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PERSPECTIVE

Laboratory testing of individuals with severe α_1-antitrypsin deficiency in three European centres

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ABSTRACT: α_1-Antitrypsin (AT) deficiency is a hereditary disorder that may lead to early-onset emphysema, and chronic liver disease later in life. Although there are validated methods for testing, the vast majority of α_1-AT-deficient individuals remain undiagnosed. Recommendations have been published for the testing and diagnosis of α_1-AT deficiency; however, guidelines on best practice are not well established.

In our article, we review the developments in diagnostic techniques that have taken place in recent years, and describe the practices used in our three European centres. The determination of the level of α_1-AT and genotyping are reported as the main diagnostic steps, whereas isoelectric focusing (also referred to as phenotyping) is reserved for confirmatory analysis.

The following recommendations for best practice are put forward: detection of all PiZZ and other severe deficiency individuals; automated genotyping; preparation of reference standards; quality control programmes; development of standard operating procedure documents; and standardised methods for the collection of dried blood samples. Closer cooperation between laboratories and the sharing of knowledge are recommended, with the objectives of improving the efficiency of the diagnosis of severe α_1-AT deficiency, increasing the numbers of individuals who are detected with the disorder, and assisting the establishment of new patient identification programmes.

KEYWORDS: Algorithm, α_1-antitrypsin deficiency, chronic liver disease, chronic obstructive pulmonary disease, diagnostic procedures, emphysema

Deficiency of α_1-antitrypsin (α_1-AT) is one of the most common hereditary disorders worldwide. The main physiological role of α_1-AT (also known as alpha-1 proteinase inhibitor) is to protect lung tissue from damage by proteolytic enzymes, in particular neutrophil elastase. Synthesis of α_1-AT takes place predominantly within the liver. In α_1-AT deficiency, polymerisation of the α_1-AT glycoprotein leads to its accumulation within the hepatocytes, and reduced levels are released into the circulation. When the concentration of pulmonary α_1-AT is low, neutrophil elastase is not neutralised by α_1-AT and can destroy lung tissue, resulting in early-onset emphysema, particularly in smokers. A further clinical manifestation of specific mutations of the SERPINA1 gene that encodes for α_1-AT is liver disease, presenting in infancy as hepatitis and jaundice, and as cirrhosis in children and adults.

Synthesis of α_1-AT is controlled by the codominant expression of two alleles, and individuals with α_1-AT deficiency have inherited two abnormal proteinase inhibitor (Pi) alleles at the α_1-AT gene locus. The principal normal allele is the M allele, and the PiMM genotype is present in 94–96% of Caucasians [1]. The most common deficient variant is the Z allele, and >95% of individuals with severe α_1-AT deficiency carry the PiZZ genotype [2]. The serum α_1-AT levels measured in individuals with the PiMM and PiZZ genotypes are 20–48 μM (1.5–3.5 $g \cdot L^{-1}$) and 2.5–7 μM (0.20–0.45 $g \cdot L^{-1}$), respectively, and a concentration of <11 μM (0.80 $g \cdot L^{-1}$-radial immunodiffusion, or 0.50 $g \cdot L^{-1}$-nephelometry, see below) is associated with an increased risk of emphysema [3].

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The great majority of individuals with α_1-AT deficiency have not been recognised; however, validated methods for the measurement of the levels of α_1-AT in the blood, and for the definitive diagnosis of α_1-AT deficiency through determination of the individual's genetic composition at the Pi locus, have been available for many years. The World Health Organization (WHO) and the American Thoracic Society (ATS), in collaboration with the European Respiratory Society (ERS), have produced reports [3, 4] that include recommendations for screening and testing for α_1-AT deficiency using these well-established procedures. However, the WHO and ATS/ERS recommendations do not provide best practice guidelines on the implementation of a screening or diagnostic programme and, as yet, an ideal algorithm of laboratory procedures for case detection of α_1-AT deficiency carriers has not been established.

Since the most recent publication of the ATS/ERS recommendations in 2003, there have been further advances in the techniques available for the diagnosis of α_1-AT deficiency. Our article will describe existing practices being used at our three European centres that have expertise in the testing and diagnosis of the disorder. Our laboratories have taken advantage of, and utilised, the improvements in diagnostic methodology. We wish to share our experience with the objectives of enhancing the quality and efficiency of the laboratory diagnosis of α_1-AT deficiency, and facilitating the establishment of successful diagnostic programmes to detect greater numbers of affected individuals.

