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Alpha-1 deficiency in severe asthma patients

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SUMMARY

INTRODUCTION: Alpha-1 antitrypsin (AAT) deficiency, an autosomal co-dominant condition, decreases protein concentration and activity at both serum and tissue levels. Few studies investigated whether the type of *SERPINA1* gene phenotype in patients with severe asthma can influence symptoms and disease control during follow-up.

OBJECTIVE: To assess whether the presence of a non-MM genotype of *SERPINA1* in patients with severe asthma is associated with disease control, systemic and airway inflammation, lung function and comorbidities prevalence compared to severe asthma patients with a homozygous genotype (MM).

METHODS: Asthmatic patients belonging to Global Initiative for Asthma (GINA) step 5 were retrospectively analysed in an Italian reference asthma clinic. We collected clinical, biological and functional variables at baseline and for the three following years.

RESULTS: Out of 73 patients enrolled, 14 (19.18%) were non-MM and 59 (80.8%) were MM. Asthmatics with non-MM genotype had lower serum AAT concentration (P¼0.004) and higher emphysema prevalence than the MM group (P ¼ 0.003) at baseline. During follow up, only MM patients showed a significant improvement of both ACQ-6 score ($P < 0.0001$) and eosinophilic systemic inflammation ($P < 0.0001$).

CONCLUSIONS: Our findings emphasise the importance of a screening for AAT deficiency in severe asthma, as alleles mutation may influence patient's follow-up.

KEY WORDS: severe asthma; alpha-1 antitrypsin deficiency; lung function decline; inflammation

Alpha-1 Antitrypsin (AAT), is a glycoprotein encoded by the *SERPINA1* gene located on chromosome 14 at the cytogenetic locus 14q32.12. The gene is organised into 6 introns and 7 exons, 4 of which are coding (II, III, IV, V) and 3 non-coding (IA, IB, IC).[1] The mature protein consists of 394 amino acids, with a molecular weight of 52 kDa and a half-life in blood of 4–5 days.

It is produced mainly in the liver and in smaller quantities in pancreas, lung alveolar cells and enterocytes. In the lungs, AAT mainly inhibits neutrophil elastase in order to avoid the damage of pulmonary alveoli, leading to emphysema.[2,3] Mutations in the *SERPINA1* gene may cause decreased serum concentration of the AAT protein.[4] To date, nearly 300 protein-changing variants in the AAT gene have been identified, and the most common are the Z allele, found in >1% of people with European ancestry, and the S allele.[5] Affected people may be homozygous (two copies of the same pathological allele), compound heterozygous (two different pathological alleles), or simple heterozygous (one pathological and one healthy allele, i.e., M).[5]

As alpha-1 antitrypsin deficiency (AATD) is a codominant disease, each allele contributes 50% of the total circulating enzyme inhibitor: MM homozygous patients have normal AAT level, and non-MM patients have intermediate or severe AATD. In other words, individuals with a pathological allele in heterozygosity have intermediate AATD,[6] while homozygous and compound heterozygous patients have severe AATD with AAT serum levels 50 mg/dL.[7] AAT levels may be considered normal if they are >110 mg/dL. The AAT cut-off set by each laboratory varies, and generally lies between 100 mg/dL and 120 mg/dL.[8] A recent paper proved that an optimal decisional cut-off of 110 mg/dL can discriminate between PI*MM and any other genotypes carrying at least one S or Z allele, with a 78.3% sensitivity and 86.4% specificity.[9] In patients with normal C-reactive protein (CRP) levels and AAT 110 mg/dL, second-level investigations should be performed, including phenotyping, genotyping and gene sequencing.[10]

Several published papers indicate that asthma is a common comorbidity in patients with AATD, as AAT inhibits neutrophil elastase-induced airway

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inflammation.[11,12] Its reduced activity may predispose to the development of chronic airway hyperreactivity as a result of the increased release of chemotactic agents by neutrophils.[13]

Numerous studies have investigated the relationship between AATD and severe asthma, but the results are contradictory, and no study ever described airways and systemic inflammation in severe asthma patients in non-MM heterozygosity.[14–16] To date, the WHO recommends quantitative screening of AAT levels for adults and adolescents asthma patients.[17]

Although the association between asthma and AATD is well documented, a review by Pini et al. highlights the need for targeted studies to investigate the biological mechanisms underlying this relationship, with focus on non-MM heterozygosity.[18]

For this reason, we hypothesised that patients diagnosed with severe asthma with pathological genetic mutations on the *SERPINA1* gene may exhibit clinical, biological and functional characteristics that differentiate them from severe asthma patients without genetic mutations.

