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Review

AAT as a diagnostic tool

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Abstract

Serum alpha-1-antitrypsin (AAT) concentration can be affected by both inflammatory and non-inflammatory conditions. This paper characterizes the nature of AAT in physiology and pathologic deficiency and increasing states. The relationships between the AAT concentration in different clinical materials (serum, urine, faeces) and various diseases connected with different organs were analyzed.

Keywords: Alpha-1-antitrypsin quantifications; Alpha-1-antitrypsin deficiency; Acute phase protein; Protease–antiprotease imbalance; Vascular diseases; Protein-losing enteropathy

Contents

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1. Introduction

Precisely 40 years have passed since Laurell and Eriksson [1] associated alpha-1-antitrypsin (AAT) deficiency in serum with the development of premature emphysema. Since then, a great deal of progress has been accomplished in many aspects of this condition. A growing interest in the worldwide epidemiology of AAT deficiency, and the recognition of the broad spectrum of associated pulmonary and extrapulmonary manifestations have given rise to the need for the creation of standardized and uniform biochemical and molecular diagnostic tools for screening for this condition. A better understanding of the pathophysiological mechanisms leading to development of pulmonary emphysema, liver and vascular diseases, and another complications associated with AAT deficiency, provided the basis for the development of therapeutic strategies, with special emphasis on AAT replacement therapy [2–5].

The creation of a common database of subjects recognized in a standardized way, with the participation of world-renowned experts, was charged to the Alpha One International Registry (AIR), a scientific foundation established to comply with a World Health Organization recommendation to develop a multinational registry of AAT deficiency. One commitment of the Alpha One International Registry members, belonging to 15 national registries, is to meet every 2 years in an open scientific and clinical update on AAT deficiency [3,5]. The basic aim of this work is early diagnosis of individuals, who have AAT deficiency, but who have not yet been diagnosed. Undiagnosed individuals are losing opportunities for important lifestyle changes and therapies to prevent the onset or progression of lung disease. Furthermore, the large number of undiagnosed individuals has made it difficult to define the natural history of the disease, since the health status of such individuals is largely unknown [6,7].

The prevalence of AAT deficiency, a potentially lethal disease, is generally found to vary between different geographic areas and different racial groups. It is a common genetic disorder that affects all major racial subgroups worldwide: African blacks, Arabs and Jews in the Middle East, Caucasians worldwide, central Asians, far east Asians, and southeast Asians [8–10]. Deficient subjects receiving AAT augmentation therapy had a lower mortality rate compared with subjects, who were never treated [5,8]. The cost of providing augmentation therapy with human pooled AAT is expensive—approximately US$36 471–52 000/year for a 70-kg patient. The total costs for individuals with the PiZZ phenotype exceeded those for individuals with a non-PiZZ phenotype [4,11]. Therefore, establishment of a threshold value for serum AAT deficiency, which threatens the normal functioning of the organism, and precise measurement of serum AAT concentrations play an important role in the clinical evaluation of pathophysiologic processes.

This paper characterizes nature of AAT in physiology and in pathologic deficiency and increasing states. The relationships between the AAT concentrations in different clinical materials (serum, urine, faeces) and various diseases connected with different organs were analyzed.

2. Normal serum AAT concentration

AAT found in the highest concentrations in plasma diffuses into tissue spaces to protect tissues from being harmed by enzymes released from cells when they are injured and inflamed [12]. Severe deficiency of AAT results in an imbalance between proteinases and inhibitors within the lungs and also in vascular manifestations, including cervical artery dissection and aneurysms [3,13]. It is a 52-kDa glycoprotein synthesized chiefly in the liver, and to a lesser extent by macrophages and neutrophils, to neutralize the degradation effects of proteases. It is the main blood-borne serine protease inhibitor of a broad range of proteases: destructive neutrophil proteases includ-

ing elastase, catepsin G, proteinase 3, pancreatic elastase and trypsin, chymotrypsin and skin and synovial collagenases. Its primary function is the inhibition of neutrophil elastase [9,14–17].

