OUTCOME OF CHRONIC DELTA HEPATITIS IN ITALY: A LONG-TERM COHORT STUDY

Grazia Anna Niro[1], Antonina Smedile[2], Antonio Massimo Ippolito[1], Alessia Ciancio[2], Rosanna Fontana[1], Antonella Olivero[2], Maria Rosa Valvano[1], Maria Lorena Abate[2], Domenica Gioffreda[1], Paolo Caviglia[2], Mario Rizzetto[2], Angelo Andriulli[1]

Divisions of Gastroenterology, ñCasa Sollievo Sofferenzaò Hospital, IRCCS, San .Giovanni Rotondo [1], and ñSan Giovanni Battistaò Hospital, University of Turin, Turin [2], Italy

Short title: Outcome of chronic HDV infection

Index terms: delta hepatitis, delta hepatitis infection, chronic liver disease, outcome, HDV, HBV, cirrhosis, interferon, HCC.

Correspondence to:
Grazia Anna Niro, M.D.,
IRCCS ñCasa Sollievo della Sofferenzaò, Gastroenterology Unit
Viale Cappuccini 1,
71013 San Giovanni Rotondo (FG), Italy.
TEL: + 39 0882 410263
FAX: +39 0882 835411
e-mail: g.niro@operapadrepio.it

Electronic word counts:

Abstract: 241

Text: 4502

Number of Figures: 4

Number of Tables: 5

Abbreviations: HDV, hepatitis delta virus; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; anti-HDV, antibody to hepatitis delta virus; HIV, human immunodeficiency virus; IgG anti HDV, IgG antibody to hepatitis delta virus; IgM anti HDV, IgM antibody to hepatitis delta virus; PCR, polymerase chain reaction; HCV, hepatitis C virus; ALT, alanine aminotransferase; AST, aspartate aminotransferase; IU, International Units; HBsAg, hepatitis B surface antigen; anti-HBs, antibody to hepatitis B surface antigen; anti-HBe, antibody to hepatitis B e antigen; CT computerized tomography.

ABSTRACT

Background/Aim: To investigate the impact of HDV infection on morbidity and mortality of patients.

Patients/Methods: Retrospective study on 188 patients, included into a program of periodic surveillance until 2008. Demographic data, stage of liver disease, treatment efficacy, development of liver complications (ascites, oesophageal bleeding, encephalopathy), and survival were registered. Cox regression analysis was carried out to determine the impact of viral and patient features on survival.

Results: At baseline, 126 patients (67%) tested positive for serum IgM anti-HDV antibodies, 171 (91%) for anti-HBe, 175 (91%) for serum HDV-RNA, and 61 (33%) for serum HBV-DNA. Eighty-two patients (43%) had chronic hepatitis at histology, the remaining 106 individuals a clinical/histological diagnosis of cirrhosis. Ninety-six patients received interferon (n=90), or lamivudine (n=6) therapy, and 27 of them (30%) attained a sustained response. During follow up 21 patients with chronic hepatitis progressed to cirrhosis. Of the 127 cirrhotic patients, hepatic decompensation occurred in 42 patients (33%), and hepatocellular carcinoma in 17 (13%). The 5- and 10- year survivals free of events were 96.8% and 81.9% for patients with chronic hepatitis, and 83.9% and 59.4% for cirrhotics ($p<0.01$). At multivariate analysis, no antiviral therapy ($p=0.01$), cirrhosis at presentation ($p<0.01$), and male sex ($p=0.03$) independently predicted a worse outcome.

Conclusion: HDV liver disease lasts several decades. Half of patients who develop cirrhosis advances to liver failure. At present interferon therapy is recommended as soon as possible to slow or alter the natural course of the liver disease.

INTRODUCTION

The hepatitis D virus (HDV) is a single stranded RNA virus that requires a helper function provided by the hepatitis B virus (HBV) for replication (1). Analysis of HDV isolates from different regions of the world revealed eight phylogenetically distinct genotypes. Distinct geographic distribution has become apparent, with genotype I being the most frequent (2). Moreover, the pathogenicity of HDV genotypes has been reported as highly variable (3).

Since the 1990s, the circulation of HDV in several countries of the world has declined steadily, due essentially to the implementation of vaccine immunoprophylaxis against HBV, a strategy that is depriving the defective HDV virus of the HBV network necessary to propagate its infection (4). In Europe this decline sustained the expectation that hepatitis D would be brought under control. However, surveys in England (5), Germany (6), and Italy (7) have revealed the decline has not continued in most recent years, with a consistent reservoir of HDV remaining in immigrants from many parts of the world.

Along with the substantial decline in the epidemiology of HDV, the clinical scenario of hepatitis D has also changed. The clinical perception of HDV infection is moving from a severe, rapidly progressive disease leading to end-stage liver failure or hepatocellular carcinoma (HCC) in few years, to a slowly progressive liver cirrhosis for which liver transplantation represents the ultimate therapeutic option (8). In Italy, over an observation period of up to 28 years, chronic HDV infection evolved to cirrhosis in 30% of patients, with clinical decompensation being the first dominant complication (9).

