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Treatment of hepatitis delta virus genotype 3 infection with peg-interferon and entecavir

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SUMMARY

Objectives: Hepatitis delta virus (HDV) is recognized as the most pathogenic and infectious among the hepatotropic viruses. Studies on the treatment of HDV have predominantly included European patients and carriers of genotype 1 (HDV-1) in their clinical protocols. For the Amazon region, data show that infected individuals have mainly Native American ancestry and that >90% of HDV carriers have the genotype 3 (HDV-3). Thus combined therapy clinical protocols do not adequately address the treatment of these patients.

Methods: A prospective, non-randomized study was conducted in which 22 patients received 180 μg of pegylated interferon alpha 2a (PEG-IFN) plus entecavir at a dose of 0.5 mg for 48 weeks, with a subsequent 24-week follow-up. Throughout treatment, the patients were monitored for biochemical responses and the kinetics of hepatitis B virus (HBV) and HDV viral loads.

Results: Of the 22 patients treated, 15 presented normal alanine aminotransferase values at the end of treatment ($p = 0.002$). At week 24 of treatment, 86.4% of the patients did not present detectable HDV-RNA; at week 48, the rate of negative patients increased to >95% and remained the same after 6 months. With regard to HBV, only two patients (9%) still presented detectable HBV genetic material at the end of treatment, suggesting the effectiveness of combined therapy in combating the two viruses.

Conclusions: These findings support the use of this effective therapeutic protocol for HDV-3 in patients of non-European ethnicity and suggest a possible 'easy to treat' variant when compared to HDV-1.

1. Introduction

Hepatitis delta virus (HDV) was identified by Rizzetto et al. upon examination of the liver biopsies of patients infected with hepatitis B virus (HBV) presenting abnormal symptoms of severe liver disease.[1] This virus is recognized as the most pathogenic and infectious among the hepatotropic viruses.[2]

Millions of people are susceptible to infection as a result of already being carriers of HBV, and as the replication of HDV cannot be inhibited with the same drugs used for mono-infected patients, the search for an effective treatment is essential. Some authors have reported the efficacy and safety of pegylated interferon (PEG-IFN) use in patients with chronic hepatitis delta. However, the results obtained after treatment were extremely variable, making it difficult to assess the true impact of interferon (standard or pegylated) on the course of this severe liver disease.[3–6]

The fact that combination therapy increases the effectiveness in the treatment of co-infections of HBV and hepatitis C virus (HCV),[7] led to the investigation of whether or not the association of conventional interferon alpha (IFN-α) with lamivudine (or ribavirin) would also be beneficial for the treatment of chronic hepatitis delta. A small increase in the virological response was obtained in patients treated with conventional IFN-α plus lamivudine (28%) compared to those treated with IFN alone (17%), but the difference was not statistically significant.[8] Based on this evidence, new articles have emerged suggesting that combination therapy for HBV and HDV is

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the best way to treat hepatitis delta.[9] The association of PEG-IFN with oral antivirals used in the treatment of HBV infection has become an alternative therapeutic protocol (named combined therapy), and some clinical studies have suggested several advantages when compared to IFN treatment alone.[10–12] The mechanisms involved in this approach are not known, but it is suggested that the suppression of viral replication for prolonged periods decreases the chances of hepatic decompensation.[10]

These prior studies have two important shortcomings. The first is with respect to the population studied. Although the virus presents a worldwide distribution, the patients recruited in already published studies have predominantly been Caucasian.[12–17] Since Brazil shows a more heterogeneous trend in ethnic groups, the present study population comprised Caucasians, Native Americans, and blacks.[18,19]

The second concerns the prevailing HDV genotype, which could interfere with combination therapy. It is known that the different genotypes can lead to different clinical outcomes and that HDV genotype 3 (HDV-3) is associated with more severe forms of the disease.[20] Despite the apparent participation of the genotype in cases of fulminant hepatitis, the influence of the genotype on the course of treatment has not been verified as with other hepatotropic infections (i.e., HBV and HCV); these other hepatotropic infections have presented different responses to treatment with PEG-IFN according to the viral genotype.[21–23]

As there is no effective therapy for chronic hepatitis delta patients and patients in the authors' outpatient setting have a different epidemiological profile from those included in other reports, it was sought to assess the efficacy of treatment with PEG-IFN combined with a nucleoside analog (entecavir) in the treatment of chronic hepatitis delta, as well as to correlate the response to treatment with genotype 3 of the virus (HDV-3).