METHODS

A retrospective analysis was conducted in Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Maugeri, Tradate, Italy, from 2018 to 2023. Demographic, clinical and biological data were collected in a de-identified database. Approval from the IRCCS Maugeri Ethical Committee was obtained (N° 2722 CE, 26/04/2023). The study was performed according to the Declaration of Helsinki.

Study populations

Between September 2018 and January 2021, seventy-three outpatients with severe asthma in the clinically stable phase underwent a quantitative analysis of the serum level of AAT and subsequent genotyping. The diagnosis of asthma was made in accordance with the Global Strategy for Asthma Management and Prevention (GINA) guidelines.

Participants in the study were patients who met the following inclusion criteria: age >18 years, both sexes, with stable severe asthma, and who had signed an informed consent to medical genetics. The presence of liver disease, a known history of AATD and exacerbation in the month prior to the visit were exclusion criteria.

Based on the genetic test results, the study population was consecutively enrolled and subsequently divided into two groups: 59 patients in MM homozygosity and 14 patients in non-MM heterozygosity.

Measurements

The Asthma Control Questionnaire 6 (ACQ-6) was used in all patients to assess symptoms and disease control. All patients were interviewed about their comorbidities, therapies and smoking habits in order to record the number of packs/years of smoking. Atopy, according to the World Allergy Organisation (WAO) definition, was assessed by prick tests for the most common inhalant allergens and also the concentration of total and specific immunoglobulin E in serum (dust mites: *Dermatophagoides pteronissinus and farinae,* pollens: *Ambrosia elatior, Betula verrucosa, Corylus avellana, Parietaria officinalis,* Graminaceae mix, *Olea europaea, Alms incana;* moulds: *Altentaria alternata* and *Aspergillus fumigatus;* cat and dog epithelia) were determined.

Quantitative analysis of AAT was performed using a turbidimetric method (normal serum AAT range: 90–200 mg/dL). A concomitant normal serum CRP concentration was used as an internal control for the analysis. Genetic analysis for *SERPINA1* gene sequencing was in accordance with recent guidelines.[10] Patient blood samples used for the genetic analysis were run through the AlphaKit® QuickScreen (Diagnostic Grifols, Barcelona, Spain) test. The sample was analysed at the specialised centre at the Fondazione IRCCS Policlinico San Matteo in Pavia. Blood eosinophils and neutrophils (expressed as cells/mm^3 and as % of white blood cells) were measured as part of the complete blood and differential count.

Patients performed spirometry tests (MIR MiniSpir; MIR, Rome, Italy), and both forced expiratory volume in the 1 sec (FEV_1) and forced vital capacity (FVC) were recorded and expressed as absolute values (in litres, L) and as a percentage of a predicted value (% predicted). The FEV_1 and FVC values were also recorded as a ratio. At least three measurements were taken for each spirometric test and each lung volume variable to ensure reproducibility of the data. The induced sputum test was performed according to ATS/ERS guidelines to study bronchial inflammation.[19] The fraction of exhaled nitric oxide (FeNO) was also measured in all patients (Vivatmo Pro; Bosch by Cosmed, Rome, Italy).

In addition, asthma control, lung function decline and systemic inflammation were evaluated every year during a three-year follow-up.

Statistical analysis

An ad hoc electronic form was created to collect all study variables. Qualitative variables were described with absolute and relative (percentages) frequencies, whereas quantitative variables were shown in based on their normal or non-normal distribution as mean (standard deviations [SD]) or median (interquartile ranges [IQR]), respectively. A Kolmogorov–Smirnov test was used to assess the normality of distribution in all variables. Comparisons between two groups for qualitative and quantitative variables were assessed with the v^2 or Fisher's exact test when appropriate, Student *t*-test and Mann–Whitney in case of parametric or nonparametric distribution. The analysis of

Table 1. Gene variants analysis of AADT patients.