Although substantial advances have been made in the treatment of chronic viral hepatitis, therapy for chronic hepatitis D is not yet satisfactory. In practice, monotherapy with conventional or pegylated interferons remains the only available option and may achieve remission of disease in 20-50% of cases depending on dosage and duration of treatment. However, the rate of HDV-RNA clearance remains unsatisfactory (10-13), and the capability of treatment to change the course of disease remains unclear. Therefore, the identification of factors influencing the outcome could significantly improve the management of HDV chronic hepatitis.

We studied virologic patterns, influence of treatment, occurrence of major complications, and survival in a cohort of patients with chronic HDV infection, who were consecutively enrolled in a program of periodic surveillance.

Patients and Methods

Patients

We investigated all patients with HDV infection who were admitted to the Liver Units of San Giovanni Rotondo and Turin, Italy, from 1991 to 2005 and who agreed to be entered into a program of surveillance. Patients were enrolled if they met the following criteria: (1) HBsAg-positive in serum, (2) serum positive HDV antibodies (IgG-anti-HDV) for at least 6 months, (3) chronic hepatitis or cirrhosis at histological and/or clinical evaluation, (4) no evidence of HIV co-infection, (5) alcohol intake of less than 40 g/day, and (6) absence of clinical and/or ultrasound signs of hepatic decompensation or HCC. The length of time elapsed from the initial diagnosis of HDV infection to enrolment was also evaluated.

Diagnosis of HDV infection

The diagnosis of HDV infection relied on serum anti-HDV IgG and HDV-RNA detection in HBsAg carriers. Tissue HDAg staining was commonly performed by an immunofluorescence technique in liver tissue sections obtained by percutaneous biopsy. Two criteria were deemed necessary in order to define an active HDV infection: detection of HDV antigen (HDAg) in liver tissue and/or of serum IgM-anti-HDV antibodies, and presence of HDV-RNA in blood.

PCR Analysis of Viral Nucleic Acids

Semi-quantitative detection of HDV-RNA in serum was performed as previously described (14) by single and nested PCR amplifications of a highly conserved region of the HDV genome, by using primers selected among genotype I of HDV. The amplified region corresponded to the C-terminal portion of the hepatitis delta antigen (HDAg) coding region, and included the RNA editing site and the polyadenylation signal. The sensitivity of the PCR assays was approximately 1000 genomes for single PCR, and 10 genomes for nested PCR. To avoid false-negative results, which might be consequent to genetic variability of HDV virions, a second region was amplified in negative samples. The second amplified region targeted the ribozyme region of the genome, shown as the most conserved region among different genotypes (15).

Serum HBV-DNA levels were determined by a commercial quantitative PCR assay (Amplicor HBV Monitor Test, Roche Diagnostics, GmbH Mannheim, Germany), with a sensitivity threshold of about 400 copies/mL (78 UI/mL). Before PCR-based assay became available, HBV-DNA was detected by a sandwich capture hybridization assay (Digene Diagnostics) with a lower detection limit of 5 pg/ml (286.000 UI/mL). HCV-RNA was detected by standard amplification assay (TMA, Bayer Diagnostics, Tarrytown, NY, USA).

Independently from serum levels, detectable HDV viremia was assumed as indicative of an active HDV infection. For HBV-DNA, the conventional cut-off level of 2000 UI/ml was used to identify active HBV infection (16). Active HCV infection was defined on the basis of serum HCV RNA values above 600 IU/ml (17).

Other Laboratory and Virological Testing

Routine serological tests, as serum alanine (ALT) and aspartate (AST) aminotransferase, direct and total bilirubin, albumin, prothrombin time, complete blood counts, and Ŭ-fetoprotein were assessed by standard methods. Serologic markers of HBV, HDV, HCV, and HIV were tested by commercial enzyme immunoassays (Abbott Laboratories, North Chicago IL; Sorin Biomedica, Saluggia, Italy; Ortho Diagnostic Systems, Raritan, NJ).

Definition of Response to Treatment

The virological response was defined as loss of serum HDV-RNA at the end of treatment (end-of-therapy virological response) or during the post-therapy follow-up (sustained virological response). Relapse was defined as reappearance of serum HDV-RNA after treatment. A biochemical response was defined as normal serum alanine aminotransferase levels at end of treatment (end-of-therapy biochemical response) or during follow-up (sustained biochemical response). A relapse was defined as an increase in serum alanine aminotransferase levels to more than 1.5 times the upper limit of normal after a biochemical response.

Definition of Cirrhosis.

Liver biopsy was proposed to define grading and staging of liver disease at enrolment and before antiviral treatment. Histological activity was evaluated by the modified Ishak score (18). In patients who did not consent to liver biopsy, cirrhosis was diagnosed based on platelet count Ò 100,000 mm^3, on ultrasound features of surface nodularity, splenomegaly, and portal vein diameter > 13 mm (19), and in detecting esophageal varices at endoscopy. Compensated cirrhosis was defined by absence of ascites, jaundice, portal-systemic encephalopathy or variceal bleeding. Correspondingly, decompensated cirrhosis was defined as presence of any of these complications.