2. Methods

2.1. Study population and experimental design

This study was approved by the local ethics committee (process number CAAE 0012.0.046.000-11, 2011/Set/09). Informed consent was obtained from each patient.

A prospective, non-randomized study was performed with a treatment regimen of 48 weeks in duration for 22 selected patients (Figure 1). Throughout the treatment period, 180 μg of pegylated interferon alpha 2a (PEG-IFN) was administered subcutaneously once a week along with the nucleotide analog entecavir at an oral dose of 0.5 mg daily.

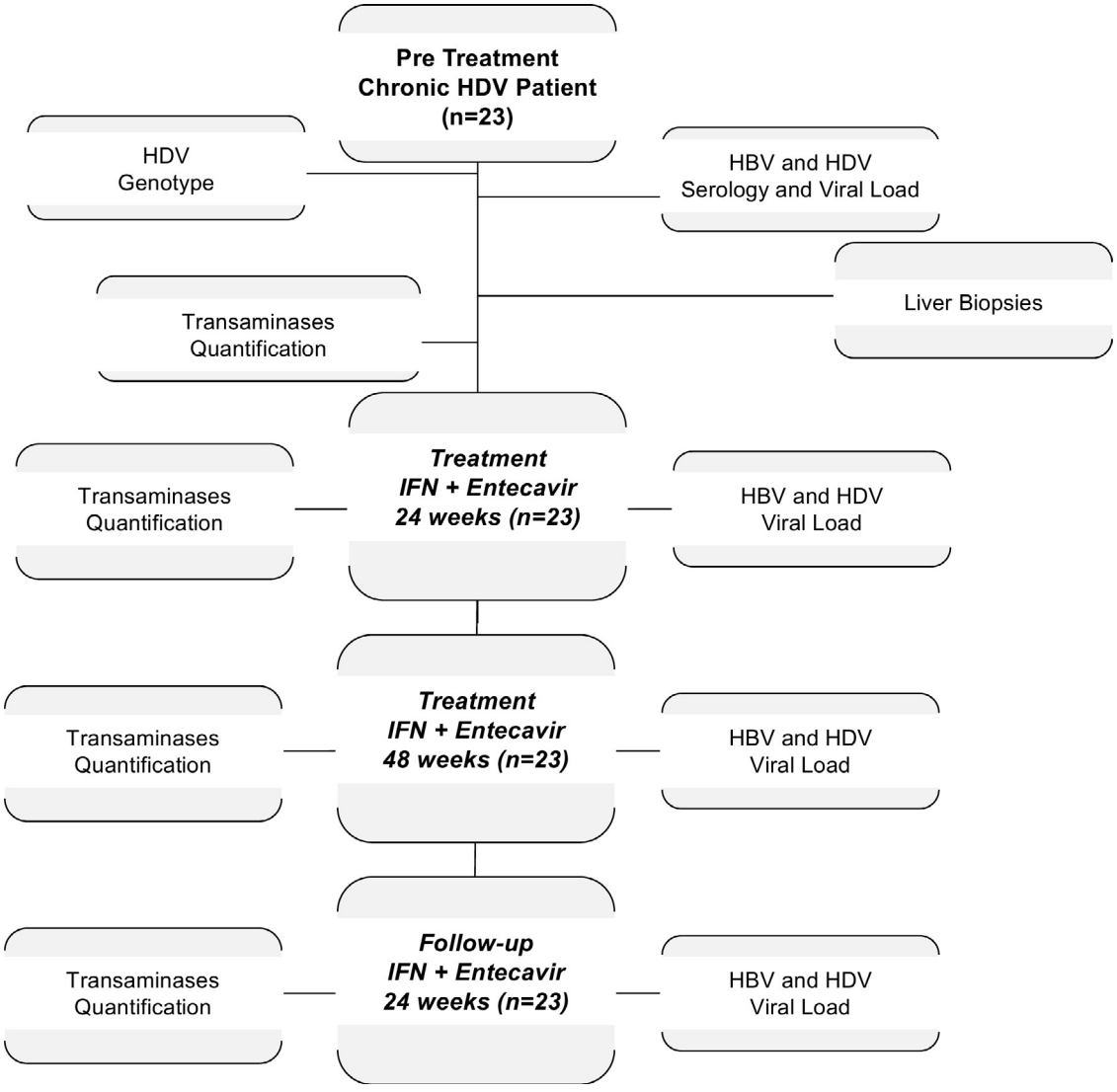

Figure 1. Flowchart showing the monitoring of patients during the therapeutic protocol.

