

Trends in Diagnosis of Alpha-1 Antitrypsin Deficiency Between 2015 and 2019 in a Reference Laboratory

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Background: Alpha-1 antitrypsin deficiency (AATD) remains largely underdiagnosed despite recommendations of healthcare institutions and programmes designed to increase awareness. The objective was to analyse the trends in AATD diagnosis during the last 5 years in a Spanish AATD reference laboratory.

Methods: This was a retrospective revision of all alpha-1 antitrypsin (AAT) determinations undertaken in our laboratory from 2015 to 2019. We analysed the number of AAT determinations performed and described the characteristics of the individuals tested, as well as the medical specialties and the reasons for requesting AAT determination.

Results: A total of 3507 determinations were performed, of which 5.5% corresponded to children. A significant increase in the number of AAT determinations was observed from 349 in 2015 to 872 in 2019. Among the samples, 57.6% carried an intermediate AATD (50–119 mg/dL) and 2.4% severe deficiency (<50 mg/dL). The most frequent phenotype in severe AATD individuals was PI*ZZ (78.5%), and aminotransferase levels were above normal in around 43% of children and 30% of adults. Respiratory specialists requested the highest number of AAT determinations (31.5%) followed by digestive diseases and internal medicine (27.5%) and primary care physicians (19.7%). The main reason for AAT determination in severe AATD adults was chronic obstructive pulmonary disease (41.7%), but reasons for requesting AAT determination were not reported in up to 41.7% of adults and 58.3% of children.

Conclusion: There is an increase in the frequency of AATD testing despite the rate of AAT determination remaining low. Awareness about AAT is probably increasing, but the reason for testing is not always clear.

Keywords: alpha-1 antitrypsin deficiency, diagnosis, screening, lung disease

Introduction

Alpha-1 antitrypsin deficiency (AATD) is an autosomal codominant hereditary disorder characterised by low serum levels of alpha-1 antitrypsin (AAT). At a clinical level, AATD is not properly a disease, but rather a predisposition for the development of pulmonary emphysema in adults and liver disease, especially in children.¹

AAT is a serine protease inhibitor encoded by the *SERPINA1* gene. Over 150 mutations have been described in *SERPINA1*. According to the isoelectric focusing (IEF) pattern, the most common non-disease-causing allele is designated PI*M, and the two most frequently deficient alleles (>95% of patients with AATD) are PI*S

and PI*Z.² The PI*Z variant, as well as other rare deficient variants such as PI*Siiyama and PI*Mmalton can form intracellular polymers in hepatocytes, the primary source of AAT synthesis, leading to reduced levels of circulating blood AAT.^{3,4} AAT levels in patients with the Pi*ZZ phenotype are approximately 15% that of normal values.¹

Laboratory testing for this deficiency comprises three strategies: serum or plasma AAT quantification, AAT protein phenotyping and genotyping.⁵ AATD is typically diagnosed by measurement of protein levels by nephelometry in routine blood analysis.⁵ If AAT quantification, corrected by inflammatory status measured by C-reactive protein (CRP), is below the normal range, AATD should be confirmed by assessing the genotype, protein phenotype, or a combination of both.^{5,6}

Guidelines from national and international societies, such as the World Health Organization,⁷ The Spanish Society of Pneumology and Thoracic Surgery⁸ and the European Respiratory Society,¹ recommend AAT testing, at least once in a lifetime, for all patients with chronic obstructive pulmonary disease (COPD), emphysema, or adults with asthma and irreversible airflow obstruction, and liver disease of unknown aetiology.

However, the number of patients diagnosed with AATD is much lower than expected according to epidemiologic studies⁹ and most patients are not correctly diagnosed until late in the course of their pulmonary or liver disease, after many visits to multiple physicians.^{10,11}

Studies based on databases of medical records are increasingly used in clinical research, and since they represent real-life clinical practice, they may help to detect gaps or inadequacies in diagnosis and treatment.¹² In this context, we performed the present study with the aim of exploring the patterns of AATD diagnosis and their evolution over the last 5 years. The results will help to design programmes to increase awareness about the diagnosis of AATD.

Method

Study Design

This was a retrospective review of all AATD determinations carried out at the clinical laboratory of Vall d'Hebron Barcelona Hospital Campus. This central core laboratory processes one of the largest number of samples of all laboratories in the Spanish public health system (around 6000 patients and 25,000 samples daily). Moreover, it is also a reference laboratory for AATD and performs

phenotyping and genotyping of AATD samples from other hospitals in Catalonia. The data analysed include a 5-year period from January 2015 to December 2019. The main objective of this study was to analyse the trends in AATD diagnosis during the last 5 years by measuring the number of requests for AAT quantification in blood samples received at the central core laboratory and their results in terms of blood concentrations as well as the phenotype and/or genotype in samples demonstrating AATD. A secondary objective was to explore the medical specialities of the physicians sending samples and the reasons for AAT determinations.

