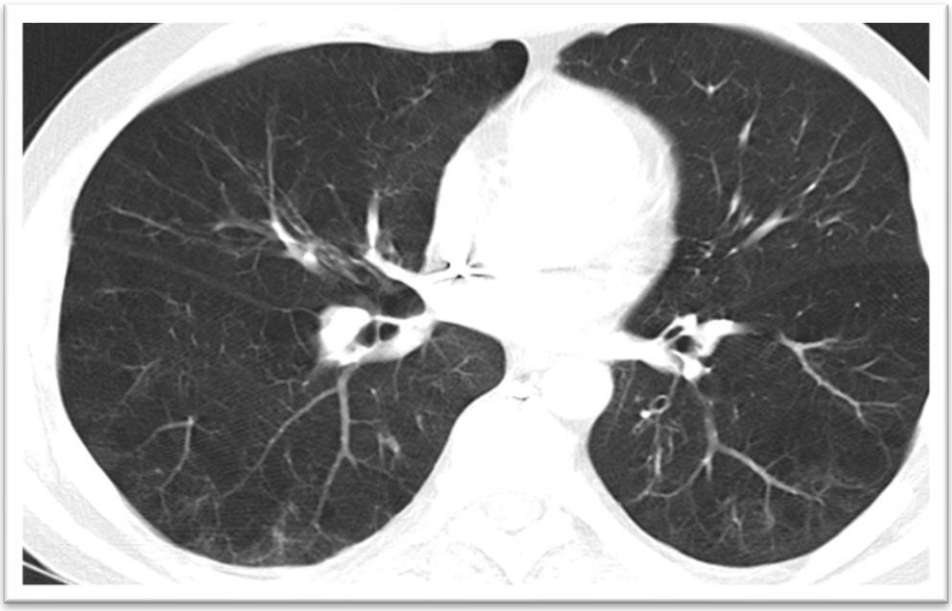

Figure 1. Chest computed tomography scan showing emphysema in a patient with AATD

1.1.2 Epidemiology

AATD is considered one of the most common genetic diseases in adults (9) with an estimated prevalence of 1 in 2000 to 3000 live births in Europe (10). Although more than 50 deficient alleles associated to AATD are known, in the clinical practice 96% of individuals with AATD present Pi*ZZ genotype and only 4% combinations of Z, S, rare and null variants (11, 12). The distribution of worldwide frequencies of Pi*Z is represented on figure 2.

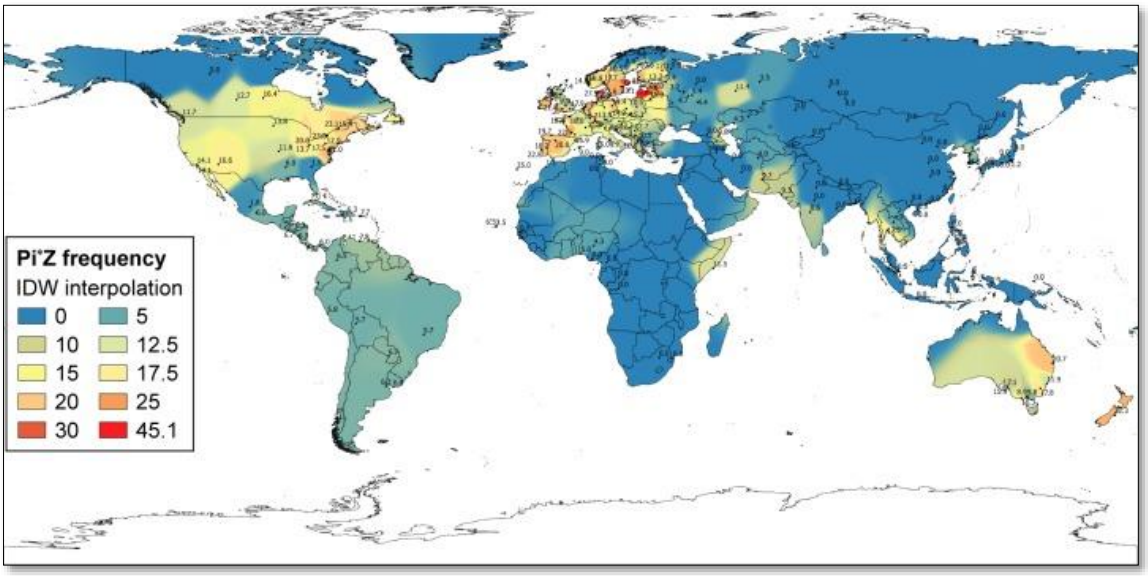

Figure 2. Distribution of worldwide frequencies of Pi*Z (3)

Among European countries there is a high variation in the prevalence of the Z allele, being more frequent in the Northwest of the continent while the S allele is more prevalent in the Iberian Peninsula. In Spain the prevalence of the S and Z alleles is 104 per 1000 inhabitants and 17 per 1000 inhabitants respectively. It is estimated that approximately 12 000 individuals present Pi*ZZ genotype and 145 000 Pi*SZ. As for Pi*MZ and Pi*SS, it is predicted almost one and a half million of individuals with these genotypes (13). Finally, regarding rare and null variants, in the Spanish Registry of AATD (REDAAT for the Spanish acronym: Registro español de deficit de alfa-1 antitripsina), they constitute the 4.5% of the registered cases (14).

1.1.3 Pathophysiology of AATD

1.1.3.1 Molecular and genetic basis of AATD

The AAT is a 52kDa circulating glycoprotein of medium size, water soluble, with a plasma half-life of 5 days. The molecule is encoded on chromosome 14q31 32.1, and is

composed of three β- sheets (A-C) and presents a mobile reactive loop. Within the loop, there are The P1-P1´residues of methionine serine and act as a pseudosubstrate for neutrophil elastase (15). After the cleavage between the enzyme and the P1-P1´ peptide, the proteinase is inactivated and posteriorly this conformation of AAT bound is recognised by hepatic receptors and cleared from the circulation.

Although this "bait" action performed by AAT is an effective inhibitor of serine proteases, it also constitutes the main point of mutations.

Regarding the Z mutation of AAT (Glu342Lys), it is produced at residue P17 at the head of the β- sheet and the base of the mobile reactive loop. The mutation opens the β-sheet A and favors the insertion of the reactive loop of a second AAT molecule to form a dimer (16-19). Posteriorly, this formation of dimers can extend to the formation of polymers within the endoplasmic reticulum of the hepatocytes and form inclusion bodies.

1.1.3.2 Polymerization and liver disease in AATD

In the presence of AATD, the RNA messenger transfers the genetic information to the ribosomes where the abnormal AAT is codified and posteriorly dimerises and polymerises (20). In extreme cases, these polymers can form inclusion bodies in the hepatocytes which activate cytoplasm and nucleus mechanisms and stimulate apoptosis and accelerated repair in response to cellular stress. This continued cellular stress conducts to liver fibrosis, cirrhosis and hepatocarcinoma.

In the Z mutation, 85-90% of the AAT suffers intracellular polymerisation in contrary to the S mutation where polymerization only occurs in the 60%. Moreover, the polymerization of the S protein is slower and can be degraded easier without forming

inclusion bodies. Homozygous individuals to the S allele do not develop liver disease due to the lack of polymer inclusions in their hepatocytes.

In Pi*SZ individuals, it has been described heteropolymers formed by S and Z proteins. For this reason, Pi*SZ individuals can develop liver pathology as severe as homozygous to the Z allele. Liver heteropolymers have also been described in individuals with Pi* IZ genotype and are considered to be associated to the development of cirrhosis (21).

Regarding the Pi*MZ genotype, hepatocytes present lower polymer accumulation and therefore are less likely to develop liver diseases (22).

1.1.3.3 Polymerization and emphysema in AATD

It is well known that tobacco exposure constitutes the main risk factor for the development of emphysema. However the mechanism of lung injury in AATD is not completely understood. It has been accepted that the retention of mutated AAT in the liver is the first step of injury. The polymerization of AAT within the hepatocytes induces the drop of AAT serum and tissue levels, which in combination with its reduced inhibitory capacity, generates an imbalance of protease-antiprotease in the lung. This disequilibrium allows an abnormal overexpression of neutrophil elastase which perpetuates a chronic inflammatory process and posteriorly induces irreversible destruction of pulmonary alveoli (22).

In the past years, it has been demonstrated that not only the hepatic polymerization of the mutated AAT is implicated in the pathophysiology of emphysema. Currently, it is considered that in order to the develop emphysema, the interaction of other proteinases than the neutrophil elastase is needed. However, the neutrophil elastase remains the main proteinase of the cascade and the regulator of the expression of other proteases (23).

Among other mechanisms of lung injury, it is known that the AAT loses its antiapoptotic capacity when mutated. In normal conditions, the AAT prevents the apoptosis of pulmonary endothelial cells through the inhibition of caspases and the reduction of oxidative stress. However, when mutated, the AAT losses its antiapoptotic capacity leading to high levels of oxidative stress and therefore contributing to the development of emphysema (24).

Moreover, it has been described the presence of polymers of mutated AAT within endothelial bronchial cells. These extracellular polymers act as chemotactic and stimulate human neutrophils which favour the inflammation of the airway and posteriorly cell apoptosis and tissue injury (25, 26). In addition, it has been demonstrated that cigarette smoke also increases the concentration of AAT polymers within alveolar lavage (27, 28).

1.1.3.4 Circulating polymers of AAT

Extracellular polymers of AAT have been described in lung lavage, in skin in patients with panniculitis and in the kidney in patients with vasculitis (29). Moreover, in the past years, it has been demonstrated the presence of polymers in serum samples of homozygous and heterozygous patients. These circulating polymers (CP) are formed within the ER and traffic through this organelle to posteriorly be secreted by the Golgi compartment (30) and become undetectable after liver transplantation (31).

In the study performed by Tan et al, they observed the presence of CP of AAT in serum samples of AATD Pi*ZZ patients and mixed phenotypes after secretion to the circulation from liver.

Although the presence of CP have been demonstrated, little is known about the association of the concentration of CP and the severity of the liver and lung disease in patients with AATD.

1.1.4 Diagnosis

1.1.4.1 Laboratory diagnosis

AATD is one of the most common hereditary diseases diagnosed in adulthood, however, it continues to be under diagnosed given to its varied form of clinical presentation and to the lack of knowledge of the disease by physicians (32).

According the World Health Organization (WHO), AATD should be ruled out in every patient with Chronic Obstructive Pulmonary Disease (COPD) (33).

The laboratory diagnosis is performed by a combination of quantitative measurement of AAT by nephelometry and phenotype characterisation by isoelectric focusing (34). In the past years, the molecular analysis of the AAT gene or genotype has been the reference standard for identifying rare variants associated with AATD (35). Recently, Belmonte et al. (36), have proposed a new diagnostic algorithm according to AAT serum concentrations (figure 3).

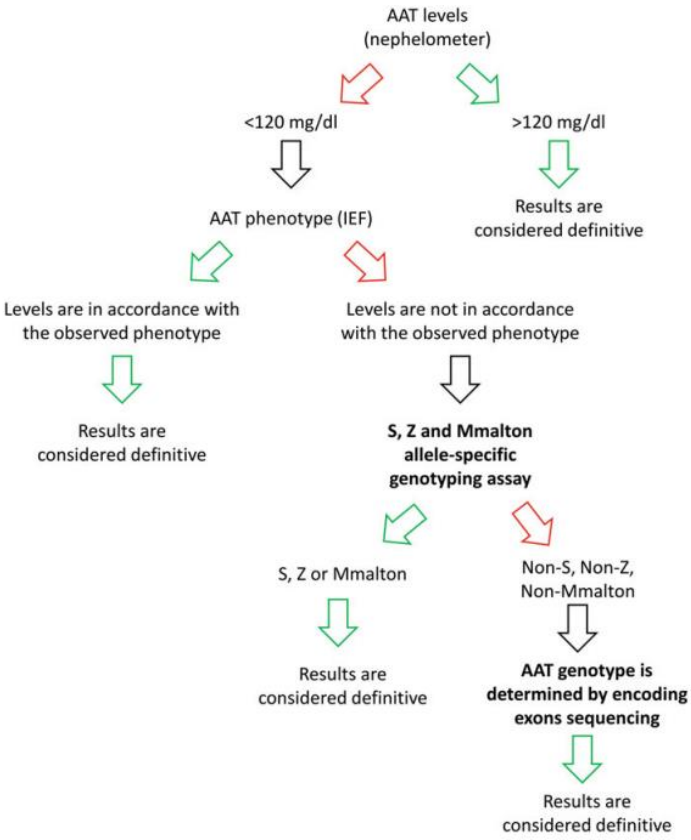

Figure 3. Belmonte et al proposed algorithm for the diagnosis of AATD (36)

1.1.4.2 Clinical diagnosis and follow-up

AATD predisposes to the development of different diseases along the patient´s life, however, the most common clinical manifestations are pulmonary and liver diseases: pulmonary emphysema and liver diseases such as neonatal cholestasis, juvenile hepatitis, liver cirrhosis in children and adults and hepatocarcinoma (37, 38).

The clinical manifestations may vary according the AATD phenotype. It is known that Pi*ZZ patients are more prone to develop liver and lung disease, followed by Pi*SZ individuals and rare variants (12).

1.1.4.2.1 AATD and lung disease

The clinical manifestations of lung disease can be variable among patients. Firstly, patients with AATD and lung disease were described as young individuals with early onset COPD with basal emphysema as the most common radiological finding. However, in the past years this heterogeneity has lead to the WHO and Scientific societies to recommend testing every patient with the diagnosis of COPD or adult-onset asthma (33, 39).

After performing the diagnosis of AATD, annual measurement of lung function post-bronchodilator Forced Expiratory Volumen in the first second (FEV_1) and gas transfer should be carried out in patients in order to detect disease progression. Imaging tests such as computerized thoracic (CT) scan as part of routine follow-up still requires further validation (39).

1.1.4.2.2 AATD and liver disease

Liver damage is then caused by this protein accumulation, inducing apoptosis of the hepatocytes and a compensatory hepatocyte proliferation that eventually produces liver fibrosis that can evolve to cirrhosis or hepatocellular carcinoma (40, 41). Most patients with liver disease are homozygous for the deficient Z allele (Pi*ZZ), although different degrees of liver involvement have been described in heterozygotes (Pi*SZ and Pi*MZ), especially if associated with other co-factors such as alcohol consumption or metabolic syndrome (42, 43).

Currently there is no non-invasive gold standard technique for the screening and early diagnosis of liver disease in patients with AATD (44). In clinical practice, liver

enzymes are routinely checked, while liver ultrasound is performed if necessary. However, it has been observed that transaminase levels have a low sensitivity to identify liver disease, and they correlate little with the degree of liver disease, especially in adulthood (45). Serum biomarkers and image devices based on elastography technique have been developed to overcome this problem and to assess the presence of fibrosis in liver diseases of different etiologies (46, 47).

Recently, there has been increasing interest in the use of elastographic methods, such as transient elastography, for screening liver disease in AATD patients (48, 49, 50). However, the screening and management of asymptomatic liver disease in AATD may differ among centers due to a lack of consensus or guidelines.

1.1.5 Treatment

Since 1987 a purified preparation of AAT from donor plasma is available for intravenous administration (51). The aim of this treatment is to raise AAT serum levels and in lung tissue in order to prevent the destruction of the lung and therefore to slow the progression of emphysema.

It has been demonstrated that augmentation therapy can achieve and maintain protective AAT levels in both blood and lung tissue (52). Moreover, large studies have shown that patients treated with augmentation therapy have a slower decline in FEV_1 and a reduction in mortality compared to those not receiving this treatment (52, 53). In addition, other studies have shown a reduction in lung density decline in treated patients when compared to those untreated (54, 55).

In the RAPID study, patients with AATD were recruited and randomized to receive either augmentation therapy or placebo and followed for over 2 years by CT

densitometry. This study showed that augmentation therapy was effective in reducing loss of lung tissue. Moreover, patients that received placebo during two years, agreed to receive augmentation therapy for the next two years, and once again a reduction in the rate of decline in lung density was also observed. However, the initial loss of long tissue during the two years of placebo was not recovered (56).

2. HYPOTHESIS

The polymerization of mutated AAT is involved in the pathogenesis of the lung and liver disease, therefore the CP of AAT could be helpful as a biomarker of the severity of the damage produced.

The utilization of transient liver elastography could be useful for the diagnosis of liver disease of patients with AATD.

3. OBJECTIVES

3.1 Main objective

To investigate the role of the polymerization of the mutated AAT in the pathogenesis of liver and lung disease.

3.2 Secondary objectives

- To determine the concentrations of CP of AAT in individuals with different AATD genotypes.

- To investigate the association between CP of AAT and the severity of lung disease in patients with AATD homozygous and heterozygous for the Z allele.

- To describe the association between CP of AAT and the severity of liver disease defined by transient elastography in patients with AATD homozygous and heterozygous for the Z allele.

- To evaluate the utility of transient elastography for the identification of liver disease in individuals with AATD and its association with biomarkers of liver function.

4. COMPENDIUM OF PUBLICATIONS

4.1 Article 1 :

Núñez A, Belmonte I, Miranda E, Barrecheguren M, Farago G, Loeb E, Pons M, Rodríguez-Frías F, Gabriel-Medina P, Rodríguez E, Genescà J, Miravitlles M, Esquinas C. Association between circulating alpha-1 antitrypsin polymers and lung and liver disease. Respir Res. 2021 Sep 15;22(1):244. doi: 10.1186/s12931-021-01842-5. Erratum in: Respir Res. 2021 Nov 1;22(1):283.