EPIDEMIOLOGY

Epidemiological studies suggest that ~100,000 individuals in the USA have α_1-AT deficiency [3], as have a similar number in Europe [2]. The epidemiology and geographical distribution of α_1-AT deficiency have been reviewed by many authors [2, 5–8]. It is estimated that there are 3.4 million individuals worldwide with the PiZZ, PiSZ or PiSS genotypes, and 116 million individuals who are heterozygous for the disorder with the PiMZ or PiMS genotypes [7]. Targeted screening studies have been carried out in several countries to detect cases of α_1-AT deficiency by determining the frequency of the Z and S alleles in samples from individuals presenting with chronic obstructive pulmonary disease (COPD) [9–13].

CLINICAL RECOGNITION OF α_1-AT DEFICIENCY AND EXISTING RECOMMENDATIONS FOR TESTING

Despite the fact that α_1-AT deficiency was first reported in the 1960s, the number of individuals diagnosed with the disorder is only a small proportion of that predicted from screening studies and estimates of gene frequency. <10% of individuals in the USA who have α_1-AT deficiency have been identified [13–15], and it is estimated that up to 3% of patients with COPD in the USA may have undiagnosed α_1-AT deficiency [4]. A survey of 305,009 expected cases of α_1-AT deficiency in eight other countries has shown that only 1,068 of these cases were actually diagnosed [8]. Prolonged delays in making the diagnosis are also commonplace; whereas most individuals are never diagnosed, a study carried out among a small subset of individuals who were eventually diagnosed found that a mean of 7.2 yrs elapsed between the onset of symptoms and the diagnosis of α_1-AT deficiency, and that 44% of individuals reported seeing at least three physicians before diagnosis [16]. More recently, an average diagnostic delay of 8.3 yrs has been reported [15].

There are at least two reasons to explain why α_1-AT deficiency is under-recognised by the medical community. First, many physicians are unfamiliar with the disorder and not aware that pulmonary conditions, similar to those in individuals presenting with ordinary (*i.e.* smoking-related) COPD may in fact be due to α_1-AT deficiency. They may also be unaware that symptoms of liver disease can also result from α_1-AT deficiency. Secondly, many subjects with severe α_1-AT deficiency may not have developed clinically significant impairment of lung function and may have no clinical symptoms [8].

In their statement on standards for the diagnosis and management of α_1-AT deficiency [3], the ATS/ERS provided recommendations regarding whether genetic testing should or should not be carried out. These were graded according to the level of supportive evidence for such testing, taking into account both the benefits of testing and the adverse issues involved (psychological effects on the individual, economic costs and ethical considerations). The clinical settings for which genetic testing was definitely recommended (a type A recommendation) are shown in table 1. The ATS/ERS also advised that every individual with a low α_1-AT level should be tested once by either phenotyping (isoelectric focusing) or genotyping.

The WHO has recommended that all individuals with COPD, adults and adolescents with asthma, and all individuals with a family history of the disorder should be screened once for α_1-AT deficiency using a quantitative test [4]. All subjects with abnormal levels of α_1-AT in the blood should subsequently undergo phenotyping.

Unfortunately the guidelines described above for the testing of α_1-AT deficiency, in particular the recommendation to test all symptomatic adults with COPD, are not routinely followed. In order to help physicians recognise the disease, the ATS and ERS have emphasised the following conditions that should prompt suspicion of α_1-AT deficiency: early-onset COPD (age <45 yrs); emphysema in the absence of a recognised risk factor (*e.g.* smoking); emphysema with prominent basilar hyperlucency; bronchiectasis without evident aetiology; unexplained liver disease; vasculitic syndromes (anti-proteinase-3-vasculitis, necrotising panniculitis) [3]. α_1-AT deficiency should also be suspected in individuals with a family history of emphysema, bronchiectasis, liver disease, panniculitis, or the disorder itself. However, we must keep in mind that the real goal that has to be achieved is to test all patients with COPD at least once in their lives [3, 4].

