What to start with in first line treatment of chronic hepatitis B patients: an Italian multicentre observational cohort, HBV-RER study group

Gianluca Cuomo[1], Vanni Borghi[1], Tiziana Giuberti[2], Pietro Andreone[3], Marco Massari[4], Erica Villa[5], Antonello Pietrangelo[6], Gabriella Verucchi[7], Fabio Levantesi[8], Carlo Ferrari[2]

[1]Infectious Diseases Unit, Azienda Ospedaliero Universitaria di Modena, Modena, Italy;
[2]Infectious Diseases and Hepatology Unit, Azienda Ospedaliero Universitaria di Parma, Parma, Italy:
[3]Internal Medicine Unit, Azienda Ospedaliero Universitaria di Bologna, Bologna, Italy;
[4]Infectious Diseases Unit, IRCCS - ASMN Reggio Emilia, Reggio Emilia, Italy;
[5]Gastroenterology Unit, Azienda Ospedaliero Universitaria di Modena, Modena, Italy;
[6]Internal Medicine Unit, Azienda Ospedaliero Universitaria Modena, Modena, Italy;
[7]Infectious Diseases Unit, Azienda Ospedaliero Universitaria di Bologna, Bologna, Italy;
[8]Internal Medicine Unit, Ospedale di Bentivoglio, Bologna, Italy

SUMMARY

Treatment options for chronic hepatitis B (CHB) are pegylated interferon (Peg-IFN) in minimal/mild liver fibrosis and nucleot(s)ide analogues (NUC) in more advanced disease. Since little is known about their use in daily clinical practice, we conducted a multicentre prospective study to investigate treatment regimens applied to naïve CHB patients in real life. HBV-RER is an observational multicentre Italian network that collect clinic and virologic data of patients with CHB. Among the 2527 CHB patients seen during the study period (2009-2012), 502 patients started a first line antiviral treatment. Liver biopsy was performed in 30.9% of the patients, with high levels of fibrosis being detected in 19.4% of them. In 216 patients (43%) Peg-IFN was used as first-line therapy while the remaining patients started NUC therapy (entecavir and tenofovir in 75%, lamivudine in 15%, telbivudine and adefovir 10%). By multivariate logistic regression, an age under 40 (OR 0.92, 95%IC 0.90-0.94; p<0.001) and the execution of liver biopsy (OR 3.83; 95%IQR 1.76-8.36; p<0.001) were the only determinants of choice between Peg-IFN vs NUC. Peg-IFN was expected to be used in first-line treatment for CHB in 70% of the patients based on Italian recommendations, but a much lower proportion of patients were actually treated with Peg-IFN with a limited use of the biopsy. Thus, in daily clinical practice physicians prefer to use NUCs, presumably because of their optimal tolerability and anti-viral efficacy, even if they frequently require life-long treatment as opposed to the short duration of Peg-IFN.

Keywords: HBV, pegylated interferon, chronic hepatitis B.

INTRODUCTION

Approximately 350-400 million people around the world are chronic hepatitis B surface antigen (HBsAg) carriers. Chronic hepatitis B virus (HBV) infection is characterized by a wide spectrum of clinical conditions, ranging from chronic inactive carriage of the virus to pro-

