Research Article

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The long-term benefits of nucleos(t)ide analogs in compensated HBV cirrhotic patients with no or small esophageal varices: A 12-year prospective cohort study

Pietro Lampertico[1], Federica Invernizzi[1], Mauro Viganò[2], Alessandro Loglio[1], Giampaolo Mangia[1], Floriana Facchetti[1], Massimo Primignani[1], Manol Jovani[1], Massimo Iavarone[1], Mirella Fraquelli[3], Giovanni Casazza[4], Roberto de Franchis[5], Massimo Colombo[1,*]

[1]*"A.M. and A. Migliavacca" Center for Liver Disease, Division of Gastroenterology and Hepatology, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Università degli Studi di Milano, Milan, Italy;* [2]*Division of Hepatology, Ospedale San Giuseppe, Università degli Studi di Milano, Italy;* [3]*Division of Gastroenterology and Endoscopy, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Università degli Studi di Milano, Milan, Italy;* [4]*Department of Biomedical and Clinical Sciences, Ospedale Luigi Sacco, Università degli Studi di Milano, Milan, Italy;* [5]*Division of Gastroenterology, Ospedale Luigi Sacco, Università degli Studi di Milano, Milan, Italy*

Background & Aims: Esophageal varices (EV) are a marker of disease severity in compensated cirrhosis due to hepatitis B virus (HBV) which predicts also the risk of hepatocellular carcinoma (HCC), clinical decompensation and anticipated liver related death. The dynamics and prognostic significance of EV in patients under long-term HBV suppression by nucleos(t)ide analogs (NUC), are poorly known.

Methods: A standardized protocol (Baveno) including 414 upper gastrointestinal (GI) endoscopies was applied to 107 HBeAg-negative compensated cirrhotic patients (93% Child-Pugh A) during a median of 12 (range 2 to 17) years of NUC therapy. Patients who initially started on lamivudine (LMV) and then developed resistance (LMV-R), were rescued by early administration of adefovir, or were switched to tenofovir. Surveillance included serum HBV DNA every three months and abdominal ultrasound every six months.

Results: Twenty-seven patients had baseline F1 EV which regressed in 18, remained unchanged in eight and progressed in one patient; the 12-year cumulative incidence of EV regression was 83% (95% CI: 52–92%). *De novo* F1/F2 EV developed in 6/80 patients with a 12-year cumulative incidence of 10% (95% CI: 5–20%). Six of seven patients with *de novo* varices or progression of pre-existing varices had either a clinical breakthrough due to LMV-R and/or developed a HCC. No bleedings from ruptured EV occurred, 12 patients died (9 HCC) and 15 were transplanted (13 HCC): the 12-year cumulative incidence of HCC and overall survival was 33% (95% CI: 24–42%) and 76% (95% CI: 67–83%), respectively.

Conclusions: Long-term pharmacological suppression of HBV in HBeAg-seronegative patients with compensated cirrhosis leads to a significant regression of pre-existing EV accompanied by a negligible risk of developing *de novo* EV.

Keywords: Hepatitis B virus; Cirrhosis; Esophageal varices; Nucleos(t)ide analogs; Gastrointestinal bleeding; Hepatocellular carcinoma; HBsAg clearance; Transient elastography.

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* Corresponding author. Address: Division of Gastroenterology and Hepatology, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Università di Milano, Via F. Sforza 35, 20122 Milan, Italy. Tel.: +39 0255035432; fax: +39 0250320700.
E-mail address: massimo.colombo@unimi.it (M. Colombo).

Abbreviations: EV, esophageal varices; NUC, nucleos(t)ide analogs; GI, gastrointestinal; LMV, lamivudine; LMV-R, lamivudine resistance; HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HVPG, hepatic venous pressure gradient; EGDS, esophago-gastroduodenoscopy; PHG, portal hypertensive gastropathy; GOV, gastro-esophageal varices; IGV, isolated gastric varices; LT, liver transplantation; RWM, red wale marks; HIV, human immunodeficiency virus; US, abdominal ultrasound.