Disease Progression

In patients presenting with chronic hepatitis, progression to cirrhosis was defined by liver histology (F4) and/or platelet count, ultrasound and endoscopic features, as previously described. In those presenting with cirrhosis, disease progression was evaluated by increased Child-Pugh class,

development of clinical decompensation, or HCC. HCC was diagnosed based on the use of contrast imaging with computerized tomography (CT) scan, magnetic resonance imaging, and second generation contrast ultrasound. In patients undergoing liver biopsy, HCC was classified according to international criteria (20). Complications of cirrhosis such as ascites, jaundice, hepatic encephalopathy, and gastrointestinal bleeding were diagnosed and treated according to established criteria.

Statistical Analysis

Baseline characteristics and measures of clinical and demographic predictors (age, gender, etc.) are described as proportions or means and standard deviations. Separate Kaplan Meier survival analyses were presented for patients with chronic hepatitis and cirrhosis. Differences in survival times were assessed with a log-rank test. The effect of several risk factors on survival free of decompensation or HCC were evaluated by the Cox proportional hazards regression model. The results were expressed in terms of hazard ratios, 95% confidence intervals (CI), and Wald χ statistics p values. All analyses were performed by using SPSS.

RESULTS

Baseline demographics and serologic/virological profile of patients

One hundred and eighty-eight patients (56 females and 132 males) with a mean age of 50 +/- 15 years represented the study population. Length of time elapsed from the initial diagnosis of HDV infection to enrolment into current investigation was 9.6 ± 7.5 years. Mean length of follow-up following the initial encounter was 7.8 ± 4.1 years (range 3-17 years). All but 17 patients were of Italian descendent (140 from Southern Italy and 31 from Northern Italy); of non-Italian patients, 16 were immigrants from Romania and one from Egypt. Eleven patients had at least one other relative included into the study. The route of HDV acquisition remained unrecognized in 96 patients (51%), but followed a blood transfusion in 4 (2%), and was associated to intravenous drug use and multiple sexual partners in 24 (13%); of remaining patients, 62 (33%) recalled a family history of chronic HDV infection, and 2 (1%) a professional exposure (Table 1).

Infection of HDV was diagnosed by serum HDV RNA detection in 175 patients (93%), with high (>1,000 copies/ml) viremia levels in 120 of them; in the remaining 13 non viremic patients, the diagnosis relied on repeated anti-HDV positivity. All patients were infected by HDV genotype I and tested HBsAg positive; 17 (9%) patients were serum HBeAg positive, and 171 (91%) negative. Sixty-one patients (50 anti-HBe positive and 11 HBeAg positive) had detectable circulating levels of HBV-DNA, and in 50 of them with high (>2000 UI/ml) viremia Eighteen patients tested positive for serum anti-HCV antibodies (Table 1), and 4 of them had also circulating HCV RNA. HCV genotype 3a was identified in 3 patients, genotype 1b in the remaining one.

Table 1 reports demographic and clinical data of patients at baseline. Characteristics were not statistically different in patients with chronic hepatitis and cirrhosis.

The interrelationship between active HBV and active HDV infection, as apparent from serum viremia levels, is shown in Table 2. By taking as active HDV infection a serum HDV RNA level Ó10 genomes/ml, and as active HBV infection a serum HBV DNA level >2000 UI/ml, the two viruses appeared to be concomitantly replicating in 43 patients (23%), whereas HDV replication was shown capable to inactive HBV in 132 patients (70%); in only a minority of patients (4%) HBV replication inhibited HDV. In the remaining 6 patients (3%) the two viruses were not detectable into the circulation.

Histological and Clinical Diagnosis

Liver histology was available for 108 patients and showed mean inflammatory and fibrotic scores of 8.7 ± 2.9 (range 2-13) and 4.1 ± 1.3 (range 2-6), respectively. Of the remaining 80 patients

who refused a liver biopsy, a chronic hepatitis was clinically ascertained in 22 patients and a stable cirrhosis in the remaining 58 patients (Table 3).

Treatment

Overall, 96 patients (51%) received antiviral treatment, 63 of them were treated with conventional interferon at doses ranging from 6 and 9 million units thrice weekly for 12 months as a mean; 27 patients were treated with peg-interferon alpha 2b at a dosage of 1.5 εg/kg weekly for 72 weeks, given as monotherapy in 21 patients or in combination with 800 mg daily of ribavirin in the remaining 6 patients; 6 patients received lamivudine monotherapy at a dosage of 100 mg/day for 12 or 24 months. Of the 90 interferon-treated patients, 61 (68%) were treated with a single course, 26 (29%) underwent a second course of either standard interferon (19 patients) or pegylated interferon (7 patients), and 3 patients (3%) were treated with 3 courses (Table 4). Overall, 27 patients (30%) attained a sustained response, 13 of 47 patients (28%) with chronic hepatitis at baseline, and 14 of 43 (33%) cirrhotics at enrolment. Among the 6 patients treated with lamivudine, all of them HBV-DNA and HDV-RNA positive at treatment start, no single patient experienced viral clearance during or after treatment. Ninety-two patients (49%) were never treated due to refusal (n=23, 25% of untreated), low platelet counts (n=41, 45%), or persistently normal serum transaminases levels (n=28, 30%).

