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Review article
Pregledni članak

ALPHA-1-ANTITRYPSIN DEFICIENCY – MOLECULAR BASIS, CLINICAL PRESENTATION, THERAPEUTIC OPTIONS AND AN INTEGRATIVE APPROACH IN DIAGNOSTICS

DEFICIJENCIJA ALFA-1-ANTITRIPSINA – MOLEKULSKE OSNOVE, KLINIČKE MANIFESTACIJE, TERAPIJSKE MOGUĆNOSTI I INTEGRATIVNI PRISTUP U DIJAGNOSTICI

Anđelo Beletić[1], Aleksandra Dudvarski-Ilić[2], Branislava Milenković[2], Ljudmila Nagorni-Obradović[2], Mila Ljujić[3], Valentina Đorđević[3], Dragica Radojković[3], Nada Majkić-Singh[4]

[1]Center for Medical Biochemistry, Clinical Center of Serbia, Belgrade, Serbia
[2]School of Medicine University of Belgrade and Clinic for Pulmonology, Clinical Center of Serbia, Belgrade, Serbia
[3]Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Serbia
[4]Society of Medical Biochemists of Serbia, Belgrade, Serbia

Summary

The primary role of alpha-1-antitrypsin (AAT), encoded by the highly polymorphic SERPINA1 gene, is to protect the lung parenchyma from proteolysis by neutrophil elastase. AAT deficiency (AATD) is an autosomal recessive disease, considered as the most important genetic cause of liver disease in children and emphysema in adults. According to frequency, deficient alleles can be classified as »common« (Z and S) and »rare« (Mmalton, Mheerlen, Mprocida etc). Type, intensity and onset of clinical disease associated with AATD occur as a result of interaction between AATD and additional genetic and acquired factors (tobacco smoking, air pollution exposure etc). The most frequent clinical manifestations include premature emphysema, chronic hepatitis, cirrhosis and hepatocellular carcinoma. Epidemiological studies highlight the need for improvement in diagnostic efficiency for AATD. It is recommended for a diagnostic approach to integrate precise, internationally recognized clinical criteria and a standardized laboratory protocol, based on a combination of biochemical and molecular methods. The predilection site of clinical manifestations guides the therapeutic approach. Augmentation therapy is possible in lung disease, while currently the only specific measure in patients with severe liver failure due to AATD is transplantation. In all patients, preventive measures, ammeliorating the deleterious effects of

Kratak sadržaj

Primarna uloga alfa-1-antitripsina (AAT) jeste da zaštiti plućni parenhim od proteolize dejstvom neutrofilne elastaze. Njegovu biosintezu kontroliše izuzetno polimorfni gen SERPINA1. Deficijencija AAT (AATD) jeste autozomalno recesivno oboljenje i smatra se najčešćim genetskim uzrokom oboljenja jetre kod dece i emfizema kod odraslih. Prema učestalosti, deficijentni aleli se mogu podeliti na »česte« (Z i S) i »retke« (Mmalton, Mheerlen, Mprocida itd.). Za vrstu, intenzitet i vremenski period u kome se razvijaju kliničke manifestacije smatra se odgovornim interakcija AATD i dodatnih genetskih i stečenih faktora rizika (pušenje, izloženost aerozagađenju i sl.). Kod obolelih se najčešće javljaju preuranjen emfizem, hronični hepatitis, ciroza i hepatocelularni karcinom. Epidemiološke studije naglašavaju potrebu povećanja dijagnostičke efikasnosti kod AATD. Preporučuje se da dijagnostički pristup integriše precizne, međunarodno identifikovane, kliničke kriterijume i standardizovan laboratorijski protokol, zasnovan na biohemijskim i molekularno-biološkim metodama. Terapijski pristup zavisi od vrste kliničkih manifestacija. Kod pulmoloških bolesnika je moguće primeniti terapiju nadoknade, dok kod osoba sa terminalnom fazom oštećenja jetre uzrokovanog AATD transplantacija trenutno predstavlja jedinu specifičnu terapiju. Kod svih obo-

Address for correspondence:

Anđelo Beletić
Center for Medical Biochemistry
Clinical Center of Serbia
Višegradska 26, 11000 Belgrade, Serbia
Phone: + 381 60 15 11 083
e-mail: andjelo.beletic@yahoo.com

