Hepatitis B coinfection in HIV-positive patients starting antiretroviral therapy

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PhD Thesis

I, Ivor Huw Orpwood Price, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Abstract

Evidence is limited to support decisions on treatment and monitoring requirements in the management of hepatitis B virus (HBV) and HIV coinfected patients. The antiretroviral drugs lamivudine (3TC), emtricitabine (FTC) and tenofovir (TDF) are also active against HBV.

To assess the evidence for using TDF with 3TC/FTC to suppress HBV viral replication in coinfected patients we performed a systematic review and meta-analysis of HBV viral suppression from published and unpublished reports. We then carried out a sub-study of the DART trial (a randomised controlled trial of HIV treatment strategy in Africa) to examine HBV epidemiology, viral suppression and associations between HBV coinfection and liver status, immunosuppression (CD4 cell count) and death.

The meta-analysis found: the proportion of coinfected patients with suppressed HBV replication after one year of TDF treatment was 57.4%, rising to 85.6% at three years; that prior or concomitant 3TC exposure had no effect; but that little data was available beyond three years follow-up.

55.2% of the DART population had evidence of HBV exposure and 9.3% had current infection (detectable HBsAg). HBeAg status and HBV viral load (HBV VL), but not exposure or current infection, were associated with immunosuppression. After 48 weeks, HBV suppression was achieved in 81 (56.6%) of 143 with detectable HBV DNA at baseline. Suppression was associated with baseline HBeAg status and HBV VL but not TDF/3TC versus 3TC alone. Suppression once achieved was durable regardless of which treatment was given. If not suppressed at 48 weeks, most treated with both 3TC and TDF suppressed by the end of follow-up, but not those treated with 3TC alone. Coinfection was associated with an increased risk of exacerbations of liver inflammation, HIV progression and death, but deaths were not usually liver-related.

These studies have implications for the management of HBV coinfection in resource-poor settings.

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Equations

Abbreviations

3TC	Lamivudine
ABC	Abacavir
ADV	Adefovir
ALT	Alanine transaminase
Anti-HBc	Antibody to hepatitis B core antigen
Anti-HBe	Antibody to hepatitis B "e" antigen
Anti-HBs	Antibody to hepatitis B surface antigen
aOR	Adjusted odds ratio
API	Age and platelets index
APRI	AST to platelet ratio index
ART	Antiretroviral therapy
AST	Aspartate transaminase
AUROC	Area under the receiver operating curve
AZT	Zidovudine
BLQ	Below the level of quantification
BMI	Body mass index
CI	Confidence interval
CDM	Clinically driven monitoring
CHB	Chronic hepatitis B
CNS	Central nervous system
CT	Continuous treatment arm of STI study
D4T	Stavudine
DART	Development of Antiretroviral Therapy
DNA	Deoxyribonucleic acid
ECLIA	Electrochemoluminescence assay
ECM	Extracellular matrix
EFV	Efavirenz
EIA	Enzyme-linked Immunoassay
ENT	Entecavir
FIB-4	Fibrosis-4 index
FTC	Emtricitabine
GGT	Gamma-glutamyl transferase
HAART	Highly-active antiretroviral therapy
HBcAg	Hepatitis B core antigen
HBeAg	Hepatitis B "e" antigen
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus / hepatitis B
HCV	Hepatitis C virus / hepatitis C
HIV	Human immunodeficiency virus

IQR	Interquartile range
IRD	Immune reconstitution disease
IRR	Incidence rate ratio
IU/mL, U/L	International units per millilitre, units per litre
JCRC	Joint Clinical Research Centre, Kampala
LCM	Laboratory and clinical monitoring
NVP	Nevirapine
OR	Odds ratio
ORF	Open reading frame
PLT	Platelets
RNA	Ribonucleic acid
ROC	Receiver operating curve
RTV	Ritonavir
SE	Standard error
STI	Structured treatment interruption
TB	Tuberculosis
TDF	Tenofovir
ULN	Upper limit of normal
VL	Viral load
WHO	World Health Organisation

1 Background

1.1 Epidemiology

Over the last three decades, since its discovery, human immunodeficiency virus (HIV) infection has grown to become one of the most important infectious diseases in the world; an estimated 35.3 million people were living with HIV in 2012 and 1.6 million died of AIDS-related causes in 2012 [1].

However, in patients with access to treatment, rates both of progression to AIDS and of HIV-related deaths declined rapidly after the introduction of Highly Active Antiretroviral Therapy (HAART) in 1996 and HIV is now managed as a chronic disease [2-5]. UNAIDS estimates that the total number of AIDS-related deaths worldwide peaked at about 2.3 million in 2005 and has been declining since [6].

Hepatitis B virus (HBV) infection is one of the most common infections worldwide with one third of the world's population showing evidence of prior infection [7]. Approximately 360 million people worldwide have chronic HBV (CHB), defined as the presence in blood of HBV surface antigen (HBsAg) or HBV DNA for a period of at least six months [8]. HBV-related liver disease progresses through a process of inflammation and fibrosis and leads to death via cirrhosis and liver failure or via hepatocellular carcinoma (HCC), being one of the top 10 infectious causes of death worldwide [9]. It has been estimated that HBV caused 786,000 deaths in 2010, most through cirrhosis (40%) and hepatocellular carcinoma (43%) [10]. The risk of an individual infected with HBV failing to control the virus and developing CHB is related to the age at which infection is contracted with rates of chronicity ranging from 80 to 90% in infants born to HBeAg positive mothers, 23 to 73% in young children and 5 to 10% in adults (reviewed by Hyams [11]) and, to a lesser extent, to the sex of the individual, with males being more likely to remain chronically infected [11-14].

The natural history of CHB infection classically passes through some or all of four stages, namely (i) immunotolerance, (ii) immunoactivation, (iii) inactive carrier and (iv) reactivation [15]. In those infected at or near birth, the first two phases tend to be prolonged until the third or fourth decade [16, 17] while those infected later in childhood may have a very brief or absent immunotolerant phase [18, 19].

Data on the prevalence of hepatitis B and C (HCV) in HIV-positive adults in sub-Saharan Africa was recently reviewed by Barth [20]. 60 studies with at least 20 HIV-infected adults were included. The range of HBsAg prevalence was 3.9 to 70.3% while the median prevalence was 12.1% and the mean 14.9%. Some studies in the meta-

analysis included HIV-negative patients and the risk ratio for HBsAg positivity in HIV-positive patients compared to HIV-negative was 1.4 (95% confidence interval 1.2 to 1.7).

By comparison the cumulative prevalence of HBsAg (proportion ever having had detectable HBsAg) in the UK HIV-positive population has been estimated to be 6.9% [21].

Epidemiology of Hepatitis B and HIV in Uganda and Zimbabwe

The prevalence of CHB varies worldwide but sub-Saharan Africa, where two thirds of those with HIV live, is an area of high CHB prevalence [22].

In sub-Saharan Africa few children are infected with hepatitis B at or before birth. There are no relevant data from Uganda or Zimbabwe but in a study in northern Namibia Botha found only 1% of children under 6 months positive on HBsAg testing but a dramatic increase occurred at around 11 months of age with 13% positive thereafter. Only 37% of children positive for HBsAg had mothers who were also positive for HBsAg, indicating transmission from other sources [23].

Global distribution of age-specific HBsAg prevalence was recently estimated regionally by the World Health Organisation [24]. Estimates in adults (>20 years) for 2005 were between 4.0 and 6.8% for females and 4.3 and 6.8% for males in East sub-Saharan Africa (includes Uganda) and between 5.4 and 8.4% for females and 4.2 and 6.4% for males in Southern sub-Saharan Africa (includes Zimbabwe).

The earliest estimates of HBV prevalence in what is now Zimbabwe were performed by Cruickshank in the early 1970s with HBsAg prevalence in blood donors of African ethnicity in Harare 3.6 to 4.0% and in Bulawayo 4.4%. In blood donors of European ethnicity the prevalence was 0.2%. In rural areas the prevalence varied from 0% in Nyanga to 5.6% in Kariba [25, 26]. In 1978 Goldsmid also noted ethnic differences, this time in the army, with no cases of HBsAg found in 564 soldiers identified as Asian, coloured or European whereas the prevalence in those identified as African was 7.6% In a subset, the performance of cross-over immune-electrophoresis (CIEP) and direct haemagglutination tests were compared with the prevalence being estimated as 6.3% with the former and 12.5% with the latter, raising the possibility of underestimation of prevalence in the earlier studies which had also used the CIEP method [27]. A national seroprevalence survey in 1985 found higher rates in healthy volunteers of between 13.5% (Kariba) and 19.7% (Masvingo) with 13.7% in Harare [28]. A second national seroprevalence survey in 1996 found an overall prevalence of 15.4% with 19.6% in Harare but wide regional differences from 2.8% to 36.8% [29]. A study in 1999 of

pregnant women delivering at Harare Maternity Hospital found 25.0% carried HBsAg [30]. The latest data comes from 2010. A study in pregnant women which also examined HIV-1 prevalence found that rates in Harare were lower than in previous studies: 2.4% in HIV-negative and 5.8% in HIV-positive women [31]. Another study, a multinational cohort from 2 randomised controlled trials, found HBsAg in 11.0% of HIV-positive participants in Zimbabwe [32].

HBV seroprevalence was also first studied in Uganda in the early 1970s. HBsAg was detected in 2.1 to 3.1% of inpatients in Kampala [33, 34]. Since then studies have found wide variations in prevalence and differences in distribution (Table 2). For example, in the largest study prevalence was 23.9% in the northeast region and 3.8% in the southwest [35]. Significant gender differences have been found in some studies but no difference found in others.

Immunisation against HBV is effective and its inclusion in childhood schedules has been recommended by the World Health Organization since 1992. It was introduced into schedules in Zimbabwe in 2000 and Uganda in 2002 [36]. The latest estimated coverage from the World Health Organization is that in Uganda 78% of the target population (infants), and in Zimbabwe 95%, have received a third dose of HBV vaccine [37, 38].

UNAIDS estimated the prevalence of HIV in adults aged 15 to 49 years to be 6.5% in Uganda and 14.3% in Zimbabwe in 2009 compared to 7.0% and 23.7% respectively in 2001 [39].

Figure 1: Hepatitis B prevalence in Zimbabwe – locations
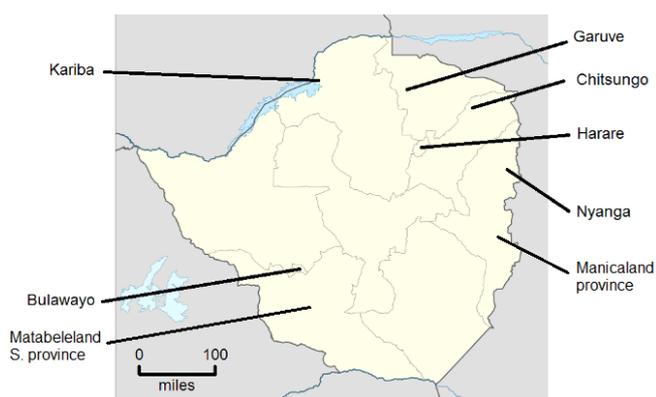
NordNordWest/Wikipedia [40]

Table 1: Hepatitis B prevalence in Zimbabwe

Author	Year	Location	Group	HIV	n	HBsAg %	anti-HBc %
Cruickshank[25]	1971	Harare	BD	NR	25	4.0	
Cruickshank[26]	1972	Harare	BD – Af	NR	3,986	3.6	
		Harare	BD – Af forces		387	7.2	
		Harare	BD – Eu		1,275	0.2	
		Bulawayo	BD		228	4.4	
		Nyanga	HS and Pt		71	0.0	
		Chitsungo	Villagers		652	1.5	
		Chitsunga	Villagers		169	4.7	
		Kariba	Villagers		144	5.6	
Goldsmid[27]	1978		Army – non-Af	NR	564	0.0	
			Army – Af		262	8.3	
			Army – Af		96	[a]7.6	
			Army – Af		96	[b]12.5	
Tswana[28]	1985	Masvingo	HV	NR	147	19.7	
		Gweru	HV		116	14.7	
		Kariba	HV		245	13.5	
		Wedza	HV		220	16.8	
		Harare	HV		539	13.7	
		Kadoma	HV		198	17.2	
		Nationwide	HV		1,471	13.7	
Emmanuel[41]	1988	Harare	HS	NR	226	6.6	48.7
Mvere[42]	1996	Harare	BD	NR	198	3.5	
Tswana[29]	1996	Harare	HV	NR	209	19.6	
		Manicaland	HV		381	9.2	
		Matabele S	HV		281	21.7	
		Nationwide	HV		3,394	15.4	44.6
Madzime[30]	1999	Harare	Pregnant	NR	984	25.0	
Mavenyengwa[31]	2010	Chitsungo	Pregnant	All	59		42.4
		Chitsungo	Pregnant	Neg	50	4.0	
		Chitsungo	Pregnant	Pos	9	0.0	
		Guruve	Pregnant	All	163		34.4
		Guruve	Pregnant	Neg	98	3.1	
		Guruve	Pregnant	Pos	34	2.9	
		Harare	Pregnant	All	181		24.3
		Harare	Pregnant	Neg	126	2.4	
		Harare	Pregnant	Pos	52	5.8	
Thio[32]	2010	Harare	Pt	Pos	227	11.0	

[a] cross-over immuno-electrophoresis. [b] direct haemaglutination test (Hepa-test). BD: blood donors. Af: African. Eu: European. HS: hospital staff. Pt: patients. HV: healthy volunteers. NR: not recorded.

Figure 2: Hepatitis B prevalence in Uganda – locations
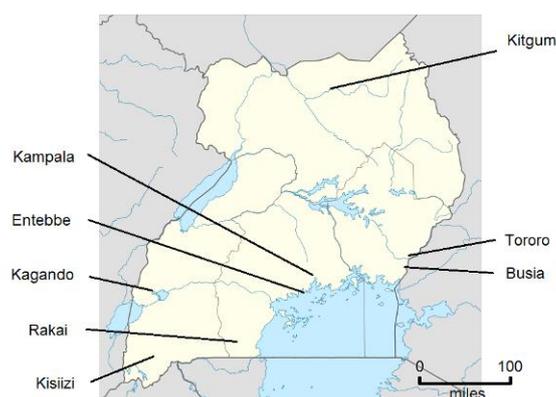
NordNordWest/Wikipedia [43]

Table 2: Hepatitis B prevalence in Uganda

Author	Year	Location	Group	HIV	n	HBsAg %	anti-HBc %
Maynard[33]	1970	Kampala	IP	NR	143	2.1	
Anthony[34]	1972	Kampala	IP	NR	224	3.1	
Sadikali[44]	1973	Kampala	IP	NR	213	3.3	
Hudson[45]	1988	Kagando and Kisiizi	OP	All	206	5.3	
				Neg	138		58.7
				Pos	15		80.0
de Lalla[46]	1990	Kitgum	Pt, preg, forces	NR	358	10.0	
			Male		213	12.7	
			Female		145	6.2	
Opio [47]	1994	Entebbe	OP		1,429	15.7	42.9
				Neg	1,020	14.6	37.5
				Pos	409	18.6	55.4
Nakwagala[48]	2002	Kampala	OP	All	258	15.5	53.5
			OP	Neg	129	13.2	41.9
			OP	Pos	129	17.8	65.1
			Bantu	NR	238	13.9	
			Nilotic/Hamites	NR	20	35.0	
Pido[49]	2005	Kampala	Medical students	NR	182	11.0	65.9
Braka[50]	2006	Nationwide	HCW	NR	311	9.0	55.9
Pirillo[51]	2007	Kampala	Pregnant	Pos	164	4.9	
Weidle[52]	2008	Tororo/Busia	ART initiation	Pos	545	23.5	67.3
Bwogi[35]	2009	Nationwide	Survey	NR	5,875	10.3	52.3
			Male		2,656	11.8	53.6
			Female		3,219	9.1	51.2
		North east			473	23.9	87.5
		Central [a]			1,023	6.2	38.6
		Kampala			363	5.3	31.9
		Southwest			780	3.8	24.9
Ocama[53]	2010	Kampala	ART initiation	Pos	470	8.9	
Seremba[54]	2010	Kampala	IP	All	380	14.5	
			IP – male	All	169	14.8	
			IP – female	All	211	14.2	
			IP	Neg	186	10.8	
			IP	Pos	194	18.0	
Ziraba[55]	2010	Kampala	HCW		370	8.1	48.1
			Male		98	9.2	46.3
			Female		272	7.7	53.1
Stabinski[56]	2011	Rakai	Community		438	4.8	

[a] includes Entebbe & Rakai. IP: In-patients. OP: Out-patients. Pt: Patients. Preg: pregnant. HCW: health care workers. NR: not recorded. ART: antiretroviral therapy.