ID	Variant name	Mutation	Consequences
1	S	p.E288V - E264V - c.863A>T rs17580	Increased turnover; decreased inhibitory activity mild protein deficiency
2	S	p.E288V - E264V - c.863A>T rs17580	Increased turnover; decreased inhibitory activity mild protein deficiency
3	$Q0_{Clayton}$	c.1152-1158insC –p.400Ter	No protein production
4	S	p.E288V - E264V - c.863A>T rs17580	Increased turnover; decreased inhibitory activity mild protein deficiency
5	S	p.E288V - E264V - c.863A>T rs17580	Increased turnover; decreased inhibitory activity mild protein deficiency
6	S	p.E288V - E264V - c.863A>T rs17580	Increased turnover; decreased inhibitory activity mild protein deficiency
7	S	p.E288V - E264V - c.863A>T rs17580	Increased turnover; decreased inhibitory activity mild protein deficiency
8	F	p.R247C – R223C- c.739C>T rs28929470	Normal circulating protein levels, but dysfunctional
9	$M_{Whitstable}$	g.11640del26bp, insGG	Truncated protein, protein deficiency
10	S	p.E288V - E264V - c.863A>T rs17580	Increased turnover; decreased inhibitory activity mild protein deficiency
11	S_{Munich}	p.S354F – S330F- c.1061C>T rs201788603)	Mild protein deficiency
12	S	p.E288V - E264V - c.863A>T rs17580	Increased turnover; decreased inhibitory activity mild protein deficiency
13	Z	p.E366K -E342K- c.1096G>A rs28929474	Polymerisation, decreased inhibitory activity; protein deficiency
14	$M_{Whitstable}$	g.11640del26bp, insGG	Truncated protein, protein deficiency

AADT ¼ alpha-1 antitrypsin deficiency.

variance test was applied to evaluate the lung function decline intra-group. The allele frequencies were determined by direct gene count method. The observed genotype frequencies were tested for Hardy–Weinberg equilibrium using the v^2 test, after applying the Yates correction due to the presence of rare genotypes.

$P < 0.05$ was considered statistically significant. Stata v17 (Stata Corp, College Station, TX, USA) was used for every statistical computation.

RESULTS

Seventy-three patients with severe asthma (68.5% female) were included in the study. At baseline all subjects were treated with high doses of beclomethasone hydrofluoroalkane (HFA) equivalent, respectively 48/73 and 30/73 were treated with long-acting muscarinic antagonist (LAMA) and/or leukotriene receptor antagonist therapy (LTRA); no patient was treated with biological therapy for severe asthma (anti-interleukin [IL] 5 or anti-IL 5R or anti-IL4/IL13 or anti-IgE). Although all patients involved were eligible for at least one biological drug only 67/73 (91.7%) accepted the therapy.

Seventy-two patients (98.6%) had at least one comorbidity: nasal polyposis (43 patients, 58.9%), hypertension (33 patients, 45.2%), rhinitis (29 patients, 39.7%), gastroesophageal reflux (29 patients, 39.7%) and bronchiectasis (17 patients, 23.3%) were the most common comorbidities. Of 73 patients, 14 (19.2%) showed a non-MM genotype. The results of gene variants analysis and their consequences on protein function and concentration are shown in Table 1.

Among 14 patients that showed a non-MM genotype, the majority (8/14, 57.2%) carried out the S mutation variant, 2 (14.4%) the $M_{Whitstable}$ variant, and the others carried out $0_{Clayton}$, S_{Munich}, F and Z variants (7.1% each). Calculation of the expected genotypic frequencies from the allelic frequencies of the sample allowed us to demonstrate that our sample population is at the Hardy-Weinberg equilibrium ($v^2_{[6]}$ ¼0.866; P ¼ 0.96) and they are listed in Table 2. The clinical and demographic characteristics, stratified by SERPINA1 genotype, including atopy and comorbidities of all patients are presented in Table 3.

The study population presents homogeneous characteristics between the two study groups: there are no statistically significant differences in body mass index (BMI) and age of onset of respiratory symptoms, as well as in asthma control (number of exacerbations per year and ACQ-6 scores).