Non-standard abbreviations: AATD, alpha-1-antitrypsin deficiency; AAT, alpha-1-antitrypsin; SERPIN, serine protease inhibitor; NE, neutrophil elastase; PR-3, proteinase 3; RCL, reactive center loop; TNF, tumor necrosis factor; MMP, matrix metaloproteinase; COPD, chronic obstructive pulmonary disease; AFP, alpha fetoprotein.

habits and environmental factors are recommended. Introduction of gene therapy is expected to additionally improve health outcomes in affected persons. Current results with an integrative AATD diagnostic strategy in the Serbian population are highly encouraging, prompting towards its further implementation in common medical practice with the ultimate goal to establish a national register of affected individuals.

Keywords: alpha-1-antitrypsin deficiency, molecular basis, clinical presentation, integrative approach in diagnostics

lelih je neophodno preventivno uticati na smanjenje štetnog uticaja životnih navika i faktora sredine. Očekuje se da zdravstveni ishodi kod obolelih budu značajno unapređeni uvođenjem genske terapije. Dosadašnji rezultati istraživanja efikasnosti integrativnog pristupa detekciji AATD u populaciji Srbije su ohrabrujući i upućuju na potrebu njegovog omasovljenja, čime bi se ostvarili uslovi za formiranje nacionalnog registra obolelih.

Ključne reči: deficijencija alfa-1-antitripsina, molekularne osnove, klinička prezentacija, integrativni pristup u dijagnostici

Introduction

Alpha-1-antitrypsin deficiency (AATD), an autosomal recessive disorder primarily affecting the lungs and liver, is considered as one of the most frequent potentially lethal genetic diseases among Caucasians. During the past sixty years, since its discovery by Laurell and Ericsson, significant improvements have been achieved in understanding its molecular pathology, clinical manifestations, epidemiology and therapeutic options. Nevertheless, only 6% of affected individuals have been diagnosed, which illustrates that its diagnostic efficiency is not satisfactory, thus urging for introduction of an integrative approach in AATD diagnostics in order to enable appropriate therapeutic and preventive interventions (1, 2).

The aim of this article is to briefly present contemporary and widely accepted concepts about AATD, together with experiences gained in implementing an integrative diagnostic strategy for detecting AATD in the Serbian population.

Biochemistry and physiology of AAT

Alpha-1-antitrypsin (AAT) is the archetype of the SERPIN protein family, the members of which control many inflammatory cascades, mainly through tightly grasping and inhibiting serine proteases, such as neutrophil elastase (NE), cathepsin G and proteinase 3 (PR-3) (3). It is a monomeric glycoprotein with a molecular mass of approximately 51 kDa (4). Among 13 structural domains of the AAT molecule, the »reactive center loop« (RCL) is responsible for physiological functions (5). Comparison of the AAT inhibitory affinity towards different serine proteases reveals that the highest is observed for NE, followed by PR-3, rennin, urokinase, plasmin, thrombin, cathepsins, caspase 3, kallikreins etc, while the inhibition of trypsin is of no physiological importance (6). Its synthesis primarily occurs in hepatocytes, although it can be evidenced to a lesser extent in monocytes, macrophages, neutrophils, epithelial cells in the lungs and intestines, renal parenchyma, δ-cells of pancreatic islets etc. The most important stimulators of AAT synthesis are IL-6, IL-1, TNF-α and endotoxins. AAT secreted into blood is a mixture of different isoforms, which differ according to the structure of carbohydrate chains and the N-terminal end of the molecule (4).

From a physiological viewpoint, AAT represents a major defence against the elastolytic burden in the lower respiratory tract, owing to its ability to inhibit NE. Due to relatively small molecular mass, AAT easily diffuses from the bloodstream into the interstitial space, where the continuous release of NE occurs. Acting as a pseudosubstrate, the RCL »traps« NE, causing conformational changes in the emerging complex, which results in irreversible inhibition of NE activity and subsequent degradation of both AAT and NE, in hepatocytes or Kupffer's cells (7). Additional antiinflammatory effects, unrelated to NE inhibiton, such as modulation of neutrophils' chemotaxis and regulation of cytokine expression, have also been reported (6, 8). The spectrum of AAT physiological functions has been broadened by experimental evidence of its regulatory role in apoptosis, insulin secretion, iron metabolism, and immune response to various bacteria and HIV virus (6).