Corresponding author
Gianluca Cuomo
E-mail: cuomo.gianluca@policlinico.mo.it

gressive forms of chronic hepatitis B (CHB) that can evolve to liver cirrhosis and hepatocellular carcinoma (HCC) [1].
Presence or absence of hepatitis B e antigen (HBeAg) in the serum allow to distinguish HBeAg positive and negative forms of CHB. Different therapeutic strategies are available at present to achieve a sustained suppression of HBV replication, with the goal of preventing the progression of the disease to cirrhosis and end-stage liver disease (ESLD) [2].
The improvement of histological activity induced by therapy is associated with a low risk of developing cirrhosis and to a reduction of HCC risk, especially in non-cirrhotic patients [3]. The ideal end point of therapy is the HBsAg loss, which is rarely achieved with the current therapeutic options [4].
There are two main strategies of therapy for chronic hepatitis B (CHB), which are active in both HBeAg positive and negative forms of disease: a treatment of finite duration with Peg-Interferon (Peg-IFN) or nucleot(s)ide analogues (NUC) (the latter only for HBeAg positive CHB), or a long-term treatment with NUCs. International guidelines allow the use of Peg-IFN from a stage of minimal liver fibrosis up to a stage of well compensated cirrhosis and Italian guidelines recommend its use as a first line therapy in these clinical conditions [5-7].
NUCs can be used in all stages of HBV-related disease, including decompensated cirrhosis (NUCs are the only therapeutic option for ESLD). According to Italian guidelines, long-term ("indefinite duration") treatment with NUCs can be considered as first line therapy in alternative to Peg-IFN in patients starting from a stage of moderate fibrosis (METAVIR F≥2 or Ishak S≥3) when Peg-IFN is contraindicated [7].
Since little is known about the use of anti-HBV agents in the daily clinical practice, we conducted a multicenter prospective observational study to investigate treatment regimens applied to naïve CHB patients in real life in the Emilia Romagna Region, Italy, during the period 2009-2012 and to assess whether sharing a standardized protocol for the management of chronic HBV infection could help in making the behavior of the clinicians in the different regional centers more homogeneous and more adherent to available guidelines [8].

PATIENTS AND METHODS

The HBV-RER Study Group (RER=Regione Emilia Romagna) is an observational multicenter Italian network established with the support of a regional health service grant (Emilia Romagna region, Italy). The main purpose of the Study group was to improve the quality of the clinical practice in the management of HBV infections using a shared protocol of diagnosis and treatment of HBV infected patients in all clinical centers of the region. The protocol was generated by a scientific committee comprising representatives of the regional groups following the largest cohorts of HBV infected patients, and thus more expert in the treatment of chronic HBV infection, starting from available guidelines; it was finally approved by the central regional health agency before publication.
The purpose of the final document was to identify shared criteria for the management and treatment of chronic hepatitis B in adults based on the best evidence available and their benefit-risk profile. The scientific committee suggest that the "best practice" to treat a patient with CHB was to determine the grade of fibrosis using a liver biopsy and to start, when possible, with pegylated interferon in presence of mild fibrosis.
The network was supported by a web-based database, activated in January 2009, for the collection of clinic and virologic data of the patients with CHB followed in 33 different medical units (infectious diseases, gastroenterology, hepatology, internal medicine).
All HBsAg positive patients who had at least one medical evaluation in one of the participating centers from the 1st of January 2009 to the 31st of December 2012 were considered to be eligible for the enrollment in the study. Since the protocol for the clinical management of HBV infected patients was licensed and shared with the different centers in May 2011 and then implemented in the following months by local meetings involving the physicians of all clinical centers, data relative to the periods January 2009 - June 2011 and July 2011 - December 2012 were taken as indicative of the behaviors followed pre- and post-protocol application.
HIV positive patient were excluded. Data about demographic, hematologic and virologic parameters were recorded, as well as the fibrosis score

when a liver biopsy was available. Anti-HBV treatments (started before enrolment or during the study period) were reported. Patients were enrolled in the observational study irrespective of the stage of HBV infection (inactive carrier, CHB, compensated or decompensated cirrhosis, HCC) or of the presence of co-infections (HDV, HCV). By histological assessment of liver fibrosis and by clinical evaluation, the HBV-associated liver disease was classified as inactive HBsAg carriage, CHB or cirrhosis. Also the FIB4 score, a non-invasive scoring system based on several laboratory tests that help to estimate the amount of liver fibrosis, assessed by the following formula, was used [9, 10]:

$$\frac{\text{Age (years)} \times \text{AST level (U/L)}}{\text{Platelets count } (10^9/\text{L}) \times \sqrt{\text{ALT level (U/L)}}}$$

A FIB4 value <1.6 was considered as indicative of no fibrosis, between 1.6 and 3.6 as mild fibrosis, and >3.6 as cirrhosis; it was calculated for each patient independently from the execution of liver biopsy.
In this study we analyzed patients enrolled in the HBV-RER network that were HBsAg positive, without co-infections and that started anti-viral therapy after January 1st 2009, as a first line treatment.