Selection of Samples

All samples requesting AAT determination during the study period were included. In accordance to the results of AAT quantification, samples were classified as follows based on the reference values of our laboratory: normal (AAT levels ≥ 120 mg/dL), intermediate deficiency (AAT levels between 119 mg/dL and 50 mg/dL) and severe deficiency (AAT levels < 50 mg/dL), as indicated by Ferrarotti et al.¹³ All samples showing AAT deficiency were phenotyped by IEF using serum or plasma and the Hydragel 18 A1AT Isofocusing kit (Sebia, Evry, France).¹⁴ Genotyping was performed in case of discrepancy between AAT levels and the phenotype observed. PI*S, PI*Z and PI*Mmalton alleles were genotyped by an allele-specific genotyping assay using real-time polymerase chain reaction and specific probes.¹⁵ Discordant cases were analysed by sequencing the entire encoding region of the *SERPINA1* gene. Serum, plasma, whole blood or buccal swap samples were used indifferently for genotyping methods.¹⁶

Data about request origin and the reason for the request for AAT determination were collected, when available. Since one of the main consequences of AATD is liver disease, we also tested some biochemical markers of liver damage available in routine blood analysis, such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and calculated the Fibrosis-4 (FIB-4) score as $(\text{patient age} \times \text{AST (IU/L)}) / (\text{platelet count} \times 10^9/\text{L}) \times \text{ALT}^{-1/2}$ (IU/L).¹⁷ The FIB-4 index can accurately differentiate coinfecting human immunodeficiency virus/hepatitis C virus patients with no or moderate hepatic fibrosis (score < 1.45) from those with advanced fibrosis (score > 3.25).¹⁸ A recent study showed that this index also has prognostic implications in patients with COPD.¹⁹ However, the cutoff values for liver disease associated

with AATD remain undefined,²⁰ and therefore, we used the score described above to classify our patients.

As indications for AAT investigation may differ by age, the individuals studied were classified as children (<15 years of age) and adults (≥ 15 years old), according to the paediatric age in our centre, and were analysed separately.

Classification of Clinical Specialty of Requesting Physicians

Clinical specialties requesting AAT determinations in adults were classified into 5 groups: 1) respiratory disease, 2) digestive disease and internal medicine, 3) primary care, 4) external centres and 5) others. The respiratory disease group included the departments of pneumology, the respiratory system and cystic fibrosis. Digestive disease and internal medicine were included together because the Liver Unit is independent from the Digestive Diseases Department. This group also included specialists in the digestive system, gastroenterology, hepatobiliary surgery and liver transplant units. The primary care group included primary care centres of the Catalan Health Institute of the catchment area of our hospital. The external centres group included other hospitals in Catalonia that sent samples to our laboratory because AAT determination was not available. The remaining specialties were included in others.

Regarding children, all the samples were classified together in a group called paediatrics.

Reason for Requesting AAT Determination

The reasons for requesting AAT determination in adults were classified into 6 groups: 1) COPD, which included COPD, emphysema or bronchitis; 2) bronchiectasis, constituted by bronchiectasis and cystic fibrosis; 3) asthma; 4) liver disease, which included liver fibrosis, cirrhosis, hepatitis, liver failure, hepatocellular carcinoma (HCC), non-alcoholic fatty liver disease, transaminase, bilirubin or gamma-glutamyl transferase above reference values; 5) others, when the requests were related to pathologies not associated with AATD; and 6) unknown.

Regarding children, the reasons for requesting AAT determination were divided into the same 6 groups, but with two slight differences: the COPD group was changed to a group called bronchitis, which included bronchitis as a reason of requesting AAT determination. In the liver

disease group, in addition to the clinical manifestations described in adults, three AATD-associated complications which are clinically relevant in infants were added: cholestatic hepatitis, poor feeding and poor growth.

Statistical Analysis

Descriptive analysis of all the samples collected during the study period was performed separately for children and adults. Qualitative variables were described as absolute frequencies and/or the respective percentages. Mean and standard deviation were calculated for quantitative variables. Liver parameters, such as transaminases, and samples with above normal levels were presented as multiples of the upper limit of normal range (ULN) and percentages calculated based on the ranges established in our centre: AST (10–35 U/L) and ALT (7–35 U/L) for adults and AST (15–60 U/L) and ALT (7–35 U/L) for children. FIB-4 has been validated in adults older than 18 years, and therefore, it was calculated only in patients of this age group. Results are shown as percentages divided into three score ranges: <1.45, 1.45–3.25 and >3.25. Comparisons of percentages among groups were performed by the Chi-squared test. A p value < 0.05 was considered significant. Statistical analyses were performed with STATA version 14 (StataCorp, College Station, TX, USA).

Results

Trends in AAT Determinations

A total of 3507 serum AAT determinations were performed during the 5 years of the study, of which 192 (5.5%) corresponded to children (Figure 1). Of these, 2019 (57.6%) samples had intermediate AAT deficiency, with 1912 (54.5%) corresponding to adults and 107 (3.1%) to children. The number of samples with severe deficiency was 84 (2.4%), 72 (2.1%) of which were from adults and 12 (0.3%) from children.

In general, the number of AAT determinations showed a gradual increase during the study period (Table 1 and Figure 2). As shown in Table 2, the clinical specialty with the highest increase was primary care, leading to a 5.4-fold increase regarding the number of AAT determinations requested in 2015.

Phenotype/Genotype of Samples

Of a total of 2103 deficient samples, 1978 (94%; 1899 intermediate and 79 severe deficiency) were phenotyped and the remaining 125 (5.9%) could not be phenotyped/

