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Hepatotoxicity of Antiretrovirals
Incidence, Mechanisms and Management

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Abstract

Hepatotoxicity is a relevant adverse effect derived from the use of antiretrovirals that may increase the morbidity and mortality among treated HIV-infected patients and challenges the treatment of HIV infection. Although several antiretrovirals have been reported to cause fatal acute hepatitis, they most often cause an asymptomatic elevation of transaminase levels. In addition to ruling out a variety of processes not related to the use of antiretrovirals or to the HIV infection, for appropriate management of the complication it is necessary to deduce the possible pathogenic mechanisms of the hepatotoxicity. Among these mechanisms, direct drug toxicity, immune reconstitution in the presence of hepatitis C virus (HCV) and/or hepatitis B virus (HBV) co-infections, hypersensitivity reactions with liver involvement and mitochondrial toxicity play a major role, although several other pathogenic pathways may be involved. Liver toxicity is more frequent among subjects with chronic HCV and/or HCB co-infections and alcohol users. Complex immune changes that alter the response against hepatitis virus antigens might be involved in the elevation of transaminase levels after suppression of the HIV replication by highly active antiretroviral therapy (HAART) in patients co-infected with HCV/HBV. The contribution of each particular drug to the development of hepatotoxicity in a HAART regimen is difficult to determine. The incidence of liver toxicity is not well known for most of the antiretrovirals. Although it is most often mild, fatal cases of acute hepatitis

linked to the use of HAART have been reported across all families of antiretrovirals. Acute hepatitis is related to hypersensitivity reactions in the case of non-nucleosides and to mitochondrial toxicity in the case of nucleoside analogues. Alcohol intake and use of other drugs are other co-factors that increase the incidence of transaminase level elevation among HIV-infected patients. The management of liver toxicity is based mainly on its clinical impact, severity and pathogenic mechanism. Although low-grade HAART-related hepatotoxicity most often spontaneously resolves, severe grades may require discontinuation of the antiretrovirals, for example when there is liver decompensation, hypersensitivity reaction or lactic acidosis.

Highly active antiretroviral therapy (HAART) has dramatically changed the course of HIV infection management, having decreased the morbidity and mortality derived from classical opportunistic infections. As a counterweight to this positive impact, antiretroviral therapy (ART) has adverse effects that challenge the management of HIV-infected patients. Among these, liver toxicity deserves special attention since it often leads to the discontinuation of HAART, particularly in patients co-infected with hepatitis C virus (HCV) and/or hepatitis B virus (HBV). The mechanisms involved in HAART-derived liver toxicity are not well understood, which makes its management more difficult.

In this review, the incidence and clinical impact of antiretroviral-induced liver toxicity are evaluated. Furthermore, the mechanisms involved are explored with the aim of helping clinicians with the management of this complication. The medical literature was reviewed using Medline, selecting English articles up to October 2003. 'Liver toxicity', 'hepatotoxicity', 'HAART', and 'antiretroviral' were the main keywords used for the search.

1. Clinical Impact

HAART-linked hepatotoxicity is an adverse effect that has become evident over the past few years with the widespread use of HAART and the availability of more drugs, some of which are likely to be more hepatotoxic than others. In a recent American study that evaluated the causes of death of HIV-infected individuals, discontinuation of ART due to hepatotoxicity increased from 6% in 1996 to 31.8% in 1998–1999 among these fatal cases.[1] Liver toxicity generates medical visits, work-up exams and frequent hospital admissions, all of which increase expenses. In addition, hepatotoxicity hampers the maintenance of HIV suppression over time.

The severity of liver toxicity ranges from the absence of symptoms to liver decompensation, and the outcome ranges from spontaneous resolution to liver failure and death.[2] Some authors have identified the rise in liver transaminase levels within the first months of ART as a risk factor for mortality among HIV-infected subjects.[3] In some cases, drug-induced liver toxicity may contribute to the mortality of HIV-infected individuals not related to the HIV infection. In a recent study, severe hepatotoxicity with acute hepatic necrosis in HIV-positive patients caused 2% of deaths due to hepatitis or other liver diseases, mainly among patients with prior liver disease.[4] Thus, although hepatotoxic drugs may not be sufficient to cause fatal liver disease *per se*, it could lead to liver failure by aggravating pre-existing liver disease.

Drug-related liver toxicity has impacted on the recommendations for ART in certain scenarios. Thus, according to recent recommendations from the Centers for Disease Control and Prevention,[5] the use of nevirapine should be avoided as part of post-exposure prophylaxis regimens. These recommendations are based on the occurrence of fulminant hepatitis in two cases and severe liver toxicity in 12 other healthy subjects who received a HAART regimen containing nevirapine after HIV exposure.[6,7] However, nevirapine seems to be safe when administered to mother and child as a single dose for the prevention of mother-to-child HIV transmission.[8]

2. Definition, Severity and Patterns of Liver Injury

The clinician thinks of liver damage when abnormalities in liver function tests are seen. The allocation of these abnormalities to a defined pattern of liver damage is essential to the diagnostic and work-up processes. The three main patterns of liver abnormalities, namely cholestatic, hepatocellular and mixed injuries, can be indentified by the relative proportions of these tests compared with the limits of normal of each test. Thus, if alanine aminotransferase (ALT) is raised >8-fold the limits of normal while alkaline phosphatase is raised only 1.5-fold, the pattern is classified as hepatocellular.