2.1. Population variances of serum AAT

Both age and gender are important covariates to normal AAT ranges and necessitate separate ranges [18]. The gender-specific regression equation for AAT median values along with the age range has been described. AAT measurements fitted between the 10th and 99th centiles. The poor fit at the lower centiles is probably due to the effect of genetic variations. AAT levels varied somewhat with age, rising slightly beyond age 55; males followed a pattern similar to that for females [19]. AAT values change little during the first decade of life [18]. The interval width between the 5th and 95th centiles appears to be broader in young children compared with teenagers and adults [19]. The availability of a reliable set of reference data for both sexes and throughout life facilitates a more reliable incorporation of serum protein values into diagnostic and prognostic patient evaluation [18].

2.2. Standardized measurement of serum AAT concentration

Normal ranges of serum AAT vary significantly among various clinical laboratories, which sometimes leads to misdiagnosis of normal states, deficiency or rising levels. Since different commercial standards vary, they should be compared to reliable reference materials: the Certified Reference Material (CRM) 470 (International Reference Preparation for Proteins in Human Serum (RPPHS)) [19]. Based on a highly purified α_1-antitrypsin standard, the ranges (5th to 95th percentile) for AAT serum levels of the common phenotypes are as follows: MM, 20–53 μmol/l; SS, 20–48 μmol/l; ZZ, 3.4–7.0 μmol/l; MZ, 15–42 μmol l/l; MS, 18–52 μmol/l; and SZ, 10–23 μmol/l [20].

Serum AAT concentration can be affected by both inflammatory and non-inflammatory conditions [18,19]. The problem has become more acute with the availability of augmentation therapy for AAT deficiency, since physicians now make therapeutic decisions based on the deficiency state [20].

3. Increase in serum AAT concentration—an inflammation-sensitive plasma protein

AAT, an acute phase reactant, is up-regulated during acute phase response to tissue necrosis and inflammation. Serum levels increase in rheumatoid arthritis, bacterial infections, vasculitis, carcinomatosis and following estrogen level rises after puberty, during pregnancy or in conjunction with contraceptive medication use [9,15,18]. Recent publications have also shown that even relatively minor changes in the level of AAT as an acute phase protein are associated with the development of arterial hypertension and an increased risk of cardiovascular disease [21,22].

Only after the confusing effects of non-inflammatory conditions are taken into account can AAT measurement be used to detect and stage inflammatory processes. In patients with acute AAT deficiency, an acute phase status does not induce a significant increase in the serum AAT level, and although AAT concentrations rise in inflammatory conditions of the lungs, they remain below levels observed in non-deficient subjects [5]. Measurement of AAT, whose value rarely increases more than fourfold, can be used as an indicator of the risk of pulmonary and neonatal liver disease, if they are discordantly very low in comparison with other acute phase proteins [18].

4. Genetically determined serum AAT deficiency

The level of AAT in serum or plasma is controlled by a co-dominant gene located on chromosome 14q32.1, namely the Pi locus of the protease inhibitor. More than 70 letter code allele variations have been developed and more than 10 are associated with the deficient phenotypes [9,14–16,23]. As AAT phenotypes can be classified as "at risk" and "not at risk" for the development of emphysema, an AAT serum level of less than or equal to 11 μmol/l is used as the serum AAT level that defines AAT deficiency [6,14,20].

The following four AAT groups were generated in a tested population [9,13–16,18,24,25]:

The first—most common genotype—is considered normal (PiMM), making up 93.3% of the tested population.

The second—with moderately reduced rates (averaging 80% of normal values for PiMS, 60% for PiMZ and several other AAT phenotypes), occurring in 3.5% of the population.
The third—comprising 3.1% of the population, produces only about 40% of normal values (PiSZ).
The fourth—this group is rare (about 0.1% or less) and produces very low levels of the protein (20% or less for PiZZ and other very rare genotypes Pi Snull, Pi Znull and Pi null/null).

4.1. The sequence of diagnostic procedures for detecting AAT deficiency

In the past, serum protein electrophoresis was considered to be a useful diagnostic test for the disease, in that a visually determined absence of the alpha-band was an excellent correlate of AAT deficiency. AAT deficiency can be detected incidentally, on occasions when serum protein electrophoresis is performed for an unrelated reason [7].