At baseline, the AAT serum level was significantly lower in the non-MM patients in comparison to the MM patients (P ¼ 0.003) and the presence of emphysema (evaluated with computed tomography scan) was more frequently observed in non-MM

Table 2. Allele frequencies of the study population.

	MM	MS	MZ	$MQ0_{Clayton}$	MF	$MM_{Whitstable}$	MS_{Munich}
Observed	59	8	1	1	1	2	1
Expected	59.7	7.9	0.9	0.9	0.9	1.8	0.9
Allele frequencies	0.904	0.055	0.007	0.007	0.007	0.014	0.007

Table 3. Descriptive analysis at baseline stratified by SERPINA1 genotype.

Variables	Homozygous (MM) (*n* ¼ 59) *n* (%)	Heterozygous (non-MM) (*n* ¼ 14) *n* (%)	*P*-value
Females	42 (71.2)	8 (57.1)	0.31
AAT serum level			
AAT level (Fleming), mg/dl, mean ± SD	1.43 ±0.24	1.18 ±0.19	0.003*
≤49	0 (0.0)	1 (7.1)	0.01*
50–100 mg/dl	4 (6.8)	4 (28.6)	
101–159 mg/dl	46 (78.0)	9 (64.3)	
≥160 mg/dl	9 (15.3)	0 (0.0)	
CRP, mg/dl, median [IQR]	0.13 [0.06–0.22]	0.15 [0.09–0.21]	0.48
Age of onset of respiratory symptoms, median [IQR]	39 [23–50]	40 [28–50]	0.63
BMI, kg/m^2, median [IQR]	24.7 [22.9–27.9]	24.1 [21.6–27.7]	0.50
Pack-year, median [IQR]	8.0 [3.5–19.0]	3.0 [2.0–30.0]	0.824
Total IgE, kU/L, mg/dl, mean ± SD	362.01 ±353.52	389.94 ±785.47	0.84
Extrinsic asthma	37 (62.7)	8 (57.1)	0.70
Nasal polyposis	36 (61.0)	7 (50.0)	0.45
Rhinitis	21 (35.6)	8 (57.1)	0.14
GERD	25 (42.4)	4 (28.6)	0.38
Hypertension	27 (45.8)	6 (42.9)	0.84
Bronchiectasis	14 (23.7)	3 (21.4)	1.00
Emphysema	1 (1.7)	4 (28.6)	0.004*
Number of exacerbations in the previous year, median [IQR]	2 [1–2]	2 [0–2]	0.97
ACQ-6 score, median [IQR]	0.5 [0.0–1.3]	0.5 [0.2–1.3]	0.77
FeNO, median [IQR]	50.5 [27–70]	48 [24–69]	0.85
Neutrophils IS, %, median [IQR]	31.3 [8.8–60.2]	42.1 [11.4–62.6]	0.71
Eosinophils IS, %, median [IQR]	31.6 [4.0–69.3]	24.3 [0.6–41.6]	0.25
Blood leucocytes, median [IQR]	7.2 [6.5–8.4]	8.4 [6.1–9.9]	0.29
Blood neutrophils, %, mg/dl, mean ± SD	54 ±9.1	58.2 ±14.0	0.22
Blood neutrophils count, median [IQR]	3,836 [3,227–5,026]	4,135 [3,021–5,952]	0.66
Blood eosinophils, %, median [IQR]	6.4 [3.4–8.4]	7 [3.5–10.4]	0.56
Blood eosinophils count, median [IQR]	449.4 [227.4–672.4]	581 [342.8–675.0]	0.83
NLR, median [IQR]	1.8 [1.4–2.4]	1.9 [1.6–3.2]	0.44
FEV_1, %, mg/dl, mean ± SD	83.1 ±23.6	90.3 ±22.8	0.31
FVC, %, mg/dl, mean ± SD	98.6 ±19.0	101.3 ±25.0	0.65
FEV_1/FVC, %, mg/dl, mean ± SD	69.1 ±11.3	71.6 ±10.3	0.45

* Statistically significant.
AAT ¼alpha1-antitrypsin; SD ¼standard deviation; CRP ¼C-reactive protein; IQR ¼interquartile range; BMI ¼body mass index; IgE ¼immunoglobulin E; GERD ¼ gastroesophageal reflux disease; ACQ ¼Asthma Control Questionnaire; FeNO ¼fractional exhaled nitric oxide; IS ¼induced sputum; NLR ¼blood neutrophils/ blood lymphocytes; FEV_1 ¼ forced expiratory volume in 1 sec; FVC ¼ forced vital capacity.

patients (P ¼ 0.004). No statistical differences regarding lung function, systemic and airway inflammation were found (Table 3).

