Alpha 1 Proteinase Inhibitor

CLINICAL USE AND FUTURE TRENDS

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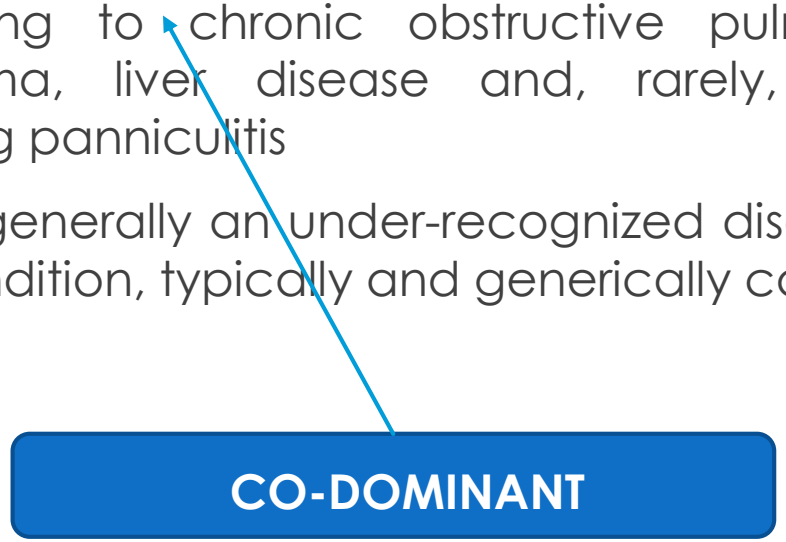
European Alpha-1 Clinical Research Collaboration (EARCO) – European Respiratory Society (ERS)

Outline

- Genetic, epidemiology and clinical features of Alpha 1 Antitrypsin Deficiency
- Unveil the hidden cohort of alpha 1 deficient subjects: is there any room for population screening?
- Augmentation therapy with alpha 1 proteinase inhibitor: expected results in the «conventional» patient
- New insights on the physiology and pathophysiology of Alpha 1 Antitrypsin
- Potential future applications of alpha 1 proteinase inhibitor

ALPHA 1 ANTITRYPSIN DEFICIENCY

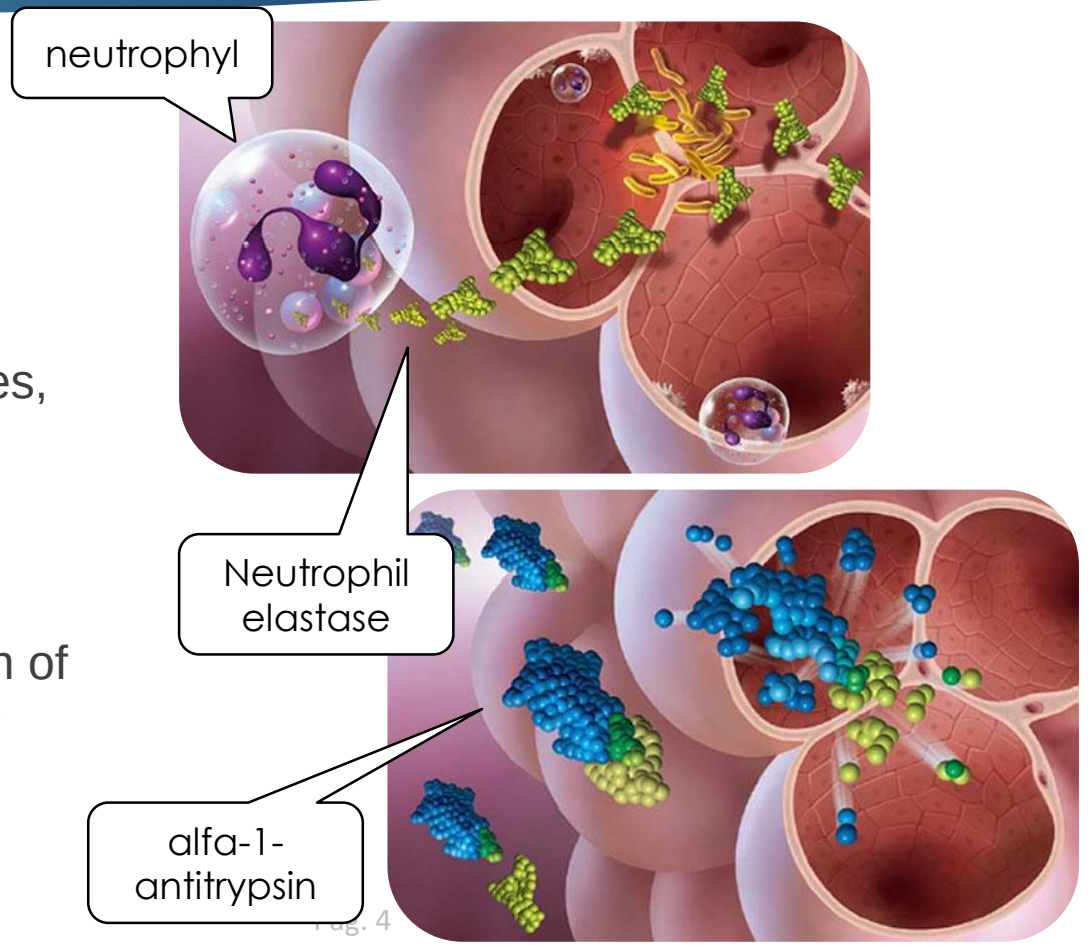
- Alpha-1-antitrypsin deficiency (**A1ATD, OMIM #613490**) is a genetic autosomal recessive disease characterized by low serum levels of A1AT, predisposing to chronic obstructive pulmonary disease (COPD) or emphysema, liver disease and, rarely, multi-organ vasculitis and necrotizing panniculitis
- A1ATD is generally an under-recognized disease because it is considered a rare condition, typically and generically categorized as COPD.



Alfa-1-antitrypsin

Properties *:

- 52 kDa plasma glycoprotein
- produced by hepatocytes contributes to almost all of the circulating AAT, although it is also produced in smaller quantities by other cells such as monocytes, macrophages, pulmonary alveolar cells and intestinal epithelial cells
- characterised primarily by its function as an extracellular protease inhibitor of neutrophil elastase (NE)
- AAT is abundant in the plasma with a mean concentration of 1.3 g/L (range 0.9–1.75 g/L) and a plasma half-life of 4–5 days



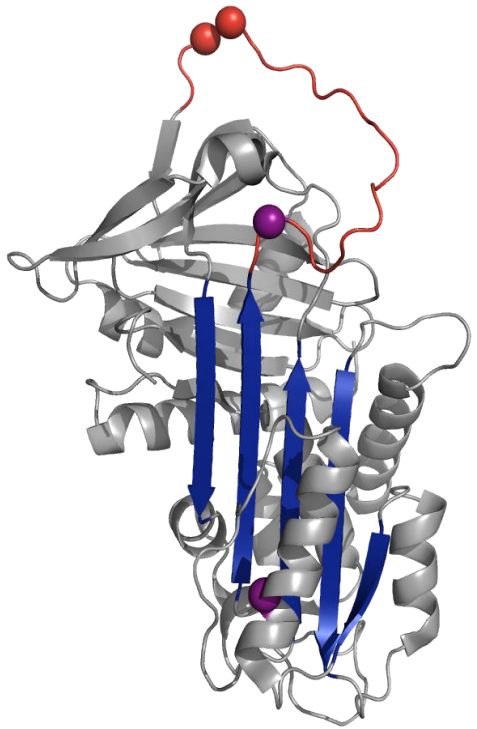
** ATS/ERS statement: standards for the diagnosis and management of individuals with alpha-1 antitrypsin deficiency. Am J Respir Crit Care Med 2003; 168:818-900*

***Alves Costa C., Santos C. Alpha-1 antitrypsin deficiency. The experience of Pulido Valente Hospital with augumentation therapy. Rev Port Pneumol. 2009; XV(3): 473-481*

Alfa-1-antitrypsin

Genetic

- encoded by the SERPINA1 on chromosome 14q32.1-32
- SERPINA1 is also polymorphic with over 200 mutations recognised to date that reduce plasma AAT levels by altering protein production and folding, or influencing the glycosylation status of AAT
- mutations are classified by their phenotypic expression and electrophoretic mobility during isoelectric focusing; PiM (medium), PiS (slow), and PiZ (very slow)
- The most severe deficiency states are defined by AAT plasma levels less than 35% of the mean expected value (11 μM or 50 mg/dL measured by nephelometry). This is commonly as a result of a point mutation causing an amino acid change from glutamic acid to lysine at position 342 (Glu342Lys), which is referred to as the Z allele.
- Additionally reported are the PiSZ, PiSS, and rare or null allel



Per gentile concessione del Prof. David Lonas, Cambridge, UK

ATS/ERS statement: standards for the diagnosis and management of individuals with alpha-1 antitrypsin deficiency. Am J Respir Crit Care Med 2003; 168:818-900