Because of the overlap in serum AAT levels between normal individuals and carriers of AAT deficiency, immunoassay cannot reliably detect persons with abnormal phenotypes and genotypes.

Full spectrum diagnostic testing for AAT deficiency recommended by the World Health Organization can be divided into four stages: immunoassay, phenotype, genotype, and function [6,26,27].

An immunoassay test, which is reliable, inexpensive and subject to automation, is typically the first test to be performed when an individual is suspected of having AAT deficiency. This test measures the concentration (“level”) of AAT in plasma or serum.

Phenotyping is the stage 2 test, providing independent confirmation of the critically important diagnosis of AAT deficiency. This test is recommended by the WHO for individuals with abnormal immunoassay results. It is most commonly carried out by isoelectric focusing of plasma or serum proteins in a polyacrylamide gel over a narrow pH range. Phenotyping also detects and identifies unusual AAT alleles and identifies subjects heterozygous for AAT deficiency. The flaw of phenotyping is that it is a labor-intensive manual test and considerable experience and expertise are required to interpret the results. Phenotyping results are confusing in patients receiving augmentation therapy, since the test detects both infused protein and patients’ native AAT. Furthermore, it cannot detect the presence of “null” AAT genes that do not result in detectable AAT in plasma.

Genotyping is the stage 3 method for diagnosis of AAT deficiency; it provides a very precise determination of an individual’s genetic makeup at the Pi locus, based on analysis of that individual’s DNA.

Evaluation of AAT functions in serum is the stage 4 test and its aim is to detect dysfunctional alleles, which produce a proper amount of AAT; however, this protein does not fulfill its function.

4.2. Dried blood spot screening test

The search for undiagnosed subjects in large populations requires the adaptation of a suitably sensitive screening method and a simple system of specimen collection. A useful solution is the use of dried blood spot specimens, which enable both quantitative AAT detection and genetic analysis. Because of its sensitivity and excellent correlation with the standard method, the dried blood spot quantitative assay is a reliable tool for routine measurement of AAT. Storage of drying blood samples showed no in vitro destruction at room temperature over a period of 1 week [28]. In addition, an issue emerged, which had significance for clinical practice—whether the screening test should be performed only on a selected population of patients with chronic obstructive pulmonary disease and asthma or whether it should rather be directed at a large number of unselected individuals [29].

The indications for AAT deficiency serum screening are as follows: chronic bronchitis with airflow obstruction, in a “never smoker”; brochiectasis in the absence of risk factors; premature chronic obstructive lung disease (before age 50 years); predominantly basilar emphysema; unremitting asthma, especially in persons younger than 50 years; apparent risk factors for cirrhosis [14].

4.3. Treatment

Low plasma AAT concentrations are the result, not of a lack of AAT variant synthesis, but of the blocking of its processing and secretion by hepatocytes. AAT accumulates in the endoplasmic reticulum of hepatocytes as inclusions, which are easily distinguishable with periodic acid-Shiff staining [30]. This new un-

derstanding of the structural basis of AAT deficiency provides a platform for rational drug design to block polymerization in vivo and so attenuate associated liver disease. The only specific treatment for serum AAT deficiency available at present is augmentation therapy of plasma purified AAT administered intravenously [5,31].

5. AAT as a diagnostic tool in lung diseases

The clinical manifestations of AAT deficiency occur chiefly in the lungs, with a high risk of development of chronic obstructive pulmonary disease (COPD) by the third or fourth decade of life. In individuals with AAT deficiency, there is an at least 20-fold increased risk of developing emphysema. Investigators, who studied AAT deficiency over the past few years, have gained a better understanding of the processes involved in the development of emphysema and its progression [15]. There are at least 116 million carriers (PiMS and PiMZ) and 3.4 million deficiency allele combinations (PiSS, PiSZ and PiZZ) worldwide [8–10]. Population screening studies revealed that 2–3% of patients with COPD were homozygous for the most common AAT deficiency—PiZ. The greatest risk of emphysema occurs in individuals with the very rare homozygous null type, who completely lack AAT, and those with other rare types, who have only 1–2% of normal AAT levels.