Blood AAT level in healthy adults, measured by the nephelometric method, ranges from 0.9 to 2.0 g/L, with a slight increase in premenopausal women and in individuals of both genders older than 60 years (9). In children younger than 14 years, concentrations are increased and age-dependent, as shown in *Table I* (10). Increased AAT blood level is associated with acute phase response and high body estrogen content, while the decrease can be caused by genetic deficiency, increased use and urinary or gastro-intestinal loss. Pulmonary parenchyma is considered adequately protected against uncontrolled NE activity when the AAT blood level is maintained above 0.5 g/L (11 μmol/L) (9).

Molecular genetics of AATD

Alpha-1-antitrypsin deficiency (AATD) is a genetic disorder caused by the inheritance of two deficient

Table I AAT levels in children of different age (10).

Age	Level (g/L)	Age	Level (g/L)
< 1 month	1.2–3.5	< 2 years	0.9–2.5
< 6 months	1.1–2.9	< 14 years	1.1–2.8

Table II Classification of SERPINA1 alleles (data about the most frequent deficient and dysfunctional alleles are bolded) (1).

Allele	Mutation type	Cellular defect	Predilection site
Functional alleles			
M	substitution (1 bp)	/	/
$X_{Christchurch}$	Glu363Lys	/	/
Deficient alleles			
S	**Glu264Val**	**degradation**	**lungs**
Z	**Glu342Lys**	**accumulation**	**lungs, liver**
M_{malton}	Phe52del/Phe51del	accumulation	lungs, liver
S_{iiyama}	Ser53Phe	accumulation	lungs, liver
$M_{heerlen}$	Pro369Leu	degradation	lungs
$M_{procida}$	Leu41Pro	degradation	lungs
$M_{mineral\ springs}$	Gly67Glu	degradation	lungs
Null alleles			
$QO_{granite\ falls}$	Tyr160X	no iRNA	lungs
$QO_{ludwigshafen}$	Ile92Asn	no protein synthesis	
$QO_{\ hongkong}$	Leu318LeufsX17	STOP codon	lungs
$QO_{isola\ di\ procida}$	deletion 17 kb, Ex2-5	no iRNA	lungs
Dysfunctional alleles			
F	Arg223Cys	↓NE inhibition	lungs
Pittsburgh	Met358Arg	AT III activity	hemorrhagic diathesis
Z	**Glu342Lys**	**↓NE inhibition**	**lungs, liver**
$M_{mineral\ springs}$	Gly67Glu	↓NE inhibition	lungs

variants of the gene encoding for AAT, namely SERPINA1 (11). The gene is located on chromosome 14q31, spans 122 kb in length and has seven exons and six introns. SERPINA1 gene is highly polymorphic, as evidenced by more than 120 allelic variants described so far (12). Regarding the functionality of the encoded protein and clinical manifestations associated with their inheritance, alleles can be classified as functional, deficient, null and dysfunctional, as presented in *Table II* (1).

An AAT molecule encoded by a deficient allele encounters a folding problem in establishing its tertiary structure, which in turns makes it prone to intracellular degradation or accumulation, resulting in insufficient secretion into the bloodstream. In the presence of null alleles, synthesis of AAT is significantly blocked, consequentially making the AAT blood level almost immeasurable. Dysfunctional alleles encode for AAT molecules with inadequate NE inhibition activity, which is not always accompanied with a reduced AAT blood level (1).

In European populations, functional (M) alleles have frequency of 95%, while among deficient ones, those designated as Z and S are the most common, occurring with average frequencies of 1–3% and 2–3% respectively (13). Comparison of the AAT level in the blood of individuals who inherited a Z allele and healthy persons reveals a ratio of 15% for a homozygous and 60–75% for a heterozygous state. For the S allele, the same ratio approximately equals to 60% in a homozygous and to 80% in a heterozygous state (1). The rest of deficient, null and dysfunctional alleles are jointly designated as »rare alleles«.