Virological and clinical outcome

All patients were followed up for a mean of 7.8 ± 4.1 years (range 3-17 years). The serum virologic profile of patients at the last evaluation is given in Table 2: 109 patients (58%) had virological profiles stabilized over time, whereas in 79 patients the pattern of HDV and HBV viremia varied. At the last evaluation, 135 of 175 viremic patients at baseline tested HDV-RNA positive, whereas clearance of HDV viremia was documented in 40 patients, 72% of them with liver cirrhosis. HBV-DNA persisted positive in 19 out of 50 patients with HBV-DNA levels > 2000 UI/ml at baseline. Twelve patients (6%) lost serum HBsAg, 2 of them had a spontaneous clearance, while in the other 10 subjects viral clearance followed interferon treatment. Seroconversion from HBsAg to anti-HBs was documented in 2 patients, with a title of anti-HBs of 150 and 250 UI/ml, respectively, and persistently normal transaminases levels.

The clinical sequelae of patients, according to their fibrotic stage at presentation, are shown in Fig. 1. Among the 82 patients with clinical, instrumental or histological features of chronic hepatitis at baseline, 61 subjects had a stable un-progressive disease at the last visit, and in the remaining patients the liver disease progressed to the stage of cirrhosis (Fig 1). The cumulative

probability of developing cirrhosis among patients without the condition at baseline was 0.70 at 17 years, with an incidence rate of 1.23 per year. Male gender was identified as risk factor for cirrhosis development ($p = 0.02$). One hundred and twenty-seven patients had the HDV infection progressed to liver cirrhosis, 107 of them at presentation and the remaining 21 subjects during the follow up. When initially recognized, all cirrhotic patients were in Child-Pugh class A, whereas during the follow up, 8 patients progressed to class B, and 51 to class C. At the last visit, esophageal varices were detected at endoscopy in 62 patients (49%). Forty-two cirrhotic patients experienced one or several episodes of liver decompensation, with a 0.75 cumulative probability of developing decompensation at 17 years, and an incidence rate of complication of 2.47 per year. Decompensated events included ascites (36 patients), jaundice (25 patients), bleeding from esophageal varices (9 patients), and encephalopathy (12 patients). Seventeen cirrhotic patients developed a HCC, at a cumulative rate of 0.55 at 17 years, and an annual incidence rate of 1.0. At the diagnosis of HCC, patients had a mean age of 53±13 years, were in the majority males (70%), with unknown risk of infection (70%), anti-HBe positive (82%) and persistent HDV-viremia (53%). HBV-DNA was detectable in 4 patients (viremia < 2000 UI/ml).

Survival

At the end of follow-up 17 patients have died (9%). Causes of death were liver failure in 11, complications after OLT in 3 patients and liver-unrelated events in 3 (trauma in 1, sepsis after cholecystitis in 1, and malignancy in 1). One-hundred and seventy-one patients (91%) were still alive: 126 free of complications, 35 after OLT, and 10 with signs of severe liver decompensation (n=3) or HCC (n=7). Transplanted patients were followed for a mean period of 8 years ± 3.9 (mean ± SD). In 6 of them malignancies were diagnosed (colon cancer in 2 patients, breast cancer in 1, HCC in 1, non-Hodgkin lymphoma in 1, cutaneous basocellular epithelioma in 1). In 4 patients biliary-plastic procedures were performed due to bilio-digestive anastomosis strictures.

For the entire cohort of patients, the median probability of surving free of major events of liver decompensation or HCC from the initial diagnosis of HDV infection was 28 years (Fig 2). The 5- and 10- year probabilities of survival free of liver decompensation and of HCC were 96.8% and 81.9% for patients with chronic hepatitis, and 83.9% and 59.4% for those with cirrhosis ($p<0.01$) (Fig 3). For the 96 patients who received a single or several courses of antiviral therapies, the 5- and 10- survival probabilities free of major complications were 95% and 88%.

Prognostic factors of survival free of complications.

At the univariate analysis, no antiviral therapy, cirrhosis and male sex resulted significantly associated with liver decompensation or HCC development ($p<0.01$). When significant and nearly significant predictors of worse outcome of disease were included in a multivariate analysis, cirrhosis ($p<0.01$, HR 3.40 [IC 95% 1.60-7.22]), lack of treatment ($p=0.01$, HR 2.06 [IC 95% 1.17-3.62]), and male gender ($p=0.03$ HR 1.93 [IC 95% 1.08-3.48]) resulted as independent predictors of an unfavorable outcome (Table 5).

DISCUSSION

In the current study we evaluated the clinical course of a chronic HDV infection in a large cohort of patients followed up for a mean period of 8 years. The scrutinized population was homogeneous for its origin (mostly from Southern Italy), the prevailing HDV genotype (genotype I), and a low rate of HBV replication. At presentation, the cohort included 82 patients (44%) with chronic hepatitis, a fourth of them evolving to cirrhosis during the observation period, and 106 (56%) cirrhotics, half of them developing major complications during the follow-up. These data confirm the highly pathogenicity of HDV, and the prevalence of subjects with cirrhosis among HDV carriers referring to our units (3). The identification of virological, therapeutic and/or host factors able to stabilize chronic hepatitis and to avoid decompensation and HCC remains a major issue.