Núñez et al. Respir Res (2021) 22:244
https://doi.org/10.1186/s12931-021-01842-5

Respiratory Research

RESEARCH Open Access

Association between circulating alpha-1 antitrypsin polymers and lung and liver disease

Alexa Núñez[1,2†], Irene Belmonte[1†], Elena Miranda[3], Miriam Barrecheguren[1], Georgina Farago[1], Eduardo Loeb[4], Mònica Pons[5], Francisco Rodríguez-Frías[2,6,7,8], Pablo Gabriel-Medina[6], Esther Rodríguez[1], Joan Genescà[2,5,7], Marc Miravitlles[1,2,9*] and Cristina Esquinas[1,2]

Abstract

Background: Alpha-1 antitrypsin deficiency (AATD) is considered one of the most common genetic diseases and is characterised by the misfolding and polymerisation of the alpha-1 antitrypsin (AAT) protein within hepatocytes. The relevance of circulating polymers (CP) of AAT in the pathogenesis of lung and liver disease is not completely understood. Therefore, the main objective of our study was to determine whether there is an association between the levels of CP of AAT and the severity of lung and liver disease.

Method: This was a cross-sectional study in patients with different phenotypes of AATD and controls. To quantify CP, a sandwich ELISA was performed using the 2C1 monoclonal antibody against AAT polymers. Sociodemographic data, clinical characteristics, and liver and lung parameters were collected.

Results: A cohort of 70 patients was recruited: 32 Pi*ZZ (11 on augmentation therapy); 29 Z-heterozygous; 9 with other genotypes. CP were compared with a control group of 47 individuals (35 Pi*MM and 12 Pi*MS). ZZ patients had the highest concentrations of CP ($p < 0.001$) followed by Z heterozygous. The control group and patients with Pi*SS and Pi*SI had the lowest CP concentrations. Pi*ZZ also had higher levels of liver stiffness measurements (LSM) than the remaining AATD patients. Among patients with one or two Z alleles, two patients with lung and liver impairment showed the highest concentrations of CP (47.5 µg/mL), followed by those with only liver abnormality ($n = 6$, CP = 34 µg/mL), only lung ($n = 18$, CP = 26.5 µg/mL) and no abnormalities ($n = 23$, CP = 14.3 µg/mL). Differences were highly significant ($p = 0.004$).

Conclusions: Non-augmented Pi*ZZ and Z-patients with impaired lung function and increased liver stiffness presented higher levels of CP than other clinical phenotypes. Therefore, CP may help to identify patients more at risk of developing lung and liver disease and may provide some insight into the mechanisms of disease.

Keywords: Alpha-1 antitrypsin deficiency, Circulating polymers, Emphysema, Liver disease

Background

Alpha-1 antitrypsin deficiency (AATD) is considered one of the most common genetic disorders in adults [1], with a prevalence of 1 in 2000 to 3000 live births in Europe [2]. AATD is characterised by low circulating levels of the alpha-1 antitrypsin (AAT) protein caused by specific mutations in the *SERPINA1* gene resulting in a misfolded protein and intracellular liver polymerization. *SERPINA1* is highly polymorphic with at least 120 mutations having

*Correspondence: marcm@separ.es
†Alexa Núñez and Irene Belmonte contributed equally and should be considered first authors
[1] Pneumology Department, Hospital Universitari Vall d'Hebron, Vall d'Hebron Institut de Recerca (VHIR), Vall d'Hebron Barcelona Hospital Campus, P. Vall d'Hebron 119-129, 08035 Barcelona, Spain
Full list of author information is available at the end of the article

been described, and of these, 60 are deficient variants [3, 4]. The normal allele present in 95% of healthy individuals is defined as the M allele, and the most common deficient variants are S and Z [5, 6]. The alleles of *SERPINA1* are codominant, therefore, individuals heterozygous and homozygous for the Z allele have AAT plasma concentrations of 50% and 10 to 15% of normal, respectively.

In normal conditions, the protein is mainly synthesised and secreted by hepatocytes and its main function is to protect lung tissue from damage caused by proteolytic enzymes such as neutrophil elastase. In the presence of the Z allele, most of the AAT synthesised polymerises and accumulates in the lumen of the endoplasmic reticulum (ER) of liver cells as inclusion bodies. These inclusions are associated with neonatal hepatitis, cirrhosis and hepatocellular carcinoma [7]. In addition, lower concentrations of circulating AAT predispose to early onset panlobular emphysema in individuals with smoking history [8–10].

Polymers of AAT have also been identified in the bronchoalveolar lavage fluid and alveolar walls of carriers of the Z allele [11]. In a study by Alam et al. [12], it was observed that cigarette smoking accelerated polymerisation of AAT in patients homozygous for the Z allele, leading to a greater depletion of the protection against neutrophil elastase in the lung. Later, other authors found that these circulating polymers (CP) of AAT were present in serum samples of AATD Pi*ZZ and mixed phenotypes patients, probably due to secretion to the circulation from liver cells [13, 14].

However, little is known about the role of CP of AAT in the pathogenesis of disease in patients with AATD. Therefore, the aim of our study was to determine CP concentrations in individuals with different genotypes of AAT and to investigate the association between CP and the severity of lung and liver disease in patients with AATD homozygous and heterozygous for the Z allele.

Methods

This was a cross-sectional study performed in the Vall d'Hebron Hospital Campus (Barcelona, Spain), which is a reference centre for AATD [15]. Patients with moderate and severe deficiency (genotypes Pi*SS, MZ, SZ, ZZ and rare variants) were consecutively included from the AATD outpatient clinic of the Pneumology Department between January and December 2019. A control group of adults, older than 18 years with Pi*MM and Pi*MS genotypes were also consecutively recruited during the same period among those attending routine medical check-ups in the Preventive Medicine outpatient clinic in our centre.

The study was carried out according to the principles of the Declaration of Helsinki and the prevailing norms for performing investigation in humans. Data confidentiality was ensured according to the Law of Data Protection 2016/679. The study was approved by the Ethical Committee and Clinical Investigation of the Vall d'Hebron University Hospital (Barcelona, Spain) number PR (AG) 156/2016, and all the participants provided written informed consent.

Variables

Sociodemographic data and clinical characteristics were collected from all patients. Comorbidities were registered according to the Charlson comorbidity index [16]. Patients performed spirometry and values for forced expiratory volume in the 1st second (FEV_1), forced vital capacity (FVC) and the FEV_1/FVC ratio were registered. Chronic obstructive pulmonary disease (COPD) was diagnosed when the post-bronchodilator FEV_1/FVC ratio was below 0.7.

Liver stiffness measurement (LSM) was performed using transient elastography (Fibroscan 502 Touch, Echosens, Paris, France) in a fasting state according to the usual standard procedure [17]. Quality criteria were at least 10 valid measurements and an interquartile to median ratio $\leq$30%. Only valid assessments were considered for the analysis. Data were expressed in kilopascals (kPa). Normal LSM values vary between 4–6 kPa. LSM $\geq$ 6 kPa were considered abnormal and suggestive of liver disease/mild fibrosis.

Laboratory testing

Biochemical tests included determination of liver enzymes: aspartate-aminotransferase (AST), alanine-aminotransferase (ALT), gamma-glutamyl transferase and alkaline phosphatase. In addition, two fibrosis biomarkers were assessed: the fibrosis-4 (FIB-4) score and the enhanced liver fibrosis (ELF) test. The FIB-4 score was calculated as age (years) $\times$ AST [IU/L]/(platelet count [10^9/L] $\times \sqrt{}$ALT [IU/L]). The ELF test (Siemens Healthcare Diagnostics, Vienna, Austria) consists of three components: type III procollagen peptide, hyaluronic acid and tissue inhibitor of metalloproteinase-1 and is a marker of liver fibrosis [18]. In addition, fibrinogen and C-reactive protein (CRP) were determined as markers of systemic inflammation.

AAT blood levels and genotyping

Quantitative measurement of AAT levels was determined by immune nephelometry and genotyping was performed using real-time polymerase chain reaction or sequencing the entire encoding region of the *SERPINA1* gene as previously described [15].

Circulating polymers of AAT

To quantify CP, a sandwich ELISA with plasma samples was performed using the 2C1 monoclonal antibody (mAb) against AAT polymers [19]. Plates were coated overnight at room temperature with 50 µL/well of purified 2C1 mAb at 2 µg/mL. The next day, the plates were washed and incubated with 300 µL/well of blocking solution for 1 h. Standards and samples were diluted in blocking buffer, added to the plate and incubated for 2 h at room temperature. Bound polymers were detected with anti-total AAT 3C11 mAb labelled with horseradish peroxidase and incubated for 75 min, and its activity was subsequently measured in a plate reader at 450 nm using a 3,3′,5,5′-tetramethylbenzidine (TMB) substrate solution. CP (µg/mL) concentrations were determined by interpolation of absorbance values on the standard curve [20]. Monoclonal antibody 2C1 recognizes the pathological polymers formed by AAT, however, in samples with elevated AAT concentrations and the absence of polymers, a minimal amount of monomeric AAT binds to mAb 2C1 with low affinity, showing a weak background signal. In order to reduce this noise, the proportion of polymers versus the total levels of AAT (%) was determined in all samples together with total polymer concentrations (µg/mL).

Statistical analysis

Qualitative variables were described with absolute frequencies and percentages. The description of quantitative variables was performed using the mean, standard deviation (SD), median and quartiles. The Kolmogorov–Smirnov test was used to assess the normality of distributions.

The sociodemographic, clinical characteristics and CP levels (µg/mL and %) were compared according to the genotypes. In the case of quantitative variables, ANOVA tests were carried out with Bonferroni correction for multiple comparisons. The Chi-squared test (Fisher test for frequencies < 5) was used for the comparison of categorical variables. Linear relationships between clinical variables and levels of CP were also analysed using the Pearson correlation coefficient.

For all the tests, p-values < 0.05 were considered statistically significant. The statistical package R Studio (V2.5.1) was used for the analyses.

Results

Characteristics of participants

A total of 70 patients with different AAT genotypes were included. Among them, 32 (46%) were homozygous Pi*ZZ, of whom 11 were on augmentation therapy; 29 (41%) were heterozygous for the Z allele (13 Pi*MZ, 13 Pi*SZ, 1 Pi*MmaltonZ, 1 Pi*PLowelZ, 1 Pi*FZ); 4 (6%) carriers of the S allele (3 Pi*SS, 1 Pi*SI); and 5 (7%) rare variants (1 Pi*MMmattawa, 2 Pi*MMmalton, 1 Pi*SMmalton and 1 Pi*MMvall d'Hebron) (Table 1). The control group consisted of 47 individuals with a mean age of 46 years (SD = 14.1) and 17 (36.2%) were male; 35 had a normal genotype Pi*MM and 12 had a Pi*MS genotype.

Sociodemographic and clinical characteristics of patients according to the AATD genotype

Patients were divided into three groups according to their AATD genotype: (1) homozygous Pi*ZZ, (2) heterozygous for the Z allele, (3) others: Pi*SS, Pi*SI, Pi*MMmattawa, Pi*MMmalton, Pi*SMmalton and Pi*MMvall d'Hebron.

No differences were observed between groups in terms of age, body mass index or sex distribution (Table 2).

Homozygous Pi*ZZ patients had a lower FVC% (p = 0.014), a lower FEV_1% (p = 0.07) and higher percentage of COPD (p = 0.008) than the other genotypes. Moreover, Pi*ZZ individuals showed higher LSM (p = 0.018) and ELF levels (p = 0.004) compared to the remaining patients.

Regarding laboratory findings, as expected, Pi*ZZ patients presented lower AAT levels (p < 0.001). No significant differences were observed for leukocytes, platelets, liver enzymes, CRP, FIB-4 or fibrinogen concentrations among groups (Table 2).

Circulating polymer concentrations in the different AATD genotypes

As a group, the Pi*ZZ patients presented higher concentrations of CP than heterozygous patients and those with other genotypes (Table 2).

Table 1 Description of AAT variants identified in the study population

Variant	Codon change	Classification	Deficiency
M	–	Normal	Not deficient
Z	Glu342Lys	Deficient	Severe
S	Glu264Val	Deficient	Moderate
F	Arg223Cys	Deficient	Not deficient Reduced inhibitory activity
I	Arg39Cys	Deficient	Moderate
Mmalton	Phe52del (M_2)	Deficient	Severe
Mvall d'hebron	Pro369Ser	Deficient	Moderate
Plowell	Asp256Val (M_3)	Deficient	Severe
Q_0mattawa	Leu353Phefs*24 (M_{1Val})	Null	Severe

AAT alpha-1 antitrypsin, *AATD* alpha-1 antitrypsin deficiency

Table 2 Characteristics of the 70 patients included in the study

Variables	ZZ (n = 32)	Z- (n = 29)	Other (n = 9)	p value
Age, years	54.6 (15.4)	50.1 (13.5)	47 (17.6)	0.235
Sex, male (%)	20 (62.5)	16 (55.2)	3 (33.3)	0.297
BMI (kg/m^2)	24.1 (3.2)	24.7 (3.5)	24.6 (4.2)	0.683
Smokers (%)	0	6 (20.7)	1 (11.1)	0.120
Ex-smokers (%)	18 (58.1)	14 (48.3)	4 (44.4)	
Never smokers (%)	13 (41.9)	9 (31)	4 (44.4)	
Tobacco exposure (pack-years)	22.7 (14.6)	32.4 (34.1)	34 (16.7)	0.360
COPD (%)	21 (65.6)	9 (31)	2 (22)	0.008
Charlson Index	2.2 (1.6)	1.5 (1.3)	1.5 (1.9)	0.122
FVC (%)	83 (27)	100 (19)	104 (15)	0.014
FEV_1 (%)	69 (32)	92 (31.3)	99 (15)	0.007
FEV_1/FVC	63 (16)	71 (17)	76 (4.8)	0.028
LSM (kPa)	5.3 (1.2)	4.5 (1.2)	4.4 (0.9)	0.008
FIB-4	1.5 (0.9)	1.1 (0.4)	1.1 (0.6)	0.115
Augmentation therapy (%)	11 (34)	0	0	< 0.001
Haemoglobin (g/dL)	15.4 (1.4)	14.4 (1.2)	14.2 (1.2)	0.008
Leukocytes (× 10^9/L)	7.6 (2.7)	7.6 (2.8)	7.1 (1.5)	0.922
Platelets (× 10^9/L)	242 (71)	245 (54)	282 (51)	0.163
AST (IU/L)	29.9 (12.2)	24.4 (7.2)	29.6 (18.9)	0.129
ALT (IU/L)	30.4 (18.1)	25.8 (14.1)	24.9 (13.7)	0.392
ALP (IU/L)	81.6 (25.2)	78.6 (29.2)	80.5 (21.5)	0.819
GGT (U/L)	32.9 (16.1)	37.5 (51.5)	41.8 (18.8)	0.234
Proteins (g/dL)	7.1 (0.5)	7.3 (0.3)	7.2 (0.5)	0.489
AAT (mg/dL)	40 (36)	74 (25)	82 (22)	< 0.001
CRP (mg/L)	0.2 (0.2)	0.3 (0.6)	0.2 (0.2)	0.261
Fibrinogen (g/L)	3.7 (0.5)	3.8 (0.8)	3.9 (0.7)	0.353
ELF	8.7 (0.9)	8.1 (0.7)	8.6 (0.8)	0.004
AAT polymers (µg/mL)	37.4 (16.4)	13.5 (8)	2.3 (2.9)	< 0.001
AAT polymers, %	12.8 (7.2)	2.2 (2)	0.3 (0.5)	< 0.001

Values are mean (standard deviation) unless otherwise specified. *Patient group ZZ*: homozygous patients to the Z allele; -Z: heterozygous patients to the Z allele (Pi*MZ, Pi*SZ, Pi*MmaltonZ, Pi*PlowelZ, Pi*FZ); other: Pi*SS, Pi*SI, Pi*MMmattawa, Pi*MMmalton, Pi*SMmalton and Pi*MMvall d'hebron)

BMI body mass index, *COPD* Chronic obstructive pulmonary disease, *FVC* forced vital capacity, FEV_1 forced expiratory volume in the first second, *FIB-4* fibrosis-4 score, *LSM* liver stiffness measurement, *FIB-4* fibrosis-4 score, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *ALP* alkaline phosphatase, *GGT* gamma-glutamyl transferase, *AAT* alpha-1 antitrypsin, *CRP* C-reactive protein, *ELF* enhanced liver fibrosis test

Considering the different genotypes individually, the highest values were observed in augmented Pi*ZZ patients (42.9 µg/mL (SD = 16) and one Pi*FZ with 42.1 µg/mL, very close to the 34.5 µg/mL (SD = 16.2) obtained in untreated Pi*ZZ patients. The lowest values were observed in controls (1.04 µg/mL (SD = 1.73) for Pi*MM and 0.9 µg/mL (SD = 1.7) for Pi*MS) and in patients with the Pi*SS and Pi*SI genotypes. Patients heterozygous for the Z allele and other rare variants had intermediate values (Table 3 and Fig. 1). The distribution of CP in percentage followed a similar distribution among genotypes (Table 3).

Correlation between circulating polymers and parameters of lung and liver impairment in untreated Pi*ZZ and Z-heterozygous patients

In order to determine the possible relationship between CP concentrations and lung and liver alterations, we selected homozygous or heterozygous patients carrying the Z allele, excluding those on augmentation therapy. A negative, significant and weak linear relationship was found between CP concentrations and parameters of airflow obstruction; FEV_1/FVC $r = -0.32$, $p = 0.026$ and FEV_1 (%) $r = -0.31$, $p = 0.029$ (Fig. 2). Similarly, a positive and weak linear relationship was found between CP and LSM and ELF ($r = 0.39$ $p = 0.005$ and $r = 0.38$ $p = 0.007$, respectively) (Fig. 3). In contrast,

35. Kim SU, Choi GH, Han WK, Kim BK, Park JY, Kim DY, et al. What are 'true normal' liver stiffness values using FibroScan?: a prospective study in healthy living liver and kidney donors in South Korea. Liver Int. 2010;30:268–74.
36. Roulot D, Czernichow S, Le Clesiau H, Costes JL, Vergnaud AC, Beaugrand M. Liver stiffness values in apparently healthy subjects: influence of gender and metabolic syndrome. J Hepatol. 2008;48:606–13.
37. Barrecheguren M, Torres-Duran M, Casas-Maldonado F, Miravitlles M. Spanish implementation of the new international alpha-1 antitrypsin deficiency international registry: The European Alpha-1 Research Collaboration (EARCO). Arch Bronconeumol. 2021;57(2):81–2.
38. Miravitlles M, Nuñez A, Torres-Durán M, Casas-Maldonado F, Rodriguez-Hermosa JL, López-Campos JL, et al. The importance of reference centers and registries for rare diseases: the example of alpha-1 antitrypsin deficiency. COPD. 2020;17(4):346–54.