Clinical disease associated with AATD primarily occurs in individuals whose genotype contains no functional alleles and they are therefore denoted as »affected« individuals. The term AATD »carrier« refers to individuals inheriting a functional in combination with a deficient, null or dysfunctional allele. As a consequence, AATD is considered an autosomal recessive disease. Nevertheless, considering the fact that, due to the deleterious effects of various acquired risk factors, clinical manifestations may develop in »carriers«, the view that AATD is an autosomal co-dominant disease is also acceptable (1, 14).

Epidemiology of AATD

The two most important epidemiological issues in AATD are that it is a relatively common disease and that in a significant number of patients it is not detected in a timely fashion (1). The world-wide frequency is estimated to 1 affected in 3 000–5 000 persons, while the total incidence of individuals homozygous for Z and S alleles in Europeans is 0.2% and 0.3% respectively. Regarding clinical manifestations, AATD occupies the third position among potentially lethal genetic disorders among Caucasians, preceded only by cystic fibrosis and Down's syndrome (15). Estimations that AATD is diagnostically confirmed in only 6% of affected individuals, accompanied by data that the time span necessary for

identification of AATD as the cause of pulmonary or hepatic disorders averages at approximately 7 years, sufficiently illustrate the inappropriate diagnostic efficiency for AATD (1, 13).

Molecular pathology in AATD

Regarding its molecular pathology mechanisms, AATD is an example of conformational diseases, characterized by the formation of toxic protein aggregates (»toxic gain-of-function«) and loss of function. Analogous with the archetypical position of AAT among SERPINS, AATD can be regarded as the archetype of serpinopathies, a group of about 200 conformational diseases, caused or associated with mutations in the genes encoding for various SERPIN (e.g. hereditary angioedema, cirrhosis, dementia, hemorrhagic diathesis, thrombosis etc.) (16).

Due to the presence of Z mutation, the tertiary structure of the AAT molecule is distorted and the RCL of one molecule interlocks with the β pleated sheet of another, forming fibril-like polymers. Further polymerization leads to the formation of insoluble toxic inclusions, triggering ER stress and hepatocyte injury (»toxic gain-of-function«), thus representing a hallmark of AATD liver disease. Intracellular accumulation consequently diminishes anti-protease protection on the airway epithelial surface (loss-of-function) and an uncontrolled proteolytic attack is allowed (17). Additionally, polymerization of AAT locally produced in the lungs is also evidenced and significantly aggravates pulmonary manifestations and limits local administration of augmentative therapy (3).

In case of the S variant, the decreased AAT concentration results from molecular misfolding and increased molecule degradation within the hepatocytes, while the intracellular accumulation is absent (3).

Clinical manifestations of AATD

The clinical phenotype of AATD develops through complex, and still not completely understood, interactions between mutations in SERPINA1 and additional inherited and acquired factors. Homozygous presence of the Z allele or its combination with another deficient alleles is associated with severe clinical manifestations. In general, homozygous presence of the S allele is not related to significant clinical manifestations, while they may develop in individuals inheriting a combination of S and another deficient allele. It should also be emphasized that in some cases the protein encoded by a functional allele may lack functionality, due to the deleterious posttranslation effect of proteolysis, oxidation, nitrosylation etc. Additional risk factors contributing to the development of AATD clinical manifestations encompass various polymorphisms (e.g. genes encoding for NE, TNF-α, MMPs etc) and a plethora of environmental and acquired factors: smoking, air pollution, age, airways hyperreactivity, comorbidities etc. It is also observed that men are more prone to AATD manifestations development when compared to women (18).

Typically, pulmonary manifestations begin in middle-age years, while the hepatic it has been occur in childhood or old age. For a very long time it was believed that it should not be expected for both types of manifestations to develop in the same patient. Nevertheless, the results of clinical studies conducted afterwards provided enough evidence suggesting that risks for the development of different manifestations of AATD in the same persons are independent (18).

