Effectiveness of entecavir treatment and predictive factors for virologic response

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ABSTRACT

Introduction: Entecavir (ETV) is a potent inhibitor of hepatitis B virus (HBV) replication. In patients adherent to treatment, virologic remission rates of > 95 % can be maintained with entecavir at 3-5 years.

Aim and methods: A cohort study was performed, including all subjects who received ETV for chronic hepatitis B, in the South-Eastern Romania. We assessed viral response, HBeAg loss and seroconversion, HBsAg loss and seroconversion, biochemical response. Comparison of categorical data was performed by χ²-test or Fisher’s exact where applicable.

Results: Data from 533 patients were available: predominantly males (64 %), 82.6 % nucleotide naive, 23.1 % HBe-Ag positive, 78.2 % with elevated ALT, 8 % with cirrhosis. The median follow-up was 24 months (range 12-48 months). Rate of undetectable HBV DNA increased constantly from year 1 to 3, reaching 91.2 %. Positive predictive factors for virologic response were low score of fibrosis (p=0.006), low level of HBV DNA (p=0.003), while negative predictive factors were: HBe antigen positive status (p-value < 0.001), prior IFN therapy (p = 0.015). Virologic rebound was found in 7.8 % (breakthrough in 0.8 %). Rate of HBe Ag loss increases with the therapy duration, reaching 47.83 % in year 3, with two positive predictive factors: Male sex (p = 0.007), and undetectable HBV DNA at 24 weeks (p = 0.002). The percentage of HBs Ag loss was 1.31 %.

Conclusions: ETV maintained and even increased the high initial response rate (from 78 % to 91.2 %). Low score of fibrosis, low level of HBV DNA, HBe antigen negative status, absence of prior interferon therapy predict a good virologic response. Virologic rebound was found in a higher rate in our population, due probably to a poor drug compliance. Lamivudine-resistant patients usually respond well to ETV, but 15.62 % are non-responders, suspect of Entecavir resistance.

Key words: Chronic hepatitis B. HBe antigen positive. HBe antigen negative. Entecavir.

INTRODUCTION

Chronic hepatitis B virus (HBV) infection affects more than 350 million people worldwide, and is a leading cause of liver related mortality (1,2). These individuals are at increased risk of developing cirrhosis, liver failure or hepatocellular carcinoma (HCC) (3-5).

Most HBV consensus reports and guidelines recommend antiviral therapy for the immune clearance phase, especially when there is significant necroinflammatory activity and advanced fibrosis(6).

Therapy must ensure a degree of virologic suppression that will then lead to biochemical remission, histological improvement and prevention of complications. The ideal end point is HBS antigen (HBsAg) eradication, which however is infrequently achievable with the currently available anti-HBV agents. A more realistic end point is the induction of sustained or maintained virologic remission (7).

In HBe antigen (HBeAg) positive chronic hepatitis B, the initial goal of therapy is virologic response defined as HBsAg loss or seroconversion with HBV DNA below 1,000 IU/L (6).

The current available therapeutic options are interferon-based therapy, with a prolonged immunological effect (a sustained response) and long-term nucleos(t)ide analogue (NUCs) therapy, which can achieve a maintained response (8).

Entecavir (ETV) is a potent inhibitor of HBV replication (8). In HBeAg positive patients treatment should be continued until HBeAg seroconversion or until HBsAg
séroconversion, or if there is evidence of loss of efficacy. In HBeAg negative patients treatment should be continued until HBs seroconversion or if there is loss of efficacy (10-13).

ETV can reduce serum HBV DNA by 6.9 log_{10} in HBeAg-positive patients and by 5 log_{10} in HBeAg-negative patients. Although it is a more potent antiviral drug, the 1-year HBeAg seroconversion rate is not superior than other NUC (21 % at 1 year and 31 % after 2 years of ETV treatment) (14).

