Alpha 1 Antitrypsin Deficiency: Focus on Liver Disease in Young and Old

Karin Kok
Alpha 1 Antitrypsin Deficiency: Focus on Liver Disease in Young and Old

Een wetenschappelijke proeve op het gebied van de medische wetenschappen

Proefschrift

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Chapter 1

Introduction and outline of the thesis
Past-to-present
In 1963, Laurell and Eriksson, presented in the “Scandinavian Journal of Clinical and Laboratory Investigation” 5 patients with a new type of dysproteinemia, characterized by very pronounced alpha-1 antitrypsin (A1AT) deficiency. This deficiency was detected by paper electrophoresis. (Figure 1.1) A total of 3 out of these 5 patients had pulmonary disease, 1 patient had a malabsorption syndrome and the last patient had no obvious disease.1

In 1967 and 1970, they continued to establish an association between chronic obstructive lung disease and A1AT deficiency.2,3 In 1972 these authors published a key paper that described the presence of liver disease in adults with A1AT deficiency4 and in 1974 they reported a high incidence of hepatocellular carcinoma in patients with A1AT deficiency related cirrhosis.5 At that time, others showed A1AT polymers in 52% of hepatocellular carcinoma6 and furthermore, A1AT deficiency was considered as a cause of neonatal hepatitis.7 The first report on A1AT deficiency originating from the Netherlands was published in 1980 and contained interesting epidemiologic data that demonstrated that the frequency of A1AT deficiency was 8 out of 10,000 newborns.8 In 1983 the point mutation which is involved in A1AT deficiency was detected.9

Over the years, many reports concerning A1AT deficiency and its relation

with prognosis, associations with environmental factors, (patho)physiologic mechanisms and gene association studies have been published. In December 2009, a “Google-search” for A1AT deficiency resulted in no less than 1.3 million hits. Nowadays, the diagnosis A1AT deficiency is not made by a paper patchy spot as shown above, but current diagnostic tools are much more sophisticated. (Figure 1.2 and 1.3)
The gene

A1AT deficiency is a genetic disorder and the A1AT protein is encoded by the protease inhibitor (Pi) locus located on chromosome 14q32.1 (SERPINA1 gene). The A1AT protein is a member of the “SERPIN” family, similar to alpha1-antichymotrypsin, alpha2-antiplasmin, antithrombin and C1-inhibitor. The gene is 12.2 kb in length with 7 exons (4 coding and 3 non-coding) and 6 introns. (Figure 1.4) The protein encoded includes 394 amino acids. The protease inhibitor locus is highly polymorphic with approximately 123 single nucleotide polymorphisms (SNPs), resulting in numerous different A1AT isotypes that can be detected by iso-electrophoresis. The wild type allele results in a normal M-type protein. The most common mutations are E264V and E342K, resulting in the S-type protein (exon 3) and Z-type protein (exon 5) respectively. Beside the SNPs in exons, other SNPs in the enhancer sequence of the A1AT gene, involving the regulation of A1AT expression have been described.10,11

The protein

A1AT is mainly produced in the liver and reaches the lung by diffusion from the circulation or by pulmonary production in macrophages and bronchial epithelial cells.12 The A1AT protein is a serum glycoprotein acting as an acute phase protein. The major physiological function of A1AT is inhibition of proteolytic enzymes as elastase and other proteinases which are secreted by neutrophilic granulocytes during inflammatory processes. The normal A1AT protein has a tertiary structure based on a large central β-sheet, surrounded by two other sheets and a reactive centre loop. The reactive centre loop binds to proteinases and can move in and out the β-sheet. In case of a Z-type protein (thus E342K mutation) the reactive centre loop is reacting with the β-sheet of a second A1AT molecule. As a consequence, this leads to polymers that are retained in the endoplasmic reticulum of hepatocytes.11,13 (Figure 1.5)

Mechanism of liver disease

Several studies have provided evidence that the SNP, which is responsible for the Z-type A1AT reduces the stability of the molecule in its monomeric form and increases the likelihood that it will form polymers by a so-called “loop-sheet” insertion mechanism.14 Z-type A1AT polymers can be identified by electronmicroscopy as periodic-acid-Schiff stain positive and diastase-resistant inclusions within the endoplasmic reticulum of hepatocytes. (Figure 1.6 panel B) The S-type A1AT has less effect on loop sheet polymerization and accumulation. It is suggested that the process of protein degradation involves modification of misfolded proteins by the enzyme endoplasmic reticulum mannosidase I. Mannosidase inhibitors delay degradation and increase secretion of Z-type A1AT. Expression of liver disease in patients with
A1AT deficiency correlates with a delay in intracellular degradation of Z-type A1AT and accumulation of polymers. There is remarkable variability in the phenotypic expression of disease in individuals with Pi ZZ homozygosity. Apart from environmental factors such as smoking and alcohol abuse, genetic modifiers appear to be partly responsible for the variability in clinical expression of patients with A1AT deficiency. For example, SNPs in the promoter region and a variant of the endoplasmic reticulum mannosidase I (ERManI) gene were associated with an early onset of end-stage liver disease in patients with homozygous (Pi ZZ) A1AT deficiency. Others suggested that individual variations in episodes of inflammation and hence to increased synthesis of A1AT can explain the variability in severity of liver disease and its age at onset.

Mechanism of pulmonary disease
Polymerization and retention of polymers in the endoplasmic reticulum of hepatocytes, causes a decrease in the amount of serum A1AT that is available to protect the lungs against elastolytic damage. An imbalance between the A1AT protection and elastase with consequent unchecked proteolytic activity, can lead to emphysema. Either a mild increase in elastase (which might happen with smoking or lung infection) or a reduction in anti-elastase defence (which may happen in severe A1AT deficiency) can affect the elastase-anti-elastase balance unfavourably towards accelerated lung breakdown. The decreased A1AT level is not a single issue, the Z-type A1AT is about 5 time less effective as an inhibitor of elastase than normal M-type A1AT. In addition, Z-type A1AT polymers might be chemoattractants for neutrophils thereby potentially increasing lung inflammation.

Outline of the thesis
Despite progress in understanding the mechanisms of disease in A1AT deficiency, several questions with regard to the clinical consequences of A1AT deficiency are still open. First, we are interested in the clinical course of A1AT deficiency. What is the course of liver disease in A1AT deficiency with respect to the pediatric and adult population? Are there specific complications that need to be considered when treating these patients? Second, we will focus on the diagnostic strategy for establishing A1AT deficiency. Third, we wonder whether SNPs in the SERPINA1 gene modify the development, course, and treatment results of liver diseases due to various etiologies. Finally, we addressed questions regarding the clinical relevance of liver disease due to heterozygous A1AT deficiency.

This thesis aims to address these issues with a primary focus on the elements that are important for the clinical management of these patients.

Figure 1.6: Mechanisms of pulmonary and liver disease.
References

Chapter 2

Heterozygous Alpha-1 Antitrypsin Deficiency as a co-factor in the Development of Chronic Liver Disease; a Review

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Abstract

Alpha-1 antitrypsin (A1AT) is an acute phase protein that is produced in liver cells. A1AT deficiency is a hereditary disease that is defined by the hepatic production of an abnormal protein that cannot be released into the plasma. This leads to deficiency of plasma A1AT and subsequently to an impaired protection against proteases, resulting in pulmonary disease. Accumulation of the abnormal protein in hepatocytes can lead to liver damage. Serum level measurement, phenotyping and liver biopsy can be used for establishing the diagnosis.

Homozygous A1AT deficiency can cause neonatal hepatitis; in adults end stage liver disease, cirrhosis and hepatocellular carcinoma may develop. There are strong arguments to consider heterozygous A1AT deficiency as an important co-factor in the etiology of chronic liver disease. Studies have shown that A1AT heterozygosity can be considered a modifier for HCV, end stage live disease, cirrhosis and hepatocellular carcinoma. The accumulation of A1AT in the hepatocytes occurs more profoundly in a sick liver, and as a consequence it affects the natural course of the liver disease. Therapeutic options include augmentation therapy (infusion of purified human plasma A1AT) in pulmonary disease; in end stage liver disease liver transplantation is an option. In the future other interventions such as gene therapy or strategies to inhibit polymerization are promising.

Background

Alpha-1 antitrypsin (A1AT) is an acute phase protein that is produced in liver cells. It is released into the plasma in response to an inflammatory stimulus. A1AT deficiency is a hereditary disease that is defined by the hepatic production of an abnormal protein that cannot completely be released into the plasma. This leads to deficiency of plasma A1AT and subsequently to an impaired protection of the lungs against proteases. This results in pulmonary emphysema, the hepatic accumulation of the abnormal protein can lead to chronic liver disease. This review gives an update of the present knowledge of partial A1AT deficiency in relation to various liver diseases.

Genetics and (patho) physiology

The A1AT molecule is a serum glycoprotein acting as an acute phase protein. It is released during inflammatory processes from the hepatocyte which results in increased plasma concentrations. The major physiological function of this protein is the inhibition of destructive neutrophil elastase, thus protecting against pulmonary damage.1 The A1AT protein is encoded by the protease inhibitor (PI) locus located on chromosome 14q32.1. The PI locus is highly polymorphic, resulting in different A1AT isotypes that can be detected by electrophoresis. The most common allele is the M allele that results in a functionally normal protein with normal serum A1AT levels. The normal A1AT protein has a tertiary structure based on a large central ß-sheet, surrounded by two other sheets and a reactive centre loop. The reactive centre loop can move in and out of the large ß-sheet. At higher temperatures polymerization can occur between molecules due to insertion of the loop of one molecule into the large ß-sheet of the other. The point mutation, found in the Z variant destabilizes the loop-sheet polymerization of the A1AT molecule, resulting in chains of polymers that are retained in the hepatocytes. These polymers accumulate in the endoplasmic reticulum of the hepatocytes and may be recognized as PAS(+) inclusion bodies. (Figure 1.1) Only 15% of the Z-variant of A1AT can be secreted into the plasma, the other 85% accumulates in the liver. In Pi ZZ homozygous subjects, this results in a severe deficiency of serum A1AT and in accumulation of the abnormal protein in the endoplasmatic reticulum of the hepatocytes which can lead to chronic liver disease.
The S variant of the A1AT molecule, has less effect on the loop sheet polymerization. Formation of S-polymers is slower, resulting in less retention of protein in the hepatocytes compared to the Z variant. There is a mild reduction in serum A1AT levels. When S and Z variants are co-inherited, the two interact with the formation of polymers within the hepatocytes; this can lead to reduction in the serum A1AT level, inclusion of the polymers, accumulation and subsequently development of cirrhosis.

Less frequently found variants are null alleles resulting in undetectable A1AT levels due to intracellular degradation or intracellular accumulation of the protein; this is usually associated with severe pulmonary disease, but not with liver disease.2-7 (Table 2.1)

<table>
<thead>
<tr>
<th>Allele</th>
<th>Mutation</th>
<th>Cellular defect</th>
<th>Enzymatic activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>-</td>
<td>-</td>
<td>Normal</td>
</tr>
<tr>
<td>S</td>
<td>Glu264Val</td>
<td>Intracellular degradation</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Z</td>
<td>Glu342Lys</td>
<td>Intracellular accumulation</td>
<td>Low</td>
</tr>
<tr>
<td>Null</td>
<td>Different mutations</td>
<td>Most mutations no mRNA</td>
<td>Nill</td>
</tr>
</tbody>
</table>

A1AT deficiency is characterized by an imbalance between the protease neutrophil elastase and the protease inhibitor A1AT. It has been suggested that neutrophil elastase might promote the development of cancer.8 The exact carcinogenic mechanism or sequence is not known.

In a recent paper, the authors hypothesized, that in A1AT deficiency, the hepatocytes in which A1AT is accumulated are inhibited in their growth, but still do express regenerative signals. Relatively normal cells, without A1AT deposits are thereby stimulated and this chronic stimulation of regeneration may lead to the formation of neoplasms.9

Diagnostic aspects
To establish the diagnosis different methods are available. The A1AT serum level can be determined by clinical chemistry. In homozygous A1AT deficiency the level is very low. However in the heterozygous variant the A1AT level can be within the normal range, especially during an acute phase reaction. Therefore electrophoresis should be performed in any case of suspected A1AT deficiency in order to determine the phenotype. Liver biopsy is the gold standard for establishing A1AT accumulation and PAS positive, diastase resistant inclusions can be found. A specific immuno-histochemical staining can confirm the diagnosis. (Figure 2.1) It is also possible to determine the phenotype on paraffin embedded liver slides.

Figure 2.1 Liver biopsy: cirrhosis due to alpha-1 antitrypsin deficiency.
Epidemiology
Despite the fact that A1AT deficiency is a frequent disorder, it is poorly recognised in clinical practice. There are probably two main reasons, first in patients with liver disease, the diagnosis is not always considered and second not all subjects with a deficient phenotype develop liver disease, i.e. the penetrance is low.  

Given this well known underdiagnosis, the real prevalence of A1AT deficiency has mainly been determined by epidemiological methods, either using a control cohort from an epidemiological study, or through neonatal screening. The Z-allele is especially prevalent in Northern Europe, while the S allele is prevalent in Southern Europe (Table 2.2).

Neonatal / paediatric liver disease
A1AT deficiency is the most common genetic cause of liver disease in early childhood. The most common presentation is by prolonged jaundice. The stools generally contain no yellow or green pigment, indicating cholestasis and mimicking biliary atresia. All patients have hepatomegaly and about 50% also have splenomegaly. Approximately 5% of the patients present with an increased bleeding tendency. This is due to vitamin K deficiency caused by the cholestasis induced malabsorption. Less commonly children present later in childhood with hepato-splenomegaly or with cirrhosis.  

In Sweden, in 1972-1974, 200,000 neonates were screened for A1AT deficiency: 120 Pi ZZ (0.06%), 2 Pi Z-, 54 Pi SZ and 1 Pi S- children were found. Only 14 of the Pi ZZ children had prolonged jaundice, 9 of those had severe liver disease. All infants appeared healthy at six months of age. Infants with a Pi SZ phenotype had no signs of liver disease. At the age of 16 years, in 17% of Pi ZZ adolescents and in 8% of Pi SZ adolescents, elevated liver enzymes were found. The adults with liver disease in infancy were clinically healthy. At the age of 26 years, the Pi ZZ subjects were compared to Pi MM individuals. The Pi ZZ subjects had normal lung function; 4-5% of them had mild liver test abnormalities. In the Province of Bozen in Northern Italy, Pi phenotyping in umbilical cord blood was performed as a routine neonatal screening. About 5% of Pi SZ children were affected by liver involvement with elevated liver enzymes and 7% of 833 Pi MZ heterozygotes had elevated liver enzymes in early childhood. At age of 5 and 10 years, none had liver disease. The serum levels of A1AT were similar in the groups with and without liver test abnormalities; however these values had a wide range. Although these studies suggest a good prognosis for neonatal cholestasis due to A1AT-deficiency, other studies described children who developed severe liver disease.

Clinical aspects
Pulmonary disease
A1AT deficiency is associated with less inhibition of elastase resulting in pulmonary disease. A1AT homozygosity (Pi ZZ) results in a pulmonary phenotype with early onset of emphysema, asthma and bronchiectasia. A1AT deficiency results in panacinar pathology and disproportionate emphysematous involvement of the lung bases. Tobacco smoking is the most important additional risk-factor for the development of pulmonary disease. Also subjects with the Pi MZ phenotype have an increased risk for the development of pulmonary disease.

Table 2.1: Epidemiology (16-19)

<table>
<thead>
<tr>
<th>Country</th>
<th>Frequency</th>
<th>Method</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweden</td>
<td>0.06%</td>
<td>Neonates, phenotyping if serum A1AT &lt; 40%</td>
<td>1976</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>0.03%</td>
<td>S allele Neonates, phenotyping when</td>
<td>1980</td>
</tr>
<tr>
<td>France</td>
<td>0.01%</td>
<td>Control cohort epidemiological study</td>
<td>2003</td>
</tr>
<tr>
<td>Spain</td>
<td>0.02%</td>
<td>Control cohort epidemiological study</td>
<td>2003</td>
</tr>
<tr>
<td>Italy</td>
<td>0.02%</td>
<td>Control cohort epidemiological study</td>
<td>2003</td>
</tr>
<tr>
<td>Portugal</td>
<td>0.02%</td>
<td>Control cohort epidemiological study</td>
<td>2003</td>
</tr>
<tr>
<td>Australia</td>
<td>0.02%</td>
<td>Control cohort epidemiological study</td>
<td>2003</td>
</tr>
<tr>
<td>USA</td>
<td>0.02%</td>
<td>Control cohort epidemiological study</td>
<td>2003</td>
</tr>
<tr>
<td>New Zealand</td>
<td>0.05%</td>
<td>Control cohort epidemiological study</td>
<td>2003</td>
</tr>
<tr>
<td>Canada</td>
<td>0.02%</td>
<td>Control cohort epidemiological study</td>
<td>2003</td>
</tr>
</tbody>
</table>
Liver disease in homozygous A1AT deficient adults
Liver disease due to A1AT deficiency generally presents at adult age. One study reviewed adult patients with liver disease and A1AT deficiency; the mean age of the patients when liver disease became symptomatic was 58 years for the ZZ phenotype, 66 years for the SZ phenotype and 73 years for the MZ phenotype. If the liver disease was advanced at the time of diagnosis, 42% of these patients died within two years. A review of autopsy data on 94 Pi ZZ homozygous A1AT carriers showed that cirrhotic patients survived longer compared to non-cirrhotic patients. The non-cirrhotic patients had more severe lung disease and died earlier. A cohort of patients who are registered in the Alpha-1 foundation Registry (a USA foundation providing increased research and improved health for A1AT deficiency), and who had reported liver disease or jaundice (165 of the 2175 participants in the registry) completed a questionnaire. Of these patients 71% was Pi ZZ and 18% was Pi MZ, in the remainder the phenotype was unknown. Mean age at diagnosis of liver disease was 31 years (range 0-68 years), 30% had had liver transplant or were on the waiting list. Male gender and obesity were risk factors for advanced liver disease, while white race, Pi phenotype, infant jaundice, diabetes or hypercholesterolemia were not. Although this survey is the largest cohort of A1AT deficiency and liver disease in the literature, the self-selected cohort runs a risk of inclusion bias. The natural history of the disease is not completely known. The risk of cirrhosis in adults is difficult to estimate because most available data are retrospective and derived from patients known to have A1AT deficient lung disease or cirrhosis.