As in other adult-onset genetic disorders, the identification of individuals with mutations predisposing to α_1-AT deficiency can motivate these individuals to avoid risk factors for symptomatic disease, such as cigarette smoking and exposure to environmental pollutants. Studies have shown a lower frequency of smoking in adolescence among individuals identified at birth as having α_1-AT deficiency compared with matched control groups [17, 18], and an increased motivation to stop smoking among adults diagnosed with a genotype for α_1-AT deficiency [19, 20]. However, such benefits need to be

TABLE 1 Recommendations for genetic testing for α_1-antitrypsin (AT) deficiency

Testing is recommended in the following settings (type A recommendation):

1. Symptomatic adults with persistent obstruction on pulmonary function testing, with:
   a. Emphysema
   b. COPD
   c. Asthma where there is incompletely reversible airflow obstruction after aggressive treatment with bronchodilators
2. Asymptomatic individuals with persistent obstruction on pulmonary function testing with identifiable risk factors, including:
   a. Cigarette smoking
   b. Occupational exposure
3. Individuals with unexplained liver disease, including newborns, children and adults (particularly the elderly)
4. Adults with necrotising panniculitis
5. Adults and adolescents with a sibling with α_1-AT homozygosity, *i.e.* the PiZZ genotype (asymptomatic individuals who may be at high risk of having the genetic predisposition for developing α_1-AT deficiency)

COPD: chronic obstructive pulmonary disease. Data modified from [3].

weighed against psychological effects, such as an individual's anxiety about their predisposition to disease, and there are other adverse effects of genetic testing, such as discrimination by employers and the refusal of health insurance. Furthermore, in the case of a hereditary disorder, such as α_1-AT deficiency, genetic testing also has implications for family members. It is recommended that patients are provided with information on α_1-AT deficiency and that the implications (medical, social, emotional) of genetic testing are carefully explained [3].

METHODS FOR THE DIAGNOSIS OF α_1-AT DEFICIENCY

Procedures for testing for α_1-AT deficiency have been available since the 1960s, and new techniques have been introduced during the intervening years. These advances in methodology should facilitate the widespread application of more rapid, convenient and cost-effective tests for α_1-AT deficiency and thus lead to an increase in the numbers of individuals diagnosed with the disorder [21]. The different methods that have currently been validated for both testing and diagnosis of the disorder are assessed in tables 2 and 3.

Large-scale screening programmes for α_1-AT deficiency have previously been hindered by the need to draw intravenous blood and then transport the potentially bio-hazardous specimens to the testing laboratory. Over the past few years, the use of dried blood spots (DBS) has been initiated, whereby testing is carried out on a few drops of blood that have been dried onto filter paper (number 903; Schleicher & Schuell, Bioscience Inc., Keene, NH, USA). The applicability and reliability of the DBS method for α_1-AT deficiency testing have been described extensively [9–11, 21, 23–26]. The advantages and disadvantages of using whole blood samples and DBS are reviewed in table 2. The tests used for the measurement of α_1-AT levels in the blood (immunoassay techniques, nephelometry), as well as diagnostic procedures (phenotyping and genotyping methodology), are listed in table 3.

IDENTIFICATION PROGRAMMES FOR α_1-AT DEFICIENCY

The initial issues that we wanted to address were, first, ''Who should be screened?'' and, secondly, ''Should screening be carried out only by measurement of the α_1-AT concentration in the blood, or should individuals be fully diagnosed by identification of the alleles at the α_1-AT gene locus?''. We decided to call our initial testing procedures ''targeted detection'' instead of either diagnosis or screening because, clearly, the priority should be given to individuals with COPD. However, the question, ''Who should be targeted for detection?'' cannot be answered fully and depends on the resources available. The steps in targeted detection and diagnosis would include the categories presented in table 1.

Whereas methods have been validated for testing for α_1-AT deficiency, there is no one established algorithm for the detection and diagnosis of the disorder that is universally used by laboratories and countries around the world. It is important, therefore, to compare and review current procedures and initiatives being undertaken at different centres. From such sharing of knowledge, we can develop recommendations for effective methodology and diagnostic algorithms.

Testing and diagnostic algorithms

We report here the procedures currently employed in three laboratories in Europe working on national programmes for the detection of α_1-AT deficiency, namely the University of Pavia, Italy; the University of Marburg, Germany; and the

TABLE 2 Advantages and disadvantages of whole blood and dried blood spots for α_1-antitrypsin deficiency testing

	Advantages	Disadvantages
Whole serum or plasma	Whole blood (collected in EDTA) is easier to handle in the laboratory when carrying out the tests	Need to draw intravenous blood
		Potential biohazard
	Physicians feel more comfortable with whole blood as it is the conventional procedure	Requirement for packaging and shipping of liquid blood to the testing laboratory; this is both costly and an important barrier to large-scale screening efforts
Dried blood spots	Easier transport of samples	On occasion there is not enough sample for performing certain tests
	Less blood needed	
	Patients can send samples themselves	Handling is more difficult than whole blood when performing the tests
	Fast throughput, cost-effective, suitable for screening purposes	

