

SERIAL TESTING OF LIVER CHEMISTRIES AND HBV DNA DURING ANTIRETROVIRAL TREATMENT IN PATIENTS WITH HIV/HBV CO-INFECTION

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We studied the association between hepatitis B virus (HBV) replicative markers and aminotransferases values during a 18 months interval after late initiation of highly active antiretroviral therapy (HAART) in a sample of 126 teenagers with more than ten years old HBV/HIV co-infection.

Our objective was to differentiate between drug induced and disease induced alterations of liver laboratory tests. We evaluated the patients according to the degree of hepatic injury expressed by aminotransferases levels. We chosen as reference values the upper normal limit (UNL) of aminotransferases determinate in our laboratory on more than 300 samples from healthy adolescents: 24 for boys, respectively, 18 mIU/L for girls for ALT and, 30 for boys, respectively, 26 mIU/L for girls for AST. The average ALT value in HIV monoinfected subjects was 33-35 and in HIV HBV coinfecting patients was 48-50 IU/mL. The data for AST have the same trend. Significant higher values were registered for ALT than for AST, especially in coinfecting boys. The percent of patients with aminotransferase over 1,5 times UNL in the coinfecting patients was 37.1% for ALT and 29.8% for AST. Aminotransferases values up to 2 times UNL predominated in the naive patients, over this value we registered a higher percentage of treated patients. Increases in frequency of aminotransferases higher values were registered in the added PI therapy groups. Patients with mild aminotransferases increases have had in a high percent (over 45%) HBsAg present. The presence of HBeAg is not accompanied by higher ALT values. In patients with occult hepatitis, unexpectedly, we found a higher percentage of aminotransferases values over 3 times UNL. Also in these patients prevailed elevated AST values and sometimes the inverse ALT/AST ratio. ALT is the best marker of liver inflammatory activity available, but is of limited utility in predicting degree of inflammation and of no use in estimating severity of fibrosis in HIV HBV co-infected adolescents.

Key words: HIV; HBV; Co-infection; HBV replicative markers.

Abbreviations: •AIDS, acquired immunodeficiency syndrome; •ALT, alanine aminotransferase; •AST, aspartate aminotransferase; •ART, antiretrovirals; •HAART, highly active antiretroviral therapy; •HIV, human immunodeficiency virus; •HBV, hepatitis B virus; •HCV, hepatitis C virus; •HDV, hepatitis delta virus; •NRTI, nucleotide reverstranscriptase inhibitors; •NNRTI, nonnucleotide reverstranscriptase inhibitors; •PI, protease inhibitors; •UNL, upper normal limit.

INTRODUCTION

The era of highly active antiretroviral therapy (HAART) has led to declining rates of opportunistic infections and a new focus on other causes of morbidity, such as end-stage liver disease secondary to hepatitis B infection. The treatment and prevention of hepatitis B has taken on great

significance in light of the negative impact HIV has on the natural history of chronic hepatitis B^{1,2}.

Liver disease is a major cause of death now in the HAART era. It was not such a huge issue in the pre-antiretroviral era (pre-1995). Hepatitis C is the number-one problem in this regard, although hepatitis B is not far behind, because it's being ignored in a lot of patients.

Published data on the overall prevalence of HBV and HIV coinfection are incomplete. Studies of certain subgroups have identified prevalence of previous or current HBV infection of 45% in HIV-infected homosexuals aged 22–29 years (CDC, unpublished data, 1998–2000), 24% in adolescent HIV-infected males³, and 43% in HIV-infected women, including 76% among HIV-infected female IDUs. Chronic HBV infection has been identified in 6%–14% of HIV-positive persons from Western Europe⁴ and the United States, including 9%–17% of homosexuals⁵, 7%–10% of IDUs, and 4%–6% of heterosexuals⁶. In Romania, Ruță *et al.*, 2005, found a prevalence of HIV HBV coinfection above 75% in HIV infected adolescents⁷.

The course of HBV infection can be modified by the presence of HIV, with a lower incidence of jaundice and a higher incidence of chronic HBV infection. Limited data also indicate that HIV-infected patients with chronic HBV infection have an increased risk for liver-related mortality and morbidity.

Our objective was to differentiate between drug induced and disease induced alterations of liver laboratory tests in a group of HIV HBV coinfecting adolescents. Basic liver chemistry was repeatedly tested in 126 coinfecting patients with (N=115) or without (N=11) antiretroviral therapy during a follow-up interval of 18–24 months. We tried to explain the paradox of treating liver disease with potentially hepatotoxic drugs: antiretrovirals (ARV), particularly nucleotide analogs reverse transcriptase inhibitors (NRTI) and protease inhibitors (PI).

