Cholangiocarcinoma is associated with a raised enhanced liver fibrosis (ELF) score independent of primary sclerosing cholangitis.

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Abstract

Background

Cholangiocarcinoma (CCA) complicates primary sclerosing cholangitis (PSC) in 10-20% of cases, but current tools for prediction of a CCA diagnosis are inadequate. Recently we demonstrated the utility of the ELF test to stratify prognosis in PSC. We observed that patients with PSC+CCA had significantly higher ELF score than those with PSC alone. In this study, we aimed to investigate further this association in a larger cohort of PSC patients with CCA compared with patients with PSC or CCA alone.

Materials and methods

Stored sera from patients with PSC (n=119), CCA without known chronic liver disease (n=36) and PSC+CCA (n=32) underwent ELF testing. ELF score, gender, age, age at disease diagnosis, inflammatory bowel disease, PSC duration and severity, and CCA features were compared amongst the three cohorts. Factors related to an elevated ELF score were investigated.

Results

ELF score was significantly higher in patients with CCA without underlying chronic liver disease and in patients with PSC+CCA compared to those with PSC alone ($p<0.001$). In multivariate analysis, elevated ELF score was associated with the diagnosis of CCA independently of age and PSC status ($p<0.001$).

Conclusions

ELF score was elevated in patients with CCA irrespective of the presence of PSC, and independently of liver disease stage. Our results indicate that the association between high ELF score and CCA may be related to the tumour's desmoplastic nature, independent of background liver fibrosis, suggesting that ELF score could

be used to risk stratify for CCA in PSC.

Introduction

Cholangiocarcinoma (CCA) is a malignant primary liver tumour originating from cholangiocytes, the epithelial cells lining the intrahepatic and the extrahepatic bile ducts, conventionally classified according to its localization within the biliary tree (intrahepatic, hilar, distal) [1, 2]. It is a relatively rare neoplasia in the Western countries, generally occurs after the fourth decade of life, mainly in men, and has a high mortality rate [3-5].

Primary sclerosing cholangitis (PSC) is a chronic liver disease characterised by chronic inflammation of the intrahepatic and/or extrahepatic bile ducts and in general progresses through liver fibrosis to cirrhosis and its complications [6-8]. The disease is associated with an increased risk of biliary tract cancer, in particular CCA. CCA occurs in 6-20% of PSC patients and is often (in approximately one third of cases) detected within the first year of PSC diagnosis [9-11].

In a recent study, we demonstrated that the enhanced liver fibrosis (ELF) test, a non-invasive marker of fibrosis based on three circulating markers of hepatic matrix metabolism (hyaluronic acid (HA), tissue inhibitor of metalloproteinases-1 (TIMP-1) and the propeptide of type III procollagen (PIIINP) [12]) is a useful prognostic tool in PSC and correlates with transplant free survival [13]. Furthermore, we observed that PSC patients with CCA had significantly higher ELF scores than those affected by PSC alone [13]. Both these findings have been subsequently validated in a large international multicenter study [14]. It is unclear whether the association of elevated ELF test with CCA diagnosed in PSC patients is due to the advanced stage of background liver fibrosis or it is related to the biological features of CCA. Indeed, CCA is characterised by an abundant desmoplastic reaction independent of surrounding tissue fibrosis that may affect

the results of the ELF test [15]. We therefore aimed to investigate this further in a large cohort of PSC patients with CCA compared to patients with PSC or CCA alone.

Patients and Methods

PSC was diagnosed on the basis of the typical cholangiographic findings on either magnetic resonance cholangiopancreatography or endoscopic retrograde cholangiopancreatography, according to validated criteria [7, 16]. Cases of secondary cholangitis and small duct PSC were excluded. PSC duration was defined as the time from the date of diagnosis to the date of serum sampling. Diagnosis of CCA was confirmed by histology and/or cytology. CCA patients affected by a concomitant liver disease other than PSC were not included. Diagnosis of inflammatory bowel disease (IBD) was based on endoscopic and histological findings and established by the currently accepted criteria [17, 18]. The study included 30 PSC patients with CCA and 119 PSC patients without cancer from a previous study [13], as well as 38 patients with CCA (2 with PSC) collected at the Royal Free London between July 2007 and April 2009 and stored at -80 °C. ELF test was performed using using ADVIA Centaur® XP system – Siemens Healthcare Diagnostics Inc. – Tarrytown, NY, USA, and the score calculated with the published algorithm, combining TIMP-1, HA, and PIIINP values: ELF score = 2.278 + 0.851 ln(CHA) + 0.751 ln(CP3NP) + 0.394 ln(CTIMP1) [19]. Interpretation of the ELF score was based on the cut-offs suggested by the manufacturer (<7.7: mild, 7.7-9.8: moderate, ≥9.8: severe fibrosis). Mayo risk score was calculated for all patients with PSC, using the

algorithm for the revised Mayo risk score [20] considering blood tests performed at the time of the serum collection for ELF testing. Biochemistry was performed using standard routine laboratory protocols.

