CHAPTER 3

INCREASE OF SERUM GAMMA GLUTAMYLTRANSFERASE (GGT) ASSOCIATED WITH THE DEVELOPMENT OF CIRRHOTIC CYSTIC FIBROSIS LIVER DISEASE

PREDICTION OF CIRRHOTIC CYSTIC FIBROSIS LIVER DISEASE.

Submitted

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ABSTRACT

Background:

Cirrhotic liver disease (CCFLD) develops in 5-10% of CF patients. Identification of patients at risk on CCFLD is potentially beneficial for preventive treatment. We studied the evolution of liver enzymes (ALT, AST and GGT) in years preceding the diagnosis of CCFLD.

Methods:

We analyzed medical records of 277 pediatric CF patients. The time point of CCFLD diagnosis was defined as the date when the ultrasonographic macronodular liver and the clinical splenomegaly were first recorded. We analyzed liver enzymes in the 2 years preceding the diagnosis of CCFLD. We compared these results with the annual liver enzymes of no-CCFLD controls (>15 years of age and no ultrasonographic or physical signs of CCFLD).

Results:

At group level the median GGT, and not AST or ALT, of CCFLD patients, in the 2 years preceding their diagnosis, significantly higher than the median of all GGT results of no-CCFLD controls (45 vs. 17 U/l, respectively, P<0.001). The value of a single GGT result, for predicting future CCFLD, was low. However, for CF patients with a mean GGT>35 U/L, based on repeated measurements, the Odds ratio to develop CCFLD was 39 (95% CI: [9-175], sensitivity: 95%, specificity: 64%, positive predictive value: 50%).

Conclusion:

In pediatric CF patients a persistent, high-normal, serum GGT is strongly associated with the diagnosis of CCFLD within 2 years. The prognostic value of a single GGT measurement remains limited. Our results indicate that groups of patients at increased risk for CCFLD can be identified on the basis of repeated GGT measurements.
Cirrhotic cystic fibrosis liver disease (CCFLD) develops in 5-10% of cystic fibrosis patients (1). It is a serious complication of CF and patients with CCFLD tend to have a more severe CF phenotype than CF patients without liver disease (2, 3). CCFLD is characterized by extensive and often inhomogeneous cirrhosis. Clinically, CCFLD patients frequently have splenomegaly, hypersplenism and complications of portal hypertension, including variceal bleeding and ascites (4, 5). Liver synthesis and detoxification functions are often spared, and the need for primary liver transplantation for CF liver disease has remained rather rare (6, 7).

CCFLD is an acquired complication of CF and is not yet present in infancy. Most CCFLD patients develop the disease during childhood and have established disease before puberty (8). The diagnosis of CCFLD is mostly based on clinical, imaging and biochemical (liver enzymes) parameters. CCFLD patients develop splenomegaly and thrombocytopenia (9). The specific findings of hepatic nodularity and splenomegaly on ultrasound (US) are reported as reliable markers for advanced fibrosis with only limited discrepancy with liver biopsy (10). Histology of liver biopsies specimens typically shows a severe bridging type of portal fibrosis and proliferation and destruction of bile ducts (11).

The recognition CCFLD in an early phase and/or the identification of patients at risks may have relevant clinical and therapeutic consequences (12). To date the only treatment option used for CCFLD in clinical practice is ursodeoxycholic acid (UDCA)(13). It has been hypothesized that UDCA improves or recovers the compromised or obstructed bile flow in CF conditions. Theoretically, it would seem favorable to start treatment of CCFLD either in patients at risk (prevention) or an early phase of the disease. Not only UDCA, but also other bile salt analogues and anti-fibrogenic agents are currently under development and evaluated (14). Identification of patients at risk for CCFLD or in an early, preclinical stage of the disease would be of value to test the preventive and/or disease course modifying capacity of these agents.