In a comparative analysis of all licensed antiviral therapies, Entecavir was most effective in improving liver histology (56 %) and only second after tenofovir for inducing undetectable HBV DNA levels (61 %) and normalization of ALT levels (70 %). It ranked third in loss of hepatitis B surface antigen (1 %) (15). Of note is the very low resistance rate (1.2 %) observed in the nucleoside-naïve HBeAg-negative patients treated with ETV for up to 5 years.

In patients adherent to treatment, virological remission rates of > 95 % can be maintained with entecavir at 3-5 years (16,17).

Romania has intermediate prevalence of HBV chronic infection (4.4 %) (18). Data show that in most cases of chronic Romanian HBV infection, there is mixture of A and D genotypes, which are very sensitive to NUCs (19).

The objectives of this study were to assess viral response (HBV DNA); HBeAg loss and serocconversion (only in patients who are HBeAg positive, by definition); HBsAg loss and serocconversion; biochemical response (ALT) and also predictive factors for viral and biochemical response.

MATERIAL AND METHODS

A retrospective cohort study was performed, including all subjects who received ETV for chronic hepatitis B, in the South-Eastern Romania (Bucharest, Ilfov, Dambovita, Giurgiu, Ialomita, Calarasi, Braila, Arges, Buzau, Prahova). Patients were selected according to current Romanian guidelines, and they were enrolled consecutively. Their medical records were available from the National Health Insurance House.

Inclusion criteria were established according to the current Romanian guidelines which recommend therapy for HBe Ag positive-patients when serum ALT levels are more than twice the upper limit of normal (or less but with active inflammation and fibrosis on liver biopsy or Fibromax) and HBV DNA levels ≥ 20,000 IU/ml. In HBe Ag negative-patients the same criteria regarding ALT and necroinflammation/fibrosis are used, but HBV DNA more than 2,000 IU/ml. HBV DNA levels, ALT, HBs Ag, HBs Ab (HBs antibodies), HBe Ag, HBe Ab (HBe antibodies) are monitored every 6 months to check for drug compliance and resistance development. Patients with coinfection (hepatitis C virus or hepatitis D virus were excluded).

Treatment: Naïve patients received 0.5 mg/day ETV, while those refractory to lamivudine received 1 mg/day. Lamivudine-refractory was defined as persistently detectable and increasing levels of HBV DNA by PCR on lamivudine or documented YMDD mutation.

Patients with prior interferon (IFN) therapy were those pre-treated at least 6 months with Interferon (4.5-5 MU million units three times weekly) or Peg-interferon (180 micrograms weekly subcutaneous).

Viral load (HBV DNA titre) was assessed using PCR (polymerase chain reaction)-based assays, which have a detection threshold of 12 IU/mL.

Outcomes

Percentage of patients with undetectable HBV DNA (by PCR assay) at week 24, 48, 72, 96, 120, 144, 168, 192, HBV DNA drop log_{10} IU/m at week 24, 48, rate of virological breakthrough, proportion of HBeAg positive patients at baseline who lost HBeAg by week 48, 96, 144, 192, proportion of HBeAg positive patients at baseline who seroconverted by week 48, 96, 144, 192, proportion of patients with abnormal ALT at baseline who normalised at weeks 24, 48, 72, 96, 120, 144, 168, 192, proportion of patients who experienced ALT flare, HBsAg loss, HBsAg seroconversion. We also analyzed the possibility of HBe antigen seroreversion, when data available.

For evaluation of response we used the EASL (European Association for Study of the Liver) definitions: Virological response is defined as undetectable HBV DNA by a sensitive PCR assay. Partial virological response is defined as a decrease in HBV DNA of more than 1 log_{10} IU/ml (international units per milliliter) but detectable HBV DNA after at least 6 months of therapy in compliant patients. Virological breakthrough is defined as a confirmed increase in HBV DNA level of more than 1 log_{10} IU/ml compared to the nadir (lowest value) for patients with detectable HBV DNA level, or HBV DNA levels increasing to > 20 IU ml for patients with undetectable HBV DNA levels during ETV therapy; it may precede a biochemical break-through, characterised by an increase in ALT levels. The main causes of virological breakthrough on NUC therapy are poor adherence to therapy and/or selection of drug-resistant HBV variants (resistance) (7).