It is relevant to point out that many drugs increase γ-glutamyl transpeptidase (γ-GT) levels. This is often misinterpreted as a marker of liver damage, but the isolated elevation of this enzyme actually reflects enzyme induction. It should only be considered to indicate a cholestatic lesion when the increase is associated with a proportional increase in alkaline phosphatase levels. Bilirubin has not been included in table I because it can increase for a variety of reasons, such as haemolysis. Bilirubin levels always rise when cholestatic injury occurs. However, following hepatocellular damage (as is found in acute viral hepatitis or drug toxicity), a delayed elevation in bilirubin levels is often observed due to the reassociation of the regenerated hepatocytes with the bile canaliculi. This process is known as post-hepatitic cholestasis. Finally, fasting and certain drugs (e.g. indinavir and atazanavir) may increase the unconjugated fraction of bilirubin, which seems to be related in the case of indinavir to competitive inhibition of bilirubin-conjugating activity.[9]

Table I. Patterns of liver injury

Liver injury	Enzyme level changes observed
Cholestasis	Large increases in ALP and γ-GT; smaller increases in ALT and AST
Mixed	Moderate increases in ALT, AST, ALP and γ-GT
Hepatocellular	Large increases in ALT and AST; smaller increases in ALP and γ-GT

ALP = alkaline phosphatase; **ALT** = alanine aminotransferase; **AST** = asparatate aminotransferase; **γ-GT** = γ-glutamyl transpeptidase.

There is a broad variability among studies in the criteria to categorise the severity of hepatotoxicity. We propose, as the most accepted one, the AIDS Clinical Trials Group scale of liver toxicity.[10] According to this scale, patients with transaminase levels within normal limits at baseline are considered to develop hepatotoxicity when ALT and/or aspartate aminotransferase (AST) levels rise above the upper limits of normal (ULN). The score is graded as follows: grade 1 (1.25–2.5 × ULN); grade 2 (2.6–5 × ULN); grade 3 (5.1–10 × ULN) and; grade 4 (>10 × ULN). To avoid an over-representation of individuals with chronic hepatitis at baseline experiencing liver toxicity, changes in transaminase levels are recorded based on baseline values by some authors, although there is no consensus on this issue.[11] Therefore, transaminase level elevation is scored as grade 1 (1.25–2.5 × baseline); grade 2 (2.6–3.5 × baseline); grade 3 (3.6–5 × baseline) and; grade 4 (>5 × baseline) in subjects having abnormal liver enzyme values at baseline. Severe hepatic injury (the primary study outcome) is defined as a grade 3 or 4 change in AST and/or ALT levels during ART. If AST and ALT grades are discordant, the highest should be used for classification purposes.

3. Differential Diagnosis

In addition to HAART-derived hepatotoxicity, some liver diseases are often associated with HIV infection and should also be ruled out. Thus, viral hepatitis due to hepatitis A virus (HAV), HBV, HCV, and hepatitis D virus (HDV), cytomegalovirus and Epstein-Barr virus may cause hepatocellular liver injury. Bacillary angiomatosis, other bacterial infections (included mycobacteria), fungal processes and tumours such as lymphomas and Kaposi's sarcoma may involve the liver, causing a predominantly cholestatic pattern. A mixed pattern is most often seen when HAV, bacillary angiomatosis and liver steatosis occur. Drugs commonly used in HIV-infected patients can induce cholestastic (cotrimoxazole [trimethoprim/sulfamethoxazole], erythromycin, azithromycin, amoxicillin/clavulanic acid) or hepatocellular (isoniazid, phenytoin) patterns. In addition, other aetiologies of liver disease occurring in non-HIV subjects should

Table II. Risk factors for liver toxicity after initiating antiretroviral therapy (ART)

Study	No. of patients	ART	Patients with HCV/HBV co-infections (%)	CD4+ count (cells/mm3)[a]	Incidence of liver toxicity [n. (%)]	Risk factors
Rodríguez-Rosado et al.[12]	187	PI-based	58	NA	26 (14)[b]	HCV
Saves et al.[13]	748	PI-based	41	144	67 (9)	HCV, HBV, prior cytolysis
	1249	Two NRTIs	44	234	71 (6)	HCV, HBV, prior cytolysis
Sulkowski et al.[11]	211	PI-based	51	109	26 (12)	HCV, HBV, ↑CD4, ritonavir
	87	Two NRTIs	61	215	5 (6)	HCV, HBV, ↑CD4
Den Brinker et al.[14]	394	PI-based	22	150	70 (18)	HCV, HBV
Saves et al.[15]	1080	PI-based	30	290	23 (2)	HCV,HBV
Núñez et al.[16]	222	HAART (PI, NNRTI)	40	337	21 (9)	HCV, age, alcohol use
Bonfanti et al.[17]	1477	PI-based	≈50	265[c]	NA[d]	HCV, ritonavir, prior cytolysis
D'Arminio Monforte et al.[18]	1255	HAART (mainly PI)	57	327	61 (5)	HCV, HBV, prior cytolysis, prior non-HAART therapy
Aceti et al.[19]	1325	PI-based	59	≈200	42 (3)	HCV, HBV, ritonavir, no response to HAART[e]
Wit et al.[20]	560	HAART (PI, NNRTI)	19	170	35 (6)[f]	HCV, HBV, prior cytolysis, nevirapine, ritonavir (full dose), female sex, treatment naive

a Median values.

b Hepatic injury defined as at least 2-fold ALT/AST increase above baseline values.

c Mean value.

d Incidence rate for severe hepatotoxicity 2.7% (95% CI 2.6, 2.8).

e Observed after 12 months of therapy.

f Grade 4 hepatotoxicity.