The most common clinical hepatic manifestation associated with ARV therapy is asymptomatic aminotransferase elevation. However, in most studies, the rate of liver enzyme elevation in HIV HBV coinfecting treated patients has not differed significantly from that seen in naïve (untreated) subjects^{8,9}.

METHODS

The cohort consisted of 161 subjects (84 boys and 77 girls) from which 126 (78.3%) were HBV HIV coinfecting. The coinfecting patients (66 boys and 60 girls) have had the following HBV serologic status: 18 (14.3) were HBeAg positives; 70 (55.6%) were HBsAg positives and 15 (11.9%) have had occult hepatitis. All coinfecting patients tested anti HBe total present from which 31 (24.6%) developed protective anti HBs antibodies but only 23 (18.3%) have had titers above 10 IU/ml (the protective cut-off).

Other data on patients and laboratory investigations were presented in two previous papers^{7, 10}.

Laboratory evaluation of HBV infection included hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg), hepatitis B e antibody (anti-HBe), hepatitis B core antibody (anti-HBc), and HBV DNA. Hepatitis D antibodies were

assayed on HBsAg positive patients. Other causes of liver disease were also investigated, particularly hepatitis C, cytomegalovirus and Epstein Barr virus. In our series only <4% of all HIV-infected patients have had evidence of hepatitis C or D infection, which can be demonstrated by HCV-HDV antibodies and/or the presence of HCV RNA.

Alanine aminotransferase (ALT, also sometimes termed SGPT) and aspartate aminotransferase (AST, also named SGOT) and are primarily distributed in liver, kidney (ALT) and liver, heart, skeletal muscle (AST). ALT is exclusively cytoplasmic but both cytoplasmic and mitochondrial forms of AST were present in all cells. ALT is the best marker of liver inflammatory activity available, but is of limited utility in predicting degree of inflammation and of no use in estimating severity of fibrosis. Markers of hepatic functions that may indicate progression to cirrhosis are AST/ALT ratio, albumin, prothrombin time and platelets count. All these markers should be measured every 3 months. The half-life of ALT is 47±10 hours, the half-life of mitochondrial AST average 87 hours. ALT and AST activities are significantly higher in males than in females and reference intervals, upper and lower normal limits (UNL and LNL) vary with age. In most types of liver diseases ALT level is higher than that of AST (ALT/AST ratio >1).

The Consensus of Guidelines Committee authors (www.aasld.org) recommended that performance criteria for ALT and AST should be defined at the upper normal limits (UNL) of reference values and that current performance goals of catalytic tests are inadequate for general clinical use. The data in patients with stable ALT /AST suggest that total error of <10% is required at UNL for accurate detections of patients who may benefit from treatment¹¹.

Markers of synthetic hepatic functions, such as albumin and prothrombin time, provide clues as to the presence of advanced liver disease but not in advanced AIDS patients. Leucopenia and thrombocytopenia may heighten suspicion of portal hypertension. Low albumin levels are common in patients with advanced HIV infection, and thrombocytopenia may be related to HIV associated idiopathic thrombocytopenia.

Fluctuation in aminotransferases may not always be due to HBV infection. Aminotransferases may also become elevated following immune reconstitution or as a result of HAART-associated hepatotoxicity. Significant liver disease may be present even in patients with normal aminotransferases, particularly those with low CD4 counts.

HBV patients who have serum HBV DNA >10⁵ copies/mL should be considered for treatment, especially if their liver biopsy demonstrates significant liver disease. The exception to this rule is the HBeAg positive patient who has persistently normal liver function tests. A reasonable strategy for this patient is a period of observation with serial monitoring of aminotransferases; treatment can be initiated when aminotransferases become elevated to greater than 2 times upper limit of normal since the chance of a durable treatment response is then improved.

RESULTS

Description of the patients

In a series of 161 consecutive HIV infected adolescents we found 126 (78.3%) coinfecting with HBV (having antiHBc antibodies with or without

other serological markers of replicative HBV infection). Average age was 13.8 ± 1.3 years. 115 (91.3%) of the coinfecting children were under antiretroviral therapy and the remainder, 11, were naive. The average time of treatment was 13 ± 6.7 months. The treated patients were allocated to the following therapy schemes: 76% for the therapy with “2NRTI+1PI”; 13.2% for the therapy with “2NRTI+1NNRTI”; 10.8% for the therapy with “1NRTI+1NNRTI+1PI”. In the Table 1 the characteristics of the patients are presented.