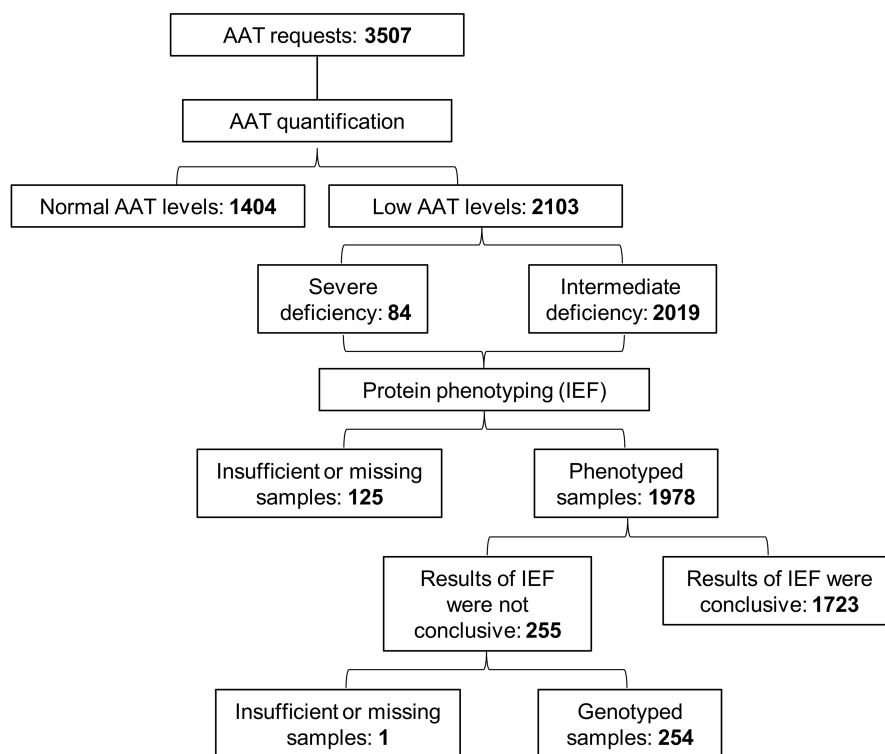


Figure 1 Diagnostic algorithm followed in the study and number of samples obtained in each step.
Abbreviations: AAT, alpha-1 antitrypsin; IEF, isoelectric focusing.

genotyped due to insufficient or missing samples. Of the total of 1978 samples phenotyped, 254 (13%) were genotyped, because the phenotyping results were not conclusive (Figure 1). PI*ZZ was the most frequent phenotype in

samples with severe deficiency (62, 78.5%), followed by 7 (8.9%) cases of the PI*SZ phenotype, 8 (10.1%) cases of rare or unknown mutations, 1 PI*SS and 1 PI*MM. The severe deficiency sample (46.1 mg/dL) showing a PI*MM

Table 1 Number of Alpha-1 Antitrypsin Determinations Performed, and Samples Detected with Deficiency by Year

	Period	Total AAT Determinations	Deficient Samples*		
			Total	Intermediate Deficiency	Severe Deficiency
Adults	2015	307	156 (50.8)	149 (48.5)	7 (2.3)
	2016	520	306 (58.8)	298 (57.3)	8 (1.5)
	2017	761	452 (59.4)	433 (56.9)	19 (2.5)
	2018	888	546 (61.5)	530 (59.7)	16 (1.8)
	2019	839	524 (62.5)	502 (59.8)	22 (2.6)
	Total adults	3315	1984 (59.8)	1912 (57.7)	72 (2.2)
Children	2015	42	19 (45.2)	18 (42.9)	1 (2.4)
	2016	44	26 (59.1)	25 (56.8)	1 (2.3)
	2017	44	29 (65.9)	27 (61.4)	2 (4.5)
	2018	29	20 (69)	18 (62.1)	2 (6.9)
	2019	33	25 (75.8)	19 (57.6)	6 (18.2)
	Total children	192	119 (62)	107 (55.7)	12 (6.3)
Total		3507	2103 (60)	2019 (57.6)	84 (2.4)

Note: *Percentages (%) refer to the total AAT determinations performed each year.

Abbreviation: AAT, alpha-1 antitrypsin.

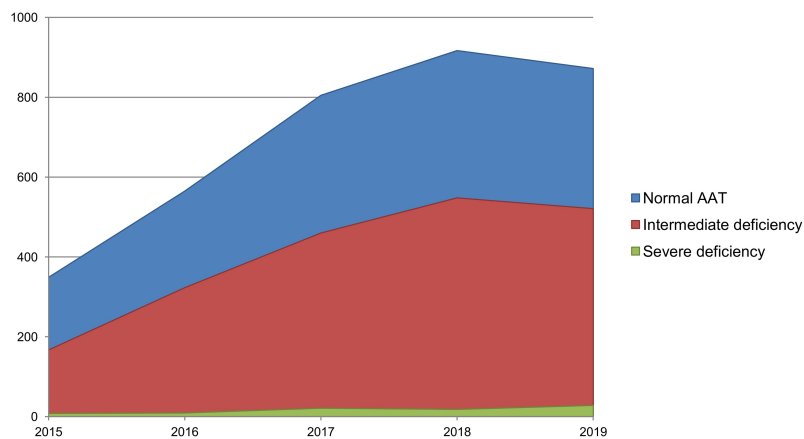


Figure 2 Trends in alpha-1 antitrypsin determinations during the study period.

phenotype was confirmed by sequencing of the four encoding exons of the *SERPINA1* gene. This sample corresponded to a child with severe liver failure not related to AATD; and the PI*SS by phenotype (47.6 mg/dL), corresponded to a sample from an external centre and could not be genotyped due to insufficient sample. Regarding intermediate deficiency, 802 (42.2%) were PI*MM and 302 (16%) PI*MZ. Interestingly, 68 (3.6%) had a rare or unknown genotype, and 10 (0.5%) were PI*ZZ. Out of these 10 PI*ZZ, 9 were patients on augmentation therapy with AAT concentrations between 53 and 74 mg/dL; these samples were sent for evaluation of trough AAT serum levels. The remaining sample came from an external centre (77 mg/dL) and no more information could be obtained. The phenotypes of the adults and children with AATD are shown in Table 3.