ALT = alanine aminotransferase; **AST** = aspartate aminotransferase; **HAART** = highly active antiretroviral therapy; **HBV** = hepatitis B virus; **HCV** = hepatitis C virus; **NA** = not available; **NNRTI** = non-nucleoside reverse transcriptase inhibitor; **NRTI** = nucleoside reverse transcriptase inhibitor; **PI** = protease inhibitor; ↑ indicates increased.

also be contemplated, including gallstones, alcohol use, recreational drug use and genetic diseases such as haemochromatosis and α-1-antitrypsin deficiency.

4. Incidence and Risk Factors

The reported incidence of severe liver toxicity after initiating HAART ranges from 2% to 18%.[11-20] Differences in the study populations, as well as in the methods used, probably account for the wide range. The risk factors associated with HAART-derived hepatotoxicity are summarised in table II.

4.1 Co-Infection with Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV)

Liver toxicity, especially severe toxicity (grades 3 and 4), is clearly more frequent in HCV and/or HBV co-infected individuals treated with HAART than in those without these co-infections.[11-20] The role of the AST/ALT ratio in the assessment of HCV-related chronic liver disease has been recently pointed out and may help to predict the risk of developing liver toxicity in patients treated with HAART.[21] The consequences of the elevation of liver enzymes after initiating HAART in co-infected patients are not well known. In certain cases an impairment of liver function, with clinical signs and symptoms, histological damage and even changes in the HCV viral load, occurs after HAART initiation.[12,22-24]

In one study, a higher risk of hepatotoxicity was found in patients carrying HCV genotype 3 (HCV-3) compared with other genotypes.[25] The clinical implications of this finding are two-fold. On one hand, the presence of HCV-3 may impact on the

selection of HAART regimen, choosing those with less potential for hepatotoxicity. On the other hand, since genotype 3 shows a higher response to interferon-α and ribavirine, anti-HCV treatment should be given prior to HAART if possible, in order to cure the HCV infection and minimise drug-induced hepatotoxicity. However, there is an ongoing debate on the issue, especially in less clear situations such as infection by non-HCV-3 genotypes and non-optimal CD4 counts.

4.2 Antiretrovirals

The results of the studies that have evaluated the risk for liver toxicity associated with the use of particular antiretroviral drugs or drug classes are conflicting. The unbalanced and often insufficient representation of some antiretrovirals in these series make it difficult to determine with accuracy the role of each particular drug in the development of liver toxicity.

The phenomenon of hepatotoxicity became more evident after the introduction of ART of high activity, which initially invariably included a protease inhibitor. However, none of the studies have been able to prove a higher potential for liver toxicity with this particular class of drugs. Among the protease inhibitors, some studies suggest that ritonavir may be more hepatotoxic,[11,17,19,20,26] although these results have not been confirmed by others.[18,27] In certain cases, ritonavir has caused fatal acute hepatitis.[28] Several cases of liver toxicity associated with the use of indinavir have also been published.[29-31] The risk of developing severe liver toxicity seems to be low with saquinavir, nelfinavir and lopinavir and rare with amprenavir.[32,33] According to a small study, the use of two protease inhibitors, often including ritonavir at low doses as a booster for the second protease inhibitor, does not seem to increase the risk of toxicity for the liver.[27]

Among the nucleoside reverse transcriptase inhibitors (NRTIs), some authors have found a lower incidence of hepatotoxicity with lamivudine and tenofovir.[16,18] However, the majority of the NRTIs can induce mitochondrial damage and therefore have a potential to induce liver injury, as will be explained in section 5.4.[34] Cases of hepatic failure have been reported in patients taking zidovudine, but didanosine and stavudine have been the most often involved in severe hepatotoxicity.[35-38] Abacavir and tenofovir, with low potential for mitochondrial damage, seem to have a safer profile regarding the liver. In patients with chronic HBV, the discontinuation of lamivudine may be accompanied by a flare of HBV replication, translated into an increase in transaminase levels.

The risk of liver toxicity associated with the non-nucleoside reverse transcriptase inhibitors (NNRTIs) is variable and involves several aspects and mechanisms. We have mentioned several cases of severe liver toxicity, some of them fatal, in subjects receiving nevirapine as part of a post-exposure prophylaxis regimen.[6,7] Likewise, in a trial assessing the NRTI emtricitabine compared with nevirapine, a higher incidence of hepatotoxicity was observed among patients receiving nevirapine.[39] Of interest, in both the post-exposure prophylaxis series,[6,7] and in the emtricitabine trial,[39] hepatotoxicity developed early in the course of treatment. In addition, hepatotoxicity predominated among Black women in the emtricitabine study.[39] These data suggest a hypersensitivity reaction caused the liver abnormalities. However, in other reports, the hepatotoxicity of nevirapine-containing regimens had a later onset (beyond the fourth month), with an increase in the cumulative incidence over time.[40,41] Therefore, it looks like there is a second mechanism through which nevirapine causes liver toxicity that is much more common than the hypersensitivity syndrome.