It is still not entirely clear whether heterozygotes for AAT deficiency are predisposed to lung disease [14,15]. Abundant evidence suggests persons with one normal (M) and one deficient (Z) gene (PiMZ heterozygotes) are at little or no risk of emphysema compared to the general population, even though their plasma levels average only 57% of normal [31]. Recent experiments involving neutrophil cell biology have explained why cases with partial deficiency (MZ heterozygotes) or moderate deficiency (SZ heterozygotes) do not display an increased risk of developing emphysema despite significantly reduced concentrations of AAT in plasma (and therefore also in the lungs) [3]. Neutrophil elastase is stored and released by exocytosis from azurophilic granules of mature, active polymorphonuclear leucocytes. The elastase concentration within the granules is approximately 5 mM, and so of an order of magnitude two times larger than normal AAT concentrations in plasma (30 μM) and the interstitium (24 μM). Elastase concentrations decrease rapidly in the direct vicinity of granules, to a level equal to AAT concentrations in most patients with normal AAT levels or with heterozygous deficiency. From this, it follows that proteolytic damage could be limited to the area where granules are present, even in cases of partial serum AAT deficiency. Only the common PiZ phenotype, with an AAT concentration of less than 10 μmol, constitutes an increased risk of the development of rapidly progressive emphysema. These theories and observations of excess neutrophil recruitment to the lungs (probably based on the release of leukotriene B4 by alveolar macrophages) explain the singular susceptibility of cases with deficiency to the development of intense and rapid lung destruction [3].

Several pathways are now recognized to cause pulmonary damage in the Z AAT deficiency homozygote [23]:

- Firstly, uncontrolled proteolytic attack;
- Secondly, more recently, it has been recognized that the Z mutation also favors the spontaneous formation of AAT loop-sheet polymers within the lungs;
- Thirdly, the AAT that is available to protect the lungs is approximately five times less effective at inhibiting neutrophil elastase than normal M AAT.

The obtained evidence indicates unequivocally that emphysema is the result of an imbalance (protease/antiprotease theory) between elastase release during phagocytosis by neutrophils present in alveoli of the lungs and antielastases, which are responsible for protecting the lungs from elastase [3,9,14].

AAT polymers may play an important role in the progression of emphysema. Formation of polymers may be accelerated by the inflammatory milieu that exists within the lungs of individuals with Z AAT deficiency. Polymerization is accelerated at low pH; cigarette smoke is mildly acidic. The presence of AAT polymers may explain the progression of lung disease in Z AAT homozygotes after smoking cessation and despite adequate intravenous replacement with plasma AAT [23].

Although the role of AAT inactivation by oxidants in vivo is still a controversial issue, it remains possible

that this mechanism may be important. AAT may be inactivated by conversion of methionine residues at or near the AAT reactive loop site by oxidants from either exogenous (cigarette smoke) or endogenous (phagocytes) sources. Release of oxygen radicals and chlorinated oxidants, which can oxidize the methionine at the active site of AAT, decreases the rate of association of the inhibitor with neutrophil elastase 2000-fold, significantly reducing its ability to inhibit elastase activity. An alternative inactivation mechanism is proteolytic cleavage, occurring within the reactive loop site, which can be produced by enzymes from multiple sources, either endogenous (tissue metalloproteinases) or exogenous (proteinases released by microorganisms or pollens). The two inactivation mechanisms may interact, since oxidized AAT becomes rapidly susceptible to proteolytic cleavage by non-target proteinases [5,9].

Cigarette smoking contributes to destructive changes in emphysema by suppressing the proteinase inhibitory activity in human serum and by the spontaneous formation of loop-sheet polymers, inducing certain bronchoalveolar changes. Most authors emphasize that the combination of anti-proteinase deficiency and cigarette smoke can have a devastating effect on lung function. The first signs of AAT-related emphysema in smokers may occur 10–15 years earlier than in non-affected smokers [9]. According to another opinion, environmental factors, such as cigarette smoking, are not sufficient to cause manifestation of emphysema and it is likely that secondary genes exist for the lungs and liver [5].