TABLE 3 Advantages and disadvantages of quantitative tests, phenotyping, and genotyping for α_1-antitrypsin (AT) deficiency

Test	Parameter measured	Advantages	Disadvantages
Immunoassay			
Procedures	Concentration of α_1-AT in plasma or serum	Relatively inexpensive	Does not reliably detect heterozygotes
Radial immunodiffusion		Good reliability (for nephelometry)	May overestimate α_1-AT levels (radial immunodiffusion)
Rocket immunoelectrophoresis		Readily automated	
Nephelometry			
Phenotyping			
Procedure	Isoelectric points of α_1-AT isoforms (characterisation of the type of α_1-AT protein)	Independent confirmation of diagnosis (an α_1-AT allele can be identified without knowledge of the blood α_1-AT level)	Manual technique and time-consuming
Thin-layer IEF[#]		Identifies heterozygotes	Demands skill and experience
		Possibly semi-automation [22]	Requires appropriate standards (and may need to be performed in a Reference Laboratory)
			Does not detect null alleles (*i.e.* no detectable α_1-AT in the blood) and M-like alleles
			Interpretation of rare alleles is difficult
Genotyping			
Procedures	Determination of subject's genetic composition at the Pi locus. Detects genetic abnormalities	Definitive for each mutation tested with available primers	Requires specific primers for each allele
a) PCR, *e.g.* restriction fragment length polymorphism, real-time fluorescence PCR (TaqMan®), fast amplification-reverse hybridisation	Sequencing is used when PCR cannot identify both α_1-AT alleles, *i.e.* in the case of rare variants	Detects null alleles	Can be expensive (sequencing)
		Relatively inexpensive (except sequencing)	In some cases genotyping can miss rare variants, unless followed by sequencing
b) Sequencing of exonic DNA (carried out in cases where available primers fail to provide a complete diagnosis)		Can be adapted for automation (*i.e.* rapid screening for the presence of Z and S alleles)	
		Identifies conclusively specific mutations (also in patients already on augmentation therapy)	

IEF: isoelectric focusing; Pi: proteinase inhibitor. [#]: The American Thoracic Society/European Respiratory Society have positioned IEF as the ''gold standard'' for the diagnosis of α_1-AT deficiency [3].

Biochemistry Laboratory at Vall d'Hebron Hospital, Barcelona, Spain. The algorithms for targeted detection currently used by each of these centres are illustrated in figures 1–3. In Pavia (fig. 1), genotyping is performed at the first stage of the algorithm, simultaneously with the quantitative measurement of α_1-AT levels. At the other two laboratories (figs 2 and 3), determination of the α_1-AT level preceded genotyping. In a study undertaken in Barcelona, an analysis of 971 samples from COPD patients found that none of the samples with normal α_1-AT levels were from individuals carrying the PiZZ phenotype. For the reason of cost, it was decided to perform genotyping only on samples containing α_1-AT at a concentration in DBS equivalent to $<1\ g{\cdot}L^{-1}$ ($\sim$14 μM). When this procedure was undertaken on a further 1,166 samples, a reduction in cost of 30% was achieved compared with the cost that would have been incurred if all 2,137 samples had been screened for genotype [11]. The current practice recommended by the Spanish Registry, therefore, is for laboratories to determine α_1-AT levels, and then send only those samples with concentrations below normal to the Barcelona centre for genotyping to identify Z and S alleles.

Phenotyping was carried out at the Pavia and Marburg centres only on samples with an α_1-AT level previously determined as below normal, after the prior detection of a Z or S variant by genotyping, or in the case of discrepancy between α_1-AT level and genotype. By contrast, in Barcelona, phenotyping was not performed at any stage in targeted detection programmes, and is reserved only for diagnosis assessment in the same situations described for Pavia and Marburg, mainly after detection of low α_1-AT concentrations, or again in the case of discrepancy between α_1-AT level and genotype.