The degree of liver injury expressed by aminotransferases levels

We chosen as reference values the upper normal limits (UNL) of aminotransferases determined in our laboratory on more than 300 samples from HIV HBV negative adolescents between 15–19 years. The reference values, named UNL in the following text, were: 24 for boys, respectively, 18 mIU/L for girls, for ALT and, 30 for boys, respectively, 26 mIU/L for girls, for AST. The average ALT value in HIV mono-infected subjects was 33–35 and in HIV HBV coinfecting patients was 48–50 IU/ml. The data for AST were shown in Fig. 1. Significant higher values were registered for ALT especially in coinfecting boys. We analyzed the augments of these constants over the normal values by 1, 5 to 5 times the UNL.

The percent of patients with high aminotransferase values (3,5 times the upper normal limit) was double

for AST compared with ALT, respective 40% vs. 20% despite the higher percentage of patients with ALT abnormal values. The trend was kept for all patients with high levels of aminotransferase values, even for patients with $2 \times$ UNL aminotransferase values (Fig. 2). However, the percent of patients with increased ALT values was higher than the percent of patients with increased AST. The percent of patients with aminotransferase over 1,5 times UNL in the coinfecting patients was 37.1% for ALT and 29.8% for AST.

We allocated the patients with aminotransferase below $3.5 \times$ UNL to the group with mild hepatic injury, and the ones with aminotransferase values between $3.5-5 \times$ UNL to the moderate hepatotoxicity group, while the patients with aminotransferase values above $5 \times$ UNL belong to the severe hepatotoxicity group.

Moderate hepatotoxicity appears both in the antiretroviral treated group and in the naive group while severe hepatotoxicity appears only in treated patients (Table 1 and Fig. 3). The majority of patients presented mild hepatotoxicity. The highest percent of patients was encountered in patients with aminotransferase values between 1,5–2 times the UNL, especially in naive patients.

In the group of patients with aminotransferase levels 1,5–2 times UNL prevailed the naive patients, compared with the patients with aminotransferase over 5 times UNL where treated patients prevailed.

Table 1

Studied population's features

Characteristics	All patients (n=161)	Treated patients (n=137)	Naïve patients (n=24)	Mild and moderate hepatotoxicity		Severe hepatotoxicity	
				Treated (n=132)	Naïve (n=23)	Treated (n=5)	Naïve (n=1)
HIV/HBV coinfecting N; (%)	126 (78.3)	115 (91.3)	11 (8.7)	99 (78.6)	6 (4.8)	5 (4%)	0
Average age (years, range)	13.8 (8-17)	14 (9-17)	13 (8-16)	13.9 (9-16)	14 (11-16)	14.4 (14-15)	0
AIDS N; (%)	25 (19.8)	21 (16.7)	4 (3.8)	18 (14.3)	1 (0.8)	3 (2.4)	
CD4/mm ³ Average \pm SD	379 \pm 294	389 \pm 305	311 \pm 194	380 \pm 306	317 \pm 197	619 \pm 174	
RNA-HIV copies/ml Average	5×10^4	4.5×10^4	8.7×10^5	4.1×10^4	9.3×10^4	1.5×10^5	
% of patients with ALT over 1,5 times UNL	37.1	31.2	45.5	24.2	4.8	12.9	0
% of patients with AST over 1,5 times UNL	29.8	28.2	22.5	21.8	4.8	7.1	0

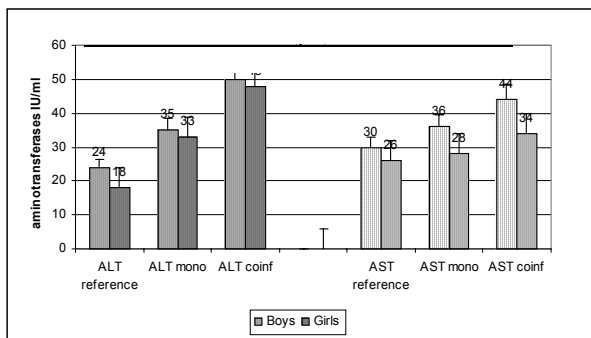


Fig. 1. The average aminotransferases values in infected patients.