Worldwide PI Z variant Gene Frequency Distribution

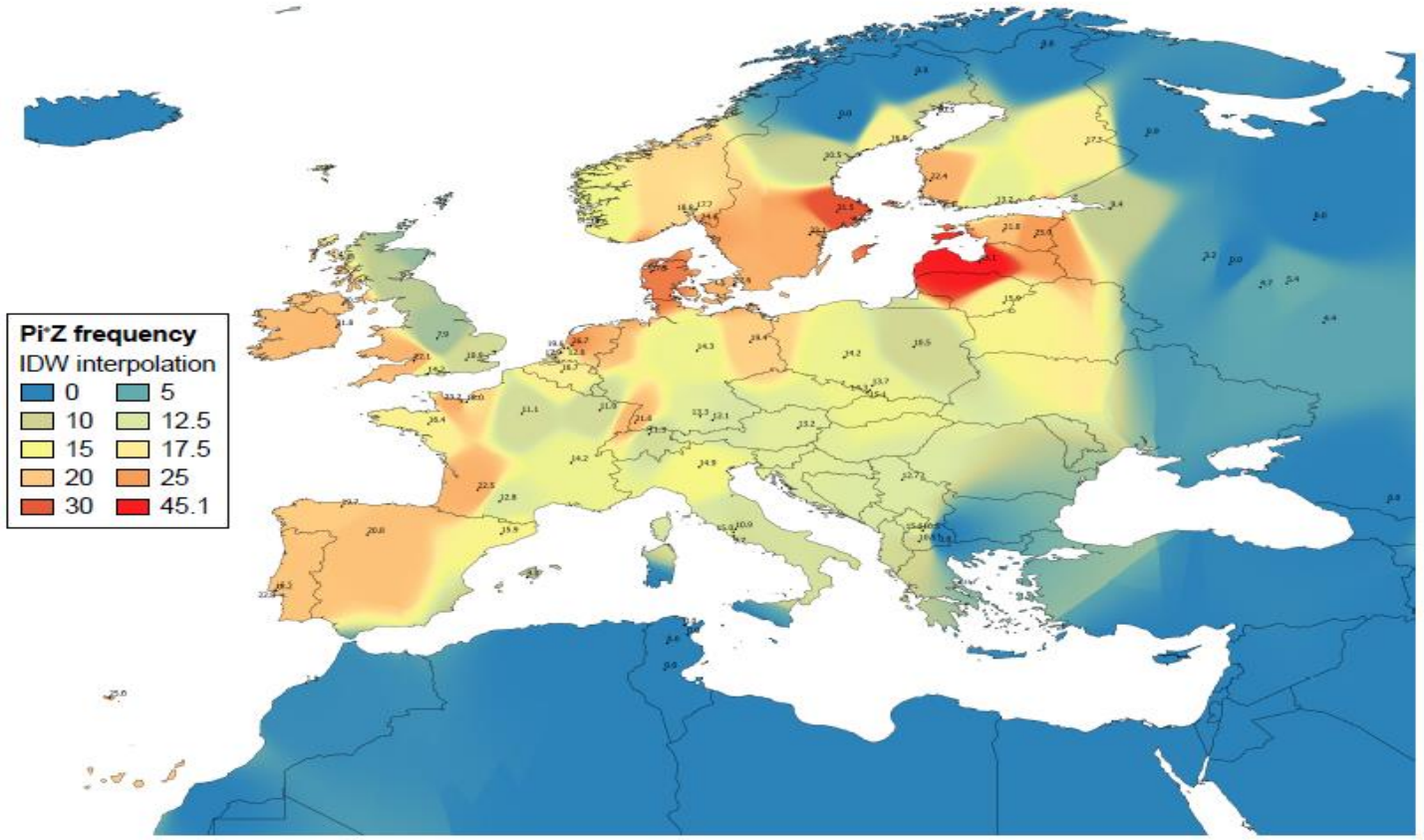


Figure 1 Distribution of Pi*Z frequencies in Europe (×1,000).
Abbreviation: IDW, inverse distance weighted.

REVIEW ARTICLE

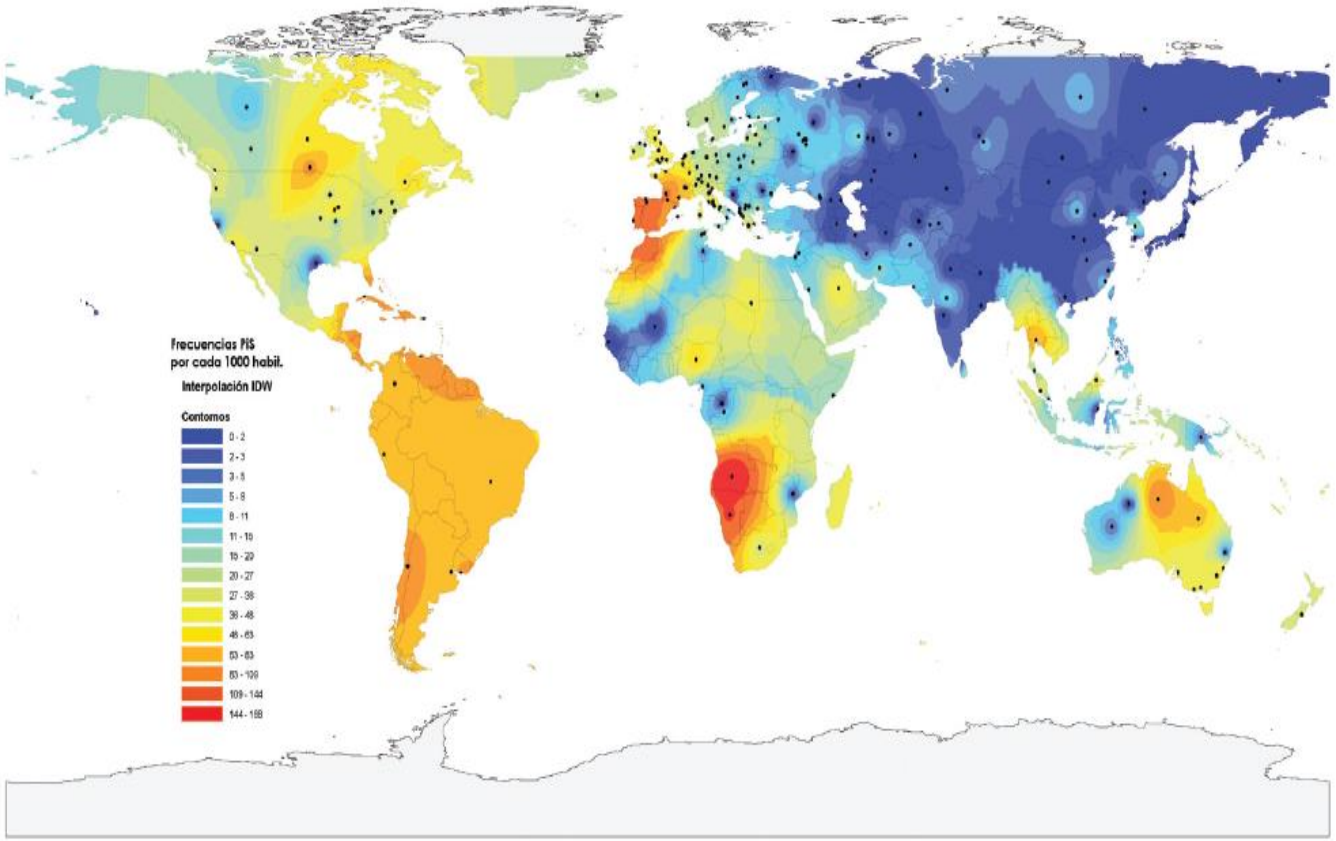
Alpha-1 Antitrypsin Deficiency: Current Perspective from Genetics to Diagnosis and Therapeutic Approaches

Simona Santangelo[1,*,#], Simone Scarlata[1,#], Luana M. Poeta[2], Adam J. Bialas[3,4], Gregorino Paone[5] and Raffaele Antonelli Incalzi[1]

[1]U.O.C. of Geriatrics, Unit of Respiratory Pathophysiology and Thoracic Endoscopy, Campus Bio Medico University and Teaching Hospital, Rome, Italy; [2]Department of Bioscience, Biotechnology and Biopharmaceutics, University of Bari, Bari, Italy; [3]Department of Pulmonology and Allergy, Medical University of Lodz, Lodz, Poland; [4]Healthy Aging Research Centre, Medical University of Lodz, Lodz, Poland; [5]Department of Cardiovascular, Respiratory, Nephrologic, Anesthesiologic and Geriatric Sciences, "Sapienza" University of Rome, Rome, Italy

The countries of Northern and Western Europe have the highest prevalence of the PI Z variant. This mutation appears to have originated in southern Scandinavia