Patients were divided in three groups: affected by PSC alone, affected by PSC and CCA (PSC+CCA) and affected by CCA without PSC or other concomitant liver disease (CCA alone). Demographics, clinical and biochemical characteristics were retrospectively recorded and compared amongst the 3 cohorts. Factors potentially related to an elevated ELF score were investigated in the overall cohort and in the subgroup of patients with PSC, with and without CCA.

Ethics

The study protocol was in accordance with the Declaration of Helsinki, following local ethical approval (London - Queen Square Research Ethics Committee, 06/Q0152/106; the Regional Committee for Research Ethics in Southeastern Norway, 2011/2572). All patients gave written informed consent.

Statistical analysis

Statistical analysis was performed using SPSS (version 20.0; IMB® SPSS®, Inc., Chicago, IL). Categorical variables are presented as frequencies (%) and were compared with the $\chi 2$ or Fisher's exact test. Continuous variables were tested for normal distribution (Kolmogorov-Smirnov test) and compared by Student t test or Mann-Whitney U test, accordingly. Normally distributed variables are reported as mean ± standard deviation (SD). Non-normally distributed data are presented as

medians (range). One-way analysis of variance (ANOVA) was used to compare means among the three groups and a post-hoc analysis was undertaken to assess the single comparisons. Multivariate binary logistic analysis was performed by logistic regression. Significance testing was 2-sided and set to ≤0.05.

Results

The study cohort consisted of 119 patients affected by PSC, 36 patients with CCA and 32 patients with both PSC and CCA. Baseline characteristics of patients are reported in **Table 1**. The differences between the three groups (PSC alone, PSC+CCA, CCA alone) are shown in **Table 2**. Male gender was more represented in all the three groups, while median age at the time of serum sampling was significantly lower in patients with PSC alone and higher in patients with CCA, with and without PSC (PSC alone < PSC+CCA < CCA alone, $p<0.001$ for all comparisons).

PSC patients with CCA were significantly older at PSC diagnosis than those without cancer ($p<0.001$) and had a significantly higher Mayo Risk Score at the time of ELF testing ($p<0.001$) (**Table 2**). A hundred and ten (74%) patients with PSC were affected by IBD, [88/119 (74%) in the PSC only group vs. 22/32 (69%) in the PSC+CCA group, $p=0.80$], but none of the patients with CCA only had a diagnosis of IBD.

Of the patients with CCA alone, location of CCA was available for 29 (80.5%) cases, and tumour stage was available in 27 (75%). Twenty-six (90%) patients had hilar and 3 (10%) intrahepatic CCA. Six (22%) patients had known metastatic disease. No statistically significant difference was found in ELF scores according

to the location or the stage (metastatic vs. non-metastatic) of the disease. Importantly, none of the patients with CCA alone was affected by a concomitant liver disease, and available imaging (ultrasound, computerised tomography scan, magnetic resonace) performed for staging of the disease did not detect features of cirrhosis or portal hypertension in this group.

ELF score

Mean ELF score in the overall cohort was 10.5 ± 1.7. Only 9 patients (4.8%), all belonging to the group with PSC only, had an ELF score compatible with the absence of significant fibrosis, while ELF score was compatible with moderate and advanced fibrosis in 68 (36.4%) and 110 (58.8%) patients, respectively. ELF score was significantly higher in patients with CCA alone and PSC+CCA compared to those with PSC alone ($p<0.001$), while no statistically significant difference was found in mean ELF score between patients with CCA alone and those with PSC and CCA (**Table 2, Figure 1**). Similarly, median levels of the individual components of the ELF test (HA, TIMP-1 and PIIINP) were significantly lower in patients with PSC alone compared to those with CCA ± PSC ($p<0.001$ in all cases), while no statistically significant difference was found between the PSC+CCA group and the CCA alone group (data not shown). Factors associated with a higher ELF score were investigated. On univariate analysis, ELF score was significantly associated with older age ($p<0.001$), diagnosis of CCA ($p<0.001$), concomitant IBD ($p<0.001$), higher Mayo risk score ($p<0.001$), more advanced age at PSC diagnosis ($p=0.02$) and PSC duration ($p=0.03$). There was not a statistically significant correlation between ELF and gender ($p=0.21$), age at CCA diagnosis ($p=0.66$) or CCA stage ($p=0.53$) (**Table 2**).