There is a growing possibility that asthma may be a contributory factor to the development of permanent airflow obstruction, both in smokers and non-smokers with AAT deficiency. The prevalence and severity of asthma increases in persons with abnormal AAT phenotypes. To address this problem, the World Health Organization has recommended that all patients with chronic obstructive lung disease, and all adults and adolescents with asthma should be screened once in their life for AAT deficiency, using a quantitative test (i.e. an immunoassay method) [3,9,15,17,28,32].

These days numerous pharmaceutical campaigns utilize the possibility of controlling the course of the disease by using human purified or transgenic AAT, antielastase drugs, which can replace AAT function, and anti-inflammatory therapy to circumvent the need for anti-proteinase protection of the lungs. Intravenous therapy restores and maintains the AAT concentration in the circulation above 11 μM [5].

6. Serum AAT deficiency in liver diseases

In contrast to the pathobiology of lung disease, liver injury in AAT deficiency increases not as a result of serum protease inhibitor deficiency, but because of pathological polymerization of AAT variants prior to their secretion from hepatocytes [30,32,33]. The mutant, deficient Z variant of AAT undergoes conformational rearrangement, forming polymers that accumulate within the endoplasmic reticulum to cause chronic liver disease. Although many AAT deficiency variants have been described, only two other AAT mutations have been associated similarly with plasma deficiency and hepatic inclusions: AAT Siiyama (most common in Japan) and AAT Mmalton (most common in Sardinia) [23].

The pathognomic histologic feature is the presence within hepatocytes of PAS-positive, diastase-resistant granules, which tend to be located in hepatocytes adjacent to the portal zones [26]. PiZ immunohistochemistry is an easy, highly specific method used to detect this metabolic defect in liver biopsies [34]. The AAT polymerization process, also referred to as loop-sheet polymerization, is common to other AAT-related inhibitors (serpins) and has recently been described in the neuron-specific protein ‘‘neuroserpin’’, underlying familial encephalopathy with neuronal inclusion bodies [30,35]. Such polymers have proinflammatory properties and may therefore contribute to tissue injury. Liver damage is found only with those variants that aggregate within liver cells and the acuity of liver disease is a reflection of the magnitude of accumulation. It may be summarized that a protein, of which there is a deficiency in the organism, is simultaneously hepatotoxic [5,15,23,26,30,32,33].

AAT deficiency is the most common genetic cause of liver disease in children and it predisposes adults to chronic liver disease and hepatocellular carcinoma [5,15,23,33]. There is no evidence that children with AAT deficiency-related liver disease are prone to developing lung disease in adulthood [5].

In infants, inclusion bodies may not become detectable until 3 months of age and are found less

frequently in patients heterozygous for the PiZ allele [26]. Children carrying the AAT SS or SZ genotypes are not at risk of developing chronic liver disease [5].

AAT deficiency is the commonest metabolic disease leading to liver transplantation in children. Approximately 10–15% of the PiZZ population develops liver disease, 5% of them will require liver transplantation within the first 4 years of life [30,36].

Patients with heterozygous AAT deficiency of the PiZ type bear an increased risk for chronic liver disease. If at all, this genetic defect will become clinically relevant only in middle-aged adults or the elderly. Nationwide prospective screening studies carried out in Sweden by Sveger [7] have documented that only 10–15% of the PiZZ population developed clinically significant liver disease over the first 20 years of life. These data indicate that other genetic traits and/or environmental factors predispose a subgroup of PiZZ individuals to liver injury [15,33,34].

Molecular studies have shown that the formation of PAS globules is secondary to abnormal loop-sheet polymerization, which is temperature- and concentration-dependent [23,26]. Environmental factors, such as infection, pyrexia and inflammation, would increase the polymerization process, leading to further promotion of hepatic toxicity. The possibility that other environmental factors (such as viral hepatitis, iron overload, excessive ethanol intake) put patients with the PiZZ phenotype at risk for liver damage has been suggested [15,23,26]. The variability in the severity of liver disease and age of onset among patients with AAT deficiency can be explained, in part, by individual variations in episodes of inflammation and therefore, increased AAT synthesis [30].

7. AAT as a diagnostic tool in renal diseases

As a circulating anionic plasma protein (pI 4,5), AAT is electrostatically bound in vivo to positively charged moieties in normal, and especially in diabetic basement membranes. The presence of abnormal PiZ protein in the subendothelial region of the glomerular basement membrane in AAT deficient patients with glomerulonephritis suggests a possible role for this protein in the pathogenesis of this lesion [37,38].