Worldwide PI S variant Gene Frequency Distribution



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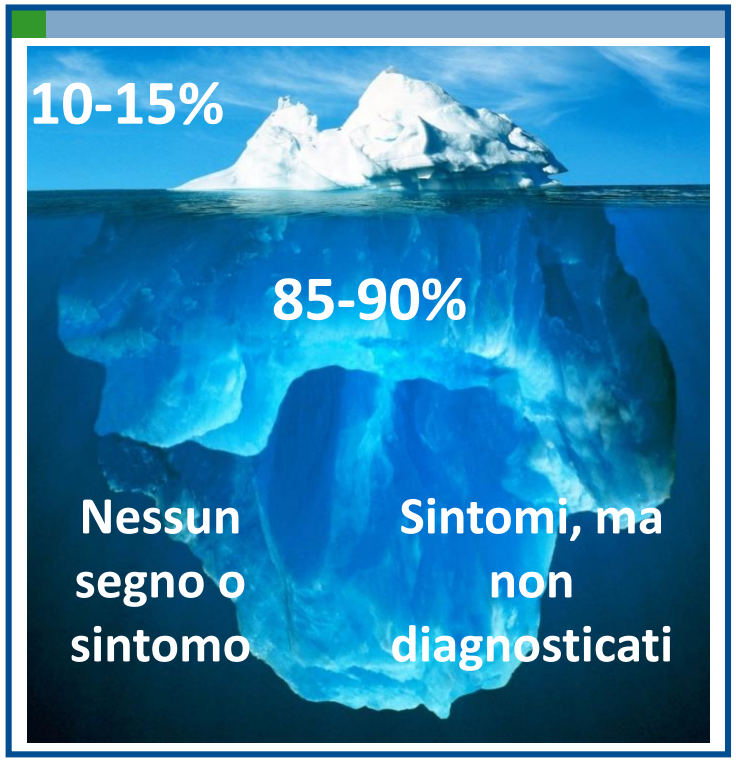
The population of southern Europe (peak in the Iberian peninsula) has the highest prevalence of the PI S variant

Mean Gene Frequency in the main geographic regions of the world

Geographic Region	Cohorts: N°/Size	Mean Gene Frequency - PI S	Mean Gene Frequency - PI Z
Northern Europe	38/21,005	0.0176	0.0153
Southern Europe	77/33,769	0.0564	0.0125
North America	43/33,147	0.0328	0.0092
Australia and New Zealand	15/8,243	0.0395	0.0151
Africa	23/3,887	0.0310	0.0048
Far East Asia	24/8,685	0.0007	0.0004
Central Asia	48/6,151	0.0043	0.0040

Alpha 1 antitrypsin deficiency: an underdiagnosed condition

- Frequency of severe deficiency (PiZZ) in Europe[1]: approx. 1:5,000
- To date, only 10 to 15 % of all carriers of homozygosis have been diagnosed
- A mean 6-8 years delay occurs from symptoms onset to final diagnosis[2]



[1]*Blanco I et al., Eur Respir J 2006 ;*
[2]Khonlein T et al., Ther Adv Respir Dis, 2010

Lack of knowledge and awareness between the health care providers

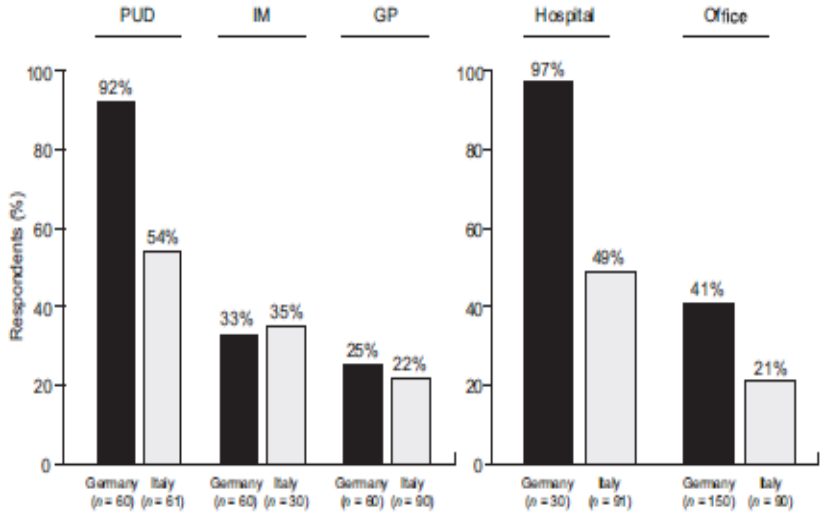


Figure 4 Physicians currently testing for AAT deficiency.
Percentage of respondents from three different disciplines in Germany and Italy who answered *"Yes"* to the question, *"Do you currently test for AAT deficiency?"*.
PUD – pulmonologists; IM – internal medicine specialists; GP – general practitioners.

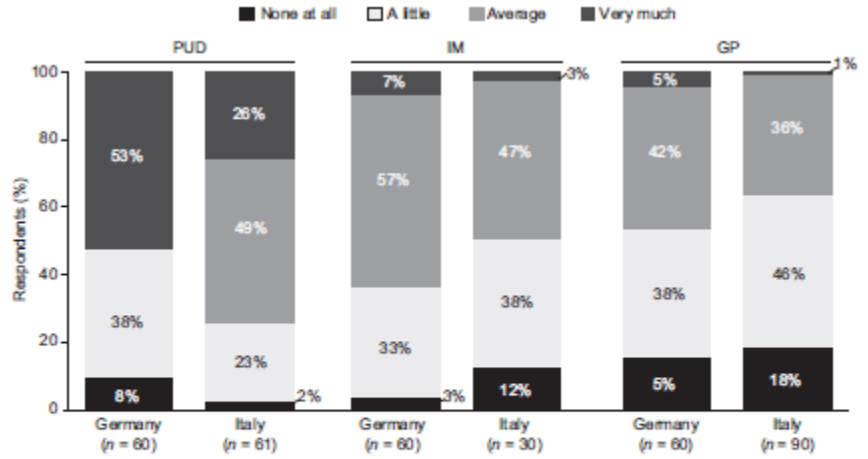
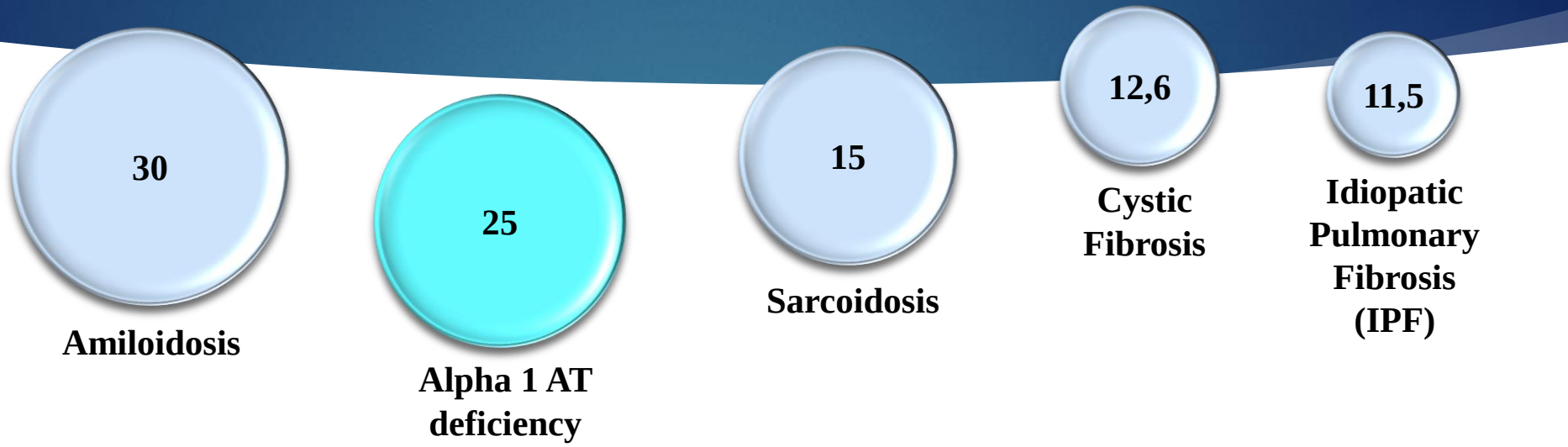


Figure 3 Knowledge of lung disease caused by AAT deficiency, by specialty.
Percentage of respondents from three different disciplines in Germany and Italy who gave the answer indicated to the question, *"How much do you know about lung disease caused by AAT deficiency?"*.
PUD – pulmonologists; IM – internal medicine specialists; GP – general practitioners.

Prevalence of rare diseases with clinical manifestations in the lungs



#/100.000 inhab.