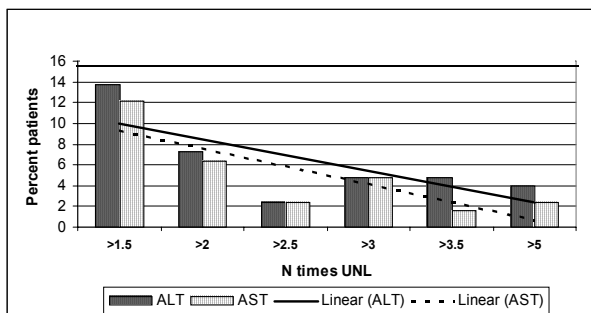


Fig. 2. The ranking of aminotransferases levels in HIV HBV coinfecting patients.

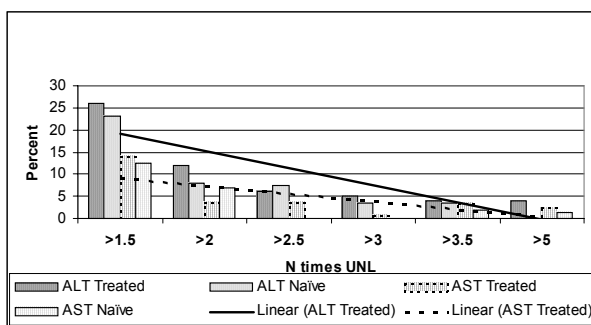


Fig. 3. Differences in aminotransferases values between treated and naïve coinfecting patients.

The trend of increased aminotransferases values in treated patients vs. naïve patients

Aminotransferases values up to 2 times UNL predominated in the naïve patients, over this value we registered a higher percentage of treated patients. Analyzing the values of aminotransferase according to the treatment schemes, we noticed that the patients treated with 2NRTI+1NNRTI did not presented any type of increase in aminotransferase. Increases in frequency of aminotransferases higher values were registered in the added PI therapy groups: for the 1NRTI+1NNRTI+1PI, the patients presented only mild increases of the

aminotransferases up to 2 times the UNL, while the patients treated with 2NRTI+1PI, presented mild, moderate and severe increases in the aminotransferase levels (Fig. 4).

If for up to 1,5 times UNL, the percentage of patients for AST, respective, for ALT were resembling: 14,95%, respective, 16,82%, for over 2 times UNL ALT values, the percentage of patients was double, triple or quadruple. A percent of 0, 93%, for AST vs. 1, 87%, for ALT, from the patients with 2NRTI+1PI, presented severe hepatotoxicity with aminotransferase values over 5 times UNL.

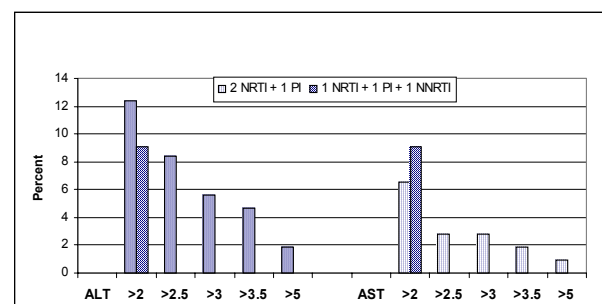


Fig. 4. The distribution of treated patients with high ALT or AST values according to therapy group.

The patients with mild hepatotoxicity, with aminotransferases under 3,5 times UNL were spliced in to treated and naïve groups. There are no differences between the two groups in respect with their immune status. The average values of the CD4 and the moderate aminotransferase values were quite similar in the both groups (Table 1). However, in the untreated group the percentage of patients with AIDS was more than double, 36, 8% vs. 16,7%, and the average value of the HIV viral load was of $9,3 \times 10^4$ copies ARN HIV/ml vs. $4,13 \times 10^4$ copies ARN-HIV/mL.

In patients with moderate and severe hepatotoxicity (with aminotransferase levels over 3,5 times UNL) the effect of the antiretroviral therapy was more easily to notice. The patients treated with antiretrovirals have normal levels of CD4 cells and a viral load at least 5 times lower than the naïve patients.

The incidence of hepatic injuries

From the total of 124 coinfecting patients, evaluated at least three times over 18 months interval, 5 (4%) developed extremely higher values

of both aminotransferases (over 100 IU/ml) with ascendant trend. We evaluated the possible causes of the aminotransferase increases. For this purpose we analyzed the patient's medical records and noticed that no patient presented symptoms that are common to severe adverse drug reactions and there were no interruption of the ARV therapy. The average aminotransferase values for these patients, at the moment of diagnosis, was 147 ± 21 for the AST (range 118–190) and, 135 ± 16 mIU/L (range 108–184) for ALT.

