High dose intravenous AAT and plasma neutrophil derived fibrinogen fragments

A recent review by Stoller and Aboussouan presented the current understanding of intravenous augmentation therapy for α1-antitrypsin (AAT) deficiency. Their criteria for demonstrating efficacy of this therapy did not include evidence of protection against lung tissue destruction. Such studies would show that sufficient levels of AAT are reached in the lungs to allow inhibition of neutrophil derived enzymes before they degrade elastic fibres to cause alveolar destruction—the hallmark of emphysema. To date, no such evidence has been presented. A study with the currently recommended augmentation regimen using assays of elastin degradation products failed to show any efficacy. The authors argued that the duration of treatment was probably too short, but one may also argue that the dose was too low.

Ever since the introduction of AAT augmentation therapy, no clinical benefit has been demonstrated in a randomised clinical trial. However, the direct health care costs associated with AAT deficiency are high. As no effect of this treatment on lung function has been proven, the question arises as to whether the currently recommended dose is high enough to achieve the desired biochemical and clinical effect.

We studied the effect of two different doses of intravenous AAT on neutrophil mediated proteolysis. Plasma levels of large fibrinogen/fragments formed by neutrophil elastase mediated degradation (PMN-FDP) were measured. These fragments are significantly higher in the plasma of subjects with AAT deficiency than in healthy controls, indicating an imbalance in the protease-antiprotease ratio in vivo at sites of inflammation where fibrinogen is deposited. Although not disease specific, fibrinogen is present at sites of inflammation and, as such, is relevant for patients with AAT deficiency who have increased inflammation in their lungs, even in the absence of a smoking habit.

Twenty subjects with the ZZ phenotype of AAT and emphysema volunteered to participate in the study. Written informed consent was obtained and the study was approved by the ethical board of Leiden University Medical Center. The study consisted of two parts. Firstly, 10 patients (forced expiratory volume in 1 second (FEV1) <65% predicted) were randomised (1:1) to receive either a single infusion of 250 mg/kg AAT (a dose currently used for monthly infusion) or no treatment. AAT was supplied by Laboratoire François du Fractonnement et des Biotechnologies, Lille, France. In all 10 patients blood samples were taken on the days indicated in fig 1A. In the second part of the study the other 10 patients (FEV1 <65% predicted) were randomised (1:1) to receive either two infusions of 250 mg/kg AAT 1 week apart or no treatment and blood samples were taken. In addition, plasma was taken once from 20 healthy controls.

As shown in fig 1A, the levels of PMN-FDP fragments decreased in patients given the currently used dose but did not reach levels seen in normal individuals. In contrast, doubling the dose of AAT resulted in normal levels of fragments and these levels were maintained for 10 days. PMN-FDP fragments from untreated patients ranged from 109 ng/ml to 179 ng/ml, whereas those from healthy controls ranged from 9 ng/ml to 25 ng/ml.

These results suggest that fibrinogen fragments may serve as a marker for inflammation induced proteolysis in the lung in vivo and that their formation can be inhibited with higher doses of AAT than the currently recommended dose for augmentation. Furthermore, our results suggest that the currently applied dose may not be high enough to produce a protective effect on the decline in lung function in individuals with type Z deficiency of AAT. To justify the cost of this expensive treatment, assessment of the efficacy on the basis of biochemical markers of neutrophil mediated alveolar destruction in these patients is indicated; this is feasible with our assay and other improved assays.

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The authors have no competing interests concerning the content of this article.

References


Authors’ reply

Drs Stolk and Nieuwenhuizen present important findings regarding the effect of high dose augmentation therapy on plasma fibrinogen degradation fragments in 20 subjects with PiZZ α1-antitrypsin (AAT) deficiency. Their findings are interesting for two reasons: (1) they examine the effects of doses of augmentation therapy higher than have conventionally been given, and (2) they observed a reduction in PMN-FDP fragments in the group receiving two infusions of augmentation therapy at 250 mg/kg compared with the group receiving a single infusion, thereby supporting the possibility that higher dose augmentation therapy confers benefit.

However, as the authors point out, PMN-FDP is not a specific measure of elastolysis and so, in our view, cannot yet be advanced as evidence of definitive protection against lung destruction in AAT deficiency. Still, their findings invite further study of the dose-response effectiveness of higher dose augmentation therapy, ideally using conventional and emerging measures of lung destruction including detailed pulmonary function tests and chest CT densitometry.

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CFTR mutations and polymorphisms in adults with disseminated bronchiectasis: a controversial issue

The recently published paper by King et al.1 reported the results of our study of 19 Serbian patients with disseminated bronchiectasis (DB) of unknown cause for whom complete screening of the CFTR gene was performed. Our patients consisted of four men and 15 women of mean age 54.5 years (range 24–79); the mean age at onset of the disease was 38.8 years. The diagnosis was based on high resolution computed tomographic (HRCT) scanning. Known and common causes of bronchiectasis such as pulmonary ciliary dyskinesia, immunodeficiency, and α1-antitrypsin deficiency were excluded. Most of the patients had Pseudomonas aerogen-osa isolated from their sputum. Pulmonary function tests were performed in 16 of the 19 patients. The remaining three were unable to undergo these tests because of the severity of their disease. Mean (SD) forced vital capacity (FVC) was 66.6 (20.5)% of predicted and mean (SD) forced expiratory volume in 1 second (FEV1) was 55.3 (24.0)% of predicted.

