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## Alpha-1 antitrypsin deficiency in a French General Hospital: fortuitous detection rather than efficient screening

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### Abstract

**Introduction:** We studied the characteristics of the screening procedure for alpha-1 antitrypsin at Nevers Hospital (France), together with the performance of serum protein gel electrophoresis for the fortuitous detection of patients with deficiency.

**Material and methods:** We carried out a retrospective study of requests for alpha-1 antitrypsin determination referred to the laboratory during 3 years. We compared these requests with the numbers of patients seen at the hospital and requiring screening according to international recommendations. In parallel, we reviewed all the serum protein gel electrophoresis results obtained during the same period.

**Results:** The laboratory received 102 direct requests for alpha-1 antitrypsin determination, whereas more than 1397 patients presented an indication for screening. No case of alpha-1 antitrypsin deficiency was detected among the 102 patients screened. In parallel, 5551 serum protein gel electrophoresis analyses were carried out at the laboratory. A decrease in the size of the alpha-1 globulin fraction was detected in 68 patients. Seventeen of these patients underwent alpha-1 antitrypsin determinations and 14 were found to have alpha-1 antitrypsin deficiency.

**Conclusion:** Alpha-1 antitrypsin deficiency was more frequently detected fortuitously, by electrophoresis, than through efficient screening. The exploration of alpha-1 globulin deficiencies by serum protein gel electrophoresis thus appears to be still a particularly efficient approach to the detection of alpha-1 antitrypsin deficiency and should be carried out systematically. Furthermore, the testing of all patients with an indication for screening according to international recommendations should be encouraged.

**Key words:** alpha-1 antitrypsin deficiency, serum protein electrophoresis, agar gel electrophoresis, PI phenotype, screening

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### Introduction

Carl-Bertil Laurell, a biochemist at the University of Malmö, was one of the pioneers of the development of serum protein electrophoresis (SPE) as a diagnostic tool in medicine. In 1961, he introduced the use of agarose gels, in place of paper, as a support for the migration and separation of proteins, a “standard” technique still widely used in medical laboratories. In 1963, he identified the first patients with severe deficien-

cies of alpha-1 antitrypsin (AAT), the principal plasma protease inhibitor, based on the absence of a serum alpha-1 fraction on SPE. In collaboration with Sten Erikson, he subsequently demonstrated the relationship between AAT deficiency and the development of emphysema [1].

Today, the biological diagnosis of AAT deficiency is based on sensitive and specific quantifications of plasma AAT by immunoturbidimetry or nephelometry, which is then followed by isoelectric focusing to phenotype the most com-

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mon variants, or genotyping [2]. In France, these examinations are carried out by a few specialized laboratories, but testing is accessible nationwide, via subcontracting through a network of hospital and private medical laboratories. Nevertheless, AAT deficiency remains underdiagnosed in France and worldwide, despite the existence of international recommendations for screening [2]. It is estimated, for example, that only 5 to 10% of the 7,500 to 10,000 patients with the PiZ phenotype, the most severe form of this deficiency, have been identified in France [3, 4], and that this condition is diagnosed on average 10 years after the appearance of the first symptoms [5]. In Poland, the number of patients with the PiZ phenotype is estimated to be between 4,189 and 7,000 [6].

Serum protein electrophoresis remains as relevant as ever and is still widely prescribed for the exploration of various diseases (hematological, liver, renal and inflammatory diseases, for example).

The widespread use of this technique may still allow the fortuitous discovery of patients with AAT deficiency. We studied the characteristics of screening for AAT deficiency at Nevers Hospital (NH), and the performance of SPE for the incidental discovery of AAT deficiency in patients.

### Material and methods

We retrospectively reviewed all requests for AAT determination ( $\pm$  typing) received by the laboratory during 3 years (May 2012 to May 2015) (subcontracted tests), noting the reasons for the prescription of the tests and their results. We compared the number of tests performed with the number of patients hospitalized at the NH eligible for screening in accordance with international recommendations or already diagnosed as having AAT deficiency, by performing statistical queries in the PMSI of the hospital (PMSI, *programme de médicalisation des systèmes d'information*; the hospital medico-administrative database).