This clinical study was performed on an outpatient basis under strict supervision by the research team. The following inclusion criteria were established: (1) age >18 and <70 years, (2) serological diagnosis of infection with HBV and HDV, (3) PCR positive for genetic material from HDV, (4) no antiviral therapy used in the past 6 months, (5) the patient had to present a floating or persistent elevation of alanine aminotransferase (ALT) on at least two occasions in the 3 months leading up to the start of treatment, (6) the patient had to present compensated liver disease classified as Child–Pugh A <7 points or MELD <12, (7) the patient had to be negative for hepatocellular carcinoma, and (8) the patient had to present laboratory criteria that would allow the use of PEG-IFN.

Exclusion criteria were the following: (1) pregnant women or those who did not acknowledge contraceptive control, (2) patients who had been treated with PEG-IFN in the 6 months preceding the start of the study, (3) patients who had used nucleos(t)ide analogs in the 12 weeks preceding the study, (4) patients who had undergone anticancer or immunomodulatory therapies in the 5 years prior to the start of the study, (5) those with a positive test for hepatitis A virus (HAV) IgM antibodies, (6) those testing positive for antibodies to HIV 1 or 2 (anti-HIV), (7) patients with a history or clinical evidence of other concomitant liver disease, (8) those with autoimmune diseases, alcoholic liver disease, or exposure to toxins, (9) alcohol consumption higher than 30 g per day, (10) those with decompensated liver cirrhosis, (11) any other ongoing decompensated disease such as heart disease, diabetes mellitus, etc., (12) hemophiliac patients, (13) patients with psychiatric disorders considered serious (evaluation with psychiatric report), (14) patients with chronic renal failure, (15) patients with neutropenia, carriers of hemoglobinopathies, and/or hemolytic anemia, and (16) patients with other diseases that would prevent them from complying with the established protocol.

2.2. Clinical monitoring

A treatment period of 48 weeks was completed and a subsequent 24-week follow-up was performed. Possible adverse reactions to medications such as itching, nausea, vomiting, diarrhea, headache, and leukopenia were also monitored, and the patients were assessed and treated according to the reaction presented.

2.3. Hematological and biochemical tests

Patients were monitored as outpatients and, when necessary, complete blood count, prothrombin time, direct bilirubin, creatinine, alkaline phosphatase, gamma-glutamyl transferase, urea, aspartate aminotransferase, and blood glucose tests were performed.

In addition to these procedures, the patients had blood collected in the pre-treatment weeks and at 4, 12, 24, and 48 weeks post-treatment for the quantification of the ALT enzyme (normal value considered to be below 35 U/l).

2.4. Serological tests

Immediately before starting treatment, all patients were tested for the detection of the following viral markers using the ELISA technique in accordance with the manufacturer's instructions: hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg), hepatitis B e antibody (anti-HBe), and total HDV antibodies (anti-HDV).

2.5. Histological evaluation

Twenty-two patients underwent percutaneous liver core needle biopsy blindly. All liver specimens were fragments larger than 2 cm; these were fixed in 10% formalin and later embedded in paraffin. The histopathological technique consisted of serial histological sections stained with hematoxylin–eosin, complemented with separate sections stained with the following special stains: PAS (periodic acid–Schiff) with diastase, reticulin, picrosirius, Perls, and orcein.[24] The material collected was satisfactory for 18 patients. The liver biopsy results of these 18 patients were reviewed according to the METAVIR classification,[25] which is able to evaluate the different degrees of inflammation and fibrosis.