The intra-group analysis showed that MM patients had a better ACQ-6 score, with values that were statistically significant in comparison with the baseline (T0 vs. T12: $P < 0.0001$; T0 vs. T24: $P < 0.0001$; T0 vs. T36: $P < 0.0001$), while for the non-MM patients, the ACQ-6 score was not significantly lower.

Considering systemic inflammation, a significant decrease of the blood eosinophils count was observed only in the MM patients for the whole follow-up period (T0 vs. T12: P < 0.0001; T0 vs. T24: $P <$ 0.0001; T0 vs. T36: $P < 0.0001$). In both groups the FEV_1/FVC improved (not significantly) during the 36 months of follow-up (Table 4).

DISCUSSION

AAT deficiency is an autosomal codominant genetic condition that predisposes to the onset of chronic pulmonary disease.[4,20] An accurate phenotyping and, possibly, endotyping are clinically fundamental to identify the best treatment option for each patient with severe asthma.

In the present study we evaluate severe asthmatic patients who differ for the *SERPINA1* genotype (MM homozygosity and non-MM heterozygosity). At baseline, the clinical-functional and inflammatory parameters of the two patient groups were assessed and compared with each other. In agreement with the data in the literature, a statistically significant difference emerged only for serum AAT concentration and the presence of emphysema.[4] Although the smoking history is an important determinant of lung diseases, no differences in the pack year calculation was found between the two groups.

According to Eden et al.,[21] 57% of severe asthmatic patients with AATD were atopic and about the 10% showed the MS genotype. The most interesting results emerged from the data collected during follow-up. In particular, MM patients reached a better asthma control during the three-years follow-up, with better ACQ-6 score and a reduction of eosinophilic systemic inflammation. For non-MM patients, although an improvement in both systemic inflammation and symptoms control was observed, the differences from baseline were not statistically significant, probably due to the presence of mutated alleles. This data could be explained by looking at the airway and systemic

Table 4. Data analysis at 3 years of follow-up.