The role of AAT deficiency in the development of glomerular lesions is still controversial. Glomerular lesions were noted in 79% of AAT patients with the PiZZ phenotype, including mesangiocapillary glomerulonephritis, mesangial proliferative glomerulonephritis and endocapillary proliferative glomerulonephritis with segmental necrosis [39]. According to Montanelli [12], low plasma levels or low AAT functional activity do not seem to correlate with the expression of renal damage, indicating that the nephropathy is not a direct and single expression of the protein deficiency. Authors speculate that nephropathy associated with AAT deficiency, phenotype PiZZ, may be immune complex mediated, resulting from an abnormal immune response to a circulating neoantigen, PiZ protein, following hepatic parenchymal destruction.

This hypothesis would also account for the predominance of renal pathology among AAT patients with liver disease as opposed to those with pulmonary manifestations [40].

7.1. AAT as a parameter in urine

The molecular masses of AAT (56 kDa) and albumin (68 kDa) are close to the threshold value of the size of particles, which pass through systemic barriers. An increase in mean albumin and AAT urinary excretion was demonstrated, compared with the control group, both in patients with essential hypertension and with secondary hypertension. The increase in mean AAT urinary excretion demonstrated in hypertension could suggest a generalized participation of this protein in pathological vascular changes. The highest increases in mean AAT urinary excretion in renovascular hypertension may be connected with local changes in the kidney [41].

8. AAT as a diagnostic tool in cardiovascular diseases

AAT is the main proteinase inhibitor in human plasma and has previously been suggested as a guardian of vascular tissue. It is bound to the surface of endothelial cells and may diffuse into the arterial wall from the circulation or be produced locally in the arterial wall [20]. Elastin is a substrate for elastase and the amount of elastin in the vasculature is likely to

affect distensibility of blood vessels [42]. Published data demonstrate the effect of both AAT deficiency and increased AAT serum concentrations on cardiovascular changes.

Since, in AAT deficiency, elastase may attack elastin in the arterial wall, a hypothesis has been proposed, namely that AAT deficiency is associated with reduced blood pressure, and thus with a reduced risk of ischemic cerebrovascular and ischemic heart disease and with increased life expectancy [25,42]. AAT deficiency may have stronger and earlier effects in men than in women [21,25,43]. On the other hand, an increase in the serum concentrations of AAT, as a plasma protein highly sensitive to inflammation, is associated with elevated blood pressure and increased incidences of myocardial infarction and stroke [21,22]. Although hypertension is strongly associated with stroke and infarction, there are great differences between hypertensive men with similar blood pressure values [21,22]. This association could prove useful for earlier identification of the risk of development of cardiovascular diseases among men [22].

Inflammatory phenomena at sites of atherosclerotic plaques are increasingly thought to be major determinants of the progression and clinical outcome of atherosclerotic disease. Destruction of elastic tissue in arteries may be accelerated by local inflammation [25]. AAT has been reported to exert anti-inflammatory activity [44]. This protein may be associated with the development of atherosclerosis or destabilized atherosclerotic plaques [21,22]. AAT deficiency could lead to fewer cleaved fragments of AAT in atherosclerotic plaques and thereby reduce atherosclerotic inflammation and the risk of ischemic heart disease [25].

Since AAT deficiency may protect against cardiovascular disease, rather than promote it, there is no obvious clinical relevance in assessing genotypes and levels.

9. AAT in arterial aneurysm

AAT helps maintain the integrity of elastic and collagen fibers [13]. A disequilibrium between proteolytic enzymes and protease inhibitors may contribute to some pathogenesis in the arterial wall, leading to structural abnormalities of the extracellular matrix and increasing the susceptibility of the vessel wall to additional short-lived trigger mechanisms [25,45–47]. AAT <90 mg/dl was associated with cervical artery dissections independent of age, sex, or vascular risk factors [13]. Protease–antiprotease imbalance may contribute to the formation of cerebral aneurysms and suggest a future role for plasma protease assays in attempting to identify cerebral aneurysms before hemorrhage occurs [48]. An abdominal aortic aneurysm is characterized by dilatation of the aorta and involves the expansion and thinning of all the layers of the arterial wall [47]. The presence and risk of rupture of splenic artery aneurysms may be greater in patients with AAT deficiency [46]. The PiZ deficiency allele frequency in the London intracranial aneurysms data was eightfold higher than in controls [49].