Replicative activity of HDV and HBV may influence the course of disease (3, 21). At baseline HDV-RNA resulted positive in 91% of our patients, as tested by the semi-quantitative PCR, and viremia was detectable either in patients with chronic hepatitis and cirrhosis. Changes in HDV replication have been reported during the follow up of patients with HDV chronic disease (9,15). In our series 40 patients cleared viremia, either spontaneously or after interferon therapy, and 2/3 of patients were cirrhotics. Previous observation would suggest that the earlier the HDV infection is cleared the more relevant would be the impact of viral clearance of the overall prognosis of the liver disease: once the stage of liver cirrhosis has developed, the viral clearance will have limited influence on further course of the liver disease. The dynamic inter-relationship between HDV and HBV requires serial determinations of viremia levels better define which of the two viruses might be responsible for the liver damage. In agreement with a previous report (17), at the two time-points evaluated in the present investigation viremia levels of the two viruses fluctuated widely, an observation that could be related to either intrinsic mechanisms of reciprocal HDV-HBV inhibition or to the variable progression of the underlying liver disease (21). Seroclearance of HBsAg in patients with HDV chronic infection represents the ultimate step in the process of HDV clearance (22). Indeed, as long as the HBsAg is produced, HDV can thrive and sustain replication and liver disease. Twelve patients lost serum HBsAg (6%) in our cohort , and the clearance occurred mostly in patients with chronic hepatitis, and was favorably influenced by the interferon therapy.

Our study showed that previous antiviral treatment with interferon was significantly associated with a less aggressive outcome. The role of interferon therapy in patients with chronic HDV infection is controversial. Available trials indicate that high doses of interferon and a long duration of therapy were associated with biochemical response and improvement in liver histology

(23). The implementation of pegylated-IFN-Ŭhas substantially improved the pharmacokinetic and pharmacodynamic profiles of standard IFN-Ŭand has provided better compliance for the long-term treatment required in chronic hepatitis D. Crucial challenges remain related to the selection of patients to treat, the definition of response and the duration of treatment. We suggest treatment with Peg-IFN at high doses for patients with compensated chronic hepatitis D as soon as diagnosis is made. Response should be evaluated based on decrease in serum of HDV-RNA and therapy should be continued for at least 1 year before a patient is defined as non responder. Moreover, regression of fibrosis and amelioration of clinical outcome has been reported in patients with sustained benefit from therapy-induced HDV clearance. (24). In keeping with previous data (24), the majority of our treated patients maintained a stable disease and only a minority of them evolved toward liver decompensation or developed HCC. Of interest, a favorable outcome was observed either in patients who cleared HDV viremia after interferon therapy and in those still viremic at end of the study. In the last group of patients we are tempted to relate this favorable outcome to a reduction in HDV levels, which could not be quantified with available method.

We could confirm previous observations on a mild course of the HDV infection in the long run: overall, episodes of major live-related events of decompensation were observed in 42 (22%) of 188 infected patients during the 8 years of follow-up. In addition, the median survival free of major complications was in direct relation with the underlying stage of the liver disease at baseline: ascites, gastrointestinal bleeding, encephalopathy and/or jaundice occurred in 37 (35%) out of 106 cirrhotics. A recent study from Romeo et al. (9) reported at least one episode of liver decompensation in 54 of 299 patients under observation, with a comparable percentage of 18%. Previously Fattovich et al. (25), analyzing a smaller cohort of patients, reported decompensation in 12 of 39 (31%) HDV-positive cirrhotic patients.

Seventeen patients in our cohort (9%) were diagnosed with HCC , and 9 of them were transplanted. The mean age of these patients was 53±13 years and persistent HBV replication was observed in 29%. Romeo et al. (9) diagnosed HCC in 46 out of 299 pts (15%) and reported the chance of developing HCC to be influenced by previous treatment with interferon and persistent HBV replication. The carcinogenetic role of HDV by itself remains unclear, mostly due to the powerful role of HBV and its ability to integrate into the host genome (affecting cellular signaling and growth control), but data concerning to the oncogenetic power of HDV by itself are discordant. Indeed, in areas where HBV-related HCC is endemic, such as Taiwan, few cases of HCC have been related to an ongoing HDV infection (26). On the contrary, in Italy anti-HDV positive cirrhotics were reported at increased risk for HCC compared to anti-HDV negative patients. (25)

HDV genotype I has been worldwide associated with a highly pathogenic potential (3). In our area, where this genotype is almost exclusively represented (14), a third of patients cleared the HBsAg and maintained a preserved liver functional reserve; it is worth mentioning that 17% of patients with HBsAg clearance seroconverted to anti-HBs development during the long run.

In conclusion, main finding of our study was the observation that interferon therapy modified the outcome of patients with chronic HDV-related liver disease by reducing the occurrence of major complications. In our cohort of patients, HCC was not the major complication, while ascites and gastrointestinal bleeding prevailed in drawing patients toward death and OLT.

