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REVIEW ARTICLE

Update on and future perspectives for the diagnosis of alpha-1 antitrypsin deficiency in Brazil

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ABSTRACT

Alpha-1 antitrypsin deficiency (AATD) is a rare genetic disorder caused by a mutation in the *SERPINA1* gene, which encodes the protease inhibitor alpha-1 antitrypsin (AAT). Severe AATD predisposes individuals to COPD and liver disease. Early diagnosis is essential for implementing preventive measures and limiting the disease burden. Although national and international guidelines for the diagnosis and management of AATD have been available for 20 years, more than 85% of cases go undiagnosed and therefore untreated. In Brazil, reasons for the underdiagnosis of AATD include a lack of awareness of the condition among physicians, a racially diverse population, serum AAT levels being assessed in a limited number of individuals, and lack of convenient diagnostic tools. The diagnosis of AATD is based on laboratory test results. The standard diagnostic approach involves the assessment of serum AAT levels, followed by phenotyping, genotyping, gene sequencing, or combinations of those, to detect the specific mutation. Over the past 10 years, new techniques have been developed, offering a rapid, minimally invasive, reliable alternative to traditional testing methods. One such test available in Brazil is the A1AT Genotyping Test, which simultaneously analyzes the 14 most prevalent AATD mutations, using DNA extracted from a buccal swab or dried blood spot. Such advances may contribute to overcoming the problem of underdiagnosis in Brazil and elsewhere, as well as being likely to increase the rate detection of AATD and therefore mitigate the harmful effects of delayed diagnosis.

Keywords: alpha 1-antitrypsin deficiency/diagnosis; alpha 1-antitrypsin deficiency/genetics; Genotyping techniques.

INTRODUCTION

Alpha-1 antitrypsin deficiency (AATD) is a rare genetic disorder, albeit the most common hereditary disorder in adults.[1,2] The mutation originates in the *SERPINA1* gene, which encodes alpha-1 antitrypsin (AAT), the most abundant protease inhibitor in human serum.[1] AATD is characterized by a reduction in serum AAT concentrations and is associated with an increased risk of lung disease (e.g., COPD, bronchiectasis), liver disease (e.g., chronic hepatitis, cirrhosis), and other less common conditions.[3-5]

AAT is a member of the serine protease inhibitor superfamily.[6,7] Synthesized mainly by hepatocytes (≥ 80%), AAT is also found in the lung, kidney, and intestine.[8] The main function of AAT is to inhibit neutrophil elastase to protect the lung from excessive proteolytic degradation of elastin and other connective tissue components, as well as from external factors, such as smoking.[6,7] AAT also inhibits numerous other proteolytic enzymes, providing more than 90% of the antiprotease capacity in serum.[6,7] Evidence in recent years has indicated that AAT also has broad-spectrum anti-inflammatory, immunomodulatory, and antimicrobial properties.[6,7]

Early diagnosis of AATD is a priority because it enables implementation of preventive measures, such as avoidance of smoking and of exposure to environmental pollutants, and identifies candidates for therapeutic intervention.[9] Early diagnosis can modify the natural history of AATD and dramatically improve patient outcomes.[10] In clinical practice, however, AATD is largely underdiagnosed due to low clinical suspicion, as well as lack of knowledge about the disease and of appropriate diagnostic tests.[11-13] An estimated 85% of individuals with AATD go undiagnosed,[11] and a significant proportion of individuals are diagnosed at advanced age after years of symptoms and multiple physician visits.[12]

The Latin American Project for the Investigation of Obstructive Lung Disease[14] found spirometric evidence

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of persistent airflow obstruction in 15.8% of the sampled population in Brazil (963 adults > 40 years of age in the city of São Paulo), of whom 12.5% had never been exposed to tobacco smoke, suggesting that other risk factors (e.g., AATD) may have been involved and undiagnosed. Reasons for underdiagnosis of AATD in Brazil include a lack of awareness of the condition among physicians, particularly because a laboratory diagnosis is the only method of identifying AATD in individuals with COPD[15]; a racially diverse population, which may cause individuals of European ancestry, who have a higher frequency of alleles involved in early lung changes, to be overlooked[16]; and, until recently, the lack of rapid and convenient diagnostic methods.[9]

This review study provides an update on the diagnosis of AATD, including tools available in Brazil, and features a diagnostic algorithm that may assist in confirming suspected cases of AATD.