In parallel, we reviewed all SPE tests performed in the laboratory over the same period. Gel electrophoresis was performed on a Hydrasys-Hyrys device (Sebia). For all tests in which the alpha-1 globulin fraction was smaller than the value considered normal according to laboratory standards ( $< 1$  g/L, rounded to the nearest decimal place), in the absence of an obvious cause (hepatocellular insufficiency, severe undernutrition, protein leakage), a comment was recorded concerning the possibility of AAT deficiency requiring confirmation by quantitative determination.

This study has been declared to the CNIL, the French Commission for Data Protection & Civil Liberties (No. 1860860).

### Results

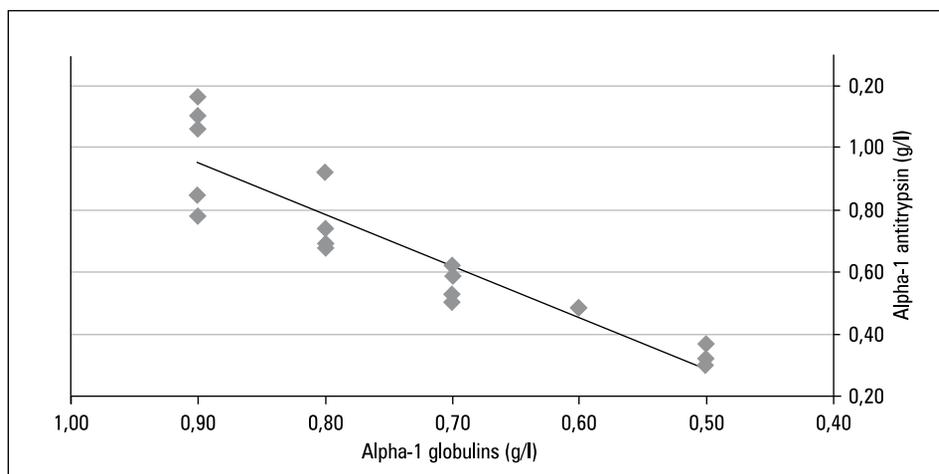
The laboratory received 119 requests, including 102 direct requests for AAT determination, during the study period. Overall, 28 of the tests were performed for the exploration of liver disorders, 70 for respiratory disorders (32 for emphysema, 28 for chronic obstructive pulmonary disease (COPD), 5 for asthma, 5 for sleep apnea, and 2 for pneumothorax), 1 in the context of vasculitis, and 3 for unknown indications (not recorded). No cases of AAT deficiency were detected in the 102 directly requested AAT determinations. Over the same period, 1,397 patients admitted to the hospital had an indication for screening: 1,180 had COPD, 147 had pulmonary emphysema, 38 had partially irreversible asthma, 27 had bronchiectasis of unknown etiology, four had anti-PR3 antibody-positive vasculitis and one had panniculitis. It was not possible to determine the number of patients with unexplained liver disease or bronchial obstruction. One patient had already been diagnosed with AAT deficiency (PiMZ).

In parallel, 5,551 SPE tests were performed at the laboratory, on serum samples from 4,406 patients. The alpha-1 globulin fraction was abnormally small in 68 patients. In response to the comment added to the results concerning the possibility of a deficiency, 17 subjects underwent AAT assays (and typing by isoelectric focusing in cases of confirmed deficiency; Fig. 1). AAT deficiency (AAT concentration  $< 0.93$  g/L, the reference value of the subcontractor laboratory) was confirmed in 14 of the 17 patients (82%; Fig. 1). The deficiency phenotypes were as follows: two PiZZ, four PiSZ, one PiSS, five PiMZ, and two PiMS. The results for the alpha-1 globulin fraction and AAT determination for these 17 patients are summarized in Figure 1, and the characteristics of the 14 subjects with AAT deficiencies are presented in Table 1.

