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Research Article

Serum endocan levels do not predict hepatocellular carcinoma development in cirrhosis

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Abstract. Objective: Cirrhotic patients are at increased risk of developing liver malignancy with a dismal impact on prognosis. Novel accurate biomarkers are vital to increase early detection, monitor recurrence and thus improve outcome. We aimed to determine if increased concentrations of endocan, a novel biomarker of angiogenesis investigated in hepatocellular carcinoma (HCC), can detect very early liver malignancy in a cohort of prospectively enrolled patients with cirrhosis. Material and method: We conducted a retrospective analysis of a prospective cohort of patients with cirrhosis and no evidence of HCC according to ultrasound criteria on enrolment. Patients were followed-up every 6 months through abdominal imaging and routine blood tests. Endocan was measured from serum samples taken at baseline by commercially available ELISA kits. Results. 85 cirrhotic patients (mean age 59±10 years; 36 female) followed up for a median of 18 months were analyzed. The etiology of cirrhosis was chronic alcohol abuse in 33 patients, HCV in 32, HBV in 12 and mixed/other in 8 patients. 46 were Child A and 39 Child B/C with a median MELD score of 12±5 for the cohort. During follow-up 5 patients (5.8%) developed HCC and 3 of them died. There was no significant difference between patients with and without HCC regarding endocan (p=0.9). Conclusion. Endocan levels are significantly increased in cirrhosis but do not predict the development of hepatocellular carcinoma during follow-up.

Key Words: cirrhosis; liver neoplasms; endocan; follow-up studies; ultrasound.

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Introduction

Cirrhotic patients are at increased risk of developing liver malignancy with a dismal impact on prognosis (EASL Clinical Practice Guidelines 2018). Current epidemiological trends suggest that the incidence of hepatocellular carcinoma is on the rise, particularly due to an increase in the incidence of non-alcoholic fatty liver disease (NAFLD) (Lee et al 2021) and the medical costs and socio-economic burden related to HCC are a significant burden for the healthcare system (Flores et al 2021, Fouad et al 2021). As a direct consequence, a host of novel biomarkers have been studied with the aim of increasing early detection, monitor recurrence, predict disease response to therapy and thus, ultimately, improve patient-related outcomes (Huang et al 2021, Zhang et al 2020, Liu et al 2021).

Current screening strategies for hepatocellular carcinoma (HCC), based on a combination of transabdominal ultrasound and serum alpha-fetoprotein (AFP), offer a modest benefit in terms of cancer-related mortality reduction (Moon et al 2018, Su et al 2021, Lersritwimanmaen et al 2018). However, the utility of AFP in predicting new-onset or recurrence of HCC development in cirrhotic patients is limited (Liang et al 2021, Ungtrakul et al 2016) and the high-quality examination required of a correct screening ultrasound in liver disease is not always accessible. As a result, novel non-invasive biomarkers which can detect early HCC with good accuracy are needed.

We hypothesized that endocan, a biomarker of angiogenesis which our group has recently investigated in liver cirrhosis (Voiosu et al 2018), could aid in detection of very early hepatocellular carcinoma. We aimed to determine if increased serum levels of endocan at baseline are associated with the development of liver malignancy in a cohort of prospectively enrolled patients with cirrhosis.

Materials and method

We conducted a retrospective analysis of an inception cohort of 85 consecutive patients with clinical or histological evidence of liver cirrhosis who were enrolled in a prospective manner for a follow-up study at Colentina Clinical Hospital between November 2013 and May 2015. Patients with severe comorbidities (e.g. severe lung or heart disease), confirmed malignancies, active infections, acute hepatitis and renal failure were excluded. All patients were evaluated at the index visit thorough medical history, complete physical examination, routine blood work and Child-Pugh and Model for end-stage liver disease (MELD) scores were used to assess the severity of cirrhosis. Concurrent medication, especially use of betablockers and aldosterone antagonists was recorded in the study files and updated during follow-up. Patients were examined through abdominal ultrasound at enrollment in order to exclude potentially relevant liver nodules. To increase the quality of the ultrasound and prevent missed lesions, the evaluation was performed while on a low-sodium diet and, where applicable, 48 hours after large-volume paracentesis. Paracentesis that revealed malignant cells was considered as an exclusion criterion for this study.