GENETICS

The *SERPINA1* gene is located on the long arm of chromosome 14 (14q31-32) and is transmitted by simple autosomal codominant Mendelian inheritance through two alleles, one from each parent.[6,7] Approximately 125 variants of the *SERPINA1* gene have been identified which, for clinical purposes, are classified as normal, deficient, null, and dysfunctional.[7]

The normal allele is Pi*M. The most common deficiency alleles are Pi*S and Pi*Z, which encode abnormal proteins that undergo polymerization in the liver. The normal genotype Pi*MM is present in approximately 80-95% of the population and expresses 100% of serum AAT. The five deficient genotypes (Pi*MS, Pi*SS, Pi*MZ, Pi*SZ, and Pi*ZZ) are present in the remaining 5-20% of the population and express 80%, 60%, 55%, 40%, and 15% of serum AAT, respectively.[4] In addition, there are about 25 rare deficiency alleles that express low amounts of AAT, and 25 null alleles that express undetectable amounts (< 1%) of AAT.[7] Recent studies have indicated that certain epigenetic mechanisms may account, at least in part, for differences in the clinical expression of lung disease in patients with the deficient PI*ZZ and PI*SZ genotypes.[17,18]

EPIDEMIOLOGY

AATD affects mainly Whites of European heritage. The estimated prevalence of the most common severe genotype (Pi*ZZ) is 1:2,000-5,000 individuals in Europe, and 1:5,000-7,000 individuals of European descent residing in countries such as Canada, the United States, Australia, and New Zealand.[19] Epidemiological studies estimate Pi*Z genotype frequencies by using cohort and prevalence studies to develop inverse distance weighted interpolation maps that provide information about genotype distribution worldwide. According to this method, there are an estimated 6,000 individuals with the Pi*ZZ genotype in Brazil.[7,20] Another perspective is to determine the proportion of patients with COPD who are affected by AATD. A recent epidemiological study reported that the prevalence of Pi*ZZ/prevalence of COPD ratio in Europe was 0.12% (0.08-0.24%), differences being wide among the countries.[21] Numbers may be even higher in other countries; in Argentina, for example, the prevalence of AATD (Pi*ZZ or Pi*SZ) among COPD patients > 40 years of age was found to be 0.83%.[22]

Due to the absence of specific studies, little is known about the epidemiology of "rare" and "null" AATD alleles,[7] which may be more prevalent than previously assumed. A retrospective review of 3,511 AATD genetic studies performed in the laboratory of the Spanish Registry of Patients with Alpha-1 Antitrypsin Deficiency, from 1998 to 2010, detected 1.6% of cases with rare AAT alleles, most commonly Pi*I and Pi*Mmalton.[23]

Brazil has a racially diverse population that includes immigrants from European countries. Although epidemiological data on the prevalence of AATD in the general population in Brazil are lacking,[24] a cross-sectional study involving 926 COPD patients from five different regions of Brazil found an overall prevalence of 2.8% for AATD and 0.8% for the Pi*ZZ genotype.[16] These figures align with estimates that severe AATD is responsible for 0.1% to 1% of COPD cases[21,22] and reinforce the need for vigilance and increased screening for AATD in the population with COPD in Brazil.[16]

CLINICAL MANIFESTATIONS

AATD predisposes patients to various diseases; low serum AAT levels, other genetic characteristics, and environmental influences contribute to disease development and progression.[25] The major clinical manifestations of severe AATD are lung disease (emphysema) and liver disease (chronic hepatitis, cirrhosis, and hepatoma). Lung disease occurs when serum AAT levels are insufficient to overcome the relatively excessive action of neutrophil elastase—the so-called 'protease-antiprotease imbalance'—which results in degradation of elastin and other extracellular matrix components of the lower respiratory tract.[4] Liver disease occurs as a complication of intrahepatocytic accumulation of unsecreted, polymerized AAT.[4] Less common conditions associated with AATD include neutrophilic panniculitis and systemic vasculitis (typically granulomatosis with polyangiitis).[4,25-27]

In patients with AATD-associated lung disease, the most common physiological impairment is chronic airflow obstruction, demonstrated by a post-bronchodilator FEV_1/FVC ratio < 0.7, reduced FEV_1, and decreased DLCO. Air trapping is common, and a degree of hypoxemia may be present, even in mild or moderate cases. Emphysema in AATD is predominantly located in the lower lobes, although it may be found in the upper lobes in some individuals.

[28] Patients with the most severe forms of AATD have airflow obstruction and reduced DLCO; the decline in DLCO is greater than is that in FEV_1 in severe disease, and, therefore, DLCO might be a more appropriate test for patient follow-up.[29] Given the heterogeneity of the clinical and functional expression of AATD, initial assessment of the lung disease associated with AATD must include complete evaluation of the respiratory physiology, exercise capacity, symptom intensity, and disease impact, as well as performance of HRCT of the chest. Blood gas analysis may be part of a more comprehensive evaluation in certain cases in which oxygen saturation is low.[28]

Liver disease associated with the Pi*ZZ phenotype has two forms of presentation, one in early childhood (e.g., neonatal cholestasis) and one in adulthood, when some individuals (not necessarily those with previous liver disease during childhood) develop chronic liver disease that progresses to fibrosis.[4] An analysis of the 2019 Swedish registry data found a prevalence of any liver disease of 10% among 1,595 Pi*ZZ individuals.[30] Male gender, age over 50 years, and repeatedly elevated liver function test results were consistently associated with an increased risk of liver disease in adulthood.[30,31] Previously, a retrospective study[32] based on 17 autopsied individuals diagnosed with AATD in the city of Malmö, Sweden, between 1963 and 1982, found a prevalence of cirrhosis of 41% and a prevalence of primary liver cancer of 29%. The significantly higher risk in males suggested a possible additive effect of exogenous factors (e.g., alcohol consumption and exposure to occupational toxins).