2.6. Viral load

For this study, the methodology described by dos Santos et al. was used to determine the HBV load (sensitivity of 20 IU/ml).[26] To monitor HDV, the quantification method described by Botelho-Souza et al. was used (sensitivity of 75 copies/ml of viral RNA).[27] Some of the samples were sent at random to the Genome Center in São Paulo for external control and to validate the results.

2.7. Genotyping of HDV

For the determination of the HDV genotype, all of the previous procedures described by Botelho-Souza et al. were followed, including primers, cycle conditions, nucleotide sequencing, and phylogenetic analysis.[18]

2.8. Statistical analysis

GraphPad Prism 5 (GraphPad Software, La Jolla, CA, USA) was used for the statistical analysis, as well as the design of the graphs. Quantitative values were recorded as medians and statistical differences were assessed using several parametric and non-parametric tests, including column statistics, the *t*-test, Pearson's correlation, and two-way analysis of variance (ANOVA), as appropriate. For analyses of qualitative data, the Chi-square test was used. Differences were considered significant at $p < 0.05$.

3. Results

3.1. Patient population

Twenty-two patient began treatment between July 2011 and July 2012. Detailed patient characteristics are presented in Table 1. All patients were treated simultaneously for HBV and HDV for 48 weeks. Of these 22 patients, none abandoned treatment or died before completion at week 48. No patient failed to attend follow-up during the 24 post-treatment weeks. Because this is an endemic region there was no association with risk factors such as the use of intravenous drugs, blood transfusions, or tattoos. No patient was working as a healthcare professional.

Serological tests were performed pre-treatment and all patients presented positive HBsAg, negative hepatitis B surface antibodies (anti-HBs), negative HBeAg, positive anti-HBe, positive anti-HDV IgG, negative HCV antibodies (anti-HCV), and negative anti-HIV.

3.2. Dual therapy side effects

The most common adverse reactions were related to the administration of PEG-IFN. Arthralgia, myalgia, and asthenia were reported in all patients. Weight loss was observed in 83% of patients, with a mean weight loss of 5 kg during treatment. Headache, epigastric pain, and fever were reported by 50% of patients, and other less frequent symptoms such as diarrhea and dizziness were also mentioned. The drugs used for symptomatic relief, when present, were dipyrone (at a dose of 500 mg orally every 6 h for those with no history of allergy to this medication),

Table 1
Patient characteristics

Patient characteristics	Number	%
Sex		
Male	14	61
Female	9	39
Race		
White	10	44
African	1	4
Native American	12	52
HCV/HIV infections	0	0
Age, years		
Median	45	
Range	18–70	
Biopsy characteristics	**Number**	**%**
Activity (A)		
A 1	3	16
A 2	10	55
A 3	5	29
Fibrosis (F)		
F 1	7	39
F 2	5	28
F 3	5	28
F 4	1	5
Pre-treatment features	**Number**	**%**
HBV viral load, log IU/ml	2.10[a]	
HDV viral load, log copies/ml	3.78[a]	
ALT, IU/l	98.00[a]	
Alpha-fetoprotein, up to 8.5 ng/ml	22	100
HDV genotype		
I	0	0
III	22	100
Other	0	0
Previous treatment	0	0

HCV, hepatitis C virus; HIV, human immunodeficiency virus; HBV, hepatitis B virus; HDV, hepatitis delta virus; ALT, alanine aminotransferase.
[a] Mean values.

paracetamol (at a dose of 750 mg orally every 8 h), omeprazole (at a dose of 40 mg orally once daily), and metoclopramide (at a dose of 10 mg orally every 8 h).

Throughout treatment, the patients were monitored at the frequency stipulated in the protocol. No reported adverse events were considered serious enough to require dose adjustment or justify the suspension of medications.

3.3. Treatment outcomes

3.3.1. Biochemical response

Due to laboratory differences, ALT value results of less than 35 U/l were treated as normal. Of the 22 patients treated, 15 presented normal values at the end of treatment and 14 out of 15 maintained a biochemical response after 24 weeks of follow-up. Comparing the pre- and post-treatment analyses, a p-value of 0.0015 was obtained (Figure 2A).