REFERENCES

1) Rizzetto M, Canese MG, Aricò S, Crivelli O, Trepo C, Bonino F, et al. Immunofluorescence detection of a new antigen-antibody system (delta/anti-delta) associated with hepatitis B virus in liver and in serum of HBsAg carriers. Gut 1977; 18: 997-1003.
2) Dény P. Hepatitis delta virus genetic variability: from genotype I, II, III to eight major clades? Curr Top Microbiol Immunol 2006; 307: 151-171.
3) Su CW, Huang YH, Huo TI, Shih HH, Sheen IJ, Chen SW, et al. Genotypes and viremia of hepatitis B and D viruses are associated with outcomes of chronic hepatitis D patients. Gastroenterology 2006; 130: 1625-1635.
4) Ciancio A, Rizzetto M. Clinical patterns, epidemiology and disease burden of hepatitis D virus chronic liver disease. in Margolis HS, Alter MJ, Liang TJ, et al. (editors). Proceedings of the 10th International symposium on viral hepatitis and liver disease- Atlanta GA: International Medical Press, 2002; p. 271-75.
5) Cross TJS, Rizzi P, Horner M, Jolly A, Hussain MJ, Smith HM, et al. The increasing prevalence of hepatitis delta virus (HDV) infection in South London. J Med Virol 2008; 80:277-282.
6) Wedemeyer H, Heidrich B, Manns MP. Hepatitis D virus infection not a vanishing disease in Europe! Hepatology 2007; 45:1331-1332.
7) Gaeta GB, Stroffolini T, Smedile A, Niro G, Mele A. Hepatitis delta in Europe: vanishing or refreshing? Hepatology 2007; 46: 1312-1313.
8) Rosina F, Conoscitore P, Cuppone R, Rocca G, Giuliani A, Cozzolongo R, et al. Changing pattern of chronic hepatitis D in Southern Europe. Gastroenterology 1999; 117: 161-166.

9) Romeo R, Del Ninno E, Rumi M, Russo A, Sangiovanni A, De Franchis R, et al. A 28-year study of the course of hepatitis delta infection: a risk factor for cirrhosis and hepatocellular carcinoma. Gastroenterology 2009; 136:1629-1638.
10) Farci P. Delta hepatitis: an update. J Hepatol 2003; 39: S212-S219.
11) Niro GA, Rosina F, Rizzetto M. Treatment of hepatitis D. J Viral Hepat 2005;12: 2-9.
12) Castelnau C, Le Gal F, Ripault MP, Gordien E, Martinot-Peignoux M, Boyer N, et al. Efficacy of Peginterferon alpha-2b in chronic hepatitis delta : relevance of quantitative RT-PCR for follow-up. Hepatology 2006; 44: 728-735.
13) Niro GA, Ciancio A, Gaeta GB, Smedile A, Marrone A, Olivero O, et al. Pegylated interferon alpha-2b as monotherapy or in combination with ribavirin in Chronic Hepatitis Delta. Hepatology 2006; 44: 713-720.
14) Niro GA, Smedile A, Andriulli A, Rizzetto M, Gerin JL, Casey JL. The predominance of Hepatitis Delta Virus Genotype I among chronically infected Italian patients. Hepatology 1997; 25: 728-733.
15) Le Gal F, Gordien E, Affolabi D, Hanslik T, Alloui C, Dény P, et al. Quantification of Hepatitis Delta Virus RNA in Serum by Consensus Real-Time PCR indicates different patterns of virological response to Interferon therapy in chronically infected patients. J Clin Microbiol 2005; 43: 2363-2369.
16) Carosi G, Rizzetto M. Treatment of chronic hepatitis B: recommendations from an Italian workshop. Dig Liv Dis 2008; 40: 603-617.
17) Raimondo G, Brunetto MR, Pontisso P, Smedile A, Maina AM, Saitta C, et al. AISF Cooperative Group. Longitudinal evaluation reveals a complex spectrum of virological profiles in Hepatitis B virus/Hepatitis C virus-coinfected patients.
18) Ishak KG. Pathologic features of chronic hepatitis. A review and update. Am J Clin Pathol 2000; 113: 40-55.
19) Symonovsky V. The diagnosis of cirrhosis by high resolution ultrasound of the liver surface. The British Journal of Radiology 1999; 72: 29-34.
20) Peters RL. Pathology of hepatocellular carcinoma. in Okuda K, Peters RL, (editors). Hepatocellular carcinoma. New York: John Wiley, 1976: 107-168.
21) Wu JC, Chen TZ, Huang YS, Yen FS, Ting LT, Sheng WY, et al. Natural history of hepatitis D viral superinfection: significance of viremia detected by polymerase chain reaction. Gastroenterology 1995; 108: 796-802.
22) Niro GA, Gravinese E, Martini E, Garrubba M, Facciorusso D, Conoscitore P et al. Clearance of Hepatitis B Surface Antigen in Chronic Carriers with Hepatitis Delta

Antibodies. Liver 2001; 21: 254-259.

23) Farci P, Mandas A, Coiana A, Lai ME, Desmet V, Van Eyken P et al. Treatment of chronic hepatitis D with interferon alfa.2a. N Engl J Med 1994; 330: 88-94.

24) Farci P, Roskam T, Chessa L, Peddis G, Mazzoleni AP, Scioscia R et al. Long-term benefit of interferon alpha therapy of chronic hepatitis D: regression of advanced hepatic fibrosis. Gastroenterology 2004; 126; 1740-1749.