Heterozygous A1AT deficiency and liver disease
Although the role of homozygous A1AT in liver disease is established, the association between heterozygous A1AT deficiency and chronic liver disease is still subject to ongoing investigation. Several studies however showed an association between heterozygous A1AT deficiency and chronic liver disease. In 1981 a study showed the association of Pi MZ and liver disease. A total of 1055 liver biopsies was screened for A1AT depositions in hepatocytes and 34 patients with these inclusions were found. These patients were phenotyped, the prevalence of phenotype Pi MZ in the whole biopsy group was 2.4%. In the subgroup of patients with liver cirrhosis, 9% had a Pi MZ phenotype. A percentage of 21 % of patients with cryptogenic cirrhosis and chronic hepatitis had a Pi MZ phenotype. This was significantly increased compared to other causes of cirrhosis. The prognosis of the Pi MZ cirrhotic patients was poor, most patients died within one year. More recently patients with end stage liver disease, in work-up for liver transplantation were investigated. Pi MZ was found in 7.3- 8.2%, compared to 2.8% in the control population. A heterozygous phenotype was more prevalent in patients with hepatitis C, alcoholic liver disease, cryptogenic cirrhosis and hepatocellular carcinoma. In one study consecutive liver biopsies and autopsies were screened for Pi Z deposits. In the biopsy group 3.4% of cases were Pi MZ phenotyped, whereas in the autopsy group this was 1.8%. In biopsies from older people heterozygous for A1AT, more fibrosis and more Pi Z deposits were found; the liver involvement seems to be age dependent. In case of coexisting liver disease, patients presented with more inflammation, more fibrosis and more Pi Z deposits than the biopsies without concomitant liver disease. We described 3 patients with alcoholic liver disease and a rapidly deteriorating clinical course, resulting in the patients’ death. All 3 patients were found to be heterozygous for A1AT. To summarize: These studies showed that various liver diseases influence the A1AT accumulation and that the A1AT accumulation influences the course of the liver disease. The risk of developing liver cirrhosis is increased in patients with heterozygous A1AT deficiency, also without coexisting liver disease. The exact impact and involvement of liver disease by heterozygous A1AT deficiency are unknown. Further research is needed to provide these data.

Heterozygous A1AT deficiency and coexisting hepatitis C virus infection
The role of A1AT deficiency in the severity and the course of liver disease in chronic hepatitis C virus (HCV) infection is not clear, despite the fact that several studies analyzed the association of HCV-induced liver disease and A1AT deficiency. In Austria 1865 patients referred for the evaluation of chronic liver disease were analyzed, 9% had a deficient phenotype. From these patients with cirrhosis, 62% was HCV positive, 33% had evidence for HBV
A1AT deficiency and chronic liver disease: review

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Infection, 41% abuse of alcohol and 12% had features of autoimmune liver disease. Out of 53 cirrhotic A1AT deficient patients, only 5 had no coexisting liver disease. The authors concluded that the risk for chronic liver disease is increased in patients with the Pi Z gene, because they may have increased susceptibility to viral infection or additional factors, necessary to induce chronic liver disease.34 The same authors investigated the prognosis of patients with A1AT deficiency. Some 54 patients with A1AT deficiency had evidence of chronic liver disease, 78% showed positive viral markers (hepatitis B or hepatitis C); this was compared to 106 patients with A1AT deficiency without chronic liver disease, without signs of additional viral infection. Life expectancy in A1AT deficient patients was significantly lower in patients with chronic liver disease in comparison to patients without chronic liver disease.35 Patients with end stage liver disease, in work-up for liver transplantation were also investigated. In the HCV patients Pi MZ was found in 10-13%, compared to 2.8% in the control population. This suggests that an abnormal heterozygous phenotype is a co-factor in the development of chronic liver disease in HCV.30,31 In contrast, other studies showed no association of hepatitis C infection and A1AT deficiency.36-38 To conclude: the results of these studies are controversial. Some studies showed a higher incidence of A1AT deficiency in HCV infection and an increased susceptibility to viral infections in A1AT deficiency and other studies do not. Different methods to determine the A1AT state were used. Further research on the influence of A1AT deficiency in the course of HCV infection and vice versa is necessary.

Heterozygous A1AT deficiency and hepatocellular carcinoma

Established risk factors for hepatocellular carcinoma (HCC) include amongst others chronic hepatitis B, HCV infection and alcoholic liver cirrhosis. Several studies investigated the correlation between A1AT deficiency and HCC.35,40 In 1986 it was suggested for the first time that men with A1AT deficiency may be at risk for cirrhosis and HCC. Autopsy was performed in 16 adult patients with A1AT deficiency. In 5 out of these 16 patients, a HCC was found.40,41 In 317 HCC, Pi Z deposits were found in 6% compared to 1.8% in the control group.

In heterozygous A1AT deficiency, HCC had also developed in non-cirrhotic livers and were frequently characterized by cholangiocellular differentiation. In patients with A1AT deficiency bile duct lesions were frequently found. This might reflect a predisposition for the liver tissue for developing tumours with cholangiolar differentiation in A1AT deficiency.43,44 In contrast to these studies, others did not show an association between A1AT deficiency and hepatocellular carcinoma, although carcinomas in non-cirrhotic livers were Pi MZ associated.45-48 To summarize: several studies are performed to investigate the relation between A1AT deficiency and hepatocellular carcinoma. The outcomes are not uniform. In our opinion studies with large cohorts of patients with hepatocellular carcinoma are the most reliable; these studies did find an association.

A1AT deficiency and associations with other diseases

A1AT deficiency is not only associated with liver and lung disease. Associations with panniculitis, nephrotic syndrome, intracranial aneurysm, hereditary haemochromatosis and celiac disease have been described.49-62

Therapy

Most therapeutic strategies in the treatment of A1AT deficiency are directed towards pulmonary disease. Infusion of purified pooled human plasma A1AT is known as augmentation therapy. The goal of this treatment is to raise and maintain serum A1AT concentrations above the protective threshold. Data from different studies suggest that intravenous augmentation therapy has a positive biochemical and clinical effect. The therapy is expensive (US $ 28,075-65,973 per year).7 Different concepts have been studied to prevent the polymerization and accumulation of the A1AT protein. New peptides that block the polymerization of the Z protein have been developed.63,64 Gene therapy by injecting adeno-associated virus carrying the human A1AT gene is another promising concept.65 Liver transplantation is used in end stage liver disease and results in acquisition of the donor phenotype, a rise in serum levels of A1AT and prevention of associated diseases.66
Conclusion

Alpha-1 antitrypsin deficiency is an autosomal recessive disorder that can lead to chronic pulmonary disease and liver disease. The liver disease is caused by accumulation of an abnormal, polymerized protein. Deficient phenotypes are present worldwide. Homozygous A1AT deficiency in children can cause neonatal hepatitis. In adults homozygous patients are at risk for developing end stage liver disease, cirrhosis and hepatocellular carcinoma. Heterozygous A1AT deficiency is probably an important co-factor in the etiology of chronic liver disease, as several studies showed associations with HCV, end stage liver disease, cirrhosis and HCC. Screening on A1AT deficiency should be done routinely, A1AT serum levels may be used, but phenotyping is crucial, as serum levels may be not reflect true deficiencies (due to inflammation serum levels can be false normal). Especially in cryptogenic chronic liver disease and in liver disease that deteriorates faster than may be expected, A1AT deficiency may be of clinical significance as a (co)-factor. Clinical research is needed in A1AT-related liver disease to investigate the association between heterozygous A1AT deficiency and the presentation and course of liver diseases. We believe that current data are insufficient to decide on the pro’s and con’s of screening on hepatocellular carcinoma in A1AT deficiency. Therapeutic options in A1AT deficiency include augmentation therapy in pulmonary disease; in end stage liver disease liver transplantation is an option. Gene therapy or strategies to inhibit polymerization are promising.

References


Chapter 3

Prognosis of neonatal cholestasis
due to Alpha-1 Antitrypsin Deficiency

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Richard A de Vries
Roderick HJ Houwen
Abstract

**Background:** Alpha-1 antitrypsin (A1AT) deficiency is the most common genetic cause of liver disease in early childhood. Several studies, mostly from transplantation centers, have reported a poor outcome of patients with neonatal cholestasis due to A1AT deficiency. We retrospectively studied the natural course of liver disease in a cohort of patients with neonatal cholestasis due to A1AT deficiency.

**Methods:** We studied neonates who were diagnosed with a ZZ or SZ phenotype within the first 3 months of life and who had neonatal cholestasis. Patients born in the Netherlands between January 1991 and December 2006 were included. Clinical and biochemical parameters at presentation were recorded. Outcome was reported at end of follow-up and in 5- and 10-years follow-up cohorts.

**Results:** In this 16 years time frame 50 patients were identified. A total of 15 neonates presented with vitamin K deficiency bleeding. During follow-up 5 (10%) patients died and 2 (4%) patients received liver transplantation. At the end of follow-up 12% had cirrhosis, 62% had abnormal liver enzymes and/or hepatosplenomegaly and 12% had no biochemical or clinical abnormalities. Survival without liver transplantation was 87% in the 5-years follow-up cohort and 73% in the 10-years follow-up cohort.

**Conclusions:** In our study, the natural outcome of patients with neonatal cholestasis due to A1AT deficiency is better than was suggested in studies originating from transplantation centers, but similar to other cohort studies.

Background

A1AT deficiency is a hereditary disease characterized by the hepatic production of an abnormal A1AT protein that cannot completely be released into the plasma. This leads to deficiency of serum A1AT and subsequently to an impaired protection of the lungs against proteases resulting in pulmonary emphysema; the hepatic accumulation of the abnormal protein can lead to liver disease. The A1AT protein is encoded by the protease inhibitor (Pi) locus located on the long arm of chromosome 14q31-32.3. This locus is highly polymorphic, and so far more than 100 different A1AT isotypes have been identified, which can be detected by iso-electrophoresis of the protein or mutational analysis. The most common M allele results in a functional normal protein with normal serum A1AT levels. Two frequent variants p.glu342lys (denoted as Z allele) and the p.glu264val (commonly referred to as the S allele) are encountered in Western Europe.2 The point mutation found in the Z variant destabilizes the loop-sheet polymerization3 of the A1AT molecule, resulting in chains of polymers that are retained in the liver cells.4 In Pi ZZ homozygous subjects, this results in a severe deficiency of serum A1AT and in accumulation of the abnormal protein in the endoplasmatic reticulum of the hepatocytes, which may lead to symptomatic liver disease.5 The S variant of the A1AT molecule has less deleterious effect on the protein. Consequently liver disease is uncommon, and only seen in SZ patients.6,7 A1AT deficiency type Pi ZZ is the most common genetic cause of liver disease in early childhood. Symptomatic patients generally present with neonatal cholestasis, or less commonly later in childhood with hepatosplenomegaly or cirrhosis.6,8,9 In Sweden 200,000 neonates were screened for A1AT deficiency; 120 patients with Pi ZZ were identified but only 22 of these children presented with neonatal cholestasis.10 After 4 years follow-up 4 patients had died (18%); 3 patients due to their liver disease and 1 patient of aplastic anemia. This patient had cirrhosis at autopsy.11-13 However in other studies up to 50% of the patients with A1AT deficiency related neonatal cholestasis either died or had to be transplanted.14-18 Because these studies were mainly done in transplantation centres, they may not be representative for the natural course of A1AT deficiency related neonatal cholestasis.
Therefore we set out to study the natural course of liver disease due to A1AT deficiency in all children presenting with neonatal cholestasis in the Netherlands born between January 1991 and December 2006.

**Methods**

Patients were included when they were born between January 1st, 1991 and December 31st, 2006 and had presented with neonatal cholestasis due to A1AT deficiency. Patients were identified by searching the databases of the 5 centers in the Netherlands where phenotyping or genotyping for A1AT deficiency is routinely performed. Files of all patients, that had genotyping and/or phenotyping done within the first three months of life were reviewed by contacting the referring physician. Subsequently, a clinical database was established that contained the following data: date of birth, sex, age at presentation, birth weight, presenting symptoms, A1AT-phenotype, duration of follow-up with biochemical markers and clinical outcome.

**Results**

**Patients**

A total of 56 children who were diagnosed with a Pi ZZ of Pi SZ phenotype were analyzed; 4 children were sibs of known patients and had no evidence of neonatal cholestasis; they were excluded from further analysis. Therefore 52 patients met the criteria for inclusion in this study. Two of these patients, for whom clinical and biochemical data at presentation and follow-up were unavailable, had to be excluded. Our cohort therefore consists of 50 patients (32 boys, 18 girls). Pi ZZ phenotype was found in 49 children; one child had a Pi SZ phenotype.

**Presentation**

The median age at presentation was 5 weeks (range 0–12 weeks). Median birth weight was 3205 grams (n=43, range: 1675–4595 grams). The initial presentation within the group of 50 patients, was jaundice in 26 patients, 9 children presented initially with failure to thrive, which could subsequently be attributed to malabsorption due to cholestasis, while 15 patients presented with clinical signs of vitamin K dependent hemorrhagic disease. Intracranial hemorrhage was the presenting symptom in 7/15 children. Base-line patient and laboratory characteristics are outlined in Table 3.1.

**Overall outcome**

Median follow-up time was 6.1 years (range: 1 month – 15 years). In this group of 50 patients 5 neonates died (10%). Two boys died as a result of decompensated liver failure at an age of 26 weeks and 49 weeks respectively. One boy died at an age of 26 months after screening for living related transplantation which was refused by his parents. Furthermore, 1 boy, born in 2005, died at an age of 6 weeks as a result of massive intracranial hemorrhage. Lastly, 1 girl, born in 1991, died at an age of 7 months after reconstruction of an atrio-ventricular septum defect; she developed extensive cardiac and respiratory complications. Given the unrelated cause of death this patient was excluded from further outcome analysis. Two girls (4%) underwent orthotopic liver transplantation (OLT) at age 1 respectively 7 years. After transplantation these children had no evidence for liver disease. At last follow-up six patients had clinical or histological signs of cirrhosis, but were clinically stable; laboratory data showed normal synthetic capacity of the liver. Of the remaining 37 children, 6 patients had elevated liver enzymes with hepatomegaly without splenomegaly and

| Table 3.1: Baseline characteristics in 50 neonates with alpha-1 antitrypsin deficiency |
|------------------------------------------|--------|------------------|------------------|
| Total number of patients                | 50     |
| Male/female                             | 32/18  |
| Median age at presentation in weeks (range) | 5 (0-12) |
| Median AST (range) (n<45 IU/l)          | 89 (17-2106) |
| Median ALT (range) (n<45 IU/l)          | 54 (14-1490) |
| Median GGT (range) (n<45 IU/l)          | 457 (80-2526) |
| Median total bilirubin (range) (n<17 µmol/l) | 106 (18-490) |

*Baseline characteristics and biochemical results of 50 patients with neonatal cholestasis due to A1AT deficiency.*
Several studies have reported the outcome of liver disease in children with A1AT deficiency. Most of these studies were done in transplantation centers, suggesting that up to 50% of this group has to be transplanted, with a substantial proportion of cirrhosis in the remainder.  

**Discussion**

Several studies have reported the outcome of liver disease in children with A1AT deficiency. Most of these studies were done in transplantation centers, suggesting that up to 50% of this group has to be transplanted, with a substantial proportion of cirrhosis in the remainder.

**5-years and 10-years follow-up cohorts**

Five-years outcome could be analyzed in 32 children born before 2001. In this group 3 children died and 1 child underwent liver transplantation; consequently, 28/32 (87%) survived without liver transplantation. A total of 22 out of these 28 patients had elevated liver enzymes, of whom 4 had clinical signs of cirrhosis; the remaining 6 children had normal liver enzymes. Ten-years outcome could be analyzed in 15 children born before 1996. In this group 2 children died and 6 children had normal liver enzymes. Ten-years outcome could be analyzed in 22 out of these 28 children born before 2001. In this group 5 children died and 5 children had normal liver function tests. Our data are compared with the data as could be retrieved from the Sveger studies.  