Diagnosis of CCFLD is presently only possible in the cirrhotic phase of the disease. To date no reliable diagnostic tool or measurement are established to predict patients at risk for CCFLD or to recognize the early phase of the disease. Different clinical signs are suggested to be related to the development of CCFLD. However, no strong relations are established between, for example, hepatic US findings and the risk for the development of CCFLD (15, 16).

Liver enzymes such as the transaminases AST (aspartate transaminase) and ALT (alanine transaminases) and gamma-glutamyl tranpeptidase (GGT) are frequently evaluated during routine clinical checkups of CF patients (17). Elevated AST, ALT, and GGT are sometimes regarded as indicators for the presence or development of CFLD. Colombo et al. reported that UDCA treatment in CF patients frequently corrects the elevation of transaminases (18). It
needs to be realized, however, that elevation of liver enzymes occurs frequently and transiently in CF (19). These elevations of transaminases could be induced by hepatotoxic therapies like antibiotics or be a para-infectious phenomenon associated with pulmonary exacerbation.

The relation between liver enzymes and the presence of CCFLD was first studied by Potter et al. (17). These scientists related liver biopsy findings of 43 CF patients to their (ALT, AST and GGT levels at the time of biopsy. The biopsies were performed based on clinical indications for liver disease including hepatomegaly, abnormal liver function tests, splenomegaly or esophageal varices. Potter et al. found liver fibrosis (grades≤3) in 37% of their biopsies and that GGT, at the time of the biopsy, did not correlate with the presence of fibrosis. Unfortunately, no historical biochemical results were available to include in the analysis. Therefore, the results of this study could not address the role of liver enzymes during the development of CCFLD. Lindblad et al. reported on a historical cohort of liver biopsy results in 41 CF patients (4). Nine out of these 41 patients had histology proven cirrhosis, of which 5 indeed had clinical signs of cirrhosis. In this cohort the sensitivity of liver enzymes was 100% and the specificity 41% for the presence of moderate or severe fibrosis and cirrhosis.

In this retrospective, controlled, study we aimed to identify potential biochemical risk factors for the future development of CCFLD. Therefore we focused evolution of liver enzymes (ALT, AST and GGT) in years preceding the diagnosis of CCFLD in patients with already established cirrhosis.

**METHODS**

**Study cohort**

We performed a retrospective analysis in a cohort that contained all pediatric CF patients (2 – 18 years) from the Cystic Fibrosis centers of the University Medical Center Utrecht and the Beatrix Children’s Hospital, University Medical Center Groningen, The Netherlands (reference date January 1st 2007). According to the clinical protocols patients were seen and evaluated at least yearly in our CF centers. The annual medical checkup included blood testing for ALT, AST and GGT and ultrasonography of liver and spleen.

**Study method**

We defined the existence of cirrhosis as the ultrasonographic appearance of multilobular macronodularity of the liver and the presence of splenomegaly (10). For this purpose we reviewed the annual radiology reports for the ultrasonographic description of macronodularity. Additionally we reviewed medical records for reported an enlarged spleen
Increase of serum GGT associated with the development of cirrhotic cystic fibrosis liver disease

on physical examination or ultrasonographic enlarged spleen compared to the maximum references spleen size according to age (20). We reviewed all biochemistry results for ALT, AST and GGT. We defined the upper limit of normal for AST, ALT and GGT of 50 U/l (21). Since liver enzymes could be transiently elevated due to neonatal cholestasis in CF patients, unrelated to the development of CCFLD, we excluded liver enzymes results obtained before the age of 2 years. Based on these results we categorized patients into 4 groups:

A) Macronodularity and splenomegaly

B) Macronodularity without splenomegaly

C) Splenomegaly without macronodularity

D) No established macronodularity or splenomegaly

CCFLD study group

We considered group A (macronodularity and splenomegaly) as patients with an established diagnosis of CCFLD. We defined the date of CCFLD diagnosis as the first evaluation date on which the patient met the CCFLD criteria of ultrasonographic liver macronodularity and splenomegaly.