25) Fattovich G, Giustina G, Christensen E, Pantalena M, Zagni I, Realdi G et al. Influence of hepatitis delta virus infection on morbidity and mortality in compensated cirrhosis type B. Gut 2000; 46: 420-426.

26) Chen DS, Lai MY, Sung JL. Delta agent infection in patients with chronic liver diseases and hepatocellular carcinoma ï an infrequent finding in Taiwan. Hepatology 1984; 4:502-3.

TAB 1. Demographic and clinical data of enrolled patients at baseline.

	All Patients	Chronic Hepatitis	Cirrhosis
Chronic Hepatitis (no, %)	82 (44)	82 (44)	NA
Cirrhosis (no, %)	106 (56)	NA	106 (56)
Age (years mean ± SD)	50 ± 15	48 ± 16	51 ± 14
Gender, male (no,%)	132 (70)	58 (70)	74 (70)
Known Risk Factors of transmission (no,%)	92 (49)	44 (54)	48 (46)
BMI (kg/m2 , mean ± SD)	26.3 ± 3.7	26.4 ± 3.6	26 ± 4
Glycemia (mg%, mean ± SD)	93 ± 17	93 ± 21	93 ± 13
ALT (mean ± SD)	116 ± 105	110 ± 86	107 ± 87
Anti-HCV +ve (no, %)	18 (9.6)	6 (7)	12 (11)
HDV-RNA +ve (no, %)	175 (93)	77 (94)	98 (93)
HDV-RNA > 1000 genomes/ml (no, %)	97 (52)	49 (60)	48 (45)
HBV-DNA +ve (no, %)	61 (32)	31 (39)	30 (28)

TAB 2. Distribution of 188 HDV/HBV-positive patients according to longitudinal virological profiles.

	Baseline	Last Observation			
	N°of Cases %	Active HDV/ Active HBV	Active HDV/ Inactive HBV	Inactive HDV/ Active HBV	Inactive HDV/HBV
Active HDV/Active HBV	43 (23%)	10	25	-	8
Active HDV/Inactive HBV	132 (70%)	4	94	1	33
Inactive HDV/Active HBV	7 (4%)	-	-	2	5
Inactive HDV and HBV	6 (3%)	1	1	1	3

Table 3. Outcome according with stage of fibrosis at liver histology or clinical diagnosis at baseline.

	Progression to Cirrhosis	Alive with HCC or Decompensation	OLT	Death	HBsAg Clearance
Fibrosis at Liver Histology:					
Ishak Score 0-2 N=13	-	-	-	-	7
3-4 N=47	21	1	6	1	2
5-6 N=48	NA	4	12	5	2
Clinical Chronic Hepatitis N=22	-	-	-	-	1
Clinical Cirrhosis N=58	NA	5	20	5	0
Total N=188	21	10	38	11	0

NA Not applicable

Table 4. Outcome of untreated and treated patients either with Lamivudine or Ó1 cycle of Interferon.

	Treatment	Baseline CH	Baseline Cirrhosis	SVR	HBs-Ag clearance	Decomp. or HCC
Treated Pts N=96	Lam MonoTx n=6	3	3	0	0	3
	IFN 1 cycle n=61	29	32	18	8	11
	IFN > 1 cycle n=29	18	11	9	2	1
Untreated Pts N=92	No	32	60	NA	2	44

Table 5. Univariate and multivariate survival analysis of variables associated with outcome free of decompensation or HCC.

	HCC or decompensated	No HCC or decompensated	Univariate Analysis	Multivariate Analysis			
Variables	n=59	n=129	p value	p value	HR	95% CI	
						Lower	Upper
Gender Male	42	90	0,05	0,03	1,93	1,08	3,48
Age <=50	26	69	0,98	-			
Unknown Risk of Transmission	34	62	0,56	-			
HCV	3	15	0,28	-			
HDV-RNA persistence	37	98	0,60	-			
HBVDNA +ve at baseline	19	42	0,79	-			
No Treatment	41	51	< 0,01	0,01	2,06	1,17	3,62
Cirrhosis at Baseline	51	55	< 0,01	< 0,01	3,40	1,60	7,22

FIGURE LEGENDS

Fig 1. Outcome of Patients with chronic hepatitis and cirrhosis at baseline.

Fig 2. Overall survival free of major complications from the initial diagnosis of HBV/HDV co infection.

Fig 3. Probability of survival free of major complications according to staging of chronic hepatitis and cirrhosis at enrolment. Eight complications were observed in patients with chronic hepatitis vs 51 events in cirrhotic patients.

Fig 4. Probability of survival free of major complications in treated patients.