Several retrospective studies have evaluated the development of hepatotoxicity linked to the use of NNRTIs (table III). In some series, the incidence of liver toxicity is not higher compared with other antiretrovirals.[40,42,43] This is especially true in populations with a low prevalence of chronic HCV infection.[42] Although some authors have found a higher risk of liver toxicity for nevirapine compared with efavirenz,[44,45] others did not do so.[42] It has to be highlighted that all these noncontrolled and retrospective studies assessed very heterogeneous groups of patients and, therefore, may have important biases. The concurrence of other changes in the ART regimens makes it very hard to ascribe liver toxicity to a particular drug.

It is interesting that one of the studies did not find cross-hepatotoxicity between nevirapine and efavirenz.[44] In that same study, the morbidity and

Table III. Severe hepatotoxicity in patients treated with non-nucleoside reverse transcriptase inhibitors (NNRTIs): incidence, timing and risk factors

Study	Patients with HCV/HBV co-infections (%)	NNRTI	No. of patients	Incidence (n [(%)])	Timing	Risk factors
Martínez et al.[40]	46/9	Nevirapine	610	76 (12.5[a])	4% at 3 months	HCV, prolonged ART
Palmon et al.[42]	12/9	Nevirapine	141	2 (1.4)	Days 16, 98 and 468	
		Efavirenz	91	1 (1.1)		
		Delavirdine	40	0 (0)		
Sulkowski et al.[44]	43/8	Nevirapine	256	40 (15.6)	Median 137 days	Nevirapine, HCV, HBV, PI, ↑CD4
		Efavirenz	312	25 (8[b])	Median 100 days	
Martín-Carbonero et al.[45]	45/0	Nevirapine	162	19 (12)	Median 5.5 months	Nevirapine, HCV, female sex, alcohol use
		Efavirenz	136	5 (4[b])	Median 5.5 months	
De Maat et al.[43]		Nevirapine	174	59 (3.4)		HBV, PIs

a Hepatotoxicity defined as an increase in ALT or AST ≥3-fold above baseline values.

b Statistically significant differences.

ALT = alanine aminotransferase; **ART** = antiretroviral therapy; **AST** = aspartate aminotransferase; **HBV** = hepatitis B virus; **HCV** = hepatitis C virus; **PI** = protease inhibitor; ↑ indicates increased.

mortality derived from liver toxicity among patients taking nevirapine or efavirenz was similar. Moreover, in a study assessing nevirapine hepatotoxicity, transaminase levels decreased in many of the patients who continued taking the same treatment.[40]

Taken together, all these data suggest that NNRTIs have a greater risk of inducing immunoallergic reactions involving the liver soon after the initiation of therapy. With prolonged therapy, especially in patients co-infected with HBV and/or HCV, NNRTIs show a trend of causing a slight increase in the cumulative incidence of hepatotoxicity, which may spontaneously abate over time. Only on rare occasions is liver toxicity serious. In particular, morbidity and mortality linked to the use of nevirapine has not been proven to be superior to those of other antiretrovirals.

4.3 Other Factors

Heavy alcohol intake has also been identified as a risk factor for severe hepatotoxicity in patients taking antiretrovirals.[16,45] This finding is relevant, since it highlights the deleterious effects that alcohol has in individuals with an underlying chronic liver disease.[46] Individuals initiating HAART, especially those with chronic hepatitis, should be strongly advised to avoid alcohol abuse.

Several authors have identified other risk factors for the development of HAART-derived liver toxicity, such as the prior presence of elevated levels of transaminases,[13,17,18,20] older age,[16] female sex,[19,45] prior monotherapy,[18] first ART,[20] lack of response to HAART (only observed at 12 months)[19] and an increase in the CD4+ count after HAART initiation.[11,44] This last association suggests that immune mechanisms might be involved in the early elevation of transaminase levels after beginning HAART in patients co-infected with hepatitis viruses, which will be discussed in section 5.6.

5. Mechanisms of Liver Toxicity

Despite the numerous published studies on antiretrovirals and hepatotoxicity, many unanswered questions still remain, in particular those related to the mechanisms involved. The attribution of transaminase level elevation to one drug is often hard to establish, especially when it is included in a multiple-drug regimen, as in the case of HAART, not to mention other possible treatments taken by patients with HIV infection. The clinical correlations vary and the lesion patterns are similar to those of other liver diseases. In addition, in a significant percentage of HIV-positive patients, HCV and/or HBV infections co-exist and modify the impact of antiretrovirals on the liver. The possible mechanisms in-

volved in the development of hepatotoxicity associated with the use of antiretrovirals, with their most relevant clinical features and supporting published data, are summarised in table IV. It is probable that multiple pathogenic pathways simultaneously concur in some patients, as it is difficult to identify the exact mechanisms involved in the development of hepatotoxicity.