Publisher's Note

4.2 Article 2:

Pons M, Núñez A, Esquinas C, Torres-Durán M, Rodríguez-Hermosa JL, Calle M, Tubio-Pérez R, Belmonte I, Rodríguez-Frías F, Rodríguez E, Genescà J, Miravitlles M, Barrecheguren M. Utility of Transient Elastography for the Screening of Liver Disease in Patients with Alpha1-Antitrypsin Deficiency. J Clin Med. 2021 Apr 16;10(8):1724. doi: 10.3390/jcm10081724. PMID: 33923569; PMCID: PMC8073267

Journal of
Clinical Medicine

MDPI

Article

Utility of Transient Elastography for the Screening of Liver Disease in Patients with Alpha1-Antitrypsin Deficiency

Mònica Pons [1,†], Alexa Núñez [2,3,†], Cristina Esquinas [2,3], Maria Torres-Durán [4], Juan Luis Rodriguez-Hermosa [5], Myriam Calle [5], Ramón Tubio-Pérez [4], Irene Belmonte [2], Francisco Rodriguez-Frias [6,7], Esther Rodriguez [2], Joan Genescà [1,7], Marc Miravitlles [2,3,8,*] and Miriam Barrecheguren [2]

1 Liver Unit, Department of Internal Medicine, Hospital Universitari Vall d'Hebron, Vall d'Hebron Institut de Recerca (VHIR), Vall d'Hebron Barcelona Hospital Campus, 08035 Barcelona, Spain; monica_xina@hotmail.com (M.P.); jgenesca@vhebron.net (J.G.)
2 Pneumology Department, Hospital Universitari Vall d'Hebron/Vall d'Hebron Institut de Recerca (VHIR), Vall d'Hebron Barcelona Hospital Campus, 08035 Barcelona, Spain; alexagnd01@hotmail.com (A.N.); crise4@hotmail.com (C.E.); irbelmu@gmail.com (I.B.); estherod@vhebron.net (E.R.); mbarrech@vhebron.net (M.B.)
3 Department of Medicine, Universitat Autònoma de Barcelona, 08193 Barcelona, Spain
4 Pneumology Department, University Hospital Complex of Vigo, Instituto de Investigación Biomédica Galicia Sur, 36213 Vigo, Spain; mtordur@yahoo.es (M.T.-D.); ramon.antonio.tubio.perez@sergas.es (R.T.-P.)
5 Pneumology Department, Instituto de Investigación Sanitaria del Hospital Clínico San Carlos (IdISSC), Hospital Clínico de San Carlos, Departamento de Medicina, Facultad de Medicina, Universidad Complutense de Madrid, 28040 Madrid, Spain; jlrhermosa@yahoo.es (J.L.R.-H.); mcallerubio@gmail.com (M.C.)
6 Department of Clinical Biochemistry, Hospital Universitari Vall d'Hebron, Vall d'Hebron Barcelona Hospital Campus, 08035 Barcelona, Spain; frarodri@gmail.com
7 Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBEREHD), 28029 Madrid, Spain
8 Centro de Investigación Biomédica en Red de Enfermedades Respiratorias (CIBERES), 28029 Madrid, Spain
* Correspondence: marcm@separ.es
† Both authors have contributed equally and should be considered first authors.

check for updates

Citation: Pons, M.; Núñez, A.; Esquinas, C.; Torres-Durán, M.; Rodriguez-Hermosa, J.L.; Calle, M.; Tubio-Pérez, R.; Belmonte, I.; Rodriguez-Frías, F.; Rodriguez, E.; et al. Utility of Transient Elastography for the Screening of Liver Disease in Patients with Alpha1-Antitrypsin Deficiency. *J. Clin. Med.* **2021**, *10*, 1724. https://doi.org/10.3390/jcm10081724

Academic Editor: Francisco Dasí

Received: 5 March 2021
Accepted: 13 April 2021
Published: 16 April 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract: Screening of liver disease in alpha-1 antitrypsin deficiency (AATD) is usually carried out with liver enzymes, with low sensitivity. We conducted a multicenter cross-sectional study aiming to describe the utility of transient elastography for the identification of liver disease in patients with AATD. A total of 148 AATD patients were included. Among these, 54.7% were Pi*ZZ and 45.3% were heterozygous for the Z allele. Between 4.9% and 16.5% of patients had abnormal liver enzymes, without differences among genotypes. Liver stiffness measurement (LSM) was significantly higher in Pi*ZZ individuals than in heterozygous Z (5.6 vs. 4.6 kPa; $p = 0.001$). In total, in 8 (5%) individuals LSM was >7.5 kPa, considered significant liver fibrosis, and ≥10 kPa in 3 (1.9%) all being Pi*ZZ. Elevated liver enzymes were more frequently observed in patients with LSM > 7.5 kPa, but in 5 out of 8 of these patients all liver enzymes were within normal range. In patients with AATD, the presence of abnormal liver enzymes is frequent; however, most of these patients do not present significant liver fibrosis. Transient elastography can help to identify patients with liver fibrosis even with normal liver enzymes and should be performed in all Z-allele carriers to screen for liver disease.

Keywords: alpha1-antitrypsin deficiency; liver disease; transient elastography

1. Introduction

Alpha1-antitrypsin deficiency (AATD) is caused by a specific mutation of the SERPINA 1 gene which results in abnormal production and low circulating levels of alpha1-antitrypsin (AAT). It is one of the most common genetic diseases in adulthood and is associated with an increased risk of developing pulmonary emphysema and liver disease [1,2].

AAT is a protein synthesized and secreted mainly by hepatocytes, the main function of which is to protect lung tissue from damage caused by proteolytic enzymes such as neutrophil elastase [2]. AAT is a highly polymorphic protein with more than 120 variants, including about 60 deficient alleles. The normal allele, present in more than 95% of normal subjects, is called M [1,2]. The most frequent deficient alleles are S and Z, and they are found in 10% and 2% of the Spanish population, respectively [3–6].

The Z variant presents an alteration in its tertiary structure that facilitates misfolding of the protein and gives rise to the spontaneous formation of polymers, leading to the accumulation of the protein in the endoplasmic reticulum of the hepatocytes [7,8]. Liver damage is then caused by this protein accumulation, inducing apoptosis of the hepatocytes and a compensatory hepatocyte proliferation that eventually produces liver fibrosis that can evolve to cirrhosis or hepatocellular carcinoma [8,9]. Most patients with liver disease are homozygous for the deficient Z allele (Pi*ZZ), although different degrees of liver involvement have been described in heterozygotes (Pi*SZ and Pi*MZ), especially if associated with other co-factors such as alcohol consumption or metabolic syndrome [10,11].

Currently there is no non-invasive gold standard technique for the screening and early diagnosis of liver disease in patients with AATD [12]. In clinical practice, liver enzymes are routinely checked, while liver ultrasound is performed if necessary. However, it has been observed that transaminase levels have a low sensitivity to identify liver disease, and they correlate little with the degree of liver disease, especially in adulthood [13]. Serum biomarkers and image devices based on elastography technique have been developed to overcome this problem and to assess the presence of fibrosis in liver diseases of different etiologies [14,15].

Recently, there has been increasing interest in the use of elastographic methods, such as transient elastography, for screening liver disease in AATD patients [16–18]. However, the screening and management of asymptomatic liver disease in AATD can differ among centers due to a lack of consensus or guidelines. Therefore, the aim of our study was to describe the utility of transient elastography for the identification of liver disease in patients with AATD.

2. Materials and Methods

This was a multicenter cross-sectional study including patients older than 18 years with mild, moderate, and severe AATD (Pi*MS, SS, MZ, SZ, ZZ, and rare variants) consecutively recruited from the outpatient Pneumology Clinics of three AATD reference centers in Spain (Vall d'Hebron University Hospital, Barcelona, University Hospital Complex of Vigo, and Hospital Clinico San Carlos, Madrid) from 1 April 2017 to 1 January 2020. As part of the assessment of patients with AATD, all of them were offered blood analysis, full lung function tests, and transient elastography, and the only exclusion criterion was to refuse to sign informed consent. The study was approved by the Vall d'He- bron Hospital Ethics Committee (Barcelona, Spain), number PR(AG)335/2016, and all patients provided written informed consent.

2.1. Variables

During the first visit, a complete physical examination was performed in all patients with special interest in signs of chronic liver disease such as splenomegaly, jaundice, or palmar erythema. Sociodemographic and clinical characteristics were collected and other parameters such as body mass index (BMI), lung function tests (forced expiratory volume in the first second (FEV1), FEV1/forced ventilatory capacity (FVC), and carbon monoxide transfer coefficient (KCO)), comorbidities, treatments, and AAT augmentation therapy were reported. Diagnosis of chronic obstructive pulmonary disease (COPD) was established when the post-bronchodilator FEV1/FVC ratio was below 0.7.

Blood samples were obtained for determination of liver function tests: Aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), international normalized ratio (INR), platelet count,

and albumin. In addition, the Fibrosis-4 (FIB-4) score was calculated as age (years) × AST [IU/L]/(platelet count [109/L] × √ALT [IU/L]) and AST-to-platelet ratio index (APRI) as (AST [IU/L]/40 IU/L)/platelet count [109/L] × 100. Patients were classified according to the previously established FIB-4 cut-offs of <1.45 with a high negative predictive value for ruling out advanced fibrosis and >3.25 with a high specificity and a 65% positive predictive value for ruling in advanced fibrosis [19]. For APRI, we used the cut-off <0.5 for excluding cirrhosis (high negative predictive value) and >1.0 as a high specific cut-off for predicting cirrhosis [20].

The Enhanced Liver Fibrosis (ELF) test (Siemens Healthcare Diagnostics, Vienna, Austria) was available as a biomarker of liver fibrosis in one of the centers. The ELF test is a panel of markers that consists of 3 components: Type III procollagen peptide, hyaluronic acid, and tissue inhibitor of metalloproteinase-1. We explored the manufacturer-recommended 9.8 cut-off to rule in advanced fibrosis [21].

2.2. Liver Stiffness Measurement by Transient Elastography

Liver stiffness measurements (LSM) were performed in a fasting state using a Fibroscan 502 Touch (Echosens, Paris, France) using the M or XL probe as per device indication. Quality criteria used in all centers were at least 10 valid measurements and an interquartile-to-median ratio ≤ 30%. The LSM technique was carried out in accordance with the European Association for Study of the Liver (EASL) clinical guidelines [22].

Results were expressed in kilopascals (kPa). Normal liver stiffness values are around 5 kPa. Transient elastography has good re-producibility and has good diagnostic performance for estimating liver fibrosis. However, the accuracy is not as good for detecting significant fibrosis compared to advanced fibrosis or cirrhosis [22,23]. Since there are no specific LSM cut-offs for AATD liver disease, a LSM > 7.5 kPa was used as suggestive of significant fibrosis and ≥10 kPa was suggestive of advanced fibrosis according to previously established cut-offs in other liver diseases (mainly viral etiologies and alcoholic liver disease) [22,24].

The presence of steatosis was assessed by the controlled attenuation parameter (CAP) and results were expressed in decibel per meter (dB/m). The cut-off >268 dB/m was used as an indicator of moderate steatosis, and for severe steatosis the cut-off was >280 dB/m [25].

2.3. Statistical Analysis

Qualitative variables were described with absolute frequencies and percentages. The description of quantitative variables was performed using the mean, standard deviation (SD) or median, and interquartile range (IQR). The Kolmogorov–Smirnov test was used to assess the normality of distributions.

Patient characteristics were compared according to genotypes and other clinical conditions. In the case of quantitative variables, the Student's *t*-test for normally distributed variables or the Mann–Whitney U-test if normality was not assumed was used, while ANOVA tests were performed in the case of variables with more than 2 categories. The Chi-squared test (Fisher test for frequencies < 5) was used for the comparison of categorical variables. A linear relationship between quantitative variables, in particular between surrogates of liver disease (LSM, CAP and FIB-4) and spirometric markers of airflow obstruction (FEV1(%) and FEV1/FVC), were analyzed using Spearman tests. For all the tests, *p*-values < 0.05 were considered statistically significant. The statistical package R Studio (V2.5.1) was used for the analyses.

3. Results

3.1. Demographic and Clinical Findings

A total of 148 AATD patients were included from January 2017 to December 2019. Among these, 81 (54.7%) were homozygous Pi*ZZ and 67 (45.3%) were heterozygous for the Z allele (29 Pi*SZ, 35 Pi*MZ, 1 Pi*FZ, 1 Pi*PlowellZ, 1 Pi*MmaltonZ).

The mean age was 52.5 and 57 years for heterozygous and Pi*ZZ, respectively, and 50% of the patients were male. Liver disease in infancy was reported as the cause of the diagnosis of AATD in 19.4% and 11.1% of heterozygous and homozygous patients, although there were no patients with an active diagnosis of liver disease at the time of the study. COPD was diagnosed in 22.7% of heterozygous subjects and up to 70% for Pi*ZZ patients. Consequently, the mean FEV1 (%) was significantly lower in Pi*ZZ compared with heterozygous (69% (SD: 30.5%) versus 92.9% (SD: 27.6%); $p < 0.001$). The baseline characteristics of the global population and the two genotype groups are shown in Table 1.

Table 1. Baseline characteristics of the patients included by AAT genotype.

	ZZ (n = 81)	Heterozygous Z (n = 67)	*p*-Value
Age	57.0 (14.4)	52.5 (14.5)	0.051 [1]
Sex, men	41 (50.6%)	34 (50.7%)	0.985 [2]
BMI	25.1 (3.9)	24.0 (7.0)	0.398 [1]
Smoking exposure:			0.010 [2]
Active	43 (53.1%)	22 (32.8%)	
Former smoker	7 (8.6%)	16 (23.9%)	
Never smoker	31 (38.3%)	29 (43.3%)	
Alcohol consumption	19 (23.5%)	19 (28.4%)	0.991 [2]
Diabetes mellitus	0 (0%)	2 (3.0%)	0.203 [2]
Hypertension	14 (17.5%)	16 (23.9%)	0.453 [2]
AAT levels, mg/dL	33.3 (61.9)	71.9 (20.8)	<0.001 [1]
Reason for diagnosis:			0.002 [2]
Liver disease	9 (11.1%)	13 (19.4%)	
Lung disease	52 (64.2%)	23 (34.3%)	
Family study	17 (21.0%)	28 (41.8%)	
Other	3 (3.7%)	3 (4.5%)	
COPD	57 (70.4%)	15 (22.7%)	<0.001 [2]
Asthma	5 (7.8%)	14 (21.2%)	0.056 [2]
Neonatal jaundice	6 (7.4%)	3 (4.5%)	0.513 [2]
FVC, L	3.6 (1.5)	3.9 (1.1)	0.197 [1]
FVC, %	90.0 (28.5)	99.8 (19.8)	0.033 [1]
FEV1, L	2.1 (1.2)	3.0 (1.2)	<0.001 [1]
FEV1, %	69.0 (30.5)	92.9 (27.6)	<0.001 [1]
FEV1/FVC	0.6 (0.2)	0.7 (0.2)	0.001 [1]
KCO, %	51.0 (32.5)	58.9 (36.7)	0.231 [1]

Footnote: BMI: Body mass index; COPD: Chronic obstructive pulmonary disease; FVC: Forced ventilatory capacity; FEV1: Forced expiratory volume in 1 s; KCO: Transfer coefficient of the lung for carbon monoxide; AAT: Alpha-1 antitrypsin. [1] Mann–Whitney U-test *p*-value, [2] Chi-squared *p*-value.

3.2. Clinical and Laboratory Signs of Liver Disease

Thirty-two patients (21.6%) had abnormal liver enzymes. The distribution of values showed significant differences only in AST values, which were significantly higher in Pi*ZZ patients (29.2 UI/L (SD: 15.4) vs. 25.0 UI/L (SD: 8.0; $p = 0.029$). The most frequent pattern was an elevation in GGT (14.9% of patients). Pi*ZZ patients had a higher FIB-4 score compared to heterozygous Z (1.6 (SD: 0.8) vs. 1.2 (SD:0.5); $p < 0.001$). Only 5 patients

had FIB-4 > 3.25 and all were Pi*ZZ. The APRI score was higher in Pi*ZZ patients than in heterozygous Z (0.35 (SD: 0.18) vs. 0.27 (SD: 0.09); $p = 0.007$), but most of the patients had APRI values < 0.5, excluding advanced fibrosis or cirrhosis, and only one Pi*ZZ patient had an APRI score > 1.0. The ELF score was obtained in 52 patients (27 Pi*ZZ and 25 Pi*Z patients). Pi*ZZ had significantly higher values compared to Pi*Z phenotypes (8.6 (SD: 0.8) vs. 8 (SD: 0.6); $p = 0.007$). Only 1 Pi*ZZ patient showed values above the cut-off of 9.8 (Table 2).

Table 2. Results of blood analysis and transient elastography in patients with different AAT genotypes.