The most frequent genotype among patients with lung disease associated with AATD is ZZ (more than 95%), followed by SZ (1). Epidemiological data suggest that patients homozygous for the Z allele represent 1–2% of the total population of patients with COPD, while similar evidence is lacking for patients with the MZ and MS genotype. The most prominent issue in their anamnesis is relatively young age, ranging from 30 to 40 years, and frequent exacerbations. The course of disease is progressive, leading to development of panacinar emphysema, which might be expected when the AAT blood level falls below 0.5 g/L. The mechanism of emphysema development has been most extensively studied in patients homozygous for the Z allele, revealing that quantitative and qualitative deficiency act jointly. Due to intrahepatic polymerization, only small amounts of AAT reach the lung interstitium, thus increasing the quantity of uninhibited NE and possibility for elastin degradation. Qualitative deficiency of »mutated« AAT molecules, represented by decreased affinity for NE and increased aggregation diathesis, additionally blunts anti-elastolytic defence and potentiates neutrophils' chemotaxis into lung interstitium. If the patient is a smoker, emphysema development is accelerated because components of tobacco smoke facilitate AAT aggregation and oxidatively damage the RCL. Also, the possible enrolment of synchronic apoptosis in a large number of alveolar cells, enabled through constant activation of NF-kB by AAT polymers in the lungs, should not be neglected, especially because it can explain the panlobular nature of emphysema associated with AATD (7). Although suggested, an explicit causative relationship between AATD and asthma or bronchiectasis has not been established (6).

According to currently available data, hepatic manifestations have been associated with homozygous inheritance of Z, Siiyama and Mmalton alleles. In contrast to emphysema development, risk for hepatic injury in individuals with the MZ genotype is significant (18). In a certain number of newborns homozygous for the Z allele, cholestatic jaundice and hepatitis may develop immediately after birth. During childhood, affected individuals may encounter failure to thrive, increased activities of ALT and AST, hepatomegaly, or even cirrhosis and acute liver insufficiency. Nevertheless, it has to be emphasized that at least 50% of individuals

homozygous for the Z allele exhibit no signs of liver disease during childhood. In adults, symptoms of liver disease associated with AATD are extremely heterogeneous, varying from asymptomatic individuals, through patients with an unspecific increase in the activity of liver-specific enzymes to development of cirrhosis and hepatocellular carcinoma (18).

Paniculitis and certain types of vasculitis should be mentioned as less frequent manifestations of AATD, developing as the consequence of insufficient inhibition of membrane serine proteases and PR-3 respectively. In both pathological entities, proinflammatory effects of circulating polymers of mutant AAT molecules play a very important role. Additional associations of AATD and numerous diseases or conditions, such as rheumatoid arthritis, inflammatory bowel disease, pancreatitis, diabetes mellitus, atherosclerosis, various tumours etc, have emerged, but their nature, intensity and importance need further investigation (6).

Diagnostic strategy for AATD

Timely detection of AATD is of enormous importance for affected individuals because it offers the possibility of therapeutic and preventive interventions, as well as genetic counselling. At the same time, efforts should also be made to ensure that the established diagnostic strategy is cost-effective, so advantage should be given to targeted detection instead of population-based designs (2, 21). In accordance with the principles of evidence-based medicine, investigation of AATD is primarily indicated in conditions listed in *Table III*. Screening programs are only recommended for populations with an AATD incidence higher than 1:1 500 (19, 20).

Regarding the heterogeneity and uncertain specificity of associated clinical disease, AATD detection is mostly based on laboratory tests, enrolling quantitative analysis, SERPINA1 genotyping, AAT phenotyping using isolectric focussing and functional tests (19). Principles and performances of the available tests are presented in *Table IV* (9, 21, 22).

Table III Primary indications for AATD investigation (19, 20).

Lung disease	patients developing emphysema before the age of 45 years
	patients developing COPD before the age of 60 years
	patients with COPD with no traditional risk factors
	asymptomatic adults with constant airway obstruction and risk factors
Liver disease	unknown etiology
Other diseases	paniculitis
	vasculitis with no traditional risk factors
Familial testing	brothers and sisters of individuals homozygous for deficient alleles

Table IV Laboratory tests in evaluation of AATD (9, 21, 22).