CLINICAL SUSPICION

The risk of developing lung and liver disease varies according to the AATD genotype (homozygous or heterozygous combinations of deficient and null alleles). Individuals with serum AAT levels < 50 mg/dL (< 11 μM) are at a higher risk of pulmonary disease, the majority (> 90%) being Pi*ZZ homozygotes or having rare or null genotypes.[25] For reasons yet to be clear, 30-50% of the individuals with the PI*ZZ genotype do not develop lung disease during their lifetime or have only minor symptoms. This variable disease expressivity, not accounted for by risk factors such as smoking, suggests the presence of as yet unidentified genetic disease modifiers.[17,18] The risk of liver disease is higher in individuals who are homozygous or heterozygous for alleles associated with intrahepatocyte polymerization (e.g., Z, Mmalton, and Siiyama).[27,31]

The time to the onset of respiratory symptoms in AATD varies considerably, but, in general, symptoms tend not to appear before adulthood. The decline in pulmonary function depends on factors such as exposure to tobacco smoke or environmental pollutants, occupational exposure to toxins, coexisting asthma, lower respiratory tract infections, and predisposing family factors.[25,33] Although respiratory symptoms may appear in smokers about 35 years of age and nonsmokers about 45 years of age, in the real-world setting, the average age at the diagnosis of AATD is usually above 50-55 years, irrespective of smoking history.[34] Primary symptoms are dyspnea on exertion, wheezing, and increased cough and phlegm.[25,33]

A lack of awareness of AATD is the major barrier to diagnosis. Because clinical manifestations of AATD-related lung disease are indistinguishable from those of COPD, a laboratory diagnosis is required.[35] The WHO and scientific societies, such as the American Thoracic Society, the European Respiratory Society, and the Spanish Society of Pulmonology and Thoracic Surgery, as well as the Spanish Guidelines for COPD and the GOLD, recommend that all COPD patients be tested for AATD at least once in their lifetime regardless of their smoking history or age.[4,10,36-38] Other candidates for AATD testing are patients with bronchiectasis, severe bronchial asthma showing progressive bronchial obstruction or evidence of pulmonary emphysema, unexplained liver disease at any age, systemic vasculitis, or neutrophilic panniculitis (Chart 1).[2,25] Predispositional testing should be undertaken in first degree relatives (siblings, children, and parents) and partners (for family genome purposes) of individuals with AATD.[4,10,36-38]

The underdiagnosis of AATD in Brazil highlights the need for testing COPD patients in accordance with recommendations of international guidelines.[9] Three studies undertaken in Brazil found that systematic screening for AATD in COPD patients increased the chances of identifying patients with mutations in the *SERPINA1* gene.[16,39,40]

LABORATORY DIAGNOSIS

Standard diagnostic methods

The standard approach to diagnosing AATD centers around determining AAT concentration in blood, usually by nephelometry, and then identifying specific alleles by studying the phenotype and/or genotype.[2,5,41]

The reference value for serum AAT level determined by nephelometry in healthy adults is 116-232 mg/dL (21-41 μmol/L).[42] However, because AAT is an acute phase reactant, along with C-reactive protein (CRP) and amyloid A, its plasma levels increase in response to inflammatory or infectious stimuli.[6,7,25] Moreover, because COPD is associated with systemic inflammation, AAT levels can be elevated in COPD patients when compared with age-matched controls, thus increasing the challenges of identifying possible heterozygotes among the COPD population.[43,44] Although the presence of inflammation does not generally influence a diagnosis of AATD in Pi*ZZ homozygotes, in order not to miss carriers or other patients with a deficiency, it may be useful to take a more general approach by measuring CRP and AAT levels at the same time. A normal level of CRP confirms that AAT levels are true and not falsely elevated. If the

Chart 1. Candidates for determination of alpha-1 antitrypsin levels.[a]

Individuals with COPD
Adults with bronchiectasis in whom the most common causes have been ruled out
Adults with bronchial asthma who develop progressive bronchial obstruction or show evidence of pulmonary emphysema
Blood relatives of patients with diagnosed AATD
Individuals with many family members presenting with dyspnea and chronic cough
Individuals with liver disease of unknown cause
Individuals in whom protein profile analysis shows absence of alpha-1 glycoprotein peak
Individuals with panniculitis or vasculitis of unknown cause

Adapted from Miravitlles et al.[2] and the Portuguese consensus document for the management of alpha-1-antitrypsin deficiency.[25] AATD: alpha-1 antitrypsin deficiency. [a]Routine determination of serum AAT levels is not recommended.

level of CRP is increased, AAT levels may be falsely elevated, which requires a repeat measurement of AAT levels under conditions of clinical stability.[45] A simple and practical recommendation is to measure AAT concentrations when the patient is free from inflammation or infection.

