

Letter to the Editor

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Improving the diagnosis of AATD with aid of serum protein electrophoresis: a prospective, multicentre, validation study

<https://doi.org/10.1515/cclm-2024-0176>

Received February 5, 2024; accepted February 22, 2024;

published online March 5, 2024

Keywords: alpha-1 antitrypsin deficiency; alpha1-globulin; serum proteins; screening

To the Editor,

Alpha-1 antitrypsin deficiency (AATD) is a heritable condition characterized by a deficiency in alpha-1 antitrypsin

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(AAT), a critical circulating protease inhibitor. This condition predisposes individuals to the deleterious effects of unopposed neutrophil elastase, culminating in heightened susceptibility to pulmonary and hepatic pathologies [1].

AAT exerts its primary role in safeguarding the pulmonary parenchyma against proteolytic damage induced by neutrophil elastase. Genetically conditioned deficiency results in a compromised AAT synthesis, thereby precipitating an underwhelming representation of functional AAT in the systemic circulation. Consequently, individuals harboring *AATD* gene mutations confront elevated susceptibility to diseases typified by chronic obstructive pulmonary disease (COPD) and hepatic disorders. Timely identification and therapeutic intervention are imperative to attenuate the progression of these maladies [2].

Notably, AATD remains underdiagnosed despite its clinical significance [3]. In this context, the potential of alpha-1 band serum electrophoresis evaluation assumes pivotal significance as an instrumental modality for population screening.

This technique, grounded in the principle of serum protein electrophoretic separation, demarcates the migration of AAT within the alpha-1 region [3, 4]. Recently, Scarlata and colleagues have already demonstrated, in a single center, that an algorithm based on the alpha1-globulin fraction of Serum Protein Electrophoresis (SPE) is effective for population screening and allows for early detection of patients affected with AATD variants [5]. Similar results have been obtained soon after as single institution's experience [6].

We therefore propose to strengthen the scientific evidence on the effectiveness of the use of the alpha1-globulin band in protein electrophoresis for the early diagnosis and population screening of AATD, through a multicenter design conducted in four Italian hospitals.

The study was organized as multicenter project with four Laboratory Units with the respective Pulmonology Units in Italian hospitals namely: Monaldi Hospital, Naples (Center 1); AOR Villa Sofia Cervello, Palermo (Center 2); Santa

Maria della Misericordia Hospital, Perugia (Center 3); Fondazione Policlinico Universitario Campus Bio-Medico, Rome (Center 4).

In the period spanning June 2022–December 2022, the laboratories checked all the serum protein electrophoresis consecutively performed (84,270) and selected 461 subjects with the following criteria: (a) adults aged 30–70 years; AND (b) alpha1-globulin value <2.9 % OR presence of evident splitting of the alpha1 fraction, attributable to heterozygous subjects, even in the case of values between 2.9 and 4.9 %; AND (c) exclusion of subjects with “false” decreases in the alpha1 fraction due to the presence of large monoclonal components or patients with nephrotic syndrome.

The Laboratory Units of Perugia and Rome confirmed of AAT deficiency by specific protein assays: a1antitripsina (Siemens) and Abbott Alinity C (Abbott Laboratories, IL, USA) A1-Antitrypsin immunoturbidimetric assay, respectively. The selected subjects were notified to the respective Pulmonology Units, that contacted them by offering further testing to correctly diagnose AATD. In the total 92 subjects accepting it, samples were collected and shipped to the Center for Diagnosis of Inherited Alpha1-Antitrypsin Deficiency, IRCCS San Matteo Hospital Foundation (Pavia) for first and/or second level investigations [3]. In particular, the Pulmonology Units of Perugia and Rome collected buccal swabs to perform genetic analysis, whereas the Pulmonology Units of Naples and Palermo collected Dried Blood Specimens (DBS) to perform both biochemical and genetic investigations. Table 1 reports details of recruitments according to the different Centers.