The risk factors for the elevated aminotransferase level

As shown in Table 1 at baseline there were differences between patients with mild and severe hepatotoxicity. So, the five ARV treated patients whose level of AST or ALT exceeded more than 5 times UNL presented HBeAg positive chronic hepatitis B and were females. We identified the following independent risk factors for the higher aminotransferase level: chronic infection with HBV, high levels of ADN VHB (over 10^9 copies/mL), the use of a powerful therapy regimen (salvage therapy type as a first line therapy without a treatment beforehand with NRTI), recent treatment with nevirapine, the use of ritonavir as a booster in association with lopinavir, initial higher level of ALT and the feminine sex. At the moment of establishing the degree of hepatic injuries none of them was infected with HCV or HDV. Even if all the patients were HBV infected, lamivudine was used only in one patient.

The prevalence of HBV infectivity markers according to ALT and AST levels in coinfecting patients

We analyzed HBV infectivity markers and the degree of hepatic inflammatory lesions expressed by aminotransferases level. Patients with mild aminotransferases increases have had in a high percent (over 45%) HBsAg present (Fig. 5). The presence of HBeAg is not accompanied by higher ALT values. In patients with occult hepatitis, unexpectedly, we found a higher percentage of aminotransferases values over 3 times UNL. Also in these patients prevail elevated AST values and sometimes the inverse ALT/AST ratio (data not shown).

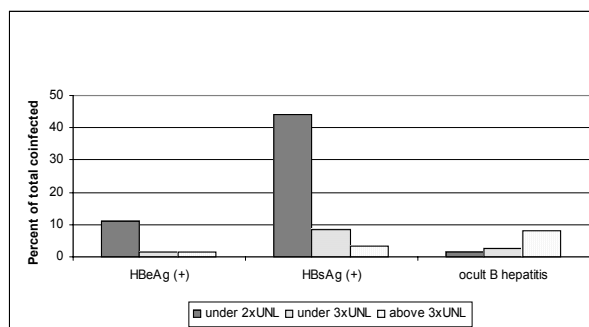


Fig. 5. The prevalence of HBV infectivity markers according to ALT levels in HIV/HBV coinfecting patients.

If we analyze separately the prevalence of HBV infectivity markers in treated or untreated patients, according to the aminotransferase levels, we notice that treated subjects have higher frequency of elevated aminotransferases. The long term use on of ritonavir (200 mg bid) for boosting effect of other PI was associated with the elevated aminotransferase levels. In the 112 patients treated with boosted ritonavir the following PI were associated: amprenavir, saquinavir, lopinavir. Another PI, indinavir, proved to have a protector effect against developing severe hepatotoxicity, probably due to the fact that the regimens that contains it never included nevirapine or/and high ritonavir doses.

In order to investigate the evolution of chronic HBV infection in patients treated with antiretroviral regimen that includes lamivudine, we investigated 19 children with chronic HBV infection. All of them have had aminotransferase values under 1.5 times UNL and undetectable HBV viral load (<1000 copies/ml – kit detection limit). In spite the fact that four gave up lamivudine they did not develop increases in aminotransferase values more than 1.4 times UNL,

From 39 HBV chronically infected patients that had never been treated with lamivudine 12 (31%) presented aminotransferase boosts during the follow up on an average level of 3 times UNL (range 2–6.7) and an average value of HBV viral load of 4.5×10^6 genome copies/mL.

DISCUSSIONS

Standardization of aminotransferase values is a priority need for monitoring HIV infected patients under ART. Our laboratory established separate UNL for boys and girls. Also, unexpectedly elevated ALT and/or AST were evaluated by

repeat testing. It is known that young people engaging in strenuous physical exercise have higher aminotransferase values. In some cases repeat have been performed after a period of rest.

Persistence of increased ALT for an interval more than 6 months after an episode of hepatitis flare or elevation of ALT (without another explanation) on more than one occasion over a similar interval in a coinfecting patient under ART rise the problem of differential diagnosis between hepatitis induced liver injuries or drug induced hepatotoxicity. In the Table 2 main characteristics of both conditions were synthesized.

Table 2

Differential diagnosis between hepatitis induced liver injuries or drug induced hepatotoxicity.

Characteristics	Hepatitis flare	Drug injury
Peak ALT (N × UNL)	10–40	>40
AST/ALT ratio	<1	Early >1
Peak bilirubine (mg/dL)	<15	<5
Prothrombin Time prolongation	<3 sec	>5 sec

In viral hepatitis serological markers and viral load are the most reliable indicators of resolution. HBV DNA is an important determinant of duration of therapy. In treated patients, HBV DNA should be used to document viral clearance. In untreated patients, replicative serological markers (HBsAg and HBeAg) should be monitored periodically to determine viral clearance¹².

