The E genotype of hepatitis B: Clinical and virological characteristics, and response to interferon

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Summary

Objectives

10 hepatitis B virus (HBV) genotypes are known with different geographic distribution and response to interferon (IFN) therapy. The E genotype is the more prevalent genotype in West and Central Africa, but few data about response to IFN are available.

We describe the epidemiological and clinical characteristics in a cohort of patients immigrants from Africa in our country with HBV E genotype chronic hepatitis infection (CHB).

Methods

63 patients with CHB and E genotype were included; 41 with CHB and low viral load were treated with PEG-IFN monotherapy; 10 with CHB and high viral load with sequential approach (entecavir and PEG-IFN). 12 patients with inactive CHB were followed with blood sample and abdomen ultrasonography every six months.

Results

The virological response in the monotherapy group was 17.9%. Hepatitis B surface antigen (HBsAg) loss was observed in 1 patient (2.5%); 56 patients (88%) showed at the time of diagnosis of CHB another infectious diseases that required specific treatment before PEG-IFN; this treatment was also affected by an higher incidence of side-effects (>50%). All patients with high viremia showed a primary non-response to PEG-IFN.

Conclusions

The HBV E genotype evidences the worse response to PEG-IFN and maybe requires novel treatment options.

Introduction

CHB represents a major health problem with approximately 400 million carriers worldwide (1). The persistence HBV infection can worsen to cirrhosis, hepatic decompensation and hepatocellular carcinoma (HCC) (2). The serum HBV-DNA level was identified as the main risk factor for liver cirrhosis and hepatocellular carcinoma (3). thus the main goal of CHB treatment is the suppression of viral load (4) since the complete eradication of HBV infection is impossible to obtain because of covalently closed circular DNA (cccDNA) which is responsible for persistent hepatocytes infection even in patients with a loss of serum HBV DNA (5). Several hepatitis B viral factors as viral load and viral mutations are related to clinical outcomes, while the HBV genotype has been object of study in the last years with respect to the influence on the infection natural course and the response to the treatment (6). Currently at least 10 HBV genotypes are known (A–J) with several subtypes, defined by a divergence in the HBV-DNA >8% for genotypes and 4–8% for subtypes (7) Geographic distribution of HBV genotypes is well defined, and differences in modes of transmission, virological and serological outcomes and response to IFN therapy are reported in several studies (8) The treatment with nucleos(t)ide analogues (NUCs), instead, seems not to be influenced by HBV genotypes (9, 10).The main studies which analyzed the role of HBV genotype in the treatment with NUCs

mostly dealt with genotypes A, B, C and D (11), while only 7 patients with E genotype were reported in the study of Westland et al. (12); others studies about treatment response to IFN showed only data according to A, B, C and D genotypes (11). Only few data about treatment response to IFN were reported in patients with HBV E genotype (10, 13). This genotype is present only in Africa (14) and is most prevalent in west-Africa, above all in Gambia, Mali, Burkina Faso, Ghana, Togo, Benin, Congo, Central African Republic, Senegal, Ivory Coast, Namibia and Angola (15); the HBV A genotype is predominant in east, north and south Africa; differently to A, HBV E genotype doesn't have subtypes described (16). In Europe the E genotype was only found in sporadic cases of immigrants (17). Interestingly, the E genotype shows a lower genetic diversity than A genotype, and this might suggest a more recent evolutionary history and introduction; the estimate time of appearance of this genotype was described in 200 years approximately (18). This is also confirmed by the evidence that in the afro-american population the HBV A genotype is the most prevalent genotype and the spread of E genotype should have followed the slave trade; the source is still unknown, but seems to be related to animal reservoir, such woodchuck and chimpanzees (18). Until now few data about the treatment of CHB with E genotype were available. We describe the clinical and epidemiological data in a cohort of patients immigrants from Africa in our country with HBV E genotype and CHB.