1.2 Hepatitis serology testing

Serological tests for Hepatitis B detect either viral antigens or antibodies against those antigens. Surface antigen is found on the virion envelope and also makes up the sphere and filament forms found in the blood of individuals with HBV infection. It is produced in 3 forms of varying length from the S domain of the viral DNA; the S domain alone, the S plus pre-S1 and the S plus pre-S1 and pre-S2. The C open reading frame (ORF) codes for the core antigen and the e antigen. The core antigen is a structural protein that forms the 27 nm nucleocapsid. The e antigen is derived from the same ORF but with transcription starting at a different codon. It is non-structural and is found independent of the virus particles. Its function is unknown though it may play an important role in mother to child transmission [57]. Tests used clinically to detect and assess HBV infection include surface antigen (HBsAg), e antigen (HBeAg) and antibodies against the surface (anti-HBs), e (anti-HBe) and core (anti-HBc) antigens.

Anti-HBc appears at around 2 months after infection in the majority of patients and persists for life. It is therefore a marker of ever having been infected. The HBV proteins HBsAg and HBeAg are markers of on-going infection and are not found if an infection is cleared. In those who clear HBV, anti-HBs usually becomes detectable around 4 to 5 months after HBV acquisition as the infection resolves and HBsAg and HBV viral load become undetectable [58].

HBeAg, discovered by Magnius [59], is a marker of a state with high levels of viral replication, high viral load and high infectivity [60]. It appears in blood with HBsAg early during acute infection and disappears if the infection resolves [61]. In CHB infection, individuals usually seroconvert from HBeAg positive to anti-HBe positive between the second and fourth decades of life and then have a lower HBV viral load, low or normal ALT and a lower probability of progression [61].

1.3 Increasing importance of HBV coinfection in HIV infection

In countries with widespread access to HAART, rates of HIV-related deaths are declining [3-5] and although deaths from AIDS are still more common, a high [62, 63] and increasing [64] proportion of deaths in HIV-infected individuals have been from liver disease, which is commonly due to coinfection with HBV or HCV (Figure 3) [62, 65].

Figure 3: Cause of death in 3 large multi-centre HIV cohort studies
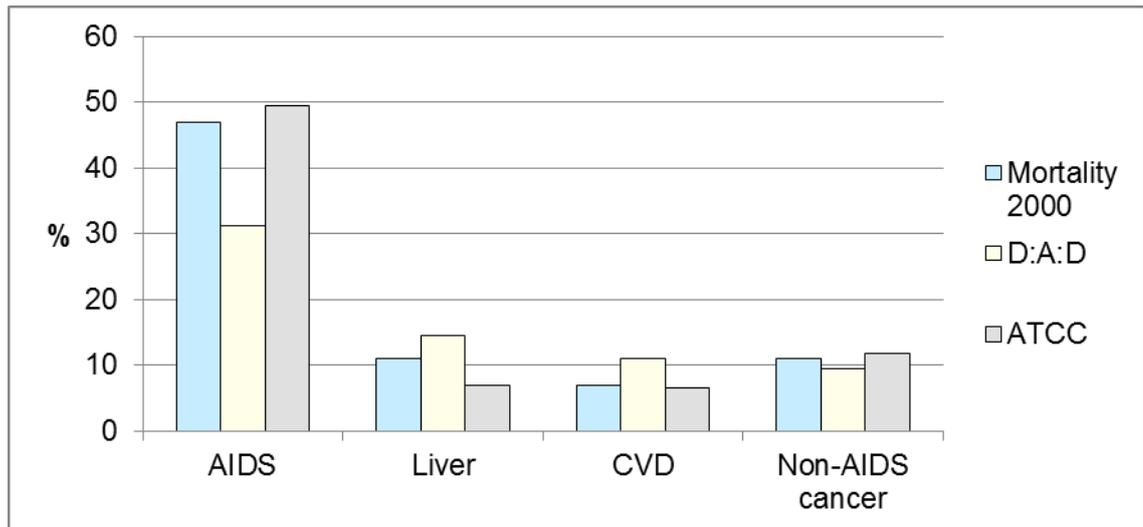
References: Mortality 2000 [66], D:A:D [62], ATCC [67]

A French study that attempted to identify a cause for all deaths occurring in 2000 in HIV-infected individuals found viral hepatitis to be the second most common cause of death after AIDS, causing 11% of deaths, with HBV responsible for 2%. 47% died of AIDS-related causes (23% non-Hodgkin's lymphoma), 11% of non-AIDS and non-hepatitis cancers, 7% of cardiovascular disease, 6% of bacterial infections, 4% of suicide and 1% of adverse reaction to antiretroviral medication [66].

Liver-related disease was also the most common non-AIDS-related cause of death in an analysis of data (1999-2004) from 11 cohorts in Europe, Australia and the United States, causing 15% of deaths. AIDS caused 31%, cardiovascular disease 11% and non-AIDS cancers 9%. Liver-related death was found to be associated with viral hepatitis infection; 76% of liver-related deaths occurred in patients with HBV and/or HCV coinfection. Causes of liver-related deaths included hepatic failure, hepatocellular carcinoma, variceal haemorrhage and drug toxicity [62].

Another multi-cohort analysis (1996-2006) found a rather lower proportion of deaths were attributable to liver disease. 13 cohorts in Europe and North America were included and liver disease was responsible for 7.0% of deaths, being the fifth most common cause of death after AIDS, non-AIDS malignancies, non-AIDS infections and violence and/or drug-related causes. In those who contracted HIV via injecting drug use the hazard ratio (HR) for liver death was 6, suggesting again that the relative importance of liver death in HIV is related to the prevalence of viral hepatitis coinfection [67].

At a single site in the United States of America, Bica found liver disease as a cause of death to be second to AIDS (23% after 49% with AIDS) but of note found the rate increased from 12% in 1991 to 50% in 1998-99 (p=0.003) [64].

Similar results from Catalonia over the period 1997-2004 showed death due to liver-related causes second (23%) to deaths due to AIDS (40%) with the proportion of deaths that were liver-related increasing from 8% in 1997 to 41% in 2004 [68].

1.4 Interactions between infection with HIV and HBV

Despite the announcement of the discovery of a novel virus, later to be called HIV, in May 1983 [69], Ravenholt postulated in October of that year that HBV was itself the cause of AIDS. This was because of the shared epidemiological pattern, the overlapping risk groups and routes of transmission and the development of liver disease after a similar latent period [70]. In the same edition of The Lancet McDonald suggested that the infectious agent of HIV could be a "strand of nucleic acid residing within HBsAg" much like delta virus [71]. The virology of HIV has since been characterised and HIV and HBV are distinct viruses with very different life-cycles and pathology. They do however have similarities and clinical manifestations of infection with the two viruses interact. The mechanisms of such interaction are complex and incompletely elucidated.

1.4.1 Effect of HBV infection on HIV-related disease

One proposed mechanism whereby HBV could exacerbate HIV-related disease is via the increase in HIV replication seen to be caused by the product of the X gene of HBV [72].

Studies examining the effect of HBV infection on progression of HIV disease have given conflicting results. Most have shown no effect of HBV status on progression to AIDS [63, 73-83], CD4 rise on HAART [74, 75, 79, 80, 82, 84-88] (including when examined at 3 months and later [63, 89]), HIV virological suppression on HAART [63, 78, 79, 81-87, 89] or on HIV-related deaths [62, 79, 81, 90, 91].

In contrast some studies have found an increased rate of AIDS [92-94], HIV virological failure [80] or HIV-related death [76, 80, 82, 86, 94, 95] and a reduced CD4 rise on treatment [81, 96].

A meta-analysis including data from 12 studies concluded that HBV has no effect on progression to AIDS (effect estimate, calculated from pooled risk ratio, incidence rate ratio (IRR), odds ratio (OR), or HR using a random effects model, 0.93, 95% CI 0.75 to 1.15). All-cause mortality was increased (effect estimate 1.36, 95% CI 1.12 to 1.64) but although deaths were not stratified by cause the authors did suggest that reduced survival could be due to liver disease secondary to HBV infection rather than to an increase in HIV-related deaths [83].

Of course there is a risk of confounding factors being responsible for the association found between hepatitis infection and death. For example, in the SMART study HBV or HCV coinfection increased the risk of non-AIDS death but the most common causes of these deaths were "unknown", substance abuse and non-AIDS malignancy while only 2 out of 37 deaths were hepatic and neither of these had HBV [97].

These studies were carried out on populations in Europe, North America, Australia, the Asia-Pacific region and in South Africa and overall suggest that any effect of HBV status on HIV disease is small if it exists at all.

1.4.2 Effect of HIV infection on HBV-related disease

In contrast to the lack of effect that HBV has on HIV disease, HIV affects HBV disease at several points in its natural history including the probability of remaining chronically infected, the level of HBV replication, e antigen status, liver inflammation, fibrosis, response to treatment and liver-related death.

Progression from acute to chronic HBV

HIV infection decreases the chance that HBV will be cleared after infection. Hadler followed HIV-positive and negative men in the USA who contracted HBV while enrolled into a HBV vaccine trial and found that the proportion developing CHB was 7% in the HIV-negative men and 33% in the HIV-positive (aOR 8.0, p<0.001) [98]. These figures are similar to those from Australia published by Bodsworth who found 4% of HIV-negative and 23% of HIV-positive men who have sex with men (MSM) developed CHB after infection (p=0.026) [99].

HBV DNA level

In a study of 132 French MSM, Colin found coinfected patients to have higher HBV DNA levels (p=0.01) [100] though this finding was not replicated in a larger French population of 477 men and women with HBV infection which found no significant difference in DNA level between HIV-positive and negative patients [101]. However in the second study more of the coinfected patients received anti-HBV treatment which may explain the contrast with the earlier study in which patients were untreated.

Immunopathogenesis

The innate immune response to early HBV infection involves natural killer (NK) cells which have anti-HBV effects and anti-hepatocyte effects but HBV infection itself appears to have immunosuppressive activity. In a study of 8 HIV-negative and 3 HIV-positive patients with acute HBV infection [102] NK activity was lower in HBV infected individuals than in uninfected controls and was similar in 2 of those with HIV. These 2 had CD4 counts of 495 and 576. The third HIV-positive patient had a CD4 count of 12

and in contrast in his samples NK activity was markedly raised. This patient had a fulminant hepatitis and died. Thus severe HIV-associated immunodeficiency may dramatically alter the course of acute HBV.

Adaptive immune responses also differ in HIV-positive patients. In patients immune to HBV (anti-HBs and anti-HBc positive), HBV-specific CD8+ T cell responses were lower in those HIV-positive than in those HIV-negative and were found to increase after treatment with HAART in 2 of 4 patients examined [103].

Hepatic stellate cells in the absence of injury produce type IV collagen which is a component of normal basement membrane. These cells can express cell surface markers CD4, CCR5 and CXCR4 and when activated can be infected by HIV, though this may in fact be independent of such cell surface markers. Once infected by HIV, stellate cells switch to producing type I collagen and secrete the pro-inflammatory cytokine MCP-1 [104]. MCP-1 secretion and stellate cell chemotaxis are also increased directly by the HIV surface molecule gp120 [105]. Thus HBV may activate stellate cells, facilitating HIV infection of these cells which leads to increased inflammation and fibrosis.

Evidence for HIV infection of hepatocytes is unclear but it has been shown that gp120 and HIV virions can both induce hepatocyte apoptosis [106] and sensitise hepatocytes to apoptosis in response to other causes of injury [107]. Apoptosis may thus be one pathological mechanism whereby HIV infection increases liver damage.

Only one case of liver disease thought to be directly due to HBV in a patient coinfected with HIV has been reported [108] and usually HBV is not cytopathic, the damage to the liver from HBV infection being due to the immune response. It may therefore be expected that in immunosuppressive disease such as HIV the degree of liver damage may be less.

Consistent with this is the finding that despite a higher number of hepatocytes showing HBeAg or HBcAg on microscopic examination of liver biopsy specimens [100, 109], coinfected patients had lower alanine transaminase (ALT) levels (p=0.0001) [100]. However albumin levels were also lower (suggesting advanced liver disease) and the proportion with cirrhosis higher in HIV-positive patients. Lower ALT is associated with reduced liver inflammation as a result of less active liver disease but is also associated with reduced numbers of remaining hepatocytes in individuals with very advanced liver disease.

HBeAg to anti-HBe seroconversion

Coinfected patients are more likely to have persistent HBeAg. In a study that followed 152 MSM with detectable HBsAg over a mean follow-up period of 2.8 years, Gilson found the rate of HBeAg loss to be lower in the coinfected patients with a relative hazard of 0.39 (95% CI 0.16 to 0.94) compared with HIV-negative men [77].

Similarly other studies have shown lower rates of HBeAg loss [101] and anti-HBe seroconversion [101, 110] and higher rates of seroreversion (from anti-HBe positive / HBeAg negative to anti-HBe negative / HBeAg positive) [110].

Pre-core mutants

Mutations in the HBV pre-core region are associated with HBeAg negative CHB and a high risk of progression to liver fibrosis, failure and death [111]. Whether they occur more frequently in HIV-coinfected patients is unclear. In a prospective study of HIV-positive patients with CHB, Revill found a mutation that gave rise to shortened precore/core proteins and higher HBV DNA levels and which occurred at a higher rate in coinfected than in HBV monoinfected patients [112].

However in another prospective cohort, Cassino found that coinfected patients were less likely to have basal core promoter mutations and that precore mutations were no more likely than in monoinfected patients [113].

Liver flares

A flare is an acute worsening of liver disease and is marked by a rise in transaminases (precise definitions vary – see section 1.7.3). The underlying pathological mechanism in HBV-infected patients is a cytotoxic T-cell response against HBV-infected hepatocytes. In coinfected patients a flare may arise in several different clinical situations and may be fatal [114-116]. Flares associated with treatment may be related to an adverse drug reaction or to an improvement in immune status (immune reconstitution disease, IRD), which typically occur soon after ART initiation, or to a rise in HBV DNA which may occur when treatment is withdrawn or when treatment resistance arises. Flares may also occur on HBeAg positive to anti-HBe positive seroconversion as hepatocytes producing HBeAg are cleared by the immune system.

Treatment-related flares are discussed in more detail in section 1.7.3.

Liver disease progression and mortality

In some studies cirrhosis has been found at higher rates in coinfected patients [100, 101] while other studies have failed to show such an association [110, 117].

In those coinfected with HIV and HBV, rates of liver-related death are much higher than in those with mono-infection or without either infection [118]. Rates in those uninfected, HBV monoinfected, HIV monoinfected and HBV/HIV coinfected were 0.0, 0.8, 1.7 and 14.2 per 1,000 person years respectively in a prospective cohort of MSM (Figure 4).