Liver Parameters

Serum aminotransferase quantification was requested in 2312 (69.7%) adults and 144 (75%) children. Up to 38.2% of children and 25.3% of adults had elevated AST levels,

and the percentages for ALT were 48.6% and 34.6%, respectively. However, the number of samples with increases >2 ULN were only 4.5% for AST and 9.6% for ALT in adults and 14.5% and 26.3% in children, respectively (Table 4).

The FIB-4 index was calculated in 2218 (66.9%) adults. Scores > 3.25 were observed in 3.5% of individuals with normal AAT levels, 5.6% of those with intermediate AATD ($p=0.023$ compared with normal AAT) and 9.3% of individuals with severe AATD ($p=0.031$ compared with normal AAT) (Figure 3). Samples coming from requests of specialists in digestive disease and internal medicine were those with a higher percentage of elevated aminotransferase values and FIB-4 scores > 3.25 (Table 5).

Clinical Specialties

The highest number of requests was received from specialists in respiratory diseases with 31.5% of total AAT determinations, closely followed by specialists in digestive diseases and internal medicine with 27.5% and primary care physicians with 19.7% (Figure 4). Blood samples from paediatric patients had the highest diagnostic yield

Table 2 Number of Alpha-1 Antitrypsin Determinations Performed by Each Clinical Specialty Every Year of the Study Period

Clinical Specialty	Total AAT Requested				
	2015	2016	2017	2018	2019
Respiratory disease	134	156 (1.2)	296 (2.2)	298 (2.2)	221 (1.6)
Digestive disease and internal medicine	78	152 (1.9)	204 (2.6)	275 (3.5)	257 (3.3)
Primary care	38	108 (2.8)	137 (3.6)	201 (5.3)	207 (5.4)
External centres	45	80 (1.8)	79 (1.8)	63 (1.4)	111 (2.5)
Paediatrics	42	44 (1)	44 (1)	29 (0.7)	33 (0.8)
Others	12	24 (2)	45 (3.8)	51 (4.3)	43 (3.6)

Note: Data are expressed as n (X-fold increase) regarding number of AAT determinations requested in 2015 by each clinical specialty.

Abbreviation: AAT, alpha-1 antitrypsin.

Table 3 Phenotypes Obtained in Adults and Children Classified into Intermediate and Severe Deficiency

Phenotype	Adults		Children	
	Intermediate Deficiency	Severe Deficiency	Intermediate Deficiency	Severe Deficiency
Common				
M/M	769 (42.8)	–	33 (32.7)	1
M/S	527 (29.3)	–	22 (21.8)	–
M/Z	282 (15.7)	–	20 (19.8)	–
S/Z	72 (4)	7	14 (13.8)	–
S/S	77 (4.3)	1	5	–
Z/Z	10 (0.6)	51 (76.1)	–	11 (91.7)
Rare				
M/Phe52del ^a	16 (0.9)	–	–	–
S/Phe52del	1	1	–	–
Z/Phe52del	–	4	–	–
M/I	17 (0.9)	–	–	–
S/I	3	–	–	–
M/PLowell	9	–	5	–
S/PLowell	2	–	–	–
Z/PLowell	–	2	–	–
Z/F	1	–	–	–
M/Q0ourém	4	–	–	–
M/Leu353Phe_fsX24 ^b	1	–	–	–
M/Q0clayton	4	–	2	–
M/Mvallhebron	1	–	–	–
S/Ybarcelona	1	–	–	–
Heterozygous u.m	1	1	–	–
Total	1798	67	101	12

Notes: Data higher than 10 are expressed as n (%) regarding total phenotyped/genotyped samples of each deficiency group. ^aPhe52del: Mmalton or Mpalermo variant (uncertain background); ^bLeu353Phe_fsX24: Q0mattawa or Q0ourém variant (uncertain background).

Abbreviation: u.m, uncharacterized mutation.

Table 4 Values of Serum Aminotransferases Expressed as Multiples of the Upper Limit of Normal and Classified into Normal AAT Levels, Intermediate or Severe Deficiency

Adults		N ^a	≤ 1.5 ULN		> 1.5 ULN		> 2 ULN		> 3 ULN		> 5 ULN	
			AST	ALT	AST	ALT	AST	ALT	AST	ALT	AST	ALT
	Normal AAT	818	765	733	53	85	27	52	15	28	6	12
	Intermediate deficiency	1438	1300	1147	138	291	76	169	27	70	12	20
	Severe deficiency	56	53	50	3	6	2	1	1	0	0	0
Children	Normal AAT	53	35	25	18	28	16	22	11	18	5	11
	Intermediate deficiency	79	71	59	8	20	3	12	3	6	2	2
	Severe deficiency	12	10	8	2	4	2	4	2	2	2	1

Note: N^aTotal of samples with aminotransferases in each deficiency group.

Abbreviations: AAT, alpha-1 antitrypsin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ULN, upper limit of normal.

for individuals with severe deficiency (6.3%), followed by samples referred by respiratory specialists, in which severe AAT was detected in 3.5% of individuals. Intermediate deficiency was found in 86% of AAT determinations

requested by specialists in digestive diseases and internal medicine. Table 6 shows the number of individuals characterised by each clinical specialty and classified into AAT normal levels, intermediate and severe deficiency.