No-CCFLD control group

From group D (no macronodularity and no splenomegaly) we selected a no-CCFLD control group. This group consisted of CF patients that, at the age of 15 years, had never developed any signs of CCFLD (e.g. both normal liver ultrasounds and no reported splenomegaly). Of this group we use all the annually collected liver enzymes results after the second year of life.

Statistics

For statistical analysis, we used IBM SPSS version 20. For comparison of the nominal variables, we use the Chi square testing or Fischer exact, when appropriate. For comparison of the continuous variables, we used the Mann–Whitney U test. We use receiver operating characteristic (ROC) to determine AOC and the cutoff value. We used contingency tables to analyze frequency distribution and risk ratios.
Figure 1. Schematic representation of the composition of the studies population. Group A are patients with cirrhotic cystic fibrosis liver disease (CCFLD) defined as ultrasonographic macronodularity and splenomegaly, Group B are patients with macronodularity and no splenomegaly, group C are patients with splenomegaly without macro nodular disturbances of the liver on US and group D with no signs of CCFLD. Group D is referred to as the reference population. The study group contained all patients of group A of whom biochemistry results were available in period 2 years prior to the date of diagnosis of CCFLD. The control group was defined as CF patients out of the reference population that had not developed any signs of CCFLD (e.g. normal liver ultra sound and no splenomegaly) at the age of 15 years.
RESULTS

Study cohort

The total study cohort consisted of 277 pediatric CF patients (Figure 1). Nineteen (7%) patients met the criteria for established CCFLD (Group A). Additionally we identified patients with either ultrasonographic signs of macronodularity but no splenomegaly [group B: N=10 (4%)], or presence of splenomegaly but no macronodularity on liver ultrasound [group C: N=12 (4%)]. Two hundred thirty six (85%) patients had not displayed any signs of ultrasonographic macronodularity or splenomegaly (group D, reference group). In the current, retrospective, study we found a relatively high and variable age of diagnosis for CF. This is explained by the fact that in the Netherlands a nationwide neonatal CF screening program was introduced only in 2011 (Table 1).

We evaluated different potential risk factors or factors reported being related with the development of cystic fibrosis related liver disease (Table 1). Almost all patients with signs of liver involvement had started UDCA treatment. We found a significantly increased prevalence of DIOS in the two patients groups with macro-nodular abnormalities on liver ultrasound (group A and B). In patients with only splenomegaly (group C), on the other hand, the prevalence of DIOS was low.

We analyzed the relationships between liver enzymes and prevalence of liver involvement in CF (Table 1). We did this by evaluating the proportion of patients per study group, in which any liver enzymes results had been above the upper limit of normal (AST, ALT and GGT > 50 U/l). We found no significant higher proportion of patients with increased liver transaminases (AST or ALT) in the study groups with any signs of liver disease (group A, B and C) compared to group D. However, in the CCFLD patient (group A) we did observe a significantly higher proportion of patients with a GGT above the upper limit of normal of 50 U/l compared to the patients without any signs of liver disease (group D). The latter indicative for a potential relation between GGT elevations and the development or presence of CCFLD.

CCFLD study group

We evaluated the age of presentation of CCFLD (group A) in the study population. We determined the date of diagnosis as the first day patients met the defined criteria of CCLFD. The peak incidence of CF liver disease was around the age of 10 years. We found no patients who developed CCFLD before the age of 5 or after the age of 15 years. The median age at presentation of CCFLD in our population was 10 years. Almost all (95%) CCFLD patients from group A were treated with UDCA.
### Table 1. Description of clinical symptoms and biochemistry data of total studied pediatric CF population.