Figure 4: Effect of HBsAg and HIV status on liver-related mortality
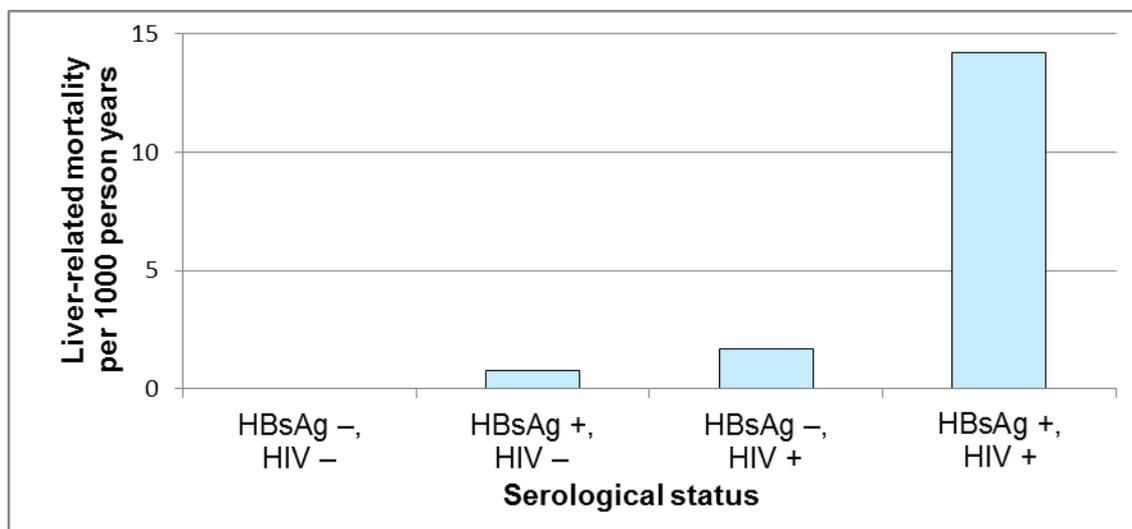
Reference: Thio [118]

Response to treatment

HIV coinfection decreases the rate of response to interferon treatment. In a randomised controlled trial of interferon-α2a for the treatment of CHB 33% of the HIV-negative patients responded to therapy (defined as sustained loss of HBV DNA and HBeAg) while none of the HIV-positive ones did [109]. Similar results were found in another randomised controlled trial of interferon-α2a in which 41% of HIV-negative patients lost HBeAg and 17% lost HBV DNA while again no HIV-positive patients responded [119] and another in which 39% of HIV-negative patients lost HBeAg and HBV DNA versus no HIV-positive patients [120].

In another randomised trial the relative risk of a response to interferon (defined as loss of HBV DNA and HBeAg and appearance of anti-HBe) was 0.22 in HIV-positive patients though confidence intervals on this result were wide (95% CI, 0.03 to 1.78) [121].

In a French cohort study, 26 HIV-positive and 50 HIV-negative men were treated with interferon-α2b for six months. Loss of HBV DNA occurred in 27% of those HIV-positive and 56% of those HIV-negative and HBe seroconversion occurred in 11.5% *vs*. 28% respectively, although these differences were not statistically significant [110].

Another cohort study comparing HIV-positive and negative patients demonstrated a marked reduction in response associated with HIV. After a course of interferon of at

least 12 weeks, 1 of 23 (4.3%) HIV-positive and 42 of 91 (46.2%) HIV-negative had a sustained loss of HBeAg and HBV DNA [122].

1.5 Treatment

While HIV and HBV are not closely related, with HIV being a ssRNA-RT (single-stranded, RNA reverse transcriptase) retrovirus (group VII) and HBV being a dsDNA-RT (double-stranded, DNA reverse transcriptase) virus (group VI), and have very different life cycles, they both make use of a reverse transcriptase enzyme and some drugs which target reverse transcriptase have activity against both viruses.

1.5.1 Treatment of HIV infection

Drugs currently licensed for the treatment of HIV fall into six classes. In patients who have not received prior therapy and virological testing shows no drug resistance, guidelines recommend the use of two nucleoside (or nucleotide) reverse transcriptase inhibitors (NRTI) together with either a non-nucleoside reverse transcriptase inhibitor (NNRTI), a protease inhibitor (PI) boosted with low-dose ritonavir (RTV) or an integrase inhibitor [123, 124].

The choice of NRTIs is wide but in general either lamivudine (3TC) or emtricitabine (FTC) will be used with tenofovir (TDF) or alternatively with either zidovudine (AZT) or abacavir (ABC).

1.5.2 Treatment of HBV infection

Seven drugs are available to treat HBV [125] and are of two classes: NRTI and interferons.

NRTI act on the HBV DNA-polymerase at one or more of three steps: priming, minus strand synthesis and plus strand synthesis. They have differing potencies and patterns of resistance. All six NRTIs act on minus strand synthesis. ADV and TDF also inhibit priming, 3TC and clevudine inhibit plus strand synthesis and entecavir (ENT) and telbivudine inhibit all three stages.

While a course of interferon of finite duration may lead to a prolonged HBV DNA suppression, response rates are low in the context of HIV coinfection (section 1.4.2). In HIV-negative patients response rates have been shown to vary by HBV genotype, with response (HBeAg and DNA clearance) rates by genotype as follows: A 49 to 66%, B 39 to 41%, C 15 to 17%, D 25 to 26%, E 36%, F/H 50%, G 20% [126-130].

In the past, treatment of HBV has been stratified according to HBeAg status, but recent guidelines advocate that treatment decisions be made on the basis of liver

inflammation (ALT) and fibrosis and on HBV DNA level with long-term viral suppression the goal [131]. Rates of HBV viral suppression are high with NRTI treatment. Due to the interactions between HIV and HBV and the dual action of many treatments (and therefore the risk of promoting HIV resistance), current recommendations state that coinfected patients should start HAART with a regimen including TDF and 3TC or FTC if their CD4 count is <500 cells/mm^3. Patients with a higher CD4 count requiring HBV treatment are also recommended to start HAART with TDF and 3TC or FTC but adefovir (ADV) or interferon (acting only on HBV) can be used in patients who do not want or are unable to take TDF as part of HAART [131, 132].

1.6 Benefits of treatment in HBV/HIV coinfection

Treatment guidelines in Europe and North America recommend that HBV status is determined for all HIV-infected patients [133, 134]. However HBV testing is not routine in resource-poor settings and there is a lack of data on the effect of HBV coinfection on clinical outcomes [135].

1.6.1 HBV suppression

Studies have shown the ability of 3TC [136, 137], TDF [138-140], ADV [141], 3TC plus ADV [142], 3TC plus telbivudine [143], 3TC plus TDF [114, 139, 140, 144, 145] and FTC plus TDF [117, 140, 146] to suppress HBV in HIV-coinfected patients.

TDF with 3TC has been shown to be more effective than 3TC alone in naïve patients [114, 139, 144, 147] and also to be effective in the presence of 3TC resistance [139, 144].

There is very limited data from randomised controlled trials comparing TDF as the only HBV-active drug with TDF used in combination with 3TC or FTC. In one such trial (with three arms: 3TC, TDF and 3TC plus TDF), Nelson examined suppression at 48 weeks and found no difference between groups in terms of the proportion with HBV DNA <400 copies/mL at 48 weeks [148].

In a second randomised controlled trial, Matthews compared TDF with TDF plus 3TC in naïve patients and also found no difference in suppression rates at 48 weeks [114].

1.6.2 Liver disease progression and mortality

The risk of cirrhosis [149] and hepatocellular carcinoma [150] are related to the level of HBV DNA and suppression of HBV has been shown to reduce the risk of disease progression [151].

Improved liver function tests and reduced degree of cirrhosis

Although the development of cirrhosis has been regarded as an irreversible step in the progression of chronic liver disease, there have been reports showing that improvement is possible. In one case, the liver biopsy improved from Metavir F4 (cirrhosis) to Metavir F1 after 3 years of HBV suppression on TDF-containing HAART [152]. Such reports are of course subject to the limitations inherent in the small sample provided by percutaneous liver biopsy. However liver function tests can also improve in coinfected patients with cirrhosis, as reported in one study of 7 patients treated with TDF in whom HBV DNA was suppressed [153].

In another study, 141 coinfected patients were followed on TDF. In those with the most severe fibrosis at baseline (F3 or F4) there was a rapid improvement in fibrosis score (Fibrometer, which uses age, platelets, prothrombin index, aspartate aminotranseferase (AST), alpha-2-microglobulin, urea and hyaluronic acid [154]) during the first year and a steady decline in degree of fibrosis up to 3 years [155].

An extreme case was described by Guiterrez in which a HBV/HIV coinfected patient who had been put on a waiting list for liver transplantation with advanced liver disease (including jaundice, ascites and gastrointestinal bleeding) started treatment including TDF plus 3TC and 45 months later had improved to such a degree that he was taken off the transplant list [156].

Decreased mortality

Highly active antiretroviral therapy (HAART) has greatly improved the prognosis for patients infected with HIV, with dramatic declines in the incidence of AIDS and the rate of death [157]. However, with this decline in AIDS-related causes of death, liver disease has emerged as one of the most common causes of death in HIV-positive individuals [158] and is associated with HBV or HCV coinfection in most cases [62]. This is primarily due to a lowering of the risk of death in those on HAART rather than HAART causing liver deaths. For example, the SMART study showed that the incidence of liver disease was lower in those taking HAART continuously compared to those who took it intermittently, despite relatively high CD4 counts [159]; this protective effect was also seen in HBV and HCV coinfected patients [97].

1.6.3 Seroconversion to anti-HBe or anti-HBs

Typically, HBV replication in carriers falls after a period of immune tolerance (with HBeAg-positivity), with seroconversion to anti-HBe and a reduced risk of liver disease. HIV coinfection decreases the rate of seroconversion and increases reactivation to HBeAg-positivity and the emergence of immune-escape mutations.

In a study reported in 1996, Benhamou described 40 patients treated with 3TC or 3TC/AZT (not HAART) for 1 year, stratified according to baseline HBV DNA level (greater or less than 5 pg/mL, or approximately 1.4 x10^6 copies/mL). Of those with high HBV DNA, 5 (17%) lost HBeAg, 3 (10%) of whom developed anti-HBe, all within the first 6 months of treatment [136].

In Kosi's study, HBeAg seroconversion was higher (though the difference was not statistically significant) in patients treated with TDF/FTC than in those treated with 3TC or FTC alone (14.5% *vs.* 9.2%, p=0.29) [160].

Seroconversion of HBsAg to anti-HBs is the most favourable end-point for those with HBV infection and represents resolved infection and (in the absence of profound immunosuppression) lifelong immunity.

In Benhamou's study above, of the 10 with low HBV DNA at baseline, 3 lost HBsAg, 2 of whom seroconverted to anti-HBs. None of the 30 with high HBV DNA lost HBsAg [136].

Piroth followed 17 patients with HBV/HIV coinfection over three years from 3TC-containing HAART initiation. One had HBsAg seroconversion with loss of HBsAg and appearance of anti-HBs [161].

In a retrospective cohort analysis of HBV/HIV infected patients, Kosi found a higher rate of cumulative annual loss of HBsAg (of 6.6% in HBeAg positive patients and 7.9% in HBeAg negative patients) and the rate was higher (though again the difference was not statistically significant) with TDF plus FTC than with 3TC or FTC (10.3% *vs.* 6.0%, p=0.23) [160, 162].

1.7 Limitations of treatment in HBV/HIV coinfection

1.7.1 Resistance

Since several drugs used to treat HBV also have activity against the reverse transcriptase of HIV they may induce resistance mutations in HIV. Thus these drugs should only be used in patients who are on fully suppressive HIV therapy. However HIV resistance mutations have been found in patients with HBV treated with 3TC, TDF and ENT [163]. In contrast, K65R was not found in seven patients treated with ADV monotherapy in Spain and Germany [164].

HIV treatment guidelines recommend the use of combinations of drugs with different classes (and therefore resistance mutations), at least until HIV is fully suppressed, in order to limit the development of resistance. However whether combination therapy is

of benefit in treatment of HBV is still unclear. Guidelines for the treatment of coinfected patients generally recommend using TDF plus either 3TC or FTC in combination as two drugs with HBV activity [125, 134]. However in the past, until TDF became available and widely used, 3TC monotherapy has been the norm. Very little data has been published comparing combination therapy with monotherapy and none show a significantly higher rate of suppression (for detail see meta-analysis results, chapter 3).

Lamivudine resistance

HBV resistance to lamivudine is well recognised in coinfected patients and commonly arises with mutations at codon 204 in the YMDD (tyrosine methionine aspartate aspartate) active site of the HBV reverse transcriptase or at codon 180. In a study of 30 coinfected patients with resistance after 3TC monotherapy the mean duration of 3TC treatment before the emergence of resistance was 3.6 years [165].

HBV resistance in coinfected patients was first described by Benhamou in 1999. He followed 66 patients with detectable HBV DNA (with a lower level of detection of about 140,000 IU/mL) who were treated with 3TC as the only active drug against HBV reverse transcriptase. Although after 2 months 86% had undetectable HBV DNA, only 47% remained suppressed after 2 years and only 9% after 4 years of treatment. Of 24 patients tested, 22 had detectable resistance mutations [166].

Some other studies have also found high rates of HBV resistance after 1 to 4 years of treatment, for example in the CAESAR study 5 (38%) of 13 had resistance after 1 year [167] while in a cohort study including patients from Australia and the USA mutations were found in 50% of those treated for less than 2 years and in 94% of those treated for over 4 years [168].

In contrast, recently published data gives a far lower rate of resistance acquisition. A prospective HIV cohort in Thailand included 30 HBV coinfected patients (63% HBeAg positive) who commenced 3TC-containing HAART. HBV suppression, defined as an undetectable HBV VL (the lower limit of detection was 50 copies/mL) was achieved by 20 at 12 months (100% of those HBeAg negative and 47% of those HBeAg positive) and by 1 more at 38 months. Two others suppressed during the first year but had rebounded by 12 months. Of the 23 that reached HBV suppression, 18 (78%) maintained suppression to the end of follow-up. The cumulative rate of maintained response to 3TC was 80% at 4 years [169].

When used in combination with TDF, 3TC resistance is less common though it has been detected [144, 170, 171].

Tenofovir resistance

The mutation A194T was detected in 2 patients treated with TDF for over 6 months and was examined *in vitro* by site-directed mutagenesis, both alone and in conjunction with L180M and M204V. The triple mutant was found to have a >10 fold change in the IC_{50} for TDF [171]. A second *in vitro* study confirmed this decrease in susceptibility both alone and with other (3TC) resistance mutations and calculated the fold change to be between 5 and 6. Of particular note, the replicative capacity of HBV was reduced by A194T with and without L180M and/or M204V, whereas this decrease was reversed by concomitant mutations in the pre-core or basal core promoter regions [172].

Other studies of patients treated with TDF have failed to show any TDF resistance mutations [114, 117, 145, 173].

1.7.2 HBsAg changes

The HBV genome contains overlapping reading frames and mutations rtV173L and rtM204V/I that may arise in response to 3TC pressure are also sE164D and sI195M or sW196S/L/stop in the HBsAg gene. These HBsAg mutations reduce binding affinity of antibody against HBsAg *in vitro* [174]. rtV191I also causes a stop in the surface gene (sW182stop) [170]. These stop codons alter the conformation of surface antigen and may allow vaccine-escape [175]. Other surface gene mutations that arise in coinfected patients during 3TC therapy include sT114R, sP120T, sP120Q, sK122K/N, sQ129R, sM133I, sF134L, sT143M and sD144E [168, 176].

Stop codons in the surface gene may also interfere with HBV testing. HBsAg is frequently used to screen patients and products for the presence of HBV and such a modification in HBsAg may lead to false negative screening test results and to transmission of HBV [177]. Newer assays avoid this by including more than one epitope.

1.7.3 Flares

Exposure to HAART is in itself a risk factor for liver disease. Most antiretroviral medications are capable of causing liver damage, with in particular high-dose RTV [178] and nevirapine (NVP) [179] having a higher risk and although RTV is no longer used at high dose, NVP is still frequently used in developing countries. A recent systematic review that included over 30,000 adults and children starting first-line HAART in 8 randomised controlled trials and 26 cohort studies found that 8.4% of those treated with NVP suffered hepatotoxicity with 3.2% having a severe (grade 3 or grade 4) reaction. The incidence was lower in those treated with EFV, but hepatotoxicity was still reported for 3.6% and severe hepatotoxicity for 2.3% [180].