In general, the initial sample tested is in the form of a DBS, with whole blood samples being collected only for confirmatory testing and for DNA sequencing. Sequencing of exonic DNA was normally the final procedure carried out to determine the actual variant present in the sample when a polymerase chain reaction (PCR) was unable to provide a complete identification of both α_1-AT alleles. We advocate that a primary aim of any diagnosis programme should be to detect all individuals with the ZZ phenotype. Detection of the S allele is a secondary goal if resources are limited. Genotyping using PCR has the advantage that a definitive answer is given, for example, as to whether Z or S alleles are present or not. The PCR methodology can be adapted for automation, *i.e.* rapid screening for the presence of S and Z alleles [27–29]. An automated genotyping procedure may well be cheaper than

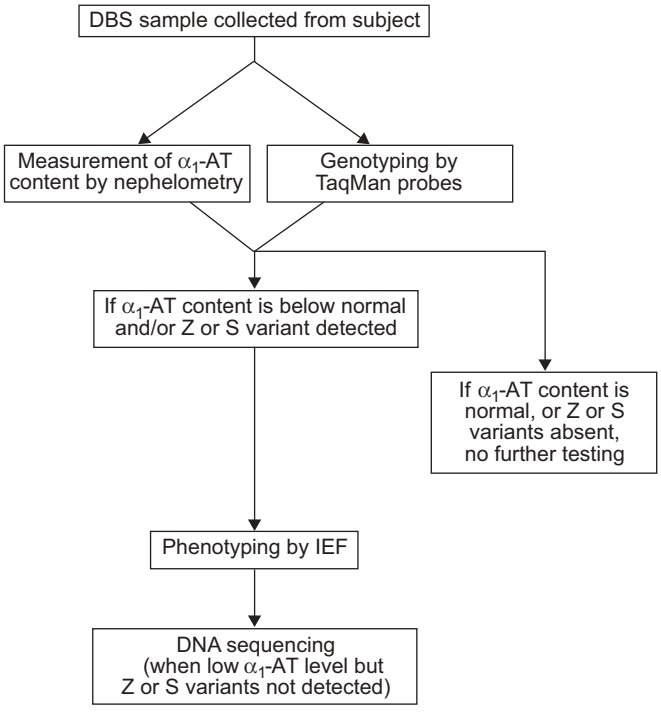

FIGURE 1. Testing algorithm from University of Pavia, Pavia, Italy. DBS: dried blood spot; α_1-AT: α_1-antitrypsin; IEF: isoelectric focusing.

other methods for screening higher numbers of individuals. However, such an approach would not detect rare variants, and in these cases, further sequencing analysis would be needed to obtain a definitive diagnosis.

Detection rates for α_1-AT deficiency alleles

Detection rates at each laboratory cannot be compared because they mostly depend on the characteristics of the patients screened, and only in part on the algorithm, as far as the cut-off of plasma α_1-AT level is concerned [25]. In routine practice, the detection of Z and S variants (present in both homozygous and heterozygous individuals) was observed in ~30–35% of the total number of samples that have been tested by genotyping in Pavia, and in 12% of genotyped samples in Marburg. In Barcelona, the detection programme reserves genotyping for those samples with low concentrations of α_1-AT, which represent ~7–10% of the total samples according to the predefined threshold. Z and S variants were detected in 40% of these genotyped samples. Sequencing is performed for confirmation of the deficient allele when Z and S variants are not detected. Non-Z and non-S rare deficient alleles were detected in a further 10% of all genotyped samples in Pavia. In Marburg, three novel mutations and 45 rare genes have been detected among the 96 samples that have been sequenced. More data about the individual results of the detection programmes are available in the original publications [9, 11, 12, 30, 31].

Sample collection

We recognise that improvements could be made to the collection of samples before they arrive at the laboratory for testing. Although shipment of DBS is easier and less costly than whole blood, and individuals can send the samples