Statistical analysis
Statistical analysis was done by median and interquartile range (IQR) for continuous variables, and of frequency for categorical variables. In univariate analysis between different groups, continuous variables were compared using non-parametric analysis (Mann Whitney) and categorical variables were compared using the Chi-square test. A P-value less than 0.05 was considered to be statistically significant. Multivariate analysis was performed using the stepwise logistic regression method.
The variables with p≤0.05 at the univariate analysis plus age and gender entered in the multivariate model. Analysis was performed by using IBM-SPSS (v. 20) statistical software.
The study was approved by the Institutional Review Board of all the Centers participating to the HBV-RER Study and was conducted in accordance with provisions of the Declaration of Helsinki and Good Clinical Practice guidelines.

RESULTS

A total of 2527 HBsAg positive patients were enrolled in the study during the four years observational period: 1325 of them had never been treated before, while 1202 had previously been treated or were on treatment at the time of enrollment. 67% of the patients were males and 80% were Caucasian. 62% were born in Italy, 14% come from Western Europe, and 9% from Asia. Median age was 47 years (IQR 36-59). Observation period of the cohort was 4880 person-years, with a median number of observation of 2.9 visit/year/patient (IQR 2.0-4.5).
528 patients started anti-viral therapy after January 1st 2009, as a first line treatment, and were defined as naïve. 26 of them were excluded from analysis, because delta (15 patients) or HCV (4 patients) co-infected; 7 patients were excluded because first line treatments were based on experimental protocols. The characteristics of the 502 naïve patients analyzed are summarized in Table 1. 73.1% of them were male, 38.6% were not Italian, 80.1% were Caucasian; 18.9% were HBeAg positive.
The median age was 49 years (IQR 38-58). Liver biopsy was performed in 30.9% of the patients and a high level of fibrosis was detected in 19.4% of them (defined as Ishak score S4-S6) [11]. 121 patients (24.1%) had a FIB4 score consistent with liver cirrhosis (>3.6).
In order to understand whether the generation of a shared protocol could have an effect on the clinical behaviour of the different centres, we then analysed separately the periods before and after publication and implementation of the protocol, as shown in Table 1. Data concerning patients (gender, p=0.320; age, p<0.256; origin, p=0.763) and virologic characteristics (presence of HBeAg, p=0.341) did not differ in the two different periods; only quantitative HBV DNA showed a slightly significant statistical difference (p=0.040) between the two periods. Liver biopsy was performed in 32.4% of the patients in the first period of observation and in 29.7% after the consensus statements (p=0.510). There were no statistically significant differences between the two period in terms of FIB4 score (p=0.560). Furthermore no statistically significant differences were shown with respect to the rate of the choice of first line treatment with Peg-IFN or NUCs (p=0.615) in the

Table 1 - General characteristic of the naïve patients, included in the HBV-RER study, stratified by period of protocol.

Characteristics	*Jan 2009 - Jun 2010 No. 219*	*Jul 2010 - Dec 2012 No. 283*	*Total No. 502*	*p value*
Gender				
Male	165 (75.3%)	202 (71.4%)	367 (73.1%)	0.320
Origin Foreign (not Italian)	83 (37.9)%	111 (39.2 %)	194 (38.6%)	0.763
Race Caucasian Asian Black Hispanic	174 (79.5%) 28 (12.8%) 16 (7.3%) 1 (0.5%)	228 (80.6%) 24 (8.5%) 31 (11.0%) -	402 (80.1%) 52 (10.4%) 47 (9.4%) 1 (0.2%)	0.153
Age*	47 (38-58)	50 (38-59)	49(38-58)	0.256§
HBeAg Positive Negative Missing	47 (21.5%) 162 (74.0%) 10 (4.6%)	48 (18.1%) 217 (76.7%) 18 (6.4%)	95 (18.9%) 379 (75.5%) 28 (5.6%)	0.341
ALT UI/ml*	66.0 (33.0 – 120.0)	54.0 (31.0-110.0)	58.0 (31.0-113.0)	0.164§
*HBV DNA** log UI/ml	5.12 (2.97-6.66)	4.48 (2.34-6.27)	4.75 (2.62-6.49)	w0.040§
Liver Biopsy Performed Not-performed	71 (32.4%) 148 (67.6%)	84 (29.7%) 199 (70.3%)	155 (30.9%) 347 (69.1%)	0.510
Fibrosis S0-S1 S2-S3 S4-S6 NC°	19 (26.8%) 32 (45.1%) 11 (15.5%) 9 (12.7%)	24 (28.6%) 28 (33.3%) 19 (22.6%) 13 (15.5%)	43 (27.7%) 60 (38.7%) 30 (19.4%) 22 (14.2%)	0.451
FIB4 <1.6 ≥1.6 - ≤3.6 >3.6	101(46.1%) 65 (29.7%) 53 (24.2.%)	119 (42.1%) 96 (33.9%) 68 (24.0%)	220 (43.8%) 161 (32.1%) 121 (24.1%)	0.560
Therapy Peg-IFN NUC	97 (44.3%) 122 (55.7%)	119 (42.0%) 164 (58.0%)	216 (43.0%) 286 (57.0%)	0.615