In patients coinfected with HBV and HIV the risk of liver damage on commencing HAART has been shown to be higher. However previous estimates of the rate of significant liver damage have varied and studies have been limited by the use of different case definitions, low patient numbers and the lack of patients from Africa, where the majority of coinfected patients live.

Different definitions of flare have been used with the most common being the AIDS Clinical Trials Group grade 3 definition: a rise in ALT or AST to five times the upper limit of normal (ULN) [181]. The ULN is typically around 40 IU/L (see section 1.8.1) and on occasion an absolute level of 200 IU/L to represent five times ULN has been used [182, 183]. Sometimes additional criteria are used to avoid misclassifying patients with raised transaminases at baseline, for example, a rise to 3.6 times the baseline value [179] or requiring a rise of at least 100 IU/L [184, 185]. Generally a single value above the threshold identifies a flare but it has been argued that a better definition is the detection of ALT >200 IU/L on two occasions at least 2 weeks apart to exclude transient rises [186].

Previous studies have found wide variation in rates of flares in HBV/HIV coinfected patients starting HAART ranging up to 50% (see Table 3). Of note, rates in many studies in Africa have been low (0 to 3%) though African studies have found rates up to 22.5%. However very few of the studies listed in Table 3 have found deaths associated with flares and when they have occurred have rarely been due to liver disease [85, 187] and often to other causes (such as Kaposi's Sarcoma or renal failure [184, 187]).

Table 3: Liver flares in HBV/HIV coinfected patients starting HAART

Source	Publ. year	Country	Flare definition	Follow-up (days)	n / N	Incid. (%)
Saves [188]	1999	France	5x ULN[a]	393[f]	15 / 87	20.8
Saves [189]	2000	France	5x ULN	150[g]	5 / 29	17.2
Piroth [161]	2000	France	NR	1,095	3 / 17	17.6
Sulkowski [178]	2000	USA	5x ULN[b]	167-182[f]	2 / 12	16.7
den Brinker [184]	2000	Netherlands	5x ULN[c]	480[f]	13 / 29	44.8
Bonfanti [190]	2001	Italy	5x ULN	534[g]	14 / 97	14.4
Nunez [191]	2001	Spain	5x ULN[b]	245[g]	3 / 11	27.3
Monforte [182]	2001	Italy	200 IU/L	540[f]	8 / 91	8.8
Wit [192]	2002	Netherlands	10x ULN[c]	1,095[f]	15 / 49	30.6
Law [185]	2003	Thailand	5x ULN[c]	336	9 / 60	15.0
Livry [193]	2003	France	2.5x ULN	570[f]	6 / 12	50.0
Hoffmann [187]	2007	South Africa	5x ULN[b]	239[f]	18 / 80	22.5
Ofotokun [194]	2007	USA	2.5x ULN	672	43 / 84 [e]	51.2
Matthews [114]	2008	Thailand	5x ULN[d]	336	9 / 36	25.0
Weidle [52]	2008	Uganda	5x ULN	720	0 / 128	0.0
Idoko [87]	2009	Nigeria	5x ULN	336	8 / 262	3.1
Moore [195]	2010	Malawi	5x ULN	350	1 / 42 [e]	2.4
Mbougua [196]	2010	Cameroon	5x ULN	720[f]	0 / 14	0.0
Kalyesubula [197]	2011	Uganda	2.5x ULN	98	0 / 7	0.0
Matthews [85]	2011	South Africa	5x ULN	120	10 / 106	9.4
Wang [198]	2012	China	5x ULN	336	6 / 65	9.2
Hawkins [199]	2012	Tanzania	5x ULN	554[f]	19 / 1,071	1.8

a   or >200 IU/L
b   or >3.5x baseline if raised at baseline
c   and increase of >100 IU/L
d   or increase of >100 IU/L if raised at baseline
e   HBV and HCV combined
f   median
g   mean

Publ.: publication. Incid.: incidence. NR: not recorded. Shading; studies in sub-Saharan Africa.

Further evidence of liver flares in HIV/HBV coinfected patients includes a cohort of 2,947 patients, 6.4% of whom were HBsAg seropositive, in which HBsAg had a HR of 6.0 (p=0.0001) for grade 4 liver events [200]. In another study of 755 patients starting HAART, HBsAg seropositivity had an OR of 3.2 (95% CI, 0.9 to 9.09, p=0.04) for severe hepatitis (defined as an increase in ALT or AST to >10x ULN or 5 times baseline if markedly abnormal) [201]. These studies did not report the proportion experiencing flare and so are not listed in Table 3.

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It is often difficult if not impossible to determine the cause of a flare on starting HAART, whether adverse drug reaction, HBV suppression or immune reconstitution, all of which have been proposed.

Adverse drug reaction

Some studies have analysed the relative probabilities of flares on different antiretroviral treatment regimes. Drug-related flares (ALT or AST >5x ULN) were examined by Sulkowski in HIV-positive patients starting a new antiretroviral therapy regimen including nucleoside analogues and/or protease inhibitors. RTV (given at treatment dose rather than the lower dose used to boost other protease inhibitors) was the only drug identified as increasing the risk of hepatotoxicity (relative risk 5.6, 95% CI 2.1 to 15.9) and, while chronic HBV or HCV infection was associated with an increased risk in those not prescribed RTV (relative risk 3.7; 95% CI, 1.0 to 11.8), only 2.7% of the patients investigated had HBV as opposed to 52% with HCV [178].

In another study Sulkowski examined patients exposed to non-nucleoside therapy, of whom 8% had HBV and 43% had HCV. A flare was seen in 16% prescribed NVP and 8% prescribed EFV and, in an adjusted analysis, chronic HBV or HCV infection was associated with an increased relative risk (2.1, 95% CI 1.1-3.9) [179].

In Thailand a pooled analysis of 8 randomised controlled trials found the relative risk of severe hepatotoxicity (ALT >5x ULN) of HBsAg was 3.2 (95% CI 1.1-9.0, p=0.003) and NNRTI therapy had a relative risk of 9.8 (95% CI, 3.0-31.5, p=0.0001) whereas there was no effect of protease inhibitor therapy, with or without RTV [185].

Drug interactions may further increase the risk of hepatotoxicity. In a retrospective cohort study of South African patients starting 3TC with AZT and efavirenz (EFV), treatment for tuberculosis (TB) increased the risk of a flare (>5x ULN) 8.5 fold [187].

Flares on suppression of HBV

It has been suggested that liver flares can arise as HBV DNA VL is declining and becoming suppressed. For example, in ACTG5127 HBV/HIV coinfected patients on stable HAART were randomised to receive either TDF or ADV and rates of ALT rise to >5x ULN were 11% with TDF and 12% with ADV [141].

Immune reconstitution disease

In the Tenofovir in Coinfection trial (TICO), flares (defined as ALT >5x ULN, or a rise of >100 IU/L if elevated at baseline) occurred in 25% of patients after a median of 8 weeks on treatment. 11% of flares resulted in death [114]. Further immunological analysis suggests an immune reconstitution response to a high antigenic burden

(correlated with HBV DNA) as the cause of early on-treatment flares in coinfected patients [202].

Also supporting the immune theory of early on-treatment flares is the fact that flares have been found to be more common in patients who start therapy with a lower CD4 count [187] and have been seen in association with rapid rise in CD4 on starting HAART [115].

Treatment breakthrough/interruption

Flares have been described in HIV-negative patients with HBV, for example Honkoop described a patient (HIV status not mentioned but presumably negative) who had been treated with 3TC for six months after which HBV DNA was undetectable. On stopping 3TC he had a flare with a rise in HBV DNA and a dramatic rise in ALT to 100 times the ULN. ALT resolved with HBeAg to anti-HBe seroconversion and a fall in HBV DNA [203]. Lim described three cases of patients with fatal flares on treatment cessation [204]. In one retrospective cohort study, 10% experienced flares (defined as ALT >10x ULN) on NRTI treatment. Three quarters of these flares were associated with development of resistance and occurred after a median of 18 months on treatment and only in patients taking 3TC or ADV. 10% of flares resulted in the patient's death. Treatment discontinuation flares occurred in 8% after a median of 15 weeks and were followed by HBeAg loss in some patients. Unlike on treatment flares, no discontinuation flares were fatal [205].

In coinfected patients, Bessesen described 5 cases in which flares arose, 2 (1 fatal) upon 3TC cessation and 3 upon development of resistance at codons 180 and 204 (528 and 552 using the older nomenclature) while on 3TC as the only HBV-active drug [206]. A patient with a flare associated with a rise in HBV VL on treatment was also described in the CAESAR study [167].

The STACCATO trial was designed to investigate CD4-guided structured treatment interruptions in a Thai population. Patients were treated with TDF plus FTC. There were 8 HBV/HIV coinfected patients who had a treatment interruption and 1 (12.5%) of these had a flare, which resolved with reintroduction of treatment [146].

A retrospective analysis of coinfected patients in the Swiss HIV Cohort Study found liver enzyme elevations in 42 (29%) of 147 stopping 3TC, however only 5% were grade 3 or 4 (>5x ULN). 1 (0.7%) resulted in death [116]. The time to maximum ALT was up to 133 days after treatment interruption. Grade 4 flares were most common around 40 days, but occurred up to 76 days, after treatment interruption

A higher rate was found in a Dutch retrospective cohort study; 22% of HBsAg seropositive patients stopping 3TC experienced a grade 4 liver enzyme elevation (ALT and/or AST >10x ULN and 100 IU/L higher than baseline) within 16 weeks, though none died [192].

Seroconversion

Flares may also be associated with HBeAg loss, anti-HBe seroconversion and/or HBsAg loss [114, 205].

The function of HBeAg is unknown but it has been suggested that it is immunosuppressive through favouring tolerance of HBV, either by acting in the thymus or by inducing apoptosis (reviewed in Milich [207]). Levels of HBeAg decline on initiation of nucleoside treatment and may become undetectable after 8 to 24 weeks [208]. Flares may arise as immunotolerance induced by HBeAg declines and the immune system better recognises infected hepatocytes, even without anti-HBe production.

1.8 Liver status determination

1.8.1 Alanine aminotransferase

Alanine aminotransferase (ALT) is an enzyme that catalyses the reaction shown in Figure 5.

Figure 5: Reaction catalysed by alanine transaminase

glutamate   +   pyruvate   ⇌   α-ketoglutarate   +   alanine
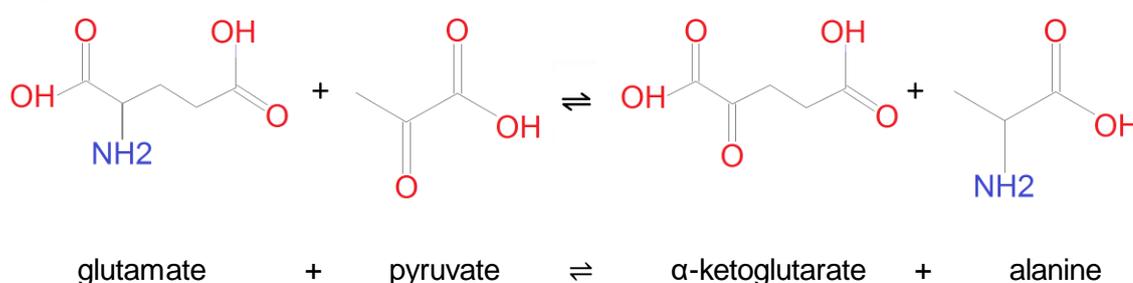
It is mainly found in hepatocytes where the concentration is around 3,000 times higher than in serum. On damage to hepatocytes, ALT is released into the blood and as such it is a marker of liver inflammation. Common causes of raised ALT include hepatotoxic drugs, alcohol, non-alcoholic fatty liver disease and viral hepatitis with other causes including autoimmune hepatitis, haemochromatosis, Wilson's Disease and alpha-1-antitrypsin deficiency.

The ULN for ALT is traditionally based upon the local population and, since males generally have higher ALT than females, may take sex into account. However, although ALT also varies by age and body mass index (BMI), these are not taken into

consideration when setting normal ranges. The ULN may be determined using a statistical definition, being the upper 97.5[th] percentile of ALT in healthy subjects. This has generally resulted in the ULN being between 30 and 50 IU/L. Using the same statistical method applied to a low-risk blood donor population (who tested negative for HBsAg, anti-HCV, anti-HIV-1/2 and syphilis and without behavioural contraindication to donation) resulted in lower ULN of 19 IU/L for women and 30 IU/L for men [209] and these limits are recommended for use in HBV-infected individuals by some experts [210]. The ULN may also be defined by following individuals over time and calculating the risk of disease or death. One such study which followed 94,000 men for 8 years and used death certificates to allocate cause of death determined the ULN for men to be 30 IU/L and although the researchers were unable to reliably estimate a limit for women they believed it would be lower [211].

1.8.2 Fibrosis scoring

The progression of HBV-related liver disease involves an increase in fibrosis; isolated fibrotic areas around portal tracts become confluent (bridging) and then the architecture of the liver is replaced with fibrotic tissue. The normal blood flow from the hepatic portal vein through the venous sinuses is disrupted with shunting and reduction in the ability of the liver to remove toxins or to produce clotting factors. The evidence for increased severity of HBV-related fibrosis with HIV coinfection is mixed (section 1.4.2).

Liver biopsy is the gold standard method to determine the degree of liver fibrosis. However it has associated risks; mortality rates are around 0.01 to 0.1%, mostly from intraperitoneal bleeding [212, 213]. Biopsy may also be inaccurate due to sampling error. A biopsy should ideally be 30 mm long (after shrinkage due to formalin fixation), be taken with a needle of at least 1.6 mm gauge and contain at least 11 portal tracts [212]. However liver fibrosis does not occur uniformly and a biopsy of this size (volume about 35 mm^3) may sample only 1/50,000 of the liver and cirrhosis may be missed in 10 to 30% of cases [214]. Histological examination of biopsy specimens requires specialised training and laboratory facilities. Liver biopsy may not be appropriate in all patients in whom fibrosis estimation may be of use. In particular, contraindications to liver biopsy include extrahepatic biliary obstruction, bacterial cholangitis, cystic lesions in the liver and abnormal coagulation indices (raised prothrombin time (>6 seconds) or low platelets (<60,000/mm^3)) or recent antiplatelet drugs (e.g. aspirin).

Transient elastometry (TE, FibroScan®) is a non-invasive alternative to biopsy which measures liver stiffness, which increases with fibrosis. The Fibroscan probe delivers a physical impulse to the skin which is transmitted through the liver. The speed with which this impulse passes through tissues is proportional to the square root of the

stiffness and is measured by an ultrasound probe. It is quick and safe and measures a larger volume of liver (approximately 3 cm^3 with a standard "M" probe). However use of TE requires specialist training and the equipment required is expensive and not widely available.

The levels of some markers in blood have been shown to correlate with the degree of liver fibrosis. These can be categorised into direct and indirect markers of liver fibrosis on the basis of whether they reflect the metabolism of hepatic extracellular matrix (ECM) (Table 4).