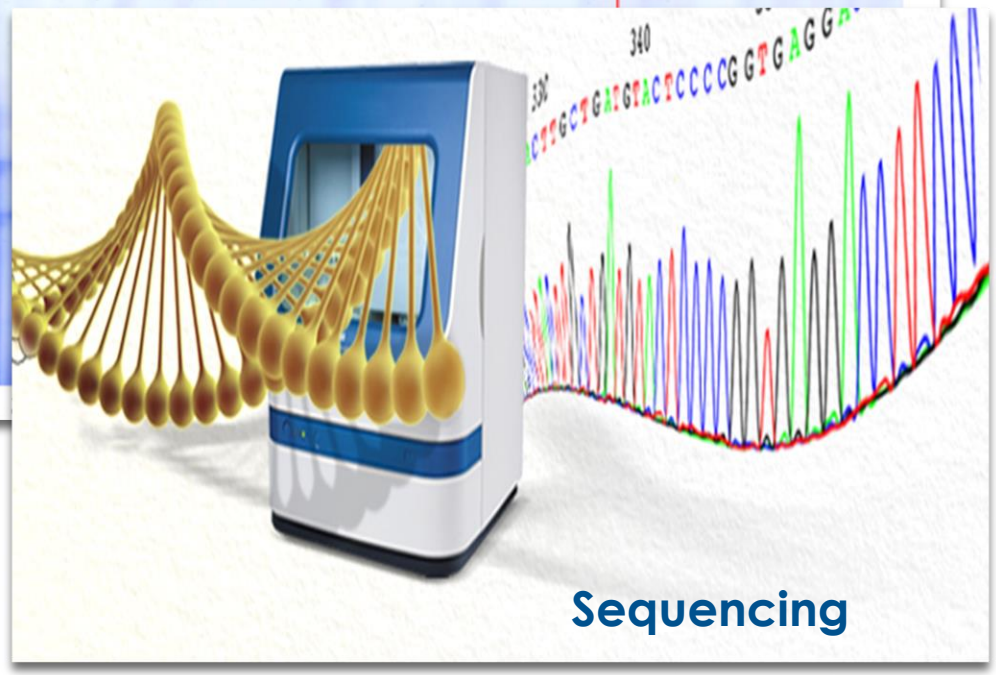
Prevalenza delle malattie rare, dati bibliografici Orphanet Maggio 2014

Diagnostic workflow

Nefelometry

Phenotyping (IEF)

Genotyping



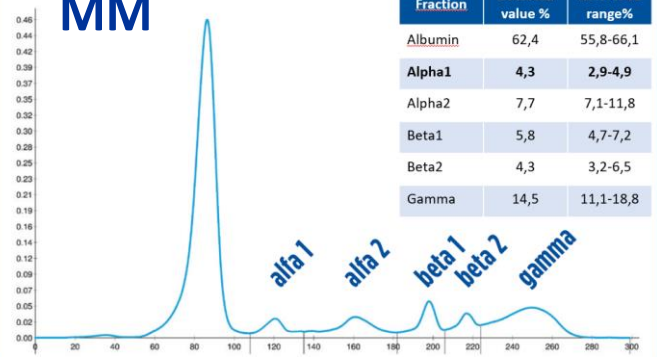
Test	Strenghts	Limitations
Nephelometry, radial immunodiffusion, Rocket immunoelectrophoresis	Measurement of circulating A1AT Inexpensive, available in most local areas, automated, good reliability	It does not detect heterozygous individuals. It can overestimate the AAT levels
Phenotyping (IEF)	It identifies many alleles of AAT based on the electrophoretic migration pattern	It does not identify null alleles and M-like alleles, laborious, difficult to interpret
Genotyping	Molecular identification of PI Z and PI S variants. Semi-automation possible	It does not identify some rare null variants. Long analysis times (3-4 days)
Sequencing	Identification of rare variants	Expensive and with long analysis times

Is there any room for population screening?

Simone Scarlata*, Simona Santangelo, Ilaria Ferrarotti, Angelo Guido Corsico, Stefania Ottaviani, Panaiotis Finamore, Davide Fontana, Marc Miravitlles and Raffaele Antonelli Incalzi

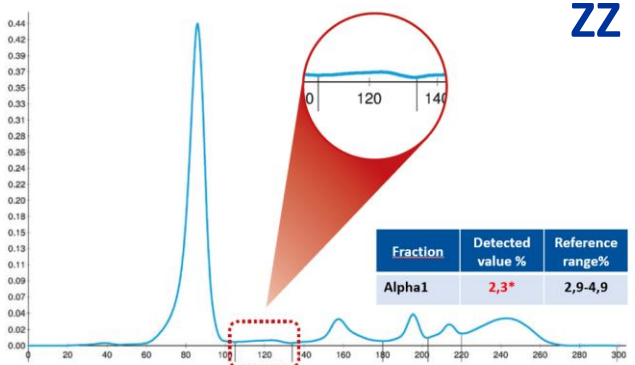
Electrophoretic α_1-globulin for screening of α_1-antitrypsin deficient variants

MM



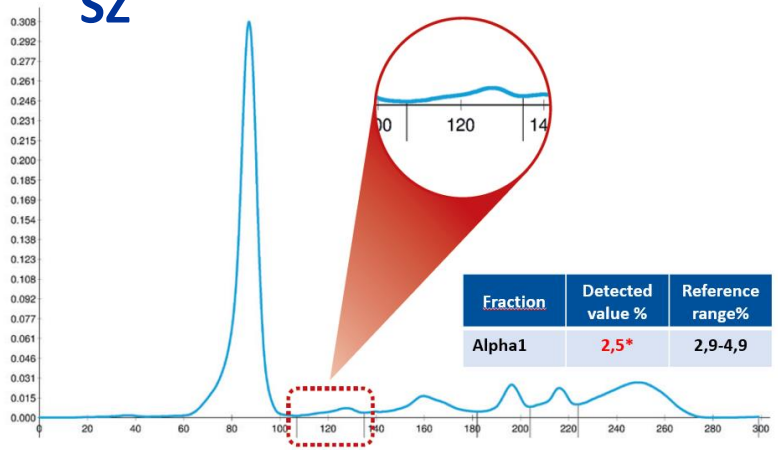
Fraction	Detected value %	Reference range%
Albumin	62,4	55,8-66,1
Alpha1	**4,3**	**2,9-4,9**
Alpha2	7,7	7,1-11,8
Beta1	5,8	4,7-7,2
Beta2	4,3	3,2-6,5
Gamma	14,5	11,1-18,8

ZZ

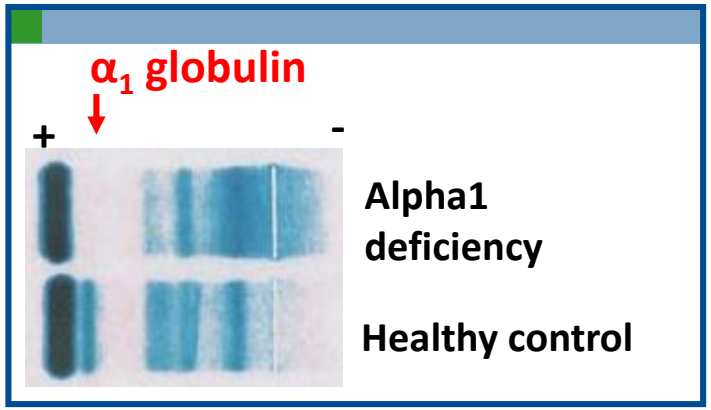


Fraction	Detected value %	Reference range%
Alpha1	**2,3***	**2,9-4,9**

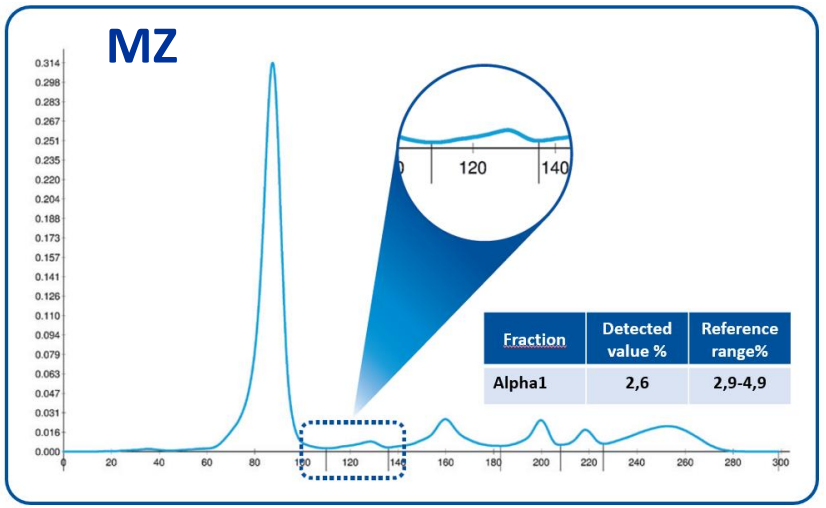
SZ



Fraction	Detected value %	Reference range%
Alpha1	**2,5***	**2,9-4,9**



MZ



Fraction	Detected value %	Reference range%
Alpha1	**2,6**	**2,9-4,9**

Is there any room for population screening?