On multivariate logistic regression analysis, the only variable independently correlated with an elevated ELF score (≥9.8) was the diagnosis of CCA (OR 5.85, 95% CI 2.28-14.95, p<0.001) (**Table 2**). To further investigate its influence on ELF values, patients' age was dichotomized (<60 and ≥60 years). On univariate analysis, an age ≥60 years was significantly associated with an ELF value of ≥9.8 (p<0.001). On multivariate analysis, only a diagnosis of CCA maintained its independent correlation with an elevated ELF test (OR 6.63, 95% CI 2.64-16.63, p<0.001) (data not shown).

In the subgroup of patients affected by PSC, factors significantly associated with an ELF score ≥9.8, on univariate analysis, were age at ELF test (p=0.04), Mayo risk score (p<0.001) and diagnosis of CCA (p<0.001). On multivariate logistic regression analysis, Mayo risk score (OR 8.14, 95% CI 3.87-17.09, p<0.001), the diagnosis of CCA (OR 5.36, 95% CI 1.21-23.78, p=0.027) and older age at ELF test (OR 1.07, 95% CI 1.03-1.11, p=0.001) all remained significantly associated with an elevated value of ELF score (**Table 3**).

To study the role of ELF as a potential predictor of CCA in PSC, an analysis including only the patients with PSC (with and without CCA) was undertaken. In the subgroup of patients with PSC, the diagnosis of CCA was associated, on univariate analysis, with older age (p<0.001), more advanced age at PSC diagnosis (p<0.001), higher Mayo risk score (p<0.001) and an ELF score of 9.8 or higher (p<0.001) (**Table 4**). ELF optimal cut off for the diagnosis of CCA was 10.1 [AUC 74% (95%CI 0.64-0.83, p<0.001), 81% sensitivity, 60% specificity].

In multivariate analysis, only ELF score ≥9.8 (OR 4.91, 95%CI 1.19-20.21, p=0.027) and older age at PSC diagnosis (OR 1.09, 95%CI 1.00-1.18, p=0.048) were independently associated with CCA **(Table 4)**. When categorization of age

(<60 and ≥60 years) was applied, an age ≥60 years was not significantly associated to the diagnosis of CCA (p=0.078) in the univariate analysis, while ELF score ≥9.8 and older age at PSC diagnosis were confirmed to be the only factors independently associated with the diagnosis of CCA (**Table 4**).

Discussion

To our knowledge, this is the first study investigating the utility of liver fibrosis markers (ELF score) in CCA. In this cohort of patients with PSC, PSC with CCA and CCA alone, we found that ELF score was significantly elevated in patients affected by CCA, irrespective of the presence of PSC. On multivariate analysis, a diagnosis of CCA was the only factor independently associated with an elevated ELF score, irrespective of patients' age and stage of the liver disease.
Furthermore, in the subgroup of patients affected by PSC, an ELF score ≥9.8 was found to be an independent predictor of CCA.

PSC is a severe, progressive cholestatic liver disease that lacks effective treatment other than liver transplantation, with a highly variable natural history and a lack of validated prognostic tools [21]. This is particularly true for CCA, which is a significant threat to these patients in the absence of diagnostic tests for surveillance and early detection [11]. Cholangiocarcinoma is a malignant primary liver tumour originating from the bile ducts, with a generally poor prognosis and a high rate of mortality at 1 year [9, 22]. Generally rare, CCA is a major threat to PSC patients, with a reported 400-fold increased risk to develop CCA in PSC compared to the general population [23] and a lifetime incidence of up to 20% [24]. Current biomarkers such as serum Ca19-9 have low positive predictive value

and are inadequate for surveillance of PSC patients for CCA [23, 25], hence the hunt for better biomarkers or combinations of predictors are required.

The ELF test is a commercially available, well validated test which has demonstrated good diagnostic accuracy for the detection of moderate and severe fibrosis and proven good prognostic performance for predicting clinical outcomes in viral hepatitis, primary biliary cholangitis and non-alcoholic fatty liver disease/steato-hepatitis [26-28]. Recently, several reports have demonstrated the utility of ELF as a prognostic marker in PSC, showing that a higher ELF test is associated with reduced transplant-free survival [13, 14, 29]. Incidental findings of elevated ELF scores in patients with CCA amongst the PSC patients in these studies raised questions regarding the basis for this association, and in particular whether it was related to more advanced liver disease in PSC+CCA patients compared to patients with PSC alone or whether ELF score reflects some biological property of the tumours, thus introducing ELF test as a putative risk stratification tool in CCA surveillance.