AAT and CRP measurements in plasma were performed in DBS samples by sandwich ELISA by using ThunderBolt® Analyzer (Gold Standard Diagnostics), a fully automated multiplate open platform. The ELISA kit for AAT quantification (ImmuChrom GmbH) and for CRP quantification (Alpha Diagnostic International) were used according to the manufacturers' protocols [7].

DNA was extracted from DBS and buccal swabs by using an automatic extractor (QIAcube) and the QIAamp DNA Mini Kit or Investigator Kit (QIAGEN), as previously reported [8].

Once extracted, all DNA were genotyped by applying AAT Genotyping Test [8]. According to the current diagnostic algorithm [9], when necessary, SERPINA1 gene was sequenced as recently described [10].

All the statistical analyses were performed by the software MedCalc (MedCalc Software Ltd, Belgium).

By applying the diagnostic algorithm for AATD to the 92 samples, 8 resulted with both pathogenic alleles (respectively, PI*ZZ, PI*SS, PI*S/Mmalton, PI*V/Mprocida, PI*Z/Smunich, PI*Mwurzberg/Smunich, PI*S/I, PI*Z/Mwurzberg), 49 with one pathogenic allele (namely, 18 PI*MZ, 7 PI*MS, 6 PI*M/Mmalton, 4 PI*M/Plowell, 4 PI*M/Q0ourèm, 2 PI*M/Q0perugia, 2 PI*MV, 2 PI*M/Mprocida, 1PI*M/Mwurzberg, 1 PI*M/Q0isoladiprocida, 1 PI*M/Mwhitstable, 1 PI*M/Mrouèn) and 37 without pathogenic alleles.

Details of genotypes, alpha1-globulin % and AAT plasma concentration according to the different Centers are reported in Table 2.

In general, among the subjects who agreed to undergo genetic testing, a high positive detection rate was achieved (59.8 %). The rate was quite consistent among centers (52.6 , 77.8, 59.5 and 55.5 % in Center 1, 2, 3 and 4, respectively), thus indicating that locally performed confirmation assay of protein deficiency did not affected patient selection. Moreover, also the use of different procedures for sample collection (DBSs and buccal swabs) did not influence the final results.

To restrict the inclusion criteria, the optimal threshold for alpha1-globulin percentage was determined by plotting a receiver operating curve (ROC). To this end, all samples with at least one pathological SERPINA1 allele were assumed to be positive. The ROC curve identified ≤ 2.5 as the optimal cut-off value of percentage of alpha1-globulin band in SPE for suspecting genetic AAT deficiency (35.9 % sensitivity and 80 % specificity) and ≤ 2.3 for highly recommended diagnosis of AATD (19.3 % sensitivity and 100 % specificity).

The potential advantages of implementing the use of SPE for screening purposes are manifold: first, population screening for AATD would assume heightened significance by virtue of the demonstrable salubrious effects of early intervention. Mitigation strategies such as smoking cessation, the avoidance of occupational peril, and judicious medical management are quintessential in the amelioration of disease progression. Furthermore, the early identification of AATD-affected individuals would also permit the optimization of these interventions to yield more efficacious outcomes. Additionally, the scope of utility intrinsic to alpha band serum electrophoresis evaluation might transcend individualized patient care. It could engender the accrual of invaluable data repositories germane to epidemiological investigations by delineating the prevalence and

Table 1: Recruitment details.

	Center 1	Center 2	Center 3	Center 4	Total
SPEs screened, n	29,792	14,214	33,255	7,009	84,270
SPEs selected, n (% of screened)	167 (0.56)	98 (0.69)	156 (0.47)	40 (0.57)	461 (0.55)
Subject collected for further analysis, n (% of selected)	19 (11.4)	9 (9.1)	37 (23.7)	27 (67.5)	92 (19.9)

Table 2: Frequencies of samples according the combination of alleles and α -1 globulin % and AAT plasma concentration.