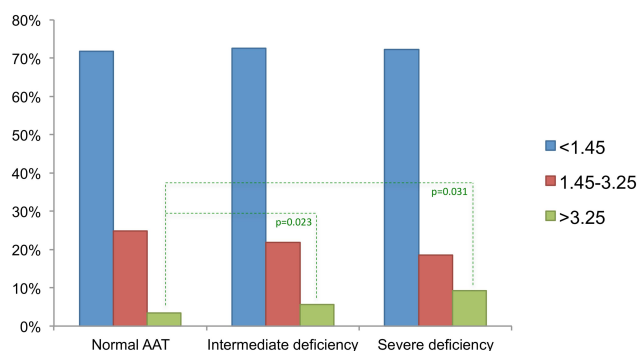


Figure 3 Percentage of adults with normal AAT levels, intermediate and severe deficiency divided into three score ranges of FIB-4 index: <1.45, 1.45–3.25 and >3.25.

Abbreviation: AAT, alpha-1 antitrypsin.

Reasons for Requesting AAT Determination

In adults, documented lung disease (COPD, asthma and bronchiectasis) was indicated as the reason for AAT determination in 17.1% of samples, followed by liver (16.1%), and unknown with 41.7%. In contrast, in children, documented lung disease accounted for 7.9%, liver 27.1% and unknown 58.3% (Figure 5). Samples from digestive and internal medicine had the highest frequency of unknown reasons (43%) and samples from primary care the highest percentage of others (39%).

Discussion

We report the trends in testing for AATD and the proportion of different clinical specialties requesting AAT determinations during the last five years in a Spanish reference centre specialized in AATD diagnosis and treatment. The results show that the number of AAT determinations performed in our catchment area has significantly increased during the 5 years of the study. All specialties contributed to this increase, but the largest increase was observed for samples from primary care. However, in most cases we could not identify the suspected diagnosis or the reason for requesting the test.

The Clinical Laboratory of the Vall d'Hebron Barcelona Hospital Campus is one of the largest laboratories in Spain

and receives analytical requests corresponding to around 6000 patients daily. Although AAT testing is only recommended once in a lifetime and the number of AAT determinations increased along the study period, we consider that around 3500 requests in five years are a low number compared to the number of samples processed in the lab and the clinical indications, which include highly prevalent diseases, such as COPD, adult asthma or liver disease. However, other large database studies have observed similar trends. Recently, Soriano et al²¹ analysed trends in AATD testing and diagnosis in primary care between 1990 and 2014 in the United Kingdom. They found the incidence of AATD diagnosis to have generally increased, but they also identified a low frequency of AATD testing, in which only 2.2% of patients diagnosed with COPD before the age of 60 years were tested. In our area, Barrecheguren et al²² identified an increase in AATD testing in primary care over two periods (2007–2008 and 2010–2011). However, the study showed that the number of AAT determinations performed was low, ranging from 4.33 per 10,000 inhabitants in 2007 to 6.85 per 10,000 in 2008, with no increase after 2008. Similar to our results, the reasons for requesting AAT determination remained unclear in most cases, and only 13% of the adults tested had AATD-associated respiratory diseases.

We observed a large increase in AAT determinations during the study period with a total of 84 (2.4%) individuals being diagnosed with severe deficiency. Considering that the prevalence of severe AATD (specifically PI*ZZ) among the general population in Spain is only 0.03%²³ and among COPD patients in Europe it is 0.12%,²⁴ a detection rate of 2.4% for severe deficiency can be considered a good diagnostic yield. Moreover, more than half (57.6%) of the individuals tested carried an intermediate deficiency, which is highly relevant for the individual in order to prevent disease progression and to initiate family screening for early detection of affected relatives.

Although AATD is one of the most common congenital disorders, it still remains significantly underdiagnosed.^{1,25} A number of reasons may explain this, including differences in testing methods and the interpretation of results

Table 5 Elevated Aminotransferases and FIB-4 Index Regarding the Clinical Specialties of Requesting Physicians

Clinical Specialty	AST >35 (U/L)	ALT >35 (U/L)	FIB-4 >3.25
Respiratory disease	95/790 (12%)	103/790 (13%)	18/770 (2.3%)
Digestive disease and internal medicine	328/942 (34.8%)	442/942 (46.9%)	74/912 (8.1%)
Primary care	124/438 (28.3%)	207/438 (47.3%)	6/402 (1.5%)

Note: Data are expressed as number of samples with elevated liver parameters divided by total of samples for each clinical specialty.

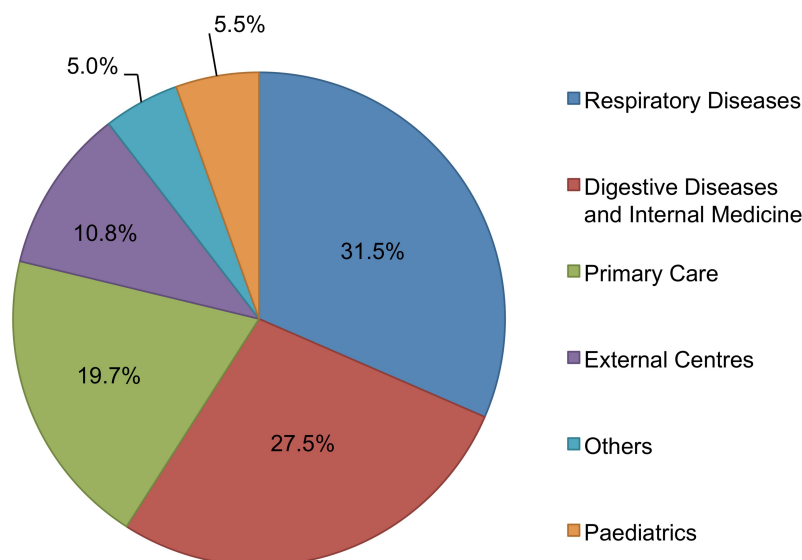


Figure 4 Percentage of alpha-1 antitrypsin determinations requested by different clinical specialties.