Patients and methods

Patient population

We retrospectively included in this study all the patients affected by CHB with HBV E genotype, diagnosed at our Infectious Diseases Unit in Turin from 2005 to 2010. This is a population of young immigrants from Central-Africa that through the desert of Sahara have arrived in Libya, where they have been imprisoned for many years under precarious hygienic and sanitary conditions. Main inclusion criteria were: HBsAg positive with any HBV-DNA and ALT value and HBV E genotype, follow-up period of at least 2 years. We excluded all patients with coinfection (HCV, HDV and HIV), with incomplete course of therapy (drop-out) or follow-up, and absence of validated outcome or available sample DNA.

Study end points

According to EASL guidelines (4) we defined the “end of treatment virological response” as HBV-DNA <2000 IU/mL (10,000 copies/mL) at the end of therapy; the “sustained virological response” as HBV-DNA <2000 IU/mL (10,000 copies/mL) at 12 months after the end of therapy. We evaluated the serological response according to HBsAg loss and anti-HBs appearance (at the end of therapy or during the follow-up).

The virological and serological response were studied according to the role of IL28-B genotype.

Treatment options

All the patients with active CHB have begun the treatment; PEG-IFN α2a monotherapy was administered at dose of 180 μcg/week in 39 patients with active CHB and baseline viral load <106 IU/mL; 2 patients refused IFN therapy and were treated only with entecavir (ETV). Among the patients with active CHB and high HBV-DNA, 7 were treated with a sequential therapy using ETV for 12 weeks before the PEG-IFN administration, ETV + PEG-IFN association for 12 weeks, then PEG-IFN monotherapy for 24 weeks. 3 patients evidenced the presence of HCC at the first assessment and started the therapy with tenofovir disoproxil fumarate (TDF). All the patients with inactive CHB were followed every six months with blood examination and ultrasonography.

Assays

Serum HBV-DNA levels were quantified with the Real Time PCR COBAS AmpliPrep/COBAS TaqMan HBV Test 2.0 (Roche Molecular Systems, NJ, USA). HBV genotypes were determined with the INNOLIPA reverse hybridization assays (INNOGENETICS, Belgium). HBsAg, HBeAg, anti-HBs, anti-HBe were detected by the Elecsys instrumental platform (Roche Diagnostics, Italy); qHBsAg was quantified with ARCHITECT HBsAg (Abbott Diagnostics, Ireland). Fibrosis stage (F) was determined with Fibroscan using the Metavir score. Under-weight condition was defined using the body mass index (BMI) <20. Neutropenia was defined as absolute neutrophil count <2000/µL. The severity of neutropenia was assessed according to National Cancer Institute criteria: grade 1: 2000–1500/µL, grade 2: 1500–1000/µL, grade 3: 1000–500/µL, grade 4: <500/µL. Anemia was defined as reduction of haemoglobin below the normal value; mild anemia as <13 g/dL (men) or <12 g/dL (women), moderate <10 g/dL, severe <7 g/dL.

IL-28B genotyping

Genomic DNA was isolated from blood samples. Patients who agreed to undergo genetic analyses and for whom blood samples were available were genotyped for rs12979860, rs8099917 and rs12980275 IL-28B polymorphisms with TaqMan Drug Metabolism Genotyping Assays (TaqMan MGM probes, FAM and VIC dye-labeled, Applied Biosystems by Life Technologies, Carlsbad, California, US), using a real-time polymerase chain reaction allelic discrimination system (Bio-Rad Real-time thermal cycler CFX96) using a standard procedure (primers, probes, and PCR conditions available on request).

Statistical analysis

For descriptive statistics, continuous variables were summarized as median (Inter-quartile range (IQR): 25th to 75th percentiles). Categorical variables were described as frequency and percentage. All data were assessed for normality using a Shapiro–Wilk test and categorical data were compared using a Mann Whitney or Kruskal–Wallis statistical test. To investigate continuous data, a Spearman Rank correlation was utilized. The association was calculated using the $\chi 2$-test. Statistical analyses were conducted by using SPSS software package ver. 20.0 (Chicago, IL, USA).