Protein phenotyping uses isoelectric focusing (IEF) electrophoresis to identify the most common AAT variants (S, Z, M, and others) present in the sample. Although IEF is the biochemical gold standard for detecting AATD variants, it requires significant expertise in interpretation and has limitations.[46] Most notably, neither does the method identify all pathological mutations present in the sample, nor does it identify null variants that produce no protein. In cases when a phenotype study does not permit a diagnosis (e.g., null, rare, and very rare variants), genotyping must be performed.

Genotyping uses PCR probes to identify the most common AATD alleles, mainly S and Z, but also others depending on available primers. Gene sequencing may be necessary in cases when a null or deficient variant other than S and Z is suspected.[2,5] Rapid genotyping methods can be used to search for the most common alleles Pi*S and Pi*Z, although misdiagnosis is possible because the methods do not include rare and null alleles.[2] Molecular analysis with direct sequencing of the *SERPINA1* gene can be used in order to identify rare alleles and null variants and to characterize new variants.[2] The technique involves complete analysis of DNA sequences of AAT-coding exons on the *SERPINA1* gene. Occasionally, it may also be necessary to study the intronic and regulatory sequences of the gene.[2,47,48]

In Brazil, some groups have proposed that AATD screening be included in the heel prick test performed routinely in newborns for conditions such as cystic fibrosis and sickle cell anemia, although opponents of the proposal argue that routine AAT measurements in newborns identifying a genetic deficiency could place a psychological burden on the affected children.

New diagnostic methods

New diagnostic methods have been developed and offer a simpler and more portable alternative to plasma/serum samples to conduct AATD testing. For example, dried blood spot specimens provide enough sample to measure AAT levels and to perform IEF electrophoresis phenotyping, providing a sufficient quantity and quality of DNA to detect Pi*S and Pi*Z alleles in a single real-time PCR and direct sequencing.[49] In Spain, a nationwide AATD case detection program conducted with COPD patients using dried blood spot specimens concluded that the screening method was feasible, simple, quick, and cost-efficient for use in this at-risk population.[50,51]

In Brazil, the dried blood spot method for measuring AAT concentrations was developed in 2011. In 2013, an immunonephelometric assay was validated to be used in serum samples and dried blood spots from COPD patients.[52] The cutoff point of 2.02 mg/dL (97% CI: 1.45-2.64 mg/dL) for dried blood spots had a sensitivity of 100%, a specificity of 95.7%, a positive predictive value of 27.2%, and a negative predictive value of 100% for establishing a diagnosis of AATD. Using the maximum value in the confidence interval as a cutoff point reduced the possibility of false-negative results. Although there was only a moderate correlation (r = 0.45) between AAT levels in serum samples and dried blood spots, it was concluded that dried blood spots were a useful alternative to serum samples to screen patients for AATD in Brazil, because the method provides rapid and minimally invasive screening at a low cost.[52]

New genotyping methods, such as the A1AT Genotyping Test (Progenika Biopharma S.A., Derio, Spain), include the analysis of rare and null alleles. This point-of-care test enables simultaneous detection and identification of the 14 most common allelic variants and their associated alleles in exons II, III, and V of the *SERPINA1* gene (Chart 2).[53] The test involves PCR amplification of genomic DNA extracted from blood (whole or dried blood spot) or saliva samples, followed by hybridization with allele-specific probes using Luminex xMAP (Luminex Corp., Austin, TX, USA) technology for high-throughput nucleic acid detection.[53] Two kits are available for sample collection for the A1AT Genotyping Test, a buccal swab kit (ORAcollect DNA; DNA Genotek, Ottawa, ON, Canada) and a dried blood spot kit (AlphaKit+;

Progenika Biopharma). The buccal swab kit is more commonly used in Brazil. The test is minimally invasive, does not require drying time, and can be transported by regular mail because DNA integrity is maintained at room temperature. The buccal swab sample remains stable for two months.[54]

The A1AT Genotyping Test offers worldwide coverage by including some of the more common rare (Mmalton, Mprocida, I, F) and ultra-rare allelic variants among the 14 allelic variants selected.[55] The absence of any of the 14 mutations in the test is reported as an "undetected variant" and suggests that the genotype may be Pi*MM (normal genotype).[56] In cases where serum AAT levels are below 50 mg/dL and none of the 14 mutations are detected, the gene is automatically sequenced by the manufacturer (Progenika Biopharma) to detect rare variants that might not have been included in the test.