	ZZ (n = 81)	Heterozygous Z (n = 67)	*p*-Value
Laboratory findings			
Platelet count, $\times 10^9$/L	222 (59)	239 (61)	0.074 [1]
INR	1.0 (0.2)	1.0 (0.1)	0.067 [1]
Bilirubin, mg/dL	0.8 (0.5)	0.7 (0.3)	0.158
AST, IU/L	29.2 (15.4)	25.0 (8.0)	0.029 [1]
AST > ULN	4 (4.9%)	4 (6%)	0.869 [2]
ALT, IU/L	26.6 (22.6)	26.1 (13.4)	0.967 [1]
ALT > ULN	6 (7.4%)	5 (7.5%)	0.952 [2]
ALP, IU/L	78.2 (29.6)	81.8 (21)	0.412 [1]
ALP > ULN	6 (7.4%)	2 (3%)	0.294 [2]
GGT, IU/L	36.2 (33.9)	31.1 (29.4)	0.336 [1]
GGT > ULN	13 (16.5%)	9 (13.6%)	0.637 [2]
Albumin, g/dL	4.3 (0.6)	4.4 (0.3)	0.044 [1]
Cholesterol, mg/dL	207 (35)	198 (36)	0.161
FIB-4	1.6 (0.8)	1.2 (0.5)	<0.001
FIB-4 < 1.45	38 (47.5%)	51 (78.5%)	<0.001 [2]
FIB-4 > 3.25	5 (6.2%)	0	0.065 [2]
APRI	0.35 (0.18)	0.27 (0.09)	<0.001 [1]
APRI < 0.5	67 (83)	64 (91)	0.023 [2]
APRI > 1.0	1 (1.2)	0	0.956
ELF, n = 60	8.6 (0.8)	8 (0.6)	0.007 [1]
Transient elastography			
LSM	5.6 (2.4)	4.6 (1.2)	0.001 [1]
LSM > 7.5 kPa	8 (9.9%)	0	0.040 [2]
LSM ≥ 10 kPa	3 (3.7%)	0	
CAP	256 (59)	253 (50)	0.252 [1]
CAP 268–280 dB/m	7 (8.6%)	4 (6%)	0.807 [2]
CAP > 280 dB/m	26 (32.1%)	21 (31.3%)	

Footnote: INR: International normalized ratio; ULN: Upper limit of normal; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; GGT: Gamma-glutamyl transferase; FIB-4: Fibrosis 4; APRI: AST to platelet ratio index; ELF: Enhanced liver fibrosis; LSM: Liver stiffness measurement; CAP: Controlled attenuation parameter. [1] Mann–Whitney U-test *p*-value, [2] Chi-squared *p*-value.

3.3. Transient Elastography

The mean LSM was significantly higher in Pi*ZZ individuals than in heterozygous Z (5.6 (SD: 2.5) kPa vs. 4.6 (SD:1.2) kPa, respectively; $p = 0.007$). In total, LSM was >7.5 kPa in 8 (5%) individuals and ≥10 kPa in 3 (1.9%), all being Pi*ZZ (Figure 1). By lowering the cut-off of LSM to >7.1 kPa as suggested in other studies [11], we found 10 Pi*ZZ patients (12.3%) and 3 heterozygous patients (4.5%), two of whom were Pi*SZ patients with LSM 7.3 kPa, and one was a Pi*MZ patient with LSM 7.5 kPa.

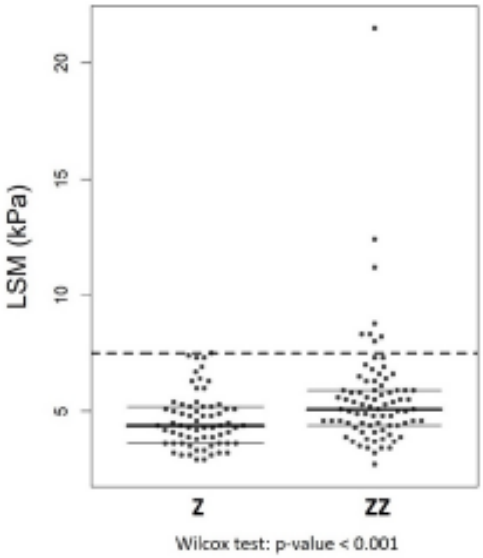

Figure 1. Comparison of mean LSM values by phenotype.

Using the LSM > 8.45 kPa cut-off of the study by Clark et al. [13], we would have identified 4 Pi*ZZ patients (4.9%) suggestive of having F ≥ 3.

Almost one-third of the patients had severe steatosis according to CAP values > 280 dB/m, with no significant differences between homozygous and heterozygous patients. (Table 2).

3.4. Characteristics of Pi*ZZ Patients According to LSM Values

Pi*ZZ individuals with LSM > 7.5 kPa were older and had a higher BMI. Two-thirds consumed alcohol, and all had COPD (versus 67% in patients with LSM ≤ 7.5 kPa; $p = 0.097$).

Elevated liver enzymes were more frequently observed in patients with LSM > 7.5 kPa. Twenty-five percent of patients with LSM > 7.5 kPa had elevated AST values compared to 2.7% in patients with LSM ≤ 7.5 kPa ($p = 0.048$), and 37.5% of patients with LSM > 7.5 kPa had elevated GGT compared to 14.1% of patients with LSM ≤ 7.5 kPa ($p = 0.120$) (Table 3, Figure 2). Conversely, 11/61 patients (18%) had at least one elevated liver enzyme but with normal LSM values (LSM < 6 kPa). Correlations between LSM and liver enzymes were only significant, albeit weakly, between LSM and AST (0.311 ($p < 0.001$)), and LSM and GGT (0.389 ($p < 0.001$)).

Table 3. Comparison between Pi*ZZ individuals based on liver stiffness (LSM) and diagnosis of chronic obstructive pulmonary disease (COPD).

	LSM ≤ 7.5 (n = 73)	LSM > 7.5 (n = 8)	*p*-Value	No COPD (n = 24)	COPD (n = 57)	*p*-Value
Age	56.2 (14.5)	64.9 (11.4)	0.076	46.2 (14.5)	61.6 (11.7)	<0.001 [1]
Sex, men	37 (50.7%)	4 (50%)	1.00	11 (45.8%)	30 (52.6%)	0.752 [2]
BMI	24.6 (3.4)	29.0 (5.3)	0.056	24.2 (3.7)	25.4 (3.9)	0.186 [1]
Smoking exposure:			0.527			<0.001 [2]
Active	37 (50.7%)	6 (75%)		7 (29.2%)	36 (63.2%)	
Former smoker	7 (9.6%)	0 (0%)		0 (0%)	7 (12.3%)	
Never smoker	29 (39.7%)	2 (25%)		17 (70.8%)	14 (24.6%)	
Alcohol consumption	16 (24.2%)	3 (60%)	0.115	5 (25%)	14 (27.2)	1.000 [2]
Hypertension	10 (13.9%)	4 (50%)	0.028	1 (4.2%)	13 (23.2%)	0.054 [2]
COPD	49 (67.1%)	8 (100%)	0.097	0	57 (100%)	0.001 [2]
Neonatal jaundice	6 (8.2%)	0 (0%)	1.000	4 (16.7%)	2 (3.5%)	0.060 [2]
FEV1, %	70.4 (30.7)	56.4 (27.3)	0.205	99.6 (13.1)	56.2 (26.3)	<0.001 [1]
Laboratory findings:						
Platelet count, $\times 10^9$/L	224 (60)	202 (49)	0.267	210 (48)	226 (62)	0.214 [1]
INR	1.0 (0.2)	1.1 (0.1)	0.378	1.0 (0.1)	1.1 (0.2)	0.040 [1]
Bilirubin, mg/dL	0.8 (0.5)	0.6 (0.2)	0.262	1.0 (0.9)	0.7 (0.2)	0.143 [1]
AST, UI/L	27.2 (10.1)	47.6 (34.7)	0.141	27.2 (10.6)	30.0 (16.9)	0.375 [1]
AST > ULN *	2 (2.7%)	2 (25%)	0.048	1 (4.2%)	3 (5.3%)	0.675 [2]
ALT, UI/L	24.2 (14.2)	48.8 (55.8)	0.254	25.5 (14.4)	27.1 (25.3)	0.719 [1]
ALT > ULN *	4 (5.5%)	2 (25.0%)	0.108	3 (12.5%)	3 (5.3%)	0.226 [2]
ALP, UI/L	78.5 (30.9)	75.9 (14.8)	0.816	70.3 (31)	81.4 (28.7)	0.130 [1]
ALP > ULN *	6 (8.5%)	0 (0%)	1.000	2 (8.3%)	4 (7.0%)	1.000 [2]
GGT, UI/L	31.8 (19.3)	75.6 (84.1)	<0.001	33.2 (22.2)	37.2 (37.7)	0.685 [1]
GGT > ULN *	10 (14.1%)	3 (37.5%)	0.120	5 (20.8%)	8 (14%)	0.589 [2]
Albumin, g/dL	4.3 (0.6)	4.4 (0.3)	0.615	4.5 (0.3)	4.2 (0.6)	0.004 [1]
Cholesterol, mg/dL	206 (35)	208 (39)	0.901	205 (39)	207 (34)	0.824 [1]
FIB-4	1.5 (0.8)	2.2 (0.7)	0.032	1.3 (0.8)	1.7 (0.8)	0.046 [1]
FIB-4 < 1.45:	37 (50.7%)	1 (12.5%)	0.059	15 (62.5%)	23 (40.4%)	0.077 [2]
FIB-4 > 3.25:	4 (5.5%)	1 (12.5%)	0.418	1 (4.2%)	4 (7%)	1.000 [2]
APRI	0.33 (0.1)	0.56 (0.3)	<0.001	0.35 (0.17)	0.35 (0.19)	0.992 [1]
Transient elastography						
LSM	5.0 (1.1)	10.8 (4.6)	0.009	5.3 (1.1)	5.7 (2.8)	0.361 [1]
CAP	249 (56)	318 (48)	0.004	233 (56)	266 (58)	0.023 [1]
LSM > 7.5 kPa:	0	8 (100%)	NA	0	8 (14.0%)	0.097 [2]

Footnote: BMI: Body mass index; COPD: Chronic obstructive pulmonary disease; FEV1: Forced expiratory volume in 1 s; AAT: Alpha-1 antitrypsin; INR: International normalized ratio; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; GGT: Gamma-glutamyl transferase; ULN: Upper limit of normal; FIB-4: Fibrosis 4; APRI: AST to platelet ratio index; ELF: Enhanced liver fibrosis; LSM: Liver stiffness measurement; CAP: Controlled attenuation parameter. *: Upper limit of normal according to sex-specific cut-offs: For AST and ALT: >35 IU/L in female, >50 IU/L in male; for ALP: >120 IU/L for both genders; for GGT: >38 IU/L in females and >55 IU/L in males. [1] Mann–Whitney U-test *p*-value, [2] Chi-squared *p*-value.

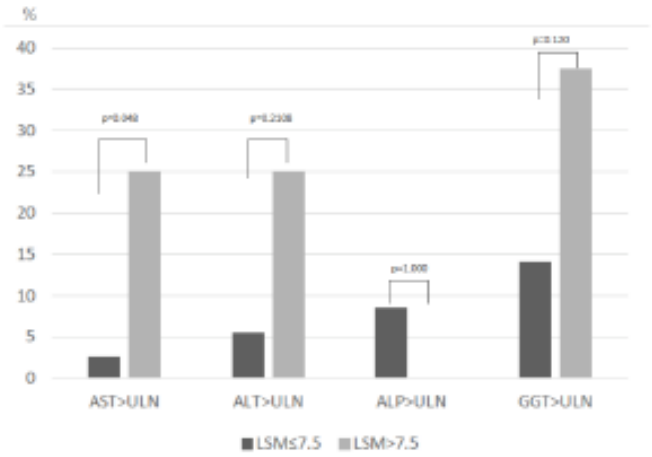

Figure 2. All individuals from the cohort with liver enzymes above the highest level of normal based on LSM values. UPN: Upper limit of normal for GGT: >38 IU/L in females and >55 IU/L in males.

Among the 8 patients with LSM > 7.5, 3 had GGT above the normal limit and 1 also had a FIB-4 score > 3.25 (Figure 3). The FIB-4 score (2.2 (SD: 0.7) versus 1.5 (SD: 0.8); $p = 0.032$), as well as CAP measurement (317.9 (SD: 48) dB/m vs. 249.6 (SD: 56.5) dB/m; $p = 0.004$), were also higher in Pi*ZZ patients with LSM > 7.5 kPa (Table 3). Severe steatosis, with CAP > 280 dB/m, was present in 6 patients (75%) with LSM > 7.5 kPa compared to 20 patients (27.4%) with LSM < 7.5 kPa ($p = 0.041$).

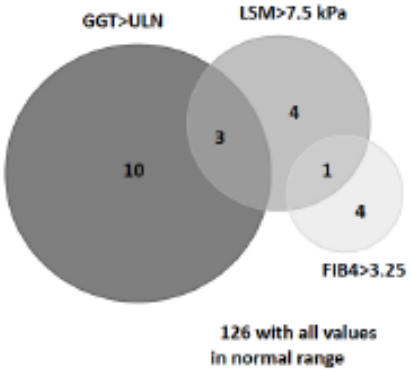

Figure 3. Relation between elevated GGT, FIB4, and LSM in Pi*ZZ patients. GGT: Gamma-glutamyl transferase; FIB-4: Fibrosis 4; LSM: Liver stiffness measurement; UPL: Upper limit of normal (according to sex-specific cut-offs: for GGT: >38 IU/L in females and >55 IU/L in males).

The APRI was higher in Pi*ZZ patients with LSM > 7.5 kPa than in those with LSM ≤ 7.5 kPa (0.56 vs. 0.33, $p < 0.001$). The APRI had a significant correlation with LSM ($r = 0.353$, $p = 0.030$).

3.5. Comparison between Pi*ZZ Patients with or without COPD

Fifty-seven Pi*ZZ patients (70.4%) had COPD. Pi*ZZ patients with COPD were older and more frequently had a history of smoking compared with non-COPD individuals. As expected, they had worse lung function with a lower FEV1 (1.6 (SD: 0.8) L vs. 3.5 (SD: 1) L; $p < 0.001$) and KCO (%) (43.7% (SD: 30.8%) vs. 68% (SD: 30.4%); $p = 0.003$).

Regarding the liver study, no differences were observed in transaminase levels, but the FIB-4 score was higher in COPD patients (1.7 (SD: 0.8) vs. 1.2 (SD: 0.8); $p = 0.046$). More

individuals in the COPD group had a LSM > 7.5 kPa (14% vs. 0%; $p = 0.097$) and they also had higher CAP values (265.9 (SD: 58.3) dB/m vs. 233.5 (SD: 55.8) dB/m; $p = 0.023$) (Table 3). Significant, albeit weak, correlations were found between FIB-4 and FEV1 (mL) ($r = -0.350$, $p = 0.002$), and CAP and FEV1 (mL) and FEV1(%) ($r = -0.391$, $p < 0.001$ and $r = -0.306$, $p = 0.006$, respectively). No significant correlations were found between LSM or ELF and measures of airflow obstruction.

4. Discussion

In our study population, we found that 10% of Pi*ZZ individuals had transient elastography results suggestive of liver fibrosis, but none of the heterozygous individuals reached the suggested threshold. Although individuals with higher LSM had higher transaminase levels and FIB-4 scores, normal levels of these biomarkers did not reliably rule out liver disease, since some of the patients with normal values had high LSM values. All patients with high LSM also had COPD.

Transient elastography is a non-invasive tool that has proven to be useful in the diagnosis of liver fibrosis of different etiologies. More recently, its utility has also been explored in AATD-related liver disease with promising results [16–18,26]. Although different cut-offs have been proposed, there is no validated cut-off of LSM for AATD liver disease. In a study including 94 Pi*ZZ patients with paired LSM and liver biopsies, Clark et al. [26] observed that cut-offs of 5.54 and 8.45 kPa had the highest accuracy for detecting significant fibrosis (≥F2) and advanced fibrosis (≥F3), respectively. However, these cut-offs had a low specificity and a low positive predictive value. Hamesch et al. [17] increased the cut-off for significant fibrosis to >7.1 kPa in order to increase the positive predictive value, confirming the presence of ≥F2 in 22 out of 23 patients with liver biopsies [27], while Guillaud et al. [16] suggested an LSM > 7.2 kPa for significant fibrosis and LSM > 14 kPa for cirrhosis. In another study in 75 patients with AATD, the investigators offered a liver biopsy to all individuals with a LSM > 6 or altered liver enzymes in combination with an abnormal ultrasound. Among the 11 biopsies analyzed, they found that the LSM scores in patients with moderate or severe fibrosis were >8 kPa [18]. According to these results and the cut-offs previously established in other etiologies, we chose an arbitrary cut-off of LSM > 7.5 kPa as suggestive of significant fibrosis, and LSM ≥ 10 kPa as advanced fibrosis/cirrhosis. In our sample, there were two Pi*SZ patients with LSM = 7.3 kPa, one of whom was overweight and had diabetes mellitus and increased GGT values, and the other was a Pi*MZ patient with LSM = 7.5 kPa without other identified risk factors of liver disease. Since the etiology of liver disease has an impact on LSM and the data on AATD induced liver disease are limited [28], further studies are needed to validate the best LSM cut-off for screening of liver disease in AATD.

Ten percent of Pi*ZZ patients in our cohort had LSM > 7.5 kPa, similar to the prevalence of liver fibrosis reported in initial studies in AATD patients, which varied from 10–15% in clinical studies [29,30] to 37% in autopsy studies [31]. More recently, with the development of transient elastography, there has been growing interest in the early detection of liver disease in AATD. The study by Guillaud et al. [16] described 5 patients (18%) with LSM suggestive of significant fibrosis and 2 patients (7%) with LSM suggestive of advanced liver fibrosis/cirrhosis. Other studies have reported a higher prevalence; Hamesch et al. [17] described a prevalence of liver fibrosis of 23.6% among 403 Pi*ZZ individuals and observed that liver disease was 9 to 20 times more frequent in this population compared to non-AAT-deficient individuals. In a cohort of COPD Pi*ZZ patients referred for lung transplantation, Morer et al. [32] found that 13% of patients had significant fibrosis (F2) and 8% advanced fibrosis (≥F3). Similar to these numbers, 8 (14%) of our COPD Pi*ZZ patients had LSM > 7.5 kPa, while in 3 (5.7%) LSM was higher than 10 kPa, suggesting the presence of advanced fibrosis.