Type	Principles and performance
Quantitative test	– principle: blood AAT level is measured using immunoassays – variants: nephelometry, *turbidimetry*, *RID* – samples: serum or heparinised plasma, blood spots – advantages: automated, low costs – constraints: genotype corresponding levels may overlap (*Table V*) (3), acquired states exert significant impact on results
Genotyping	– principle: direct SERPINA1 testing combining PCR with molecular techniques – variants: PSM, RFLP, ASO hybridization, SSCP, DGGE and RT-PCR for Z and S allele/Sequencing for null and rare alleles – samples: blood with sodium citrate as anticoagulant, bucal swabs, blood spots – advantages: relatively fast, unambiguous results, multiplex design, automated – constraints: »diagnosis of exclusion« (except sequencing), costs (sequencing)
Phenotyping	– principle: typing of AAT molecules according to pI at pH gradient 4.0–5.0 – variants: isoelectric focussing on polyacrylamide or agarose gel – samples: serum or blood spots after reductive pretreatment – advantages: simultaneous detection of all AAT variants secreted into blood (former »golden standard« for detecting AATD) – constraints: interpretation is rather complicated, time consuming, iatrogenic interferences, only semiautomation is possible
Functional test	– principle: measurement of serum's capacity to inhibit elastase or trypsin – constraints: rather low specificity – application: functional characterisation of newly discovered variants

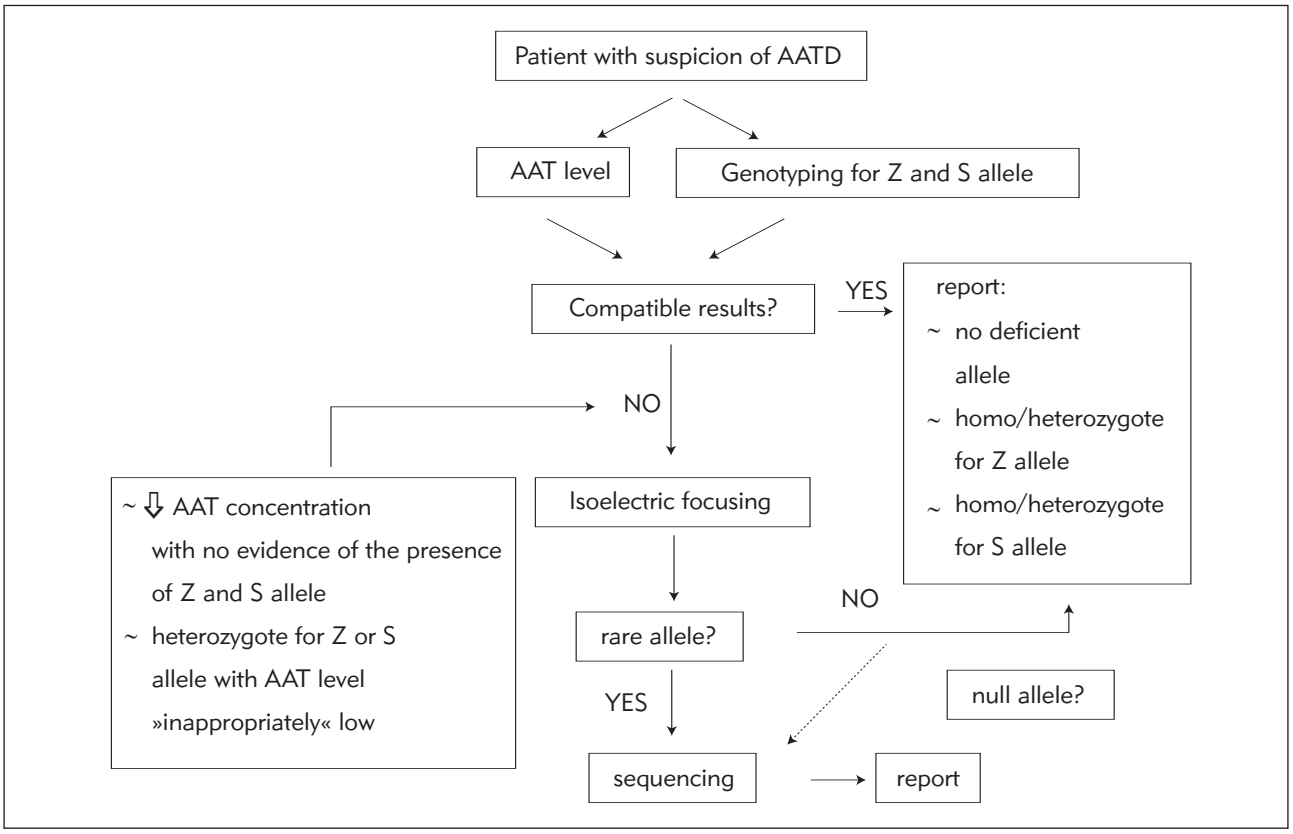

Figure 1 Integrative algorithm for AATD detection.