Regarding fluctuations in the enzyme (Figure 2B), it was found that the desired values began to be achieved after 48 weeks of treatment and remained in tests conducted at 24 weeks after the completion of treatment. Among the patients who did not achieve normal values, six showed a reduction in ALT at the end of treatment, four showed a steady increase of between 10 and 50 U/l during treatment, and ALT levels remained stable in two (data not shown).

3.3.2. Kinetics of viral loads

The HDV and HBV viral loads of the patients were evaluated at time points considered important to evaluate the prognosis.[17] Thus, the patient viral loads were analyzed during the pre-treatment period, at 24 weeks after the start of the treatment (week 24), at 48 weeks after the start of treatment (week 48), and at 24 weeks after the end of treatment, as detailed in Table 2.

At week 24, 82.6% of the patients no longer presented detectable HDV-RNA, and at the subsequent time points, the rate of negative patients increased to values >95%, remaining the same at 6 months after treatment completion. Only one subject was positive until the end of treatment, but the amount of genetic material detected for this patient at 24 weeks after the completion of treatment was about half the pre-treatment value. Figure 3 shows the dynamics of the HDV viral load tracking for each individual patient.

With regard to HBV, 15 patients (60%) had replicating HBV; at week 24, the number of patients with a detectable viral load decreased to nine individuals. At the end of treatment, only two patients (9%) still presented detectable genetic material, suggesting the effectiveness of combined therapy in combating the two viruses.

Figure 4 shows the kinetics of the two viruses, suggesting that combination therapy of PEG-IFN with entecavir inhibits HBV from multiplying when the amount of HDV decreases.

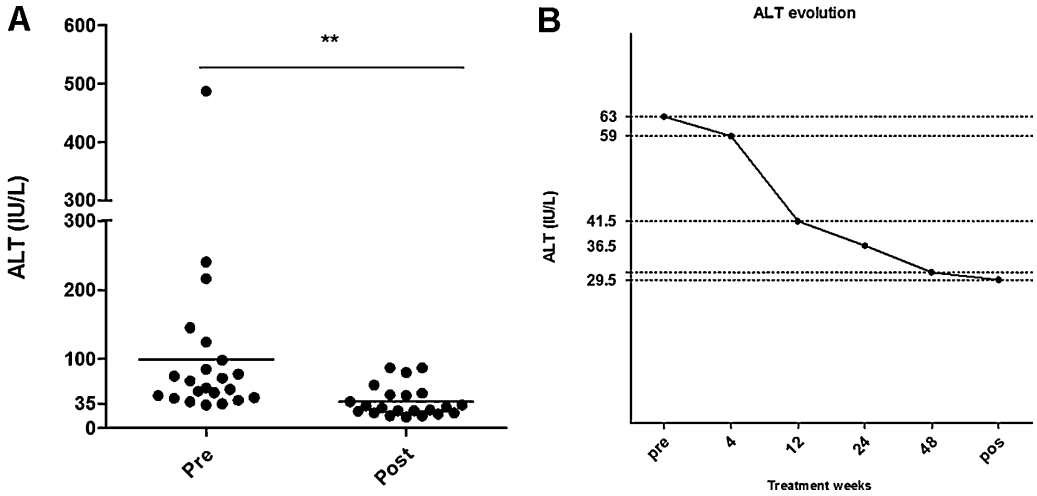

Figure 2. Reduction in the amount of alanine aminotransferase (ALT; IU/l) measured in the patients' serum. (A) Quantification of patients pre-treatment and at 6 months post-treatment; the Wilcoxon test was used ($p = 0.002$). (B) The median of all 22 treated patients whose sera were quantified in the weeks before treatment, at 4 weeks, 12 weeks, 24 weeks, and 48 weeks of treatment, and at 6 months after the end of treatment; Pearson's correlation test showed a significant p-value of 0.0296.