In our cohort, Pi*MZ individuals had lower values of LSM compared to Pi*ZZ individuals. The mean LSM was 4.7 kPa for the 34 Pi*MZ patients included. None of these patients had values above 7.5, and only one had LSM = 7.5 kPa. In this patient, other

co-factors for liver disease such as obesity, alcohol consumption, or metabolic syndrome were not found. The incidence of liver disease could be higher in heterozygous Z than in the general population, although some authors have hypothesized that while the Pi*MZ genotype acts as a disease modifier, it is not sufficient per se to trigger clinically relevant liver impairment [33]. In a study that analyzed 1184 individuals with non-alcoholic fatty liver disease (NAFLD) and 2462 with chronic alcohol misuse, the Z variant increased the risk of patients with NAFLD to develop cirrhosis and was more frequently present in alcohol misusers with cirrhosis compared to those without significant liver injury [34]. In contrast, a recent analysis of data from the European alpha-1 liver cohort showed that 10% out 419 Pi*MZ had LSM values $\geq$ 7.1 kPa compared with 4% of non-Z carriers. After adjusting for potential confounders, Pi*MZ individuals still had significantly higher odds for LSM $\geq$ 7.1 kPa [12]. There is agreement that, in coexistence with other risk factors, and especially in the context of alcohol misuse or NAFLD, Z carriage is a strong risk factor for the development of cirrhosis [17,18] and may also lead to faster hepatic decompensations [35]. In our cohort, 60% of Pi*ZZ patients with LSM > 7.5 kPa had some alcohol consumption and had a higher BMI than those with LSM $\leq$ 7.5 kPa, and, therefore, these factors could have contributed to the progression of liver disease.

Liver enzymes have often been used to screen liver disease in AATD in clinical practice [36]. In our cohort, elevated liver enzymes and FIB-4 were more frequently observed in patients with LSM > 7.5 kPa, but normal levels were also frequently present in patients with high LSM. In fact, liver enzyme alterations ranged from only 25% of cases for AST and ALT to 37.5% for GGT in Pi*ZZ patients with LSM > 7.5 kPa. Patients with fibrosis or even cirrhosis may present normal serum liver enzymes [11], and this has also been observed in Pi*ZZ individuals [13,17]. On the other hand, up to 10% of AATD patients with normal liver function tests and ultrasound may have increased LSM values [16]. Furthermore, an increase in ALT has a low sensitivity for identifying liver disease in AATD individuals [13,15]. In the European alpha-1 liver cohort, heterozygous Pi*MZ carriers also had higher serum transaminases compared to non-carriers, although this percentage varied from 5.4% to 28.6% and was higher in individuals older than 50 years [12].

The relationship between lung and liver disease in individuals with AATD is controversial. The first series of patients with the deficiency suggested that lung and liver disease rarely coexisted in AATD, and liver disease was more frequently reported in AATD never smokers compared to smokers [37,38]. However, more recent studies using new diagnostic techniques have reported more frequent coexistence of the alterations in both organs [39]. In this line, all of our patients with elevated LSM also had COPD, although the correlation between lung function and LSM was not significant. Moreover, recruiting patients from respiratory departments may have influenced the high prevalence of COPD among patients with elevated LSM; although they were also older, with higher BMI and with a higher frequency of alcohol misuse compared with patients with normal LSM. Therefore, a clear relationship between elevated LSM and lung disease cannot be established from our results.

Our study had some limitations. First, the identification of liver fibrosis was only made by transient elastography as we did not perform liver biopsies. However, as there are no specific treatments for AATD liver disease to date, the performance of an invasive diagnostic technique in otherwise asymptomatic patients may not be justified. Second, this was a cross-sectional study, and data on the evolution of LSM over time were not available. Third, the design of our study did not allow us to investigate a causal relationship between AATD and liver alterations. Our sample size was not big enough for a multivariate analysis adjusted for known confounders of increased liver fibrosis. However, the study had some strengths: We recruited individuals from three reference centers, and, considering that AATD is a rare disease, we reported information from a large series of patients with homozygous and heterozygous AATD.

In conclusion, the results of this study support the assessment of liver disease in all AATD Pi*ZZ individuals and heterozygous Pi*Z individuals with additional liver risk

28. Behairy, B.-S.; Sira, M.M.; Zalata, K.R.; Salama, E.-S.E.; Abd-Allah, M.A. Transient elastography compared to liver biopsy and morphometry for predicting fibrosis in pediatric chronic liver disease: Does etiology matter? *World J. Gastroenterol.* **2016**, *22*, 4238–4249. [CrossRef] [PubMed]
29. Cox, D.W.; Smyth, S. Risk for liver disease in adults with alpha 1-antitrypsin deficiency. *Am. J. Med.* **1983**, *74*, 221–227. [CrossRef]
30. Tanash, H.A.; Piitulainen, E. Liver disease in adults with severe alpha-1-antitrypsin deficiency. *J. Gastroenterol.* **2019**, *54*, 541–548. [CrossRef] [PubMed]
31. Fairbanks, K.D.; Tavill, A.S. Liver disease in alpha 1-antitrypsin deficiency: A review. *Am. J. Gastroenterol.* **2008**, *103*, 2136–2141. [CrossRef] [PubMed]
32. Morer, L.; Choudat, L.; Dauriat, G.; Durand, F.; Cazals-Hatem, D.; Thabut, G.; Brugière, O.; Castier, Y.; Mal, H. Liver involvement in patients with PiZZ-emphysema, candidates for lung transplantation. *Am. J. Transplant.* **2017**, *17*, 1389–1395. [CrossRef]
33. Fromme, M.; Oliverius, M.; Strnad, P. DEFI-ALFA: The French key to the alpha1 mystery? *Liver Int.* **2019**, *39*, 1019–1021. [CrossRef] [PubMed]
34. Strnad, P.; Buch, S.; Hamesch, K.; Fischer, J.; Rosendahl, J.; Schmelz, R.; Brueckner, S.; Brosch, M.; Heimes, C.V.; Woditsch, V. Heterozygous carriage of the alpha1-antitrypsin Pi*Z variant increases the risk to develop liver cirrhosis. *Gut* **2019**, *68*, 1099–1107. [CrossRef] [PubMed]
35. Schaefer, B.; Mandorfer, M.; Viveiros, A.; Finkenstedt, A.; Ferenci, P.; Schneeberger, S.; Tilg, H.; Zoller, H. Heterozygosity for the alpha-1-antitrypsin Z allele in cirrhosis is associated with more advanced disease. *Liver Transpl.* **2018**, *24*, 744–751. [CrossRef]
36. Hernández Pérez, J.M.; Blanco, I.; Sánchez Medina, J.A.; Díaz Hernández, L.; Pérez Pérez, J.A. Serum Levels of Glutamate-Pyruvate Transaminase, Glutamate-Oxaloacetate Transaminase and Gamma-Glutamyl Transferase in 1494 Patients with Various Genotypes for the Alpha-1 Antitrypsin Gene. *J. Clin. Med.* **2020**, *9*, 3923. [CrossRef] [PubMed]
37. Stoller, J.K.; Tomashefski, J., Jr.; Crystal, R.G.; Arroliga, A.; Strange, C.; Killian, D.N.; Schluchter, M.D.; Wiedemann, H.P. Mortality in individuals with severe deficiency of alpha1-antitrypsin: Findings from the National Heart, Lung, and Blood Institute Registry. *Chest* **2005**, *127*, 1196–1204. [PubMed]
38. Tanash, H.A.; Nilsson, P.M.; Nilsson, J.A.; Piitulainen, E. Clinical course and prognosis of never-smokers with severe alpha-1-antitrypsin deficiency (PiZZ). *Thorax* **2008**, *63*, 1091–1095. [CrossRef] [PubMed]
39. Dawwas, M.F.; Davies, S.E.; Griffiths, W.J.H.; Lomas, D.A.; Alexander, G.J. Prevalence and risk factors for liver involvement in individuals with PiZZ-related lung disease. *Am. J. Respir. Crit. Care Med.* **2013**, *187*, 502–508. [CrossRef] [PubMed]
40. Barrecheguren, M.; Torres-Duran, M.; Casas-Maldonado, F.; Miravitlles, M. Spanish implementation of the new international alpha-1 anitrypsin deficiency international registry: The European Alpha-1 Research Collaboration (EARCO). *Arch. Bronconeumol.* **2021**, *57*, 81–82. [CrossRef] [PubMed]

5. OVERALL SUMMARY OF RESULTS

5.1 Circulating polymer concentrations in the different AATD genotypes

A total of 70 patients with different AAT genotypes were included. Among them, 32 (46%) were homozygous Pi*ZZ, of whom 11 were on augmentation therapy; 29 (41%) were heterozygous for the Z allele (13 Pi*MZ, 13 Pi*SZ, 1 Pi*MmaltonZ, 1 Pi*PLowelZ, 1 Pi*FZ); 4 (6%) carriers of the S allele (3 Pi*SS, 1 Pi*SI); and 5 (7%) rare variants (1 Pi* Q_0mattawa, 2 Pi*MMmalton, 1 Pi*SMmalton and 1 Pi*MMvall d´Hebron) (Table 1). The control group consisted of 47 individuals, 35 had a normal genotype Pi*MM and 12 had a Pi*MS genotype.

Considering the different genotypes individually, the highest values were observed in augmented Pi*ZZ patients (42.9 μg/mL (SD= 16) and one Pi*FZ with 42.1 μg/mL, very close to the 34.5 μg/mL (SD=16.2) obtained in untreated Pi*ZZ patients. The lowest values were observed in controls (1.04 μg/mL (SD=1.73) for Pi*MM and 0.9 μg/mL (SD=1.7) for Pi*MS) and in patients with the Pi*SS and Pi*SI genotypes. Patients heterozygous for the Z allele and other rare variants had intermediate values (Table 3 and Figure 1). The distribution of CP in percentage followed a similar distribution among genotypes (Table 3).

Table 3 Circulating polymer concentrations of the different AATD genotypes

AATD genotype	AAT polymers (µg/mL)	AAT polymers (%)	AAT (mg/dL)
Patients (n = 70)			
Pi*ZZ treated (n = 11)	42.9 (16)	9.2 (9.6)	73.5 (46.9)
Pi*FZ (n = 1)	42.1 (–)	6.2 (–)	67.7 (–)
Pi*ZZ untreated (n = 21)	34.5 (16.2)	14.7 (4.8)	23.1 (5.9)
Pi*MmaltonZ (n = 1)	22.8 (–)	10.2 (–)	22.2 (–)
Pi*PlowellZ (n = 1)	15.8 (–)	4.5 (–)	35.3 (–)
Pi*SZ (n = 13)	14.2 (4.2)	2.39 (0.6)	58.8 (8.3)
Pi*MZ (n = 13)	9.78 (6.3)	0.98 (0.5)	97 (16.5)
Pi*SMmalton (n = 1)	6.9 (–)	1.45 (–)	47.9 (–)
Pi*MMmattawa (n = 1)	5.5 (–)	0.8 (–)	66.9 (–)
Pi*MMvall d'hebron (n = 1)	4.1 (–)	0.3 (–)	126 (–)
Pi*MMmalton (n = 2)	2.3 (3.2)	0.2 (0.3)	80.7 (18.5)
Pi*SS (n = 3)	0	0	84.1 (10.5)
Pi*SI (n = 1)	0	0	85.0 (–)
Controls (n = 47)			
Pi*MM (n = 35)	1.04 (1.73)	0.06 (0.1)	172.7 (34.3)
Pi*MS (n = 12)	0.9 (1.7)	0.06 (0.1)	142.7 (20.1)

Values are mean (standard deviation)

AATD alpha-1 antitrypsin deficiency, *AAT* alpha-1 antitrypsin

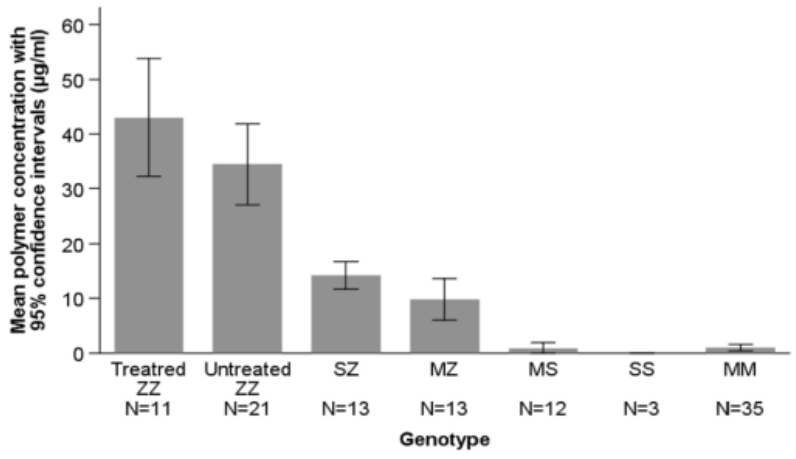

Fig. 1 Concentrations of circulating polymers according to AATD genotypes. Differences between: treated and untreated Pi*ZZ p = 0.092; all Pi*ZZ vs. Pi*SZ p = 0.021; all Pi*ZZ vs. Pi*MZ p = 0.001; all Pi*ZZ vs. Pi*MS p < 0.001; all Pi*ZZ vs. Pi*SS p < 0.001; all Pi*ZZ vs. Pi*MM p < 0.001; Pi*SZ vs. Pi*MM p < 0.001; Pi*MZ vs. Pi*MM p = 0.001. (ANOVA with Bonferroni correction for multiple comparisons)

5.2 Correlation between circulating polymers and parameters of lung and liver impairment in untreated Pi*ZZ and Z- heterozygous patients.

In order to determine the possible relationship between CP concentrations and lung and liver alterations, we selected homozygous or heterozygous patients carrying the Z allele, excluding those on augmentation therapy. A negative, significant and weak linear relationship was found between CP concentrations and parameters of airflow obstruction; FEV_1/FVC r= -0.32, p=0.026 and FEV_1 (%) r= -0.31, p=0.029 (Figure 2).

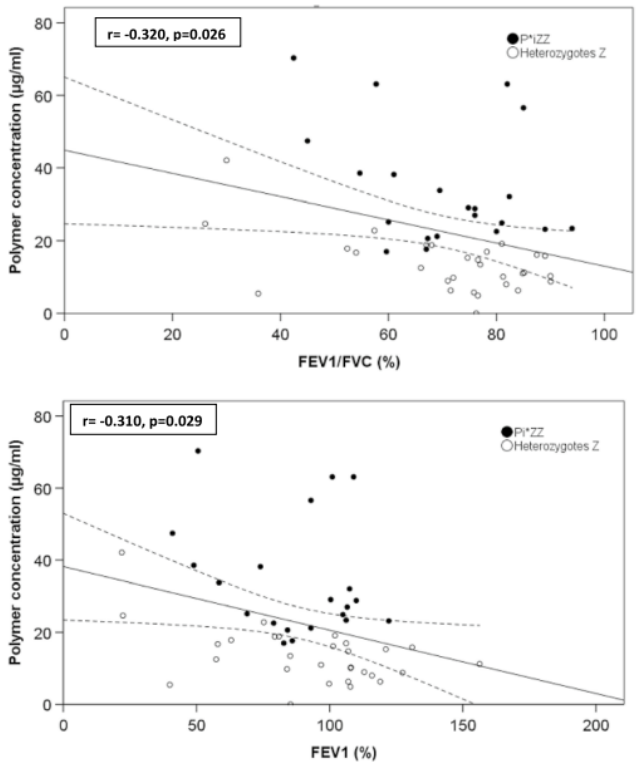

Fig. 2 **a** Correlation between circulating polymers and FEV_1/FVC in homozygous and heterozygous Z patients. **b** Correlation between circulating polymers and FEV_1 (%) in homozygous and heterozygous Z patients. FEV_1: forced expiratory volume in the 1st second; FVC: forced vital capacity; r indicates Pearson correlation coefficient; Linear regression fit (solid line) and 95% confidence interval (dashed line) of circulating polymer concentrations compared with FEV_1/FVC and FEV_1 (%) values

Similarly, a positive and weak linear relationship was found between CP and LSM and ELF (r= 0.39 p=0.005 and r=0.38 p=0.007, respectively) (Figure 3).

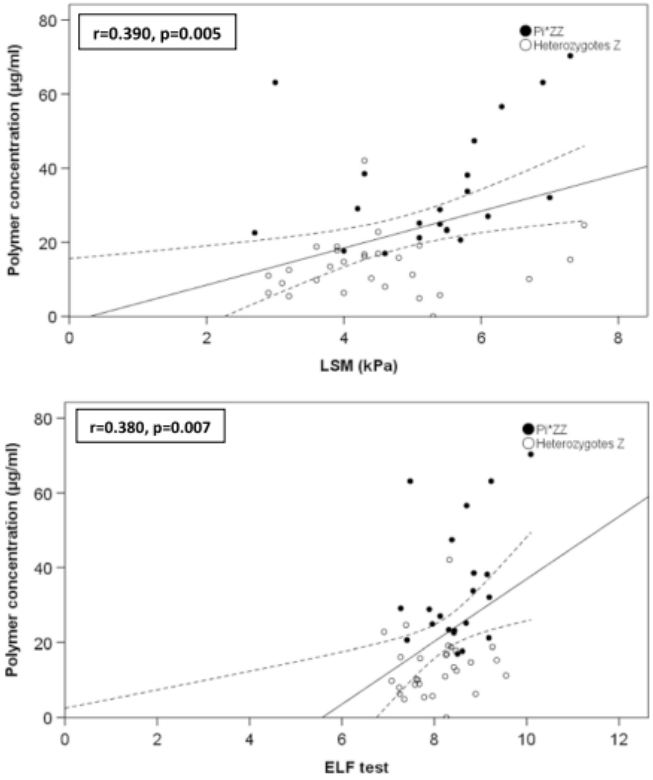

Fig. 3 **a** Correlation between circulating polymers and LSM in homozygous and heterozygous Z patients. **b** Correlation between circulating polymers and ELF test in homozygous and heterozygous Z patients. LSM: liver stiffness measurements; r indicates Pearson correlation coefficient; Linear regression fit (solid line) and 95% confidence interval (dashed line) of polymer concentrations compared with LSM and ELF tests

5.3 Circulating polymers concentrations in patients according to the combined presence of lung and liver disease

The same group of unaugmented patients with one or two Z alleles was divided into 4 subgroups according to lung and liver impairment, using the cutoff of $FEV_1/FVC < 0.7$ as diagnostic of COPD and LSM ≥6 kPa cut-off as suggestive of mild liver fibrosis. One patient was excluded from analysis due to missing data of LSM.