Results

Baseline characteristics

We presented a cohort of 63 patients affected by CHB with HBV E genotype. All patients were immigrants from west-Africa in Italy. The baseline characteristics of this population were reported in Table 1. 41 patients (63.9%) have an active CHB with HBV-DNA >2000 IU/mL and ALT >37 IU/mL, 10 (16.4%) have an active CHB with high viral load, defined as HBV-DNA >106 IU/mL and 12 (19.7%) have an inactive CHB with HBV-DNA between 20 and 2000 IU/mL and normal or ALT <2UNL. 7 patients (11.1%) have an HBeAg+, 5 (7.9%) have a cirrhosis. In Fig. 1 was showed the geographical origin of our patients.

Table 1. Baseline characteristics of the study population.

Characteristics	Patients with CHB and E genotype (n = 63)		
	Active CHB n, (%)	Active CHB and high viremia n, (%)	Inactive CHB n, (%)
	41 (63.9)	10 (16.4)	12 (19.7)
Age (yr) median [IQR]; (Range)	36.9 [28.6–42.9] (24–56)	29.2 [26.5–37.3] (21–39)	25.5 [19.3–34.5] (18–36)
Male sexn (%)	36 (92.3)	10 (100)	12 (100)
BMI median [IQR]; (Range)	21.0 [19.5–22.0] (17.0–27.0)	21.4 [18.3–21.9] (17.2–24.5)	20.8 [17.6–23.4] (17.0–24.0)
Metavir scoren (%)			
F0	1 (2.6)	0 (0)	4 (33.3)
F1	10 (25.6)	0 (0)	6 (50)
F2	16 (41.0)	2 (20)	2 (16.7)
F3	11 (28.2)	4 (40)	0 (0)
F4	1 (2.6)	4 (40)	0 (0)
HBeAg + n (%)	2 (4.8)	5 (50)	0 (0)
HBV-DNA BL (Log IU/mL) median [IQR]; (Range)	4.49 [3.91–5.22] (3.50–6.16)	7.4 [6.1–8.0] (6.0–8.1)	2.81 [2.55–2.90] (2.33–2.96)
qHBsAg BL (Log IU/mL) median [IQR]; (Range)	3.82 [3.63–	4.3 [4.1–4.4]	3.51 [3.31–3.75]

Characteristics	Patients with CHB and E genotype (n = 63)		
	Active CHB n, (%)	Active CHB and high viremia n, (%)	Inactive CHB n, (%)
	41 (63.9)	10 (16.4)	12 (19.7)
	3.99] (3.26–4.49)	(3.8–5)	(2.96–4.05)
ALT BL (IU/mL) median [IQR]; (Range)	72 [59–91] (43–148)	114 [61.0–165.5] (34–268)	37 [25–41] (23–44)