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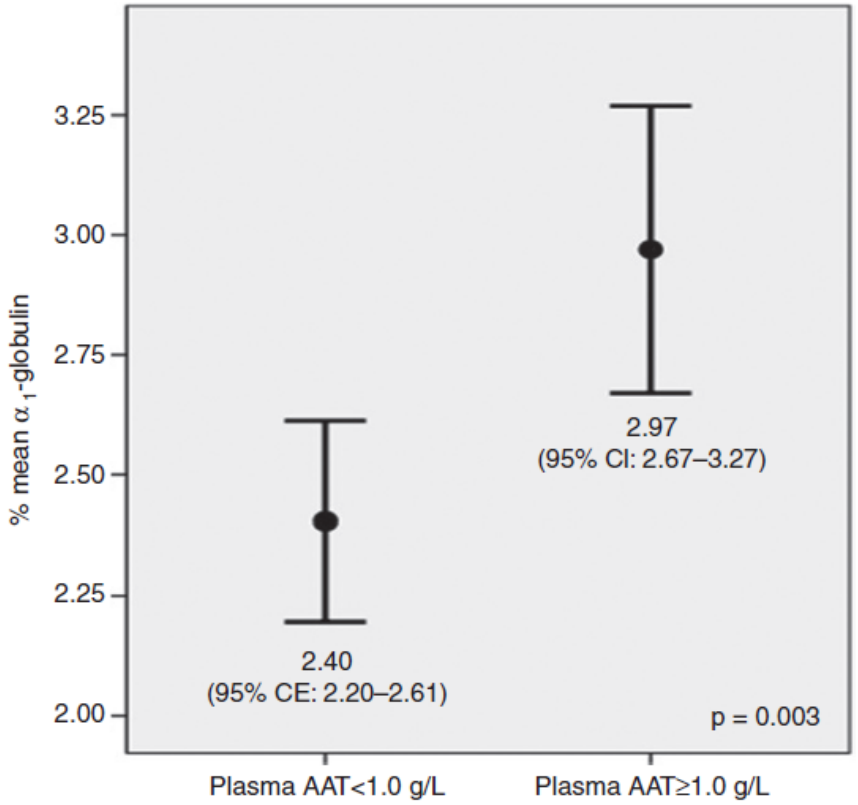
Electrophoretic α_1-globulin for screening of α_1-antitrypsin deficient variants

Table 1: Summary of population screening (PS) groups (A, B and C) with genetic AATD and related detection rates compared with data from clinical screening.

	Group A	Group B	Group C	Total	Aggregated (A+B+C+FS)	Clinical screening (CS)	Aggregated (CS+FS)
Number of screened subjects	63	5	14	82	97	113	127
Number of genetic AATD	38	2	2	42	49	18	24
Detection rate, %	60.3	40.0	14.2	51.2	50.5	15.9	18.9

Group A, α_1-globulin <2.6%; Group B, α_1-globulin 2.6%–2.9% and AST/GOT: >37 U/L and ALT/GPT: >78 U/L; Group C, α_1-globulin %: 2.9–4.6% and AST/GOT: >37 U/L and ALT/GPT: >78 U/L and ESR > 34 mm/h and CRP > 3 mg/L; AATD, α_1-antitrypsin-deficient; CS, clinical screening; FS, family screening.



Conclusions: A composite algorithm primarily based on the α_1-globulin fraction could effectively identify carriers of *Pi*M* gene mutation. This approach, not requiring clinical
evaluation or AAT serum determination, seems suitable for clinical and epidemiological purposes.

Augmentation therapy with alpha 1 proteinase inhibitor

Intravenous infusion of alpha1-proteinase inhibitor, prepared from a human plasma pool of normal donors, has the following objectives:

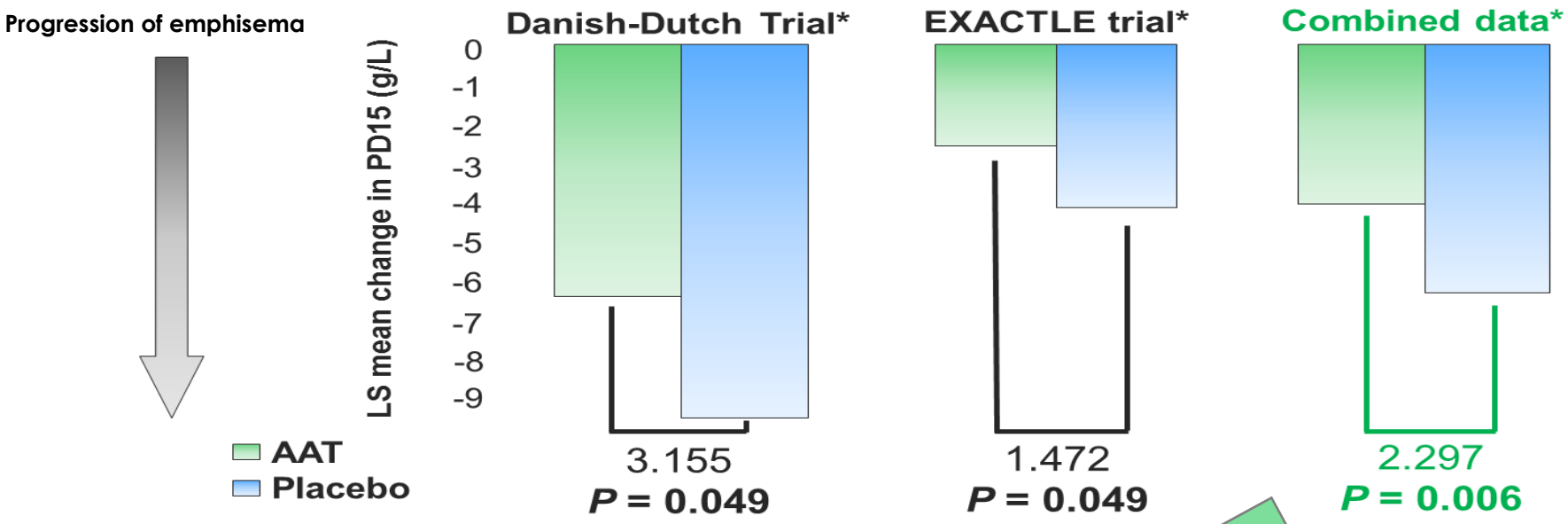
1. **increase and maintain serum AAT levels above the threshold of 50mg / dl ***
2. **slow down the progression of emphysema**
3. **reduce the number of exacerbations**
4. **improve the duration and quality of life**

To date, replacement therapy has only been approved in adults with clinical evidence of obstructive pulmonary disease due to DAAT and is currently indicated for those phenotypes that are most likely associated with lung disease (e.g. PiZZ, PiZ (null), Pi (null) (null) and PiSZ and rare variants).

- Çatipoglu S. Stoller JK. Chest Med. 2016 set; 37 (3): 487-504
- Teschler H. Eur Respir Rev. 2015 Mar; 24 (135): 46-51
- Vidal R. et al. Arch Bronconeumol. 2006;42(12):645-59

* se misurata con Nefelometria

Significant reduction in the progression of emphysema measured on CT in patients treated with replacement therapy

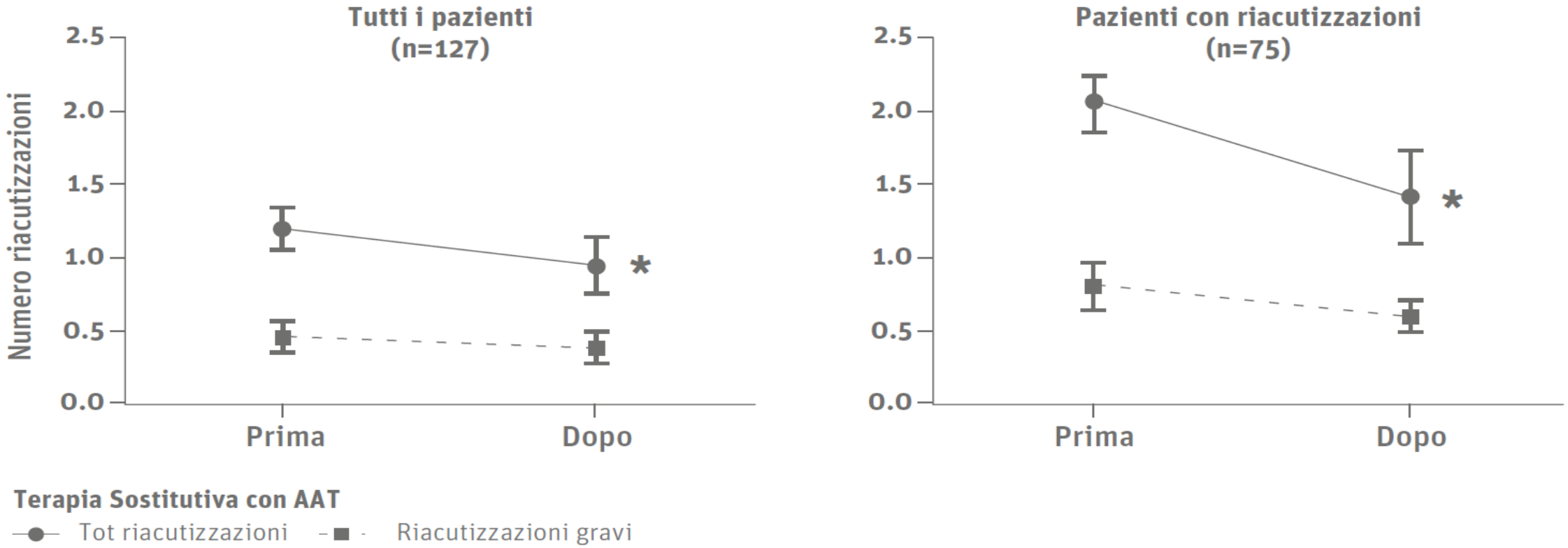


Reduction of lung tissue loss vs. placebo

36%

Stockley RA et al. Respir Res 2010; Oct 5;11(1).