There was a gradient of CP concentrations, with the highest concentration in two patients with both lung and liver impairment (mean= 47.5 µg/ml), followed by six patients with liver abnormality only (mean CP=34 µg/ml) and 18 with lung impairment only (mean CP=26.5 µg/ml). Those with no abnormalities had the lowest CP concentrations (Table 4). Differences were significant in terms of CP between the 4 groups (p=0.004).

Table 4 Polymer concentrations and clinical characteristics: liver and/or lung disease

Patients heterozygous for the Z allele and untreated patients with the Pi*ZZ genotype (n = 49)				
	No COPD and LSM < 6 kPa (n = 23)	**COPD and LSM < 6 kPa (n = 18)**	**No COPD and LSM ≥ 6 kPa (n = 6)**	**COPD and LSM ≥ 6 kPa (n = 2)**
AAT (mg/dL)	61.1 (33.2)	39.4 (22.8)	47.5 (38.3)	76.1 (56.4)
AAT polymers (µg/mL)	14.4 (8)	26.5 (14.4)	34.0 (21.6)	47.5 (32.8)
AAT polymers (%)	4.7 (5.4)	9.8 (7.5)	11.2 (7.9)	10.8 (12.2)
Untreated patients with Pi*ZZ genotype (n = 21)				
	No COPD and LSM < 6 kPa (n = 6)	**COPD and LSM < 6 kPa (n = 10)**	**No COPD and LSM ≥ 6 kPa (n = 4)**	**COPD and LSM ≥ 6 kPa (n = 1)**
AAT (mg/dL)	19.4 (3.8)	22.4 (3.6)	27.4 (7.6)	36 (–)
AAT polymers (µg/mL)	25.3 (2.9)	32.3 (14.9)	44.7 (17.8)	70.3 (–)
AAT polymers (%)	13.2 (1.6)	14.5 (6.5)	16 (3.3)	19.4 (–)

COPD is defined as $FEV_1/FVC < 0.7$

LSM liver stiffness measurement, *AAT* alpha-1 antitrypsin

5.4 Clinical and laboratory biomarkers of liver disease

From a total of 148 AATD patients, thirty-two patients (21.6%) had abnormal liver enzymes. The distribution of values showed significant differences only in AST values, which were significantly higher in Pi*ZZ patients (29.2 UI/L (SD: 15.4) vs. 25.0 UI/L (SD: 8.0; p = 0.029). The most frequent pattern was an elevation in GGT (14.9% of patients). Pi*ZZ patients had a higher FIB-4 score compared to heterozygous Z (1.6 (SD: 0.8) vs. 1.2 (SD: 0.5); p < 0.001). Only 5 patients had FIB-4 > 3.25 and all were Pi*ZZ. The APRI score was higher in Pi*ZZ patients than in heterozygous Z (0.35 (SD: 0.18) vs. 0.27 (SD: 0.09); p = 0.007), but most of the patients had APRI values < 0.5, excluding advanced fibrosis or cirrhosis, and only one Pi*ZZ patient had an APRI score > 1.0. The ELF score was obtained in 52 patients (27 Pi*ZZ and 25 Pi*Z patients). Pi*ZZ had significantly higher values compared to Pi*Z phenotypes (8.6 (SD: 0.8) vs. 8 (SD: 0.6); p = 0.007). Only 1 Pi*ZZ patient showed values above the cut-off of 9.8 (Table 2).

5.5 Transient elastography

The mean LSM was significantly higher in Pi*ZZ individuals than in heterozygous Z (5.6 (SD: 2.5) kPa vs. 4.6 (SD:1.2) kPa, respectively; p = 0.007). In total, LSM was >7.5 kPa in 8 (5%) individuals and ≥10 kPa in 3 (1.9%), all being Pi*ZZ (Figure 1). By lowering the cut-off of LSM to >7.1 kPa as suggested in other studies (11), we found 10 Pi*ZZ patients (12.3%) and 3 heterozygous patients (4.5%), two of whom were Pi*SZ patients with LSM 7.3 kPa, and one was a Pi*MZ patient with LSM 7.5 kPa.

Using the LSM > 8.45 kPa cut-off of the study by Clark et al., we would have identified 4 Pi*ZZ patients (4.9%) suggestive of having F ≥ 3. Almost one-third of the patients

had severe steatosis according to CAP values > 280 dB/m, with no significant differences between homozygous and heterozygous patients. (Table 2)

Table 2. Results of blood analysis and transient elastography in patients with different AAT genotypes.

	ZZ (n = 81)	Heterozygous Z (n = 67)	p-Value
Laboratory findings			
Platelet count, $\times 10^9$/L	222 (59)	239 (61)	0.074 [1]
INR	1.0 (0.2)	1.0 (0.1)	0.067 [1]
Bilirubin, mg/dL	0.8 (0.5)	0.7 (0.3)	0.158
AST, IU/L	29.2 (15.4)	25.0 (8.0)	0.029 [1]
AST > ULN	4 (4.9%)	4 (6%)	0.869 [2]
ALT, IU/L	26.6 (22.6)	26.1 (13.4)	0.967 [1]
ALT > ULN	6 (7.4%)	5 (7.5%)	0.952 [2]
ALP, IU/L	78.2 (29.6)	81.8 (21)	0.412 [1]
ALP > ULN	6 (7.4%)	2 (3%)	0.294 [2]
GGT, IU/L	36.2 (33.9)	31.1 (29.4)	0.336 [1]
GGT > ULN	13 (16.5%)	9 (13.6%)	0.637 [2]
Albumin, g/dL	4.3 (0.6)	4.4 (0.3)	0.044 [1]
Cholesterol, mg/dL	207 (35)	198 (36)	0.161
FIB-4	1.6 (0.8)	1.2 (0.5)	<0.001
FIB-4 < 1.45	38 (47.5%)	51 (78.5%)	<0.001 [2]
FIB-4 > 3.25	5 (6.2%)	0	0.065 [2]
APRI	0.35 (0.18)	0.27 (0.09)	<0.001 [1]
APRI < 0.5	67 (83)	64 (91)	0.023 [2]
APRI > 1.0	1 (1.2)	0	0.956
ELF, n = 60	8.6 (0.8)	8 (0.6)	0.007 [1]
Transient elastography			
LSM	5.6 (2.4)	4.6 (1.2)	0.001 [1]
LSM > 7.5 kPa	8 (9.9%)	0	0.040 [2]
LSM $\geq$ 10 kPa	3 (3.7%)	0	
CAP	256 (59)	253 (50)	0.252 [1]
CAP 268–280 dB/m	7 (8.6%)	4 (6%)	0.807 [2]
CAP > 280 dB/m	26 (32.1%)	21 (31.3%)	

Footnote: INR: International normalized ratio; ULN: Upper limit of normal; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; GGT: Gamma-glutamyl transferase; FIB-4: Fibrosis 4; APRI: AST to platelet ratio index; ELF: Enhanced liver fibrosis; LSM: Liver stiffness measurement; CAP: Controlled attenuation parameter. [1] Mann–Whitney U-test p-value, [2] Chi-squared p-value.

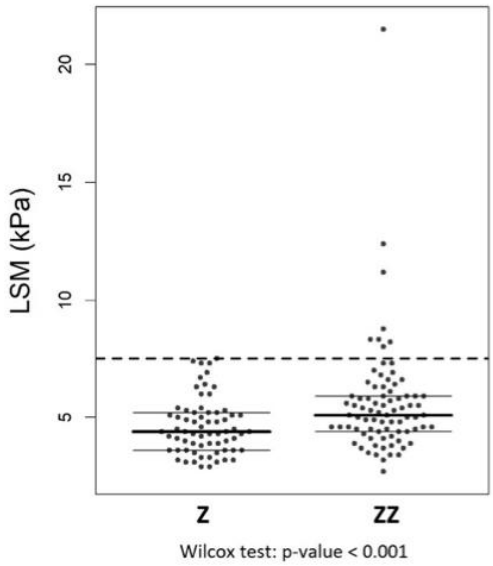

Figure 1. Comparison of mean LSM values by phenotype.

5.6 Characteristics of Pi*ZZ patients according to LSM values

Two thirds consumed alcohol, and all had COPD (versus 67% in patients with LSM ≤ 7.5 kPa; p = 0.097). Elevated liver enzymes were more frequently observed in patients with LSM > 7.5 kPa. Twenty-five percent of patients with LSM > 7.5 kPa had elevated AST values compared to 2.7% in patients with LSM ≤ 7.5 kPa (p = 0.048), and 37.5% of patients with LSM > 7.5 kPa had elevated GGT compared to 14.1% of patients with LSM ≤ 7.5 kPa (p = 0.120) (Table 3). Conversely, 11/61 patients (18%) had at least one elevated liver enzyme but with normal LSM values (LSM < 6 kPa). Correlations between LSM and liver enzymes were only significant, albeit weakly, between LSM and AST (0.311 (p < 0.001)), and LSM and GGT (0.389 (p < 0.001)).

Table 3. Comparison between Pi*ZZ individuals based on liver stiffness (LSM) and diagnosis of chronic obstructive pulmonary disease (COPD).

	LSM ≤ 7.5 (*n* = 73)	LSM > 7.5 (*n* = 8)	*p*-Value	No COPD (*n* = 24)	COPD (*n* = 57)	*p*-Value
Age	56.2 (14.5)	64.9 (11.4)	0.076	46.2 (14.5)	61.6 (11.7)	<0.001 [1]
Sex, men	37 (50.7%)	4 (50%)	1.00	11 (45.8%)	30 (52.6%)	0.752 [2]
BMI	24.6 (3.4)	29.0 (5.3)	0.056	24.2 (3.7)	25.4 (3.9)	0.186 [1]
Smoking exposure:			0.527			<0.001 [2]
Active	37 (50.7%)	6 (75%)		7 (29.2%)	36 (63.2%)	
Former smoker	7 (9.6%)	0 (0%)		0 (0%)	7 (12.3%)	
Never smoker	29 (39.7%)	2 (25%)		17 (70.8%)	14 (24.6%)	
Alcohol consumption	16 (24.2%)	3 (60%)	0.115	5 (25%)	14 (27.2)	1.000 [2]
Hypertension	10 (13.9%)	4 (50%)	0.028	1 (4.2%)	13 (23.2%)	0.054 [2]
COPD	49 (67.1%)	8 (100%)	0.097	0	57 (100%)	0.001 [2]
Neonatal jaundice	6 (8.2%)	0 (0%)	1.000	4 (16.7%)	2 (3.5%)	0.060 [2]
FEV1, %	70.4 (30.7)	56.4 (27.3)	0.205	99.6 (13.1)	56.2 (26.3)	<0.001 [1]
Laboratory findings:						
Platelet count, $\times 10^9$/L	224 (60)	202 (49)	0.267	210 (48)	226 (62)	0.214 [1]
INR	1.0 (0.2)	1.1 (0.1)	0.378	1.0 (0.1)	1.1 (0.2)	0.040 [1]
Bilirubin, mg/dL	0.8 (0.5)	0.6 (0.2)	0.262	1.0 (0.9)	0.7 (0.2)	0.143 [1]
AST, UI/L	27.2 (10.1)	47.6 (34.7)	0.141	27.2 (10.6)	30.0 (16.9)	0.375 [1]
AST > ULN *	2 (2.7%)	2 (25%)	0.048	1 (4.2%)	3 (5.3%)	0.675 [2]
ALT, UI/L	24.2 (14.2)	48.8 (55.8)	0.254	25.5 (14.4)	27.1 (25.3)	0.719 [1]
ALT > ULN *	4 (5.5%)	2 (25.0%)	0.108	3 (12.5%)	3 (5.3%)	0.226 [2]
ALP, UI/L	78.5 (30.9)	75.9 (14.8)	0.816	70.3 (31)	81.4 (28.7)	0.130 [1]
ALP > ULN *	6 (8.5%)	0 (0%)	1.000	2 (8.3%)	4 (7.0%)	1.000 [2]
GGT, UI/L	31.8 (19.3)	75.6 (84.1)	<0.001	33.2 (22.2)	37.2 (37.7)	0.685 [1]
GGT > ULN *	10 (14.1%)	3 (37.5%)	0.120	5 (20.8%)	8 (14%)	0.589 [2]
Albumin, g/dL	4.3 (0.6)	4.4 (0.3)	0.615	4.5 (0.3)	4.2 (0.6)	0.004 [1]
Cholesterol, mg/dL	206 (35)	208 (39)	0.901	205 (39)	207 (34)	0.824 [1]
FIB-4	1.5 (0.8)	2.2 (0.7)	0.032	1.3 (0.8)	1.7 (0.8)	0.046 [1]
FIB-4 < 1.45:	37 (50.7%)	1 (12.5%)	0.059	15 (62.5%)	23 (40.4%)	0.077 [2]
FIB-4 > 3.25:	4 (5.5%)	1 (12.5%)	0.418	1 (4.2%)	4 (7%)	1.000 [2]
APRI	0.33 (0.1)	0.56 (0.3)	<0.001	0.35 (0.17)	0.35 (0.19)	0.992 [1]
Transient elastography						
LSM	5.0 (1.1)	10.8 (4.6)	0.009	5.3 (1.1)	5.7 (2.8)	0.361 [1]
CAP	249 (56)	318 (48)	0.004	233 (56)	266 (58)	0.023 [1]
LSM > 7.5 kPa:	0	8 (100%)	NA	0	8 (14.0%)	0.097 [2]

Footnote: BMI: Body mass index; COPD: Chronic obstructive pulmonary disease; FEV1: Forced expiratory volume in 1 s; AAT: Alpha-1 antitrypsin; INR: International normalized ratio; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; GGT: Gamma-glutamyl transferase; ULN: Upper limit of normal; FIB-4: Fibrosis 4; APRI: AST to platelet ratio index; ELF: Enhanced liver fibrosis; LSM: Liver stiffness measurement; CAP: Controlled attenuation parameter. *: Upper limit of normal according to sex-specific cut-offs: For AST and ALT: >35 IU/L in female, >50 IU/L in male; for ALP: >120 IU/L for both genders; for GGT: >38 IU/L in females and >55 IU/L in males. [1] Mann–Whitney U-test *p*-value, [2] Chi-squared *p*-value.

Among the 8 patients with LSM > 7.5, 3 had GGT above the normal limit and 1 also had a FIB-4 score > 3.25 (Figure 3). The FIB-4 score (2.2 (SD: 0.7) versus 1.5 (SD: 0.8); p = 0.032), as well as CAP measurement (317.9 (SD: 48) dB/m vs. 249.6 (SD: 56.5) dB/m; p = 0.004), were also higher in Pi*ZZ patients with LSM > 7.5 kPa (Table 3). Severe steatosis, with CAP > 280 dB/m, was present in 6 patients (75%) with LSM > 7.5 kPa compared to 20 patients (27.4%) with LSM < 7.5 kPa (p = 0.041).

The APRI was higher in Pi*ZZ patients with LSM > 7.5 kPa than in those with LSM ≤ 7.5 kPa (0.56 vs. 0.33, p < 0.001). The APRI had a significant correlation with LSM (r = 0.353, p = 0.030).

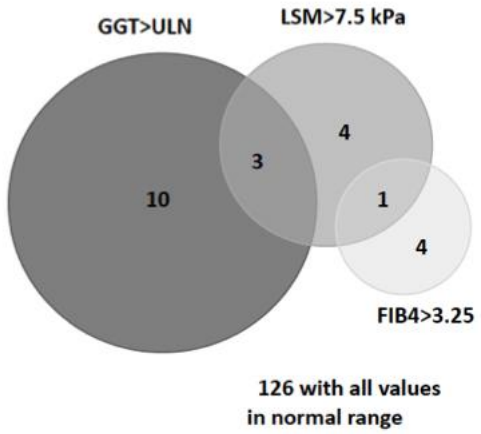

Figure 3. Relation between elevated GGT, FIB4, and LSM in Pi*ZZ patients. GGT: Gamma-glutamyl transferase; FIB-4: Fibrosis 4; LSM: Liver stiffness measurement; UPL: Upper limit of normal (according to sex-specific cut-offs: for GGT: >38 IU/L in females and >55 IU/L in males).

5.7 Comparison of liver findings between Pi*ZZ patients with or without COPD

Fifty-seven Pi*ZZ patients (70.4%) had COPD. Pi*ZZ patients with COPD were older and more frequently had a history of smoking compared with non-COPD individuals. As expected, they had worse lung function with a lower FEV_1 (1.6 (SD: 0.8) L vs. 3.5 (SD: 1) L; $p < 0.001$) and KCO (%) (43.7% (SD: 30.8%) vs. 68% (SD: 30.4%); $p = 0.003$). Regarding the liver study, no differences were observed in transaminase levels, but the FIB-4 score was higher in COPD patients (1.7 (SD: 0.8) vs. 1.2 (SD: 0.8); $p = 0.046$). More individuals in the COPD group had a LSM > 7.5 kPa (14% vs. 0%; $p = 0.097$) and they also had higher CAP values (265.9 (SD: 58.3) dB/m vs. 233.5 (SD: 55.8) dB/m; $p = 0.023$) (Table 3). Significant, albeit weak, correlations were found between FIB-4 and FEV_1 (mL) ($r = -0.350$, $p = 0.002$), and CAP and FEV_1 (mL) and FEV_1(%) ($r = -0.391$, $p < 0.001$ and $r = -0.306$, $p = 0.006$, respectively). No significant correlations were found between LSM or ELF and measures of airflow obstruction.

6. OVERALL SUMMARY OF THE DISCUSION

The results of this thesis show that overall Pi*ZZ patients presented the highest levels of CP of AAT, followed by heterozygous Z patients and individuals with rare variants. The lowest CP concentrations were observed in controls with Pi*MM and Pi*MS genotypes and patients carrying the S allele, with undetectable levels in the few Pi*SS patients analysed. Moreover, CP concentrations were significantly higher in patients with both lung and liver disease and correlated with the degree of alteration in lung function and liver stiffness.