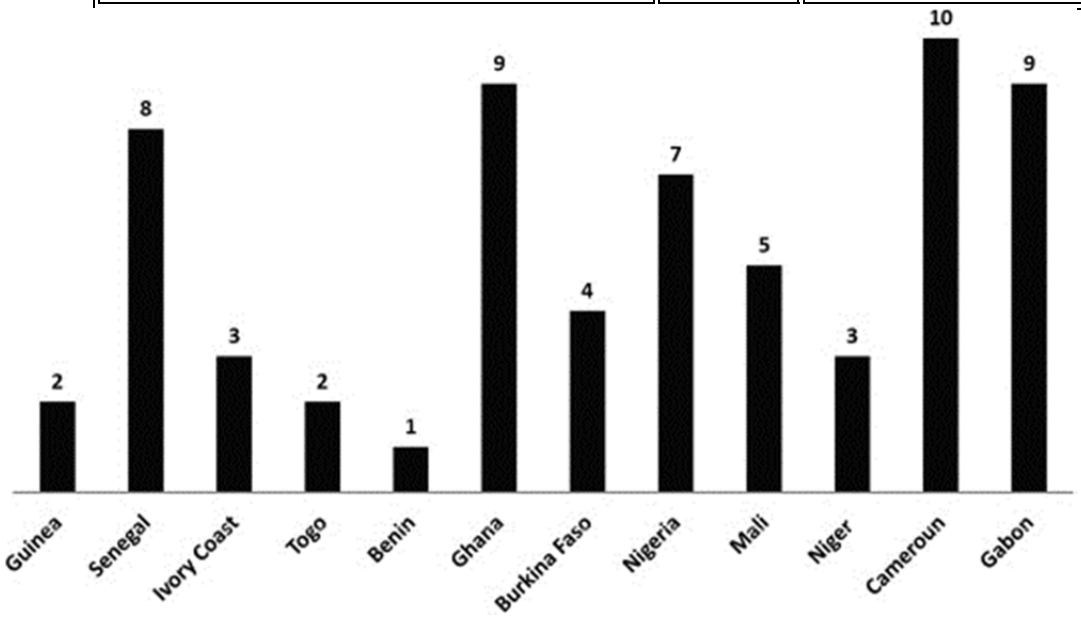

Figure 1.

Geographical origin of study population reported with number of patients for each birth country.

IL28B genotype distribution

The distribution of IL28B genotypes in our patients was depicted in Fig. 2; CC genotype of rs12979860 was present in only 12 patients (19%), while TC in 41 (65%) was the more prevalent genotype. We noticed a difference between rs12979860 CC distribution in HBV D and E genotype; in our cohort of 66 patients with CHB and D genotype the number of CC carriers (n = 36, 54.5%) was significantly higher than in E genotype ($p = 0.013$). No differences were reported in the rs8099917 genotypic distribution. Only 2 patients (3.1%) were classified discordantly to rs12979860 and rs12980275 frequencies. No significant differences were found between IL28B polymorphisms and the severity of CHB or active CHB and inactive carriers.

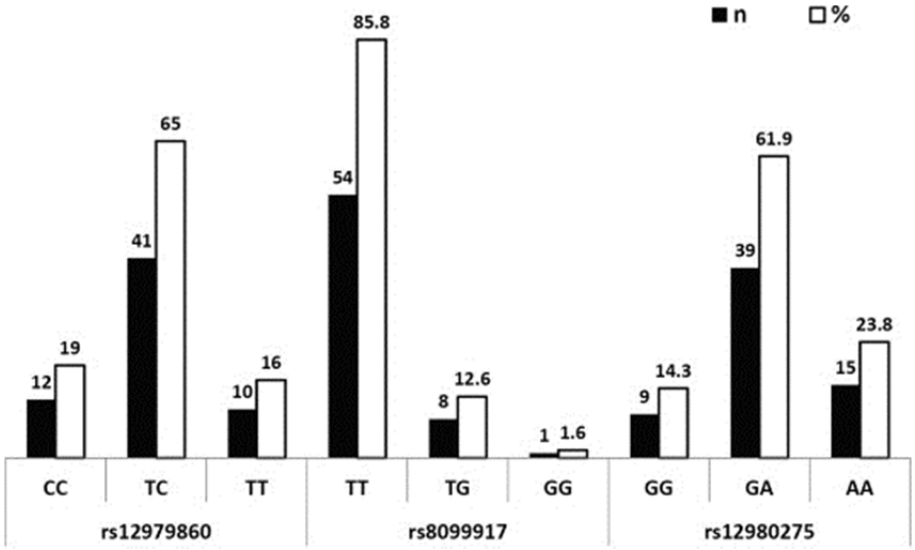

Figure 2.

Distribution (n and %) of the interleukin 28B polymorphisms (rs12979860, rs8099917 and rs12980275) in the study population.