We collected serum samples from all patients at the index visit and refrigerated them at -80°C. The endocan measurements were made with a commercial sandwich ELISA kit from Lunginnov© (Lille, France), according to the manufacturer's instructions (results expressed as nanograms per mililter ng/mL). Samples from controls were diluted two-fold and patient samples were diluted four-fold; all samples were assayed in duplicate. We used a minimum level of detection for the assay of 0.132 ng/mL. The absorbance was measured at 450 nm on a spectrophotometer (BioRad, Hercules, CA, USA) and a 4-parameter logistic regression curve was used for standard curve fitting analysis.

The initial study included a rigorous telephonic follow-up at every 3 months up to 24 months (median 12 months) and standard of care evaluations including abdominal ultrasound every 6 months, according to our protocol for HCC screening. All liver disease-related complications were recorded at each visit. Three patients were lost to follow-up and one received a liver transplant during the study period.

The main outcome of interest for this study was the development of ultrasound-detected HCC which was confirmed through typical aspect on contrast-enhanced cross-sectional imaging. Liver nodules of uncertain significance or under 1 cm in diameter were followed-up according to protocols but were not counted as HCC in this analysis. None of the nodules of uncertain significance were considered to require biopsy for diagnostic purposes during the analyzed study period.

Statistical analysis was performed using the SPSS 16 software (Chicago, Il, USA). Results are reported as means and standard deviations for variables with a normal distribution, and median, minimum and maximum respectively for variables with a non-normal distribution. Univariate analysis was conducted using the Chi-square test for nominal variables, nonparametric tests for variables with nonnormal distribution (Mann Whitney U) and student t-test for variables with normal distribution. Analysis was 2-tailed, with a $p<0.05$ considered statistically significant.

We obtained written informed consent from each patient before inclusion. This study was approved by the local ethics committee (Colentina Hospital Ethical Committee for Clinical Research) and respected the principles of the 1975 Declaration of Helsinki.

Results

Eighty-five patients with cirrhosis (mean age 59±10 years; 36 female), followed-up for a median of 18 months, were included in the final analysis. The etiology of cirrhosis was alcohol-related in 33 patients, HCV in 32, HBV in 12 and mixed/other in 8 patients. Most patients had compensated liver disease at baseline evaluation based on their Child-Pugh score (46/85), with further demographic data presented in Table 1.

During follow-up 5 patients (5.8%) developed HCC and 3 of them died. Endocan median levels at baseline were not significantly higher in patients who developed HCC compared to those who did not develop HCC: 5.88 (5.76-6.72) ng/mL vs 6.70 (3.96-9.74) ng/mL, p=0.9, Mann-Whitney U) (Figure 1).

Overall, 22 patients in the entire cohort died during follow-up. In this subgroup, baseline endocan levels were not different between patients who had developed HCC by the time of their death compared to patients who did not;5.8 (3.8-9.1) ng/mL vs. 7.8 (5.8-9.8) ng/mL, p=0.13, Mann-Whitney U.

However, endocan levels were significantly higher in patients who suffered any adverse event (decompensation of liver disease, development of HCC or death) compared to those who did not:4.5 (5.1-10.4) ng/mL vs. 3.6 (3.4-8.4) ng/mL, p=0.029, Mann-Whitney U).

Table 1. Baseline characteristics of the study population

Age (years, SD)	**59.3 (±10.4)**
Gender (male / female)	49/36
Etiology of liver disease:	
Alcohol-related liver disease	33
HCV	32
HBV	12
Mixed viral	5
Other	3
Smoker (yes/no)	29/56
BMI (kg/m², SD)	26.8 (±4.5)
Child-Pugh Class	
A	46
B	17
C	22
MELD Score	12.7 (±4.9)
Betablocker use (yes/no)	25/60
Spironolactone use (yes/no)	37/48
Follow-up lenth (months, SD)	16.7 (±6.7)
Endocan concentration (ng/mL, SD)	7.4 (±4.3)

SD – standard deviation, kg/m2 – kilogram per square meter, HCV – hepatitis C virus, HBV – hepatitis B virus, BMI – body mass index, MELD – model for end-stage liver disease, ng – nanogram, mL - milliliter