and poor awareness of the disease. Studies from different countries found low knowledge and unsatisfactory practice related to AATD by physicians at all levels, from primary care to internal medicine specialists and pulmonologists.^{26,27} In a survey carried out in Spain and Portugal,²⁸ physicians reported referring patients to other specialists and the erroneous perception of the high cost of testing as the main reasons for not testing for AATD. However, the increasing requests for AATD diagnosis obtained in our study and others suggest that awareness about AATD is probably increasing and barriers to AATD testing are progressively being overcome. This might be due to more detection programs,²⁹ the use of simpler testing approaches, such as buccal swabs or rapid point-of-care testing,³⁰ educational programmes provided by reference centres and electronic alerts to encourage guideline-based testing for AATD.^{31,32}

During the study period, 1978 samples were phenotyped and/or genotyped. As expected, homozygous PI*ZZ made up the largest number of individuals with severe deficiency, followed by PI*SZ and heterozygous carrying Z/rare variants. In individuals with intermediate deficiency, the main phenotypes detected were PI*MM, PI*MS PI*MZ, and the combination of a rare allele with the normal M variant was the predominant rare phenotype observed. These results concur with the retrospective revision of 3511 AATD determinations performed in 2012, in which 1.6% of samples carried rare AAT alleles³³ and among these variants, PI*I and PI*Mmalton represented 54% of cases. Interestingly, the number of rare variants detected increased in our study to 3.8% due to the higher number of genotyping tests performed, but we observed exactly the same proportion of the most frequent rare alleles PI*I and PI*Mmalton, which again accounted for 54.7% of all rare AATD alleles characterised.

Table 6 Number of Alpha-1 Antitrypsin Determinations Requested by Each Clinical Specialty Divided into Normal AAT Levels, Intermediate and Severe Deficiency

Clinical Specialty	Total AAT Tests Requested	Normal AAT	Intermediate Deficiency	Severe Deficiency
Respiratory disease	1105	720 (65.2)	346 (31.3)	39 (3.5)
Digestive disease and internal medicine	966	121 (12.5)	831 (86)	14 (1.4)
Primary care	691	221 (32)	463 (67)	7 (1)
External centres	378	195 (51.6)	174 (46)	9 (2.4)
Paediatrics	192	73 (38)	107 (55.7)	12 (6.3)
Others	175	74 (42.3)	98 (56)	3 (1.7)

Note: Data are expressed as n (%) regarding total number of AAT determinations requested by each clinical specialty.

Abbreviation: AAT, alpha-1 antitrypsin.

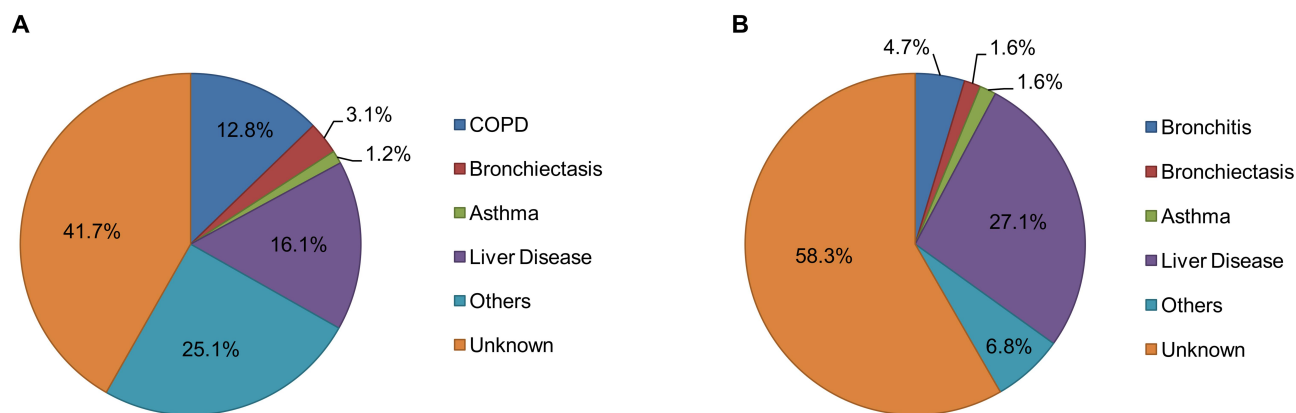


Figure 5 Percentage of reasons for requesting alpha-1 antitrypsin determination in (A) adults and (B) children.

Serum aminotransferase levels were elevated in a large number of samples; however, the number of samples with clinically significant increases was below 10% in adults and around 25% in children, reflecting the different reasons for AAT determinations, with predominant liver disease in children. The percentages of individuals with a FIB-4 index > 3.25 rose according to the severity of AATD. These data suggest that FIB-4 might be sensitive to liver damage in AATD, but larger prospective studies are needed to confirm this.²⁰

The Spanish Registry of Patients with Alpha-1 Antitrypsin Deficiency (REDAAT), mainly constituted by respiratory specialists, has recently established a diagnostic circuit with direct genotyping of buccal swabs.^{34,35} This circuit may have reduced the number of requests of blood analyses by respiratory specialists, which may account at least in part for the lack of increase in the number of determinations during the last year in our study. It is also of note that almost no samples were referred from family screening, because most family screenings in our centre are conducted by direct genotyping from buccal swabs.³⁴

Our study has some limitations: first, in many cases, we could not identify the reasons for requesting the determination. Second, we have used a threshold of 50 mg/dL as indicated by Ferrarotti et al¹³ and as used in previous reports by our group,^{5,22} however, this threshold is arbitrary, and other authors have suggested values around 57 mg/dL. Third, we could not correct AAT serum levels by level of inflammation demonstrated by simultaneous quantification of CRP.^{36–39} With our diagnostic protocol, all PI*ZZ or null or rare alleles would be identified, irrespective of the simultaneous determination of CRP levels, but we might miss

some heterozygotes that could show normal AAT serum levels due to increased inflammation.