Co-morbidity and extrahepatic manifestations present in the study population

Co-morbidity and extrahepatic manifestations were showed in Fig. 3. Fifteen patients (23.8%) evidenced the presence of type II cryoglobulins without symptoms; in 3 (4.7%) patients without cirrhosis was diagnosed an HCC before the start of the treatment (2 with solitary tumor, 1 multifocal). At the time of the diagnosis, 56 patients (88.8%) presented other infectious diseases that required a specific treatment; 3 patients (4.7%) were infected by Schistosomiasis, 5 (7.9) had an uncomplicated Plasmodium falciparum malaria, 6 (9.5%) latent syphilis, 11 (17.4%) intestinal amebiasis, 4 (6.3) toxocariasis, 14 (22.2) active pulmonary tuberculosis, 6 (9.5%) extra-pulmonary tuberculosis, 7 (11.1%) urethritis non-gonococcal; 38 patients (60.3%) showed an under-weight condition due to malnutrition.

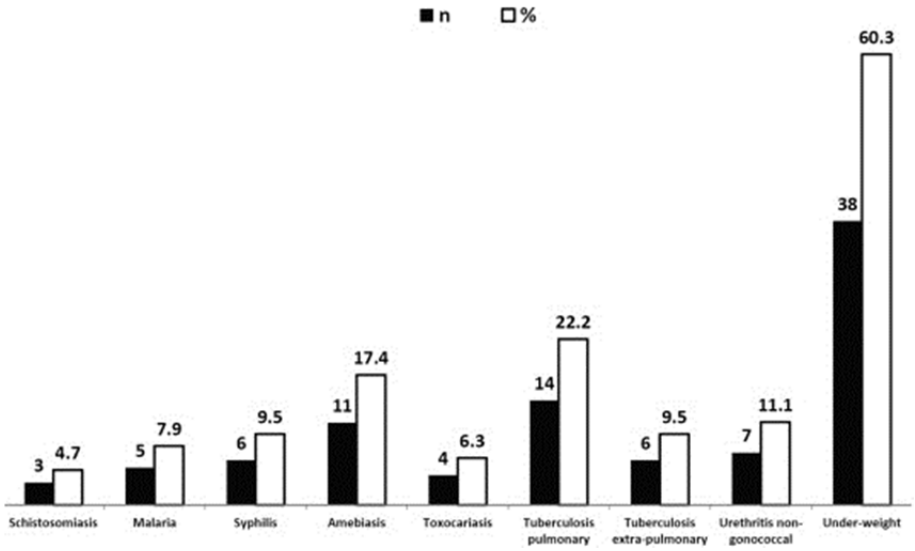

Figure 3.

Infectious diseases and co-morbidities present in the study population at the time of diagnosis of CHB.

Treatment response to PEG-IFN

Monotherapy with PEG-IFN α2a was administered in 39 patients with baseline viral load <106 IU/mL at the dose of 180 μcg/week. Median duration of the treatment was 48 weeks [IQR: 24–48]; (range: 12–72); 5 (12.8%) patients interrupted the treatment after 12 weeks for grade 4 neutropenia; 10 (25.6%) stopped the therapy at 24 weeks: 6 (15.3%) for side-effects (weakness, fatigue, depression/anxiety), 4 (10.2%) for poor response to the treatment (HBV-DNA decrease <1 Log IU/mL and qHBsAg decrease <0.5 Log IU/mL) (Table 3). 24 patients (61.5%) completed the 48 weeks of PEG-IFN and 3 (7.7%) prolonged to 72 weeks for the good compliance and higher decline of qHBsAg after 48 weeks. In the HBeAg-negative patients, the "end-of-treatment virological response" was achieved in 4 (10.2) patients; the "sustained virological response" was obtained in 3 (7.6%) patients that prolonged the course of treatment to 72 weeks; the global virological response was insofar 17.9%. In these patients qHBsAg level dropped and remained below 2 Log IU/mL. In 2 patients with HBeAg-positive the seroconversion to anti-HBe has been reached after end of treatment; 1 of these patients cleared the HBsAg (2.5%) after 1 years. None of the patients with HBeAg-negative cleared the HBsAg.