The whole coding region and intronic boundaries of the CFTR gene were analysed by direct sequencing of the PCR products obtained with primers of exons-specific primers. Genomic DNA from all the patients was extracted from peripheral blood and genomic DNA was isolated from sputum in three patients. Polymerase chain reaction (PCR) was performed in a multiplex reaction with amplification of all 27 exons and flanking sequences. PCR products were subjected to agarose gel electrophoresis and fragments were purified. Direct sequencing was performed using specific primers for the identified fragments. In addition, we performed the PSM method.3 The IVS8-5T, IVS8-7T, and IVS8-9T alleles of the CFTR gene were detected using the PSM method.3

The IVS8-5T allele was not found in any of the 19 patients with DB. The cumulative allelic frequency of mutations in this group of patients was 7.9% (3/38 chromosomes). The IVS8-5T allele was not found in any of the patients. Controversial data on the role of the 5T variant in patients with bronchiectasis have been reported. Pignatti and coworkers1 analysed 16 patients with bronchiectasis and suggested that the 5T variant had a similar role to that described in the congenital bilateral absence of vas deferens (CBVD) phenotype. However, later studies4,5 did not find a higher frequency of the 5T variant in patients with bronchiectasis.

In contrast to previous reports,6 the frequency of CFTR mutations in patients with DB was not significantly higher than in our general population (2.17%, unpublished data, 2005). Because of the small sample size, these results are preliminary and need to be confirmed in a large study, but the strength of our study lies in the strict clinical selection of patients and the fact that the complete coding region of the CFTR gene was screened.

In adults with asthma, a recent paper by Casals and coworkers7 has found a reduced risk of developing asthma than homozygous wild types.

Since heterozygous individuals may have altered disease susceptibility, we were interested in finding the inheritance pattern of this mutation in the asthmatic families. We therefore recruited 10 families (56 individuals) of the CCR5Δ32 heterozygous pro-band. Genotyping indicated that the mutation segregated in Mendelian fashion. In the process we found two homozygous for this deletion (first report from the Indian subcontinent). Furthermore, to establish the trend of CCR5Δ32 in asthmatic families from other parts of India, 36 families from other parts of India, 36 families from north-west and 48 families (147 individuals) from the north-west were also genotyped. Only two members of one family from north-west India were heterozygous for CCR5Δ32 mutation while no homo/heterozygous mutants were observed from north-east India.

We suggest that CCR5Δ32 is associated with asthma but its low frequency may delay the progress in establishing the role of CCR5 in predicting susceptibility to asthma. Nevertheless, our findings have important implications in understanding the global distribution of CCR5Δ32 and its possible impact on the susceptibility to developing polygenic and biological diseases including asthma and AIDS.

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References


CCRS Δ32 deletion and atopic asthma in India

Chromekine receptor 5 (CCRS) contributes to the generation of a Th1 immune response by interacting with agonists such as RANTES, MIP-1α, and MIP-1β. A 32 base pair deletion (Δ32) in CCRS has been proposed to protect individuals against HIV infection and to bias the immune system towards a Th2-driven response, thus affecting the susceptibility to developing allergic diseases such as asthma. In a study in Scottish children, Hall et al reported an association of CCRSΔ32 with a reduced risk of asthma7 but found no such association in adults with asthma.8 In addition, no association was detected with atopy or asthma/wheeze in two other studies.9

We examined the potential role for this deletion in the pathogenesis of asthma by an association study in a genetically untapped Indian population. Patients were diagnosed with asthma on the basis of the National Asthma Education and Prevention (Expert Panel Report 2) guidelines. Written consent was obtained from individuals participating in the study. Genomic DNA from patients with atopic asthma (mean SD age 54.2 (5.6) years) and healthy controls (27 (14.6) years) from Northern India was screened for CCRSΔ32 deletion. We found that only 11 of 367 controls were heterozygous for the mutation compared with 17 of 215 with atopic asthma. However, we failed to detect any homozygous individual in either group in preliminary analysis. In contrast to previous reports, individuals heterozygous for CCRSΔ32 showed a three times greater risk of developing asthma than homozygous wild types.

Since heterozygous individuals may have altered disease susceptibility, we were interested in finding the inheritance pattern of this mutation in the asthmatic families. We therefore recruited 10 families (56 individuals) of the CCRSΔ32 heterozygous pro-band. Genotyping indicated that the mutation segregated in Mendelian fashion. In the process we found two homozygous for this deletion (first report from the Indian subcontinent). Furthermore, to establish the trend of CCRSΔ32 in asthmatic families from other parts of India, 36 families from north-west and 48 families (147 individuals) from the north-west were also genotyped. Only two members of one family from north-west India were heterozygous for CCRSΔ32 deletion while no homo/heterozygous mutants were observed from north-east India.

We suggest that CCRSΔ32 is associated with asthma but its low frequency may delay the progress in establishing the role of CCRS in predicting susceptibility to asthma. Nevertheless, our findings have important implications in understanding the global distribution of CCRSΔ32 and its possible impact on the susceptibility to developing polygenic and biological diseases including asthma and AIDS.

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References

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