Introduction

Progression of portal hypertension is a well-established predictor of poor outcome in patients with cirrhosis of any etiology, as it is associated with an increased lifetime risk of clinical decompensation, development of HCC and anticipated liver death [1,2]. In addition, the functional derangement of the liver associated to portal hypertension limits patient access to cytotoxic therapies and local ablative or surgical treatment of HCC, which in the era of oral antiviral therapy for HBV infection, has become the relevant cause of death in this population [3–5]. In treatment naïve patients with cirrhosis of any etiology, esophageal varices (EV), a common hallmark of portal hypertension, develop and progress in size at a yearly incidence of approximately 10% and up to 30%, respectively, thus setting the stage for a life threatening risk of gastrointestinal (GI) bleeding [6–15]. As permanent suppression of virus replication by nucleos(t)ide analogs (NUC) has become an achievable endpoint in virtually all HBV patients, evidence is mounting that long-term NUC therapy effectively delays clinical progression of HBV and prevents clinical decompensation [3,4,16–18]. In a landmark study where Asian pacific patients with

advanced liver fibrosis due to HBV were randomized to receive lamivudine (LMV) or placebo, active therapy was shown to cause a significant reduction of the risk of GI bleeding from EV, however, without providing any insight on whether development or progression of EV were attenuated in parallel [19]. Circumstantial evidence, indeed, suggests that this may be the case in patients undergoing successful HBV therapy: re-analysis of the roll-over studies with entecavir (ETV) and tenofovir (TDF) provided in fact histological evidence of either regression or amelioration of cirrhosis in the majority of patients who achieved long-term suppression of virus replication [17,20]. This was also the message of two small reports in which a short course of LMV monotherapy led to some improvement of EV accompanied by a partial reduction of hepatic venous pressure gradient (HVPG) in patients with HBV-related cirrhosis [21,22]. Limiting the translational value of these observations, however, were the lack of statistical power, patients stratification by LMV-resistance, data on baseline and on treatment dynamics of EV, inclusion of both compensated and decompensated cirrhotic patients and more importantly, a follow-up of 12–27 months only.

To gain more insights on the response of portal hypertension to NUC therapy in HBV cirrhosis, 107 patients with HBeAg-negative compensated cirrhosis entered a long-term protocol-based surveillance for EV following initial therapy with LMV where resistance to LMV (LMV-R) was managed by early add on adefovir dipivoxyl (ADV) or switching to TDF.

Patients and methods

Starting from 1997, patients with HBeAg-negative compensated cirrhosis were recruited to an investigator-initiated prospective cohort study of long-term LMV treatment where add on ADV or TDF switch was planned to treat LMV-R [16,23]. The last patient was enrolled at the beginning of 2003. Surveillance for EV was part of the protocol. All patients gave their written consent to participate in the study, which was approved by local Institutional Review Committees.

Patients

All HBeAg-negative patients with cirrhosis due to HBV who received LMV at the Liver Center, Ospedale Maggiore Policlinico, Milan and had an esophago-gastroduodenoscopy (EGDS) performed within six months before LMV therapy, were enrolled. Excluded were patients with decompensated cirrhosis (Child-Pugh >B7), a previous history of GI bleeding, liver disease of other etiology, alcohol abuse (>60 g/day for men and >40 g/day for women assessed by patient's clinical interviews), organ transplantation, evidence of extra-hepatic neoplasia, portal or splenic vein thrombosis, and severe cardiac or pulmonary disease, HCC, HCV coinfection and either medium or large varices requiring prophylaxis with betablockers or banding.

From the original cohort of 124 patients [23], ten patients were excluded, two for HCC, one for co-infection with HCV and seven for medium to large varices under prophylaxis with either betablockers (n = 5) or banding (n = 2). Among the latter patients, five had severe portal gastropathy, yet none bled from ruptured EV during follow-up. Endoscopic findings during follow-up improved in four, remained unchanged in two, and worsened in one patient who developed a HCC. Four patients underwent liver transplantation (LT) (two for HCC, two for end-stage liver disease) after 55 months of follow-up (range 12–69), whereas two patients were lost after a follow-up of 21 and 58 months, respectively. The remaining patient had EV eradicated by banding and has currently been followed up for 173 months.

Of the remaining 114 patients with F0 or F1 varices at baseline, seven were excluded owing to the lack of at least one EGDS during follow-up for the following reasons: one patient died at 12 month of follow-up due to colorectal cancer, one patient underwent LT at month 21 for HCC, one patient developed HCC at month six and was referred to another center for surgery, three patients refused follow-up upper endoscopy and one patient was lost of follow-up. The baseline clinical, virological and demographic features of the 107 compensated cirrhotic patients who were enrolled in the study were similar to those of the excluded ones (data not shown). The 107 enrolled patients were to be followed for the purpose of study analysis till the first upper GI bleeding, endoscopic or medical treatment of EV, LT, death or until December 2013.