Table V The most common SERPINA1 genotypes and corresponding AAT levels (3).

Genotype	Concentration (g/L)	Genotype	Concentration (g/L)
MM	1.45 (0.9–2.0)	SZ	0.57 (0.3–0.8)
MS	1.18 (0.7–1.6)	SS	1.03 (0.6–1.4)
MZ	0.87 (0.5–1.2)	ZZ	0.10 (0.06–0.14)

Hitherto gained experience demonstrates that an optimal diagnostic approach should be based on integrative implementation of the most relevant clinical criteria for raising a suspicion that an individual is affected by AATD, and a standardized laboratory protocol, combining biochemical and molecular techniques, as presented in *Figure 1*. In suspected patients, first-line laboratory tests should include simultaneous measurement of the AAT level and investigation of the Z and S allele presence, using genotyping. Isoelectric focussing is considered as a reflex test, aiming to evaluate potential discrepancies between the AAT level and genotyping. If additional bands with altered mobility are visible in the gel pattern, it is wise to further investigate the presence of rare alleles using a sequencing technique. If this is not the case, the most probable cause of discrepancy can be attributed to acquired conditions that affect the AAT level. Nevertheless, caution is needed in such situations so as not to neglect the potential presence of null alleles. It is also worth emphasizing that the diagnostic process in the majority of individuals can be completed in the setting of clinical laboratories. Blood samples of patients identified as potential carriers of rare deficient alleles are referred to a specialized molecular biology laboratory for final confirmation (21, 22).

Therapeutic approach in AATD

Predilection site for clinical manifestations guides the therapeutic approach in AATD.

Augmentation is indicated in patients meeting the following criteria: functionally confirmed emphysema and homozygous presence of Z or rare allele, or their heterozygous combination. Usually, an infusion containing 60 mg/kg AAT is administered once a week. Results of clinical studies suggest that augmentation impedes degradation of lung parenchyma and might reduce mortality. The treatment efficiency is evaluated through monitoring improvement in the clinical status, the lungs function tests and increase in the AAT blood level. The only constraints of this approach

are that it is life-long treatment and implies rather high costs. For patients with no confirmed emphysema, the administration of symptomatic therapy and preventive measures, listed in *Table VI*, appears sufficient. Of course, this supportive approach is also recommended in patients with emphysema (3, 23).

If liver damage occurs in patients with AATD, augmentation is not applicable and it is even assumed that it can aggravate the condition. Therapy is not specific and in most cases includes regular evaluation of the clinical status and laboratory data (i.e. AFP level), correction of dietetic and life-style habits (alcohol reduction, reduced exposure to hepatotoxic agents, vaccination against hepatitis B virus, body mass control etc) and treatment of oesophageal varices. In the most advanced cases of cirrhosis, liver transplantation is recommended (18).

Possibilities for gene therapy in patients affected by AATD have been extensively studied in recent years (24). Despite encouraging results in animal models, amended by confirmation in three clinical and one preclinical study (25–28), its use in the treatment of pulmonary manifestations has not been officially approved yet. Up to now, the efficiency of gene therapy in the prevention of accumulation of »mutated« AAT molecules in hepatocytes has been tested only in animal models, and beside positive results, additional toxic effects have also been revealed (24).

Simultaneously with gene therapy, therapeutic application of small molecules acting as AAT aggregation inhibitors (i.e. synthetic chaperons, inhibitors of RCL interlocking among AAT molecules etc) has been investigated. Results were positive in animal models, but no efficiency in clinical studies has been reported yet. Recently, it has been evidenced that selective autophagy of AAT aggregates in human cell lines can be triggered by carbamazepine and sirolimus. Although promising, this approach needs serious and careful optimisation (29).

Table VI Symptomatic therapy and preventive measures for AATD-associated lung disease (3).

COPD therapy	Preventive measures
– bronchodilatators, corticosteroids, oxygen therapy, antibiotics – nutritive support, rehabilitation	– smoking cessation – reduced exposure to: dust, pesticides, air pollutants etc. – vaccination (influenza, *Streptococcus pneumoniae*)

Table VII AATD integrative diagnostic approach in the Serbian population – clinical indications.