Table 4: Direct and indirect serum fibrosis markers

Indirect	Liver function tests	ALT
		AST
		Alkaline phosphatase
		GGT
		Prothrombin time
		Total bilirubin
		Albumin
	Other biochemistry	Triglycerides
		Glucose
	Acute phase protein	Globulins
		α_2-macroglobulin
		Haptoglobin
		Apolipoprotein A1
	Blood cell count	Platelets
		Monocytes
		Segmented neutrophils
	Immunological	Chemokine (C-C motif) ligand 5 (CCL5)

Direct	ECM component	Hyaluronic acid
		Procollagen III N-terminal propeptide (PIIINP)
		Procollagen I
		Collagen IV
		Laminin
	ECM enzymes	Chitinase-3-like protein 1 (YKL-40)
		Matrix metalloproteinases (MMP)
		Tissue inhibitors of metalloproteinases (TIMP)
	Growth factors	Hepatocyte growth factor
		Connective tissue growth factor

GGT: Gamma-glutamyl transferase

The performance of a test for fibrosis (or any other binary outcome) can be assessed using the receiver operating curve (ROC) from which the area under the curve (AUROC) can be derived. Values of the AUROC range from 0 to 1, with higher values indicating better discrimination. While there is no clear cut-off for an AUROC that indicates a good or bad test, it has been suggested that values greater than 0.75 are clinically useful [215].

Many markers have been assessed as potentially useful measures of fibrosis, either alone or in combination with other markers and/or other variables such as age, sex,

BMI, spleen size and alcohol intake. Validation has taken place using either liver biopsy or TE and in a range of clinical conditions.

Validation of serum fibrosis scores in hepatitis B and HIV infection

HBV/HIV coinfection

Only two studies have been published assessing serum fibrosis scores in patients with HBV/HIV coinfection [216, 217]. In the first study, which included 108 patients, liver biopsy was used to validate the ability of 11 fibrosis scores to discriminate between the presence and absence of significant fibrosis (at least F2), severe fibrosis (at least F3) and cirrhosis (F4) [216]. AUROCs are given in Table 5.

Table 5: Performance of 11 serum fibrosis scores in HBV/HIV coinfected patients

	F0-1 *vs* F2-4	F0-2 *vs* F3-4	F0-3 *vs* F4
	AUROC	**AUROC**	**AUROC**
AST/ALT Ratio	0.48	0.52	0.51
FIB-4	0.74	0.77	0.8
APRI	0.73	0.76	0.76
Hui	0.67	0.67	0.67
Fibrotest®	0.77	0.8	0.87
Fibrometer®	0.74	0.83	0.89
Hepascore	0.74	0.83	0.92
Zeng	0.75	0.78	0.91
Forns	0.72	0.77	0.81
Hyaluronic Acid	0.66	0.72	0.85
SHASTA	0.65	0.68	0.75

FIB-4: fibrosis-4 index. APRI: AST to platelet ratio index. SHASTA: serum hyaluronic acid, AST and albumin.
Shading:　light grey – can be determined in Ugandan participants in DART;
　　　　　　dark grey – cannot be determined in DART participants.

The second study compared Fibrometer® score with biopsy. Fibrometer® generally performed well though it failed to detect cases of progression from stage 2 to stage 3-4 fibrosis [217].

HBV monoinfection

In patients with HBV monoinfection there have been many assessments of over 90 markers or combinations of serum markers using liver biopsy, TE or both. However only 8 validated markers are calculable from the data available from all DART participants and a further 4 from data from participants in Uganda (since AST was measured in Uganda but not in Zimbabwe) (Table 6).

Table 6: Fibrosis markers that can be derived in DART participants

All participants	Ugandan participants
Age	AST
Platelets or 1/platelets	APRI
API	AST/ALT ratio
ALT	FIB-4
Bilirubin	
Direct bilirubin	
Total bile acid	
White blood cell count	

API: Age and Platelets Index.

AUROCs published for some markers can vary widely from one study to another, for example the AUROC for platelets varies from 0.32 [218] to 0.86 [219].

HIV monoinfection

One small study recruited 24 HIV-positive patients with ALT >50 IU/L, and without HBV or HCV infection. Participants underwent TE and had APRI and FIB-4 calculated. Enrolled patients did not have advanced liver disease; no patients were found to have significant fibrosis on APRI or FIB-4 and out of 24, 21 had "mild fibrosis" and 3 "progressive fibrosis" on TE. "Moderate concordance" was found between elastometry and the serum fibrosis markers. No AUROCs were given but data published allows their calculation, although with wide confidence intervals. For APRI the AUROC for distinguishing progressive from mild fibrosis was 0.67 (95% CI 0.29 to 1.00) and for FIB-4 it was 0.76 (0.42 to 1.00) [220].

It has been shown that FIB-4 and APRI were correlated with moderate concordance (weighted kappa coefficient = 0.573) in HIV-positive patients in the Italian Standardized Management of Retroviral HIV Infection cohort and these were used as markers of liver fibrosis [221].

It has been suggested that Fibrotest (a marker using age, sex, bilirubin, GGT, haptoglobin, α_2-macroglobulin and apolipoprotein A1 [222]) is useful in predicting fibrosis in conjunction with TE [223] but data was not given on how well Fibrotest performed alone. Another study used FIB-4 as a marker for fibrosis in HIV-monoinfected and HIV/HCV coinfected women [224] and a third similarly used APRI in HIV-monoinfected patients [225].

FIB-4 has also been shown to correlate with clinical outcome in HIV-positive patients with or without viral hepatitis coinfection. In the ICONA cohort (Italian Cohort of Antiretroviral Naïve Patients) FIB-4 was strongly associated (relative hazard 4.48 per log(FIB-4) higher) with liver-related death [226].

1.9 Conclusion

HBV coinfection is an increasingly important factor in the management of HIV. Several issues remain to be clarified, in particular: the epidemiology of coinfection; the optimal treatment strategies in coinfected patients; and, the longer-term outcomes of treatment including development of resistance, morbidity and mortality.

This study is of key importance to the development of future treatment protocols for the clinical management of HBV/HIV coinfection in developing countries. The findings may have important implications for developing strategies for roll-out of HAART and for future antiviral resistance in HBV.

2 Hypothesis and aims

The primary hypothesis we set out to examine was:

> Hepatitis B viral replication is durably suppressed in individuals coinfected with HIV and treated with TDF.

To examine this hypothesis we:

1. performed a systematic review and meta-analysis of published data of HBV VL suppression in HIV coinfected individuals treated with TDF, and
2. examined HBV VL in participants treated with TDF in the DART study.

The DART study also provided an opportunity to consider other research questions relevant to the management of HBV/HIV coinfected individuals, including:

1. What is the prevalence of HBV infection in participants in the DART study?
2. Is HBV VL suppression more likely and/or more durable when treating HIV coinfected patients with TDF plus 3TC than when treating with 3TC as the only HBV-active drug?
3. What is the baseline liver status (inflammation and fibrosis) of participants in the DART study and how is it associated with HBV status and other characteristics such as age and sex?
4. How does treatment with ART affect a marker of liver inflammation (ALT) and what is the rate of liver inflammatory flares:
   a. on first-line HAART,
   b. on stopping HBV-active treatment, and
   c. during cycles of Structured Treatment Interruption of ART?
5. Is there an association between HBV status at treatment initiation and CD4 cell count at baseline and/or over time?
6. Is HBV status associated with clinical progression to a new WHO stage 4 event and/or death?
7. Do the causes of death differ by HBV status, with particular focus on liver disease related deaths?
8. Is there evidence that monitoring or treatment should depend upon a patient's HBV status, and thus that HBV testing should be performed?
9. Is there evidence that testing patients for ALT would alter a patient's management, and thus that ALT testing should be performed?

3 Systematic review and meta-analysis of HBV suppression during treatment with tenofovir

3.1 Introduction

As discussed in chapter 1 hepatitis B coinfection is common in HIV-infected individuals and liver diseases including HBV infection represent a major cause of morbidity and mortality [62]. There is evidence that suppression of HBV VL results in improved clinical prognosis [151].

TDF received approval for the treatment of HIV infection from the United States Food and Drug Administration (FDA) in October 2001 and from the European Medicines Agency in February 2002. (FDA approval for the treatment of CHB infection was granted in August 2008.) The first reports of the use of TDF in treating HBV infection were presented in 2002. Guidelines now recommend TDF in combination with 3TC or FTC as first-line therapy for patients with HIV/HBV coinfection [131, 132]. Many studies have reported on the effect of TDF, either with or without 3TC or FTC, in treatment-naïve or experienced patients, however many studies are small and with relatively short follow-up.

It is uncertain what proportion of patients achieves suppression of HBV DNA (viral load, VL) and whether those who do not initially suppress may achieve HBV suppression later. It is also unclear to what extent, if at all, those with complete suppression may relapse despite continued treatment, e.g. in case of development of resistance mutations. Finally, it remains uncertain whether sequential treatment, for example with 3TC initially and TDF later, compromises the chance of successful treatment with TDF.

A recent meta-analysis of randomised controlled trials of antiviral treatment for HBV excluded patients with HIV coinfection and only compared responses at 12 months [227]. Outcomes included both virological and biochemical responses, HBeAg loss or seroconversion to anti-HBe, serum HBsAg loss, histological improvement and serious adverse events. However only one of the studies included in that meta-analysis included patients treated with TDF [228].

We carried out a complementary meta-analysis of data from patients coinfected with HIV to answer the following questions:

i. what proportion of patients achieve HBV VL suppression on TDF?
ii. does the rate of suppression differ in those with prior 3TC experience?

iii.   does the rate of suppression differ in those treated with TDF-3TC/TDF-FTC combination therapy compared with TDF monotherapy?

iv.   how common is HBV rebound on TDF?

We were also able to use patient-level data to further examine loss from follow-up.

3.2 Methods

The systematic review was carried out following the guidance laid out in the PRISMA statement [229].

3.2.1 Search strategy and selection criteria

Studies included were those that described HBV/HIV coinfected individuals treated with TDF with or without 3TC and/or FTC for a period of at least one year and that reported quantitative results of plasma HBV VL at yearly intervals (at a minimum) while on TDF treatment. Studies included could be randomised controlled trials or prospective or retrospective cohort studies. Patients with undetectable plasma HBV VL at baseline were excluded since their inclusion gives a falsely high estimate of the effect of treatment. Baseline HBV VL data was not given for 20 patients in three studies (see Table 7). The analysis was restricted to patients on TDF treatment, with or without 3TC and/or FTC. In this analysis inclusion bias could be considerable if patients who failed to suppress either stopped taking TDF or had progressive liver disease and so dropped out. This would leave a higher proportion of patients with a good response, overestimating the treatment effect. Further analysis of individual patient data was carried out where this was available or was provided in the process of performing the current analysis (Table 7).

Web of Science, Embase and Medline were searched, including all years. Conference abstracts from The Liver Meeting (American Association for the Study of Liver Diseases), The International Liver Congress (European Association for the Study of the Liver) and the Conference on Retroviruses and Opportunistic Infections were searched for the years 2002-2010.

To search databases, a combination of key terms was used including "hepatitis", "HIV", and "tenofovir", limited to articles with human subjects and written in English (Appendix 1). Conference abstracts were searched online or by hand. Other publications that were discovered from the reference lists in publications reviewed were also included.

3.2.2 Data collection

Studies were screened initially by title and then data was collected from the full article of all published studies and from conference posters, or conference abstracts if posters were not available. Some studies which met the eligibility criteria did not include data on the number with undetectable HBV VL at one year, or information on prior or concomitant drug exposure. The authors of these studies were contacted by email and asked to provide additional data. Additional, unpublished data was obtained from the authors of 11 of the 23 sources included (Table 7). Data abstracted consisted of type of

study, source of study funding, number of HBV/HIV coinfected participants, number HBeAg positive at study entry, prior 3TC/FTC exposure, drug regimens used during study period, length of follow-up, type of HBV VL test used and lower limit of detection, numbers tested for HBV VL at yearly intervals, and numbers with undetectable HBV viral load at yearly intervals. To maximise power and in the absence of any evidence suggesting a difference in effect on HBV between 3TC and FTC, exposure to these two were grouped together. Patient-level data was available from some studies and this was used to analyse loss from follow-up.

Results were stratified by treatment into four groups. Group A consisted of patients who had no prior exposure to 3TC/FTC and who were treated with TDF without concomitant 3TC/FTC, Group B those without prior exposure to 3TC/FTC treated with TDF in combination with 3TC/FTC, Group C those with prior exposure to 3TC/FTC but treated with TDF without 3TC/FTC, and Group D those with prior exposure to 3TC/FTC treated with TDF in combination with 3TC/FTC.

3.2.3 Statistical analysis

Statistical analysis was carried out using Stata version 10.1. The main outcome measure used was the proportion of patients tested who had a HBV VL below the limit of detection at each of any available yearly time intervals. 95% confidence intervals for these proportions were calculated for each time point in each study and for the aggregate results.

To detect potential sources of bias, assay cut-off was plotted against proportion suppressed at one year. Publication bias was examined using funnel plots. Bias from patients being lost to follow-up was assessed using patient-level data in those from whom it was available.

Multilevel mixed effects logistic regression (XTMELOGIT command) was used to assess the effect of prior exposure to, and combination treatment with 3TC/FTC on the probability of viral suppression, with individual studies fitted as a random effect to account for clustering (Appendix 2). This implicitly weights each study by the amount of information it contains. Since there was no association of assay cut-off with rate of suppression the model was not adjusted for cut-off. Between-study heterogeneity was assessed by a likelihood-ratio test comparing the mixed effects model with a standard logistic regression model which did not include a factor for study. Models were re-run with an interaction term between concomitant 3TC/FTC and prior exposure to 3TC/FTC. Sensitivity analyses were performed (1) including only larger studies (reporting at least 10 patients), (2) excluding one study that was an outlier on the funnel plot [230], and (3) with a term for study design.

3.3 Results

The initial searches produced 2,110 references which, after duplicates were removed, gave 1,607 publications. Publications were then screened by title and if necessary by abstract to remove those clearly not meeting the eligibility criteria. This left 379 published articles. The full text of articles and posters was then checked for eligibility (or abstracts if the full article or poster was not available). 356 were removed as ineligible (as described in Figure 6) and 23 included in the analysis. Study characteristics are given in Table 7. Those studies for which authors were contacted and published data augmented by additional information are so labelled in Table 7.

Figure 6: Systematic review – summary of study search and inclusion

Initial search
2,110 publications identified

→ 503 duplicates removed

Screened
1,607 publications

→ 1,228 excluded on basis of title and abstract

Assessed for eligibility
379 publications
300 published articles
78 conference presentations
1 book

→ 356 removed
282 published articles
73 conference presentations
1 book

Reasons for exclusion:
115 reviews/comments
58 no/inadequate HBV viral load data
50 not tenofovir
30 no data at yearly time-point(s)
21 cross-sectional studies
20 data included elsewhere
17 case reports
17 HIV-negative
15 inclusion criteria
9 unable to separate into exposure/treatment groups
4 *in vitro* only

1 conference presentation replaced with recently published article

Included in review and analysis:
19 published articles
4 conference presentations

Table 7: Meta-analysis – characteristics of included studies

Author	Pub. year	Country	Study design	N included in meta-analysis	HBeAg positive	Baseline HBV VL test	Level of detection [a] IU/mL	Duration of follow-up	Add. data	Funding
Avihingsanon [231]	2010	Thailand	RCT	10	6/10	Yes	34	48 W	No	Ph[1]
Bani-Sadr [232]	2004	France	Prospective cohort	6	3/6	Yes	40	96 W	Yes [c]	NS
Butt [233]	2006	USA	Retrospective cohort	5	Unknown	Yes	20	36 M	No	Public[d]
de Vries-Sluijs [230]	2010	Netherlands	Prospective cohort	78	67/82	Yes	20	10-84 M	Yes [c]	Ph[1]
Dore [144]	2004	International	RCT	5	4/5	Yes	200	48 W	No	Ph[1, e]
Engell [234]	2011	USA	Retrospective cohort	24	18/31	Yes	6	24 M	No	Public[f]
Gutiérrez [156]	2008	Canada	Retrospective cohort	6	Unknown	Yes	Not given	15-45 M	No [c]	NS
Jain [235]	2007	USA	Retrospective cohort	28	27/28	Yes	400	12-24 M	Yes	NS
Kosi [162]	2012	Austria	Retrospective cohort	49	35/49	Yes	20	2-171 M	Yes [c]	NS
Kuzushita [236]	2010	Japan	Prospective cohort	16	15/16	Yes	60	6-63 M	Yes [c]	NS
Lee [237]	2009	USA	Retrospective cohort	17	34/43	7/17	[b] 100 - 200	12-63 M	Yes [c]	Public[g]
Marcelin [238]	2003	France	Retrospective cohort	10	9/10	Yes	40	12 M	No	NS
Matthews [114]	2008	Thailand	RCT	22	13/22	Yes	34	48 W	No	Ph[1]
Nelson [148]	2006	UK	RCT	39	Unknown	Yes	80	48 W	No	NS
Nüesch [146]	2008	Thailand	RCT	5	2/5	Yes	400	48-96 W	Yes [c]	Ph[2], Public[h]
Peters [141]	2006	USA	RCT	18	23/27	Yes	40	48 W	Yes	Public[i]
Quiros-Roldan [239]	2008	Italy	Retrospective cohort	10	5/10	Yes	400	63-258 W	No [c]	Public[j]
Rodriguez [240]	2010	USA	Prospective cohort	6	6/6	Yes	25	48 W	No	Ph[3]
Schmutz [139]	2006	Europe	Prospective cohort	75	75/75	Yes	200	26-206 W	Yes	NS
Stephan [241]	2005	Germany	Retrospective cohort	23	19/31	Yes	400	48 W	Yes	NS
Tan [242]	2009	UK	Retrospective cohort	39	39/39	38/39	[b] 100 - 2,000	69-290 W	Yes	None
Tuma [243]	2008	Spain	Retrospective cohort	38	Unknown	29/38	10	48 W	No	NS
van Bommel [244]	2004	Germany	Prospective cohort	21	21/21	Yes	80	72-130 W	No	Public[k]

Footnotes and abbreviations: see next page.