Variables	Baseline Median [IQR]	T12 Median [IQR]	T24 Median [IQR]	T36 Median [IQR]	*P*-value
MM patients					
FEV_1, % (*n* ¼34)	80.5 [70.5–107.0]	81.5 [67.8–105.8]	89.0 [70.8–100.3]	86.5 [71.3–103.0]	0.79
FVC, % (*n* ¼34)	102.5 [88.0–112.8]	98.5 [83.3–108.3]	92.0 [85.8–104.5]	93.0 [82.0–105.3]	0.009*
FEV_1/FVC, % (*n* ¼34)	70.5 [62.0–78.0]	73.2 [64.5–80.7]	75.8 [68.6–80.6]	75.5 [66.3–78.5]	0.61
ACQ-6 (*n* ¼39)	0.3 [0.0–1.2]	0.2 [0.0–0.3]	0.0 [0.0–0.5]	0.0 [0.0–0.3]	<0.0001†
Blood leucocytes (*n* ¼39)	6.9 [6.1–8.2]	6.3 [5.6–8.0]	6.3 [5.6–7.2]	6.9 [6.2–7.6]	0.02‡
Blood neutrophils, % (*n* ¼39)	55.2 [49.2–63.2]	58.4 [50.5–63.7]	58.4 [51.9–64.3]	59.0 [51.0–65.1]	0.49
Blood neutrophil count (*n* ¼37)	3,996 [3,151–4,906]	3,533 [2,828–4,408]	3,481 [3,048–4,127]	3,842 [3,104–4,781]	0.16
Blood eosinophils, % (*n* ¼39)	6.4 [3.3–8.2]	1 [0.5–2.2]	0.9 [0.0–1.8]	0.8 [0.4–3.1]	<0.0001§
Blood eosinophil count (*n* ¼39)	386.4 [199.3–6637.3]	58.5 [32.0–148.1]	62.3 [0.0–117.9]	62.6 [29.8–172.8]	<0.0001¶
NLR (*n* ¼37)	2.1 [1.5–2.7]	2 [1.3–2.6]	2 [1.5–2.5]	2.1 [1.5–2.5]	0.29
Non-MM patients					
FEV_1, % (*n* ¼5)	95 [76–101]	104 [88.5–122.5]	95 [80.5–122.5]	85 [78.5–97.5]	0.12
FVC, % (*n* ¼5)	109 [80.0–115.5]	109 [88.5–123.0]	99 [83.0–102.5]	102 [89.0–116.5]	0.39
FEV_1/FVC, % (*n* ¼5)	69 [67.5–80.5]	72 [68.5–80.8]	69.2 [68.5–77.6]	73.2 [68.8–78.1]	0.51
ACQ-6 (*n* ¼6)	1.1 [0.3–1.8]	0.3 [0.0–1.2]	0 [0.0–0.5]	0.1 [0.0–1.2]	0.16
Blood leucocytes (*n* ¼5)	9.1 [6.6–11.7]	6.6 [5.9–8.3]	6.9 [6.4–7.5]	6.9 [5.8–7.2]	0.27
Blood neutrophils, % (*n* ¼5)	53.1 [49.9–70.0]	54.4 [48.5–60.9]	55.5 [52.7–60.3]	54.1 [53.0–59.4]	0.32
Blood neutrophils count (*n* ¼5)	4,369 [3,461–8,476]	3,652 [3,024–4,739]	3,722 [3,520–4,390]	3749 [3052–4270]	0.32
Blood eosinophils, % (*n* ¼5)	9 [1.9–12.1]	1.4 [0.7–2.4]	0.8 [0.4–2.5]	0.8 [0.2–2.9]	0.29
Blood eosinophils count (*n* ¼5)	565 [186.8–986.4]	124.3 [48.0–153.4]	59.2 [29.9–159.9]	57.9 [14.3–174.0]	0.27
NLR (*n* ¼5)	1.7 [1.6–5.7]	1.5 [1.2–2.4]	1.7 [1.4–2.2]	1.7 [1.5–1.9]	0.32

* Baseline vs. T24: P ¼0.002; baseline vs. T36: $P < 0.0001$.
† Baseline vs. T12: $P < 0.0001$; baseline vs. T24: $P < 0.0001$; baseline vs. T36: $P < 0.0001$.
‡ Baseline vs. T12: P ¼0.005; baseline vs. T24: P ¼0.007.
§ Baseline vs. T12: $P < 0.0001$; baseline vs. T24: $P < 0.0001$; baseline vs. T36: $P < 0.0001$.
¶ Baseline vs. T12: $P < 0.0001$; baseline vs. T24: $P < 0.0001$; baseline vs. T36: $P < 0.0001$.
IQR ¼interquartile range; FEV_1 ¼forced expiratory volume in 1 sec; FVC ¼forced vital capacity; ACQ ¼Asthma Control Questionnaire; NLR ¼blood neutrophils/blood lymphocytes.

inflammation. Although, at baseline, there were no significant differences between the two groups on the distribution of inflammatory cells, the non-MM patients showed a higher number of neutrophils in the induced sputum. This phenomenon should be further studied to assess whether neutrophils play a central role in patients with severe asthma and AATD.

Our findings differ from those of a published study where severe asthma patients with AATD showed a significant lung function decline than those without AATD.[22] This condition has been mainly studied in patients suffering from chronic bronchitis, indeed AATD genotypes seem to be associated with the prevalence of chronic obstructive pulmonary disease.[23]

In agreement with the literature, although AAT serum levels were lower in the *non-MM* patients than in *MM* ones, no correlation of functional respiratory values with AAT genotypes was found.[15,24,25] These results were in contrast with what was reported by Vianello et al.,[22] however the study by Vianello et al.[22] reported that two non-smoker patients out of 7, had M1/M2 genotype.