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>macronodularity and splenomegaly</td>
<td>macronodularity no splenomegaly</td>
<td>splenomegaly no macronodularity</td>
<td>no macronodularity no splenomegaly</td>
</tr>
<tr>
<td>Patients (N)</td>
<td>19 (7%)</td>
<td>10 (4%)</td>
<td>12 (4%)</td>
<td>236 (85%)</td>
</tr>
<tr>
<td>Median age at evaluation date (years)</td>
<td>16* (8-17)</td>
<td>11 (6-15)</td>
<td>13* (8-17)</td>
<td>10 (2-17)</td>
</tr>
<tr>
<td>Median age at diagnosis CF (days)</td>
<td>61 (4-4894)</td>
<td>84 (9-1009)</td>
<td>195 (16-3511)</td>
<td>126(0-3440)</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Clinical symptoms</th>
<th>N</th>
<th>%</th>
<th>P value</th>
<th>Odds ratio (CI)</th>
<th>N</th>
<th>%</th>
<th>N</th>
<th>%</th>
<th>N</th>
<th>%</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>13</td>
<td>(68%)</td>
<td>0.092</td>
<td>7</td>
<td>(70%)</td>
<td>8</td>
<td>(67%)</td>
<td>114</td>
<td>(48%)</td>
<td>142</td>
<td>(51%)</td>
<td></td>
</tr>
<tr>
<td>UDCA use</td>
<td>18</td>
<td>(95%)</td>
<td>&gt;0.001</td>
<td>72 (9-556)</td>
<td>10</td>
<td>(100%)</td>
<td>9</td>
<td>(75%)</td>
<td>47</td>
<td>(20%)</td>
<td>84</td>
<td>(30%)</td>
</tr>
<tr>
<td>DIO5</td>
<td>4</td>
<td>(21%)</td>
<td>0.009</td>
<td>5 (1-16)</td>
<td>4*</td>
<td>(40%)</td>
<td>1</td>
<td>(8%)</td>
<td>13</td>
<td>(6%)</td>
<td>22</td>
<td>(8%)</td>
</tr>
<tr>
<td>Meconium ileus</td>
<td>5</td>
<td>(26%)</td>
<td>0.112</td>
<td>3</td>
<td>(30%)</td>
<td>3</td>
<td>(25%)</td>
<td>31</td>
<td>(13%)</td>
<td>42</td>
<td>(15%)</td>
<td></td>
</tr>
<tr>
<td>Pancreatic insufficient (PERT)</td>
<td>19</td>
<td>(100%)</td>
<td>0.621</td>
<td>10</td>
<td>(100%)</td>
<td>12</td>
<td>(100%)</td>
<td>233</td>
<td>(99%)</td>
<td>274</td>
<td>(99%)</td>
<td></td>
</tr>
<tr>
<td>Severe genotype</td>
<td>19</td>
<td>(100%)</td>
<td>0.062</td>
<td>8</td>
<td>(80%)</td>
<td>11</td>
<td>(92%)</td>
<td>199</td>
<td>(84%)</td>
<td>237</td>
<td>(86%)</td>
<td></td>
</tr>
<tr>
<td>DF508/DF508</td>
<td>11</td>
<td>(58%)</td>
<td>0.622</td>
<td>6</td>
<td>(60%)</td>
<td>9</td>
<td>(75%)</td>
<td>150</td>
<td>(64%)</td>
<td>176</td>
<td>(64%)</td>
<td></td>
</tr>
</tbody>
</table>

| Biochemistry | ASAT>50 U/l | 2 | (14%) | 0.285 | 3 | (33%) | 3 | (30%) | 33 | (17%) | 41 | (18%) |
|              | ALAT>50U/l | 2 | (14%) | 0.543 | 0 | (0%) | 2 | (20%) | 24 | (12%) | 28 | (12%) |
|              | GGT>50U/l | 6 | (43%) | <0.001 | 16 (5-52) | 2 | (22%) | 2 | (20%) | 6 | (3%) | 16 | (7%) |

1 Reference population
2 Mann–Whitney U (either group A, B or C individually compared to group D)
3 Chi square test, group (either group A, B or C individually compared to group D)
*P<0.05
4 Severe genotype defined as CFTR classes 1-3 mutations
Table 2. Comparisons of study groups for GGT sub analysis.