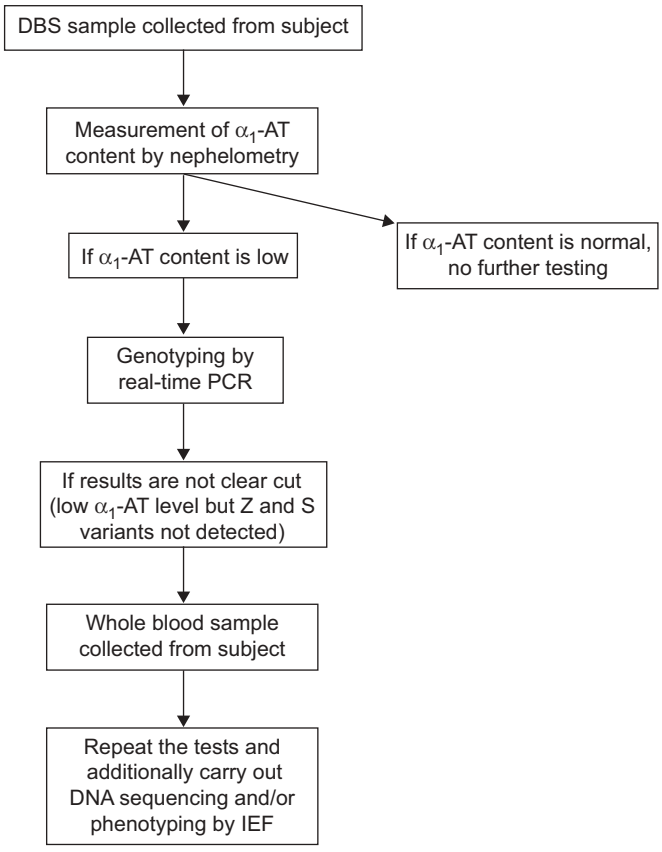

FIGURE 2. Testing algorithm for targeted detection from the Vall d'Hebron Laboratory, Barcelona, Spain. DBS: dried blood spot; α_1-AT: α_1-antitrypsin; IEF: isoelectric focusing.

themselves, problems have arisen in the DBS collection process, and up to 15% of samples are rejected. A list of recommendations and practical suggestions to improve the DBS collection process and to lower the rejection rates is provided in table 4.

Quality control and optimisation of laboratory protocol

A key issue is the establishment of effective quality control processes in the different laboratories carrying out α_1-AT deficiency testing. For example, in Germany, the laboratories are enrolled in external testing to maintain quality control of the PCR methods used. A major step would be the development of reference standards by each laboratory engaged in testing. These measures would assist the many laboratories in maintaining consistency when continuing to test samples over the longer term. With such measures in place, an additional initiative that should be considered is the development of a standard operating procedure document for quality control that would be available to laboratories setting up new programmes for α_1-AT deficiency testing. Consistency within each laboratory is regarded as fundamental, and confidence in the use of reference standards would be built up over time and with experience.

Cost analysis

Costs per sample vary depending on the protocol applied and the local costs. The mean cost per sample tested in the

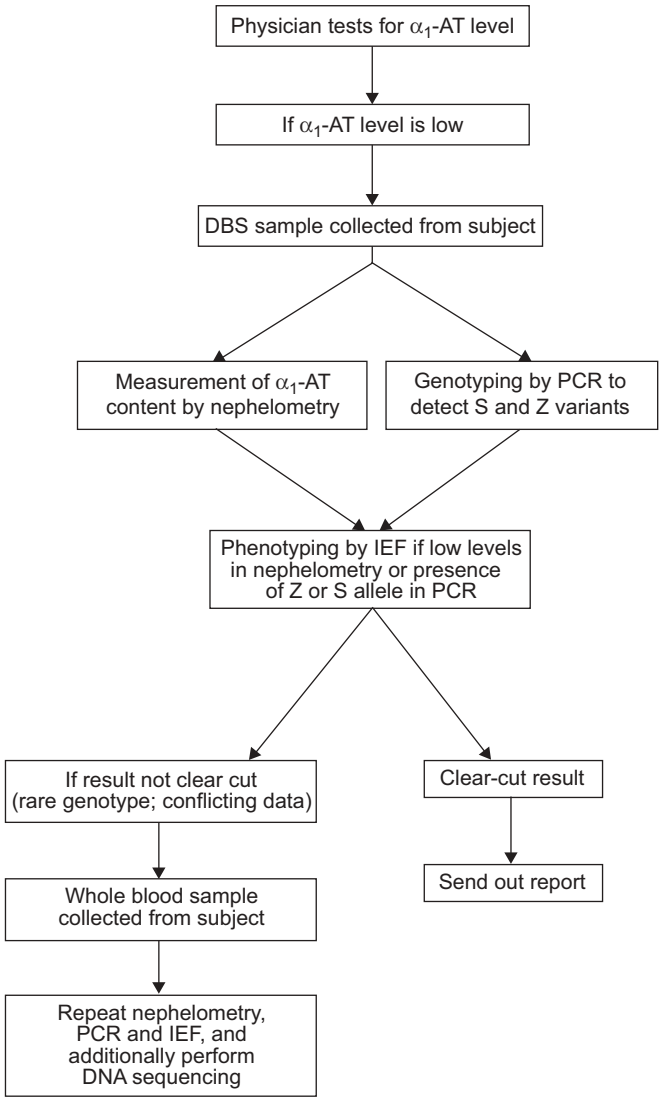

FIGURE 3. Testing algorithm from University of Marburg, Marburg, Germany. α_1-AT: α_1-antitrypsin; DBS: dried blood spot; IEF: isoelectric focusing.

detection programme in Barcelona (genotyping reserved for samples with low α_1-AT concentrations only) is €13.4. In Pavia, genotyping has a cost of €13.5 per sample, plus a further cost of €5 for determining the level of C-reactive protein, which was performed on all samples. The detection programme in Marburg has a mean cost per sample of €33. The cost of sequencing is higher and depends on the equipment, but may range from €48 to €150.