By rapidly and simultaneously detecting multiple allelic variants, the A1AT Genotyping Test reduces the diagnostic time frame and the number of samples that need to be sequenced. In Italy, investigators reported a correlation of 100% between the A1AT Genotyping Test and their own diagnostic algorithm, as well as a reduction of 66% in the diagnostic time frame for samples not requiring sequencing (which takes approximately 3 days).[55] A group from Germany reported that the use of the A1AT Genotyping Test resulted in reductions of 79% and 63.4%, respectively, in nephelometric measurements and in the number of samples requiring gene sequencing, when compared with the traditional workflow (conventional PCR), although the number of IEF electrophoresis assays was unchanged. By increasing the number of detected mutations from 2 (S and Z) to 14, Luminex-based method resulted in a median time to the diagnosis of rare genotypes of 14 days, compared with 83 days for traditional methods.[57] Recently, investigators in Spain[56] reported the initial results of an ongoing observational study evaluating a new national circuit for diagnosing AATD based on Luminex multiplex technology using online registration. The analysis included 5,803 samples from buccal swabs (85.9%) and dried blood spots (14.1%) sent by postal mail to a central laboratory. The prevalence of common allele combinations (MS: 19.0%; MZ: 14.4%; SS: 2.9%; SZ: 3.7%; and ZZ: 1.4%) aligned with previously reported estimates for Spain, and the system was effective in achieving a timely diagnosis of AATD.[56]

Diagnostic algorithm

An issue faced by all physicians treating a rare disease is the applicability of guidelines to direct management decisions that are specific to their circumstances. A recent review[58] of 15 available international AATD practice guidelines published between 1989 and 2017 identified substantial variation in management recommendations. The moderate level of agreement on "when to test" (10 statements; 41%) and "how to test" (2 statements; 56%) is thought to reflect regional variations in disease prevalence, clinical manifestations, and health care funding models.[58]

At the Ibero-Latin American forum in 2019, a new algorithm for the diagnosis of AATD was proposed (Figure 1). The algorithm was a joint development by the Spanish Registry of Patients with Alpha-1 Antitrypsin Deficiency and the Latin American Thoracic Association, and applies to regions (including Brazil) where the A1AT Genotyping Test is available. According to the algorithm, patients with COPD, first-degree relatives, and partners of patients with diagnosed AATD, as well as other high risk patients (Chart 1) should be tested for AATD. The algorithm features two pathways: a conventional testing pathway which involves screening for serum AAT levels as the first step, and an alternative pathway which involves

Chart 2. Allelic variants detected with the A1AT Genotyping Test (Progenika Biopharma, Derio, Spain).

Variant	Associated allele	Predicted AAT activity
c.187C>T	Pi*I	Reduced (slight)
c.194T>C	Pi*M procida	Reduced (severe)
c.226_228delTTC	Pi*M malton, Pi*M palermo, Pi*M nichinan	Reduced (severe)
c.230C>T	Pi*S iiyama	Reduced (severe)
c.552delC	Pi*Q0 granite falls	None (protein absent)
c.646+1G>T	Pi*Q0 west	None (protein absent)
c.721A>T	Pi*Q0 bellingham	None (protein absent)
c.739C>T	PI*F	Reduced (slight)
c.839A>T	Pi*P lowell, Pi*P duarte, Pi*Q0 cardiff, Pi*Y barcelona	Reduced (slight)
c.863A>T	Pi*S	Reduced (slight)
c.1096G>A	Pi*Z	Reduced (severe)
c.1130dupT	Pi*Q0 mattawa, Pi*Q0 ourem	None (protein absent)
c.1158dupC	Pi*Q0 clayton, Pi*Q0 saarbruecken	None (protein absent)
c.1178C>T	Pi*M heerlen	Reduced (severe)

Adapted from the U.S. Food and Drug Administration.[53] AAT: alpha-1 antitrypsin; and Pi: proteinase inhibitor.

the genetic diagnosis of the 14 allelic variants that are most commonly associated with AATD as the first step. According to the conventional pathway, serum AAT levels of < 116 mg/dL (assessed by nephelometry) are indicative of "possible AATD" and should be followed by confirmatory testing. Confirmatory tests include phenotyping and/or genotyping to identify the most common variants to establish which *SERPINA1* gene alleles are present. The alternative pathway recommends using the A1AT Genotyping Test (Progenika Biopharma) as the first step to simultaneously identify and genotype the 14 most common deficiency variants of the *SERPINA1* gene. Following a genetic diagnosis, AATD is confirmed based on serum AAT levels. In either pathway, gene sequencing (the most sensitive confirmatory test) may be required if results are discordant between the serum screening test and the genetic/phenotypic test. In a cross-sectional study in Brazil, in a sample of 926 patients who underwent quantification of AAT levels, only 3 required gene sequencing due to discordant results.[16]