5.1 Dose-Dependent Toxicity

In certain cases, a dose-dependent mechanism might be involved in the development of liver toxicity with antiretrovirals. Some authors have observed a direct correlation between plasma nevirapine concentrations and hepatotoxicity independent of the presence of HCV co-infection.[48] By further analyses, these same authors found that HCV infection *per se* was not the cause of the elevated plasma nevirapine concentrations.[47] In contrast, other authors have not found plasma nevirapine concentrations to be a prerequisite for liver toxicity.[43]

5.2 Idiosyncratic Changes in the Mechanisms of Drug Metabolism and Cytoprotection

Drugs metabolised in the liver through the cytochrome pathways may cause liver toxicity when there are polymorphisms in the enzymes.[71] Troglitazone, a drug used for the treatment of diabetes mellitus, is one of the most recent examples of drug-induced liver toxicity involving this idiosyncratic mechanism. Since many of the antiretrovirals are metabolised in the liver through the cytochrome pathways, idiosyncratic polymorphisms of the enzymatic complexes might lead to significant heterogeneity in drug metabolism, predisposing certain individuals to the development of hepatotoxicity.

Inflammatory mediators may enhance and perpetuate drug hepatotoxicity. Apoptosis inductors

Table IV. Mechanisms of hepatotoxicity linked to the use of antiretrovirals: clinical features and supporting data

Mechanism	Clinical characteristics	Supporting data and facts
Dose-dependent toxicity		Correlation between hepatotoxicity and plasma nevirapine concentrations[47] Late onset (possible cumulative toxicity)[40,42,44,45,48]
Idiosyncratic changes in the mechanisms of drug metabolism and cytoprotection		Hepatic metabolism of PI and NNRTI by cytochrome P450 enzymes Spontaneous resolution despite continuing treatment (anti-oxidative stress mechanisms)[16]
Hypersensitivity reaction	Early onset (4–6 weeks) Fever and constitutional symptoms Skin rash Multiple visceral involvement Atypical lymphocytosis and eosinophilia	Reported cases (mainly nevirapine and abacavir)[49-54]
Mitochondrial toxicity	Insidious non-specific symptoms Lactic acidosis Hepatomegaly and steatosis	Reported cases (NRTI)[36,55-59] *In vitro* studies[60]
Abnormalities of glucose metabolism	Hyperglycaemia and hyperinsulinaemia Hepatic steatosis	Involvement of insulin resistance in the pathogenesis of non-alcoholic steatohepatitis[61]
Immune reconstitution in HCV/HBV-positive patients	Low baseline CD4 counts Marked ↑ CD4 and ↓ HIV-RNA with/without ↑ HCV-RNA	Correlation with ↑CD4 counts[11,44] Reported cases[62-67] Spontaneous resolution if immune recovery persists[19,67,68]
HIV-HCV-HBV interferences	Absence of significant immune reconstitution HIV/HCV co-infection: ↓ HIV-RNA and ↑ HCV-RNA HIV/HCV/HBV co-infection: ↓ HIV-RNA and HBV-DNA, and ↑ HCV-RNA	Correlation between HIV-RNA and ↑HCV-RNA[27,69,70]

HBV = hepatitis B virus; **HCV** = hepatitis C virus; **NNRTI** = non-nucleoside reverse transcriptase inhibitor; **NRTI** = nucleoside reverse transcriptase inhibitor; ↑ indicates increased; ↓ indicates decreased.

such as Fas and tumour necrosis factor-α, which may induce liver injury by triggering hepatocyte apoptosis and necrosis, are of special interest. Some drugs may potentiate the activation of death receptors and/or intracellular stress pathways.[72]

Hepatocytes promote mechanisms of cytoprotection against the oxidative stress caused by drug metabolism. Heat-shock proteins, induced by various forms of stress including drugs, may exert cytoprotective functions that help the body to tolerate potentially damaging toxicants.[71] An increase in the levels of heat-shock proteins in individuals with polymorphisms may help the liver adapt to and minimise drug toxicity. Anti-oxidation stress mechanisms might explain the spontaneous normalisation in the levels of transaminases despite maintenance of HAART, as it occurs with isoniazid. Although still early in its development, pharmacogenomics is a new approach that may be very valuable in predicting the risk for hepatotoxicity in patients after initiation of ART.[73]

5.3 Hypersensitivity Reactions

Hypersensitivity reactions are idiosyncratic reactions of the host that are not related to the dose of the drug. Medications containing a moiety are prototypical drugs that induce these immune-mediated reactions involving the liver. Drug hypersensitivity reactions usually manifest as fever, skin rash, constitutional symptoms (malaise, fatigue, weakness, arthromyalgias), multivisceral involvement (lymphadenopathies, mucositis, pneumonitis, hepatitis, myocarditis and nephritis) and haematological abnormalities (atypical lymphocytosis and eosinophilia). They usually become apparent within the first 4–6 weeks of treatment. Immune-mediated drug reactions seem to involve the generation of neoantigens formed by the reaction of liver proteins with reactive drug metabolites.[71] Assays examining *in vitro* activation of peripheral blood mononuclear cells against a drug or its metabolites are currently under research. These assays are a promising approach to identifying susceptible individuals.[74]