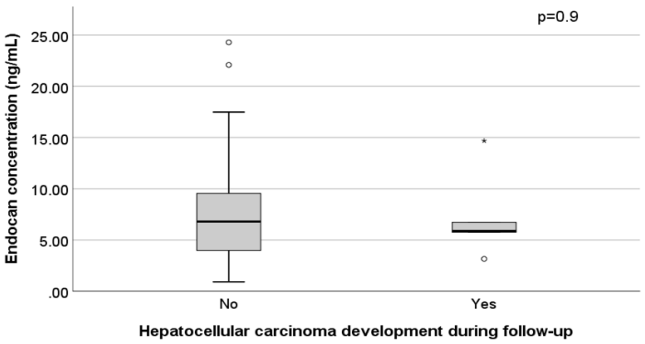

Figure 1. Incidence of HHC

Discussion

The main finding of this study is that endocan concentration are elevated in patients with cirrhosis but do not predict the development of hepatocellular carcinoma in a short follow-up period. Patients with chronic liver disease are at increased risk of hepatocellular carcinoma due to profound structural changes in liver architecture and perturbed homeostasis induced by continuous cellular aggression and inflammation (Ramakrishna et al 2013) Certain pathogens, such as the hepatitis B virus with its genomic integration into host cellular DNA, can directly lead to carcinogenesis even in pre-cirrhotic stages, but a cirrhotic liver,

regardless of etiology, is prone to neoplastic transformation. Therefore, current guidelines recommend that all patients with cirrhosis are offered inclusion in a screening program with transabdominal ultrasound performed every 6 months. However, ultrasound can be difficult to perform in patients with massive ascites or enlarged livers, is machine and operator-dependent, and can miss some early discrete lesions. Serum markers such as AFP are routinely used in some screening programs and in surveillance after treatment but its sensitivity has been shown to be limited (Zhang et al 2020). The dynamics of biomarkers according to the stage of carcinogenesis are extremely variable and a serum marker which peaks very early could prevent delays in diagnosis (Han et al 2018)

Endocan is a serum biomarker implicated in various inflammatory and vascular regulatory pathways and is also overexpressed in tumor cells (Yang et al 2015). Its overexpression is associated with aggressive tumor progression and poor outcomes in various cancers, such as breast cancer, renal cell cancer, lung cancer, gastric cancer, and pituitary adenomas as well as with tumor recurrence in patients with pancreatic neuroendocrine tumors (Lin et al 2017, Yang et al 2020). Our previous investigations have established that normal endocan levels in a healthy population are generally below 3 ng/mL (Voiosu et al 2014, Bălănescu et al 2016), and we have shown that concentrations are increased in cirrhosis. In this study, we hypothesized that a single measurement of a biomarker could identify very early stages of HCC in a population at high risk for the development of this disease. Our cohort was composed of patients in whom baseline examination did not identify suspicious liver nodules in an attempt to use endocan as a very early warning system for HCC. Only 5 patients developed HCC during the study period and they did not have significantly higher baseline endocan values compared to the other patients. Endocan seems to correlate with severity of cirrhosis and risk of severe complications but it does not appear to reflect the presence of very early carcinogenesis in the liver.

Our study has certain limitations. It is a retrospective analysis based on serum available from a biobank collected in a prospective fashion, therefore it may lack adequate power to detect a significant association between baseline endocan concentrations and the development of HCC. Although we excluded patients with acute infections and relevant documented comorbidities, it is possible that a concurrent inflammatory state, which is frequently found even in clinically-stable cirrhotic patients, may have influenced our measurements and confused the results. However, a recent study has shown that endocan can differentiate between inflammatory and neoplastic disease in other solid organ pathology, with increased concentrations being more likely in cancer (Basim et al 2020). The patients were rigorously followed up for the duration of the study, which is evident from the low drop-out rate, but the outcome of interest (HCC) and sample size is limited. Longer follow-up might have revealed a difference in the predictive role of endocan for the development of HCC and prospective studies with serial measurements of endocan are worth exploring.

In conclusion, endocan concentrations in patients with cirrhosis do not predict the development of hepatocellular carcinoma during a moderate follow-up period.

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