The vast majority of patients with AATD will benefit from genetic counseling, prevention of lung damage,

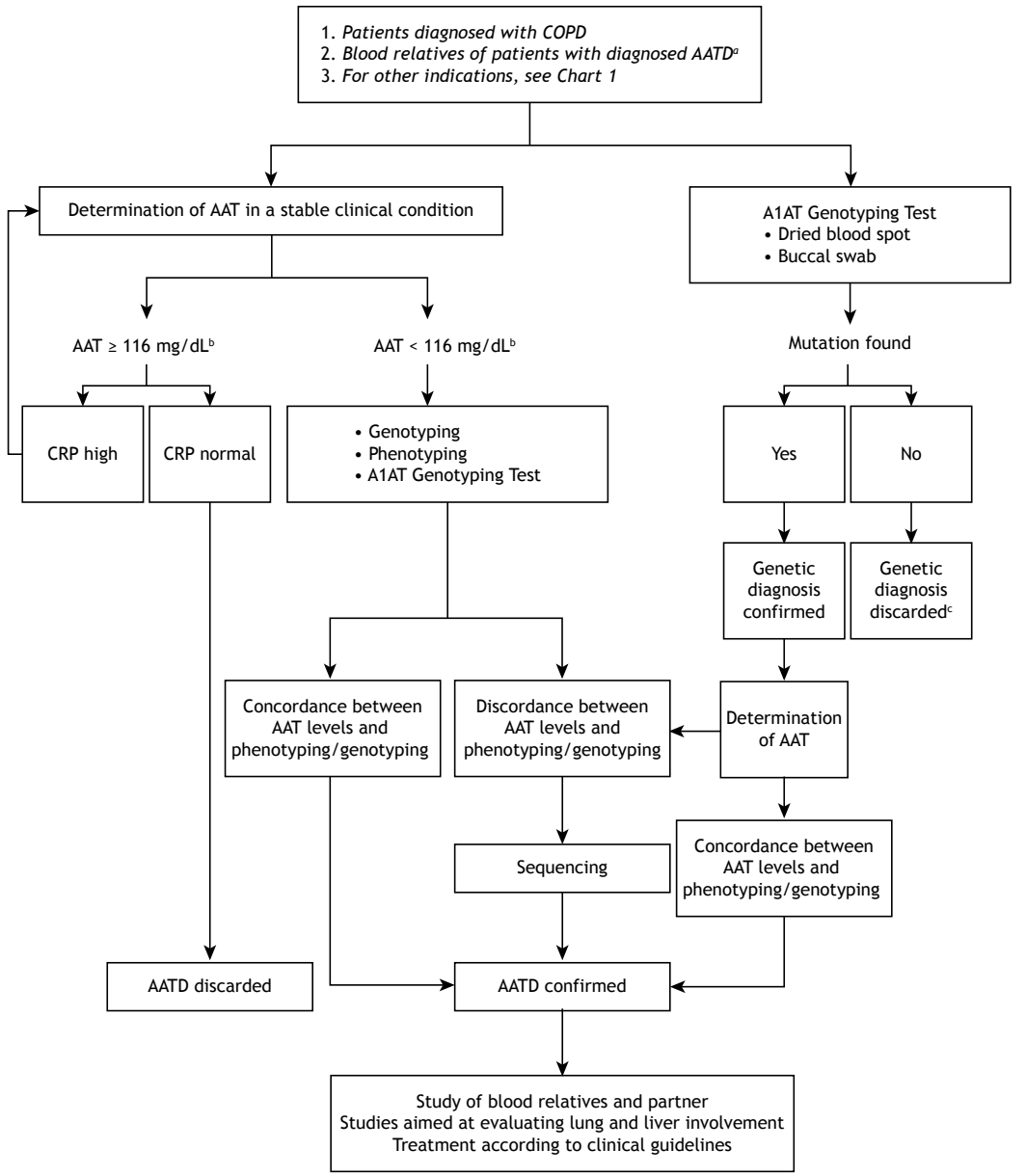

Figure 1. Alpha-1 antitrypsin deficiency diagnostic algorithm. AATD: alpha-1 antitrypsin deficiency; AAT: alpha-1 antitrypsin; and CRP: C-reactive protein. [a]In case of a patient diagnosed with AATD, investigate the partner to assess the risk of the disease in the offspring. [b]Determination in blood by nephelometry. For other techniques, apply a conversion factor. [c]If there is high clinical suspicion of AATD, determine AAT levels in a stable clinical condition.