**Table 3.2**

<table>
<thead>
<tr>
<th>Follow-up</th>
<th>Normal liver enzymes</th>
<th>Elevated ALT</th>
<th>Cirrhosis</th>
<th>OLT or death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sveger 4-year follow-up</td>
<td>4/10 (40%)</td>
<td>12/16 (75%)</td>
<td>2/18 (11%)</td>
<td>4/12 (21%)</td>
</tr>
<tr>
<td>Present study 5-year follow-up</td>
<td>6/28 (21%)</td>
<td>22/28 (79%)</td>
<td>4/28 (14%)</td>
<td>4/32 (13%)</td>
</tr>
<tr>
<td>Sveger 8-year follow-up</td>
<td>4/11 (37%)</td>
<td>7/11 (63%)</td>
<td>0/11 (0%)</td>
<td>6/22 (29%)</td>
</tr>
<tr>
<td>Present study 10-year follow-up</td>
<td>5/11 (45%)</td>
<td>6/11 (55%)</td>
<td>0/11 (0%)</td>
<td>4/15 (27%)</td>
</tr>
</tbody>
</table>

Based on our clinical experience in children with symptomatic A1AT deficiency, we had the impression that the majority of patients have a better prognosis. As neonatal cholestasis is by far the most common presentation of A1AT deficiency we set out to identify all children with this presenting symptom and born in the Netherlands from 1991-2006. Survival without liver transplantation was 87% in the 5-years follow-up cohort and 73% in the 10-years follow-up cohort. The studies initiated by Sveger more than 30 years ago, showed a similar outcome. (Table 3.2) The data of these two studies, both without obvious selection bias, are in contrast with data from transplantation centers which generally show a worse prognosis. An overview of these studies and a summary of our overall results is given. (Table 3.3)

Table 3.3: Prognosis of neonatal cholestasis due to A1AT deficiency. Results of several studies coming from transplantation centers and an overview of the results of our present study

<table>
<thead>
<tr>
<th>Study</th>
<th>Number of patients</th>
<th>Median follow-up (years)</th>
<th>Death or transplantation (%)</th>
<th>Cirrhosis (%)</th>
<th>Elevated liver enzymes and/or hepato-splenomegaly (%)</th>
<th>Normal liver function (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psacharopolous (1983)</td>
<td>67</td>
<td>3</td>
<td>19 (28%)</td>
<td>14 (21%)</td>
<td>15 (22%)</td>
<td>25 (30%)</td>
</tr>
<tr>
<td>Grishan (1988)</td>
<td>43</td>
<td>6.8</td>
<td>5 (33%)</td>
<td>3 (20%)</td>
<td>4/15 (27%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Ibarguen (1990)</td>
<td>15</td>
<td>6 (4)</td>
<td>5 (33%)</td>
<td>7 (8%)</td>
<td>6/11 (55%)</td>
<td>6/11 (55%)</td>
</tr>
<tr>
<td>Francavilla (2000)</td>
<td>9</td>
<td>7 (5)</td>
<td>3 (20%)</td>
<td>2 (14%)</td>
<td>7 (65%)</td>
<td>7 (65%)</td>
</tr>
<tr>
<td>Present study (2009)</td>
<td>30</td>
<td></td>
<td>10 (33%)</td>
<td>7 (23%)</td>
<td>12/20 (60%)</td>
<td>15 (28%)</td>
</tr>
</tbody>
</table>

1 Number of children presenting with liver disease due to A1AT deficiency; 50 children presented with neonatal cholestasis; the others presented later in childhood.

In our cohort 15 patients presented with clinical signs of vitamin K deficiency, with 7 having an intracranial hemorrhage. Although it is well known that neonatal cholestasis due to A1AT deficiency is a risk factor for hemorrhagic disease of the newborn, it was thought that this problem could be effectively prevented when giving vitamin K supplements to breastfed infants. However recent evidence suggests that the daily oral vitamin K prophylaxis for breast fed children, as recommended in the Netherlands, has a sub-optimal efficacy, especially in patients with cholestasis. Therefore this vitamin K prophylaxis schedule is now being revised. Only a minority of patients with
A1AT deficiency presents with neonatal cholestasis. Similarly, within this group, only a subgroup will progress to severe liver disease. It is tempting to speculate that, apart from environmental factors, modifier genes might be responsible for this difference. In fact a recent study showed an association between a functional SNP in the A1AT gene and liver disease. We conclude that the majority of patients presenting with neonatal cholestasis due to A1AT deficiency remains symptom free or only has elevated serum transaminases. Nevertheless, a significant proportion of patients develops cirrhosis, sometimes with end-stage liver disease; in these patients liver transplantation may be necessary.

References

Chapter 4

Vitamin K deficiency bleeding in Cholestatic Infants with Alpha-1 Antitrypsin Deficiency

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Abstract

Objective: Exclusively breast-fed infants with unrecognized cholestatic jaundice are at high risk of a vitamin K deficiency (VKD) bleeding. It is presently unknown whether this risk depends on the degree of cholestasis. Since alpha-1-antitrypsin deficiency (A1AT deficiency) induces a variable degree of cholestasis, we assessed the risk of VKD bleeding in infants with cholestatic jaundice due to A1AT deficiency.

Patients and methods: Infants with a ZZ or SZ phenotype born in the Netherlands between January 1991 and December 2006 were identified from the databases of the 5 Dutch diagnostic centres for alpha-1-antitrypsin phenotyping and/or genotyping. We determined the risk of VKD bleeding upon diagnosis of A1AT deficiency in breastfed and formula-fed infants and searched for correlations between serum levels of conjugated bilirubin and the risk of bleeding. Results: A total of 40 infants with A1ATD were studied. VKD bleeding was noted in 15/20 (75%) of breast-fed infants, compared with 0/20 of formula-fed infants with A1AT deficiency. The relative risk for VKD bleeding in breast-fed versus formula-fed infants was at least 15.8 (95% confidence interval 2.3 to 108). Conjugated bilirubin levels at diagnosis did not correlate with the risk of VKD bleeding.

Conclusions: The risk of VKD bleeding in breast-fed infants with A1AT deficiency was high and did not correlate with serum level of conjugated bilirubin at diagnosis. A similar absolute risk was previously reported in breast-fed infants with biliary atresia under the same prophylactic regimen. This confirms that – without adequate prophylaxis – the risk of VKD bleeding is uniformly high in exclusively breast-fed infants with cholestatic jaundice.

Introduction

Vitamin K prophylaxis aims to protect infants against the potentially life threatening coagulation disorder that results from severe vitamin K deficiency (VKD). In breastfed infants VKD bleeding may occur within days of birth due to the low vitamin K stores at birth, the short half life of vitamin K and the low vitamin K content of human milk. An adequate dose of vitamin K given orally or by injection at birth effectively prevents VKD bleeding in the first week of life. However, ongoing vitamin K supplementation is necessary to prevent VKD bleeding after the first week of life (late VKD bleeding) in breastfed babies having oral prophylaxis. Even with these regimens some infants do develop late VKD bleeding, usually due to unrecognized cholestasis causing vitamin K malabsorption. As late VKD bleeding is associated with intracranial haemorrhage (ICH) – resulting in significant mortality and long term morbidity – in approximately 50% of cases, it is especially important to prevent this complication. We recently reported that – despite routine vitamin K supplementation – the risk of VKD bleeding in Dutch breastfed infants with biliary atresia (BA) is approximately 80 percent. VKD bleeding has also been described in infants with various etiologies of neonatal cholestasis, but it is unknown whether the risk of VKD bleeding in these diseases is equally high. In these other causes of cholestasis (and contrary to BA) obstruction of bile flow is generally incomplete, allowing some bile to enter the duodenal lumen. Partial obstruction of bile flow is associated with lower degrees of conjugated bilirubin. Theoretically, residual bile flow could aid vitamin K absorption, and reduce the risk of VKD bleeding. Alpha-1-antitrypsin (A1AT) deficiency – the next most frequent cause of cholestatic jaundice in infancy after BA – is associated with varying degrees of liver involvement. Cholestatic jaundice is present in only approximately 1 in 10 infants with a ZZ phenotype and is even less frequent in patients with an SZ phenotype. Moreover, the degree of cholestasis in symptomatic infants can vary from mild to severe. Thus, A1AT deficiency induced cholestatic liver disease represents an attractive model to study the influence of varying degrees of cholestasis on the development of VKD bleeding at initial presentation. The objectives of this study were 1) to determine the absolute and relative risk of VKD bleeding at initial presentation of cholestatic jaundice induced by A1AT deficiency in exclusively breastfed versus formula fed infants and 2) to investigate whether varying degrees of jaundice are associated with a different risk of bleeding.
Methods

Study population
Dutch patients born from January 1991 until December 2006 presenting with neonatal cholestasis due to A1AT deficiency were identified from the databases of the 5 centres in the Netherlands involved in A1AT genotyping or phenotyping. Patients were provisionally included if a ZZ or SZ genotype or phenotype was generated within the first 6 months after birth. Subsequently, patient files were reviewed at the hospital where the patients originated to obtain relevant demographic data and clinical characteristics. The same exclusion criteria were used as described previously for BA. Briefly, exclusion criteria were:
1) gestational age less than 37 weeks, 2) birth weight below 2000 grams, 3) absence of cholestasis (with cholestasis defined as a total serum bilirubin concentration above 50 μmol/L, with a conjugated fraction of at least 20 percent). External Quality Assessment is in place throughout the Netherlands for this measurement, 4) a diagnosis of cholestasis before age 8 days. An additional exclusion criterion was the presence of an older sib with cholesstatic jaundice due to A1AT deficiency, as this should affect the advised prophylactic regimen. Infants were categorized as “breast-fed” if they had received exclusively breast feeding from birth onwards. All other infants were categorized as “formula-fed”.

Vitamin K prophylaxis
Since 1990 all infants born in the Netherlands receive an oral dose of 1 mg vitamin K directly after birth. Upon breastfeeding, parents are advised to give their child a daily oral dose of 25 μg vitamin K from the second week of life until the end of the 13th week. For daily dosing of vitamin K, a dietary supplement is used in which vitamin K is solved in arachid oil. The vitamin K prophylaxis can be stopped earlier if breastfeeding accounts for less than fifty percent of the daily intake. Formula-fed infants only receive vitamin K prophylaxis directly after birth. Thereafter, they are expected to receive sufficient amounts of vitamin K, since the formula feedings commercially available in the Netherlands contain approximately 50 μg vitamin K per litre.

Vitamin K deficiency
We calculated the prothrombin ratio (PR) at initial diagnosis to be able to compare coagulation parameters from different hospitals, and used it to assess the presence of VKD. In case of a prothrombin time (PT) in seconds the PR was determined as follows: PR = PTpatient / PTcontrol. If a PTcontrol was not determined by the laboratory, it was defined as the mean of the provided reference range. If not available, the International Normalized Ratio (INR) was used as PR. VKD was defined as a PR above 1.5 in line with our previous study. VKD bleeding was defined as bruising, bleeding or ICH in combination with a PR of more than 4 in any infant between eight days and six months of age and normalizing after administration of vitamin K. A diagnosis of ICH was made only if there was radiological confirmation. Clinical signs suggesting ICH (high pitched cry, irritable, convulsions) but without radiological confirmation were also listed, but alone were not taken as diagnostic. Life threatening VKD bleeding was defined as either an ICH or isolated respiratory and/or circulatory insufficiency.

Statistical analysis
We performed a student t-test for clinical and biochemical parameters with a normal distribution pattern to test for statistical differences between groups. Kruskal-Wallis analysis was used for parameters with a non-normal distribution. A Chi Square test was performed to determine statistical significance between groups in case of dichotomous parameters. The relative risks and 95% confidence intervals (CI) for VKD bleeding and biochemical levels of VKD were calculated. Since computation of a RR requires at least one positive case in each quadrant a dummy case was added if necessary to be able to compute a (minimal) relative risk. Approval for the study was obtained from the University Medical Center Utrecht ethics committee.

Results
Between January 1991 and December 2006, 56 patients with A1AT deficiency were added to the Dutch A1AT deficiency registry. Fifty-five infants were ZZ, one was SZ. Sixteen infants did not meet the inclusion criteria for this study,
as described in detail in table 4.1. Twenty (50%) of the remaining 40 infants were exclusively breast-fed. Infants were categorized according to the type of prophylaxis they received. Table 4.2 summarizes the clinical characteristics of breast-fed infants on daily oral prophylaxis and of formula-fed infants.

### Table 4.1: Patients and population

| Live births | 3,530,583 |
| No. of documented cases | 56 |
| Incidence of documented cases | 1.6/100,000 |

**Excluded**
- Positive family history, no cholestasis: 5
- Positive family history, cholestasis: 2
- Gestational age<37 weeks: 3
- Diagnosed prior to 1st week: 4
- Unknown feeding type: 2

**Included**
- Breast-fed: 20
- Formula-fed: 20

*derived from the central bureau of statistics [http://statline.cbs.nl]*

### Table 4.2: Patient characteristics

<table>
<thead>
<tr>
<th>Total</th>
<th>Breast-fed +25ug vitamin K daily oral n=20</th>
<th>Formula-fed n=20</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex-no. (%)</td>
<td>28 (70)</td>
<td>16 (80)</td>
<td>12 (60)</td>
</tr>
<tr>
<td>Birthweight-g*</td>
<td>3285±548</td>
<td>3388±523</td>
<td>3193±564</td>
</tr>
<tr>
<td>Age at diagnosis-days§</td>
<td>35 (28-47)</td>
<td>31 (27-42)</td>
<td>39 (30-60)</td>
</tr>
<tr>
<td>Weight at diagnosis-g*</td>
<td>3907±660</td>
<td>4134±736</td>
<td>3660±483</td>
</tr>
<tr>
<td>Bilirubin total-umol/l*</td>
<td>170±141</td>
<td>104±134</td>
<td>177±65</td>
</tr>
<tr>
<td>Bilirubin direct-umol/l*</td>
<td>69±32</td>
<td>63±25</td>
<td>74±38</td>
</tr>
<tr>
<td>ASAT-U/l§</td>
<td>98 (78-141)</td>
<td>95 (83-138)</td>
<td>100 (82-141)</td>
</tr>
<tr>
<td>ALAT-U/l§</td>
<td>57 (39-83)</td>
<td>56 (31-72)</td>
<td>61 (43-108)</td>
</tr>
<tr>
<td>GGT-U/l§</td>
<td>556 (315-874)</td>
<td>567 (320-871)</td>
<td>584 (332-918)</td>
</tr>
</tbody>
</table>

---

**Vitamin K deficiency in A1AT deficient infants at initial presentation**

VKD was evident in 16 of 20 (80%) exclusively breast-fed infants with A1AT deficiency and was associated with VKD bleeding in 15/20 (75%). Table 4.3 The PR in patients presenting with VKD bleeding was above the upper limit of quantification in 13/15 cases. Table 4.4 Life threatening consequences were found in 8/20 (40%) infants. One infant presented with respiratory insufficiency without documented ICH. In seven infants an ICH was documented and one of these infants died as a consequence. Additionally, two infants had symptoms suggestive of an ICH (convulsions in 1, irritability in 1), without radiological confirmation.

### Table 4.3: Risk of vitamin K deficiency in breast-fed and formula-fed infants with alpha-1 antitrypsin deficiency.

<table>
<thead>
<tr>
<th>Vitamin K deficiency bleeding</th>
<th>Breastfed +25ug vit K daily oral</th>
<th>Formula fed</th>
<th>P-value</th>
<th>Breastfed +25ug vit K daily oral vs formula fed</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR&gt;1.5 and PR&gt;4.0</td>
<td>16/20 (80%)</td>
<td>1/20 (5%)</td>
<td>&lt;0.001</td>
<td>16.0</td>
</tr>
<tr>
<td>Vitamin K deficiency bleeding</td>
<td>15/20 (75%)</td>
<td>0/20 (0%)</td>
<td>&lt;0.001</td>
<td>15.8*</td>
</tr>
<tr>
<td>Intracranial haemorrhage</td>
<td>7/20 (35%)</td>
<td>0/20 (0%)</td>
<td>0.008</td>
<td>7.4*</td>
</tr>
</tbody>
</table>

*Data are number/total number (%). The p value was determined with Fisher exact. + defined by PR>1.5 and PR>4.0 for severe VKD and normal thrombocyte count. * a positive dummy was added in the formula group to compute a (minimal) RR.

---

The risk of (severe) VKD was significantly lower in infants receiving formula feeding (p<0.002). None of the infants receiving formula presented with a VKD bleeding, and only one had a (mild) VKD. The minimal relative risk for VKD bleeding in breast-fed versus formula-fed infants was 15.8 (95% CI 2.3-108). This calculation was performed after adding a positive dummy, based on the assumption that the next included patient would have received formula and presented with a VKD bleeding. Breast-fed infants presented earlier than formula-fed infants, although this was not statistically significant (table 4.2). This is in line with our previous study of BA infants.11 This indicates that the high rate of VKD bleeding in breast-fed infants with A1AT deficiency accelerated recognition of cholestasis, and argues against delayed recognition as a cause for the higher risk of bleeding.