Treatment response to sequential therapy with ETV and PEG-IFN

10 patients with baseline viral load >106 IU/mL were treated with sequential approach using ETV before the start of PEG-IFN; in these patients was observed a dismal decrease of HBV-DNA during 12 weeks of ETV administration (1.9 Log IU/mL) and 12 weeks of PEG-IFN (1.2 Log IU/mL) and poor reduction of qHBsAg after 12 weeks of PEG-IFN (0.2 Log IU/mL); the PEG-IFN treatment was interrupted and they continued the ETV monotherapy. Virological response to ETV monotherapy was achieved in 6 patients (60%) after a median of 5.2 months; 4 patients (40%) with detectable viremia after 6 months of therapy (partial virological response) stopped the ETV treatment and started the TDF administration with virological response.

Side-effects during PEG-IFN treatment

The grade 1–3 of neutropenia was observed in 10 patients (25.6%) in which we performed a dose reduction of PEG-IFN or switch to standard IFN (Table 2); in the 5 patients (12.8%) with grade 4 the treatment was immediately interrupted; mild anemia was detected in 6 patients (15.3%), that has not required the reduction of PEG-IFN dose or erythropoietin-stimulating agents addiction; no severe anemia was found. Flu-like syndrome interested 17 patients (43.5%), psychiatric disorder, such anxiety and depression 14 (35.9%). 18 patients (46.1%) evidenced a weight loss >5% after treatment completion. In 15 patients (38.4%) the treatment was stopped before the expected duration for the onset of adverse events or non-response to the treatment (Table 3).

Table 2. Dose adjustment of PEG-IFN and switch to standard IFN.

Dose reduction of Pegasys®	n° (%)
135 µcg	4 (10.2)
90 µcg	1 (2.5)
Switch to standard IFN	
6 MIU 3/weekly	3 (7.7)
3 MIU 3/weekly	1 (2.5)
3 MIU daily	1 (2.5)

Table 3. Causes of treatment interruption.

Causes of treatment interruption	n° (%)
Neutropenia, grade 4	5 (12.8)
Weakness, fatigue	4 (10.2)
Depression, anxiety	2 (5.1)
Primary non-response	4 (10.2)
Total interruption	15 (38.4)

Patients with inactive CHB

The 12 patients with inactive CHB were followed every six months with blood examination and liver ultrasonography; no alcohol consumption was observed; Fibroscan was repeated every 2 years without significant changes in liver stiffness. After 4 years of follow-up 2 patients (16.6%) evidenced the progression of hepatitis status with ALT flares and HBV-DNA rise that required treatment with NAs. The other patients did not show significant changes in HBV-DNA, qHBsAg levels and liver ultrasonography or clinical symptoms. 1 patients (8.3%) developed a maxillary non-Hodgkin's lymphoma and received treatment with ETV before rituximab administration.

Treatment of other infectious diseases

The treatment of other infectious diseases present at the first diagnosis of the patients was given before the assessment of CHB; in detail, schistosomiasis was treated with praziquantel, the P. falciparum uncomplicated malaria with arthemether/lumefrantine or mefloquine, latent syphilis with benzathine pen

G, intestinal amebiasis (Entamoeba histoyitica) with metronidazole, toxocariasis with albendazole. 10 patients with active pulmonary tuberculosis (71.4%) were treated with standard regimen of therapy for 6 months (negative smear at 2 months); 4 patients (28.5%) required 9 months of treatment (positive smear at 2 months). The 6 patients with extra-pulmonary tuberculosis presented isolated cervical adenitis treated with 6 months of standard therapy. No toxicity or significant side-effects were observed during the antimycobacterial drugs administration. All the specific infectious diseases were treated and resolved before the start of PEG-IFN treatment for CHB.

Discussion

The role of HBV genotypes A–D was recently clarified according to disease progression and treatment response and the determination of genotype before starting an IFN treatment is currently recommended in the EASL guidelines (4). However, patients who carry E–J genotypes are rare so their response to treatment is still poor understood.