Biochemical breakthrough was defined by ALT levels increasing to > ULN for patients who had achieved ALT normalization under ETV treatment.

Statistical analysis

Continuous variables were resumed as median with range, because the distribution was not normal, therefore their associations were further analyzed with nonparametric correlations (Kendall’s tau_b coefficient) and Mann-
Whitney U test. For multivariable analysis, logistic regression was performed when the dependent variable was dichotomous, while the general linear model for repeated measures was used for the multivariable comparison of continuous variables with multiple measurements. Both Stata 11 (StataCorp, College Station, Texas, USA) and SPSS 16.0 (SPSS, Inc., Chicago, IL., USA) were used for data analysis. Hypothesis testing was 2-tailed, and statistical significance was defined by $p < .05$.

RESULTS

A total of 611 patients were enrolled, but 78 were lost of follow-up (stopped therapy at their own discretion, with less than 6 months duration of therapy). The baseline demographics of the remaining 533 cases are listed in table I.

All 533 patients had been followed up for 1 year, 382 and 125 and 8 had been followed up for 2 years, 3 years and 4 years respectively, at the time of study. The median duration of follow-up was 24 months (range 12-48 months).

Most of the patients were male (63.4 %). Nucleotide naïve were 440 (82.6 %), 123 patients (23.1 %) had prior exposure to interferon, 93 subjects (17.4 %) had received prior lamivudine at any time. HBe-Ag positive patients constituted 23.1 % of the population. Four hundred seventeen (78.2 %) patients had baseline elevated ALT levels. Forty-two (7.9 %) had liver cirrhosis at the beginning of ETV therapy, while the rate of comorbidities was 14.3 %. Twenty patients associated haematologic neoplasia, 6 had other solid malignant tumors, 5 with chronic renal failure (2 with kidney transplantation), 1 with systemic lupus erythematosus, 5 with reumatoid arthritis, and only one patient with liver transplantation.

Forty-eight point two per cent of patients with baseline elevated ALT had liver biopsy performed (201/417), most of them with score 1 of fibrosis (38 %) or 3 (30 %). Eighty-one percent of patients with baseline normal ALT had a liver biopsy (94/116), and again a majority of them with score 1 of fibrosis (44.7 %) or 3 (30 %) (after Ishak). Advanced degree of fibrosis correlated well with age ($p < 0.001$) and with level of ALT ($p = 0.038$), but doesn’t correlate with HBV DNA level or HBe Ag status (nonparametric Kendall’s tau_b correlation coefficient).

HBV DNA levels decreased with a median of 4.45 log at 24 weeks of therapy and 5.15 log at 48 weeks.

The rates of undetectable HBV DNA from year 1 to 3 are shown in figure 1. There was a constant increase in the rate of undetectable HBV DNA from year 1 to 3. At year 3, 114 out of 125 (91.2 %) had undetectable HBV DNA levels (< 12 IU/ml). The HBV DNA undetectable rates were 82.6 % for HBe-positive patients, and 92.2 % for HBe-negative patients.

According to baseline HBV DNA levels, patients were classified as 4-5.9, 6-7.9, and > 8 logs IU/ml. At year 3, the HBV DNA undetectable rates were respectively 95.7 %, 86.5 % and 83.3 % (Fig. 2).

Table II depicts variables that predict virologic response at week 48. Positive predictive factors for virologic response are: Low score of fibrosis, low level of HBV DNA. Negative predictive factors for virologic response are: HBe antigen positive status, prior IFN therapy. Baseline level of ALT, sex, previous lamivudine therapy had no impact on virologic response.

