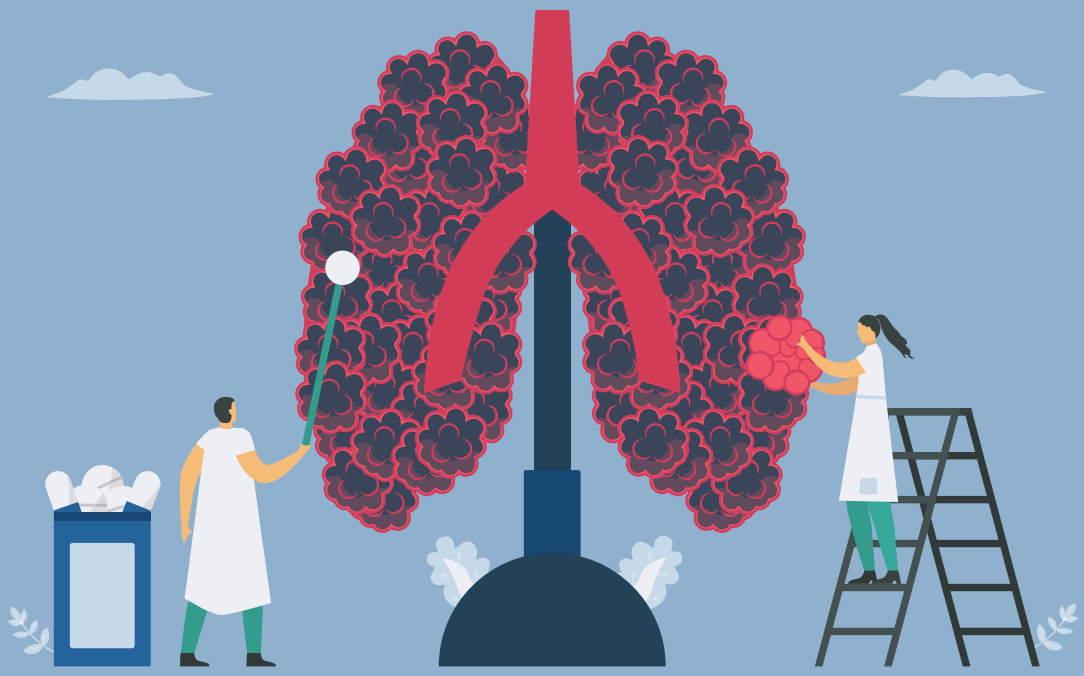

1 Alpha1-antitrypsin deficiency: pathophysiology and clinical aspects

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Alpha1-antitrypsin deficiency (AATD) is an autosomal co-dominant disease, usually underdiagnosed owing to its variable penetrance and clinical heterogeneity. The alpha1-antitrypsin (AAT) protein is encoded by the *SERPINA1* gene on chromosome 14, and its main function is to inactivate neutrophil elastase (NE) upon different insults against the lungs, including smoking.[1] AAT is a 52-kDa protein primarily secreted by hepatocytes and released into the blood circulation by the liver. It is ubiquitous in all body tissues, but its primary physiological significance is in the lungs, where it protects healthy but fragile alveolar tissue from proteolytic damage induced by different enzymes such as NE. AAT is an acute phase protein, released by the liver together with other inflammatory mediators in proinflammatory conditions. Therefore, protein levels in the circulation may vary depending on the medical condition of an individual (*i.e.*, presence of fever). Normal serum concentrations range from 1.5 to 3.5 g/L (or 20 to 48 μM).[2]

Clinical manifestations of alpha1-antitrypsin deficiency

AATD is associated with the loss of protection of the lung against proteolytic damage from NE. The most common clinical manifestations in the early onset of the disease are dyspnea and cough associated with radiological and functional evidence of panacinar emphysema and chronic obstructive pulmonary disease (COPD), more rarely bronchial asthma. The disease occurs at the age of 45 years, but its onset is accelerated in heavy smokers and in subjects exposed to severe air pollution. Liver diseases, including hepatitis, cirrhosis, hepatoma, and possibly vasculitis, represent other spectra of the clinical manifestations of AAT deficiency.[1, 2] The protease inhibitor locus that codes for AAT is highly polymorphic. Currently, over 100 genetic variants of the sequence have been cataloged. There are only two common variants related to AAT deficiency. The PI*Z allele is characterized by an E342K substitution, caused by a GAG to AAG transition in exon 5, and is associated with severe reduction of peripheral AAT levels. PI*S is identified by an E264V substitution, due to GAA to GTA transversion in exon 3, causing a mild reduction in peripheral protein levels. In Europe, the frequency of the PI*Z allele ranges from 0 to 30 per 1000, whereas the prevalence of the PI*S allele varies over a wider range, from 5 to 150 per 1000.[2, 3] The geographical distribution of these alleles outside Europe depends on colonization and migration vectors. For example, in the United States, Canada, and Australia, the frequencies among Caucasians are similar to the frequencies reported in Europe. Both deficiency alleles are rare or absent in the African and Asian populations.[4]