		Center 1	Center 2	Center 3	Center 4	Total
All collected samples	n (% of total)	19 (20.6)	9 (9.8)	37 (40.3)	27 (29.3)	92
	Mean α 1-globulin % (SD)	2.65 (0.23)	2.76 (0.15)	2.61 (0.21)	2.66 (0.24)	2.66 (0.21)
	Mean AAT, mg/dL (SD)	97.6 (23.8)	77.5 (8.5)	84.9 (20.0)	86.2 (45.2)	86.4 (26.4)
Two pathogenic alleles	n (% of total – % of center)	0	1 (12.5–11.1)	3 (37.2–8.1)	3 (37.2–11.1)	8 (8.7)
	Mean α 1-globulin % (SD)	–	2.70	2.17 (0.39)	2.53 (0.29)	2.37 (0.37)
	Mean AAT, mg/dL (SD)	–	76.6	58.8 (18.6)	51.0 (53.7)	59.1 (26.9)
One pathogenic allele	n (% of total – % of center)	10 (21.2–52.6)	6 (12.7–66.7)	19 (40.4–51.3)	12 (25.5–44.4)	47 (51.0)
	Mean α 1-globulin % (SD)	2.71 (0.41)	2.76 (0.05)	2.56 (0.21)	2.60 (0.21)	2.62 (0.26)
	Mean AAT, mg/dL (SD)	90.9 (11.0)	77.7 (9.8)	84.9 (17.8)	89.9	86.2 (24.1)
No pathogenic allele	n (% of total – % of center)	9 (24.3–47.3)	2 (5.4–22.2)	15 (40.5–40.5)	12 (32.4–44.4)	37 (40.2)
	Mean α 1-globulin % (SD)	2.73 (0.15)	2.80	2.68 (0.11)	2.9	2.71 (0.12)
	Mean AAT, mg/dL (SD)	121.0 (48.7)	ND	95.4 (16.9)	84.0	98.5 (22.8)

demographic distribution of AATD within discrete populations, thereby affording pertinent insights into public health initiatives and judicious resource allocation to ensure the provisioning of commensurate support and care for AATD-afflicted individuals.

Despite these aforementioned traits, it should be remarked that SPE cannot be considered a diagnostic tool, but a mere instrument allowing screening. Moreover, SPE is unable to differentiate between AATD protein, genetic variants and clinical phenotypes and is therefore of limited utility for the clinical management and follow up of the disease.

This study has several limitations: first, only a minority of screened subjects accepted to undergo to complete AATD diagnostic and we are not able to exclude that this might have influenced the results; indeed, since no significant differences in α 1-globulin mean levels was seen between participants and those who refused we are prone to consider this eventuality quite unlikely. Second, at the moment, we cannot provide data on the clinical relevance of our findings, as no longitudinal analysis of lung function decline rate was performed. Further research is therefore needed to clarify the impact of intermediate deficiency on lung function and COPD disease progression.

In conclusion, we confirmed previous single-center evidences [5, 6] that SPE can be successfully used for screening population of AATD, also in a multicentric fashion, leading to high positive detection rate among whom agree to undergo genetic testing. Further implementation of this approach should be taken into account as healthcare solution for individualized medicine strategies and control and rationalization of health spending.

Acknowledgments: The authors acknowledge organizational assistance from Lorenzo Dolfi Agostini (Grifols).

Research ethics: The study was carried out according to the tenets of the Declaration of Helsinki and the relevant Italian regulations for performing investigation in humans. Giving the nature of the study, no specific ethical approval was needed.

Informed consent: For subjects who agreed genetic analysis, the specific consent was obtained.

Author contributions: SS, IF designed the research project; SB, AA, FA, enrolled patients and collected clinical data; AMB, AV, FB, SO, SS, VB, MF, SA, SM performed analysis; IF analyzed data; SS, IF, AGC wrote the paper. All authors read and approved the manuscript. All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: The authors state no conflict of interest for this study.

Research funding: None declared.

Data availability: Not applicable.

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