Virologic rebound was found in 7.8 % (29/371). Patients with virologic rebound (that is an increase in HBV DNA of at least 2 log10 IU/ml compared to nadir), are most commonly men (82.8 %), young (median age 35), nucleoside-naïve (86.2 %), HBe antigen negative (82.8 %). In 90 %

<table>
<thead>
<tr>
<th>Variable</th>
<th>340 (63.8 %)</th>
<th>42 (1778)</th>
<th>123 (23.1 %)</th>
<th>96 (48-192)</th>
<th>123 (23.1 %)</th>
<th>93 (17.4 %)</th>
<th>29 (5.4 %)</th>
<th>417 (78.2 %)</th>
<th>890,000 (12-943,000,000)</th>
<th>76 (14.3 %)</th>
<th>42 (7.9 %)</th>
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<tbody>
<tr>
<td>Sex M</td>
<td>Age*</td>
<td>HBe antigen positive</td>
<td>Follow-up duration (weeks)*</td>
<td>IFN pretreated</td>
<td>LAM pretreated</td>
<td>LAM-resistant</td>
<td>ALT increased</td>
<td>Viremia (IU/ml)*</td>
<td>Comorbidities</td>
<td>Liver cirrhosis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sex M</td>
<td>Age*</td>
<td>HBe antigen positive</td>
<td>Follow-up duration (weeks)*</td>
<td>IFN pretreated</td>
<td>LAM pretreated</td>
<td>LAM-resistant</td>
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<td>Comorbidities</td>
<td>Liver cirrhosis</td>
</tr>
<tr>
<td></td>
<td>Sex M</td>
<td>Age*</td>
<td>HBe antigen positive</td>
<td>Follow-up duration (weeks)*</td>
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<td>LAM-resistant</td>
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<td>Liver cirrhosis</td>
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<td>Age*</td>
<td>HBe antigen positive</td>
<td>Follow-up duration (weeks)*</td>
<td>IFN pretreated</td>
<td>LAM pretreated</td>
<td>LAM-resistant</td>
<td>ALT increased</td>
<td>Viremia (IU/ml)*</td>
<td>Comorbidities</td>
<td>Liver cirrhosis</td>
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<td></td>
<td>Sex M</td>
<td>Age*</td>
<td>HBe antigen positive</td>
<td>Follow-up duration (weeks)*</td>
<td>IFN pretreated</td>
<td>LAM pretreated</td>
<td>LAM-resistant</td>
<td>ALT increased</td>
<td>Viremia (IU/ml)*</td>
<td>Comorbidities</td>
<td>Liver cirrhosis</td>
</tr>
</tbody>
</table>

*Median, range; IFN: Interferon; LAM: Lamivudine; ALT: Alanine amino transferase.

Fig. 1. Number of patients and rates of undetectable HBV DNA levels of the study population from year 1 to 3 (HBe-positive patients, and HBe-negative patients).
of them, next viremia was undetectable, but in 10% next viremia was increasing (that is virologic breakthrough). Thus, in total, virologic breakthrough was found in 0.8% of patients. We did not have the possibility to assess compliance in these patients, but, because of this HBV DNA pattern, we can assume that non-compliance is the problem in 90% of the cases with virologic rebound and resistance might be suspected in only 10%.

Proportion of biochemical breakthrough was 9% (48/533). Risk factors for breakthrough are: Younger age (p = 0.01), male sex (p = 0.01), increased baseline HBV DNA level (p = 0.04) (trough logistic regression).

No ALT flare was recorded among study patients. At year 3, 80.8% patients (101 out of 125), with elevated ALT levels at baseline had ALT normalization (Fig. 3). The ALT normalization rates were 91.3% (21 out of 23) for HBe-positive patients, and 78.4% (80 out of 102) HBe-negative patients at year 3.

Table III depicts variables that may have an impact on biochemical response. Male sex was a negative predictive factor for biochemical response, while advanced age was a positive predictive factor. Other variables didn’t impact on biochemical response: HBe Ag status, initial level of ALT or HBV DNA level, previous therapy, or level of fibrosis.