<table>
<thead>
<tr>
<th></th>
<th>CCFLD Study group</th>
<th>No-CCFLD Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=14, 33 data sets</td>
<td>N=36, 205 data sets</td>
</tr>
<tr>
<td>Male</td>
<td>9</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>64%</td>
<td>61%</td>
</tr>
<tr>
<td>Severe genotype*</td>
<td>14</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>72%</td>
</tr>
<tr>
<td>DF508/DF508</td>
<td>7</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>50%</td>
<td>63.9%</td>
</tr>
<tr>
<td>Meconium ileus</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>21%</td>
<td>8.3%</td>
</tr>
<tr>
<td>Pancreatic insufficient</td>
<td>14</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>DIOS</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>14%</td>
<td>3%</td>
</tr>
<tr>
<td>UDCA use</td>
<td>13</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>93%</td>
<td>22%</td>
</tr>
</tbody>
</table>

*Severe genotype defined as CFTR class1-3 mutations

No-CCFLD control group

For an additional analysis, we established a no- CCFLD control group out of the patient group D, of which we assumed that they had and would not develop CCFLD (Figure 1). By definition, they were all older than 15 years of age at the time of study evaluation. The no-CCFLD control group consisted of 36 patients (Table 2). The control group contained a slightly higher proportion of males. Meconium ileus and DIOS had also occurred in the control group. Remarkably 22% of no-CCFLD control group patients had reported use of UDCA. Since in this group no splenomegaly or macronodularity has been found at any time, the indication for UDCA most likely was increased liver enzymes, then considered as an indication for UDCA in our centers.
Figure 2. GGT in CCFLD compared to no-CCFLD CF patients. The mean gamma-glutamyl transpeptidase (GGT) results of the period 2 years prior to the diagnosis cirrhotic cystic fibrosis liver disease (CCFLD) and GGT results of no-CCFLD CF (A). Relation between alanine aminotransferase (ALT) results and corresponding GGT results of CCFLD patient (closed black dots) 2 years prior to the diagnosis CCFLD compared and all corresponding GTT results(open gray dots, obtained between age 2-15 years, from CF patients without any signs of liver disease at the age of 15 years) (B). Receiver operating curve (ROC) analysis for the relation between presence of CCFLD and GGT results within 2 years prior to the diagnosis CCFLD or no-CCFLD controls shows a AOC of 0.9±0.1; P<0.001, at a cutoff value for GGT of 21 U/l (C)
Increase of serum GGT associated with the development of cirrhotic cystic fibrosis liver disease

Table 3. Overview of effects, of different cut off values for GGT, on diagnostic testing for risk of development of CCFLD

<table>
<thead>
<tr>
<th>Cutoff value</th>
<th>Specificity</th>
<th>Sensitivity</th>
<th>Odds ratio (CI)</th>
<th>PPV</th>
<th>NPV</th>
<th>NNT</th>
</tr>
</thead>
<tbody>
<tr>
<td>GGT &gt; 20U/l</td>
<td>82</td>
<td>93</td>
<td>57 (7-1206)</td>
<td>26</td>
<td>99</td>
<td>4</td>
</tr>
<tr>
<td>GGT &gt; 25U/l</td>
<td>87</td>
<td>71</td>
<td>16 (4-68)</td>
<td>27</td>
<td>98</td>
<td>4</td>
</tr>
<tr>
<td>GGT &gt; 30U/l</td>
<td>92</td>
<td>71</td>
<td>28 (7-119)</td>
<td>37</td>
<td>98</td>
<td>3</td>
</tr>
<tr>
<td>GGT &gt; 35U/l</td>
<td>95</td>
<td>64</td>
<td>39 (9-175)</td>
<td>50</td>
<td>98</td>
<td>2</td>
</tr>
<tr>
<td>GGT &gt; 40U/l</td>
<td>96</td>
<td>50</td>
<td>28 (7-127)</td>
<td>50</td>
<td>96</td>
<td>2</td>
</tr>
</tbody>
</table>