and therapeutic intervention. Pharmacological and nonpharmacological measures for patients with AATD-associated COPD are similar to those for COPD patients without AATD.[10,37,59] Patients with severe AATD-associated COPD (serum AAT concentrations ≤ 50 mg/dL), never or former smokers, or patients with FEV_1 < 80% of the predicted value who present with impairment of lung function or progression of emphysema despite standard COPD treatment may be candidates for augmentation therapy with purified AAT,[10,37] although specific recommendations may vary by country.[58]

PATIENT ASSESSMENT: COMPLEMENTARY TESTS

After diagnosing AATD, the patient should be examined for the presence and extent of lung and liver involvement, as well as for less commonly associated conditions, such as vasculitis and panniculitis. Clinical history, physical examination, and family history must be taken into account when interpreting the results. Complementary tests to be performed in patients with COPD due to AATD are summarized in Chart 3.

Respiratory function tests

Spirometry is the basic respiratory function test to diagnose COPD. In patients with COPD due to AATD, post-bronchodilator spirometry usually shows a typical obstructive pattern, with a FEV_1/FVC ratio < 0.7, a decrease in FEV_1, and a normal or decreased FVC. In smokers with AATD, the decrease in FEV_1 accelerates in proportion to the smoking history (pack-years). The flow-volume curve shows a reduction in pulmonary flow with a typical concave morphology.[38]

Study of lung volumes in emphysematous patients shows an increase in RV and hyperinflation, translating into an increase in TLC and in the RV/TLC ratio.[38] DLCO is diminished and correlates with a loss of lung parenchyma observed by CT and with the degree of anatomical emphysema.[60,61]

A loss of lung function in patients with AATD may lead to respiratory failure. Investigation of pulmonary emphysema requires post-bronchodilator spirometry and the determination off static lung volumes and DLCO, as well as arterial blood gas analysis if SpO_2 is < 92%. A cardiopulmonary exercise test may also be required. Tolerance to effort may be limited due to airway obstruction, reduced ventilatory capacity, and dynamic hyperinflation.[62] Desaturation in a walking test or in an exercise capacity test most closely correlates with reduced quality of life in patients with AATD.[63]

Current recommendations for managing AATD include an initial clinical evaluation, full pulmonary function testing, arterial blood gas analysis in cases of low SpO_2, and, in follow-up evaluations, annual spirometry.[2,10,25,64,65]

Imaging tests

Plain chest X-rays are usually normal in the early stages of AATD but show characteristic findings of emphysema in up to 85% of cases as the disease progresses. Findings include hyperinflation with diaphragm flattening, increased retrosternal space, small heart, normal or prominent hilar arteries, and decreased caliber of peripheral vessels.[66]

As demonstrated in clinical trials to date, CT scanning expressed in terms of lung density is a useful tool to characterize lung structure and assess the impact of therapeutic interventions in AATD-associated COPD.[67] Quantitative CT provides a reader-independent estimate of the extent and severity of emphysema which correlates with various disease measures and clinical outcomes.

HRCT of the chest is more sensitive than chest X-rays in detecting early emphysematous changes and bronchiectasis. Up to 90% of the patients with severe AATD who smoke will develop emphysema in comparison with 65% of nonsmoking AATD patients.[68] Emphysema is characteristically panacinar, bilateral, and basal (Figure 2), although up to one third of the patients will have upper lobe distribution, more often found in smokers.[69] This pattern is more common in Pi*SZ heterozygotes.[70] CT has been proposed as the best method to evaluate the progression of emphysema, although its application currently remains limited to clinical studies.[71]

Liver tests

Liver function in patients with AATD can be evaluated by determining the levels of alanine transaminase, aspartate transaminase, gamma-glutamyl transferase, bilirubin, and albumin, as well as by performing coagulation tests.[30,31] Other tests, such as ultrasound, transient elastography, and magnetic resonance angiography of the liver, can also be performed when necessary and are highly sensitive for detecting liver involvement.

FINAL CONSIDERATIONS

In Brazil, as elsewhere, the presence of AATD cannot be overlooked. Early diagnosis can have a positive impact on convincing individuals with AATD to avoid smoking and minimize their exposure to environmental pollutants, potentially altering the natural history of the disease and limiting its progression. AAT augmentation therapy may be indicated in certain cases.

AATD case detection should be carried out in all patients with COPD regardless of age, sex, smoking history, or onset of respiratory symptoms. Systematic evaluation of COPD patients in Brazil has shown to be sufficiently effective and is recommended as a screening method.

New diagnostic tools, such as the A1AT Genotyping Test that uses a buccal swab or dried blood spots, can contribute to overcoming the underdiagnosis of AATD

Chart 3. Complementary tests for patients with alpha-1 antitrypsin deficiency-associated COPD.