DISCUSSION

Insufficient numbers of individuals with α_1-AT deficiency are currently being detected. The objective of our article is to facilitate the establishment of new patient identification programmes, in greater numbers of laboratories and in other countries, by sharing the experience gained at our three well-established European centres. In order to assist each new laboratory planning to set up a detection programme, our intention has been to provide a detailed description of the testing protocols and current diagnostic techniques, and to put forward our recommendations for improving laboratory practice. We should emphasise that the recommendations in our article are based on our own experience and are not official recommendations of any scientific society.

TABLE 4 Optimisation of the dried blood spot (DBS) collection process

Recommendations for best practice:

1. Procedures should be standardised for:
   a. Applying the sample to (dotting) the filter paper disc
   b. Storage of the dried sample
   c. Shipment of the sample
2. Standard lancets (possibly with an automatic trigger) should be supplied as part of the test kits
3. Ensure that the filter paper disc is thoroughly soaked through
4. The collection date should be placed directly on the filter paper disc
5. Patients sending in samples need to designate a physician who will then be informed of the test results
6. A one-page instruction sheet describing best practice for sample collection should be provided
7. The reasons why a sample has to be rejected should be explained to the physician (by either telephone call or standard letter)
8. Training sessions should be organised in hospitals to communicate best practice in handling DBS

Individuals with α_1-AT deficiency may suffer from unexplained and inadequately managed lung conditions for many years. Despite clear recommendations from the WHO and ATS/ERS on the importance of diagnosis, physicians' practices are changing minimally; many physicians remain completely unaware of the disease and may see only one person diagnosed with α_1-AT deficiency in their lifetime. In fact, α_1-AT deficiency is a condition that has been described as ''emerging from the shadows.'' Early detection of α_1-AT deficiency would enable individuals to make changes in their lifestyle (*e.g.* smoking cessation; exercising and pulmonary rehabilitation; oxygen supplementation; receiving nutritional support) to prevent the development of severe morbidity. In particular, tobacco smoking is highly correlated with the development of emphysema in subjects with α_1-AT deficiency [32–34]. Smokers may present with symptoms of pulmonary disease several years earlier (*e.g.* in the fourth decade of life [3]), and suffer a more rapid decline in lung function [35–38]. Importantly, early diagnosis of the disorder also enables individuals to receive effective treatment for α_1-AT deficiency, such as augmentation therapy [39].

Our article has reviewed validated procedures that are presently available to screen for, and to diagnose, α_1-AT deficiency. Much progress has been made in the development of these methods since testing for α_1-AT deficiency was first possible 40 yrs ago. However, a major challenge remains to utilise the techniques available to increase the numbers of individuals with α_1-AT deficiency that are actually diagnosed with the disease.

Some differences exist with regard to the procedures used by our centres, but these are relatively minor, at least for the identification of patients with severe α_1-AT deficiency. As a

TABLE 5 Strategies for increasing awareness of α_1-antitrypsin (AT) deficiency and recommendations for best practice in the establishment of new identification programmes

Increasing awareness

1. Standardised educational materials should be provided for physicians, including CME programmes
2. Include sessions on α_1-AT deficiency at chest physician and hepatologist congresses
3. The ATS/ERS and local recommendations should be disseminated
4. The development of a diagnostic algorithm for COPD patient assessment should include testing for α_1-AT deficiency
5. All education about COPD should include a recommendation to screen for α_1-AT deficiency
6. The provision of testing kits to general practitioners and to chest physicians should be increased
7. Additional patient organisations should be set up, so that patients encourage physicians to learn more about the disease
8. The importance of preventative care should be endorsed and risk factors emphasised, including education about the adverse effects of smoking
9. Use the opportunity of World COPD Day and similar events for providing information about α_1-AT deficiency
10. The availability of effective options for the treatment of diagnosed individuals should be communicated
11. Provide incentive for testing by increasing reimbursement to physicians
12. Provide incentive for testing through payment to employees (as has been recently described for cigarette smoking cessation)
13. Increase spirometry testing by primary care physicians and test individuals with airflow obstruction for α_1-AT deficiency
14. Use electronic medical record (''Physician Alert'') to prompt physicians to test for α_1-AT deficiency when pulmonary function tests show obstruction [42]