*Median and InterQuartile Range; § Mann-Whitney test; °NC: Not classifiable; ACH; ACH: Active chronic hepatitis; PIFN: Pegylated interferon; NUC: Nucleos(t)ide analogues.

periods before and after protocol implementation, as shown in Table 1.

213 patients (43%) started a first-line treatment with pegylated interferon and the remaining 286 (57%) with a nucleot(s)ide analogue. Entecavir was used in 154 (53.8%) patients, tenofovir in 60 (21.0%), lamivudine in 44 (15.4%), telbivudine in 19 (6.6%) and adefovir in 9 (3.1%). Out of 44 patients receiving lamivudine, only for 22 (50%) the drug was used as prophylaxis in onco-hematologic settings.

In order to explain whether there were some demographic or clinical characteristics in the choice of treatment, we used a univariate analysis performed on patients treated with NUCs compared to those treated with Peg-IFN, as shown in Table 2. Patients treated with Peg-IFN were significantly younger (median age 41.0 *vs* 55.0, p<0.001), more frequently not Italian (50.0% *vs* 30.1%, p<0.001) and HBeAg positive (24.8% *vs* 16.3%, p=0.022); more liver biopsies were performed in the Peg-IFN group (43.1% vs 21.7%, p<0.001) which

Table 2 - Univariate analysis between the group of patients treated with Peg-IFN and the group treated with nucleos(t)ide analogues.

Characteristics	*NUC No. 286*	*Peg-IFN No. 216*	*p value*
Male Gender	209 (73.1%)	158 (73.1%)	0.986
Age*	55.0 (45.0-64.0)	41.0 (33.0-50.0)	<0.001
Foreigns born	86 (30.1%)	108 (50.0%)	<0.001
HBeAg+	43 (16.3%)	52 (24.8%)	0.022
HBV_DNA UI/ml Log*	4.38 (2.16-6.34)	5.05 (3.35-6.6)	<0.001
ALT UI/ml*	44.5 (24.3-88.8)	78.0 (48.0-127.0)	<0.001
Liver Biopsy Performed Not- performed	 62 (21.7%) 224 (78.3%)	 93 (43.1%) 123 (56.9%)	<0.001
Fibrosis S0-S1 S2-S3 S4-S6 NC°	 15 (34.9%) 12 (20.0%) 19 (63.3%) 16 (72.7%)	 28 (65.1%) 48 (80.0%) 11 (36.7%) 6 (27.3%)	<0.001
FIB4 <1.6 ≥1.6 - 3.6≤ >3.6	 99 (34.6%) 92 (32.2%) 95 (33.2%)	 121 (56.5%) 69 (31.9%) 26 (11.6%)	<0.01

*Median and InterQuartile Range; °NC: Not classifiable; CHB: Chronic hepatitis B.

showed a significantly lower level of fibrosis (S0-S1: 65.1% *vs* 34.9%, p<0.001). Significant differences between the two groups were also observed in term of FIB4 score: a value >3.6 was reported in 33.2% of patients treated with NUCs *vs* 11.6% in Peg-IFN (p<0.001). Finally there were statistically significant differences between the two groups in terms of alanine aminotransferase (ALT) (44.5 UI/ml and 78 UI/ml in NUCs and Peg-IFN, respectively; p<0.001) and HBV-DNA levels (higher in the Peg-IFN than in the NUC group; median 4.38 log UI/mL *vs* 5.05 log UI/mL; p<0.001). A logistic regression multivariate analysis was performed to identify the clinical or virological variables associated with the choice of Peg-IFN rather than NUCs (Table 3). An age less than 40 years (OR 0.92; 95%IQR 0.90-0.94; p<0.001) and the execution of liver biopsy (OR 3.83; 95%IQR 1.76-8.36; p<0.001) were the only significant independent factors associated with the choice of Peg-IFN treatment.