CCFLD patients vs. no-CCFLD control patients

We compared GGT results of the CCFLD patients in the 2 years preceding the diagnosis CCFLD (N=28 GGT measurements) with those of the no-CCFLD control group (N=205 GGT measurements). We found that the mean GGT in the CCFLD patient group was significantly higher than in the controls (45 vs. 17 U/l, respectively, P<0.001) (Figure 2A). However the mean GGT of the CCFLD group was still within the normal-high region. Elevation of GGT in CCFLD patients appeared, in most cases, an isolated event, i.e. increase in GGT was almost never accompanied by simultaneous elevation in ALT (Figure 2B). In the CCFLD group 82% of the patients had a repeated GGT level over 30 U/l in consecutive repeated measurements versus 18% in the no-CCFLD controls. Only 14% of the patients in the no-CCFLD control group ever showed a GGT over 35 U/l, however, in the CCFLD group this was 79%. In 2 (9%) of no-CCFLD controls the GGT was never below 30 U/l (N=5/5 measurements, range 30-141U/l) despite the fact that there were no ultrasonographic or physical signs of CCFLD in these patients. In the CCFLD group 58% of patients never had a GGT below 30 U/l.

To establish a cutoff value of the GGT above which the risk for developing CCFLD would be significantly and relevantly increased, we applied a ROC analysis. We used all the GGT results of the CCFLD study group and the no-CCFLD control group. If in the CCFLD group, more than one GGT results per patients was available in the period 2 years before the diagnosis CCFLD, we used the mean value of all available results. In this manner each CCFLD patient only contributed one GGT value to the analysis. We compared these mean GGT results of CCFLD patients to all the available GGT results of the control group. We found an AOC of 0.9±0.1 (P<0.001), and determined a cutoff value for GGT of 21 U/l (Figure 3C). To establish an indication for potential clinical relevance of cutoff values, we performed cross tabulations analysis (Table 3). This analysis shows the different consequences for Odds ratio, specificity,
sensitivity, positive predictive value and negative predictive value for different, arbitrarily, GGT cutoff values. In a potential interventional trail for prevention of CCFLD the numbers need to treat based on the applied cut of values would be highly relevant. Based on the different chosen cutoff values for GGT, we calculated the theoretical numbers needed to treat, and found them to vary from 2 to 4 (Table 3).

**DISCUSSION**

Cirrhotic liver disease is a severe and chronic complication of cystic fibrosis. CF patients in early stage of disease could benefit from preventive treatment strategies. However, to date, we do not have the possibility to test and identify patients at risk for CCFLD. In this study we found that, at group level, in pediatric CF patients in the period 2 years preceding their diagnosis CCFLD the serum GGT levels are significantly higher than in CF patients who never develop CCFLD. It is notable that although generally higher than in no-CCFLD controls, also in the CCFLD group GGT results often remain within the normal and high-normal range. However, for CF patients with a mean GGT>35 U/L, based on repeated measurements, the Odds ratio to develop CCFLD was 39. These results indicate that in pediatric CF patients a persistent, high-normal, serum GGT is strongly associated with the diagnosis of CCFLD within 2 years. However the prognostic value of a single GGT measurement remains limited. Our results do indicate that groups of patients at increased risk for CCFLD can be identified on the basis of repeated GGT measurements.

Recent studies in cirrhosis research indicate that progression of fibrotic liver diseases can be stopped or even reversed by removal of the causative agent or treatment of the underlying disease (22). In particular in the field of Hepatitis B and C disease, antiviral treatment is shown to be able to reverse the severity of fibrosis (23, 24). In addition, new developments are evolving concerning the use of anti-fibrotic therapies in forms of liver disease. Although theoretically promising, to date there have not yet anti-fibrotic therapies become available for humans (25). However the scope of these positive developments indicates the rising opportunity and potential profit for preemptive treatment in CCFLD. These scientific advances also indicate the need for reliable and relevant markers to identify patients at risk for CCFLD.