Indication	Number of patients	Indication	Number of patients
Adults			
Emphysema	26	Respiratory insufficiency	1
COPD	40	Asthma	5
Chronic bronchitis	3	Pneumothorax	14
Bronchiectasis	1	Pulmonary fibrosis	2
Pulmonary infiltration	2	Familiar testing	15
Children			
Cholestasis	5	Bronchiolitis	1
Hepatitis	2	Familiar testing	3

Integrative approach in AATD detection in the Serbian population

The frequency of Z allele in the Serbian population (1.3%) is comparable to that in the populations of Central Europe, while the S allele frequency (0.66%) is the lowest among the populations of Europe, except for the Finish (30, 31). In order to improve diagnostic efficiency, an algorithm integrating precise indications for AAT testing and a standardized laboratory protocol was introduced. Standards for the diagnosis and management of individuals with AATD issued jointly by the American Thoracic Society and European Respiratory Society served as a basis for the clinical part of the algo-

Table IX Incidence of AATD and »carrier« status (given as absolute number) regarding indications for testing.

Indication	AATD (ZZ + SZ + $M_{malton}Z$)	»carrier« status (MZ + MS)
Emphysema	4 (3+0+1)	7 (6+0)
COPD	3 (3+0+0)	4 (3+1)
Chronic bronchitis	0	1 (1+0)
Bronchiectasis	0	0
Pulmonary infiltration	0	2 (1+1)
Respiratory insufficiency	0	0
Asthma	0	0
Pneumothorax	0	1 (1+0)
Pulmonary fibrosis	0	1 (1+0)
Cholestasis	0	1 (1+0)
Hepatitis	0	1 (0+1)
Bronchiolitis	0	0
Familiar testing	1 (0+1+0)	6 (6+0)

rithm (19). In establishing a standardized laboratory protocol, international recommendations were followed (21), and the PCR-rASO hybridization was included as the initial molecular-based test (32).

From January 2007 till March 2012, AATD was evaluated in 120 persons: 109 adults (58 men/36 women) and 11 children (4 boys and 7 girls). Indications for testing in adults and children are presented in *Table VII*. AATD was detected in 8 cases (6 with ZZ, 1 with SZ and 1 with MmaltonZ genotype), while 23 persons were denoted as »carriers« (19 with MZ and 4 with MS genotype). Accordingly, the frequency of Z and S alleles calculated in this clinically targeted subgroup of Serbian population was 13.7% for Z and 2.1% for the S allele. *Table IX* presents the incidence of AATD and »carrier« status regarding indications for testing.

Conclusion

During the past six decades, significant improvements in understanding both the AAT physiology and its deficiency have became evident. Mechanisms of tight regulation of NE activity are almost completely elucidated and important additional links with the control mechanisms, including regulation of apoptosis, have also been established. Structural defects in the AAT molecules, resulting from being encoded by dysfunctional SERPINA1 alleles, and the consequent cellular response, have been so extensively studied they even served as a basis for establishing new categories in molecular pathology – «sick molecules« and »conformational diseases« (16, 33). Investigation of clinical disease associated with AATD emphasized its dependence upon the complex interaction between AATD and additional genetic and acquired factors, and identified premature emphysema, chronic hepatitis, cirrhosis and hepatocellular carcinoma as the most frequent clinical manifestations. A specific therapeutic approach is guided by the predilection site of clinical disease, so augmentation is currently applicable only in patients where the lungs are affected, while for advanced liver failure, caused by AATD, transplantation represents the only approach. Health outcomes in affected individuals are expected to be significantly improved with the introduction of gene therapy into common practice. Despite all these improvements, diagnostic efficiency of AATD, as evidenced by epidemiological findings, is not satisfactory, thus prompting joint action for improvement. It is recommended for a diagnostic approach to integrate precise, internationally recognized clinical criteria, and a standardized laboratory protocol, based on a combination of biochemical and molecular methods. Current results with the integrative AATD diagnostic strategy in a Serbian population are highly encouraging, prompting towards its further implementation in common medical practice with the ultimate goal to establish a national register of affected individuals. It has to be emphasized that an inevitable prerequisite for the accomplishment of such goals is continuous teamwork of medical biochemists, molecular biologists and clinicians.

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Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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