Number of exacerbations

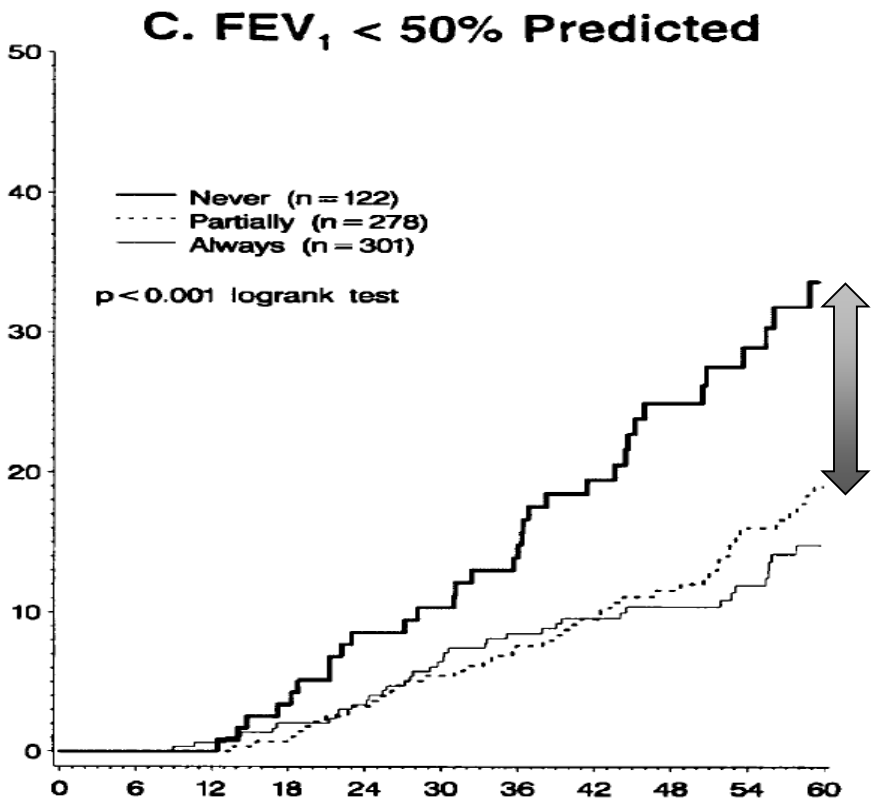


Barros T JC et al. Ther Adv Respir Dis (2012) 0(0) 1–12.

Survival and FEV_1 Decline in Individuals with Severe Deficiency of α_1-Antitrypsin

The Alpha-1-Antitrypsin Deficiency Registry Study Group*

The Alpha-1-Antitrypsin Deficiency Registry Study Group. Am J Respir Crit Care Med 1998; 158:49-59.



Substantial difference between the group of patients treated with replacement therapy and those never treated

Highly significant survival advantage ($p < 0.001$)

Pleiotropic functions of alpha-1 antitrypsin

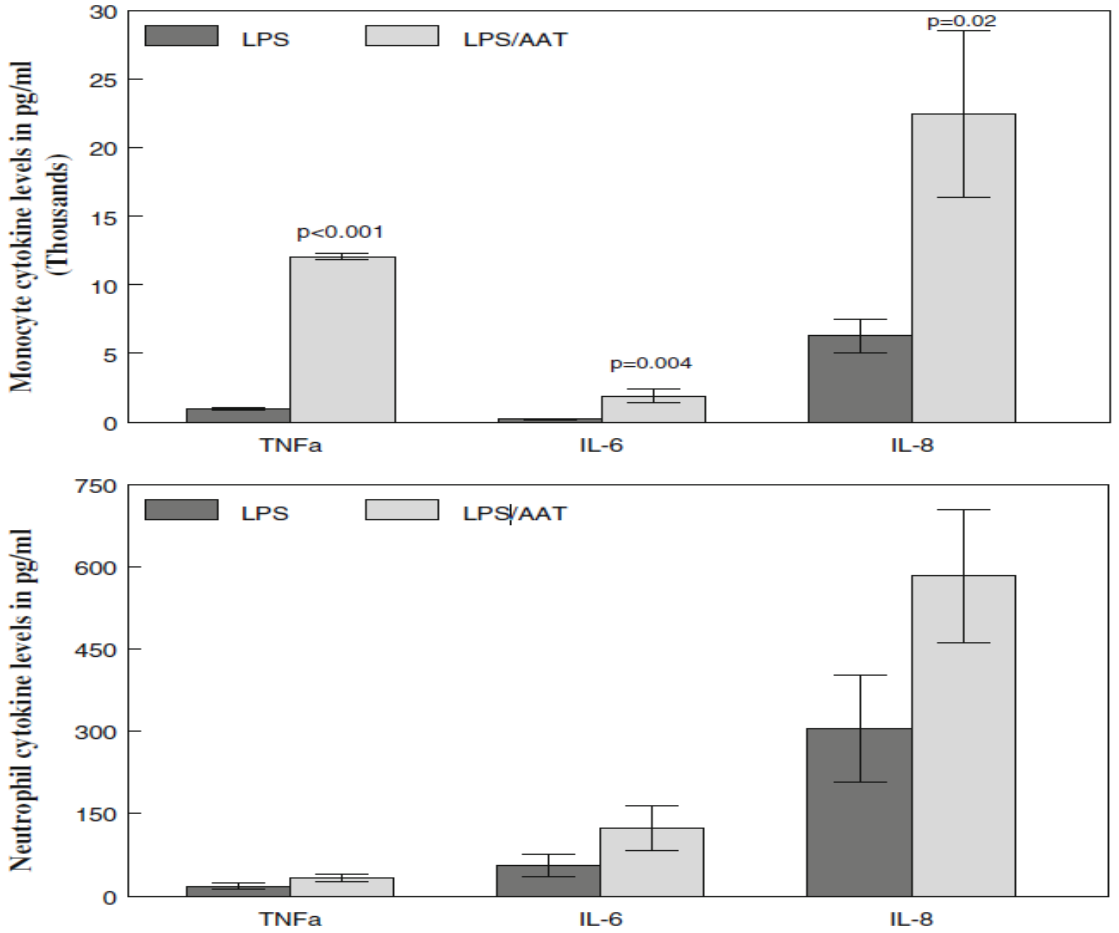
Role of Alpha-1 Antitrypsin	Function	Reference
Protease inhibitor	Anti-NE, -Cath-G and -PR3	[47]
Anti-apoptosis	Inhibition of caspase-1, caspase-3, and calpain-1	[23,25,26]
Antioxidant	Oxidative stress inhibition	[67]
Anti-inflammatory/tissue repair	Repair, fibroblast proliferation, procollagen synthesis, and activation of MAP kinase pathways	[68]
	Modulation of ADAM-17 activity	[28]
	Substrate for metalloproteinase MMP-9 activity	[69]
	Inactivation of matriptase in vitro and inhibition of epithelial sodium transport in vitro and in vivo	[70]
Antibacterial	Bacteriostasis—binding to furin (inhibits bacterial toxin activation)	[71]
Antiviral	Inhibition of HIV-1 viral cell entry	[72]
	Inhibition of SARS-CoV-2 entry by inhibiting transmembrane serine protease 2 and ADAM-17	[73,74]

Int. J. Mol. Sci. **2022**, *23*, 2441

Effects of alpha 1-antitrypsin on endotoxin-induced lung inflammation in vivo

Inflamm. Res. (2010) 59:571–578

AAT enhances the magnitude of LPS-induced specific cytokine/chemokine production, which may play an important role in **amplification and resolution of acute-phase inflammatory reactions in vivo.**