**Cholestatic jaundice and the risk of Vitamin K deficiency bleeding**

Mean total and conjugated bilirubin levels in the group of 20 breast-fed infants, as shown in table 4.2, were 104 (± 34) μmol/L and 63 (± 25) μmol/L, respectively and did not correlate with the risk of VKD or VKD bleeding.
Instead, total bilirubin levels in breast-fed infants presenting with an ICH (n=7) were significantly lower than in infants without ICH (n=13), (80 vs 116 μmol/l, p=0.02).

### Table 4.4: Infants with A1AT deficiency presenting with biochemical vitamin K deficiency.

<table>
<thead>
<tr>
<th>gender</th>
<th>Age (days)</th>
<th>bilirubin (conjugated/total)</th>
<th>PR</th>
<th>APTT (sec)</th>
<th>Normalisation after vit. K (PR if available)</th>
<th>ICH</th>
<th>other bleeding sites</th>
<th>Neurological outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>29</td>
<td>52/96</td>
<td>6.2</td>
<td>86</td>
<td>1.1</td>
<td>+</td>
<td></td>
<td>normal up to 1 yr</td>
</tr>
<tr>
<td>V</td>
<td>41</td>
<td>56/73</td>
<td>&gt;10</td>
<td>&gt;240</td>
<td>1.4</td>
<td>+</td>
<td></td>
<td>epilepsy, hematemesis, melena, umbilical, melena</td>
</tr>
<tr>
<td>M</td>
<td>35</td>
<td>86/106</td>
<td>&gt;10</td>
<td>&gt;300</td>
<td>1.0</td>
<td>+</td>
<td></td>
<td>hematemesis, melena, umbilical, melena</td>
</tr>
<tr>
<td>M</td>
<td>20</td>
<td>54/79</td>
<td>&gt;10</td>
<td>&gt;100</td>
<td>+</td>
<td>+</td>
<td>bleeding after vena puncture</td>
<td>epilepsy, hematemesis, melena</td>
</tr>
<tr>
<td>M</td>
<td>31</td>
<td>45/81</td>
<td>&gt;10</td>
<td>&gt;100</td>
<td>0.9</td>
<td>+</td>
<td>bleeding after vena puncture</td>
<td>hematemesis, melena</td>
</tr>
<tr>
<td>M</td>
<td>21</td>
<td>52/70</td>
<td>&gt;10</td>
<td>&gt;240</td>
<td>0.9</td>
<td>+</td>
<td>bleeding after vena puncture</td>
<td>neurological coma, died</td>
</tr>
<tr>
<td>V</td>
<td>37</td>
<td>30/54</td>
<td>&gt;10</td>
<td>173</td>
<td>1.5</td>
<td>+</td>
<td></td>
<td>umbilical, melena</td>
</tr>
<tr>
<td>M</td>
<td>28</td>
<td>69/112</td>
<td>&gt;10</td>
<td>&gt;240</td>
<td>1.1</td>
<td>+/-</td>
<td>irritable hematomas</td>
<td>normal</td>
</tr>
<tr>
<td>M</td>
<td>37</td>
<td>90/100</td>
<td>&gt;10</td>
<td>&gt;200</td>
<td>+</td>
<td>2,</td>
<td>convulsion bleeding after vena puncture</td>
<td>normal</td>
</tr>
<tr>
<td>M</td>
<td>27</td>
<td>30/59</td>
<td>&gt;10</td>
<td>&gt;150</td>
<td>1.1</td>
<td>+</td>
<td>bleeding after vena puncture</td>
<td>normal</td>
</tr>
<tr>
<td>M</td>
<td>13</td>
<td>36/84</td>
<td>6.4</td>
<td>170</td>
<td>1.5</td>
<td>-</td>
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<td>normal</td>
</tr>
<tr>
<td>M</td>
<td>47</td>
<td>31/110</td>
<td>&gt;10</td>
<td>&gt;150</td>
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<td>-</td>
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<tr>
<td>M</td>
<td>47</td>
<td>132/174</td>
<td>&gt;10</td>
<td>181</td>
<td>1.3</td>
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<td>85/141</td>
<td>&gt;10</td>
<td>&gt;180</td>
<td>1.0</td>
<td>-</td>
<td></td>
<td>normal</td>
</tr>
<tr>
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<td>50</td>
<td>61/127</td>
<td>&gt;10</td>
<td>171</td>
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</tr>
<tr>
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<td>1.8</td>
<td>49</td>
<td>#</td>
<td></td>
<td></td>
<td>normal</td>
</tr>
</tbody>
</table>

* Formula fed; # isolated reduction of vitamin K-dependent coagulation factors; $ respiratory insufficiency.

### Discussion

The results of this study indicate that exclusively breast-fed infants with A1AT deficiency have a high risk of developing a VKD bleeding before recognition of cholestatic jaundice under the present Dutch vitamin K prophylaxis. Of breast-fed infants, 75% presented with a VKD bleeding and 35% presented with an ICH. No VKD bleeding was documented in (partially) formula-fed infants. These findings extend our previous observations in infants with BA to a disease entity with a milder and more variable degree of conjugated hyperbilirubinemia. To our knowledge this is the first nation-wide study examining the risk of VKD bleeding in infants with A1AT deficiency. Based on the premise that A1AT phenotyping is a regular part of the workup of cholestatic jaundice in infancy we investigated the databases from the diagnostic centers performing A1AT phenotyping in the Netherlands. Out of a birth cohort of 3,138,576 infants 55 cases of Pi ZZ (and one with Pi SZ) were identified. Fifty of the Pi ZZ infants had documented cholestatic jaundice, amounting to an incidence of 1.6/100,000. This incidence is very similar to the expected incidence of 1.3/100,000, which is based on the estimated incidence of Pi ZZ in the Netherlands (1:9536) and the previously documented risk of developing cholestatic jaundice in infancy in case of Pi ZZ phenotype of 12%.

Since we aimed to estimate the risk of late VKD bleeding under the current Dutch prophylaxis we carefully excluded infants who may have received additional vitamin K, such as premature infants. Another source of misclassification may originate from the assumption that all breast-fed infants may have received the prophylaxis according to national guidelines, unless otherwise stated in patient files. However, data from other European countries indicate that adherence to a prophylactic regimen executed by parents is approximately 85%. In the group of breast-fed infants with A1AT deficiency, total and conjugated bilirubin levels (104/63 μmol/L) were significantly lower than those in breast-fed babies with BA in the Netherlands (147/98 μmol/L, p<0.001). Nevertheless, the risk of developing VKD bleeding was similar in the two groups (75 and 83%, respectively, p=0.49), as was the age at presentation (35 and 36 days, respectively). No association was found in the present study between the degree of (conjugated) hyperbilirubinemia and the risk of VKD bleeding. These findings
suggest that infants with cholestatic jaundice are at a similarly high risk of VKD bleeding, regardless of its degree. Although counter-intuitive, this notion is supported by other lines of evidence. First, VKD bleeding has been repeatedly reported in infants with minimal cholestatic jaundice (total bilirubin 21-62 μmol/L).20-23 Second, in a Japanese study investigating the incidence of subclinical VKD, highly elevated PIVKA-II levels were found in 8/22,000 infants at age 4 weeks, all of whom were breast-fed and had moderately elevated conjugated bilirubin levels (range 17-45 μmol/l).24 The incidence of cholestatic jaundice found in this study was 1/2750, similar to the reported incidence of (transitory) cholestasis in infancy25, suggesting that nearly all breast-fed infants with some degree of cholestatic jaundice developed coagulation abnormalities. It could be argued that infants with underlying cholestatic liver disease should be protected against VKD bleeding by early diagnosis and appropriate treatment, rather than by routine prophylaxis. Indeed, 2/7 cases of ICH in this report could have been prevented if doctors had rightly regarded the harmless-looking (warning) bleeds as a medical emergency and reacted with prompt parenteral administration of vitamin K. Moreover, all infants with AAT deficiency that developed VKD bleeding had bilirubin levels that are recognisable on physical examination, suggesting that earlier recognition of jaundice could help prevent VKD bleeding. However, various attempts aimed at increasing awareness and early recognition of cholestatic jaundice by parents26 and health professionals27,28 had only a modest effect on the age at diagnosis.

It has been recently suggested that the decreasing incidence of VKD bleeding in the UK is due to increased awareness of the importance of jaundice beyond two weeks of life29, although the effect of this program on the age at presentation of cholestatic jaundice has not (yet) been published. Our data in infants with AAT deficiency and BA11 indicate that the age at diagnosis would have to be reduced by approximately 3 weeks to prevent all bleedings. Theoretically, newborn screening would be an attractive option, but quantification of serum bile acids failed to separate infants with cholestatic jaundice from healthy infants.30 Therefore, under the present circumstances adequate routine vitamin K prophylaxis seems to be the most reliable way to protect all infants with unrecognized cholestasis. In previous studies vitamin K prophylaxis was shown not only to reduce the risk of VKD bleeding but also to postpone its occurrence.5,10,29 Interestingly, the age at presentation in the cohort described here is similar to the age reported in infants who received only a single oral dose at birth. Therefore, it is doubtful whether the daily oral administration of 25 microgram vitamin K in the first 3 months of life, as is customary in the Netherlands, had any effect at all in infants at the highest risk of VKD bleeding. If oral prophylaxis is to be continued in the Netherlands it seems prudent to choose a dose and formulation with proven efficacy. However, the formulation which significantly11 protected cholestatic infants is no longer commercially available. As surveillance data indicate that a mixed micellar formulation is equally effective,5,7 we suggest using this.

In conclusion, the absolute risk of VKD bleeding in exclusively breast-fed infants with cholestatic jaundice due to AAT deficiency is similar to that in infants with BA and does not correlate with the degree of conjugated hyperbilirubinemia. These findings suggest that infants with cholestatic jaundice, regardless of etiology, are at a high risk of VKD bleeding under the current Dutch regimen of vitamin K prophylaxis and change is needed. Meanwhile, doctors should be aware that early recognition of cholestatic jaundice followed by prompt treatment with vitamin K can reduce the risk of life threatening VKD bleeding in breast-fed infant.

References


Chapter 5

Influence of Alpha-1 Antitrypsin Heterozygosity on Treatment Efficacy of HCV Combination Therapy

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Abstract

Background: The role of heterozygosity for alpha-1 antitrypsin (A1AT) alleles in patients with chronic hepatitis C virus (HCV) is unclear. There is limited evidence to suggest that there is an increased prevalence of heterozygous A1AT carriers in HCV, but it is unclear how this affects treatment success.

Aim: To investigate the (1) prevalence of A1AT heterozygosity among 2 HCV cohorts and (2) its effect on treatment outcome.

Methods: We performed a retrospective cohort study using 2 different cohorts. Cohort 1 consists of 678 German HCV patients, 507 of them were treated for HCV with standard therapy. Cohort 2 consists of 370 Dutch HCV patients of which 252 HCV patients were part of a clinical trial (treatment with amantadine or placebo, in combination with PEG-interferon alfa-2b and ribavirin) while 37 HCV patients received standard therapy. We analyzed A1AT status using direct sequencing of the A1AT gene (cohort 1) or iso-electrofocusing of serum (cohort 2). In addition we measured A1AT serum levels (cohort 2).

Results: In total we included 1048 HCV patients; 986 (94%) were wildtype (Pi MM), while 61 (6%) were heterozygous for a mutant A1AT allele (41 Pi MS, 20 Pi MZ). Mean A1AT serum levels (370 patients) were lower in A1AT heterozygous patients (1.68 g/l vs. 1.36 g/l), (p<0.05) compared to wildtypes. Sustained viral response (SVR) after treatment was equal between the wildtypes and heterozygotes. (54% vs 56%)

Conclusion: We found a heterozygosity rate of 0.06, in line with healthy controls in other studies. Serum A1AT levels from A1AT heterozygous HCV patients are significantly lower compared to wildtype patients, although they do not discriminate on an individual level. Finally, SVR in A1AT wildtypes was not different from SVR in A1AT heterozygotes.

Introduction

A1AT deficiency is a hereditary disease that is characterized by the hepatic synthesis of an abnormal A1AT protein that cannot be released into the plasma completely. Accumulation of mutant A1AT protein in hepatocytes causes tissue damage and eventually ensues in liver and pulmonary disease. The A1AT protein is encoded by the protease inhibitor (Pi) locus located on the long arm of chromosome 14q31.2. This locus is highly polymorphic, and so far more than 100 different A1AT isotypes have been identified. They can be detected by iso-electrophoresis of the protein or by mutational analysis of the allele. A1AT deficiency is associated with liver and pulmonary disease, but the picture is less clear for A1AT heterozygosity.
In vitro studies showed that A1AT plays a central role in inflammation, both as a regulator of proteinase activity and as a signaling molecule for the expression of pro-inflammatory molecules. Also, A1AT has a role in viral clearance, as it inhibits human immunodeficiency virus type 1 replication. We hypothesize that A1AT heterozygosity puts the host at a higher risk for HCV and decreases viral elimination after treatment because of the decreased anti-inflammatory response. As a consequence, A1AT genotype status might be associated with the infection rate and treatment outcome. Therefore, the aim of our study was (1) to investigate the prevalence of A1AT heterozygosity in a large cohort of Dutch and German HCV patients and to assess (2) its consequence on treatment outcome.

Methods and materials

Patients
We selected two different retrospective cohorts of patients. Cohort 1 consists of 678 German HCV patients; 507 of them were treated for HCV and completed therapy. Cohort 2 consists of 370 Dutch HCV patients; 289 patients of cohort 2 were treated and completed therapy. Patients were included regardless of their degree of liver fibrosis. We did exclude patients with a hepatitis B or HIV co-infection. Our study was approved by the ethical committee.

HCV Treatment
Patients from cohort 1 were treated with interferon monotherapy, a combination of interferon and ribavirin or with PEG-interferon in combination with ribavirin. A total of 252 patients of cohort 2 were part of a nation-wide, double blind, placebo-controlled, randomised multicentre study (CIRA-study). Patients were treated with amantadine or placebo, in all cases combined with weight-based ribavirin and high-dose interferon induction therapy followed by PEG-interferon alfa-2b. The remaining 37 patients (cohort 2) were treated with standard combination therapy. Treatment was given for 24-52 weeks depending on genotype. SVR was defined as a negative qualitative HCV RNA test at 1 year after end of treatment.

Assays
A1AT status was analyzed in all patients. We performed mutational analysis (cohort 1) and iso-electric focusing and nephelometric measurement of A1AT serum levels (cohort 2).

Mutational analysis (Cohort 1)
DNA was extracted from peripheral blood leukocytes. Primer sequences used for polymerase chain reaction (PCR) were as follows: exon 5 (PiS), 5'-GATGAGGGGAAACTACACACGCTCG-3' and 5'-GGGCCTCAGTCCCAACATGG-3' and for exon 7 (PiZ), 5'-GCATAAGGCTGTGCTGACCATCGTC-3' and 5'-AGGGTTTTGTTGAACCTGACCTC-3'. An automated thermal cycler (Biometra, Göttingen, Germany) was used. A1AT heterozygosity was determined using restriction fragment length polymorphism (RFLP). Amplification of the desired products was confirmed by direct DNA sequencing. DNA sequences were analyzed by sequencing both strands. The reaction products were loaded into an ABI 373A fluorescence sequencer (Applied Biosystems, Weiterstadt, Germany).

Iso-electric focusing (Cohort 2)
Iso-electric focusing was performed in serum by iso-electric focusing (Phast-system, GE Healthcare, Munchen, Germany) and subsequent protein silver staining. Patterns were compared to MM, MZ, MS, SZ and ZZ controls. MS and MZ designated patterns were subsequently validated by an iso-electric focusing with an immuno-fixation method in an independent laboratory.

Nephelometric plasma measurement (Cohort 2)
Determination of the serum A1AT level was performed by nephelometry. The A1AT reagent was used from the Immage Immunochemistry system (Immage Beckmann-Coulter, Woerden, The Netherlands), and a calibrator with an assigned A1AT value and a human quality control sample (Biorad, Liquichek, Hercules, CA, USA) were used.

HCV testing
HCV RNA testing was performed qualitatively (Cobas amplicor HCV Monitor Test, version 2.0, detection limit 600 IU/ml, Roche Diagnostics) and quantitatively at
weeks 24, 52 and 104 (Cobas Amplicor HCV test, version 2.0; detection limit 50 IU/ml, Roche Diagnostics). Genotyping was performed by sequence analysis of the 5’ untranslated region of the HCV genome by the Visible Genetics TrueGene Hepatitis C Assay.