Test	Type	Clinical utility
Laboratory	Basic biochemistry including liver function tests and serum immunoglobulins[a]	
Respiratory	Forced spirometry	Assessment of obstructive pattern (FEV_1/FVC ratio < 0.7) and its severity (FEV_1)
	Bronchodilation test	Evaluation of reversibility of bronchial obstruction
	Lung volumes and DLCO	Evaluation of the degree of pulmonary hyperinflation and gas exchange capacity at the pulmonary level
Imaging	Chest X-ray	Basic test in all patients with respiratory symptoms
	HRCT of the chest	Confirmation of extension, location, and type of emphysema, as well as presence of bronchiectasis
	Liver ultrasound Elastography Magnetic resonance imaging	Sensitive and useful for detection of liver involvement

[a]Serum immunoglobulins: necessary to detect severe immunoglobulin A deficiency, which contraindicates treatment with intravenous alpha-1 antitrypsin.

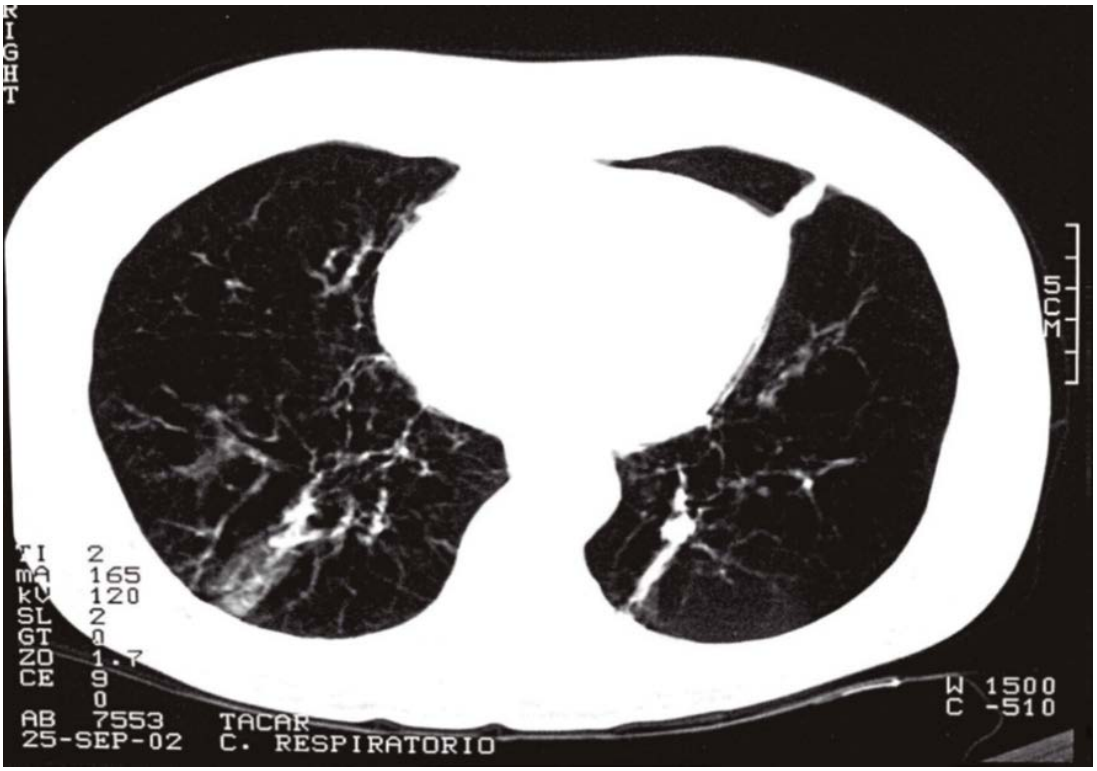

Figure 2. HRCT of the chest of a patient with pulmonary emphysema due to severe alpha-1 antitrypsin deficiency (homozygous Pi*ZZ) showing characteristic panacinar and bilateral emphysema, predominating in the pulmonary bases. Image courtesy of F Casas-Maldonado.

in Brazil, because they offer a minimally invasive, reliable, and rapid alternative to traditional methods. Complementary strategies to improve diagnosis include continuing medical education, easy access to laboratory tests, and public awareness campaigns about AATD and its clinical manifestations. Further studies about the prevalence of and screening tools for AATD would also be useful to support the implementation of efficient and cost-effective programs for the detection and management of patients with AATD in Brazil.

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AUTHOR CONTRIBUTIONS

JRJ, FCM, and MM: conception and planning of the review; interpretation of findings; drafting and revision

of preliminary and final versions; and approval of the final version. FLAF, MVCOC, and MTD: drafting and revision of preliminary and final versions; and approval of the final version.

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