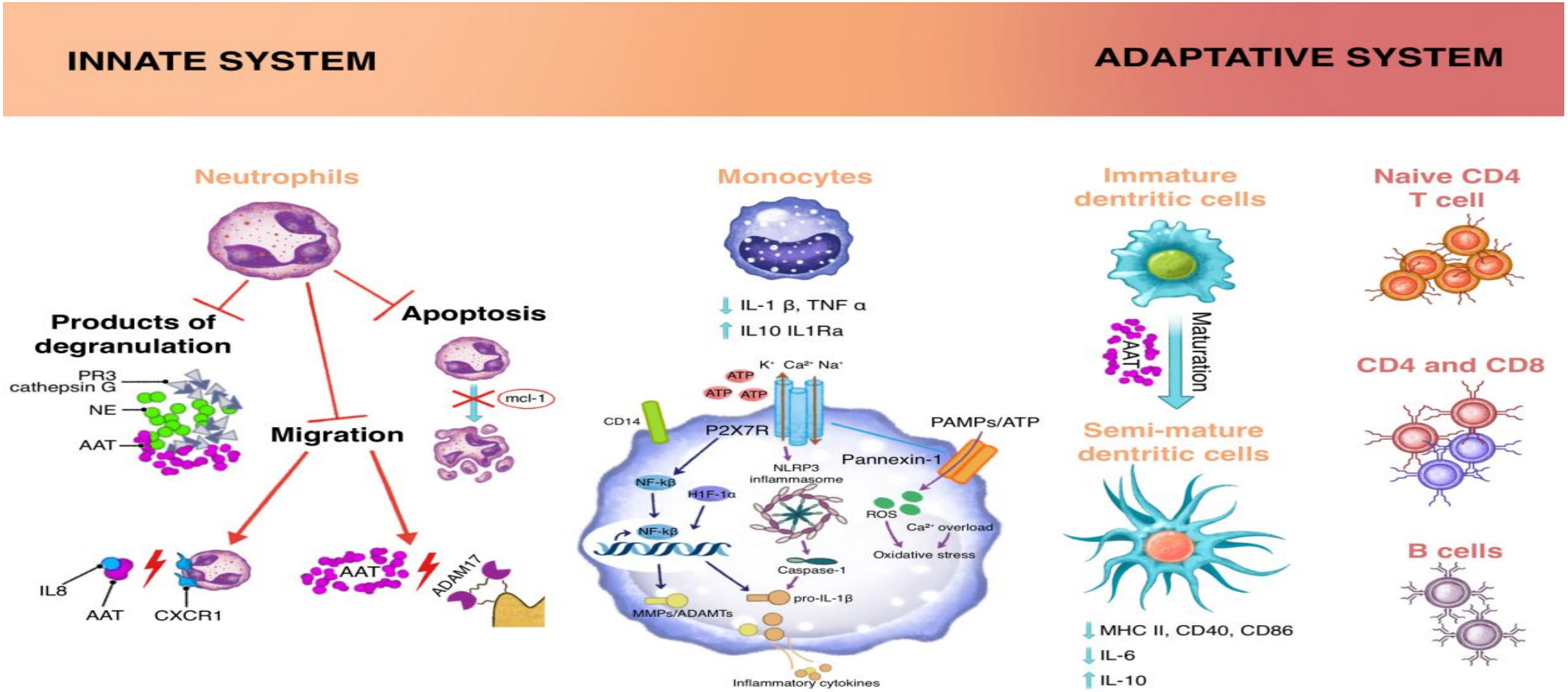


AAT immunomodulatory action: effects on innate and adaptive immune response

Immunological and homeostatic pathways of A1AT: a new therapeutic potential.

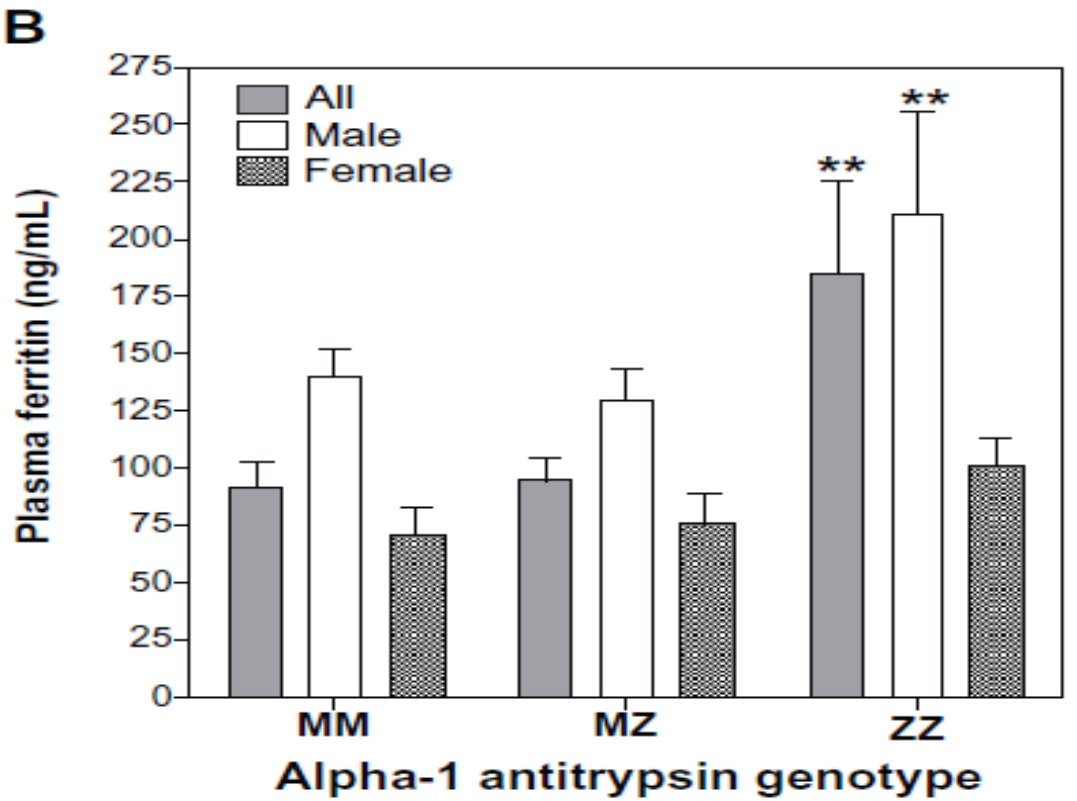
Mazzuca C, Vitiello L, Travaglini Silvia, Rigon A, Vadacca M, Zennaro D, Ramaccia M, Santangelo S, Scarlata S.

In press



Deficiency of α-1-antitrypsin influences systemic iron homeostasis

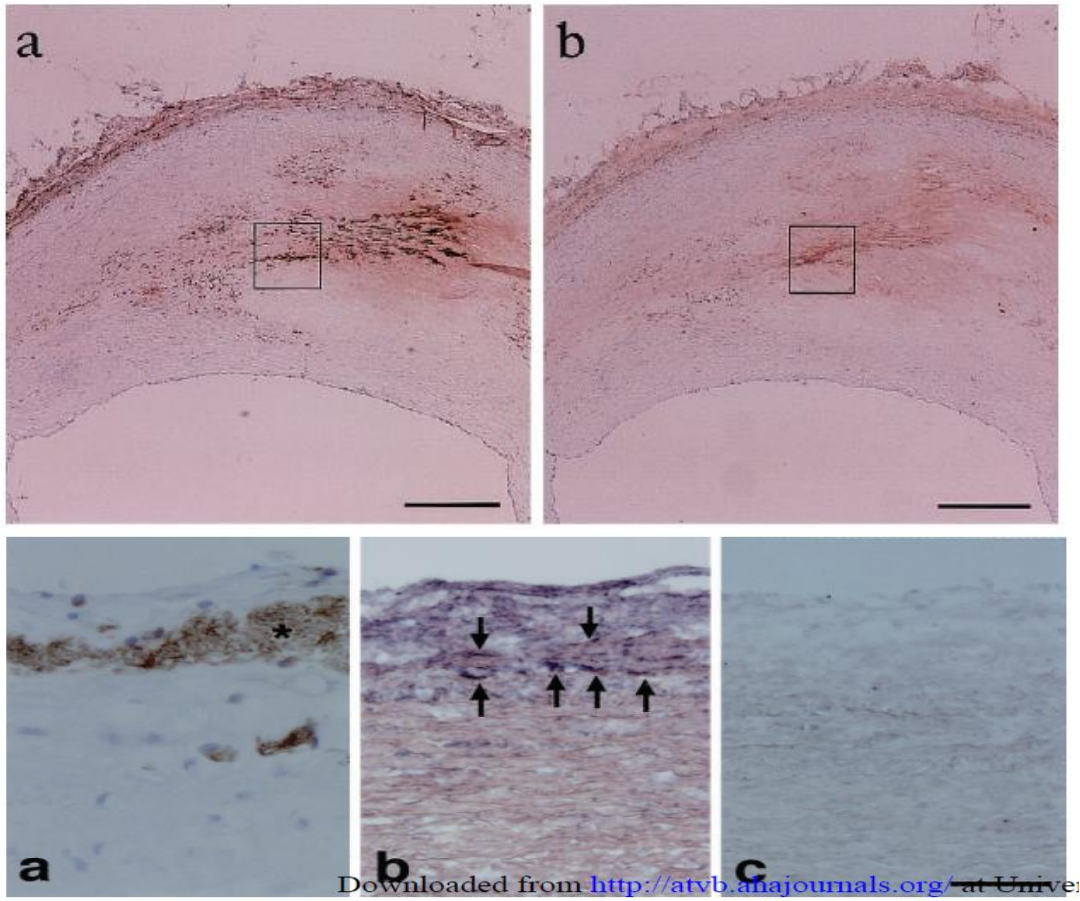
We conclude that A1AT deficiency is associated with evidence of a disruption in iron homeostasis in ZZ individuals with plasma ferritin and iron concentrations being elevated. Such a disruption in iron homeostasis can contribute to understanding the clinical presentation of A1AT deficiency.