The ELF score is based on an algorithm combining three circulating markers of hepatic matrix metabolism, reflecting the amount of fibrotic tissue in the liver [12]. In our cohort, however, ELF score was found to be significantly higher in the groups of CCA alone and PSC+CCA compared to the group of patients affected by PSC who did not have cancer. Since no patient with CCA alone had known underlying chronic liver disease, we doubt that a raised ELF reflects advanced liver fibrosis in these cases. Histopathologically, up to 95% of CCA are adenocarcinomas of moderate to poor differentiation, with characteristic mucin expression and highly desmoplastic stroma [30, 31]. The quantity of stromal desmoplasia, as well as the extracellular matrix proteins specifically produced by

cancer-associated fibroblasts have been reported as potential poor prognostic markers in CCA and have been associated with enhanced malignant behaviour of the cancer as well as to lower 1 and 3 year survival rates [15, 32]. We hypothesise that the association between high ELF score and CCA (with or without PSC) may be related to the biological nature of CCA, typically characterised by an abundant desmoplastic reaction [30, 31] likely leading to an increase of the extracellular matrix proteins turnover, or, less likely, to the tumour activation of signal pathways increasing production of the ELF components such as HA and TIMP-1 [33]. Of note, no single component of the ELF test was responsible for the augmented ELF score in patients with CCA, with HA, TIMP-1 and PIIINP all increased proportionally.

Cirrhosis of any aetiology has been reported to be an independent risk factor for CCA [34] and the Mayo risk score reflects a more advanced disease with greater liver fibrosis. The association of ELF score with patient's age is described [19, 35]. Ageing, in fact, increases susceptibility of the fibrotic response through a number of mechanisms such as reduced blood flow, increased oxidative stress, decreased number and dysfunction of mitochondria, accelerated cellular senescence and decreased regenerative ability [36]. It is also well established that ageing is directly associated with a progressive increase in the incidence of biliary tract cancer [1, 37, 38]. Thus, the significant association of an elevated ELF score with older age and higher Mayo risk score on univariate analysis is consistent. Nevertheless, in our cohort, CCA was the only factor independently associated with a high ELF score. Moreover, in the subgroup of patients affected by PSC, an ELF score ≥9.8 was an independent predictor of CCA, regardless of age and liver disease stage, although AUC was suboptimal. ELF score had in fact a good

sensitivity but a low specificity in identifying PSC patients with a diagnosis of CCA, indicating that the test cannot be used as a secure diagnostic tool, but can identify high-risk patients who should undergo further investigation including assessment of disease stage by other modalities and for evidence of CCA.

The limitations of this study include the retrospective design, the lack of information about tumour localization and stage in a number of patients, as well as the lack of a validation cohort, although this is partly remedied by supporting data from a recent multicenter study [14]. Similarly to what we observe here, in this large international cohort the subgroup of patients with PSC with CCA (n=15) was found to have significantly higher ELF test levels than patients with PSC without CCA (n=519) [median ELF 10.5 (IQR 9.2-11.8) and 9.1 (IQR 8.0-10.2), respectively; p=0.035] [14].

Finally, data about comorbidities which could potentially affect ELF values, such as cardiovascular and chronic obstructive pulmonary diseases and chronic inflammatory skin or joint disease [39], were lacking for most patients and were not included in the analysis. However, we expect the prevalence of the above-mentioned potential confouders to be very low in this population as demonstrated recently in a larger Norwegian PSC cohort [40]. Nevertheless, such comorbidities should be carefully considered in future studies.

Conclusion

The ELF score in this study was significantly elevated in patients with CCA, irrespective of the presence of PSC, and a diagnosis of CCA was associated with an elevated ELF score independent of age or liver disease stage. This suggests that

the ELF score and other more specific markers of matrix turnover may be a useful tool for identifying PSC patients at higher risk of developing CCA. This finding warrants further validation, preferably in a prospective setting.

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Figure 1. Box and whisker plots of ELF scores in patients with PSC alone, CCA alone and PSC+CCA.

Abbreviations: ELF, enhanced liver fibrosis; PSC, primary sclerosing cholangitis; CCA, cholangiocarcinoma.