Conclusion

Our study shows that the frequency of AATD testing is on the rise, despite the rate of AAT determinations in our large population (1.5 million inhabitants) remaining low. While the available data suggest that awareness about AAT is probably increasing, physicians continue to face hurdles in the testing and diagnosis of patients. An increase in physician education about AATD, regular reminders and the use of simpler testing approaches would help to overcome the barriers to AAT testing.

Ethics Approval and Informed Consent

The study was approved by the Ethics Committee of the Vall d'Hebron Hospital (Barcelona, Spain). As this was a retrospective study and all samples were anonymized, informed consent was not considered necessary.

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References

- Miravittles M, Dirksen A, Ferrarotti I, et al. European respiratory society statement: diagnosis and treatment of pulmonary disease in alpha-1 antitrypsin deficiency. *Eur Respir J*. 2017;50:1700610. doi:10.1183/13993003.00610-2017
- Greene CM, Marciniak SJ, Teckman J, et al. α 1-antitrypsin deficiency. *Nat Rev Dis Primers*. 2016;16052.
- Lomas DA, Finch JT, Seyama K, Nukiwa T, Carrell RW. Alpha 1-antitrypsin Siiyama (Ser53→Phe). Further evidence for intracellular loop-sheet polymerization. *J Biol Chem*. 1993;268:15333–15335.
- Lomas DA, Elliott PR, Sidhar SK, et al. Alpha 1-antitrypsin mmalton (Phe52-deleted) forms loop-sheet polymers in vivo. Evidence for the C sheet mechanism of polymerization. *J Biol Chem*. 1995;270:16864–16870. doi:10.1074/jbc.270.28.16864
- Miravittles M, Herr C, Ferrarotti I, et al. Laboratory testing of individuals with severe AAT deficiency in three European centres. *Eur Respir J*. 2010;35:960–968. doi:10.1183/09031936.00069709
- McElvaney NG. Diagnosing α 1-antitrypsin deficiency: how to improve the current algorithm. *Eur Respir Rev*. 2015;24:52–57. doi:10.1183/09059180.10010814
- Barker A, Brantly M, Campbell E, Carrell R, Cox DW, Dirksen AE. Alpha 1-antitrypsin deficiency: memorandum from a WHO meeting. *Bull World Health Organ*. 1997;75(5):397–415.
- Casas F, Blanco I, Martínez MT, et al. 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):185–192. doi:10.1016/j.arbres.2014.05.008
- 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. doi:10.2147/COPD.S125389
- Lara B, Blanco I, Martínez MT, et al. Spanish registry of patients with alpha-1 antitrypsin deficiency: database evaluation and population analysis. *Arch Bronconeumol*. 2017;53:13–18. doi:10.1016/j.arbres.2016.05.003
- Stoller JK. Detecting alpha-1 antitrypsin deficiency. *Ann Am Thorac Soc*. 2013;13(Suppl4):S317–S325. doi:10.1513/AnnalsATS.201506-349KV
- Miravittles M, Monteagudo M, Solntseva I, Alcázar B. Blood eosinophil counts and their variability and risk of exacerbations in COPD: a population-based study. *Arch Bronconeumol*. 2020;12:pii: S0300–2896(19)30623–4. doi:10.1016/j.arbres.2019.12.015
- Ferrarotti I, Thun GA, Zorzetto M, et al. Serum levels and genotype distribution of α 1-antitrypsin in the general population. *Thorax*. 2012;67:669–674. doi:10.1136/thoraxjnl-2011-201321
- Zerimech F, Hennache G, Bellon F, et al. Evaluation of a new Sebia isoelectrofocusing kit for alpha 1-antitrypsin phenotyping with the hydrasys system. *Clin Chem Lab Med*. 2008;46(2):260–263. doi:10.1515/CCLM.2008.036
- Belmonte I, Montoto L, Miravittles M, et al. Rapid detection of mmalton α 1-antitrypsin deficiency allele by real-time PCR and melting curves in whole blood, serum and dried blood spot samples. *Clin Chem Lab Med*. 2016;54:241–248. doi:10.1515/cclm-2015-0297
- Belmonte I, Barrecheguren M, Esquinas C, Rodríguez E, Miravittles M, Rodríguez-Frias F. Genetic diagnosis of alpha1-antitrypsin deficiency using DNA from buccal swab and serum samples. *Clin Chem Lab Med*. 2017;55(9):1276–1283. doi:10.1515/cclm-2016-0842
- Sterling RK, Lissen E, Clumeck N, et al. Development of a simple non-invasive index to predict significant fibrosis patients with HIV/HCV co-infection. *Hepatology*. 2006;43:1317–1325. doi:10.1002/hep.21178
- Vallet-Pichard A, Mallet V, Nalpas B, et al. FIB-4: an inexpensive and accurate marker of fibrosis in HCV infection. Comparison with liver biopsy and fibrotest. *Hepatology*. 2007;46(1):32–36. doi:10.1002/hep.21669
- Yong SH, Leem AY, Kim YS, et al. Hepatic fibrosis assessed using fibrosis-4 index is predictive of all-cause mortality in patients with chronic obstructive pulmonary disease. *Int J Chron Obstruct Pulmon Dis*. 2020;15:831–839. doi:10.2147/COPD.S242863
- Strnad P, McElvaney NG, Lomas DA, Longo DL. Alpha1-antitrypsin deficiency. *N Engl J Med*. 2020;382(15):1443–1455. doi:10.1056/NEJMra1910234
- Soriano JB, Lucas SJ, Jones R, et al. Trends of testing for and diagnosis of α 1-antitrypsin deficiency in the UK: more testing is needed. *Eur Respir J*. 2018;52(1):1800360. doi:10.1183/13993003.00360-2018
- Barrecheguren M, Monteagudo M, Simonet P, et al. Diagnosis of alpha-1 antitrypsin deficiency: a population-based study. *Int J Chron Obstruct Pulmon Dis*. 2016;11:999–1004.
- Blanco I, de Serres FJ, Fernandez-Bustillo E, Lara B, Miravittles M. Estimated numbers and prevalence of Pi*S and Pi*Z alleles of alpha1-antitrypsin deficiency in European countries. *Eur Respir J*. 2006;27:77–84. doi:10.1183/09031936.06.00062305
- Blanco I, Diego I, Bueno P, Pérez-Holanda S, Casas-Maldonado F, Miravittles M. Prevalence of alpha-1 antitrypsin genotypes in patients with COPD in Europe: a systematic review. *Eur Respir Rev*. 2020;29:200014. doi:10.1183/16000617.0014-2020
- Meira L, Boaventura R, Seixas S, Sucena M. Alpha-1 antitrypsin deficiency detection in a Portuguese population. *COPD*. 2018;15(1):4–9. doi:10.1080/15412555.2017.1414779
- Greulich T, Ottaviani S, Bals R, et al. Alpha1-antitrypsin deficiency - diagnostic testing and disease awareness in Germany and Italy. *Respir Med*. 2013;107:1400–1408.
- Taliercio RM, Chatburn RL, Stoller JK. Knowledge of alpha-1 antitrypsin deficiency among internal medicine house officers and respiratory therapists: results of a survey. *Respir Care*. 2010;55(3):322–327.