Statistical Analyses
The HCV characteristics (genotype) of the wild type patients and heterozygous patients were analyzed using Pearson chi-squared test and Fisher’s exact test where appropriate. The difference in age at start of treatment was analyzed using student t-test. Differences between serum levels of the wild type patients (Pi MM) and heterozygous patients (Pi MS, Pi MZ and Pi SZ) were analyzed using student t-test. We used Pearson chi-squared test for analysis of the differences in SVR between wild types and heterozygous patients and for analysis of the degree of fibrosis in wild types and heterozygotes. All statistical analyses were performed using GraphPad Prism 4 (Version 4.02, GraphPad Software Inc., San Diego, CA, USA). A two-sided p-value of < 0.05 was considered statistically significant.

Results

Genotyping
Genotyping was performed in all 1048 patients. Due to technical difficulties, iso-electro-focussing failed in a single patient, resulting in 1047 complete analyzed patients. We found 986 Pi MM (94%), 41 Pi MS (4%) and 20 Pi MZ (2%) patients. (Figure 5.1) Genotype results were within the in Hardy-Weinberg equilibrium.

Serum levels
A1AT levels were measured in serum from 370 HCV patients from cohort 2 drawn before treatment. A1AT serum levels were higher in Pi MM carriers (340 patients, mean 1.69 g/l, 95%CI 1.66-1.71 g/l) compared to Pi MS carriers (22 patients, mean 1.43 g/l, 95%CI 1.34-1.53 g/l) or Pi MZ carriers (7 patients, mean 1.14 g/l, 95%CI 0.88-1.49 g/l) respectively. The differences between the wild types and heterozygous patients were statistically significant (p<0.01); this also holds for the differences between the heterozygous Pi MS and Pi MZ patients (p<0.01). (Figure 5.2) We detected A1AT serum levels above 1.0 g/l in all heterozygous Pi MS patients and in 5/7 (71%) of the heterozygous Pi MZ patients.
Degree of fibrosis
We assessed the stage of fibrosis using the Metavir score in liver biopsies obtained from 409 patients. There was no statistically significant difference in the degree of fibrosis between wildtypes and heterozygous patients. (table 5.1)

<table>
<thead>
<tr>
<th>Metavir score</th>
<th>Pi MM</th>
<th>Pi MZ and Pi MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>F0 - F2</td>
<td>269 (70)</td>
<td>19 (73)</td>
</tr>
<tr>
<td>F3 - F4</td>
<td>114 (30)</td>
<td>7 (27)</td>
</tr>
</tbody>
</table>

*F0 = no fibrosis; F1 = minimal fibrosis; F2 = periportal fibrosis; F3 = bridging fibrosis; F4 = cirrhosis or advanced fibrosis.

HCV characteristics and treatment response
A total of 796 patients completed HCV treatment. A1AT carrier status and qualitative HCV RNA 1 year after end of treatment were not available for 10 patients (n=1 cohort 1; n=9 cohort 2). These patients were excluded from further analysis; and data from 786 HCV patients remained available for analysis. The distribution of the HCV genotypes at baseline did not differ among the groups with different A1AT genotypes. (Table 5.2)

<table>
<thead>
<tr>
<th>HCV genotype</th>
<th>Pi MM</th>
<th>Pi MZ</th>
<th>Pi MS</th>
<th>Pi SZ</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>450 (60.4)</td>
<td>10 (58.8)</td>
<td>20 (62.5)</td>
<td>0 (0)</td>
<td>p=0.89</td>
</tr>
<tr>
<td>2</td>
<td>56 (7.5)</td>
<td>1 (5.9)</td>
<td>4 (12.5)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>170 (22.7)</td>
<td>5 (29.4)</td>
<td>4 (12.5)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>34 (4.6)</td>
<td>1 (5.9)</td>
<td>2 (6.3)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>4 (0.7)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>unknown</td>
<td>34 (4.6)</td>
<td>0 (0)</td>
<td>2 (6.3)</td>
<td>1 (100)</td>
<td></td>
</tr>
</tbody>
</table>

*Mean age [yrs] | 46.6 | 47.9 | 45.5 | 59 | p=0.93 |

Table 5.2: Distribution of the HCV genotypes and age in the different A1AT states (n=795)

SVR was reached in 395/736 (54%) wild type patients, in 8/17 (47%) A1AT Pi MZ patients and in 20/32 (62%) of A1AT Pi MS patients. One Pi SZ patients did not achieve SVR. These differences were not statistically significant. (Figure 5.3) The addition of amantadine to the combination of interferon and ribavirin in patients from cohort 2 did not affect treatment response.26

Discussion
This study demonstrates that the efficacy of HCV combination treatment with (PEG)-interferon and ribavirin is independent of carrier state of A1AT, as heterozygous (Pi MZ / Pi MS / Pi SZ) HCV patients had a similar treatment response compared to wild type patients (Pi MM). In addition, we did not detect an association between A1AT heterozygosity and the degree of liver fibrosis. We found a high rate of SVR in our study population, this can be explained by the fact that we included only patients who finished treatment with (PEG)-interferon and ribavirin; we did not include patients who stopped treatment for any reason or who were lost to follow-up.
There are no previous studies documenting HCV treatment response in A1AT heterozygosity. Most of the studies in this field have focused on apparent differences in A1AT allele frequency between those with and without a certain liver disease.\textsuperscript{10,11} The common denominator is that a higher prevalence of heterozygotes is found in different causes of liver disease; this is particularly true for HCV and alcoholic liver disease in pre-transplantation patients.\textsuperscript{10,11}

We could not confirm the finding of higher A1AT heterozygosity rates in HCV patients, and our data indicate that the A1AT carrier distribution in HCV is in line with the prevalence of control populations in the Netherlands and Germany.\textsuperscript{3-5} Our results are at odds with an Egyptian study that found higher prevalence of Pi MS in HCV cirrhotic patients compared with normal controls.\textsuperscript{15} This particular A1AT allele is rare in Germany and the Netherlands.\textsuperscript{27} It is tempting to speculate why other case control studies in this field documented differences in A1AT carrier rate between patient and controls. We surmise that there are 3 possible explanations. First, the variation might depend on real population differences. On the other hand, it cannot be excluded that bias in selecting controls and patients might have affected the results. In addition, the low prevalence of patients with heterozygote genotypes might have introduced a type II error giving rise to spurious results. Serum A1AT levels were statistically significantly lower in heterozygous carriers compared to A1AT wild type patients. There was no cut-off point that discriminated between heterozygotes and wild types. In some laboratories in the Netherlands, genotyping is only performed if the serum level is below 1.0 g/l. This strategy will miss obvious cases; in our data set, all Pi MS heterozygotes and 71% of the Pi MZ heterozygotes. Previous studies also indicated the variability of A1AT serum levels in heterozygous carriers.\textsuperscript{28,29} Some case reports document A1AT heterozygotes with concomitant HCV or alcoholic liver disease\textsuperscript{31} who had consistent normal A1AT serum levels but A1AT globules in their liver biopsy specimen. It is possible that the liver inflammation is a driver for A1AT production, hence giving rise to ‘falsely’ elevated A1AT serum levels. Our study was adequately powered to detect differences in treatment outcome and to calculate the prevalence of A1AT heterozygosity in HCV patients in the Netherlands and Germany. Nevertheless, our study comes with several limitations. The lack of a control population does not allow a firm estimate of the allele frequency in relation to the background population.

While A1AT does not play a role in the HCV treatment response, it is possible that there are other important genetic factors. For example, hereditary hemochromatosis is a common genetic liver disease that appears to be over-represented in HCV patients.\textsuperscript{15} Currently, it is not known whether possession of the mutant hereditary hemochromatosis allele affects treatment response. In conclusion: We found no evidence for an increased prevalence of A1AT heterozygosity in HCV patients. Serum A1AT levels from A1AT heterozygous HCV patients are significantly lower compared to wild type patients, however they do not discriminate on an individual level. Finally, we could not confirm our hypothesis that HCV patients with A1AT heterozygosity deficiency had worse outcomes on HCV combination therapy.

References


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Chapter 6

Prevalence of Genetic Polymorphisms in the Promoter Region of the Alpha-1 Antitrypsin (SERPINA1) Gene in Chronic Liver Disease: a Case Control Study

BMC Gastroenterology 2010, 10:22
Abstract

Background: Alpha-1 antitrypsin (A1AT) deficiency, caused by the Z allele (p.E342K) and S allele (p.E264V) in the SERPINA1 gene, can induce liver and pulmonary disease. Different mechanisms appear to be responsible for the pathogenesis of these divergent disease expressions. The c.-1973T>C polymorphism located in the SERPINA1 promoter region is found more frequent in A1AT deficiency patients with liver disease compared to patients with pulmonary disease, but data are lacking regarding contribution to the development of liver diseases caused by other etiologies.

Aim: To study the prevalence of c.-1973T>C, Z allele and S allele in a cohort of patients with liver disease of various etiologies compared with healthy controls and to evaluate its effect on disease progression.

Methods: A total of 297 patients with liver disease from various etiologies and 297 age and gender matched healthy controls were included. The c.-1973T>C polymorphism and Z and S alleles of the SERPINA1 gene were analyzed by real-time PCR.

Results: c.-1973T>C was similarly distributed between patients with liver disease of various origins and healthy controls. Furthermore, the distribution of c.-1973T>C was independent from etiology subgroup. In patients with liver disease mean ages at of onset of liver disease were 44.4, 42.3 and 40.7 years for the c.-1973 T/T, T/C and C/C genotype respectively (NS). S allele heterozygosity was increased in patients with drug induced liver injury (DILI). (OR 4.3; 95%CI 1.1-17.2)

Conclusion: In our study, c.-1973T>C polymorphism was not a risk factor for liver disease of various etiologies. In addition, S allele heterozygosity might contribute to the development of DILI.

Background

Alpha-1 antitrypsin (A1AT) deficiency is a hereditary disease and can induce end-organ damage caused by defective A1AT protein processing. Liver disease in A1AT deficiency is caused by hepatic accumulation of the A1AT protein and pulmonary disease is induced by an impaired protection against neutrophil elastase due to decreased serum A1AT. The A1AT protein is encoded by the protease inhibitor (Pi) locus located on chromosome 14q32.1 (SERPINA1 gene). In Western Europe, A1AT deficiency most commonly results from presence of 2 genetic variants p.E342K (denoted as Z allele) and the p.E264V (commonly referred to as the S allele). Liver disease in A1AT deficiency has a bimodal presentation affecting children in neonatal life and, less commonly, adults in late middle life. It is unclear why A1AT deficiency leads to liver disease in some patients and lung disease in others. It appears that environmental factors are in part responsible for this difference. Pulmonary disease develops preferably in homozygous Pi ZZ persons who are tobacco smokers or are exposed to airway irritants. Indeed, smoking is an established risk factor for lung disease as A1AT deficient smokers will develop emphysema at considerable younger age, while non-smokers are at risk for liver disease developing later in life. Some 32-37% of A1AT deficient non-smoking patients will die as a result of A1AT deficiency induced liver disease. Apart from environmental factors there is some evidence that genetic factors modify the risk for A1AT deficiency related end-organ damage. For example, 72% of siblings of probands with A1AT deficiency related liver disease suffered from liver disease, which was concordant for severity in 29%, while 28% had no liver involvement. This suggests presence of genetic modifiers. Indeed a recent study identified a novel single nucleotide polymorphism (SNP) g.126076T>C (c.-1973T>C; rs8004738) in the promoter region of SERPINA1. It appeared that the SNP was enriched (15.5%) in a cohort of A1AT Pi ZZ homozygotes with liver disease relative to those with pulmonary disease (6.5%). As a result of the above-mentioned observations we hypothesized that c.-1973T>C polymorphism affects susceptibility for the progression of liver disease in patients with liver disease of various etiologies. Therefore we investigated...
the association of c.-1973T>C, p.E342K (Z allele) and p.E264V (S allele) polymorphisms in a cohort of patients with liver disease of various etiologies compared with healthy controls and evaluated its consequence on course of disease.

Methods

Patients

We recruited patients with various liver disorders, visiting the outpatient clinic of the Department of Gastroenterology and Hepatology of the Radboud University Nijmegen Medical Center. In addition, age and gender matched persons who were unrelated to our patients served as healthy controls. In the patient population, clinical and demographic data including age, sex, age at first presentation of liver disease, etiology of liver disease and presence or absence of cirrhosis were obtained. The absence of liver disease in our control population was established on the basis of self-reporting and none of the patients used any medication. Whole blood samples were stored at -20°C. Altogether the study population comprised 297 patients with various etiologies of liver disease and 297 controls. Clinical and demographic data are given. (Table 6.1) The study was approved by the local ethical committee. (Medical Ethical Committee of the Radboud University Nijmegen Medical Center.)

DNA was isolated from peripheral blood using the High Pure PCR Template preparation kit (Roche, Mannheim, Germany). The c.-1973T>C (rs8004738), p.E342K (Z allele; c.1024G>A; rs28929474) and p.E264V (S allele; c.791A>T; rs17580) polymorphisms of the SERPINA1 gene were analyzed by real-time polymerase chain reaction (PCR) using a dual-color, allele-specific discrimination assay with fluorescent labelled probes on the iCycler iQ Multicolour real-time detection system (Bio-Rad Laboratories Inc, Hercules, CA, USA). Primer and probe sequences (Sigma, St Louis, MO, USA) used for PCR and real time detection are listed in the supplementary table. All of the genotyping results in the control population were in the Hardy-Weinberg equilibrium. Haplotypes and diplotype were determined using the Partition-Ligation–Expectation-Maximization (PLEM) 1.0 software.11

Statistical analysis

Baseline characteristics, differences in allele frequency, diplotypes and haplotypes were analyzed using student t-tests, Pearson chi-squared test and Fisher’s exact test where appropriate. Odds Ratio’s (OR) were calculated for the association between the studied polymorphisms and the presence of liver disease. In addition we calculated OR’s for the association between the 3 polymorphisms and all different subgroup etiologies. We analyzed differences in age at onset of liver disease between the 3 different c.-1973 genotypes using ANOVA. All statistical analyses were performed using GraphPad Prism Version 4.02 (GraphPad Software Inc., San Diego, CA, USA). A two-sided p-value of < 0.05 was considered statistically significant. Post-hoc power calculation showed the study being adequate powered (88%) to negate our hypothesis that c.-1973T>C is associated with liver disease of various etiologies (α= 0.05, difference in group proportions 10% (20% and 30%)). Power calculations were performed using nQuery Advisor 4.0 software (Statistical Solutions Ltd, Cork, Ireland). Linkage disequilibrium (LD) values were performed with Haploview 4.0 software.
Results

c.-1973T>C polymorphism

The c.-1973T>C polymorphism distribution in 297 patients with liver disease due to various etiologies was in line with 297 healthy controls (c.-1973: T/T 32%, T/C 44% and C/C 24% in patients and T/T 30%, T/C 50% and C/C 20% in controls). (Figure 6.1) We found no association of the c.-1973T>C polymorphism with any of the distinct liver diseases investigated. Next, we observed that mean age at onset of liver disease was non-significantly lower in patients with the c.-1973 C/C allele, as mean ages of onset of liver disease were 44.4 (T/T), 42.3 (T/C) and 40.7 (C/C) years. (Figure 6.2) Further, possession of c.-1973T>C polymorphism had no influence on the presence of cirrhosis.

Z (p.E342K) allele and S (p.E264V) allele heterozygosity

We observed a similar Z allele and S allele heterozygosity rate in patients and controls (Z allele: 3.0% and 4.7%; S allele: 6.7% and 8.0%). The distribution of Z allele heterozygosity was similar among all liver diseases of various etiologies. In contrast, S allele heterozygosity was more frequently present in drug induced liver injury (DILI) compared with healthy controls (11 patients, 27% vs 8%; OR 4.27; 95%CI 1.06-17.15). A total of 8 wildtype patients developed DILI likely due to clavulanic acid (n=3), azathioprine, sulfasalazine, pantoprazole, methotrexate and quetiapine; additionally, the S allele heterozygotes had DILI caused by anaesthetic compounds (isoflurane/nestanol), clavulanic acid and celecoxib. Lastly, age at onset of liver disease was independent of Z or S allele heterozygosity as mean ages were 48.3 (Z allele), 43.2 (S allele) and 42.4 (wildtypes) years.