Table 4: Meta-analysis – characteristics of included studies – abbreviations

Pub.: publication. Add.: additional. RCT: randomised controlled trial. W: weeks. M: months. NS: not stated. Ph: pharmaceutical industry.
Pharma funding: 1: Gilead Sciences. 2: Roche. 3: GlaxoSmithKline.

a   Copies/mL converted to IU/mL by dividing by 5.
b   The limit of detection of the HBV VL assays used fell during the course of follow-up in two studies.
c   Individual patient data available.
d   National Institutes of Health/National Institute on Drug Abuse.
e   Commonwealth Department of Health and Ageing (Canberra, Australia).
f   supported in part by National Institute of Allergy and Infectious Diseases.
g   Medical Student Summer Research Training Program, supported through grants from the National Institutes of Health; Wake Forest University School of Medicine Departments, Centers, and Institutes; and private gifts.
h   Swiss National Science Foundation through the Swiss HIV Cohort Study, the Wilsdorf, Sidaide, and de Brocard Foundations, Geneva, from the Departments of Social Affairs and Economics, Geneva.
i   In part by the Adult AIDS Clinical Trials Group (ACTG) funded by the National Institute of Allergy and Infectious Diseases; virology support funding by the NIH/NIAID and the Adult ACTG Central Group; the Birmingham VA Medical Center, UAB CFAR core clinic and laboratory facilities; and NIDDK UCSF Liver Center.
j   Italian Ministry of University.
k   In part by the German BMBF Network of Competence for Viral Hepatitis (Hep Net).

Some studies included patients in more than one treatment group (for example both patients with and without prior exposure to 3TC), giving 43 study arms (Table 8).

Table 8: Meta-analysis – results included

Group	Year Author	1 S / N	2 S / N	3 S / N	4 S / N	5 S / N	6 S / N	7 S / N
A	Nelson	3 / 10						
A	Matthews	9 / 12						
A	Kosi	8 / 9	9 / 11					
A	Tan	0 / 1	1 / 1	1 / 1				
B	Dore	4 / 5						
B	Bani-Sadr	5 / 6	6 / 6					
B	Stephan	4 / 6						
B	Nelson	2 / 6						
B	Schmutz	15 / 24	15 / 17	12 / 13	4 / 5			
B	Jain	7 / 9						
B	Matthews	7 / 10						
B	Nüesch	5 / 5	2 / 2					
B	Tuma	9 / 9						
B	Kosi	8 / 12	11 / 14					
B	Lee	2 / 8	2 / 4	5 / 6	2 / 2	1 / 1		
B	Tan	3 / 6	4 / 6	2 / 4	4 / 4	3 / 3		
B	Kuzushita	9 / 14	12 / 13	8 / 8	5 / 5	5 / 5		
B	Avihingsanon	9 / 10						
B	de Vries-Sluijs	12 / 28	18 / 24	19 / 23	14 / 14	6 / 6	1 / 1	
B	Rodriguez	3 / 6						
B	Engell	6 / 10	5 / 5					
C	van Bommel	11 / 11						
C	Stephan	1 / 3						
C	Nelson	4 / 12						
C	Schmutz	27 / 48	38 / 40	30 / 32	9 / 9			
C	Lee			1 / 1				
C	Kosi	1 / 3	2 / 3					
C	Tan	0 / 2	2 / 2	1 / 1				
D	Marcelin	3 / 10						
D	van Bommel	10 / 10						
D	Stephan	8 / 14						
D	Nelson	6 / 11						
D	Peters	7 / 18						
D	Jain	10 / 19	1 / 2					
D	Gutiérrez	3 / 6	2 / 2	1 / 1				
D	Quiros-Roldan	7 / 10	8 / 9	7 / 7	5 / 5	1 / 1		
D	Tuma	22 / 29						
D	Lee	2 / 3	3 / 3	2 / 2				
D	Kosi	11 / 15	13 / 16					
D	Tan	14 / 20	12 / 15	10 / 14	7 / 8	7 / 9		
D	de Vries-Sluijs	14 / 50	34 / 49	38 / 47	33 / 38	21 / 23	8 / 8	1 / 1
D	Engell	3 / 11	4 / 13					
D	Butt	2 / 5	3 / 5	3 / 5				

S: number of HBV patients with viral suppression (below the level of detection)
N: number of patients with a HBV VL test performed
Groups: A no prior 3TC/FTC treated with TDF alone, B no prior 3TC/FTC treated with TDF and 3TC/FTC, C prior 3TC/FTC treated with TDF alone, D prior 3TC/FTC treated with TDF and 3TC/FTC.

Although data was included from six randomised controlled trials, allocation of TDF *vs*. TDF plus 3TC was randomised in only two [114, 148].

Studies used assays with widely varying cut-offs for the detection of HBV (Table 7). This could have introduced bias, with the use of more sensitive assays resulting in an apparent lower rate of suppression. However plotting the proportion undetectable against the logarithm of the cut-off value showed no clear pattern (Figure 7). The correlation coefficient between log of cut-off and proportion undetectable was 0.11. Thus the cut-off was ignored in further analyses.

Figure 7: Meta-analysis – log of HBV viral load assay cut-off against proportion undetectable at one year in each study arm
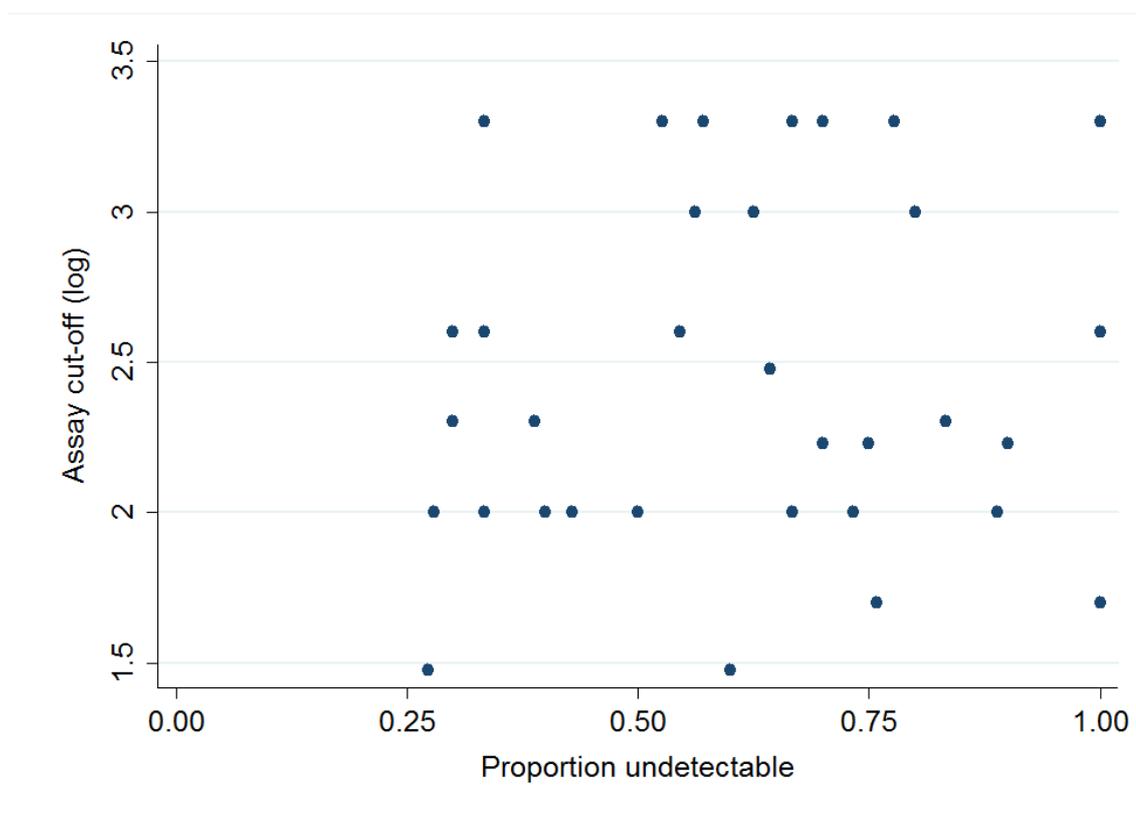
Note: Lee [237] and Tan [242] are not shown since the cut-off in these studies varied during follow-up.

The overall proportion suppressed was 57.4% (95% CI: 53.0 to 61.7%), 79.0% (95% CI: 73.6 to 83.8%), and 85.6% (95% CI: 79.2 to 90.7%) after one, two, and three years of treatment with TDF (Table 9 and Appendix 3).

Baseline HBeAg status could be determined from ten of the included studies [139, 146, 162, 230, 232, 236, 239, 240, 242, 244]. For HBeAg positive (n=251) and negative (n=38) patients respectively the proportion fully suppressed was 51.8%, 82.0%, 86.6% and 76.3%, 82.1%, 75.0% at one, two and three years (Figure 8).

Figure 8: Percentage with undetectable HBV viral load over time, by HBeAg status
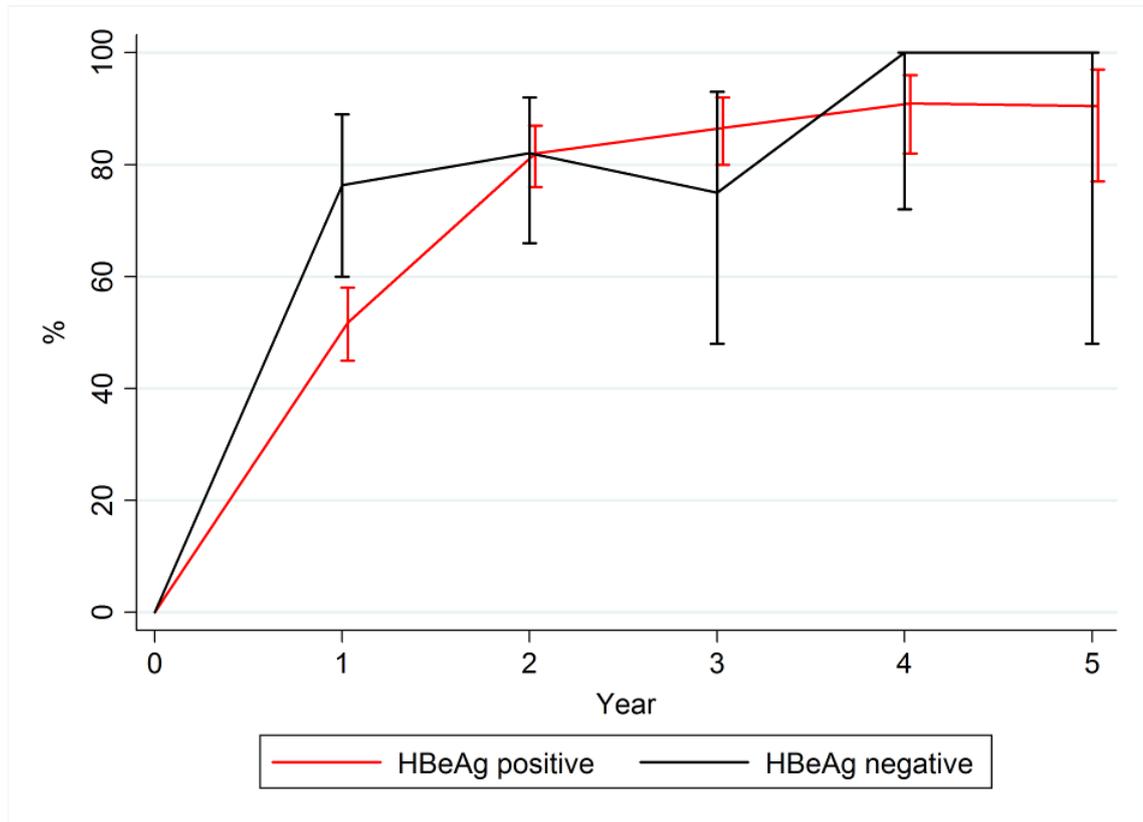
After one year of treatment, a higher proportion of HBeAg negative than HBeAg positive individuals had suppressed HBV VL (p=0.005). However, beyond one year the rates of suppression were not significantly different.

Table 10 shows the effects of prior and concomitant 3TC/FTC on virological suppression. Effects are given for all patients and also stratified by prior or concomitant treatment with 3TC/FTC as appropriate. Overall, at one year prior exposure to 3TC had an OR of 0.69 (95% CI: 0.45 to 1.08) and treatment with 3TC/FTC in addition to TDF of 1.24 (95% CI: 0.68 to 2.24), neither being statistically significant. The effect of prior exposure to 3TC/FTC was similar, but also not statistically significant, at each of one, two, and three years. The effect of concomitant treatment with 3TC/FTC favoured dual therapy at one year but TDF monotherapy at years two and three, but these effects were again not statistically significant. The OR in the stratified analyses were similar to the effects overall but with even wider confidence intervals. There was no evidence of an interaction between prior and concomitant 3TC/FTC treatment (p=0.98 at 1 year, p=0.14 at 2 years and p=0.99 at 3 years). Between-study heterogeneity, allowing for the effects of prior and concomitant 3TC/FTC treatment, was significant (p<0.01) at year 1 but not at year 2 (p=0.48) or at year 3 (p=1.0).

Table 9: Meta-analysis – suppression and effect of prior and current 3TC/FTC at yearly time points

	Number suppressed / number tested (% suppressed)									
	Group A		Group B		Group C		Group D		All	
Year	S / N	%	S / N	%	S / N	%	S / N	%	S / N	%
1	20 / 32	62.5	110 / 174	63.2	44 / 79	55.7	122 / 231	52.8	296 / 516	57.4
2	10 / 12	83.3	75 / 91	82.4	42 / 45	93.3	80 / 114	70.2	207 / 262	79.0
3	1 / 1	100	46 / 54	85.2	32 / 34	94.1	58 / 71	81.7	137 / 160	85.6

S: number of HBV VL test results showing viral suppression (below the level of detection)
N: number of patients with a HBV VL test performed
Groups: A no prior 3TC/FTC treated with TDF alone, B no prior 3TC/FTC treated with TDF and 3TC/FTC, C prior 3TC/FTC treated with TDF alone, D prior 3TC/FTC treated with TDF and 3TC/FTC.