Rate of HBe Ag dissapearance increase with the therapy duration, reaching 47.83% in year 3 of therapy (Fig. 4). Male sex is one positive predictive factor, with OR = 3.85 (CI95% = 1.44, 10.27, p = 0.007), and undetectable HBV DNA at 24 weeks another factor, with OR = 2 (CI95% = 1.12, p = 0.002), while initial HBV DNA level, ALT, age, prior antiviral therapy didn’t influence HBeAg status.

Proportion of patients HBeAg positive which seroconverted to HBe antibodies constantly increase with therapy duration, to 26.1% in year 3, advanced age being the only positive predictive factor for this phenomenon OR = 2, CI95% = 1.02, 1.15, p = 0.012 (Fig. 4).

Table II. Variables that predict virologic response (at week 48)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower score of fibrosis</td>
<td>1.5</td>
<td>1.13, 1.98</td>
<td>0.006</td>
</tr>
<tr>
<td>Lower level of HBV DNA</td>
<td>1.01</td>
<td>1.00, 1.03</td>
<td>0.003</td>
</tr>
<tr>
<td>HBe Ag+</td>
<td>0.15</td>
<td>0.07, 0.3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Previous IFN therapy</td>
<td>0.45</td>
<td>0.24, 0.86</td>
<td>0.015</td>
</tr>
<tr>
<td>Previous LAM therapy</td>
<td>1.10</td>
<td>0.51, 2.35</td>
<td>0.983</td>
</tr>
<tr>
<td>Baseline level of ALT</td>
<td>0.99</td>
<td>0.99, 1.01</td>
<td>0.345</td>
</tr>
<tr>
<td>Sex M</td>
<td>0.58</td>
<td>0.29, 1.19</td>
<td>0.757</td>
</tr>
<tr>
<td>Age</td>
<td>1.01</td>
<td>0.99, 1.04</td>
<td>0.426</td>
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</tbody>
</table>

Table III. Predictive factors for biochemical response

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex M</td>
<td>0.301</td>
<td>0.138, 0.658</td>
<td>0.003</td>
</tr>
<tr>
<td>Age</td>
<td>1.024</td>
<td>1.002, 1.047</td>
<td>0.031</td>
</tr>
<tr>
<td>Increased ALT</td>
<td>0.375</td>
<td>0.124, 1.137</td>
<td>0.083</td>
</tr>
<tr>
<td>HBe Ag positive</td>
<td>0.481</td>
<td>0.197, 1.174</td>
<td>0.108</td>
</tr>
<tr>
<td>Initial level of HBV DNA</td>
<td>1.00</td>
<td>1.00, 1.00</td>
<td>0.319</td>
</tr>
<tr>
<td>Previous LAM therapy</td>
<td>0.805</td>
<td>0.257, 2.516</td>
<td>0.709</td>
</tr>
<tr>
<td>Previous IFN therapy</td>
<td>0.95</td>
<td>0.40, 2.25</td>
<td>0.940</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>1.00</td>
<td>0.70, 1.44</td>
<td>0.969</td>
</tr>
</tbody>
</table>
Four patients (0.75 %) lost HBs Ag and developed detectable anti-HBs antibodies levels, all of them were nucleosides naïve, one with prior interferon exposure, all of them had baseline elevated ALT, with a treatment duration of 1.5–3 years. 3 HBe Ag-negative and one HBe Ag-positive, and maintained HBV DNA levels undetectable.

Three patients (0.55 %) lost HBs Ag but didn’t develop detectable anti-HBs antibodies levels. They were all nucleosides naïve, two with prior interferon exposure, all of them had baseline elevated ALT, with a treatment duration of 2-3 years, one HBe Ag-negative and two HBe Ag-positive, and maintained HBV DNA levels undetectable.

Overall, the percentage of HBs Ag loss is 1.31%.