28. Esquinas C, Barrecheguren M, Sucena M, Rodríguez E, Fernandez S, Miravittles M. Practice and knowledge about diagnosis and treatment of alpha-1 antitrypsin deficiency in Spain and Portugal. *BMC Pulm Med.* 2016;16:64. doi:10.1186/s12890-016-0222-4
29. de la Roza C, Rodríguez-Frias F, Lara B, Vidal R, Jardi R, Miravittles M. Results of a case-detection programme for alpha-1-antitrypsin deficiency in COPD patients. *Eur Respir J.* 2005;26:616–622. doi:10.1183/09031936.05.00007305
30. Greulich T, Rodríguez-Frias F, Belmonte I, Klemmer A, Vogelmeier CF, Miravittles M. Real world evaluation of a novel lateral flow assay (AlphaKit[®] QuickScreen) for the detection of alpha-1-antitrypsin deficiency. *Respir Res.* 2018;19:151. doi:10.1186/s12931-018-0826-8
31. Jain A, McCarthy K, Xu M, et al. Impact of a clinical decision support system in an electronic health record to enhance detection of alpha 1 antitrypsin deficiency. *Chest.* 2011;140(1):198–204. doi:10.1378/chest.10-1658
32. Molina J, Flor X, Garcia R, Timiraos R, Tirado-Conde G, Miravittles M. 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.* 2011;5:237–243. doi:10.1177/1753465811404919
33. Rodríguez-Frias F, Miravittles M, Vidal R, Camos S, Jardi R. Rare alpha-1-antitrypsin variants: are they really so rare? *Ther Adv Respir Dis.* 2012;6(2):79–85. doi:10.1177/1753465811434320
34. Lopez-Campos JL, Casas-Maldonado F, Torres-Duran M, et al. Results of a diagnostic procedure based on multiplex technology on dried blood spots and buccal swabs for subjects with suspected alpha-1 antitrypsin deficiency. *Arch Bronconeumol.* 2020;14:S0300–2896(20)30131–9. doi:10.1016/j.arbres.2020.04.014
35. Barrecheguren M, Torres-Duran M, Casas-Maldonado F, Miravittles M. Spanish implementation of the new international alpha-1 antitrypsin deficiency international registry: the European Alpha-1 Research Collaboration (EARCO). *Arch Bronconeumol.* 2020;pii:S0300–2896(20)30061–2. doi:10.1016/j.arbres.2020.02.003
36. Sanders CL, Ponte A, Kueppers F. The effects of inflammation on alpha 1 antitrypsin levels in a National Screening Cohort. *COPD.* 2018;15:10–16. doi:10.1080/15412555.2017.1401600
37. Janciauskiene S, DeLuca DS, Barrecheguren M, Welte T, Miravittles M. Serum levels of alpha-1-antitrypsin and their relationship with COPD in the general Spanish population. *Arch Bronconeumol.* 2020;56:76–83. doi:10.1016/j.arbres.2019.03.001
38. Ellis P, Turner A. What do alpha-1 antitrypsin levels tell us about chronic inflammation in COPD? *Arch Bronconeumol.* 2020;56(2):72–73. doi:10.1016/j.arbres.2019.06.010
39. Kueppers F. PredictAAT: accounting for inflammation in the diagnosis of alpha 1 antitrypsin deficiency. *COPD.* 2020;1–4. doi:10.1080/15412555.2020.1812055

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