### Discussion

#### Screening characteristics

The lack of detection of AAT deficiency in any of the 102 patients directly referred for testing is not entirely surprising. Despite the widespread acceptance of AAT deficiency as a common hereditary disease with a prevalence similar to that of cystic fibrosis, it would still only be expected to



**Figure 1.** Alpha-1 antitrypsin concentration vs alpha-1 globulin fraction on serum protein electrophoresis

**Table 1. Characteristics of the 14 patients with alpha-1 antitrypsin (AAT) deficiency**

Pi	n	Age	AAT concentration (g/L)	Medical history
MS	2	42-74	0,78–0,92	2: none
MZ	5	12 to 80	0,62–0,85	3: none 1: controlled asthma 1: alcoholic cirrhosis
S	1	43	0,59	none
SZ	4	78 41 59–78	0,37–0,53	1: none 1: asymptomatic but doubt on chest x-ray 2: chronic obstructive pulmonary disease
Z	2	64–68	0,30–0,32	2: emphysema

affect 2 to 3% of all cases of chronic obstructive respiratory diseases, for example [2].

Even if we take into account the known limitations of epidemiological statistics derived from administrative databases like the PMSI (e.g. lack of diagnostic accuracy, lack of exhaustiveness...) [7], the comparison of the number of requests for testing sent to the laboratory with the estimated number of patients meeting the international criteria for testing confirmed the existence of significant underscreening at our hospital. Nevertheless, the entire range of screening indications was represented. Such “case-by-case” screening has already been reported in one-off studies for most French [8] and European prescribers [9]. Prescribing practice also appears to be highly heterogeneous at our hospital, with considerable dependence on the doctor, 68% of requests emanating from a single practitioner. Nevertheless, 4 of the 6 severe cases of AAT deficiency (PiSZ and PiZZ) detected in our study had clinical histo-

ries including indications for screening according to current international recommendations, which had already been present for several years. This finding argues for the development of systematic screening in accordance with these recommendations at our institution.

### Electrophoretic performance

The dispersion of AAT levels appeared to reduce with decreasing size of the alpha-1 globulin fraction on SPE in this study, although there were too few values available to determine whether this trend was significant (Fig. 1). If confirmed, this would suggest that SPE results and quantitative determinations of AAT levels are better correlated for the most severe deficits. The three patients testing positive by SPE for whom the deficiency was not confirmed by quantitative determinations all had alpha-1 globulin fractions just below the inclusion threshold (0.9 g/l, for a threshold of < 1).

An analysis of the distribution of confirmed deficiency phenotypes within the study population revealed that their prevalence was close to that estimated for the French general population, even higher for the phenotypes associated with the most severe forms of AAT deficiency, even though only 25% of patients (17/68) underwent quantitative determinations (0.045% vs. 12.7% for PiMS, 0.1125% vs. 2% for PiMZ, 0.0225% vs. 0.5% for PiSS, 0.09% vs. 0.19% for PiSZ, 0.045% vs. 0.013% for PiZZ). The method historically developed by Laurell thus appears to have performed well in this study for the incidental detection of severe AAT deficiency. These results are consistent with those of other published studies [10]. The development of lesions is correlated with the severity of the deficiency, at least for pulmonary changes [11].

Capillary electrophoresis is now used alongside gel electrophoresis for SPE. This technique yields higher values for the alpha-1 globulin fraction because it detects orosomucoid (alpha-1-acid glycoprotein) with high sensitivity, whereas gel electrophoresis does not. This difference in sensitivity results from the high proportion of sialic acid in orosomucoid, which reduces dye binding in agarose gels but has no effect on UV absorption in capillary electrophoresis [12]. It is not, therefore, possible to detect AAT deficiency with high sensitivity solely by capillary electrophoresis-based quantification of the alpha-1 globulin fraction [13]. However, analyses of qualitative modifications to the peak relative to a reference plot can detect severe AAT deficiency (decrease in magnitude and flattening of the peak) and the presence of heterozygous variants (splitting of the peak in two) [14].

### Conclusion

In conclusion, the detection of AAT deficiency at our hospital is largely based on fortuitous discovery using SPE, rather than efficient screening. The exploration of alpha-1 globulin deficiency by SPE is informative and should be generalized. In addition, the examination of all patients with indications for screening according to the international recommendations should be encouraged. The development of filter paper-based tests and of sampling kits for direct mailing might facilitate such screening, particularly in private practice [15].

### Conflict of interest

The authors declare no conflict of interest.

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