Erhardt et al. (19) reported in a retrospective study the results of IFN treatment in 14 patients with HBV E genotype; this study is the only previous experience of treatment in patients with this genotype, but, in our opinion, it is affected by some limitations: first of all, all the treated patients were HBeAg-positive; second, it's not clear if a standard or pegylated IFN was used in these patients and the duration of treatment ranged from 6 to 18 months; third, the only outcome reported was the virological one, defined as “end of treatment response” or “sustained virological response”. In this cohort the SVR was 36%. In our study we described the treatment response (both virological and serological) to PEG-IFN monotherapy in 39 patients (37 HBeAg-negative) and to sequential therapy (ETV and PEG-IFN) in 10 patients. In the treatment with monotherapy, we showed a clear difference between HBeAg-positive and HBeAg-negative patients; in 2 HBeAg-positive patients the serological and virological response was obtained with PEG-IFN administration. In HbeAg-negative patients the global virological response was 17.9%, significantly lower to A and D genotypes (45–52%, 22–26% respectively) (20); none of these patients achieved the HbsAg loss or antiHBs seroconversion, in comparison to 14% and 2% of A and D genotypes (21).

This poor outcome response was observed also in the patients with high baseline viral load treated with ETV + PEG-IFN. The reasons for this low response to IFN are not well understood, but we would try to give some explanation. First, we treated a population of young immigrants from Africa which have crossed the desert through Mali up to Libya, traveling for months in terrible conditions and often remaining prisoners in Libya for many years contracting some infectious diseases due to overcrowding and malnutrition. For this reason, 88% of these patients reached our hospital with infectious diseases such as parasitic infections, sexual transmitted disease, tuberculosis and malaria, that required a specific treatment before the treatment of CHB with PEG-IFN. Besides, the 60.3% of the patients presented a BMI value <20 with a low serum protein level because of the undernutrition they came to during the preceding months. Therefore, this condition with multiple previous infections and bad state of nutrition could have caused an immunosuppressive condition that has brought to a low therapeutic response to PEG-IFN, despite this therapy was started when all other infectious diseases/malnutrition were treated and resolved. The role of immune-response in the control of HBV progression was clarified in previous study (22) and has been demonstrated that the weak T-cell response is associated with persistently viral replication and poor response to IFN therapy (23). The group with high viral load at baseline was treated with a combination of ETV and PEG-IFN with sequential approach, but the overall response was dismal; in this case we underline that the elevated HBV-DNA played an inhibiting action on the cellular immune-response (24) and worsened the treatment response.

Second, we observe an high incidence of side-effects during the treatment with PEG-IFN that have lowered the global tolerability; 15 patients (38.4%) presented neutropenia, 5 (12.8%) with grade 4 which required an immediate interruption of therapy; in the other 10 patients (25.6%) a dose reduction of PEG-IFN or switch to standard IFN was performed (Table 2); 11 patients (28.2%) interrupted the treatment for the side-effects due to PEG-IFN. We observed a global poor tolerance to PEG-IFN, such psychiatric disorder, weakness and weight loss, in comparison with the other patients treated in our hospital with other HBV genotype; this has probably caused a lower adherence to the therapy and accordingly a smaller effectiveness.

Third, we noticed a different distribution in comparison to the European population of rs12979860 CC genotype which was present in only 12 patients (19%), while TC in 41 (65%); this may play an important role of treatment response in our population, even if the exact influence of IL28B in the HBV therapy is still to clarify (25).

In conclusion, we underline that the HBV E genotype represents the emerging genotype in our population, with a great number of young immigrants from central-Africa affected by HBV; in these patients the IFN treatment seems to be more difficult and less effective than in D genotype for many reasons: co-morbidities, poor socio-economic conditions, side-effects and IL28B rs12979860 CC prevalence. The data presented in this paper concern the clinical management in a difficult to treat population with E genotype; further studies are required to subsequently clarify the exact role of HBV E genotype in the treatment response to PEG-IFN and in the progression of CHB in a population without other possible confounding factors.

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