For future treatment strategies, aimed at preventing CCFLD, the number of patients that need to be treated, based on the used risk factors, would be relevant. To date, CF patients often start with UDCA treatment based on persistent elevation of liver enzyme above the upper limit of normal. Liver enzymes elevations, in particular AST and ALT, are rather frequent events in CF patient. Additionally AST and ALT elevation seem not to be strongly associated with the development of the cirrhotic form of CF liver disease. Therefore many patients are probably treated with UDCA even though they do not actually carry an increased risk for the
Increase of serum GGT associated with the development of cirrhotic cystic fibrosis liver disease

development of CCFLD. Our results indicate that a cutoff value for GGT of 35 U/l could be clinically relevant to identify patient at risks for the diagnosis CCFLD. When this cutoff value is used in patient selection for preemptive treatment strategies the numbers needed to treat would be two with high sensitivity, positive and negative predictive value and a relative acceptable specificity.

For most of our CCFLD patients, GGT results of repeated measurements were available, in the 2 years preceding the diagnosis of CCFLD. In our current analysis, we used the mean of available GGT measurements as an indicator for the course of GGT in this period. However, in the ROC analysis for the no-CCFLD group we used all available GGT results per patients from the age 2-15 years including outliers. We did not correct the no-CCFLD group for any isolated single GGT elevations. Still, only two no-CCFLD patients had a mean GGT, of all their GGT results, over 30 U/l. Therefore we feel that this methodology, that involved all available GGT results of the control group, strengthens our conclusions. Based on these findings we hypothesize that in a prospective study design that includes only persistent GGT elevation in the analysis; an even more distinct difference between CCFLD and no-CCFLD can be obtained.

The current study provides additional information on the presence of (isolated) elevated liver transaminases in CF patients. In the no-CCFLD control group we found that respectively 18% and 12% of patients had recorded episodes of AST and ALT elevation above the upper limited of normal. This observation indicates that increased transaminases are rather frequent events in pediatric CF patients, not related to future development of CCFLD. Based on this observation we conclude that elevation of transaminases are not a sensitive predictive biochemical markers for development of CCFLD. It is possible that elevation of transaminases is associated with other forms of CF related liver disease like for example, non-alcoholic steatohepatitis. Elevation of transaminases can also result from secondary hepatotoxic effects of medication or clinical infectious episodes..

We are aware that our retrospective study design has potential inherent weaknesses. First a retrospective analysis of the clinical follow up may not be as accurate and complete as desired. Secondly the date of the CCFLD diagnosis cannot be pinpointed to a specific day. However the date of diagnosis used in this study is based, on the rather random date, of the annual clinical checkup. Thirdly, for the no-CCFLD control group, we used a cut-off age of 15 years. This cut-off is based on the reasonable assumption that CCFLD does not develop anymore beyond this age. However, we do realize that it cannot be ruled out that new cases of CCFLD could still develop after the age of 15 years. Based on the above-listed limitations of the current study we realize that the reported data, ideally, need confirmation in a prospective study design.

We defined CCFLD as ultrasonographic macronodularity of the liver in combination with splenomegaly. However in our analysis, we also encounter patients with either macronodularity or splenomegaly and not the combination of both. We cannot exclude that
these observations indicate an earlier phase of CCFLD development. For ultrasonographic macronodularity, without splenomegaly, the only likely explanation is an advanced stage of fibrosis, however still in the absence of relevant portal hypertension. For isolated splenomegaly the situation is different and less clear. For example, splenomegaly has been described in cystic fibrosis patients, in the absence of any signs of liver disease (26). On the other hand Mueller-Abt et al. described a case of one patient with splenomegaly as the only pathologic ultrasonographic finding in a patient with liver histology that matched cirrhosis (10).

In summary, we found evidence that in pediatric CF patients, a persistent, high-normal, serum GGT is strongly associated with the diagnosis of CCFLD within 2 years. The prognostic value of a single GGT measurement however, remains limited. Our results indicate that groups of patients at increased risk for CCFLD can be identified on the basis of repeated GGT measurements.
REFERENCE LIST