Hypersensitivity reactions have been reported relatively often with nevirapine and abacavir, both in HIV-infected patients and in subjects receiving prophylaxis after HIV exposure[49-53] but also with other antiretrovirals such as zalcitabine.[54]

5.4 Mitochondrial Toxicity

A distinctive type of hepatotoxicity involving the mitochondria may evolve into acute liver failure, albeit infrequently. The change is caused by NRTIs, most prominently fialuridine – an NRTI with anti-HBV activity, the development of which has been halted as a result of this adverse effect. The depletion of mitochondrial DNA impairs the cellular respiratory chain and inhibits pyruvate and fatty acid oxidation pathways. The main feature of the hepatic lesion is the accumulation of microvesicular steatosis in liver cells and mitochondrial depletion. This early lesion may evolve to macrovesicular steatosis with focal necrosis, fibrosis, cholestasis, proliferation of billiary ducts and Mallory bodies: a clinical picture resembling alcohol-induced liver toxicity, pregnancy steatosis or Reye's syndrome.[71]

Clinical symptoms of liver steatosis are nonspecific and insidious. These include nausea, anorexia, weight loss, weakness and fatigue and, if it evolves, jaundice and pruritus also appear. Laboratory studies have shown evidence of lactic acidosis, hypoglycaemia, hyperammonaemia and hypoalbuminaemia, while transaminase levels remain within normal limits or slightly elevated. Of interest, the underlying liver disease does not predispose to this type of lesion.[71]

NRTIs are potentially toxic for the mitochondria because of their ability to inhibit γ-DNA polymerase, the enzyme responsible for the mitochondrial DNA replication. The ability of NRTIs to inhibit mitochondrial DNA synthesis *in vitro* is in the following order: zalcitabine > didanosine > stavudine > zidovudine > lamivudine = abacavir = tenofovir.[60] Cases of lactic acidosis-steatosis have been more frequently reported in patients receiving didanosine, stavudine or zidovudine, and the outcome has often been fatal.[34,55,56] Hydroxycarbamide (hydroxyurea), used as coadjuvant treatment with didanosine to enhance its activity, seems to increase the toxic effect of some NRTIs because of the rise of intracellular levels of 5′tryphosphates products.[56,75] It is believed that cumulative exposure to NRTIs is an important factor for the development of lactic acidosis, since it usually appears after prolonged treatment, usually years, and correlates with the number of concomitant NRTIs. *In vitro* data support an

additive or synergistic long-term mitochondrial toxicity with some NRTI combinations.[76]

5.5 Metabolic Abnormalities

Steatohepatitis may cause hypertransaminasaemia. Insulin resistance is believed to be the metabolic hallmark of predisposition to non-alcoholic steatohepatitis. In the context of the lipodystrophy syndrome, HAART may cause marked abnormalities in the metabolism of both lipids and glucose, including insulin resistance.[61] Mild to moderate degrees of steatosis have been found in the liver of patients experiencing HAART-derived hepatotoxicity.[77] Thus, in some patients receiving HAART, insulin resistance and non-alcoholic steatohepatitis may contribute to the development of liver toxicity.

5.6 Immune Reconstitution in HCV- and/or HBV-Infected Patients

Liver damage induced by chronic HCV and HBV infection is mainly immune mediated. The immune deficiency caused by HIV infection is responsible for the attenuation of the inflammatory reaction in the liver of co-infected subjects. The inhibition of HIV replication with HAART leads to immune reconstitution and, consequently, the immune response to HCV and/or HBV antigens exposed in the liver cell is also restored. Thus, HAART therapy may induce the development of hypertransaminasaemia and even symptomatic hepatitis in patients with HCV[12,62-64,78,79] or HBV co-infection.[65,66] Supporting this finding, a team of researchers has found that markers of HCV-specific immune responses (HCV core-specific IgG antibody), T-cell activation and inflammation, correlated with liver damage and immune reconstitution.[80]

This syndrome of immune reconstitution typically appears in patients with very low baseline CD4 counts and/or very high levels of plasma HIV-RNA; after HAART-induced viral suppression these patients experience a rapid increase in the CD4 counts.[64] Some authors have identified an increase of >50 CD4 cells/mm^3 after initiating HAART as an independent risk factor for hepatotoxicity.[11,44] Liver histology has shown exacerbated viral hepatitis in some patients developing severe hypertransaminasaemia while receiving HAART.[77]

If the ART is continued, the immune recovery eventually allows better control of the HCV infection. As a result, the host-virus equilibrium is restored and the cytolytic damage ameliorates.[67,68] Accordingly, some authors have found a correlation between a poor immune reconstitution and hepatotoxicity at 12 months of treatment.[19] These same authors observed spontaneous resolution of the initial hypertransaminasaemia at 12 months of HAART in those patients who achieved a significant increase in their CD4 counts, suggesting an early increase in transaminase levels because of immune reconstitution and control of the HCV infection with less cytolysis subsequently.

In HBV co-infection, the HAART regimen usually includes a reverse transcriptase inhibitor with anti-HBV activity (lamivudine and/or tenofovir). As a consequence, plasma HBV levels experience a decrease and the blunting effect induced by the high levels of HBV replication disappears.[81] As a result, transaminase levels may rise as a result of the lysis of infected liver cells by the cellular immune response.