Table 10: Multivariable logistic regression analysis of effects of prior and concomitant 3TC/FTC on virological suppression

	Effect of prior 3TC/FTC						Effect of concomitant 3TC/FTC					
	Monotherapy		Dual therapy		Overall		3TC/FTC naïve		Prior 3TC/FTC exp.		Overall	
Year	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
1	0.37	0.09 to 1.59	0.64	0.39 to 1.06	0.69	0.45 to 1.08	1.13	0.40 to 3.15	2.14	0.75 to 6.12	1.24	0.68 to 2.24
2	0.80	0.06 to 11.50	0.55	0.20 to 1.49	0.69	0.35 to 1.39	0.94	0.19 to 4.70	0.23	0.03 to 1.64	0.37	0.11 to 1.30
3	-	-	0.77	0.30 to 2.03	0.75	0.29 to 1.96	-	-	0.28	0.06 to 1.96	0.25	0.05 to 1.14

Monotherapy: patients treated with TDF without concomitant 3TC/FTC, i.e. groups A and C.
Dual therapy: patients treated with TDF with concomitant 3TC/FTC, i.e. groups B and D.
3TC/FTC naïve: patients not previously exposed to 3TC/FTC before TDF treatment, i.e. groups A and B.
Prior 3TC/FTC exp.: patients previously exposed to 3TC/FTC before TDF treatment, i.e. groups C and D.
The OR comparing groups A and C and comparing groups A and B in year 3 were non-estimable as there is only one patient in group A.

The proportion suppressed increased over time and reached 91.7% (44 of 48) at 5 years (Table 8). The number of patients in follow-up at each year declined, however of the 379 patients in studies with more than one year of follow-up, individual patient data was available for 187 (49.3%). The proportion suppressed may have increased in a biased fashion if patients failing to suppress dropped out and those that were suppressed continued on therapy. However it was more likely at every time point that a later HBV VL test result was available for patients with detectable HBV than for those in whom HBV VL was fully suppressed (non-significant – data not shown).

Virological rebound on TDF was rare, with no cases seen in 16 of 23 studies which included 374 of the 550 patients in the meta-analysis. Three studies reported a single patient with an increase in HBV VL on TDF treatment [114, 239, 242], three had two patients [233, 236, 240], and one had three [230] though in three of these studies the size of the increases were not reported, in two the increases were very small (0.1 to 0.3 log), and only two had patients with an increase of at least one log (one in each study) [233, 240]. Unfortunately no discussion of these two cases was given; in particular there were no data on treatment adherence.

The funnel plot (Figure 9) shows the standard error against the proportion undetectable at one year, with the vertical line marking the summary estimate of the treatment effect (derived using fixed-effect meta-analysis) [245]. The plot is symmetrical with no suggestion of publication bias. There is larger than expected heterogeneity in the larger studies (appearing higher up on the graph with a lower standard error), with one apparent outlier with a low proportion undetectable despite large size (de Vries-Sluijs [230], Group D). Separate funnel plots of each arm in the analysis also show no publication bias (Appendix 5). Repeating the regression analysis after excluding the outlier study arm and after excluding small studies (with less than ten patients) made no material difference to the results. The model including a term for study design showed that this variable had no significant impact on the results, with p values of 0.76, 0.54 and 0.42 at 1, 2 and 3 years in the overall analysis.

Figure 9: Meta-analysis – funnel plot of standard error against proportion undetectable at one year – all study arms (with pseudo 95% confidence limits)

Funnel plot with pseudo 95% confidence limits
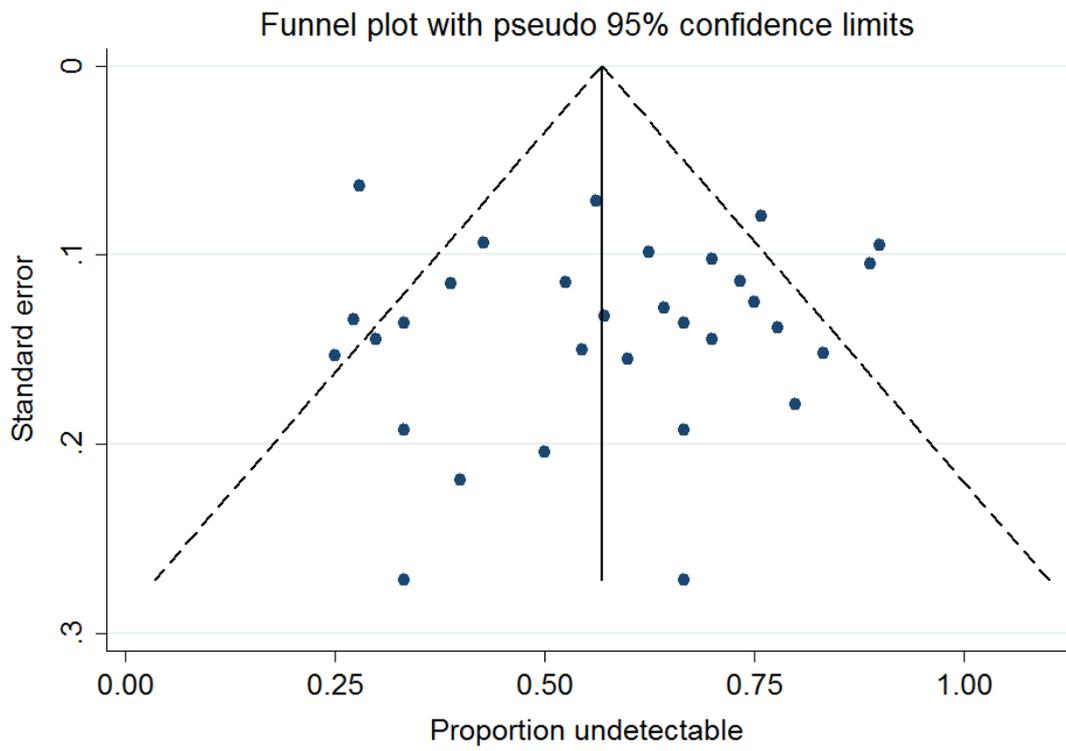
3.4 Discussion

This review of HBV/HIV coinfected patients treated with TDF demonstrates durable virological suppression of HBV replication to below the level of detection, with the proportion suppressed increasing over time, though with small numbers at later time points. Few patients experience virological failure on treatment.

However several reservations should be noted. Firstly most of the studies included were cohort studies in which patients who dropped out were not well characterised and so measurement of suppression over time could be biased. Secondly, we compared different treatment groups though allocation to these was randomised in only two studies [114, 148]. Thirdly there was little data beyond three years of treatment with the number of patients included in the meta-analysis declining rapidly over time.

The proportion with undetectable HBV at one year (59%) was lower than the proportion found in HIV-negative patients receiving TDF for treatment of HBV infection. For example, a multicentre cohort study found that, of 54 HIV-negative patients treated with TDF and FTC, 60% of whom were HBeAg positive, the probability of attaining an undetectable HBV VL was 76% at one year and 94% at two years [246]. Similarly, in a large randomised controlled trial comparing TDF with ADV, Marcellin found 93% of 250 HBeAg negative and 76% of 176 HBeAg positive patients randomised to TDF had an undetectable VL (<400 copies/mL) at 48 weeks (97% and 83% respectively of those still on TDF at 48 weeks) [228].

In the latter study, ten patients (2.3%) had virological breakthrough (defined in that study as detectable HBV after an undetectable result or an increase in HBV VL by a factor of 10 from nadir) [228]. Of the 550 patients in the current study, we identified 12 (2.4%) with a rise in HBV VL on TDF treatment (although at least five of these 12 had less than a one log rise from nadir) which is comparable. However other published data in coinfected patients have found far higher rates, for example 9 (17%) of 52 patients followed up for a median of 34 months in one retrospective cohort study (which was not included in the current meta-analysis as data on HBV VL suppression was only given at the end of follow-up and not at yearly time points) [247].

The high rate of virological suppression and low rate of breakthrough may be related to the low chance of developing TDF-resistance mutations. As described in chapter 1, in HBV/HIV coinfected patients treated with 3TC as the only drug active against HBV, resistance develops in about 90% after four years [166] whereas mutations associated with TDF resistance, such as the combination of rtL180M, rtM204V/I and rtA194T [171]

or N236T with A181V [248], have only rarely been seen and are of uncertain significance [172, 249, 250].

No statistically significant effect of prior 3TC/FTC exposure or of concomitant 3TC/FTC use was found. However the confidence intervals were wide and we could not exclude the possibility of moderately strong effects in either direction. In HIV-negative patients TDF monotherapy is as effective for HBV as combination therapy with TDF and 3TC/FTC with suppression rates (<400 copies/mL) of 81% at one year in both arms of an RCT using TDF alone or TDF/FTC combination therapy, and 88% and 85% respectively at three years [251, 252].

The main concern with sequential treatments that fail to fully suppress the VL is that resistance may develop and that cross-resistance could reduce the efficacy of subsequent drugs. TDF resistance is yet to be clearly demonstrated but it may be that the risk of cross-resistance is higher with drugs that are more similar to TDF in structure than 3TC/FTC. However HBV monoinfected patients failing to achieve virologic suppression with ADV have also been shown to respond well to TDF [253-255].

A second mechanism by which prior treatment exposure could reduce the apparent effectiveness of subsequent TDF is through introducing bias, in that patients failing one regimen for reasons other than lack of potency (such as poor adherence to therapy) may go on to fail other regimens but again, no such reduction in the effect of TDF in those with prior exposure to 3TC/FTC was found and so the effect of any such bias must be small.

As stated above, TDF received FDA approval in late 2001 and thus clinical experience to date is limited to just over one decade. Although this review includes data to a maximum of seven years, a lack of data limited the main regression analyses to three years. Patients with HIV require lifelong treatment and patients with HBV coinfection are likely to require the same. The possibility of safe discontinuation of HBV treatment may be limited to patients who clear HBsAg. However the probability of HBsAg loss is low with a rate of approximately 2.5% per year [256, 257] with the predicted median time to HBsAg seroclearance in HBeAg positive patients treated with TDF being 18 years (IQR 10 to 28 years) [258].

A limitation of this study is that it does not include analysis of the adverse effects of treatment. Future studies with longer follow-up duration will be required to determine the risk of treatment associated adverse effects, such as renal and bone toxicity, in patients exposed to TDF for many decades.

In conclusion, this meta-analysis shows that TDF suppresses HBV to undetectable levels in the majority of HBV/HIV coinfected patients, and with little virological rebound on treatment. Prior treatment with 3TC/FTC appears not to alter the efficacy of TDF treatment. Combination treatment with 3TC/FTC appears to offer no significant benefit over TDF alone.

4 Development of Antiretroviral Therapy (DART) Hepatitis Substudy

4.1 Summary of the DART study

The DART Study was an open, randomised, multi-centre trial in Africa, funded by MRC, DFID and the Rockefeller Foundation [259]. There were two main research questions, (1) is laboratory monitoring required when providing treatment for HIV and (2) can treatment be given intermittently, in order to reduce toxicity, but without reducing effectiveness?

To answer the first question there were two primary endpoints, (1) progression to a new WHO stage 4 event or death and (2) serious adverse events. This analysis was published in 2010 and found that HIV treatment can be safely given without monitoring bloods without an increase in serious or grade 3 or 4 adverse events, although from the second year of treatment such monitoring did provide a small but statistically significant reduction in disease progression to a new stage 4 event or death (Figure 10) [259].

Figure 10: Clinical disease progression and adverse events in the DART study
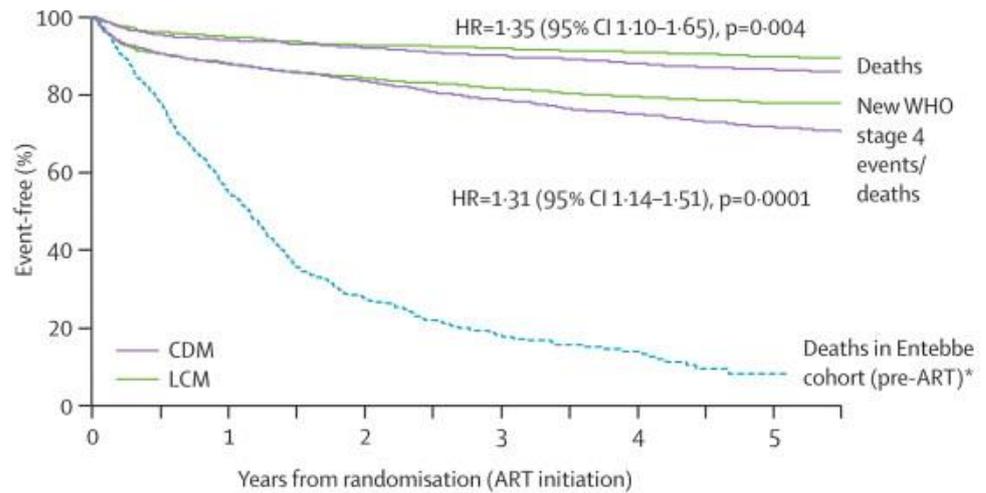
Number alive at follow-up (deaths in following interval)

LCM	1656 (82)	1552 (37)	1501 (14)	1468 (13)	1436 (16)	796 (2)
CDM	1660 (97)	1542 (32)	1494 (34)	1445 (29)	1395 (23)	749 (3)
Entebbe	516 (216)	245 (112)	108 (35)	58 (10)	21 (7)	3
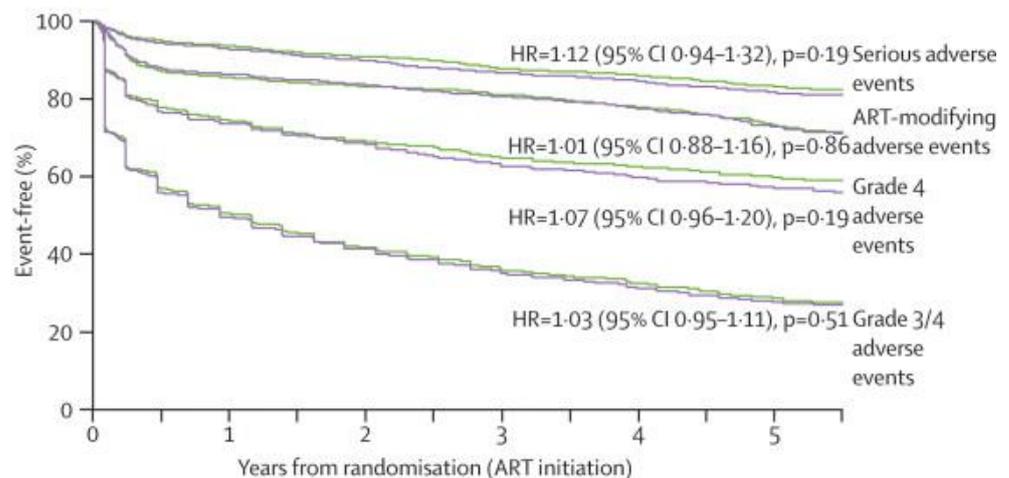
Mugyenyi P, Walker AS, Hakim J, Munderi P, Gibb DM, Kityo C, et al. Routine versus clinically driven laboratory monitoring of HIV antiretroviral therapy in Africa (DART): a randomised non-inferiority trial. Lancet. 2010;375(9709):123-31. [259]

The second question was addressed in a structured treatment interruption (STI) substudy in which 813 participants were randomised to either continuous treatment (CT) or fixed-duration treatment interruptions of 12 weeks on and 12 weeks off medication. Due to concerns with the safety of treatment interruption the STI substudy was stopped early in March 2006 [260].

The DART study is now complete, with few dropouts, archived blood samples and matching laboratory and clinical data. It provides a unique opportunity to answer some of the key questions regarding HBV/HIV coinfection.

4.1.1 Study Population

Participants were recruited at 2 sites in Uganda and 1 in Zimbabwe. These are the Joint Clinical Research Centre (JCRC), Kampala, the MRC/UVRI Uganda Research Unit on AIDS, Entebbe and the University of Zimbabwe Clinical Research Centre, Harare.