Fig.1

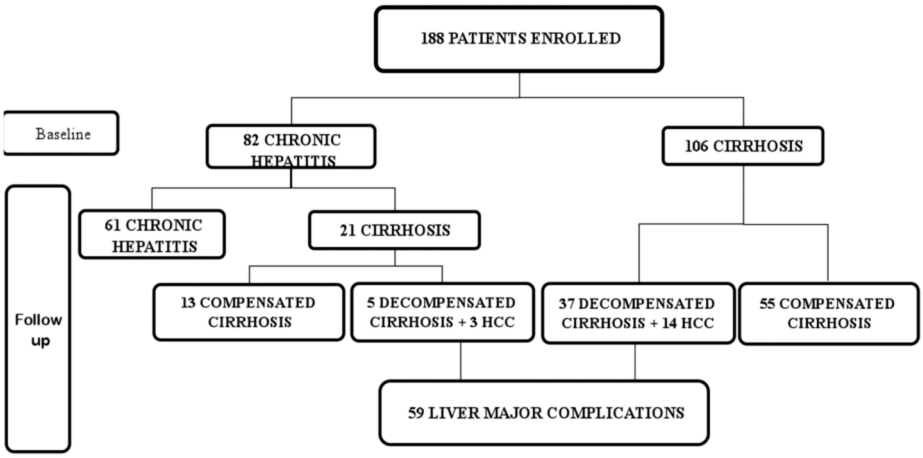

188 PATIENTS ENROLLED
Baseline
82 CHRONIC HEPATITIS
106 CIRRHOSIS
61 CHRONIC HEPATITIS
21 CIRRHOSIS
Follow up
13 COMPENSATED CIRRHOSIS
5 DECOMPENSATED CIRRHOSIS + 3 HCC
37 DECOMPENSATED CIRRHOSIS + 14 HCC
55 COMPENSATED CIRRHOSIS
59 LIVER MAJOR COMPLICATIONS

Fig.2

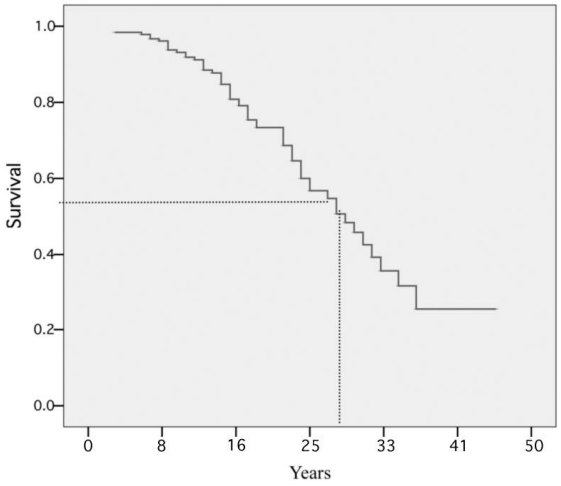


Fig.3

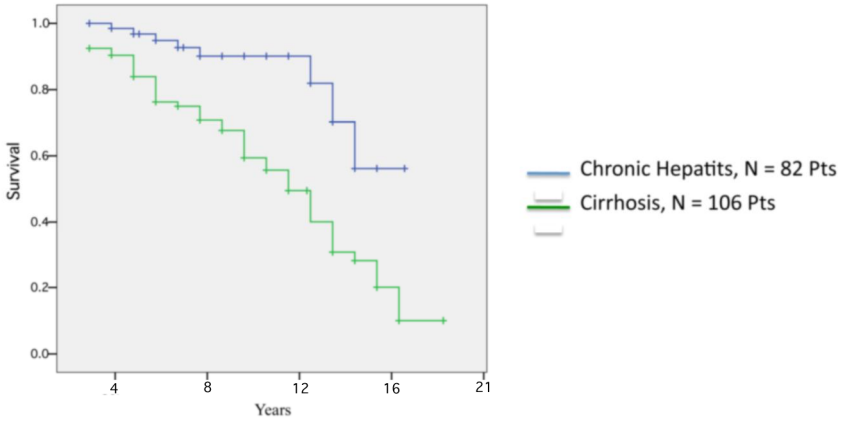
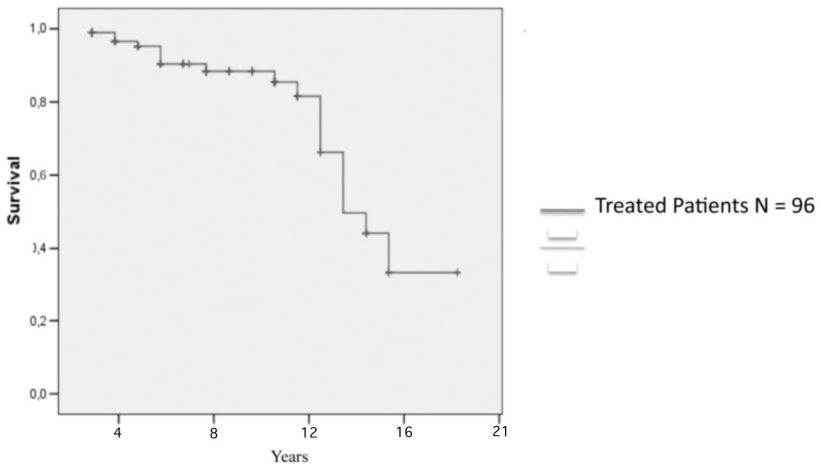

1.0
0.8
0.6
0.4
0.2
0.0
Survival
4
8
12
16
21
Years
Chronic Hepatits, N = 82 Pts
Cirrhosis, N = 106 Pts

Fig. 4


Survival
1,0
0,8
0,6
,4
0,2
0,0
4
8
12
16
21
Years
Treated Patients N = 96