Haplotyping / diplotyping

Based on the 3 polymorphisms tested, haplotype and diplotype analysis were performed. A total of 11 diplotypes could be distinguished. We could not detect differences in diplotypes between patients with liver disease of various etiologies and controls. Only the CTG haplotype was more frequent found in patients with DILI, according to the above-described association between S allele heterozygosity and DILI. (Table 6.2) Finally, linkage disequilibrium (LD) between the 3 SERPINA1 alleles was absent. This was true for patients as well as controls. (Figure 6.3)

Table 6.2: Diplotypes in patients with liver disease of various etiology and healthy controls

<table>
<thead>
<tr>
<th>Diplotype</th>
<th>Controls (%) n=297</th>
<th>Patients (%) n=297</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAG/CAG</td>
<td>130 (43.8)</td>
<td>112 (37.7)</td>
</tr>
<tr>
<td>TAG/TAG</td>
<td>78 (26.3)</td>
<td>93 (31.3)</td>
</tr>
<tr>
<td>CAG/CAG</td>
<td>51 (17.2)</td>
<td>63 (21.2)</td>
</tr>
<tr>
<td>CTG/TAG</td>
<td>11 (3.7)</td>
<td>12 (4.0)</td>
</tr>
<tr>
<td>CAG/CTG</td>
<td>8 (2.7)</td>
<td>7 (2.4)</td>
</tr>
<tr>
<td>TAG/TAA</td>
<td>8 (2.7)</td>
<td>2 (0.7)</td>
</tr>
<tr>
<td>CAG/TAA</td>
<td>5 (1.7)</td>
<td>6 (2.0)</td>
</tr>
<tr>
<td>TAG/TG</td>
<td>4 (1.3)</td>
<td>1 (0.3)</td>
</tr>
<tr>
<td>CTG/TAA</td>
<td>1 (0.3)</td>
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<tr>
<td>CAG/CTG</td>
<td>1 (0.3)</td>
<td></td>
</tr>
<tr>
<td>CAG/CAA</td>
<td>1 (0.3)</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

We show that the distribution of SERPINA1 $c.-1973T>C$ in patients with liver disease from various etiologies is similar compared to healthy controls. The genotype frequencies in the patient and control groups were in line with those from the HapMap database ($T/T$ 30%, $T/C$ 45% and $C/C$ 25%). Moreover, we found that the distribution of $c.-1973T>C$ is independent from etiology of the liver disease. We also examined whether the $c.-1973T>C$ polymorphism affected age at onset of liver disease. We found a non-significant lower age at onset in patients with the $c.-1973 C/C$ genotype compared to other $c.-1973$ genotypes. In addition, we could not demonstrate an association of $c.-1973T>C$ on severity of liver disease, e.g. cirrhosis. We also investigated other SERPINA1 variants and found that there was no increased prevalence of Z and S alleles in patients with liver disease of various etiologies compared with healthy controls, even though we observed an enrichment of S allele heterozygosity in patients with DILI.

Other investigators have studied the presence of $c.-1973T>C$ and A1AT deficiency and chronic obstructive pulmonary disease (COPD). Chappell et al. reported an enrichment of $c.-1973T>C$ in homozygous Pi ZZ neonates with hepatitis (15.5%) compared to homozygous Pi ZZ controls (adults with COPD and unaffected subjects) (6.5%). Since our study population consisted for a large extent of patients and controls with the Pi MM genotype (wildtypes) and hardly any subjects with Z and S allele heterozygosity, we cannot compare our data with the results of the above mentioned study. Another study showed a decreased prevalence of $c.-1973T>C$ in patients with COPD (48.7%) compared to controls (52.2%), suggesting a protective effect against COPD. It might be possible that $c.-1973T>C$ influences the genesis of liver disease in childhood, in line with a recently published report implicating that a variant of the endoplasmic reticulum mannosidase I (ERManI) gene is associated with an early onset of end-stage liver disease in patients with homozygous (Pi ZZ) A1AT deficiency.

Our data were in contrast with other reports as Z and S allele heterozygosity were previously associated with (end-stage) liver disease due to HCV, alcoholic liver disease and cryptogenic cirrhosis and not with DILI. We found a higher frequency of S allele heterozygosity in DILI patients. Indeed, experimental evidence supports a relation between A1AT deficiency and DILI as administration of indomethacin in a homozygous Pi ZZ mouse model leads to increased hepatic injury and a case report described prochlorperazine induced liver injury in a homozygous (Pi ZZ) A1AT deficient patient.

We could not demonstrate an association between $c.-1973T>C$ and the presence of cirrhosis. There have been several genetic case control studies that have attempted to detect associations between genetic variations and liver fibrosis. For example, the combination of angiotensinogen (ATG) gene variant $c.1-44$ and transforming growth factor beta (TGFβ2) p.R25P is associated with advanced hepatic fibrosis in obese patients with non alcoholic fatty liver disease but not in patients with other chronic liver diseases. Lastly, matrix metalloproteinase (MMP)-7(Asp-137) confers risk of liver cirrhosis.
Our study comes with limitations. Our cohort could have suffered from selection bias due to patient recruitment in a tertiary referral centre. The strength of our study is the sufficiently power to negate a 10% difference in prevalence of the c.-1973T>C polymorphism between groups. However, the study lacks power to demonstrate smaller differences and to perform a thorough subgroup analysis. Further research regarding c.-1973T>C should include homozygous Pi ZZ adults with liver disease to evaluate whether c.-1973T>C is a risk factor for a hepatic expression of A1AT deficiency.

In conclusion: We demonstrated that, in our study, c.-1973T>C polymorphism was not associated with liver disease of various etiologies. In addition, S allele heterozygosity might be a risk factor for the genesis of DILI.

References
4. Sveger T. Liver disease in alpha1-antitrypsin deficiency detected by screening of 200,000 in-
19. Kok KF, Willems JL and Drenth JPH. The cut-off value of 300 mg/dL is insufficient to detect heterozygous Alpha 1-Antitrypsin deficient Liver Disease patients. Liver Int [Epub ahead of print]
### Supplementary: Overview of primers and probes used for real time PCR reactions

<table>
<thead>
<tr>
<th>Allele</th>
<th>Forward primer</th>
<th>Reverse primer</th>
<th>Probe wildtype</th>
<th>Probe mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 allele (p.E342K)</td>
<td>AAAACATGGCCCCAGCAG</td>
<td>AGCCTTACAGTTCTCTG</td>
<td>Fam-TCACTCCTTCTGTCAG-BHQ1</td>
<td>Hex-TCACTCCTTCTGTCAG-BHQ1</td>
</tr>
<tr>
<td>5 allele (p.E264V)</td>
<td>AACATGGCTAAGAGGTGTGG</td>
<td>ATCTTCTTCCTGTGATGAG</td>
<td>FAM-CTACAGCACTGGAATGACTACCC-BHQ1</td>
<td>Hex-CTACAGCACTGGAATGACTACCC-BHQ1</td>
</tr>
<tr>
<td>c.-1973T&gt;C</td>
<td>AGGCCTGGAGGAGTTTGC</td>
<td>GAAGTCGAACAGAAGGAGGAG</td>
<td>Fam-TGGCAGCAAGCAGCTAGGCCC-BHQ1</td>
<td>Hex-TGGCAGCAAGCAGCTAGGCCC-BHQ1</td>
</tr>
</tbody>
</table>
Chapter 7

The cut-off Value of 100 mg/dL is Insufficient to Detect Heterozygous Alpha-1 Antitrypsin deficient Liver Disease Patients

Liver International 2009 Nov 30; [Epub ahead of print]

Karin F Kok
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Joost PH Drenth
Abstract

**Background:** Alpha-1 antitrypsin (A1AT) deficiency is a common disorder and leads to pulmonary and/or liver disease. Some authors indicate that testing for A1AT deficiency should start with quantification of the serum level, followed by iso-electrofocussing and/or polymerase chain reaction (PCR) for the Z and S alleles if serum levels are below a cut-off point of 100 mg/dL.

We wondered whether this diagnostic approach is suitable for the detection of A1AT deficiency in chronic liver disease.

**Patients and methods:** We studied A1AT serum levels in 3 sets of Pi MZ persons. First, 12 Pi MZ patients with liver disease due to various etiologies, second a cohort of 6 Pi MZ patients with end-stage liver cirrhosis caused by A1AT deficiency and lastly 15 Pi MZ controls.

**Results:** Mean serum levels were 106 mg/dL in patients with liver disease of various etiology, 146 mg/dL in patients with cirrhosis and 86 mg/dL in controls; p<0.01. A1AT serum levels were above the suggested threshold of 100 mg/dL in 50%, 83% and 6.7% respectively.

**Conclusion:** We showed that A1AT serum level quantification is not suitable for the detection of Pi MZ heterozygosity in patients with liver disease. We might have missed Pi MZ heterozygosity in the majority of our patients if genotyping only was performed when the A1AT serum level was below 100 mg/dL. Starting the diagnostic path with quantification of the A1AT serum level runs the risk of missing subjects with A1AT deficiency related liver disease. To prevent underdiagnosis, we propose using iso-electrofocussing or PCR rather than quantification of serum levels.

Background

Alpha-1 antitrypsin (A1AT) deficiency is common and affecting 3.4 million subjects worldwide and leads to pulmonary and/or liver disease. Most A1AT deficient patients are Pi ZZ, Pi MZ or Pi SZ genotype carriers, and A1AT deficiency is frequently not recognized. Some authors indicate that testing for A1AT deficiency should start with quantification of the serum level, followed by iso-electrofocussing and/or polymerase chain reaction (PCR) for the most common deficient alleles (Z and S) if serum levels are below a cut-off point of 100 mg/dL.

We wondered whether the diagnostic approach starting with serum level quantification is suitable for the detection of A1AT deficiency in chronic liver disease.

**Methods**

We studied A1AT serum levels in 3 sets of Pi MZ persons. First, 12 Pi MZ patients with liver disease due to various etiologies (8 HCV, 2 alcoholic liver disease, 1 AIH and 1 PSC), second a cohort of 6 Pi MZ patients with end-stage liver cirrhosis caused by histological proven A1AT deficiency and lastly Pi MZ controls (11 unaffected Pi MZ sibs of known patients and 4 Pi MZ controls found through a genetic association study). Median ages were 56, 52 and 46 years respectively (p=0.05) and gender distribution was similar in the 3 groups (male: 56%, 83% and 53% respectively). A1AT serum levels were performed using routine nephelometry, whereas A1AT carrier state was determined by iso-electrofocussing or PCR.

**Results**

Mean serum levels were 106 mg/dL in patients with liver disease of various etiology, 146 mg/dL in patients with A1AT deficiency related cirrhosis and 86 mg/dL in controls; p<0.01. (Figure 7.1) A1AT serum levels were above the suggested threshold of 100 mg/dL in 6 out of 12 (50%) patients with liver
Figure 7.1: Alpha-1 antitrypsin (A1AT) serum levels of 15 Pi MZ controls, 12 Pi MZ patients with liver disease of various etiologies and 6 Pi MZ patients with A1AT related cirrhosis.

In order to exclude that A1AT levels were falsely elevated due to a general acute phase response we measured C-reactive protein (CRP) levels in a subset of 11 subjects (3 controls, 5 patients with various liver disease and 3 cirrhotic patients). We failed to detect a correlation between A1AT levels and CRP. ($r^2 = 0.13, p=0.28$). This suggests that the acute phase response is not the major driver of the A1AT levels in our study population.

Discussion

We might have missed Pi MZ heterozygosity in the majority of our patients if genotyping only was performed when the A1AT serum level was below or similar to the cut-off point of 100 mg/dL. Our finding is corroborated by a report of a normal A1AT serum level in a Pi MZ heterozygous patient with A1AT containing globules in liver biopsy specimen and concomitant HCV.

In addition, in 11 out of 20 (55%) patients with end stage liver disease due to A1AT deficiency (Pi MZ and Pi ZZ), A1AT serum level quantification prior to liver transplantation was above the suggested cut-off point of 100 mg/dL. This suggests mere serum quantification will miss the significant number of patients with bonafide A1AT deficiency related liver disease. Lastly, another study reported the results of 512 samples referred for A1AT phenotyping. These samples were analyzed by quantification of the serum level, isoelectrofocussing and PCR. In 2% of the samples, results from the 3 tests were discordant, frequently due to presence of ‘normal’ serum levels.

We can speculate about the pathophysiologic mechanism of the normal serum levels in Pi MZ heterozygotes with liver disease. As A1AT is an acute phase protein and its production and secretion increases with inflammation, serum levels might be “falsely elevated” and are not reflecting the genotype. This is confirmed by a recent study which found that Pi MZ individuals with a CRP level > 0.8mg/dL had higher A1AT concentrations than Pi MZ individuals with lower CRP levels. It is possible that the absence of a correlation between CRP and A1AT serum levels in our population can be explained by the hepatic origin of CRP. Our dataset does not allow a firm conclusion in either direction.

Conclusion

We showed that A1AT serum level quantification is not suitable for the detection of Pi MZ heterozygosity in patients with liver disease. Starting the diagnostic path with quantification of the A1AT serum level runs the risk of missing subjects with A1AT deficiency related liver disease. To prevent underdiagnosis, we propose starting the diagnostic route with iso-electrofocussing or PCR (dependent on the local expertise) rather than quantification of serum levels.

References


Chapter 8

Liver Transplantation for Alpha-1 Antitrypsin Deficiency and the Impact of Incidental Hepatocellular Carcinoma on Post-Transplant Survival

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Joost PH Drenth

For the European Liver and Intestine Transplant Association (ELITA)
Abstract

Background: Alpha-1 antitrypsin (A1AT) deficiency is a genetic disorder that can result in lung and liver injury. Both homozygous (Pi ZZ) and heterozygous (Pi MZ and Pi SZ) disease appear to cause liver disease, however it is unknown whether heterozygous disease induces end stage liver disease and hepatocellular carcinoma (HCC). Liver transplantation (LT) is the only curative approach at this stage of liver disease.

Aim: To study (1) A1AT deficiency characteristics with respect to serum level, phenotype and concomitant liver disease (particularly HCC) and (2) patient and graft survival in LT for A1AT deficiency.

Methods: We studied 2 LT cohorts: First, LT recipients with A1AT deficiency in the Netherlands and second, a replicative cohort with data from A1AT deficiency patients collected in the European Liver Transplantation Registry (ELTR).

Results: We analyzed the medical records of 24 adult and 8 pediatric LT recipients from the Netherlands. HCC was found in 7/24 (29%) adult LT recipients; the time between 1st presentation of liver disease and LT was longer in patients with HCC. (p<0.01). The majority of HCC (4/7) was not recognized prior to LT. HCC was absent in the pediatric LT recipients. To replicate our findings we retrieved data from 317 adult and 185 pediatric European A1AT deficient LT recipients. HCC was present in 24/317 adults (8%) and presence of HCC was associated with a poor 5 years survival (69% vs. 83%, HCC patients vs non-HCC patients, p=0.01). The 15 years patient survival rates were 58% for adult LT recipients and 86% for pediatric LT recipients (p=0.001). The 15 years graft survival rates were 57% for adult LT recipients and 76% for pediatric LT recipients (p=0.02).

Conclusion: Patients with A1AT deficiency induced cirrhosis are at high risk for the development of HCC of which some were not recognized before LT. Presence of HCC predisposes for an inferior survival after LT.

Background

Alpha-1 antitrypsin (A1AT) deficiency is a genetic disorder that most frequently presents as emphysema and less commonly as liver disease. In childhood, A2AT deficiency liver disease presents as neonatal cholestasis which can lead to cirrhosis. The clinical phenotype of A1AT deficiency in adults is that of mild liver enzyme disturbances with or without advanced disease such as cirrhosis and hepatocellular carcinoma (HCC). Once diagnosed, most patients with liver cirrhosis in A1AT deficiency will develop rapid progressive liver disease. At that stage, the only curative approach is liver transplantation (LT). Theoretically, LT has a dual benefit, as it not only cures the liver disease but also restores A1AT serum levels by adopting the donor phenotype and thus offering protection against emphysema. Most LT series are small and stem from the beginning of the liver transplantation era. They often have been performed in the pediatric population and originate from single centers. A recent series from the United Network for Organ Sharing (UNOS) database reported an excellent survival in A1AT LT recipients. We investigated the clinical characteristics and prognosis after LT in both adult and pediatric patients with A1AT deficiency. We addressed the following questions. First, we were interested in clinical characteristics of patients receiving LT for A1AT deficiency. We hypothesized that the proportion of HCC cases would be low in A1AT deficient LT recipients and we tested this hypothesis in 2 different cohorts. Secondly, benefiting from the comprehensive European Liver Transplantation Registry (ELTR) we examined patient and graft survival after LT.

Methods

Our study included 2 different cohorts. First, we studied the clinical characteristics in a cohort of A1AT deficient LT recipients in the Netherlands and secondly, we studied a cohort of LT patients with A1AT deficiency that was submitted to the ELTR.

The Netherlands cohort

We analyzed the medical records of all adult and pediatric LT recipients with A1AT deficiency who were transplanted from January 1985 onwards in all 3 LT centers in the Netherlands (University Medical Center Groningen, Erasmus
Medical Center Rotterdam and Leiden University Medical Center). We used the local databases to identify LT recipients with A1AT deposits on explant histology, and were able to include 32 patients. We assessed patient demographics, date of 1st presentation of liver disease, date of LT, date of last outpatient clinic visit, results of A1AT iso-electrofocussing and A1AT serum level quantification, spirometry: Forced Expiratory Volume in 1 sec/Vital Capacity (FEV1/VC), histological report of the explant specimen, routine laboratory parameters, lifestyle characteristics such as smoking and alcohol use, concomitant liver disease and causes and dates of re-transplantation or death. When HCC was present: size, number and alpha fetoprotein level.

ELTR cohort
We retrieved data collected in the ELTR to study patient and graft survival after LT for A1AT deficiency in a large number of patients. The European Liver and Intestine Transplant Association (ELITA) board approved the study request and provided the data. We included patients who underwent LT from January 1st 1985 until May 26th 2009. Pre-LT characteristics are given in Table 8.1. One adult woman received auxiliary LT in 1987, the remaining 31 patients were treated with standard orthotopic LT.