**DISCUSSION**

This is the first cohort study of Entecavir therapy in a Romanian population. Entecavir was approved as a reimbursed medication for chronic hepatitis B in 2008, so the duration of therapy for this patients has a history of 192 weeks (almost 4 years). Non-compliance is a problem in this population, since 78 out of 611 were lost during follow-up.

When we compared our cohort study with an Italian prospective/retrospective cohort study, we find a similar male predominance, while the median age is lower in our population (42 versus 58), proportion of HBe negative patients is roughly similar (around 80 %), baseline median level of DNA HBV is similar (6 log10), while in the Italian cohort the rate of comorbidities is much higher (56 % versus 14.4 %), same as the proportion of patients with cirrhosis (49 % versus 8 %) (29).

In the cohort study of Buti M et al. (30) there was an increased proportion of males; 73 % and a slightly increased proportion of patients HBeAg-positive: 30 %, compared to our study. At baseline, the median hepatitis B virus DNA was 5.94, which is comparable to our study. At week 48, 83 % of the patients (61 % HBeAg-positive; 92 % HBeAg-negative) achieved a virological response, a much better response that in our study and 82 % (78 % HBeAg-positive; 83 % HBeAg-negative) of those with elevated baseline alanine aminotransferase showed a biochemical response, similar to our data (30).

In our study, similar with the study of Yuen et al., there were incremental increases in the rates of HBV DNA undetectability reaching 91.2 % at year 3, compared with 92.1 % in the Yuen study (23). Rates of undetectable HBV DNA are slightly lower in our cohort study compared with the Italian: 78 % versus 85 % at year 1, 84 versus 95 % at year 2, 91.2 % versus 96 % at year 3, but constantly increasing. The difference in proportion is higher regarding HBe antigen positive patients in the first 2 years of therapy, who respond slower to ETV compared with HBe negative patients, but at year 3 the proportion of HBV DNA negative patients is similar: 82.6 % in our study compared with 84 % in the Italian cohort (29).

The present study has several unique features addressing specific questions for entecavir treatment. We studied the predictive factors for virologic response and we found that low score of fibrosis, low level of HBV DNA, HBe antigen negative status, absence of prior interferon therapy predict a good virologic response. The impact of the severity of fibrosis stage might be over-appreciated because in our population the number of cirrhotic patients analyzed is low (8.1 %) and individuals with a fibrosis Ishak score > 3 were around 35 % of the subset of 297/541 who underwent liver biopsy. Baseline level of ALT, age, sex, previous Lamivudine therapy had no impact on virologic response. These data might suggest that patients with chronic hepatitis B would better benefit if they would be first treated with nucleoside-analogues, and then with interferon.

In our study, baseline HBV DNA levels predict virologic response: so, 92.1 % of patients with baseline HBV DNA levels under 8 logs copies/ml had undetectable HBV DNA at year 3, compared with 83.3 % of patients with baseline HBV DNA levels above 8 logs copies/ml, which is comparable with the proportion reported in the Yuen study: 100 % respectively 76.5 % (23).

Virologic rebound was found in 7.8 % (29/371), which is a high rate, but the breakthrough rate was low 0.8 %, comparable with other studies: ≤ 2 % in all cases (11,12,21,22). Our opinion is that virological rebound in NA naïve patients receiving entecavir is usually due to poor drug compliance (in 90 % of the cases the next HBV DNA level was undetectable). Also, other authors report that most of the patients with virologic breakthroughs are not due to the emergence of ETV-resistant mutations (12,22). Nevertheless onelimitation of the present study was that entecavir-resistant mutations were not assessed.

Proportion of HBe antigen seroconversion at year 3 is lower than in the Italian cohort: 26.1 versus 40 % (29).