Table 3 - Multivariate logistic regression analysis of determinant factors associated with the choice of Peg-IFN rather than NUCs.

	OR	*95% CI*	*p value*
Age (<40 years)	0.92	0.90-0.94	<0.001
HBeAg + vs -	1.34	0.73-2.45	0.347
Biopsy yes vs no	3.83	1.76-8.36	<0.001
Fibrosis	0.790	0.53-1.15	0.217
Fib 4	0.96	0.93-1.04	0.081
ALT	1.00	1.00-1.00	0.172
HBV-DNA log	1.08	0.98-1.19	0.125
Gender M vs F	0.92	0.55-1.53	0.733
Foreigns vs Italians	1.42	0.80-2.51	0.228

CONCLUSION

International guidelines identify distinct groups of naïve patients with different indications to treatment depending on transaminase level, HBV viral load and fibrosis stage.
The ideal goal of therapy is HBsAg clearance and seroconversion to anti-HBs, but this end-point is obtained in a very low percentage of patients treated with the different available drugs [12]. In

HBeAg-positive patients the rate of HBsAg loss after one year of therapy is 3-4% with pegylated interferon alpha, 2% with entecavir and 3% with tenofovir. In HBeAg negative patients HBsAg loss after a year is 3% with pegylated interferon alpha and 0% with entecavir or tenofovir [13-16]. The immediate objective of therapeutic interventions is suppression of HBV replication, in order to stop liver injury, prevent cirrhosis and reduce the risk of HCC.

According to Italian national guidelines, a finite duration treatment preferably with Peg-IFN remains the treatment of choice in non-cirrhotic HBeAg positive and negative patients. In HBeAg negative patients, also long-term treatment with NUC can be considered as first choice in advanced fibrosis, especially if the liver function is not well preserved. Based on these recommendations and agreement of the data obtained, Peg-IFN was expected to be used as first-line treatment for CHB with mild fibrosis (≤F2) in the about 70% of the patients enrolled in our cohort according to the data obtained using liver biopsy or FIB4 test, but, indeed, a much lower proportion of patients were actually treated with it. Similar results were obtained in a study conducted in Southern Italy on a smaller cohort where 72% of naïve patients received NUC therapy as first line treatment [17].

Histological evaluation represents an important parameter to be taken into account for the choice of treatment, according to the available recommendations.

In our study, however, only 30.9% of naïve patients underwent liver biopsy which was performed more frequently among foreign people and in patients treated with Peg-IFN compared to those treated with NUC. This percentage is lower than what reported by other authors in a European survey of CHB patients, where the liver biopsy was performed in 55% of the participants in the study [18]. By contrast, 60% of missed liver biopsies were reported in another study from USA [19]. Although the reasons why physicians did not perform liver biopsies in this study as well as in our cohort is not clear, the concern about possible complications and patient refusal likely played an important role in the clinical decision. We cannot exclude, however, that a certain number of patients in our cohort were evaluated by other surrogate markers to establish the level of fibrosis (parameters not included in the database).

At present, two potent NUCs with a high genetic barrier are available: entecavir and tenofovir. They have many advantages that justify their wide use in clinical practice irrespective of guidelines, as observed in our cohort, including the high rate of persistent viral suppression, which may prevent disease progression in a large number of patients, as well as the excellent tolerability with irrelevant side-effects [20]. The choice of the type of NUC is variable and depends exclusively by the personal decision of the physician.

In contrast, Peg-IFN in associated with a high rate of side effects on treatment and with a high rate of relapse after the end of therapy. These drawbacks are probably sufficient to push clinicians to limit its use in clinical practice despite its immunomodulatory activity which can ensure a sustained virological response after a finite duration treatment in approximately 20% of patients and HBsAg loss in 50% of them [21, 22].