Table 2
Individually assessed kinetics of HDV and HBV viral loads

Patient number	HDV, log_{10} copies/ml				HBV, log_{10} IU/ml			
	Pre-treatment	Week 24	Week 48	6 months post- treatment	Pre-treatment	Week 24	Week 48	6 months post- treatment
1	4.58	3.47	2.60	2.63	0.00	2.51	2.51	0.00
2	4.13	0.00	0.00	0.00	7.28	0.00	0.00	0.00
3	3.83	0.00	0.00	0.00	2.83	2.55	1.89	0.00
4	5.54	0.00	0.00	0.00	0.00	3.57	2.42	0.00
5	3.02	0.00	0.00	0.00	0.00	0.00	0.00	3.15
6	2.24	2.21	0.00	0.00	3.91	2.15	1.26	6.36
7	4.28	5.03	0.00	0.00	7.82	1.70	2.16	0.00
8	4.22	0.00	0.00	0.00	2.99	0.00	0.00	0.00
9	3.74	0.00	0.00	0.00	2.30	2.47	2.41	0.00
10	2.45	0.00	0.00	0.00	3.51	0.00	1.85	0.00
11	5.27	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12	3.23	0.00	0.00	0.00	0.00	0.00	0.00	0.00
13	2.06	0.00	0.00	0.00	2.37	1.78	1.88	0.00
14	3.32	0.00	0.00	0.00	2.00	0.00	0.00	0.00
15	4.31	0.00	0.00	0.00	2.76	0.00	0.00	0.00
16	5.39	0.00	0.00	0.00	0.00	0.00	0.00	0.00
17	4.64	0.00	0.00	0.00	2.32	0.00	0.00	0.00
18	3.53	0.00	0.00	0.00	2.74	0.00	0.00	0.00
19	4.85	0.00	0.00	0.00	3.04	0.00	0.00	0.00
20	3.05	0.00	0.00	0.00	1.96	0.00	0.00	0.00
21	3.77	0.00	0.00	0.00	2.58	0.00	0.00	0.00
22	4.07	0.00	0.00	0.00	0.00	1.93	2.01	0.00

HDV, hepatitis delta virus; HBV, hepatitis B virus.

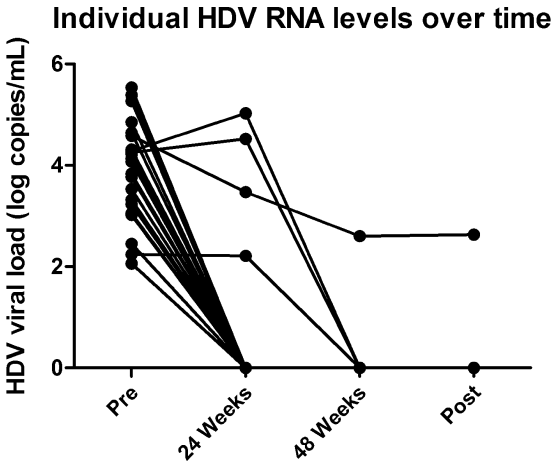

Figure 3. The individual analysis of each patient using the Wilcoxon test, showing $p < 0.0001$ between the pre-treatment week and week 24, where one can notice the absence of viral load in 18 of the 22 patients.

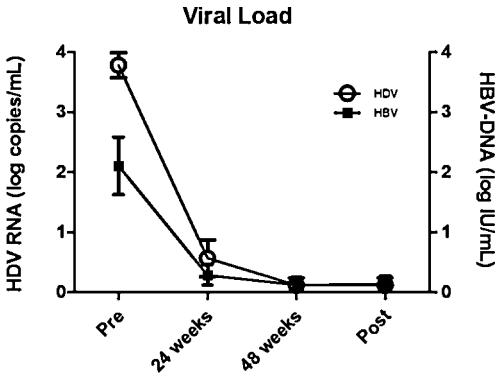

Figure 4. Monitoring of the average viral loads of HDV and HBV during the pre-treatment phase (Pre), at 24 weeks and 48 weeks of treatment, and at 6 months post-treatment (Post). The total number of patients included in these results ranged from a minimum of 18 to a maximum of 22, depending on the availability of biological material. There was no correlation between the HBV and HDV loads. However, the *t*-test showed a *p*-value of 0.0065 between the different treatment times, showing a significant reduction in viral load for both viruses over time.

4. Discussion

In the Amazon, hepatitis delta is considered a neglected disease associated with high morbidity and mortality epidemics that rapidly leads to chronicity, cirrhosis, and hepatocarcinoma.[28] It is believed that several factors lead to this clinical condition, such as poor access to health centers, low education level, poor hygiene conditions, and the high prevalence of HDV-3 in the region.[18] In addition to these factors, there is a lack of published studies on the efficacy and safety of the clinical protocol of combination therapy used in patients who are not of the European standard (male, Caucasian, with HDV -1).[12–17] This has led to a clinical gap with regard to the type of follow-up that patients from the Amazon region should follow.