Regarding the diagnosis of liver disease by transient elastography, we found that 10% of Pi*ZZ individuals had transient elastography results suggestive of liver fibrosis, but none of the heterozygous individuals reached the suggested threshold. As to liver biomarkers, individuals with higher transaminase levels and FIB-4 scores had higher LSM. However, normal levels of these biomarkers did not reliably rule out liver disease, since some of the patients with normal values had high LSM values. In addition, we observed that all patients with high LSM also had COPD.

6.1 Alpha1-antitrypsin polymers

Alpha1-antitrypsin polymers are aggregates of misfolded protein and are deposited within the ER of hepatocytes, which is the basis of the pathogenesis of liver disease in AATD (5, 66). Although most of the polymers remain as inclusion bodies in the ER of hepatocytes, some are secreted into the blood stream (30, 31). Polymers are also secreted by alveolar macrophages and have a pro-inflammatory and chemotactic role for inflammatory cells in the lung. The polymers within alveolar macrophages have no anti-elastase activity, thereby contributing to a greater imbalance of the protease-antiprotease axis (21, 25). Moreover, studies have shown that, apart from inactivating AAT by

oxidation, cigarette smoke also increases the concentration of AAT polymers within alveolar macrophages (27, 28).

6.2 Concentrations of circulating polymers in different AATD genotypes

In our study, Z homozygous patients presented the highest concentrations of CP followed by Z heterozygous patients. These data were previously observed by Tan et al. (31), who reported the highest concentrations of CP in Pi*ZZ patients, a low signal in normal Pi*MM individuals and remained undetected in S-homozygous individuals. Other studies have also reported that, among the most frequent variants, the Z mutation polymerises the most and the S the least (29, 66, 67).

Patients with the Pi*ZZ genotype on augmentation therapy presented higher CP levels than untreated patients, despite blood samples being taken just before the following dose of augmentation therapy when plasma levels of exogenous AAT are minimal. This observation confirms previous studies that demonstrate the presence of AAT polymers in the augmentation therapy preparations (68) having a direct correlation with serum levels of AAT (69). To avoid possible confounding effects caused by augmentation therapy, augmented patients were excluded from further analysis.

Association between circulating polymers and liver and lung parameters

In order to assess the relationship between CP and variables of liver and lung disease we used data from untreated homozygous or heterozygous carriers of the Z allele. We found a negative relationship between CP concentrations and airflow obstruction parameters and a significant and positive linear relationship with LSM and ELF, suggesting that higher concentrations of CP are related to lung and liver damage. These findings are in agreement with a previous study on 244 Pi*ZZ individuals, that found a

negative linear relationship between CP concentrations and the FEV_1/FVC ratio. Moreover, although that study was not designed to assess liver disease, patients who self-reported abnormal liver function, liver disease or cirrhosis had higher CP concentrations than those without a history of liver involvement (31). In a biopsy study, Mela et al. (40) found that higher polymer loads within hepatocytes were related to senescence of the cells and liver fibrosis. However, to the best of our knowledge, no other studies have related CP concentrations with LSM or ELF and this is important since transient elastography is increasingly used for the screening and follow-up of liver disease in patients with AATD (48, 70), and ELF is a systemic biomarker of liver fibrosis (60).

The importance of the polymerisation of mutated AAT in the pathogenesis of liver and lung disease in AATD has stimulated the development of new strategies of treatment for AATD based on the blockade of polymer formation (70, 72).

6.3 Transient elastography

Transient liver elastography is a non-invasive tool that has proven to be useful in the diagnosis of liver fibrosis of different etiologies. More recently, its utility has also been explored in AATD-related liver disease with promising results (48, 49, 50, 73). Although different cut-offs have been proposed, there is no validated cut-off of LSM for AATD liver disease. In a study including 94 Pi*ZZ patients with paired LSM and liver biopsies, Clark et al. (73) observed that cut-offs of 5.54 and 8.45 kPa had the highest accuracy for detecting significant fibrosis (≥F2) and advanced fibrosis (≥F3), respectively. However, these cut-offs had a low specificity and a low positive predictive value. Hamesch et al.(49) increased the cut-off for significant fibrosis to >7.1 kPa in

order to increase the positive predictive value, confirming the presence of ≥F2 in 22 out of 23 patients with liver biopsies, while Guillaud et al. (48) suggested an LSM > 7.2 kPa for significant fibrosis and LSM > 14 kPa for cirrhosis. In another study in 75 patients with AATD, the investigators offered a liver biopsy to all individuals with a LSM > 6 or altered liver enzymes in combination with an abnormal ultrasound. Among the 11 biopsies analyzed, they found that the LSM scores in patients with moderate or severe fibrosis were >8 kPa (50). According to these results and the cut-offs previously established in other etiologies, we chose an arbitrary cut-off of LSM > 7.5 kPa as suggestive of significant fibrosis, and LSM ≥ 10 kPa as advanced fibrosis/cirrhosis. In our sample, there were two Pi*SZ patients with LSM = 7.3 kPa, one of whom was overweight and had diabetes mellitus and increased GGT values, and the other was a Pi*MZ patient with LSM = 7.5 kPa without other identified risk factors of liver disease. Since the etiology of liver disease has an impact on LSM and the data on AATD induced liver disease are limited (74), further studies are needed to validate the best LSM cut-off for screening of liver disease in AATD.

6.4 Transient elastography and AATD

Ten percent of Pi*ZZ patients in our cohort had LSM > 7.5 kPa, similar to the prevalence of liver fibrosis reported in initial studies in AATD patients, which varied from 10–15% in clinical studies to 37% in autopsy studies (75, 76, 77). More recently, with the development of transient elastography, there has been growing interest in the early detection of liver disease in AATD. The study by Guillaud et al. described 5 patients (18%) with LSM suggestive of significant fibrosis and 2 patients (7%) with LSM suggestive of advanced liver fibrosis/cirrhosis (48). Other studies have reported a

higher prevalence; Hamesch et al. described a prevalence of liver fibrosis of 23.6% among 403 Pi*ZZ individuals and observed that liver disease was 9 to 20 times more frequent in this population compared to non-AAT-deficient individuals (49).

In a cohort of COPD Pi*ZZ patients referred for lung transplantation, Morer et al. found that 13% of patients had significant fibrosis (F2) and 8% advanced fibrosis (≥F3) (78). Similar to these numbers, 8 (14%) of our COPD Pi*ZZ patients had LSM > 7.5 kPa, while in 3 (5.7%) LSM was higher than 10 kPa, suggesting the presence of advanced fibrosis.

In our cohort, Pi*MZ individuals had lower values of LSM compared to Pi*ZZ individuals. The mean LSM was 4.7 kPa for the 34 Pi*MZ patients included. None of these patients had values above 7.5, and only one had LSM = 7.5 kPa. In this patient, other co-factors for liver disease such as obesity, alcohol consumption, or metabolic syndrome were not found. The incidence of liver disease could be higher in heterozygous Z than in the general population, although some authors have hypothesized that while the Pi*MZ genotype acts as a disease modifier, it is not sufficient per se to trigger clinically relevant liver impairment (79). In a study that analyzed 1184 individuals with non-alcoholic fatty liver disease (NAFLD) and 2462 with chronic alcohol misuse, the Z variant increased the risk of patients with NAFLD to develop cirrhosis and was more frequently present in alcohol misusers with cirrhosis compared to those without significant liver injury (80). In contrast, a recent analysis of data from the European alpha-1 liver cohort showed that 10% out 419 Pi*MZ had LSM values ≥ 7.1 kPa compared with 4% of non-Z carriers. After adjusting for potential confounders, Pi*MZ individuals still had significantly higher odds for LSM ≥ 7.1 kPa (44). There is agreement that, in coexistence with other risk factors, and especially in the context of alcohol misuse or NAFLD, Z carriage is a strong risk factor for the

development of cirrhosis and may also lead to faster hepatic decompensations (49, 50, 81). In our cohort, 60% of Pi*ZZ patients with LSM > 7.5 kPa had some alcohol consumption and had a higher BMI than those with LSM ≤ 7.5 kPa, and, therefore, these factors could have contributed to the progression of liver disease.

6.5 Transient elastography and liver enzymes

Liver enzymes have often been used to screen liver disease in AATD in clinical practice (82). In our cohort, elevated liver enzymes and FIB-4 were more frequently observed in patients with LSM > 7.5 kPa, but normal levels were also frequently present in patients with high LSM. In fact, liver enzyme alterations ranged from only 25% of cases for AST and ALT to 37.5% for GGT in Pi*ZZ patients with LSM > 7.5 kPa. Patients with fibrosis or even cirrhosis may present normal serum liver enzymes, and this has also been observed in Pi*ZZ individuals (45, 49, 83).

On the other hand, up to 10% of AATD patients with normal liver function tests and ultrasound may have increased LSM values (48). Furthermore, an increase in ALT has a low sensitivity for identifying liver disease in AATD individuals (45, 47). In the European alpha-1 liver cohort, heterozygous Pi*MZ carriers also had higher serum transaminases compared to non-carriers, although this percentage varied from 5.4% to 28.6% and was higher in individuals older than 50 years (44).

6.6 Lung and liver disease in AATD

The relationship between lung and liver disease in individuals with AATD is controversial. The first series of patients with the deficiency suggested that lung and liver disease rarely coexisted in AATD, and liver disease was more frequently reported

in AATD never smokers compared to smokers (84, 85). However, more recent studies using new diagnostic techniques have reported more frequent coexistence of the alterations in both organs (86). In this line, all of our patients with elevated LSM also had COPD, although the correlation between lung function and LSM was not significant. Moreover, recruiting patients from respiratory departments may have influenced the high prevalence of COPD among patients with elevated LSM; although they were also older, with higher BMI and with a higher frequency of alcohol misuse compared with patients with normal LSM. Therefore, a clear relationship between elevated LSM and lung disease cannot be established from our results.

7. CONCLUSIONS

- Pi*ZZ and heterozygous Z individuals present higher levels of CP than other AAT genotypes
- CP of AAT were associated with the presence and severity of lung and liver disease. Therefore, CP concentrations may help to identify AATD patients at greater risk of developing lung and liver disease and may provide some insight into the mechanisms of the disease.
- The assessment of liver disease in all AATD Pi*ZZ individuals and heterozygous Pi*Z individuals with additional liver risk factors should be carried out.
- Transient elastography has shown to be a valuable tool to screen for AATD liver disease.
- Due to the poor correlation between liver enzymes and other serum biomarkers and the underlying liver disease, all Z-allele carriers, even those with normal serum biomarker values, should be screened with transient elastography.
- Since AATD is a rare disease, international collaboration in large registries is needed to investigate the best screening strategy for lung and liver disease.

8. APPLICABILITY TO THE FUTURE

As described in this thesis, the polymerization of the mutated AAT protein constitutes an essencial role in the pathogenesis of lung and liver disease in AATD.

Since AATD is a rare disease, it is important to determine new biomarkers that could help in the early detection of patients that may be in risk of developing lung disease. Larger studies with larger populations should be performed in order to determine if these parameters could be used in the daily practice.

In addition, in this thesis, we determined that the transient liver elastography could be useful to the screening of liver disease in patients with AATD. As described in this thesis, liver biomarkers are not always reliable in order to detect liver disease, therefore, transient liver elastography could be helful to determine whether the patient suffers or is at risk of developing liver disease. Morever, transient liver elastography could be used for the follow-up of patients with AATD.

9. BIBLIOGRAPHIC REFERENCES

1. Stoller JK, Aboussouan LS. A review of α1-antitrypsin deficiency. Am J Respir Crit Care Med. 2012; 185: 246–259.
2. Stoller JK, Brantly M. The challenge of detecting alpha-1 antitrypsin deficiency. COPD. 2013; 10(1): 26–34.
3. Blanco I, Bueno P, Diego I, et al. Alpha-1 antitrypsin Pi*Z gene frequency and Pi*ZZ genotype numbers worldwide: an update. Int J Chron Obstruct Pulmon Dis 2017; 12: 561-569.
4. Blanco I, Bueno P, Diego I, Pérez-Holanda S, Lara B, Casas-Maldonado F, Esquinas C, Miravitlles M. Alpha-1 antitrypsin Pi*SZ genotype: estimated prevalence and number of SZ subjects worldwide. Int J Chron Obstruct Pulmon Dis 2017; 12: 1683-1694.
5. Eriksson S, Carlson J, Velez R. Risk of cirrhosis and primary liver cancer in alpha1-antitrypsin deficiency. N Engl J Med. 1986; 314: 736-739.
6. Strnad P, McElvaney NG, Lomas DA. Alpha1-Antitrypsin Deficiency. N Engl J Med. 2020; 382(15): 1443-1455.
7. Janciauskiene S, DeLuca DS, Barrecheguren M, Welte T, Miravitlles M. Serum Levels of Alpha1-antitrypsin and Their Relationship With COPD in the General Spanish Population. Arch Bronconeumol. 2020; 56: 76-83.
8. Ellis P, Turner A. What Do Alpha-1 Antitrypsin Levels Tell Us About Chronic Inflammation in COPD? Arch Bronconeumol. 2020; 56: 72-73.
9. Casas F, Blanco I, Martínez MT, Bustamante A, Miravitlles M, Cadenas S, Hernández JM, Lázaro L, Rodríguez E, Rodríguez-Frías F, Torres M, Lara B. Indications for active case searches and intravenous alpha-1 antitrypsin treatment for patients with alpha-1 antitrypsin deficiency chronic pulmonary obstructive disease: an update. Arch Bronconeumol. 2015; 51(4):185-92.

10. Luisetti M, Seersholm N. Alpha- antitrypsin deficiency. epidemiology of alpha-1 antitrypsin deficiency. Thorax. 2004; 59(2): 164-169.
11. Salahuddin P. Genetic variants of alpha1-antitrypsin. Curr Protein Pept Sci. 2010 Mar;11(2):101-17.
12. American Thoracic Society; European Respiratory Society. American Thoracic Society/European Respiratory Society statement: standards for the diagnosis and management of individuals with alpha-1 antitrypsin deficiency. Am J Respir Crit Care Med. 2003 Oct 1;168(7):818-900.
13. Blanco I, Fernández-Bustillo E, de Serres FJ, Alkassam D, Rodríguez Menéndez C. Déficit de alfa-1-antitripsina en España (variantes deficientes PI*S y PI*Z): prevalencia estimada y número de sujetos calculados para cada fenotipo [PI*S and PI*Z alpha 1-antitrypsin deficiency: estimated prevalence and number of deficient subjects in Spain]. Med Clin (Barc). 2004 Dec 4;123(20):761-5. Spanish.
14. Lara B, Martínez-Delgado B, Torres ML, Marín-Arguedas S, Bustamante A, Miravitlles M. Alpha-1-antitrypsin deficiency associated with the Mattawa variant. Arch Bronconeumol. 2013 Dec;49(12):548-50.
15. Elliott PR, Lomas DA, Carrell RW, et al. Inhibitory conformation of the reactive loop of a1-antitrypsin. Nat Struct Biol 1996;3:676–81.
16. Ryu SE, Choi HJ, Kwon KS, et al. The native strains in the hydrophobic core and flexible reactive loop of a serine protease inhibitor: crystal structure of an uncleaved a1-antitrypsin at 2.7A˚. Structure 1996;4:1181–92.
17. Elliott PR, Abrahams J-P, Lomas DA. Wildtype a1-antitrypsin is in the canonical inhibitory conformation. J Mol Biol 1998;275:419–25.

18. Elliott PR, Pei XY, Dafforn TR, et al. Topography of a 2.0A˚ structure of a1-antitrypsin reveals targets for rational drug design to prevent conformational disease. Protein Sci 2000;9:1274–81.
19. Kim SJ, Woo JR, Seo EJ, et al. A 2.1A˚ resolution structure of an uncleaved a1-antitrypsin shows variability of the reactive centre and other loops. J Mol Biol 2001;306:109–19.
20. Lee JH, Brantly M. Molecular mechanisms of alpha1-antitrypsin null alleles. Respir Med. 2000 Aug;94 Suppl C:S7-11.
21. Mahadeva R, Chang WS, Dafforn TR, Oakley DJ, Foreman RC, Calvin J, Wight DG, Lomas DA. Heteropolymerization of S, I, and Z alpha1-antitrypsin and liver cirrhosis. J Clin Invest. 1999 Apr;103(7):999-1006..
22. Lomas DA, Mahadeva R. Alpha1-antitrypsin polymerization and the serpinopathies: pathobiology and prospects for therapy. J Clin Invest. 2002 Dec;110(11):1585-90.
23. Geraghty P, Rogan MP, Greene CM, Brantly ML, O'Neill SJ, Taggart CC, McElvaney NG. Alpha-1-antitrypsin aerosolised augmentation abrogates neutrophil elastase-induced expression of cathepsin B and matrix metalloprotease 2 in vivo and in vitro. Thorax. 2008 Jul;63(7):621-6.
24. Petrache I, Fijalkowska I, Zhen L, Medler TR, Brown E, Cruz P, Choe KH, Taraseviciene-Stewart L, Scerbavicius R, Shapiro L, Zhang B, Song S, Hicklin D, Voelkel NF, Flotte T, Tuder RM. A novel antiapoptotic role for alpha1-antitrypsin in the prevention of pulmonary emphysema. Am J Respir Crit Care Med. 2006 Jun 1;173(11):1222-8.