The mechanism by which serpins are inhibited is like that of a mousetrap, with a springlike shift from a metastable to a hyperstable state. The protease attacks the reactive center loop of AAT, with the active serine of the protease forming a link to the amino acid at the base of the reactive center of AAT. The resulting cleavage of the reactive loop allows it to snap back into the main β-sheet of AAT. This spring-like movement flings the tethered protease to the opposite end of the AAT molecule, distorting its active site and altering its structure so that it can be destroyed.[5, 6]

Two hundred different mutations in serpins have been associated with different diseases. In particular, mutations affecting antithrombin confer a predisposition to thrombosis, those affecting the C1 inhibitor confer a predisposition to angioedema, and those affecting antiplasmin determine a predisposition to hemorrhage. Mutations at the reactive center result in a loss of function (*e.g.*, causing familial angioedema) or more rarely result in a change in the functions (*e.g.*, causing hemorrhagic disease). The insertion of an amino acid into the peptide loop containing the reactive center of another serpin, alpha2-antiplasmin, reduces the

distortion of the catalytic site of plasmin, allowing its release, with consequent fibrinolysis and hemorrhage. The most common cause of loss of function of serpin molecules are mutations affecting the critical mobile hinges of the molecule. These lead to spontaneous changes in conformation that allow either the insertion of the intact reactive loop into the main β-sheet, resulting in the production of an inactive "latent" form, or the insertion of the loop of one molecule into the β-sheet of the next, resulting in polymers. Polymerization occurs in AAT with the common Z variant and with mutations at the opening of the sheet, leading to lung emphysema and liver cirrhosis. Mutations at the same site in a neuron-specific serpin result in neurodegeneration and dementia.[5, 6] Hepatic polymerization of the AAT Pi ZZ protein results in both hepatocyte inclusions (an "overload" problem) and decreased peripheral concentrations (a "deficiency" problem). Low serum levels are reflected in a low lung level of AAT that is insufficient to protect the respiratory tissue from inflammation induced, for example, by cigarette smoking. Prolonged inflammation, together with yet unknown environmental or genetic factors, leads to airway and parenchymal damage resulting in lung disease. The inflammatory process of vasculitis and panniculitis may also represent the inability to modulate inflammation because of low serum and tissue levels of AAT, in combination with other cofactors yet to be determined. Within the hepatocytes, AAT polymers cause inflammation that probably plays a role in the transient neonatal jaundice seen in 11% of individuals with Pi ZZ. In most cases, this issue resolves on its own, but in others, childhood cirrhosis develops or hepatic inflammation persists. Again, yet undefined genetic or environmental factors may play a role in this persistent inflammation. With time, adult cirrhosis and hepatocellular carcinoma may occur, although the true incidence has yet to be determined. Although Pi ZZ has classically been referred to as a "deficiency," this does not explain all the facets of the diseases. Some relate to "deficiency" while others clearly reflect an "overload".[7]

Severe AATD is a well-established genetic risk factor for emphysema, accounting for approximately 1% of cases. Cigarette smoke induces significant oxidation of Z-AAT (Z mutation of AAT), which accelerates Z-AAT polymerization. The plasma deficiency and reduced inhibitory activity of Z-AAT would be exacerbated by the oxidation and polymerization of AAT within the lungs, thereby further reducing the antiproteinase screen. AAT polymers also act as a proinflammatory stimulus to attract and activate neutrophils, thereby increasing tissue damage and subsequently causing emphysema.[8, 9] These patients can be potential candidates for specific substitute therapies and lung transplantation. Compromise of alveolar and interstitial integrity in AATD depends on multiple pathways. The presence of pathogenic mutations in AAT results in secretion of reduced levels of the protein into the circulation by hepatocytes and into lung tissue by type II pneumocytes. The loss of adequate protection against elastase activity accelerates the development of emphysema, as does the loss of matrix-promoting effects of AAT on

fibroblasts. Recruitment of neutrophils may be stimulated, and their ability to kill bacteria disabled, by the effects of excess elastase on the interleukin (IL)-8/CXCR1 pathway. Intracellular accumulation of polymerized AAT within the endoplasmic reticulum of epithelial cells will damage cells and surrounding tissues. It may result in chemokine secretion, nuclear factor (NF)-κB signaling, and susceptibility to both the unfolded protein response (UPR) and apoptosis. AAT, derived from a combination of local synthesis and passive diffusion from the systemic circulation, will be concentrated within the interstitium, resulting in extracellular polymerization. Interstitial polymers will provide a local counterbalance to the chemotactic stimuli from the airspace, prolonging the interstitial transit time of neutrophils and, hence causing interstitial neutrophilia. Within the interstitium, the effects of polymers will activate neutrophils and stimulate degranulation, focusing proteolysis in the extracellular matrix and spreading the focus of tissue destruction from a centreacinar to a panacinar distribution.[10, 11]