5.7 HIV, HCV and HBV Interferences

The interference interaction between HBV, HCV and HDV is well known.[64] In a similar manner, it is possible tha t hepatitis viruses and HIV interplay reciprocal inhibitions in patients co-infected with these viruses, as some authors have pointed out.[69,81]

Previously we discussed how the suppression of HIV replication and the subsequent immune reconstitution might impact on the equilibrium between the host and HBV/HCV. However, not all changes in the HCV-RNA levels are easily explained based on the improvement in the cellular immune response induced by the ART. After suppression of the HIV replication, plasma HCV-RNA levels may either increase or decrease.[70,82] This individual evolution may explain the conflicting results obtained in the multiple studies assessing the HCV viraemia in HIV-infected patients receiving HAART.[83] Some of the patients who experienced an increase in the plasma HCV-RNA levels also experienced an in-

crease in transaminase levels due to the reactivation of HCV infection.[69]

With HBV co-infection, the picture is even more complex since the ART usually includes an anti-HBV drug and, hence, a subsequent decrease in HBV viraemia. If there is also infection by HCV, the inhibitory effect of HBV over HCV disappears and may lead to reactivation of HCV replication and an increase in transaminase levels. More studies examining viral kinetics and interferences involving 2-fold, 3-fold or 4-fold infections are needed.

6. Therapeutic Management

The three main considerations necessary for the management of transaminase level elevation after the introduction of HAART are severity, clinical impact and aetiological mechanisms. Figure 1 shows an algorithm that takes into account these three issues.

Low-grade liver toxicity most often resolves spontaneously, and just merits an expectative attitude while the antiretroviral treatment continues. In the face of severe hepatotoxicity, the presence of liver decompensation (i.e. ascites, severe ictericia and/or encephalopathy) is enough reason to stop the ART treatment.[84] Once the clinical picture resolves, a new regimen avoiding those drugs with more potential for hepatotoxicity may be tried. If complete resolution is not achieved, it is not clear what the most appropriate approach is, and each case should be evaluated on an individual basis.

In the absence of liver decompensation and co-infection by HBV and/or HCV, it is necessary to rule out an allergic reaction. Typically, skin rash, fever and non-specific symptoms develop soon after treatment initiation. If the clinical picture is consistent with a hypersensitivity reaction, the treatment should be discontinued. The role of corticosteroids in the management of these immune reactions with liver involvement is not established, despite several reports of dramatic improvement after their use in patients with acute liver failure associated with HAART initiation.[85,86] The indication for corticosteroids probably depends on the severity of the cutaneous-mucous manifestations or the presence of angioedema more than on the severity of the transaminase level elevation. The chances of developing a hypersensitivity reaction are higher if the regimen includes nevirapine or abacavir. Once the reaction has abated, a new ART combination avoiding the most suspected drugs may be introduced.

We should also consider the manifestations of lactic acidosis caused by NRTIs, which is characterised by its non-specificity, insidious onset and often the presence of liver steatosis. The condition is potentially severe, with a high mortality rate, and requires immediate interruption of the treatment. Several authors have also administered riboflavin, Levocarnitine, and antioxidants such as asorbic acid (vitamin C) and coenzyme-Q, although with poor results.[57-59] Following resolution, it is appropriate to resume HAART therapy, avoiding the NRTIs known to be more toxic to the mitochondrias.

In the absence of symptoms, special attention should be paid to the presence of other hepatotoxic factors, such as alcohol and other drugs, which may be removed. If no other factors co-exist, such as hyperinsulinaemia and hepatic steatosis, the degree of liver toxicity is the main determinant of the clinical approach. If it is severe, switching the regimen to one with less potential for liver toxicity is recommended. If it is mild to moderate, it is reasonable to continue the same treatment with a close follow-up of the liver enzymes. This is especially true when the therapeutic options are limited or there are important reasons (e.g. adherence or tolerance) to keep a particular antiretroviral regimen.

The presence of HCV and/or HBV co-infections modifies the assessment of hepatotoxicity derived from the use of antiretrovirals. The immune reconstitution, reflected in the significant increase in CD4 counts after HIV suppression, may determine enhancement of the cytolysis of liver cells in response to HCV or HBV viral antigens. The measurement of plasma HCV and/or HBV viraemia may help to assess the role of the pre-existing chronic hepatitis in the elevation of liver enzymes. A high viraemia supports the development of an immune reconstitution syndrome, and maintenance of HAART may be attempted under close follow-up of the patient, producing a spontaneous resolution over time. Finally, if there is HCV co-infection, treatment of the chronic hepatitis with pegylated interferon-α and ribavirine may be warranted if no major contraindica-