Thus, the initial design of this clinical trial was followed by a total of 22 patients who entirely fulfilled the characteristics advocated in the inclusion criteria for the selection of the project population. This sample size is similar to those used in other published studies, for example that by Grabowski et al., who assessed PEG-IFN associated with adefovir in 17 patients,[14] that by Castelnau et al., who treated hepatitis delta in 14 patients,[4] and that by Niro et al., who completed a study on PEG-IFN alone in 16 patients.[29]

Epidemiologically, the present findings shed some light on the type of patient found outside of Europe. It was observed that the patients tolerated the side effects of PEG-IFN well and did not require dose adjustments. Regarding sex, it has been reported that female sex is associated with clinical decompensation during the follow-up period;[30] however this was not corroborated in the present study since men and women responded to the treatment in the same way.

Regarding the race of the patients, it was found that Native American descendants represented more than half of the patients (52%) in this study, which may have been a differential in the high virological response rates achieved. A recent study analyzed the presence of different polymorphisms in several cytokines responsible for a predominantly Th_1 response pattern in this ethnic

group.[31] It is not known how these single nucleotide polymorphisms (SNPs) are passed to future generations, nor the influence of ancestry on the present work. However, the authors do not believe that the ethnicity factor was predominant, since the other 43% of patients who responded to treatment had different ethnicities.

Another population data point concerns cirrhotic patients. Thirty-three percent of patients in the study had advanced fibrosis (F3 and F4). These patients were clinically stable before the start of therapy, classified as Child–Pugh A and with calculated MELD scores below 12. The percentage of cirrhotic patients in the present study was higher than that reported in some other studies. Previous studies have reported varying percentages of cirrhotic patients: Castelnau et al. included 29% of patients with this condition,[4] Yurdaydin et al. studied a population in which approximately 35% had cirrhosis,[32] and Niro et al. reported approximately 75% of participating patients as being cirrhotic.[29] However, these studies reported a much lower virological response than that found in the present study (30%, 13%, and 21%, respectively), which suggests that patients with cirrhosis have a higher chance of being null or poor responders, thus presenting a lower virological response than patients without advanced fibrosis.

ALT levels are proposed to represent a good marker of the prognosis in these patients. In laboratory tests performed before treatment, it was observed that the values were above normal (>35 IU/l) in 20 out of 22 patients, indicating potential liver damage from HBV/HDV. The normalization of this biochemical parameter occurred at the end of week 48 in 14 patients, and 12 of these patients continued with ALT levels within the normal range. Other studies have presented higher values when compared to those in the present study.[4,17,33] However, in the most recently published study it can be seen that patients who responded to treatment showed lower levels.[17] The only non-responding patient, patient 1, presented a floating ALT, and post-treatment follow-up showed values of 47 IU/l. Thus, the present study also found that this increase in ALT after the end of treatment is suggestive of a relationship with increasing HBV/HDV viral loads, ultimately suggesting that liver damage continues to occur even with medication.

Regarding the monitoring of viral load, all patients who showed undetectable HDV at week 24 ($p < 0.0001$) continued to show this until treatment was completed and also during follow-up. On comparing these findings to those reported in the single article that correlates viral load and prognosis, it appears that the data are consistent with those described by the authors.[17] In addition, since there were only three patients who were still positive at week 48, and only one patient who did not become negative, it was not possible to perform a statistical analysis on exact values expected in week 24, but it is possible to suggest that the article by Keskin et al.[17] can be extrapolated to genotype 3.

These findings help strengthen the idea that combination therapy is effective in the treatment of HDV-3 in patients of non-European ethnicity. However, to obtain an answer above initial expectations, future studies should be conducted in collaboration with other places so that it can be demonstrated whether or not HDV-3 is an 'easy to treat' variant when compared to genotype 1. Regardless of future studies, this is the first study to demonstrate an effective therapeutic protocol that can be followed in patients with genotype 3.

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Conflict of interest:
None

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