Clinical heterogeneity has been demonstrated in AATD, such that clinical suspicion plays an important role in its diagnosis. Most patients will have predominant basal emphysema, and a small proportion may have upper zones of lung emphysema (which may or may not occur simultaneously with basal emphysema). Bronchiectasis is less common in AATD and often associated with emphysema. Chronic bronchitis features might be present in patients with AATD even before major structural changes are observed.[1, 6, 12] A number of genetic mutations cause AATD. The PI*ZZ genotype is the most common severe deficiency genotype and thus tends to result in the worst clinical presentation. However, milder genotypes, especially PI*SZ and PI*MZ, are also linked to the development of lung and liver disease, mainly when unhealthy behaviors are present, such as smoking and alcohol use. It has long been accepted that the Z allele, and in particular the PI*ZZ genotype, is linked to emphysema and early-onset COPD. In recent years, there has been growing interest in the relative risk conferred by genotypes causing milder deficiency, such as the S allele. The S protein forms fewer polymers than does the Z protein; therefore, it is retained less within hepatocytes, and leads to less endoplasmic reticulum protein overload. Genotype SZ has an average AAT level of 9-15 μM and risk of several diseases: COPD (related to smoking or occupational exposure), lung function decline (DLCO > FEV1), apical emphysema dominance, with less severe disease than PiZZ, and chronic liver disease. Genotype MZ has an average AAT level of 13-23 μM and a higher risk of emphysema compared with PiMM, an increased risk of COPD in smokers/ex-smokers, lung function decline (FEV1 > DLCO), higher transaminase levels, and modification of chronic liver disease (alcoholic cirrhosis, non-alcoholic liver disease, or cirrhosis). Genotype SS has an average AAT level of 14-20 μM and risk of obstructive lung diseases (COPD, asthma). Genotype MS has an average AAT of level 19-35 μM and it is not associated with the risk of lung or liver disease development.[1, 13, 14]

Extrapulmonary manifestations of alpha1-antitrypsin deficiency

AATD extrapulmonary manifestations consist mainly of liver disease. Diseases such as panniculitis and vasculitis are observed, albeit rarely. Necrotizing panniculitis and systemic vasculitis with positive c-ANCA should prompt testing for AATD, because an association between them has been established. Other reported associations of AATD from cases and small cohort studies include inflammatory bowel disease, glomerulonephritis, rheumatoid arthritis, fibromyalgia, vascular abnormalities (fibromuscular dysplasia of the arteries, abdominal and brain aneurysms, and arterial dissection), psoriasis, chronic urticaria, pancreatitis, and multiple sclerosis. Although these are rare associations, they are plausible, because AAT is anti-inflammatory and immunomodulatory; thus, in AATD, enhanced risk of inflammatory and autoimmune diseases could occur.[1, 6, 15]

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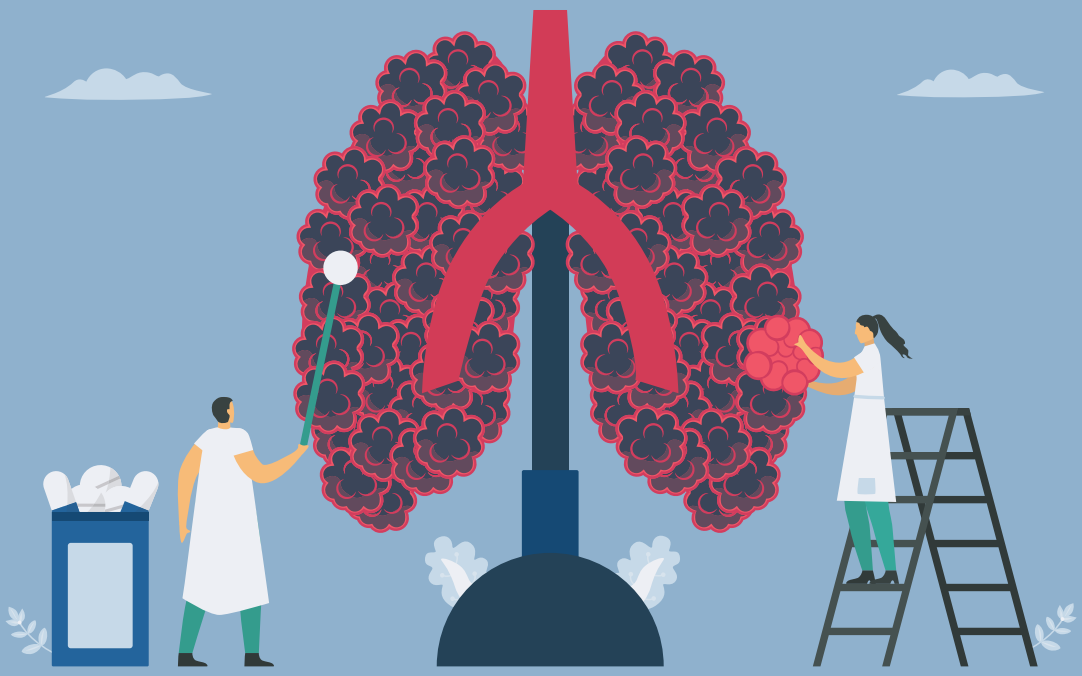