We detected HCC in 7 out of 24 (29%) adult LT recipients (71% males), and mean age (±SD) at diagnosis HCC was 50.6 (±10.3) years). The time between 1st presentation of liver disease and LT was longer in adult LT recipients with HCC compared to adult LT recipients without HCC (p<0.01). (Figure 8.1)

Table 8.1: Pre-liver transplant characteristics of 24 adult and 8 pediatric LT recipients with alpha-1 antitrypsin deficiency in the Netherlands

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Adult n=24</th>
<th>Pediatric n=8</th>
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</thead>
<tbody>
<tr>
<td>Median age at LT (yrs) (range)</td>
<td>50.6 (18.2-66.7)</td>
<td>7.1 (1.1-14.3)</td>
</tr>
<tr>
<td>Male (%)</td>
<td>19 (79%)</td>
<td>5 (63%)</td>
</tr>
<tr>
<td>Median ALT (IU/ml) (range)</td>
<td>53 (26-337)</td>
<td>124 (47-510)</td>
</tr>
<tr>
<td>Median bilirubin (umol/l) (range)</td>
<td>44 (9-800)</td>
<td>53 (10-266)</td>
</tr>
<tr>
<td>Median albumin (g/l) (range)</td>
<td>30 (16-44)</td>
<td>32 (18-41)</td>
</tr>
<tr>
<td>Prothrombin time (s) (range)</td>
<td>17 (11.8-25.3)</td>
<td>18 (12.2-25.7)</td>
</tr>
<tr>
<td>FEV1/VC &lt; 70% (%) (n=23)</td>
<td>6 (26%)</td>
<td>NA</td>
</tr>
<tr>
<td>Diabetes</td>
<td>3 (13%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Time between 1st presentation of liver disease and LT (yrs) (range)</td>
<td>7.0 (0.5-27.5)</td>
<td>7.1 (0.9-14.14)</td>
</tr>
</tbody>
</table>

| LT=liver Transplantation; A1AT=alpha-1 antitrypsin deficiency; FEV1/VC=Forced Expiratory Volume in 1 sec/Vital Capacity; NA=not available

Statistics
Descriptive statistics were performed on baseline characteristics and presented as median and range. We used Mann-Whitney-U tests, Chi-square tests and Fisher exact tests where appropriate to analyze baseline characteristics. Odds Ratio’s (OR) were calculated for gender distribution, presence of HCC, diabetes, smoking and alcohol history between patients with homozygous and heterozygous disease in the Dutch cohort. In addition, we used OR’s for calculating differences in baseline characteristics between adult and pediatric LT recipients in the ELTR cohort. For time-to-death or re-transplantation we applied the Kaplan-Meier method and compared groups using the log-rank test. All statistical analyses were performed using GraphPad Prism Version 4.02 (GraphPad Software Inc., San Diego, CA, USA). A two-sided p-value of <0.05 was considered statistically significant.

Results
The Netherlands cohort
We included 32 patients (24 adults and 8 pediatric LT recipients) who underwent LT from January 1st 1985 until May 26th 2009. Pre-LT characteristics are given in table 8.1. One adult woman received auxiliary LT in 1987, the remaining 31 patients were treated with standard orthotopic LT.

We detected HCC in 7 out of 24 (29%) adult LT recipients (71% males), and mean age (±SD) at diagnosis HCC was 50.6 (±10.3) years). The time between 1st presentation of liver disease and LT was longer in adult LT recipients with HCC compared to adult LT recipients without HCC (p<0.01). (Figure 8.1)
Age at LT and age at first presentation of liver disease were similar in patients with and without HCC. In addition, other baseline characteristics (in particular presence of homozygous or heterozygous disease) were comparable for adult LT recipients with and without HCC. In all HCC cases the diagnosis was confirmed by histological examination of the surgical explant specimen. A total of 4 out of 7 HCCs was not diagnosed prior to LT. These had an incidental HCC and had normal alpha fetoprotein levels (<10 µg/l) prior to LT. Two patients with incidental HCC had small single tumors of 8 and 15 mm, 1 patient had multifocal HCC with the larger one measuring 30 mm and 1 patient had a tumor measuring 80 mm. This tumor of 80 mm exceeded by far the Milan Criteria, though the patient was misclassified as having focal nodular hyperplasia prior to LT. All 3 patients with HCC who were recognized prior to LT had elevated alpha fetoprotein levels. We found no HCC in pediatric LT recipients, although one girl, transplanted at age 3.5 had a dysplastic node of 6 mm in her liver explant.

A1AT phenotyping was performed using iso-electrofocussing. The entire population of pediatric LT recipients was A1AT homozygous and median serum level was 0.4 g/l (range: 0.2-0.5 g/l). A1AT phenotypes in 21 adult LT recipients were: 14 Pi ZZ (homozygous) and 5 Pi MZ, 1 Pi SZ and 1 Pi Mnull (heterozygous).

Baseline characteristics were similar in homozygous and heterozygous adult LT recipients. A1AT serum levels were lower in homozygous adults compared to heterozygous adults (median 0.3 g/l vs. 1.4 g/l, p=0.001). (Figure 8.2)

Spirometry results were available in 23 adult LT recipients but not for pediatric LT recipients. A total of 6/23 (26%) adult LT recipients had signs of obstructive pulmonary disease with FEV1/VC <0.7 prior to LT. One of these patients was a recipient of a simultaneous LT and unilateral lung transplant; this patient had increased spirometry results after transplantation.

Median time of follow-up was 5.3 years (range 0.1-24) in adult and 3.4 years (range 0.7-16) in pediatric LT recipients. Five adults died after LT: due to cardiac disease (n=2, 21 and 2.5 years after LT), multi-organ-failure and in-hospital death (n=2, 0.1 and 0.4 years after LT) and metastatic melanoma (n=1, 6.4 years after LT). Re-transplantation was performed in 2 adult LT recipients because of ischemic bile duct lesions 0.2 years after LT and liver failure of unknown origin at 3.4 years after LT. Three pediatric LT recipients, all transplanted in 1989, died. Reasons of death were sepsis (n=2, 1.7 and 2.6 years after LT) and pulmonary embolism (n=1, 16 years after LT).

ELTR cohort
As we observed an unexpected high frequency of HCC in our initial cohort we sought confirmation of this observation in an independent LT cohort. This replicative cohort comes from the ELTR database and we included 317 adult and 185 pediatric LT recipients, originating from 78 centers in 20 European countries. (See for complete list: addendum) A1AT deficiency was registered as the leading indication for LT in 231 (73%) adult and 179 (97%) pediatric LT recipients and was registered as an associated disease in the remainder. The distribution in the absolute number of LT was bimodal, as we found a peak in early childhood and a second peak in middle-aged adults. (Figure 8.3)

We observed a male predominance in adult and pediatric LT recipients, but in adults this unbalanced gender distribution was more apparent (OR 1.8, 95%CI 1.2-2.7). When corrected for the presence of alcoholic liver disease, the difference was not significant (OR 1.4, 95%CI 0.95-2.2). In adult LT recipients the registered associated diseases were different compared to pediatric LT recipients as concomitant alcoholic liver disease, HCC and cryptogenic cirrhosis were more frequently reported in adults and biliary atresia was unique to pediatric LT recipients. (Table 8.2)
Figure 8.3: Age distribution among 502 patients who underwent liver transplantation due to alpha-1 antitrypsin deficiency between 1993 and 2007 according to the ELTR.

Figure 8.4: Patient survival (panel A) and graft survival (panel B) after liver transplantation in 502 patients with alpha-1 antitrypsin deficiency registered in the ELTR.

The 1-, 5-, 10- and 15-year patient survival rates were 89%, 82%, 63% and 58% for adult LT recipients and 93%, 89%, 86% and 86% for pediatric LT recipients (p=0.001 adult vs pediatric cohort). The 1-, 5-, 10- and 15-year graft survival rates were 87%, 79%, 62% and 57% for adult LT recipients and 88%, 84%, 76% and 76% for pediatric LT recipients (p=0.02 adult vs pediatric cohort). (Figure 8.4)

Table 8.2: Baseline characteristics of 502 patients, registered in the ELTR database, who underwent liver transplantation because of alpha-1 antitrypsin deficiency between 1993 and 2007

<table>
<thead>
<tr>
<th>Associated diseases (%)</th>
<th>Adult n=317</th>
<th>Pediatric n=185</th>
<th>OR (95% CI)</th>
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<tbody>
<tr>
<td>HCC</td>
<td>24 (8)</td>
<td>1 (1)</td>
<td>15 (2.0-112)†</td>
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<tr>
<td>Alcoholic liver disease</td>
<td>65 (21)</td>
<td>0 (0)</td>
<td>NA</td>
</tr>
<tr>
<td>Cryptogenic cirrhosis</td>
<td>17 (5)</td>
<td>2 (1)</td>
<td>5.2 (1.2-22.7)†</td>
</tr>
<tr>
<td>Viral hepatitis</td>
<td>11 (3)</td>
<td>1 (1)</td>
<td>1.8 (0.18-17.0)</td>
</tr>
<tr>
<td>(Semi) Acute liver failure</td>
<td>2 (1)</td>
<td>1 (1)</td>
<td>1.2 (0.11-13.1)</td>
</tr>
<tr>
<td>AIH/PSC/PBC</td>
<td>6 (2)</td>
<td>0 (0)</td>
<td>NA</td>
</tr>
<tr>
<td>Hemochromatosis</td>
<td>6 (2)</td>
<td>0 (0)</td>
<td>NA</td>
</tr>
<tr>
<td>Polycystic liver disease</td>
<td>2 (1)</td>
<td>0 (0)</td>
<td>NA</td>
</tr>
<tr>
<td>Hemangioma</td>
<td>1 (0)</td>
<td>0 (0)</td>
<td>NA</td>
</tr>
<tr>
<td>Tyrosinemia</td>
<td>1 (0)</td>
<td>0 (0)</td>
<td>NA</td>
</tr>
<tr>
<td>Biliary atresia</td>
<td>0 (0)</td>
<td>6 (3)</td>
<td>NA</td>
</tr>
<tr>
<td>Cystic fibrosis</td>
<td>0 (0)</td>
<td>1 (1)</td>
<td>NA</td>
</tr>
</tbody>
</table>

†Associated disease when A1AT deficiency was registered as indicator for LT and indicator for LT when A1AT deficiency was registered as associated disease. NA: not available. †statistic significant. =This patient was transplanted at age 20.

The 1- and 5-year patients survival rates were 86% and 69% respectively in adults with HCC (n=24, 92% male) and 89% and 83% respectively in adults without HCC (n=293, 76% male). Therefore survival in patients without HCC was superior compared to patients with HCC (p=0.01). (Figure 8.5) A total of 9 adult LT recipients with HCC died after LT, the reason of death was tumor recurrence in 5 out of 9 (56%) patients.
Discussion

This study shows a high rate of HCC in adult LT recipients due to A1AT deficiency as we found HCC in 29% of liver explants from an extensively phenotyped cohort of 32 patients. Some of these were unrecognized prior to LT (so-called incidental HCC). We confirmed these data in a larger replicative cohort and found that 24/317 (8%) had HCC in their explants. This series differs from other studies in the field as it adds several elements of novelty. First, we analyzed the frequency of HCC in a large number of LT patients with A1AT deficiency and second, we compared survival after LT in A1AT deficient patients with and without HCC.

Literature is equivocal whether A1AT deficiency represents a risk factor for HCC. Some studies indicate that HCC in patients with A1AT deficiency is related to concomitant viral hepatitis, others reported an association between HCC and the Z allele whereas others do not. The research strategies that these researchers have used to associate HCC with A1AT deficiency are diverse. There have been a number of pathology studies that detected a higher incidence of A1AT containing globules in HCC livers compared to control livers and epidemiology studies have studied whether A1AT was a covariate factor for HCC on top of underlying disorders such as HBV or HCV. Both strategies come with disadvantages as they do not accurately estimate the prevalence of HCC in patients with A1AT deficiency. Our study provides data that helps to address this issue. The high rate of HCC in our study is similar to that reported for genetic hemochromatosis (7/22, 32%). We also show that survival rates after LT in A1AT deficient adults with HCC are clearly inferior to those without HCC. Although these results are intuitive, the formal proof for this concept was absent from literature. LT recipients with HCC died due to tumor recurrence in 56% of cases. Earlier published guidelines did not recommend for or against surveillance for HCC in A1AT deficiency because a lack of data precluded an assessment of whether surveillance would be beneficial.

Our data have possible clinical implications as the risk for HCC in A1AT deficiency appears to be real, although this cohort does not allow to provide an accurate estimate.

We found that the clinical course in adult LT recipients is similar in homozygous and heterozygous patients. Separate analysis did not yield different results with respect to gender, age at first presentation of liver disease, age at LT and concomitant alcohol abuse (data not shown). This was reason for us to lump these data together. All heterozygous A1AT deficiency patients had serum levels ≥ 1.0 g/l (thus in the normal range) and as a consequence, these patients could have been missed if only serum level quantification was performed. We previously showed that A1AT serum levels were not suitable for the detection of homozygous A1AT deficiency in patients with chronic liver disease and suggested to start the diagnostic path with iso-electrofocussing or genotyping.

The ELTR based part of our study shows that LT is a successful treatment for liver disease in A1AT deficiency with excellent graft and patients’ survival in adult and pediatric LT recipients. Both graft and patients’ survival are superior in pediatric LT recipients compared to adult LT recipients. These results can be compared with the study that used the UNOS database to determine survival after LT in A1AT deficiency. This study included 406 adult and 161 pediatric LT recipients who were transplanted between 1995 and 2004. The 1- and 5-year graft and patients survival in adult and pediatric LT recipients are similar to our data. An earlier published study showed inferior results and reported a 1-year patient survival of 73% in adult and 88% in pediatric LT recipients.
survival in our cohort may be due to improvement in clinical care with time.\textsuperscript{18} The superior LT results in pediatric patients relative to adults, is known to be true for other indications such as cholestatic disease and other metabolic diseases.\textsuperscript{18} The strength of our study is the replication of data from (1) a thorough analysis of clinical characteristics in patients with A1AT deficiency who received LT in the Netherlands by a second data set from (2) a large number of European patients with a long follow-up. The limitations of our study are as follows: selection bias might be introduced as we only selected patients who received LT and therefore we cannot extrapolate our findings to all patients with A1AT deficiency related liver disease. In addition, because of the retrospective design, there were missing data.

In conclusion: first, adult patients with end-stage liver disease due A1AT deficiency, requiring LT, are at risk for the development of HCC and some are not recognized prior to LT. Patients with HCC had inferior survival outcomes. Second, baseline characteristics were similar in patients with homozygous compared to heterozygous A1AT deficiency. Third, this study showed that LT is an effective treatment of liver disease in A1AT deficiency with excellent graft and patients’ survival in adult and pediatric LT recipients.

References


17. Kok KF, Willems JL DJ. “The cut-off Value of 100 mg/dL is insufficient to detect heterozygous Alpha-1-Antitrypsin deficient Liver Disease patients.” Liver International. (Epub ahead of print)

Addendum

Centres participating in the ELITA cohort study:

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ITALY: Centro Trapianti di Fegato Bari, Prof. V. Memeo; Ospedali Riuniti di Bergamo, Dr. M. Collened; Ospedale San Martino e Cliniche Universitarie Convenzione Genova, Prof. U. Valente; Ospedale Maggiore Policlinico Milano, Prof. G. Rossi; Istituto di Chirurgia Generale Padova, Prof. A. Maffei Faccioli; Instituto Mediterraneo per i Trapianti e Terapie ad Alta Specializzazione Palermo, Prof. D. Amico; Universita Degli Studi di Roma, Prof. M. Rossi; University Hospital Udine, Prof. F. Bresadola

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SWISS: Universitysspital Bern, Prof. D. Candinas; Hospital Cantonal Universitaire de Geneve, Prof. P. Morel and Prof. G. Mentha; Universitatssspital Zurich, Prof. P. Clavien
Chapter 9
General discussion
In 2005 a total of 3 patients presented with disturbed liver enzymes. They deteriorated rapidly and all died as a result of their liver disease. All 3 patients had normal serum levels of alpha-1 antitrypsin (A1AT) and the diagnosis A1AT deficiency was only made by examination of a liver biopsy specimen. We discovered that all 3 patients were A1AT deficient and heterozygous carriers of the Pi MZ allele. This was eventually confirmed with iso-electrofocussing. In hindsight, we relied too much on the “pseudo”- normal A1AT levels and did not proceed to iso-electrofocussing or genotyping. These 3 cases spurred our interest in A1AT deficiency as a cause of liver disease. We performed an extensive literature search that aimed to address the following questions.

First, we were interested in the clinical course of the disease. Do patients with A1AT deficiency develop end-stage liver disease? What are the complications of A1AT deficiency related liver disease? And what about the characteristics and prognosis of neonatal cholestasis due to A1AT deficiency? We tried to find these answers and wrote a review of the current literature. Subsequently, we studied the clinical characteristics of neonates with cholestasis due to A1AT deficiency. We focused on prognosis and on bleeding complications due to vitamin K deficiency. Vitamin K deficiency is caused by cholestasis induced malabsorption. We detected a high proportion of vitamin K deficiency related bleeding, particularly in exclusively breast-fed infants. In addition, we studied clinical characteristics and prognosis after liver transplantation (LT) in adult and pediatric LT recipients with A1AT deficiency. We detected a high proportion of HCC in adult LT recipients and the majority was diagnosed upon examination of the explanted liver specimen. Indeed, A1AT LT recipients with HCC had an inferior survival compared to patients without HCC.