Therefore, our data could be explained considering that most of our patients were under biological therapy for severe asthma (all responders), which can reduce exacerbations and airway/systemic inflammation.[26] In support of this thesis, different studies and clinical trials demonstrated that biological therapy improves lung function while reducing the impact of exacerbations.[27,28] Indeed, the Tiffeneau-Pinelli index after 3 years changed from an obstructive condition to a normal pattern in both groups. Martín-González et al. reported the association of AATD and SERPINA1 classic variants with asthma exacerbations. Our data showed no statistically significant differences in the number of exacerbations; however, the reduced number of asthma exacerbations can be explained by the biological therapy most of our patients were undergoing during follow-up.[14]

Although airway inflammation in asthmatic patients is mainly caused by eosinophils, other mediators are also involved, including neutrophils, macrophages, and dendritic cells. A recent study suggested that AATD may cause imbalance between pro-inflammatory and anti-inflammatory factors, so that subjects with bronchial asthma and AATD are likely to suffer poor symptom control and higher rate of exacerbations.[29]

On the balance between neutrophil elastase and AAT, Vignola et al. have shown that asthmatic patients have increased levels of elastase in induced sputum[30] and elastase levels increase with the duration of asthma, causing imbalance between proteases and antiproteases, which could therefore contribute to airway remodelling, particularly in AATD patients.[31]

To our knowledge, this is the first study evaluating patients with severe asthma and non-MM heterozygosity followed-up for 3 years. We evaluated a selected and homogeneous population at the baseline in terms of disease severity, clinical and biological features. Even though we cannot extend our findings to general population, our results support the WHO recommendations to screen patients with severe asthma for AATD also at genotype level.

Among the limitations of our study, we report the relatively small sample size and the retrospective design. Despite this, the numbers of patients are similar to those reported in the literature: as of today, the WHO-recommend quantitative screening of AAT levels for all asthma patients[17] is still difficult to implement in the clinical practice.

Despite we did not observe a rapid lung function decline in patients with a non-MM genotype, routine lung function monitoring would be useful to both prevent its irreversible decline and to timely initiate the correct treatment. Larger studies are necessary to confirm the study results.

CONCLUSIONS

In conclusion, our findings highlighted the importance of a screening for AATD in patients with chronic pulmonary diseases. As neutrophils play a key role in this context, induced sputum examination could be useful in order to best phenotype these patients and choose the best treatment and follow-up for them. Last but not least, the importance of following-up of patients with severe asthma undergoing biological therapies is emphasised.

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Conflicts of interest: none declared.

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RÉSUMÉ

INTRODUCTION: La diminution de la concentration et de l'activité des protéines, à la fois dans le sérum et les tissus, est un effet du déficit en alpha-1-antitrypsine (AAT), une maladie autosomique co-dominante. Peu d'études se sont intéressées à l'influence du phénotype du gène *SERPINA1* sur les symptômes et le contrôle de la maladie chez les patients souffrant d'asthme sévère au cours du suivi.

OBJECTIF: Evaluer si la présence d'un génotype non-MM du gène *SERPINA1* chez les patients atteints d'asthme sévère est liée au contrôle de la maladie, à l'inflammation systémique et des voies respiratoires, à la fonction pulmonaire et à la prévalence des comorbidités par rapport aux patients atteints d'asthme sévère avec un génotype homozygote (MM).

MÉTHODES: Une étude rétrospective a été menée sur des patients atteints d'asthme de stade 5 selon la Global Initiative for Asthma (GINA) dans une clinique italienne spécialisée dans le traitement de cette maladie. Les données cliniques, biologiques et fonctionnelles ont été collectées au début de l'étude et sur une période de 3 ans.

RÉSULTATS: Sur les 73 patients inscrits, 14 (19,2%) étaient non-MM et 59 (80,8%) étaient MM. Les asthmatiques de génotype non-MM présentaient une concentration sérique d'AAT plus faible ($P = 0{,}004$) et une prévalence d'emphysème plus élevée que le groupe MM ($P = 0{,}003$) au début de l'étude. Pendant le suivi, seuls les patients MM ont présenté une amélioration notable du score ACQ-6 ($P < 0{,}0001$), ainsi que de l'inflammation systémique éosinophile ($P < 0{,}0001$).

CONCLUSIONS: Nos résultats mettent en évidence l'importance du dépistage du déficit en AAT dans les cas d'asthme sévère, car la mutation des allèles peut influencer le suivi du patient.