Despite a growing number of examples of associations between serum AAT deficiency and the development of aneurysms, no significant evidence of the relationship between abdominal aortic aneurysms and PiZZ polymorphism has been found [47].

10. AAT in vasculitides

The systemic necrotizing vasculitides comprise a heterogeneous group of conditions characterized by widespread focal and segmental inflammation with acute infiltrates of polymorphonuclear leucocytes, and later monocytes, and fibrinoid necrosis in blood walls and perivascular interstitial spaces [50]. AAT is a naturally occurring inhibitor of proteinase 3 (PR3) and elastase, two of the target antigens of antineutrophil cytoplasmic antibodies (ANCA). An increased incidence of AAT phenotypes associated with dysfunctional AAT or low serum levels has been reported in patients with anti–PR3 antibodies [51].

Studies evaluating AAT alleles in cohorts of patients with Wegener's granulomatosis have found the frequency of abnormal phenotypes to be increased. Determination of AAT levels and phenotypes in these patients may allow cases of AAT deficiency to be diagnosed before severe emphysema is present, and at a time when smoking cessation and replacement therapy with AAT may be beneficial [5,52].

11. AAT in panniculitis

AAT deficiency is known to be associated with panniculitis that affects children and adults. Although reports of this association are rare, the true incidence may be unappreciated owing to underdiagnosis of AAT deficiency [53–55].

12. AAT in gastrointestinal diseases

No unequivocal association exists between the etiologic role of AAT deficiency and inflammatory disease. The increased frequency of heterozygosity for the PiZ variant of AAT deficiency among patients with ulcerative colitis and in the development of duodenal ulcer in *Helicobacter pylori*-positive individuals might imply a role played by protease inhibitors in the regulation of inflammation and the immunologic response [56–58]. Data from genome-wide searches show no link between ulcerative colitis and Crohn's disease and the locus of the gene that controls AAT or other sites on chromosome 14. Statistical evaluation of the probability of simultaneous occurrence of AAT deficiency and inflammatory bowel disease in the same patient is less than 1 per 1 million individuals compared with a general population in the Midwest US [16]. Prospective and retrospective studies are needed to examine whether patients with intestinal inflammatory bowel diseases are more likely to have AAT deficiency and, conversely, whether individuals carrying the AAT deficiency genes are more likely to develop inflammatory bowel diseases [16].

It is not known whether an increase in the concentration of trypsin-2 complexed with AAT in serum may have any implications in the pathophysiology of acute pancreatitis. The presence of these complexes in the circulation could result from reabsorption of trypsin from the intestine. Most trypsin (90%) is complexed to alpha-2-macroglobulin; 10% of activated trypsin is bound to AAT in serum. Moderate increases in trypsinogen-2 and trypsin-2-AAT in serum were found in more than two-thirds of patients, in whom acute pancreatitis was a known complication of cardiac surgery with cardiopulmonary bypass [59–61]. High trypsinogen-2 and trypsin-2-AAT concentrations were found most often in patients with biliary and pancreatic cancer, and also in benign obstructive biliary disease. According to Hedström et al. [61], these parameters are new potential markers for cholangiocarcinomas.

12.1. Faecal AAT as a diagnostic tool

Abnormal faecal protein loss has been reported in many gastrointestinal diseases leading to exudation or blood loss into the lumen. Faecal AAT may be of value in patients, where the cause of hypoalbuminemia is not clinically evident [62,66].

Measurement of faecal AAT is a convenient, cheap and sensitive method of assessing gastrointestinal protein loss. Unlike albumin, it is neither degraded by intestinal proteases nor reabsorbed, and may therefore be assayed in the stool and used as a marker of protein-loosing enteropathy. Several studies showed a high sensitivity and close correlation with the results of conventional markers such as ^{51}Cr-albumin, ^{51}Cr-Cl_3, Fe-dextran, ^{67}Cr-ceruloplasmin, ^{95}Nb-albumin, and ^{131}J-serum protein, which were considered the gold standards for assessment of protein-loosing enteropathy [62–64].