Second, we focused on the diagnostic approach for detecting A1AT deficiency in liver disease. Guidelines and flow charts regarding the evaluation of A1AT deficiency proposed to start the initial evaluation with A1AT serum level quantification and performing iso-electrofocussing or genotyping only in case serum level is decreased. These flow charts are merely initiated by pulmonary centers. As serum levels >0.5 g/l represent a protective threshold above which the risk of emphysema is not increased, the cutoff point fits with the detection of A1AT deficiency in pulmonary disease. Given that aberrant A1AT phenotypes with serum levels above this protective threshold will not induce emphysema, the detection of an abnormal phenotype with a normal serum level is clinically less important for pulmonologists. In contrast, in the evaluation for hepatic disease the protective threshold is not the issue but abnormal phenotypes are relevant. We showed that the majority of liver patients with a heterozygous (Pi MZ) phenotype had serum levels above the cutoff value of 1.0 g/l. Additionally, heterozygous (Pi MZ) A1AT deficiency LT recipients had serum levels in the normal range. We concluded that relying on A1AT serum levels alone, runs the risk of missing the right diagnosis.

Third, other reports focused on associations between the Z allele and different liver diseases. The first reports describing associations between heterozygous A1AT deficiency and end-stage liver disease due to various etiologies were published in 1997 and 1998. In recent years a plethora of studies reported the results of the search for single nucleotide polymorphisms (SNPs) and associations with A1AT deficiency. The topics which were studied can be divided into 2 groups. First, studies involving the relation between SNPs and the phenotype of homozygous (Pi ZZ) A1AT deficiency. For example, the c.-1973T>C polymorphism located in the SERPINA1 promoter region (c.-1973T>C), appeared to be associated with neonatal liver disease compared to pulmonary disease in homozygous A1AT deficiency patients. Second, studies reporting SNPs in the SERPINA1 gene and their association with different liver diseases. For example, a report showed an association between the Z allele in the SERPINA1 gene and the presence of liver disease in cystic fibrosis patients. We studied the association between the presence of heterozygous A1AT deficiency and treatment outcomes in hepatitis C virus infection. Furthermore, we performed a case-control study to detect an association between c.-1973T>C and liver disease of various etiologies. We could not show an association between treatment response among wild types and patients with heterozygous A1AT deficiency and neither c.-1973T>C was associated with liver disease of various etiologies. All in all, these case-control genetic association studies require many patients and controls (mostly >500) to achieve statistical significant differences. When
General discussion

This large sample sizes are required for the detection of statistically significant differences, we question whether these findings are of major clinical relevance. Therefore, we addressed our fourth question. What is the clinical relevance of liver disease due to heterozygous A1AT deficiency? We showed that the development of chronic liver disease due to heterozygous liver disease is a serious issue. Heterozygous disease can progress to end-stage liver disease and HCC requiring liver transplantation as well as homozygous A1AT deficiency.

The studies described in this thesis might have clinical consequences as we hope that clinicians would more be aware of A1AT deficiency as cause of liver enzyme disturbances and cirrhosis. In case of unexplained liver enzyme elevations or cryptogenic cirrhosis, diagnostic tests for A1AT deficiency should be performed. To prevent under-diagnosis, they should start the diagnostic path with search for genetic mutations rather than quantify serum levels. In addition, the clinical picture of liver disease due to heterozygous A1AT deficiency is similar to homozygous A1AT deficiency. Both heterozygous and homozygous A1AT deficiency related liver disease can induce end-stage liver disease requiring liver transplantation. HCC is a common complication of liver disease in A1AT deficiency; however the majority is so-called incidental. Our findings support surveillance for HCC in A1AT deficient patients with liver disease, however the effect on survival still needs to be established.

Further research should aim at the detection of modifiers for the clinical phenotype of A1AT deficiency. In other words: why do some patients develop liver disease, others develop pulmonary disease and do some A1AT deficient subjects have no complications? Future investigation might follow research examples in cystic fibrosis, as the clinical expression of this hereditary disease is influenced by different genetic modifiers. For example, a recent study showed an association of CF transmembrane conductance regulator (CFTR) mutations and the presence of diabetes in cystic fibrosis.

References

5. Kok KF, Willems JL and Drenth JPH. The cut-off Value of 100 mg/dL is insufficient to detect heterozygous alpha1-antitrypsin deficient liver disease patients. Liver International 2009 (In press).
Summary and Conclusion
Alpha-1 antitrypsin (A1AT) deficiency is a genetic disorder which may lead to pulmonary and/or liver disease. In this thesis, the clinical consequences of A1AT deficiency are addressed. First, we described the clinical course of the disease; second the diagnostic pitfalls; third we focused on genetic associations and finally on the role of heterozygous A1AT deficiency.

Chapter 1 is an introduction to A1AT deficiency. The history of the detection of A1AT deficiency and also the SERPINA1 gene and the mutations involved in A1AT deficiency are recorded. Furthermore, we provided an outline of the physiology of the normal M-type A1AT and the pathophysiology of Z-type A1AT, which can lead to A1AT deficiency. In addition, we described the pathophysiologic mechanisms of liver and pulmonary disease. Chapter 2 is an overview of the literature regarding clinical consequences of A1AT deficiency. The clinical presentation, diagnostic strategy, prognosis and therapy of A1AT deficiency related liver disease in children and adults are reviewed.

In chapter 3 and 4, studies in neonates with A1AT deficiency are reported. In chapter 3, we studied the prognosis of neonatal cholestasis due to A1AT deficiency. We included all neonates who were born in the Netherlands between 1991 and 2006 and who were diagnosed with A1AT deficiency in the first 3 months of life. We studied 50 neonates, and 15 of them presented with vitamin K deficiency bleeding. During follow-up, 5 patients (10%) died and 2 patients (4%) had to be transplanted. At the end of follow-up, 12% had cirrhosis, 62% had liver enzyme abnormalities and/or hepatosplenomegaly and 12% had no signs of liver disease. In chapter 4, we assessed the risk of vitamin K deficiency bleeding in neonates with cholestatic jaundice due to A1AT deficiency. A total of 40 neonates were studied, 20 of them were breast-fed and received the recommended daily vitamin K prophylaxis and 20 neonates were formula-fed. Bleeding tendency was increased in breast-fed neonates compared to formula-fed neonates.

We performed 2 genetic association studies which are reported in chapter 5 and 6. In chapter 5, we described the prevalence of the Z mutation in 2 cohorts of patients with hepatitis C virus infection (together 3,048 patients). We found a heterozygosity rate (Pi MS and Pi MZ) of 0.06, in line with healthy controls in other studies. Furthermore, we could not detect an association between S and Z allele mutations and sustained viral response after treatment with (PEG-)interferon and ribavirin. Additionally, in chapter 6 we reported the results of a study to investigate the association between the c.-1973T>C mutation in the promoter region of the SERPINA1 gene and liver disease of various etiologies. We included 297 patients and 297 controls, and performed real-time PCR for the c.-1973T>C mutation and Z and S allele. We could not demonstrate that c.-1973T>C polymorphism was a risk factor for liver disease due to various etiologies. However, S allele heterozygosity was increased in patients with drug induced liver injury and therefore, might contribute to its development.

In chapter 7, we explored the diagnostic strategy for detection of A1AT deficiency. Testing for A1AT deficiency in current practice starts with serum level quantification then followed by iso-electrofocussing or genotyping when the serum level is below a cut-off point of 1.0 g/l. We had the clinical impression that we missed liver disease patients with heterozygous A1AT deficiency. Therefore, we studied A1AT serum levels in 3 sets of heterozygous (Pi MZ) persons. We included 12 patients with liver disease due to various etiologies, 6 patients with cirrhosis due to A1AT deficiency and 15 controls. Serum levels were higher in patients with cirrhosis and liver disease of various etiologies compared to unaffected controls. The majority of heterozygous Pi MZ patients might be missed when we relied on serum level quantification alone. To prevent under diagnosis, we proposed starting the diagnostic path with iso-electrofocussing or PCR rather than quantification of serum levels. In chapter 8, we assessed liver transplantation, the only curative treatment option in end-stage liver disease. We started with an analysis of the medical records of all patients who had received liver transplantation in the Netherlands. We detected a higher than expected prevalence of hepatocellular carcinoma in liver explant specimen: 29% of adult liver transplantation recipients. We sought confirmation of this finding and studied data of liver transplantation recipients with A1AT deficiency, collected in the European Liver Transplantation Registry. Similarly, we detected a high frequency of hepatocellular carcinoma. Furthermore, we could demonstrate that hepatocellular carcinoma negatively affects outcome after liver transplantation. Moreover, we showed that both homozygous and heterozygous A1AT deficiency can be associated with end-stage liver disease.
In conclusion: First, A1AT deficiency is a disorder with a low penetrance; however, in affected adults and children it is a serious issue and it can be associated with life-threatening complications. Second, to prevent under diagnosis we strongly suggest to start the diagnostic path with iso-electrofocussing or genotyping rather than serum level quantification. Finally, both heterozygous and homozygous A1AT deficiency can be associated with end-stage liver disease.
Alfa-1 antitrypsine (A1AT) deficiëntie is een erfelijke aandoening die kan leiden tot zowel long- als leverziekte. In dit proefschrift proberen we de klinische consequenties van A1AT deficiëntie te onderzoeken. Ten eerste hadden we vragen over het klinisch beloop van de ziekte, ten tweede over de diagnostische valkuilen, ten derde hebben we ons gericht op genetische associaties en ten slotte bogen we ons over de rol van heterozygote A1AT deficiëntie.

Hoofdstuk 1 is een inleiding in A1AT deficiëntie. We beschreven de geschiedenis van het herkennen van A1AT deficiëntie en tevens het SERPINA1 gen en de mutaties die betrokken zijn bij A1AT deficiëntie. We verklaren de fysiologie van het normale M-type A1AT en de patho-fysiologie van het Z-type A1AT, welke kan leiden tot A1AT deficiëntie. Daarnaast beschreven we de patho-fysiologische mechanismen van lever- en longaandoeningen. Hoofdstuk 2 is een overzicht van de literatuur met betrekking tot de klinische consequenties van A1AT deficiëntie. De klinische presentatie, het diagnostisch traject, de prognose en de therapie van leverziekten door A1AT deficiëntie bij kinderen en volwassenen komen in dit hoofdstuk aan bod.

In hoofdstuk 3 en 4 beschreven we de studies die we deden bij pasgeborenen met A1AT deficiëntie. In hoofdstuk 3 rapporteerden we de prognose van neonatale cholestase door A1AT deficiëntie. Wij includeerden alle pasgeborenen die zijn geboren in Nederland tussen 1991 en 2006 en bij wie A1AT deficiëntie is vastgesteld in de eerste 3 maanden van het leven. We vonden 50 pasgeborenen met A1AT deficiëntie en 15 van hen hebben zich initieel gepresenteerd met vitamine K deficiëntie afhankelijke bloedingen. Tijdens de follow-up zijn 5 patiënten (10%) overleden en 2 patiënten (4%) moesten worden getransplanteerd. Aan het einde van de follow-up had 12% cirrose, 62% had afwijkende leverenzymen en/of hepato-splenomegalie en 12 % had geen tekenen van leverziekte. Vervolgens werd in hoofdstuk 4 het risico op vitamine K deficiëntie afhankelijke bloedingen bij pasgeborenen met cholestatische geelzucht als gevolg van A1AT deficiëntie onderzocht. Er werden 40 pasgeborenen bestudeerd, 10 van hen kregen borstvoeding en ontvingen dagelijks de aanbevolen vitamine K profylaxe en 20 pasgeborenen kregen flesvoeding. Het risico op bloedingen was verhoogd bij pasgeborenen die borstvoeding kregen in vergelijking met pasgeborenen die flesvoeding kregen.

Hoofdstuk 7 gaat nog in op het diagnostisch traject van A1AT deficiëntie. Sommige auteurs beargumenteren dat het testen op A1AT deficiëntie zou moeten beginnen met het bepalen van een serumspiegel, gevolgd door isoelectrofocussing of genotypering als de serumspiegel kleiner is dan 1,0 g/l. We hadden klinisch de indruk dat we heterozygote leverpatiënten misten bij het volgen van die strategie. Daarom bestudeerden we A1AT serumspiegels in 3 groepen van heterozygote (Pi MZ) personen. Wij onderzochten 12 patiënten met een leverziekte door meerdere oorzaken, 6 patiënten met cirrose als gevolg van A1AT deficiëntie en 15 controles. Serumspiegels waren hoger bij patiënten met cirrose en leverziekten door verschillende oorzaken vergeleken met controles. De meerderheid van de heterozygote Pi MZ patiënten zouden zijn gemist als we alleen serumspiegels hadden bepaald. Om het missen van de diagnose te voorkomen, stelden wij voor om het diagnostische traject te starten met iso-electrofocussing of PCR in plaats van het bepalen van serumspiegels. In hoofdstuk 8 onderzochten we levertransplantatie bij patiënten met A1AT deficiëntie, dit is de enige curatieve behandeling in het eindstadium van de ziekte.
Samenvatting en Conclusie

van deze leverziekte. We begonnen met een analyse van de medische dossiers van alle patiënten die een levertransplantatie hadden ondergaan in Nederland. Tegen alle verwachtingen in, ontdekten we hepatocellulair carcinoom in 29% van de leverexplantaten bij volwassen. We zochten bevestiging van deze bevinding en bestudeerden vervolgens de gegevens van patiënten die een levertransplantatie moesten ondergaan wegens A1AT deficiëntie, zoals deze verzameld waren in het Europese levertransplantatie register. Wij vonden ook in deze patiëntengroep een groot aantal hepatocellulair carcinomen. Daarnaast konden we aantonen dat patiënten met een hepatocellulair carcinoom een slechtere overleving hadden na levertransplantatie in vergelijking met patiënten zonder hepatocellulair carcinoom. Verder hebben we laten zien dat zowel homozygote als heterozygote A1AT deficiëntie kan leiden tot eindstadium leverziekte.

Concluderend: Ten eerste, A1AT deficiëntie is een aandoening met een lage penetrantie, echter bij aangedane kinderen en volwassenen is het een serieus probleem en het kan leiden tot levensbedreigende complicaties. Ten tweede, om het missen van de diagnose te voorkomen adviseren wij om het diagnostisch traject te starten met iso-electrofocussing of genotypering in plaats van het bepalen van serumspiegels. Ten slotte, zowel heterozygote als homozygote A1AT deficiëntie kan leiden tot eindstadium leverziekte.

List of publications

Prevalence of Genetic Polymorphisms in the Promoter Region of the Alpha-1 Antitrypsin (SERPINA1) Gene in Chronic Liver Disease: a Case Control Study. BMC Gastroenterology 2010, 10:22 Kok KF, te Morsche RH, van Oijen MGH and Drenth JPH.

The cut-off Value of 100 mg/dL is insufficient to detect heterozygous Alpha-1 Antitrypsin deficient Liver Disease patients. Kok KF, Willems JL and Drenth JPH. Liver Int. 2009 Nov 30. [Epub ahead of print]


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Het project dat in dit proefschrift wordt beschreven is in meerdere fases verricht. Tijdens de opleiding tot MDL-arts leek het op een dieseltreintje dat op elk stationnetje stopt en hortend en stotend steeds weer verder gaat. Na het afronden van de opleiding, mocht ik in de “kelder”, in alle rust, ver weg van de kliniek, plaatsnemen tussen de onderzoekers. Daar kreeg het project het tempo van een Intercity, vervolgens ging de trein steeds harder rijden en werd in de laatste maanden een echte HSL. Daardoor kon het eindstation worden bereikt voordat ik in het Canisius Wilhelmina Ziekenhuis ging starten als MDL-arts.

Het afronden van het promotietraject was niet mogelijk geweest zonder de hulp van velen.

Beste professor dr. JPH Drenth, beste Joost,

Hoeveel dankwoorden kan ik op papier zetten? Eén ding is zeker, zonder jou was het dieseltreintje geen intercity geworden en was het eindstation nimmer bereikt. Ik heb genoten van onze wekelijkse bijeenkomsten die inspirerend en leerzaam waren. Je opmerkingen bij teksten waren sporadisch ontmoedigend, soms grappig (“dame, wat doet deze zin hier?”), meestal helder en altijd leerzaam.

Beste dr. MGH van Oijen, beste Martijn,

In de fase dat dit project als dieseltreintje reed, was jij degene die de motor soms iets extra brandstof gaf zodat het bleef rijden. Nadat ik bij jou in de “kelder” kwam wonen, heb je je over het project ontfermd en ben je steeds grotere rol gaan spelen. Ik ben er trots op dat ik je eerste (co)promovendus ben, velen zullen volgen.
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Curriculum Vitae