In Vivo Complex Formation of Oxidized α_1-Antitrypsin and LDL

Arterioscler Thromb Vasc Biol. 2001;21:1801-1808

AT is produced and oxidized by macrophages, then attached to LDL in the intimal layer of the arterial wall. Although AT-LDL that escapes into the blood stream can be cleared by hepatocytes, the remaining AT-LDL may be taken up by macrophages and **contribute to the lipid accumulation in arterial wall cells as the early stage of atherogenesis.**



Anti-inflammatory effect of A1AT administration in Pulmonary disease

Non AAT deficiency associated COPD/emphysema

α_1-Antitrypsin Suppresses TNF-α and MMP-12 Production by Cigarette Smoke–Stimulated Macrophages

CLINICAL RELEVANCE

This article shows that α_1-antitrypsin can suppress cigarette smoke–induced production of TNF-α and MMP-12 by alveolar macrophages, a novel mechanism that will lead to suppression of TNF-α–mediated inflammation and thus prevent emphysema.

Am J Respir Cell Mol Biol Vol 37. pp 144–151, 2007

Cystic Fibrosis

α_1-Antitrypsin inhalation reduces airway inflammation in cystic fibrosis patients

Inhalation of AAT increased AAT levels and decreased the levels of elastase activity, neutrophils, pro-inflammatory cytokines and the numbers of P. aeruginosa. However, it had no effect on lung function

Eur Respir J 2007; 29: 240–250

Asthma

Long-term augmentation therapy with alpha-1 antitrypsin in an MZ-AAT severe persistent asthma

Bronchial asthma could be due to pathogenic mechanisms related to a protease-antiprotease imbalance, what which could open new perspectives for future research on the field.

Monaldi Arch Chest Dis. 2008 Dec;69(4):178-82

Efficacy of alpha1-antitrypsin augmentation therapy in conditions other than pulmonary emphysema

Orphanet Journal of Rare Diseases 2011, **6**:14

Rheumatoid Arthritis

Alpha-1 antitrypsin protein and gene therapies decrease autoimmunity and delay arthritis development in mouse model

These results present a new drug for arthritis therapy. Human AAT protein and gene therapies are able to ameliorate and delay arthritis development and reduce autoimmunity

Journal of Translational Medicine 2011, **9**:21

Vasculites

Clinical Effect of Alpha-1 Antitrypsin Deficiency in Antineutrophil Cytoplasmic Antibody–associated Vasculitis: Results from a French Retrospective Monocentric Cohort

The potential therapeutic consequences of plasma exchange must be addressed. Prospective studies are required to assess the real effect of plasma exchange with fresh plasma replacement in patients with both ANCA-positive AAV and AAT deficiency

The Journal of Rheumatology 2019; 46:11

Fibromyalgia

Intravenous Infusions of Purified Alpha-1 Antitripsyn Effectively Controls Symptoms and Reverts Muscle Biopsy Changes in an MZ Alpha-1 Antitripsyn Deficiency and Fibromyalgia Syndrome Patient

The clinical and histopathological efficacy of AAT-Intravenous was evidenced and should open new perspectives of research and management of AAT deficiency and FMS patients

Journal of Musculoskeletal Pain, Vol. 18(2), 2010

Usefulness in autoimmune diseases

graft-versus-host disease (GVHD)

α_1-Antitrypsin infusion for treatment of steroid-resistant acute graft-versus-host disease

AAT infusion produced a high proportion of durable clinical responses in SR-aGVHD.
AAT is associated with minimal toxicity and low rates of infection in patients with SRaGVHD at significant risk for mortality.

Blood. 2018;131(12):1372-1379

Diabetes Mellitus

α1-Antitrypsin Protects β-Cells From Apoptosis

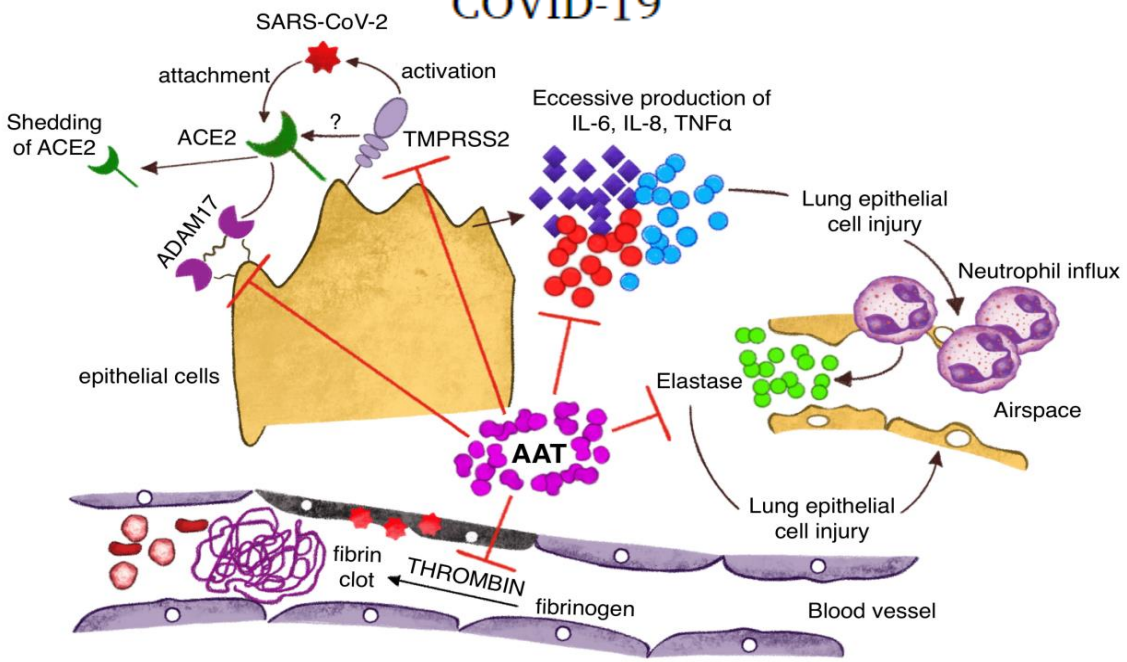
Potential role of AAT in the pathogenesis of type 1 (and possibly type 2) diabetes and support efforts with the goal of examining the potential therapeutic benefits of AAT administration for reversing and/or preventing these disorders

Diabetes 56:1316–1323 2007

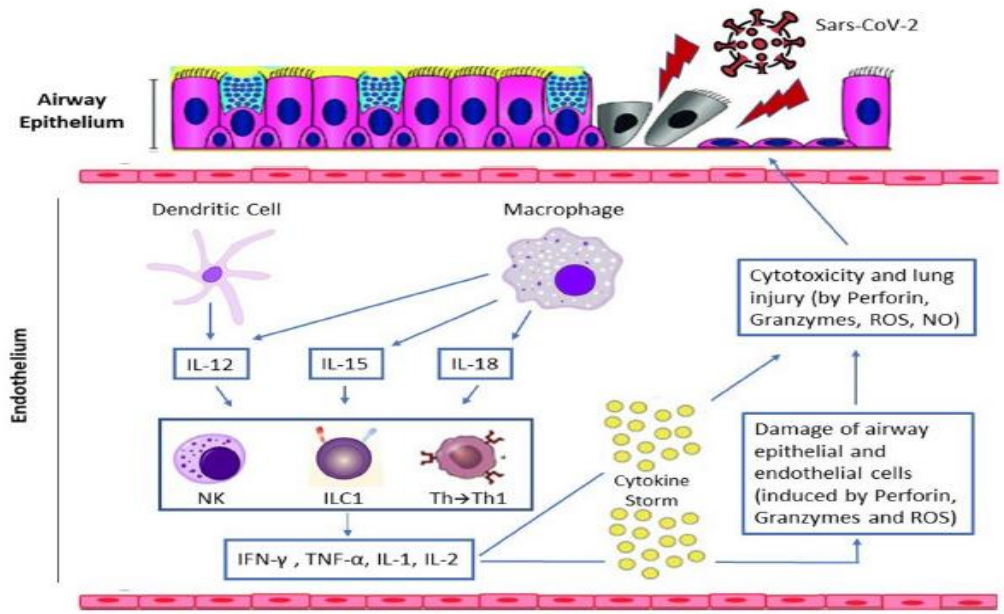
Usefulness in infectious diseases

Sars-CoV-2

Hypothesis: Alpha-1-antitrypsin is a promising treatment option for COVID-19



Medical Hypotheses 146 (2021) 110394



Conclusions

It is recognised that AAT has diverse interactions beyond protease inhibition that have been shown to facilitate beneficial anti-inflammatory and antiapoptotic responses.

Uncovering the protease and novel non-protease binding properties of AAT has led to a deeper understanding of the function of this protein in health and disease

Knowledge of the full interaction profile of AAT as it circulates in health and in deficiency states may lead to a deeper understanding of its effects, uncover novel mechanisms of action, and ultimately lead to innovative therapeutic applications of augmentation therapy in a variety of disease states

Alpha 1 Proteinase Inhibitor

CLINICAL USE AND FUTURE TRENDS

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