The value of AAT measurement in faeces in the assessment of intestinal inflammation is well established. Faecal AAT values are elevated in patients with inflammatory bowel disease. There was no significant correlation between Crohn's disease activity index and AAT [65–67].

Enhanced faecal protein loss was observed in more than 50% of children with acute and persistent diarrhea caused by various pathogens. It showed no correlation with age, duration of diarrhea or nutritional status, and did not result in any significant decrease in plasma proteins or immunoglobulins [68]. The determination of faecal AAT could provide clinically useful information regarding the difference between infectious and noninfectious diarrhea, and the activity of characterizing disease with diarrhea [63,68,69].

In HIV-positive populations, a faecal AAT value greater than 0.3 mg/g wet stool has high sensitivity and specificity for the diagnosis of intestinal Kaposi's sarcoma. The combination of faecal AAT concentration higher than 0.2 mg/g wet stool, a negative stool culture for enteric bacteria, and the absence of palatal Kaposi's sarcoma has high sensitivity and

specificity for the diagnosis of enteric cytomegalovirus infection [62].

Protein-loosing enteropathy is a serious complication common in late survivors of Fontan surgery, and in other subjects with congenital heart disease and chronic elevation of systemic venous pressure. Medical management of protein-loosing enteropathy in these patients has been only partially successful [65,70].

Mean faecal AAT concentrations increased in patients with burns covering more than 20% of the body surface area [64].

Enteric protein loss is a very frequent finding in celiac patients and the measurement of AAT clearance may be a reliable method of evaluating the activity of celiac disease, useful in following the efficacy of treatment [71].

Growing evidence exists that exposure to cow's milk elicits inflammation in the gut of infants with cow's milk allergy, irrespective of symptoms. Faecal AAT determination may be a candidate for diagnostic studies in patients with food protein-induced enterocolitis, if prospectively evaluated [72–74].

13. AAT in diabetes

Serum AAT levels in diabetes and impaired glucose tolerance increases, remains unchanged or decreases in relation to healthy controls [75]. Based on the evidence presented, it may be surmised that the elevated levels of antigenically determined serum AAT reflect the inflammatory status in diabetes. As shown by Bristow [76], an increase in AAT concentrations in diabetes occurs only in patients with bacterial infections. Moderately elevated AAT levels increased the cardiovascular risk similarly in diabetic and non-diabetic men [7]. Elevated plasma AAT was found in diabetics with overt nephropathy compared with diabetics without renal changes, and in diabetics with periodontal disease compared with their orally healthy counterparts or normal controls [76–78]. Serum AAT concentrations in pregnant diabetic women are higher than in healthy pregnant women [79].

In contrast, functional analysis of serum AAT revealed no differences between diabetic and normal populations. [76,79]. Decreases in the functional activity of this inhibitor in diabetes could be the result of formation of complexes with proteases or inactivation by free radicals [79].

AAT undergoes glycosylation more rapidly than either albumin or hemoglobin. This rapid glycosylation, combined with rapid turnover, facilitates detection of short–term changes in glycemia. AAT, unlike conventional biochemical parameters, can be assessed even after advanced hemolysis, in the postmortem diagnosis of antemortem hyperglycemic states [80].

In summary, it may be stated that the most important role of AAT as a diagnostic tool is early diagnosis of lung and liver disorders associated with a deficiency of this protein in serum. The presented evidence indicates that serum AAT deficiency is also the cause of changes and dysfunction of other organs, whose clinical manifestations may precede the progressive development of emphysema. The interpretation of changes in serum AAT concentrations should apply not only to decreases, but also to increases in the serum concentrations of this parameter. A prospective analysis of sustained slight increases in serum AAT concentrations may be useful in assessing the risk for developing arterial hypertension and cardiovascular consequences. On account of its anti-proteolytic properties and threshold molecular size, facilitating penetration of systemic barriers, AAT possesses unique properties as a diagnostic tool in stool and urine analyses.

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