Recommendations for best practice

1. Detect all individuals with the PiZZ and other severe genotypes
2. Introduce methodology to automate genotyping
3. Determine the most cost-effective approach to targeted detection
4. Laboratories should prepare their own set of reference standards
5. Laboratories should participate in a quality control programme
6. Develop a standard operating procedure document for quality control
7. Procedures for the collection of dried blood spots should be improved and better standardised

CME: continuing medical education; ATS/ERS: American Thoracic Society/European Respiratory Society; COPD: chronic obstructive pulmonary disease.

consequence, it may be that some carriers of heterozygosity with α_1-AT plasma levels above the cut-off are missed. There is increasing interest in such conditions as co-factors for lung and liver diseases, thus we can suppose that, in the future, detection programmes will be designed to include them in diagnostic flow charts. The major diagnostic steps, as shown in the algorithms, are shared by the three centres. Each of the algorithms includes genotyping, and it is considered necessary to detect all individuals with the PiZZ genotype. We agree that measurement of the level of α_1-AT in the blood and the determination of the genotype are currently the two major diagnostic techniques. We regard phenotyping by isoelectric focusing as no longer the ''gold standard'' for diagnosing α_1-AT deficiency (as had been mentioned in the ATS/ERS statement of a few years ago [3]), and consider that isoelectric focusing should be used only as a reflex tool for confirmatory analysis. Furthermore, it should be noted that two other laboratories, both in the USA, also now accept genotyping and measurement of the α_1-AT level as the main diagnostic approaches and have published the details of their algorithms, which are essentially similar to our own [40, 41].

There may be good reasons to consider adapting the algorithms used in different laboratories and countries. A key motivational factor in driving laboratories to embrace new methodology is the need to develop less expensive techniques. For large case detection programmes, simple and cheap tests are necessary. Improvements should therefore be made to the testing methodology to increase efficiency and throughput. It is recommended that the individual laboratories should carry out a cost analysis of the different methods that they are currently using, to assist in the development of the most cost-effective approach to the targeted detection. This course of action could lead to the different laboratories using a more standardised process for testing and diagnosis. Where a consensus can be achieved in developing a standardised approach, a more consistent message can be presented to physicians to encourage them to participate in patient identification.

We recommend that each laboratory use reference standards and propose that a collection of standard samples be sent out from a specialist centre to the laboratories so that they could prepare their own set of standards. Likewise, the establishment of a quality control programme is recommended for participation by existing screening and diagnostic laboratories, and also by laboratories that would begin to be involved in testing for α_1-AT deficiency. Finally, technical developments are endorsed that could improve and expedite the DBS collection process.

In order to increase the numbers of α_1-AT-deficient individuals identified, it is necessary to raise further awareness of the disease. The success of diagnostic programmes rests largely on the enthusiasm and commitment of physicians and other healthcare providers; awareness strategies should therefore include ways to encourage the interest of the medical community. It is equally important to increase awareness among α_1-AT-deficient individuals as well as more extensively, among the wider population. A key aspect of promotional campaigns to increase knowledge of α_1-AT deficiency is to emphasise the main risk factors for the disease and the consequent need for lifestyle changes by affected individuals.

As discussed above, tobacco smoking is strongly associated with the development of lung disease symptoms in individuals with α_1-AT deficiency, and smoking cessation is a principal priority in the management of the disorder [30]. There are many different ways to increase awareness of α_1-AT deficiency; these are summarised in table 5.

In summary, small and not critical differences exist in the ways that laboratories currently perform severe α_1-AT deficiency testing. However, closer cooperation, such as the sharing of protocols and technical knowledge between laboratories and countries (such as through the Alpha One International Registry (AIR) programme [43]) would enable agreement to be reached, for example, on more efficient and cost-effective methodology. Recommendations can be made as to best practices to improve the diagnosis of α_1-AT deficiency and increase the numbers of individuals identified (table 5), and our current recommendations can be used to assist laboratories in setting up programmes for α_1-AT deficiency testing in the future.

STATEMENT OF INTEREST

Statements of interest for M. Miravitlles, I. Ferrarotti, M. Luisetti and R. Bals can be found at www.erj.ersjournals.com/misc/statements.dtl

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