Upper gastrointestinal endoscopy

EGDS (Olympus GIFQ145, Hamburg, Germany) were performed within six months before LMV treatment and during follow-up according to international guidelines on portal hypertension [13–15,24,25] by two skilled endoscopists (MP, RdF), who were blinded to treatment outcome. In brief, compensated patients without varices, had endoscopy to be repeated at 2–3-year intervals in order to evaluate onset of *de novo* varices, whereas compensated patients with small varices, had endoscopy to be repeated at 1–2-year intervals to evaluate progression of varices [13,14]. EV were classified according to the North Italian Endoscopic Club criteria: location and size (F, graded 0–3) and red wale marks (RWM, graded from – to +++) [7]. Management of EV during follow-up was according to international guidelines [12–15,24,25].

Measurements

Routine laboratory analysis and serum HBV DNA were assessed every three months. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured by automated method at 37 °C (normal value ⩽40 IU/L). Commercially available enzyme immunoassays were used to determine serum antibodies to HBV surface antigen (anti-HBs), hepatitis B e antigen (HBeAg), antibodies to HBeAg (anti-HBe), anti-hepatitis Delta and anti-HIV (AXSYM, Abbott Laboratories, North Chicago, IL, USA). Anti-hepatitis C virus was assessed by a second-generation enzyme-linked immunoassay (Ortho Diagnostic System, Raritan, NJ, USA). Serum HBV DNA was measured using Versant 3.0 (branched DNA, Bayer Corporation, Tarrytown, NJ, USA) with a lower limit of quantification of 357 IU/ml till 2004, by polymerase chain reaction assay, CobasAmplicor HBV Monitor Test v2.0 (Roche Diagnostics, Mannheim, Germany) with a lower limit of quantification of 71 IU/ml from 2004 until 2008 and subsequently by real time polymerase chain reaction assay, CobasTaqMan HBV Test v2.0 (Roche Molecular Systems, Inc., Branchburg, NJ, USA) with a lower limit of quantification of 12 IU/ml. HBV genotypes and mutations in the HBV polymerase region were looked for by linear probe assays (INNO-LiPA HBV genotyping and INNO-LiPA HBV DR, Innogenetics NV, Belgium) [26].

Transient elastography (TE)

Liver stiffness (LSM) was measured using transient elastography (FibroScan® Echosens, Paris, France) by trained operators (MF, FI) as already described [27]. Starting from 2006, when the technique became available in our department, all patients were tested by Fibroscan on yearly basis. LSM, expressed in kilopascals (kPa), with at least ten successful measurements, a success rate higher than 60% and an inter-quartile ratio (IQR) less than 30%, were considered reliable. As previously reported in untreated patients with chronic HBV infection, a cut-off value ⩽9.4 kPa and >13.1 kPa had >90% sensitivity and specificity to exclude and confirm the histological diagnosis of cirrhosis, respectively [28].

Surveillance and treatment

Baseline examinations included abdominal ultrasound (US), body mass index and Child-Pugh score [29,30] whereas the surveillance protocol for HCC was according to international recommendations based on abdominal US every six months [31]. Until 2001, in patients with a newly detected liver nodule HCC diagnosis was pursed by computed-tomography (CT) followed by a US-guided fine-needle biopsy [32]; after 2005, HCC was primarily diagnosed by non-invasive internationally agreed criteria whereas biopsy was employed to diagnose nodules with an uncertain contrast pattern [33,34]. Ascites was diagnosed and treated according to international criteria, as well [35,36]. The initial treatment of HBV was LMV monotherapy 100 mg/day for all patients and drug resistant patients were rescued by adding on ADV 10 mg/day from 2003 and by switching to TDF 245 mg/day from 2008 [37–39].

Definitions

Cirrhosis was diagnosed by histology or on clinical grounds by using abdominal US features of blunted, nodular liver edge accompanied by splenomegaly (>13 cm) and $<100 \times 10^3$/μl platelets. Persistent HBV suppression meant HBV