2 Diagnosis of AATD: when to suspect and how to test

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AATD is widely underdiagnosed due to the low level of awareness among physicians. Therefore, the first issue that needs to be tackled is raising suspicion upon noticing its pathological characteristics, in a timely manner.

In the last 25 years, different recommendations[1-4] have stimulated the medical community about the importance of properly suspecting AATD in specific clusters of patients.

The recommendation that every patient diagnosed with COPD or adult-onset asthma should undergo first-level investigations for AATD, following the 1997 WHO memorandum[1] and newly restated 2017 European statement,[2] is widely accepted. In addition, patients with bronchiectasis, unexplained liver disease, panniculitis, or C-ANCA$^+$ vasculitis should also undergo AATD-specific tests according to the Medical and Scientific Advisory Committee of the Alpha-1 Foundation and 2003 ATS/ERS guidelines.[3]

Also, serum protein electrophoresis (SPE) can incidentally raise the suspicion of AATD. The normal SPE diagram identifies several serum protein fractions. After the first peak, corresponding to albumin, the second peak is due to a group of globular plasma proteins called alpha-1 globulins, mainly composed of alpha1-antitrypsin. Therefore, reductions or abnormalities in this peak should raise the suspicion of AATD deficiency or variants. A recent paper by Scarlata organized an algorithm based on the alpha-1 fraction of SPE.[5] Accordingly, up to 21,000 cases of electrophoresis were revised and 85 of them were selected to undergo biochemical and molecular analysis of AAT. As a result, 51% of cases were diagnosed as genetic AATD, with a high diagnostic rate (0.2%). Similarly, another recent paper analyzed 6.500 cases of electrophoresis and selected a sample of 360 for further analysis; 40% of them resulted in clinically relevant AATD, with a similar diagnostic rate (0.22%).[6]

SPE is a low-cost investigation for routine check-ups or for diagnosing several other conditions. Hence, more specific investigations for AATD should additionally be recommended for patients who show a quantitative decrease or morphological anomalies in the alpha-1 globulin band in SPE.

After suspecting AATD, the accurate and complete identification of AATD geno/phenotype is the basis of the clinical decisions regarding potential treatment options for individual patients, including augmentation therapy.

The determination of the AAT plasma level is the first crucial analysis. This test is widely available in most clinical laboratories and is performed by nephelometry or by the equally reliable immunoturbidimetric assay. The amount of serum AAT is directly proportional to the amount of AAT that migrates to the interstitium and epithelial lining fluid (ELF) of the lungs. Overall, ~80% of serum AAT reaches the interstitial fluid, while 10% reaches the ELF.[7] The concentration of AAT is genetically determined; nevertheless, there are biological reasons that increase the production of this protein, and other situations in which the concentration decreases. Blood levels of AAT can increase by 75-100% in response to inflammation, infection and injury. Aside from the acute-phase response, AAT increases during the third trimester of pregnancy, in advanced age, and during oral contraceptive steroid therapy. On the other hand, since AAT reaches circulating levels that are second only to albumin, all the reasons for hypoproteinemia, such as low-protein intake and high-protein depletion, reduce the concentration of AAT in the blood. Moreover, AAT is released into the circulation by liver hepatocytes; therefore, production of AAT can be decreased by liver failure. Finally, the median concentration of AAT falls during the first 6 months of life only to rise again to adult values by 12 months of age. Therefore, all the reasons that temporarily decrease or increase the blood concentration of AAT should be taken into consideration.[8]