25. Mulgrew AT, Taggart CC, Lawless MW, Greene CM, Brantly ML, O'Neill SJ, McElvaney NG. Z alpha1-antitrypsin polymerizes in the lung and acts as a neutrophil chemoattractant. Chest 2004; 125: 1952–1957.
26. Mahadeva R, Atkinson C, Li Z, Stewart S, Janciauskiene S, Kelley DG,Parmar J, Pitman R, Shapiro SD, Lomas DA. Polymers of Z alpha1-antitrypsin co-localize with neutrophils in emphysematous alveoli and are chemotactic in vivo. Am J Pathol 2005; 166: 377–386.
27. Bazzan E, Tinè M, Biondini D, Benetti R, Baraldo S, Turato G, Fagiuoli S, Sonzogni A, Rigobello C, Rea F, Calabrese F, Foschino-Barbaro MP, Miranda E, Lomas DA, Saetta M, Cosio MG. α_1-Antitrypsin Polymerizes in Alveolar Macrophages of Smokers With and Without α_1-Antitrypsin Deficiency. Chest. 2018;154(3):607-616.
28. Alam S, Li Z, Janciauskiene S, et al. Oxidation of Z a1-antitrypsin by cigarette smoke induces polymerization: a novel mechanism of early-onset emphysema. Am J Respir Cell Mol Biol 2011; 45: 261–269.
29. Gooptu B, Dickens JA, Lomas DA. The molecular and cellular pathology of α_1-antitrypsin deficiency. Trends Mol Med. 2014 Feb;20(2):116-27. doi: 10.1016/j.molmed.2013.
30. Fra A, Cosmi F, Ordoñez A, Berardelli R, Perez J, Guadagno NA, Corda L, Marciniak SJ, Lomas DA, Miranda E. Polymers of Z α1-antitrypsin are secreted in cell models of disease. Eur Respir J. 2016 Mar;47(3):1005-9. doi: 10.1183/13993003.00940-2015.
31. Tan L, Dickens JA, Demeo DL, Miranda E, Perez J, Rashid ST, Day J, Ordoñez A, Marciniak SJ, Haq I, Barker AF, Campbell EJ, Eden E, McElvaney NG, Rennard SI, Sandhaus RA, Stocks JM, Stoller JK, Strange C, Turino G, Rouhani FN,

Brantly M, Lomas DA. Circulating polymers in α1-antitrypsin deficiency. Eur Respir J. 2014 May;43(5):1501-4.

32. Esquinas, C., Barrecheguren, M., Sucena, M. *et al*. Practice and knowledge about diagnosis and treatment of alpha-1 antitrypsin deficiency in Spain and Portugal. *BMC Pulm Med* **16,** 64 (2016).

33. Barker, A., Brantly, M., Campbell, E., Carrell, R., Cox, D. W., & Dirksen, A. E. (1997). Alpha 1-antitrypsin deficiency: memorandum from a WHO meeting. *Bull World Health Organ*, *75*(5), 397-415.

34. Molina J, Flor X, Garcı´a R, Timiraos R, TiradoConde G, Miravitlles M (2011) The IDDEA project: a strategy for the detection of alpha-1 antitrypsin deficiency in COPD patients in the primary care setting. Ther Adv Respir Dis 5:237–243

35. Vidal R, Blanco I, Casas F, Jardı´ R, Miravitlles M (2006) Guidelines for the diagnosis and management of alpha-1 antitrypsin deficiency. Arch Bronconeumol 42:645–659

36. Belmonte I, Montoto L, Rodríguez-Frías F. Laboratory Diagnosis by Genotyping. Methods Mol Biol. 2017;1639:45-60.
37. Sveger T. Liver disease in alpha1-antitrypsin deficiency detected by screening of 200,000 infants. N Engl J Med. 1976 Jun 10;294(24):1316-21.
38. Stockley RA. Alpha 1-antitrypsin: more than just deficiency. Thorax. 2004 May;59(5):363-4.
39. Miravitlles M, Dirksen A, Ferrarotti I, Koblizek V, Lange P, Mahadeva R, McElvaney NG, Parr D, Piitulainen E, Roche N, Stolk J, Thabut G, Turner A, Vogelmeier C, Stockley RA. European Respiratory Society statement: diagnosis

and treatment of pulmonary disease in α_1-antitrypsin deficiency. Eur Respir J. 2017 Nov 30;50(5):1700610.

40. Mela M., Smeeton W., Davies S.E., Miranda E., Scarpini C., Coleman N., Alexander G.J.M. The Alpha-1 Antitrypsin Polymer Load Correlates with Hepatocyte Senescence, Fibrosis Stage and Liver-Related Mortality. *Chronic. Obstr. Pulm. Dis.* 2020;7:151–162.
41. Bouchecareilh M. Alpha-1 Antitrypsin Deficiency-Mediated Liver Toxicity: Why Do Some Patients Do Poorly? What Do We Know So Far? *Chronic. Obstr. Pulm. Dis.* 2020;7:172–181.
42. Teckman J.H., Jain A. Advances in alpha-1-antitrypsin deficiency liver disease. *Curr. Gastroenterol. Rep.* 2014;16:367.
43. Hamesch K., Strnad P. Non-Invasive Assessment and Management of Liver Involvement in Adults with Alpha-1 Antitrypsin Deficiency. *Chronic. Obstr. Pulm. Dis.* 2020;7:260–271.
44. Schneider C.V., Hamesch K., Gross A., Mandorfer M., Moeller L.S., Pereira V., Pons M., Kuca P., Reichert M.C., Benini F., et al. European Alpha-1 Liver Study Group. Liver Phenotypes of European Adults Heterozygous or Homozygous for Pi∗Z Variant of AAT (Pi∗MZ vs Pi∗ZZ genotype) and Noncarriers. *Gastroenterology.* 2020;159:534–548.
45. Clark V.C., Dhanasekaran R., Brantly M., Rouhani F., Schreck P., Nelson D.R. Liver test results do not identify liver disease in adults with α(1)-antitrypsin deficiency. *Clin. Gastroenterol. Hepatol.* 2012;10:1278–1283.
46. Del Poggio P., Colombo S. Is transient elastography a useful tool for screening liver disease? *World J. Gastroenterol.* 2009;15:1409–1414.

47. Kim R.G., Nguyen P., Bettencourt R., Dulai P.S., Haufe W., Hooker J., Minocha J., Valasek M.A., Aryafar H., Brenner D.A., et al. Magnetic resonance elastography identifies fibrosis in adults with alpha-1 antitrypsin deficiency liver disease: A prospective study. *Aliment. Pharmacol. Ther.* 2016;44:287–299.
48. Guillaud O., Dumortier J., Traclet J., Restier L., Joly P., Chapuis-Cellier C., Lachaux A., Mornex J.F. Assessment of Liver Fibrosis by Transient Elastography (Fibroscan®) in Patients with A1AT Deficiency. *Clin. Res. Hepatol. Gastroenterol.* 2019;43:77–81.
49. Hamesch K., Mandorfer M., Pereira V.M., Moeller L.S., Pons M., Dolman G.E., Reichert M.C., Heimes C.V., Woditsch V., Voss J., et al. European Alpha1-Liver Study Group. Liver Fibrosis and Metabolic Alterations in Adults with Alpha1 Antitrypsin Deficiency Caused by the Pi*ZZ Mutation. *Gastroenterology.* 2019;157:705–719.
50. Abbas S.H., Pickett E., Lomas D.A., Thorburn D., Gooptu B., Hurst J.R., Marshall A. Non-invasive testing for liver pathology in alpha-1 antitrypsin deficiency. *BMJ Open. Respir. Res.* 2020;7:e000820.
51. Casas F, Blanco I, Martínez MT, Bustamante A, Miravitlles M, Cadenas S, Hernández JM, Lázaro L, Rodríguez E, Rodríguez-Frías F, Torres M, Lara B. Indications for active case searches and intravenous alpha-1 antitrypsin treatment for patients with alpha-1 antitrypsin deficiency chronic pulmonary obstructive disease: an update. Arch Bronconeumol. 2015 Apr;51(4):185-92. English, Spanish.
52. Wewers MD, Casolaro MA, Sellers SE, Swayze SC, McPhaul KM, Wittes JT, Crystal RG. Replacement therapy for alpha 1-antitrypsin deficiency associated with emphysema. N Engl J Med. 1987 Apr 23;316(17):1055-62.

53. Stockley RA, Miravitlles M, Vogelmeier C; Alpha One International Registry (A.I.R.). Augmentation therapy for alpha-1 antitrypsin deficiency: towards a personalised approach. Orphanet J Rare Dis. 2013 Sep 24;8:149.
54. Dirksen A, Piitulainen E, Parr DG, Deng C, Wencker M, Shaker SB, Stockley RA. Exploring the role of CT densitometry: a randomised study of augmentation therapy in alpha1-antitrypsin deficiency. Eur Respir J. 2009 Jun;33(6):1345-53.
55. Parr DG, Dirksen A, Piitulainen E, Deng C, Wencker M, Stockley RA. Exploring the optimum approach to the use of CT densitometry in a randomised placebo-controlled study of augmentation therapy in alpha 1-antitrypsin deficiency. Respir Res. 2009 Aug 13;10(1):75.
56. Chapman KR, Burdon JG, Piitulainen E, Sandhaus RA, Seersholm N, Stocks JM, Stoel BC, Huang L, Yao Z, Edelman JM, McElvaney NG; RAPID Trial Study Group. Intravenous augmentation treatment and lung density in severe α1 antitrypsin deficiency (RAPID): a randomised, double-blind, placebo-controlled trial. Lancet. 2015 Jul 25;386(9991):360-8.
57. Miravitlles M, Herr C, Ferrarotti I, Jardi R, Rodriguez-Frias F, Luisetti M, et al. Laboratory testing of individuals with severe AAT deficiency in three European centres. *Eur Respir J.* 2010;**35**:960–968.
58. European Association for Study of Liver EASL-ALEH Clinical Practice Guidelines: Non-invasive Tests for Evaluation of Liver Disease Severity and Prognosis. *J. Hepatol.* 2015;63:237–264.
59. Sterling R.K., Lissen E., Clumeck N., Sola R., Correa M.C., Montaner J., Sulkowski M.S., Torriani F.J., Dieterich D.T., Thomas D.L., et al. Development of a simple noninvasive index to predict significant fibrosis patients with HIV/HCV co-infection. *Hepatology.* 2006;43:1317–1325.

60. Lichtinghagen R., Pietsch D., Bantel H., Manns M.P., Brand K., Bahr M.J. The Enhanced Liver Fibrosis (ELF) score: Normal values, influence factors and proposed cut-off values. *J. Hepatol.* 2013;59:236–242.
61. Miranda E, Pérez J, Ekeowa UI, Hadzic N, Kalsheker N, Gooptu B, et al. A novel monoclonal antibody to characterize pathogenic polymers in liver disease associated with alpha1-antitrypsin deficiency. *Hepatology.* 2010;**52**(3):1078–1088.
62. Lin Z.H., Xin Y.N., Dong Q.J., Wang Q., Jiang X.J., Zhan S.H., Sun Y., Xuan S.Y. Performance of the aspartate aminotransferase-to-platelet ratio index for the staging of hepatitis C-related fibrosis: An updated meta-analysis. *Hepatology.* 2011;53:726–736.
63. Mulabecirovic A., Mjelle A.B., Gilja O.H., Vesterhus M., Havre R.F. Liver elasticity in healthy individuals by two novel shear-wave elastography systems—Comparison by age, gender, BMI and number of measurements. *PLoS ONE.* 2018;13:e0203486.
64. Castera L. Non-invasive tests for liver fibrosis in NAFLD: Creating pathways between primary healthcare and liver clinics. *Liver Int.* 2020;40((Suppl. 1)):77–81.
65. Karlas T., Petroff D., Sasso M., Fan J.G., Mi Y.Q., de Lédinghen V., Kumar M., Lupsor-Platon M., Han K.H., Cardoso A.C., et al. Individual patient data meta-analysis of controlled attenuation parameter (CAP) technology for assessing steatosis. *J. Hepatol.* 2017;66:1022–1030. doi: 10.1016/j.jhep.2016.12.022.
66. Laffranchi M, Elliston EL, Miranda E, Perez J, Ronzoni R, Jagger AM, et al. Intrahepatic heteropolymerization of M and Z alpha-1-antitrypsin. *JCI Insight.* 2020;**5**(14):e135459.
67. Ekeowa UI, Marciniak SJ, Lomas DA. Alpha1- antitrypsin deficiency and inflammation. *Expert Rev Clin Immunol.* 2011;**7**(2):243–252.

68. Boerema DJ, An B, Gandhi RP, Papineau R, Regnier E, Wilder A, et al. Biochemical comparison of four commercially available human α_1-proteinase inhibitors for treatment of α_1-antitrypsin deficiency. *Biologicals.* 2017;**50**:63–72.
69. Schmid ST, Koepke J, Dresel M, Hattesohl A, Frenzel E, Perez J, et al. The effects of weekly augmentation therapy in patients with PiZZ α1-antitrypsin deficiency. *Int J Chron Obstruct Pulmon Dis.* 2012;**7**:687–696.
70. Pons M, Núñez A, Esquinas C, Torres-Durán M, Rodríguez-Hermosa JL, Calle M, et al. Utility of transient elastography for the screening of liver disease in patients with alpha1-antitrypsin deficiency. *J Clin Med.* 2021;**10**(8):1724.
71. Ordóñez A, Pérez J, Tan L, Dickens JA, Motamedi-Shad N, Irving JA, et al. A single-chain variable fragment intrabody prevents intracellular polymerization of Z α1-antitrypsin while allowing its antiproteinase activity. *FASEB J.* 2015;**29**(6):2667–2678.
72. Lomas DA, Irving JA, Arico-Muendel C, Belyanskaya S, Brewster A, Brown M, et al. Development of a small molecule that corrects misfolding and increases secretion of Z α_1-antitrypsin. *EMBO Mol Med.* 2021;**13**(3):e13167.
73. Clark V.C., Marek G., Liu C., Collinsworth A., Shuster J., Kurtz T., Nolte J., Brantly M. Clinical and histologic features of adults with alpha-1 antitrypsin deficiency in a non-cirrhotic cohort. *J. Hepatol.* 2018;69:1357–1364.
74. Behairy B.-S., Sira M.M., Zalata K.R., Salama E.-S.E., Abd-Allah M.A. Transient elastography compared to liver biopsy and morphometry for predicting fibrosis in pediatric chronic liver disease: Does etiology matter? *World J. Gastroenterol.* 2016;22:4238–4249.
75. Cox D.W., Smyth S. Risk for liver disease in adults with alpha 1-antitrypsin deficiency. *Am. J. Med.* 1983;74:221–227. doi: 10.1016/0002-9343(83)90615-0.

76. Tanash H.A., Piitulainen E. Liver disease in adults with severe alpha-1-antitrypsin deficiency. *J. Gastroenterol.* 2019;54:541–548. doi: 10.1007/s00535-019-01548-y.
77. Fairbanks K.D., Tavill A.S. Liver disease in alpha 1-antitrypsin deficiency: A review. *Am. J. Gastroenterol.* 2008;103:2136–2141.
78. Morer L., Choudat L., Dauriat G., Durand F., Cazals-Hatem D., Thabut G., Brugière O., Castier Y., Mal H. Liver involvement in patients with PiZZ-emphysema, candidates for lung transplantation. *Am. J. Transplant.* 2017;17:1389–1395.
79. Fromme M., Oliverius M., Strnad P. DEFI-ALFA: The French key to the alpha1 mystery? *Liver Int.* 2019;39:1019–1021.
80. Strnad P., Buch S., Hamesch K., Fischer J., Rosendahl J., Schmelz R., Brueckner S., Brosch M., Heimes C.V., Woditsch V. Heterozygous carriage of the alpha1-antitrypsin Pi*Z variant increases the risk to develop liver cirrhosis. *Gut.* 2019;68:1099–1107.
81. Schaefer B., Mandorfer M., Viveiros A., Finkenstedt A., Ferenci P., Schneeberger S., Tilg H., Zoller H. Heterozygosity for the alpha-1-antitrypsin Z allele in cirrhosis is associated with more advanced disease. *Liver Transpl.* 2018;24:744–751.
82. Hernández Pérez J.M., Blanco I., Sánchez Medina J.A., Díaz Hernández L., Pérez Pérez J.A. Serum Levels of Glutamate-Pyruvate Transaminase, Glutamate-Oxaloacetate Transaminase and Gamma-Glutamyl Transferase in 1494 Patients with Various Genotypes for the Alpha-1 Antitrypsin Gene. *J. Clin. Med.* 2020;9:3923.
83. Hamesch K., Strnad P. Non-Invasive Assessment and Management of Liver Involvement in Adults with Alpha-1 Antitrypsin Deficiency. *Chronic. Obstr. Pulm.*

84. Stoller J.K., Tomashefski J., Jr., Crystal R.G., Arroliga A., Strange C., Killian D.N., Schluchter M.D., Wiedemann H.P. Mortality in individuals with severe deficiency of alpha1-antitrypsin: Findings from the National Heart, Lung, and Blood Institute Registry. *Chest.* 2005;127:1196–1204.
85. Tanash H.A., Nilsson P.M., Nilsson J.A., Piitulainen E. Clinical course and prognosis of never-smokers with severe alpha-1-antitrypsin deficiency (PiZZ) *Thorax.* 2008;63:1091–1095.
86. Dawwas M.F., Davies S.E., Griffiths W.J.H., Lomas D.A., Alexander G.J. Prevalence and risk factors for liver involvement in individuals with PiZZ-related lung disease. *Am. J. Respir. Crit. Care Med.* 2013;187:502–508.
87. Colombo S, Belloli L, Zaccanelli M, Badia E, Jamoletti C, Buonocore M, et al. Normal liver stiffness and its determinants in healthy blood donors. *Dig Liver Dis.* 2011;**43**:231–236.
88. Kim SU, Choi GH, Han WK, Kim BK, Park JY, Kim DY, et al. What are 'true normal' liver stiffness values using FibroScan?: a prospective study in healthy living liver and kidney donors in South Korea. *Liver Int.* 2010;**30**:268–274.
89. Roulot D, Czernichow S, Le Clesiau H, Costes JL, Vergnaud AC, Beaugrand M. Liver stiffness values in apparently healthy subjects: influence of gender and metabolic syndrome. *J Hepatol.* 2008;**48**:606–613.
90. Barrecheguren M, Torres-Duran M, Casas-Maldonado F, Miravitlles M. Spanish implementation of the new international alpha-1 antitrypsin deficiency international registry: The European Alpha-1 Research Collaboration (EARCO) *Arch Bronconeumol.* 2021;**57**(2):81–82.
91. Miravitlles M, Nuñez A, Torres-Durán M, Casas-Maldonado F, Rodríguez-Hermosa JL, López-Campos JL, et al. The importance of reference centers and

registries for rare diseases: the example of alpha-1 antitrypsin deficiency. *COPD*. 2020;**17**(4):346–354. doi: 10.1080/15412555.2020.1795824.