The incidence of very elevated values of aminotransferases in the studied population was 12.9% for ALT and 7.1% for AST in antiretroviral experimented patients versus zero in untreated subjects. Beside the toxicity generated by antiretrovirals we did not identify other causes of the higher aminotransferase levels. The risk factors for antiretrovirals severe hepatotoxicity (which is equivalent of a raise at least 5 times UNL) were: recent use of nevirapine and protease inhibitors boosted therapy. The coinfecting patients without lamivudine (a well known HBV inhibitor) in the ARV regimen, presented a high risk of elevated aminotransferases. In all patients with moderate and severe hepatotoxicity the occurrence of at least three risk factors was registered at the diagnostic time. The use of ritonavir boosting along with other protease inhibitors, especially with lopinavir, seems to induce the most severe risk of hepatotoxicity.

From the 37 children that were treated with NNRTI (14 with nevirapine and 23 with efavirenz), 29% presented high aminotransferase such as: for efavirenz treated children 26% had an average increase of 1.7 times over UNL (range 1.3–2.5), while for the nevirapine treated children 35.7% presented high aminotransferase with an average increase of 2.9 over UNL (range 1.2–6.2). In general, the patients that received NNRTI presented a moderate risk of hepatotoxicity, but some associations of nevirapine, especially with didanosine, stavudine and kaletra, may increase this risk up to severe hepatotoxicity development. Regarding the hepatotoxic effect of nevirapine, we could not associate this drug with the hypersensitivity reaction.

One of the possible explanation of elevated aminotransferases is immune restoration. The pretreated patients with regimens that contain NRTI presented a significant increase of the CD4 cells and PI association at therapy regimen will not further improve their immune status. In the studied population, 78.6%, from the 132 patients with mild hepatotoxicity were pre-treated with NRTI and presented an average value of 380 ± 306 cells/mm³. The patients with moderate or severe hepatotoxicity experienced a strong immune reconstruction. In one study that prospectively evaluated the efficacy of lamivudine for HBV infection in patients initiating lamivudine-containing HAART for HIV disease, HBV DNA was suppressed in 57 of 66 patients after two months of treatment. However 22 of 24 patients developed lamivudine resistance with evidence of mutations in the YMDD motif of HBV after five years of treatment, mean rate of 20 percent per year. The development of lamivudine resistance in HIV/HBV co-infected patients has been associated with duration of treatment but no relationship between baseline HBV DNA level, aminotransferases, or CD4 cell count has been seen^{13, 14}.

The onset of lamivudine resistance or the withdrawal of lamivudine therapy has been associated with exacerbations of hepatitis and hepatic failure^{15, 16}. In patients who are treated with lamivudine for HIV infection, drug holidays can have devastating effects if the patient and the clinician are unaware of the patient's underlying chronic HBV status.

The final decision to treat the coinfecting patient for hepatitis B should also include the ability of the patient to adhere to a medical regimen and

willingness to attend clinic for regular medical evaluations while on therapy. Treatment for HBV can lead to flares of aminotransferases so it is important to educate the patient regarding the need for careful follow-up.

CONCLUSIONS

The use of a potent ARV regimen, such as salvage therapy as a first line therapy, is associated with a high risk of severe hepatotoxicity. All the HIV HBV coinfecting patients that initially had as a first line therapy a regimen only with NRTI presented mild hepatotoxicity. A potent antiretroviral regimen can induce the severe hepatotoxicity. In almost all cases with aminotransferases blips there are no symptoms and the interruption of the therapy do not influence them. We did not associate always the presence of viral hepatitis with poor prognosis, but lamivudine is recommended when HBV infection exists.

The baseline level of viral load and its modifications can't be asset as a risk factor. The feminine sex is more predisposed to achieve flares of aminotransferases for unclear reasons.

The best discriminant values for recognizing drug induced acute hepatic injury appear to be values as high as 200 mIU/mL for AST and 300 mIU/mL for ALT^{17,18}. We confirmed that aminotrasferases are more related to the cause of hepatic injury, rather than to severity¹⁹. There is a weak correlation between aminotransferase activities and bilirubine in viral hepatitis and none in toxic hepatic injury. Peak aminotransferase bear no relationship to prognosis and may fall with worsening of the patients condition.

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