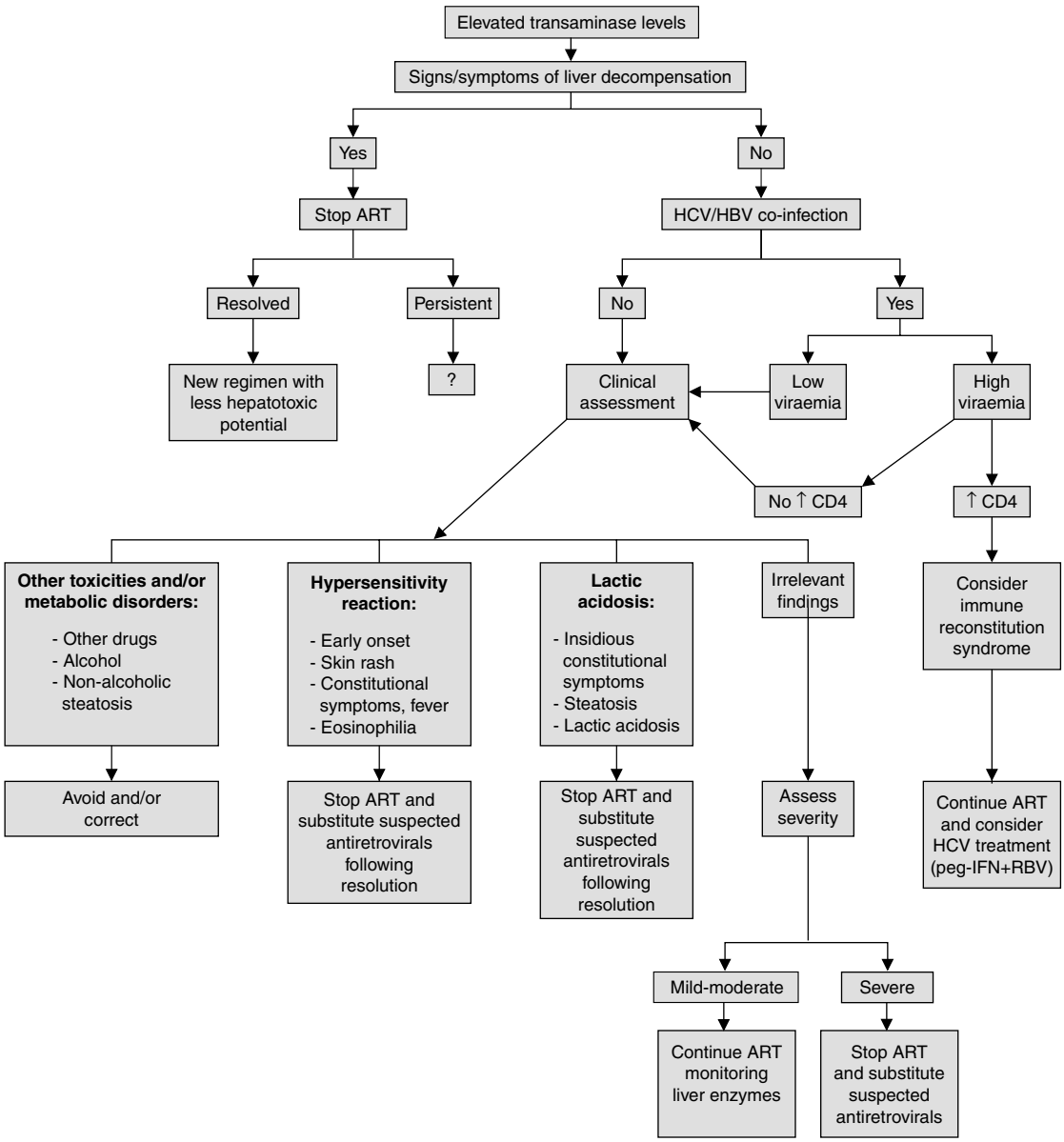

Fig. 1. Proposed algorithm for the management of antiretroviral hepatotoxicity. **ART** = antiretroviral therapy; **HBV** = hepatitis B virus; **HCV** = hepatitis C virus; **peg-IFN** = pegylated interferon; **RBV** = ribavirin; ↑ indicates increased.

tions concur, especially when the HCV genotype is a favourable one for response (i.e. 2 and 3).[77]

If the elevation of transaminase levels occurs in the medium to long term in the context of HBV co-infection, it is more probable that the patient has received lamivudine and resistance mutations have developed, which explains a rebound in HBV DNA. In this case, it is appropriate to switch to tenofovir or other therapeutic alternatives such as interferon-α.[87] However, discontinuation of lamivudine be-

cause of incorrect adherence may be another reason explaining the elevation of transaminase levels in this setting.

Although the proposed algorithm (figure 1) provides a theoretical framework to guide treatment of antiretroviral-induced hepatotoxicity, actual cases are complex and it is not possible to establish fixed compartments among the pathogenic mechanisms. In many cases, it is not possible to ascertain the causes of the hepatotoxicity, which complicates management. Moreover, it is probable that in some cases several mechanisms are involved.

7. Conclusion

Liver toxicity develops in a significant number of patients initiating HAART. The phenomenon currently represents a relevant cause of morbidity and mortality in HIV-infected individuals. HCV and/or HBV co-infection favour the development of liver toxicity linked to the antiretrovirals. Several pathogenic mechanisms may explain the elevation of transaminase levels after initiating HAART, including direct drug toxicity, immune reconstitution in the presence of HCV and/or HBV co-infections, hypersensitivity reactions with liver involvement and mitochondrial toxicity. The management of hepatotoxicity mainly depends on its clinical impact, severity and pathogenic mechanisms.

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