In conclusion, our study indicates that in real practice behaviours adopted by clinicians are only partially in line with guideline recommendations and that this attitude cannot be corrected through the elaboration and implementation of specific guidelines. It was surprising to see how little effective was any effort to change or tailor local behaviours in terms of anti-HBV therapy. It was otherwise surprising the choice for example of use as a first line treatment (not as prophylaxis) of lamivudine in 22 patients, that we know is old and less effective drug, associated with an high level of resistance [23]. This could be explained by the fact that these patients came for the large part from small clinic centers with low expertise in the hepatitis management.

In general, physicians prefer to use NUCs, presumably because of their optimal tolerability and anti-viral efficacy, even if they frequently require a life-long treatment, as opposed to the short duration of Peg-IFN therapy. Indeed, a durable viral suppression can prevent disease progression and decompensation, and can reduce the risk of HCC and death [24]. Thus, optimal profile of safety and strong effect on virus replication prevail in the physicians' evaluation over the possibility to obtain a definitive control of infection through a short treatment duration, because this outcome is achievable only in a limited proportion of patients at the price of disturbing severe side effects. In addition, the study points out that clinicians show

poor adherence not only to international but also to local guidelines, even if they have previously been discussed and shared. This is apparently an incorrect behavior but it probably reflects the evidence that in the daily clinical practice a certain number of patients present some baseline parameters, that lead clinicians to expect a low rate of response. These parameters are represented for example by an high viremia, low ALT values, unfavorable genotypes, advanced age, associated comorbidities, even if they have never been confirmed in controlled studies as negative predictors of response.

Conflict of interest
The authors have no conflicts of interest to disclose.

ACKNOWLEDGEMENTS
HBV RER investigators: AOU di Parma - Malattie Infettive ed Epatologia, C. Ferrari - T. Giuberti; AOU di Modena - Gastroenterologia, E. Villa; AOU di Bologna - Medicina Interna, P. Andreone; AOU di Modena - Medicina II, A. Pietrangelo - G. Abbati; ASMN di Reggio Emilia - Malattie Infettive, G. Magnani - M. Massari; AOU di Bologna - Malattie Infettive, G. Verucchi; AUSL di Forlì - Malattie Infettive, C. Cancellieri; AUSL di Forlì - Gastroenterologia, S. Ricca Rosellini; AUSL di Bologna - Bentivoglio - Medicina Interna, F. Levantesi; AOU di Bologna - G. Mazzella; AUSL di Piacenza - Malattie Infettive, D. Sacchini; ASMN di Reggio Emilia - Gastroenterologia, G. Fornaciari; AOU di Modena - Malattie Infettive, C. Mussini - V. Borghi - G. Cuomo; AUSL di Faenza - Epatologia e Trapianto di Fegato, F. Foschi; AOU di Ferrara - Malattie Infettive, M. Libanore- S.D. Carradori; AOU di Ferrara - Malattie Infettive e Tropicali, C. Contini; AUSL di Rimini - Medicina 2, G. Ballardini; AUSL di Bologna - Gastroenterologia, S. Macchia; AOU di Ferrara - Gastroenterologia ed Endoscopia Digestiva, S. Boccia; AOU di Modena - Medicina I, C. Vandelli; AUSL di Ravenna - Malattie Infettive, P. Bassi - M. Zanotti; AUSL di Modena - Medicina Metabolica, P. Loria; AUSL di Piacenza - Gastroenterologia ed Epatologia, G. Sbolli - F. Fornari; AUSL di Modena - Carpi - Gastroenterologia e Centro Studi Fegato, P.V. Di Maira - S. Bellentani; AUSL di Rimini - Malattie Infettive, M. Arlotti; AUSL di Cesena - Malattie Infettive, C. Grosso; AOU di Bologna - Medicina Interna, G. Bolondi; AUSL di Cesena - Gastroenterologia, P. Pazzi; AUSL di Reggio Emilia - Guastalla - Medicina e Geriatria, A. Mazzocchi; AUSL di Faenza - Malattie Infettive, A. Govoni; AUSL di Bologna - Imola - Gastroenterologia, P. Fusaroli; AUSL di Ravenna - Lugo - Medicina Interna e Amb. Epatologico, P.L. Giacomoni; AOU di Bologna - Medicina Clinica, M. Lenzi; AUSL di Fidenza - Vaio, G. Pedretti.

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