In a randomized LAM-controlled ETV trial in HBeAg-positive patients, the HBV DNA levels (< 80 vs. > 80 IU/mL)
at week 24 were useful in predicting the HbeAg seroconversion rates (30 % vs. 17 %) and undetectable HBV DNA (96 % vs. 50 %) at week 52 of ETV therapy (11). In our cohort we found two predictive factors for HBe antigen disappearance: Male sex and undetectable HBV DNA at 24 weeks. Another study reports as pre-treatment factors predictive of anti-HBe seroconversion in HBeAg-positive CHB low viral load (HBV DNA below 2 x 10^6 IU/ml), high serum ALT levels, high activity scores on liver biopsy (26), but in our study only advanced age predicted anti-HBe seroconversion.

The measurement of HBV-DNA at week 24 of therapy is considered essential in the management of both HBeAg-positive and HBeAg-negative patients, because it is the main predictor of subsequent treatment efficacy in terms of HBeAg seroconversion in HBeAg positive patients, and of subsequent resistance (20).

In other studies, the proportion of HBeAg positive patients at baseline treated with ETV who seroconverted by week 48 was 15-21 % (11,22), which is comparable with our results: 19.5 %. Our data shows, in accordance with other studies, that HBe antigen seroconversion rates tend to increase with any prolongation of therapy exceeding cumulative rates close to 30 % at three years (14).

Proportion of patients with HBsAg loss in our study is 1.31 %, comparable with the data found in the literature: < 1 % in one study (10) and 2 % in others (12,30). Analysis of these patients shows that: They were all nucleosides naïve, three out of seven with prior interferon exposure, all of them had baseline elevated ALT, with a treatment duration of 2-3 years, four HBe Ag-negative and three HBe Ag-positive, and maintained HBV DNA levels undetectable.

If we analyse only HBe antigen positive patients, the proportion of HBs antigen loss at year three of therapy was 10.3 %, which is much lower than the rate reported by Lampertico for this category of patients: 20 % (29).

Proportion (%) of patients with a biochemical response at week 48 is 91.2 %, which is comparable with the rate reported in other studies: 68-94 % (11,12,22).

We found no ALT flare, but data in the literature report a proportion (%) of patients experiencing an ALT flare up to week 48 varied between 1-4 % (10,11,22).

Other studies report a cumulative rate of entecavir-resistant mutations of 1.2 % at year 3 in lamivudine naïve subjects (23), however, in our population we don’t have sequencing data on individuals with viral rebound or breakthrough. In the Yuen study, three patients out of 222 experienced virologic breakthrough, one with resistance development, one with subsequent HBe Ag seroconversion, and one with subsequent decline in HBV DNA (23). There is a high genetic barrier to resistance of entecavir that requires the initial selection of rtM204I/V mutations (lamivudine-resistant mutations), followed by additional “signature” entecavir-associated mutations at the rtI169, rtM250, rtT184 or rtS202 loci (25).

There were 32 lamivudine-resistant (documented by resistance study or increasing viremia under LAM therapy) patients included in our study, and all of them received 1.0 mg day ETV. Most of them, 19/32 (59.37 %) had a good response: Non-detectable HBV DNA level at 48 weeks of therapy, one with virologic rebound, 8/32 (25 %) presented a slow response: HBV DNA level became undetectable after 48 weeks of therapy and remained undetectable, however 5/32 (15.62 %) were non-responders: HBV DNA level never became undetectable, was either slowly decreasing (3 cases), either rising (2 cases). They are the only patients included in the present study who are suspect of entecavir resistance, but we don’t have data on HBV sequencing. These data are comparable with data in the literature: Approximately 35 % of LAM-resistant patients develop resistance to entecavir 1.0 mg daily within 3 years of being switched to ETV (23). ETV has been licensed for the treatment of patients with LAM resistance, but its cumulative long term resistance rate is rather high (> 50 % at 5 years) in this clinical setting because the pre-existing LAM resistant HBV strains increase dramatically the probability of ETV resistance (27). Therefore, ETV is a less attractive long-term therapeutic option for patients with LAM resistance (28).

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