Inclusion criteria to DART were: age at least 18 years; CD4 <200 cells/mm^3; naïve to antiretroviral therapy except for exposure for the prevention of mother-to-child transmission. Exclusion criteria were: likely to be unable to attend follow-up; likely to have poor compliance; acute infection including intense-phase of TB treatment; malignancy requiring chemotherapy; laboratory test result indicative of contraindication to ART (including ALT >5x ULN); pregnancy; breastfeeding.

Patients (n=3,316) were randomised to care including laboratory monitoring of CD4 (LCM), or relying on clinically driven monitoring (CDM) in which arm laboratory results were not returned to clinicians unless they had been requested or the result showed evidence of a grade 4 abnormality.

4.1.2 Consent and ethical approval

Patients gave informed consent to participate in the main study and substudies. Ethics approval was obtained from bodies in Uganda, Zimbabwe and the United Kingdom.

4.1.3 Timing and follow-up

The first DART participant was randomised on 15th January 2003 and the last on 28th October 2004. Maximum follow-up was for 2,129 days (almost six years). The last day of follow-up was 31st December 2008.

4.1.4 Samples

Samples were taken and stored at baseline, 4 weeks, and every 3 to 6 months subsequently.

4.1.5 Antiretroviral treatment

Patients all received AZT and 3TC (Combivir) and either TDF (n=2,469), NVP (n=547) or ABC (n=300). Drug allocation was not randomised, except 1:1 to ABC or NVP in a sub-group of 600 Ugandan patients. Although other treatment allocations were not randomised, group baseline characteristics have been shown to be similar [259]. Approximately 10% switched to second-line HAART with switches made on the basis of clinical progression (new or recurrent WHO stage 4 event or stage 3 event if clinician so decided) or, in the LCM arm, if CD4 was confirmed lower than 100 cells/mm^3 on ART [259].

3TC and TDF have potent activity against hepatitis B [261, 262]. ABC has been shown to have weak activity against HBV [263]. The other antiretroviral drugs used in DART have no activity against HBV.

4.1.6 Data available

Laboratory test data available from DART includes CD4 and liver function tests (LFTs) on samples taken at the time-points described above.

Clinical events data was recorded monthly and is also available. Deaths were recorded by clinicians on a standardised form and reviewed by the members of the Endpoint Review Committee, who were blinded with respect to study arm.

4.1.7 Baseline characteristics

Baseline characteristics of the DART population are shown in Table 11. There were more female than male participants and this difference was more pronounced in the Ugandan sites. There were fewer participants under the age of 30 in Zimbabwe. WHO stage 4 disease was more common in JCRC than either Entebbe or Zimbabwe.

Table 11: Baseline characteristics of the DART population

	Entebbe N	%	JCRC N	%	Harare N	%	p
Total	1,020		1,297		999		
Sex							<0.001
Male	335	32.8	404	31.1	421	42.1	
Female	685	67.2	893	68.9	578	57.9	
Age							0.004
18-29	196	19.2	201	15.5	135	13.5	
30-34	251	24.6	309	23.8	236	23.6	
35-39	253	24.8	331	25.5	264	26.4	
40-44	163	16.0	253	19.5	192	19.2	
45-49	91	8.9	118	9.1	104	10.4	
50-	66	6.5	85	6.6	68	6.8	
CD4							0.09
0-49	330	32.4	465	35.9	314	31.4	
50-99	220	21.6	314	24.2	251	25.1	
100-149	242	23.7	282	21.7	235	23.5	
150-199	228	22.4	236	18.2	199	19.9	
Stage							<0.001
2	290	28.4	174	13.4	209	20.9	
3	532	52.2	731	56.4	601	60.2	
4	198	19.4	392	30.2	189	18.9	
Drug							<0.001
TDF	720	70.6	997	76.9	752	75.3	
ABC	149	14.6	151	11.6	0	0	
NVP	151	14.8	149	11.5	247	24.7	

TDF: tenofovir. ABC: Abacavir. NVP: Nevirapine.

4.2 Hepatitis serology testing

In the hepatitis substudy of DART, we aimed to examine the degree and durability of HBV viral suppression. We were also able to examine the epidemiology and clinical correlates of HBV coinfection and implications for testing and monitoring (see Hypothesis and aims – section 2).

We attempted to retrieve and test baseline samples from all participants for antibody to HCV (anti-HCV) and for HBV serological markers according to the algorithm below (Figure 11).

Figure 11: Algorithm for HBV serology testing
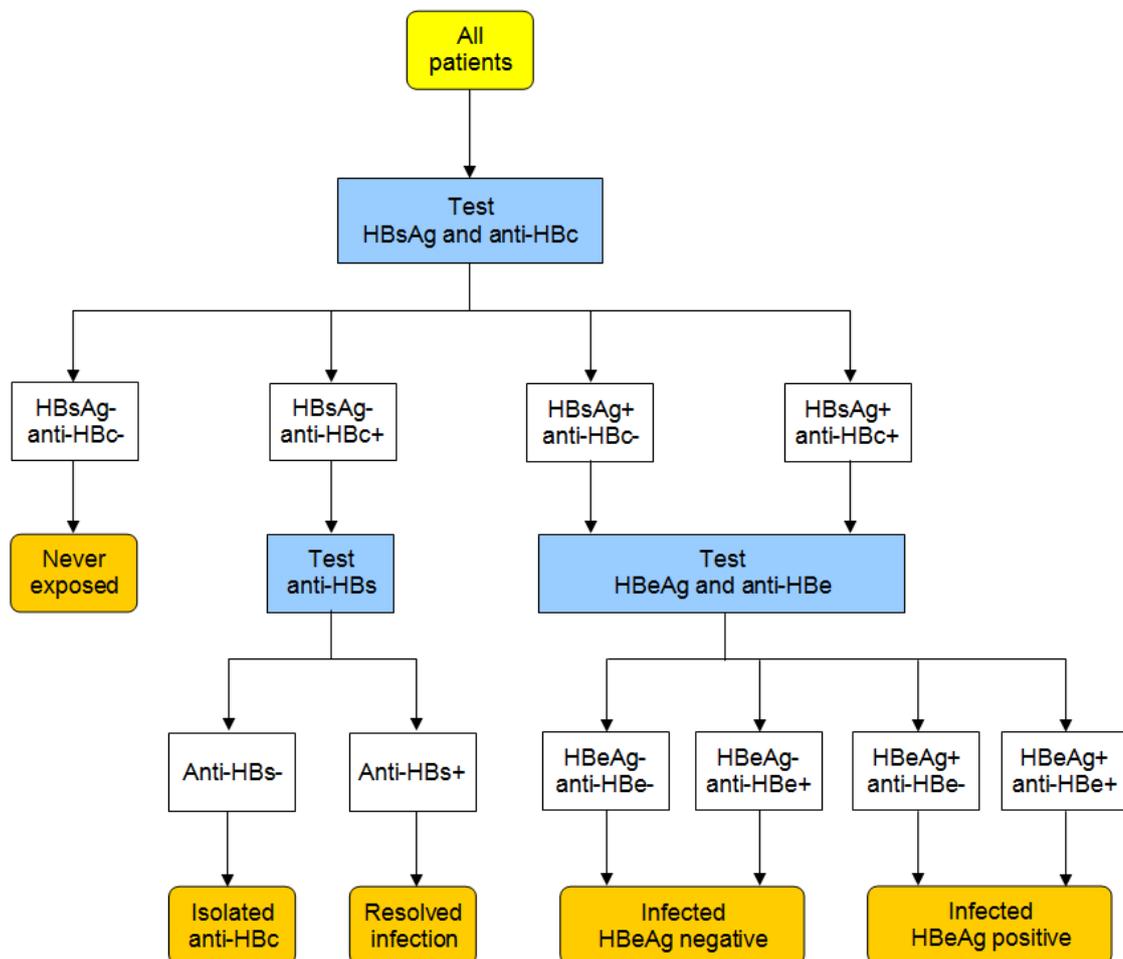
HBsAg: HBV surface antigen. anti-HBc: antibody to HBV core antigen. anti-HBs: antibody to HBV surface antigen. HBeAg: HBV "e" antigen. anti-HBe: antibody to HBV "e" antigen.

Baseline samples were initially tested for HBsAg and anti-HBc. Those that tested positive for anti-HBc but negative for HBsAg were then tested for anti-HBs and those that tested positive for HBsAg were tested for HBeAg, anti-HBe and HBV DNA.

Anti-HBc, HBsAg, anti-HBs and anti-HCV testing was done at all three sites. Anti-HBe and HBeAg testing was done at JCRC and Harare with samples from Entebbe sent to JCRC.

All assays used for HBV serology were commercial enzyme immunoassays and quality control used standardised controls supplied with test kits. HBsAg assays used were known to be unaffected by recognised HBsAg mutants.

For HBsAg, anti-HBs and anti-HBc Entebbe and Harare used Murex assays which are based on an enzyme immunoassay (EIA) method. JCRC used Roche Elecsys which is an electrochemoluminescence assay (ECLIA). All three sites followed a positive screening HBsAg test with a confirmatory neutralisation test (Appendix 5).

Testing for hepatitis B e markers used separate assays for HBeAg and anti-HBe. Harare used Murex EIA while JCRC used Roche Elecsys ECLIA.

For HCV a third generation EIA assay by Innotest was used in Uganda while Murex EIA was used in Zimbabwe.

HBsAg testing was performed on 37 participants during the follow-up period of DART. Of these, 3 (8.1%) had a positive result. No other hepatitis serology testing was undertaken. Of the 34 with a negative HBsAg result in DART, 32 had negative results when tested in the hepatitis substudy. Of the 3 with a positive result in DART, 2 were positive and 1 negative in the substudy. In all analyses the baseline result from testing undertaken as part of the substudy was used.

4.3 Hepatitis B DNA viral load testing

4.3.1 Viral load testing methods

Hepatitis B DNA viral load testing followed the algorithm below (Figure 12). All participants with positive HBsAg had hepatitis B DNA viral load testing at baseline and of the last sample taken before any change in HBV-active treatment, i.e. stopping or interrupting either 3TC or TDF for ≥30 days or starting TDF in a participant treated initially with NVP or ABC. Those participants who had HBV VL quantifiable at baseline had testing of samples taken at week 48 with the last sample only being tested if this was after 96 weeks. Participants at JCRC with quantifiable HBV VL at baseline had additional testing at weeks 4, 12 and 24.

Figure 12: Algorithm for HBV viral load (DNA) testing
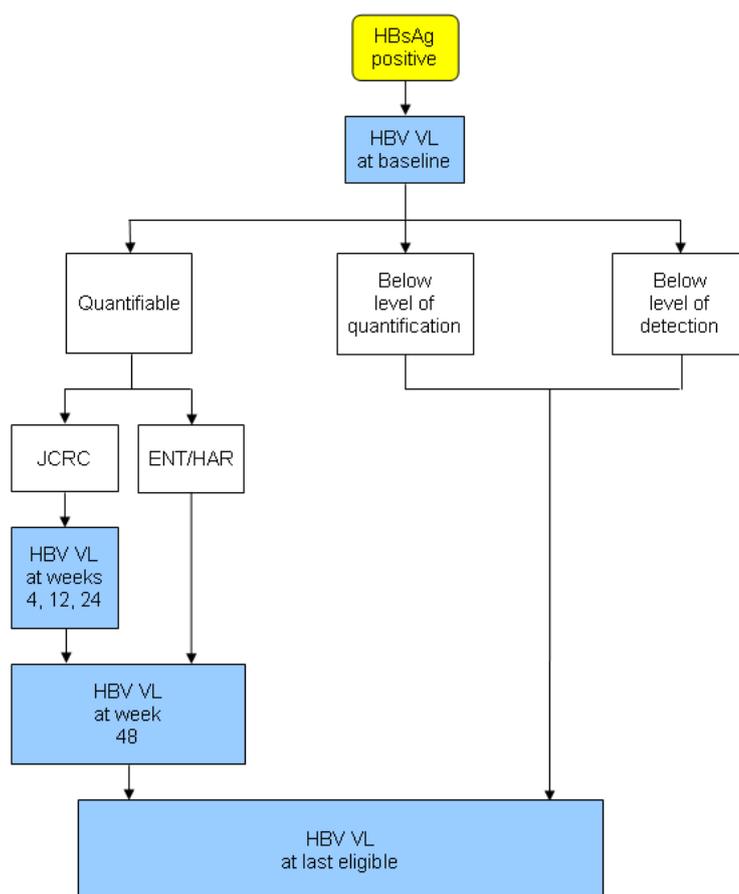
HBsAg: HBV surface antigen. VL: viral load. JCRC: Joint Clinical Research Centre, Kampala, Uganda. ENT: MRC/UVRI Uganda Research Unit on AIDS, Entebbe, Uganda. HAR: University of Zimbabwe Clinical Research Centre, Harare, Zimbabwe.

HBV DNA VL tests were performed at JCRC and Harare, with samples sent from Entebbe to JCRC for testing. JCRC used Roche Cobas Ampliprep/Cobas TaqMan while Harare used Abbott Realti*m*e HBV after manually preparing samples using the *m*Sample Preparation System$_{DNA}$.

The interpretation of these tests as used (in IU/mL) was as given below (Table 12).

Table 12: Interpretation of HBV DNA viral load test results

Test	Not detected	Below level of quantification IU/mL	Linear range IU/mL	Upper limit of quantification IU/mL
Roche TaqMan	Target not detected	<12	54.5 to 110×10^6	110×10^6
Abbott Realti*me*	Target not detected	<10	10 to $1,000 \times 10^6$	$1,000 \times 10^6$

Baseline samples at JCRC were diluted 1:4 giving a range of 48 to 440×10^6 IU/mL. IU can be converted to copies by multiplying by 3.41 (Abbott) or 5.82 (Roche). If HBV VL was reported as above the range of the assay the value used in analyses was the upper limit of the assay range.

Quality control of HBV DNA viral load testing was performed at JCRC and Harare using reference samples supplied by the United Kingdom National External Quality Assessment Service (UKNEQAS).

4.4 Statistical analysis

All analyses were performed using Stata version 12 (StataCorp LP, College Station, Texas, USA).

All logarithms used are to base 10.

Prevalence of variables was given using proportions and shown on histograms, stratified appropriately. Changes in variables over time were shown graphically with 95% confidence intervals (Stata ci command) also stratified appropriately (e.g. the change in ALT was shown stratified by HBsAg status and first-line drug regimen).

Medians were compared using the Kruskal-Wallis equality-of-populations rank test. Associations with categorical variables were examined using Chi squared or Fisher's exact test, as appropriate according to cell size. Chi-squared test for trend (Stata command ptrend) was used to examine associations with ordinal variables (age group, baseline CD4 cell count group and WHO stage of HIV disease). Unadjusted (univariable) and adjusted (multivariable) logistic regression were used to determine factors associated with binary outcome variables (e.g. prevalence of HBsAg) and unadjusted and adjusted linear regression to examine associations with numerical outcome variables, transformed as necessary to approximate normal distributions (e.g. the logarithm to base 10 of ALT). Multivariable models included all covariates of interest whether or not their effect was statistically significant i.e. no forward or backward selection approaches were used. This was vindicated by the large number of observations relative to the number of covariates, so that the number of degrees of freedom was not an issue. All variables included in models are shown in the results. Categorical variables included in multivariable models were examined with global p-values comparing across groups.

Concern that correlation between WHO stage and CD4 count at baseline could distort the conclusions of models was examined by comparing the standard errors (SE) of the coefficients of both factors in univariable and multivariable regressions of one variable, namely positive anti-HBc test result. As can be seen in Table 13, the standard errors were very similar and so it was concluded that collinearity was not a problem.

Table 13: Coefficients and standard errors of baseline WHO stage and CD4 count in logistic regression of anti-HBc

	Univariable		Multivariable	
	Coefficient	**SE**	**Coefficient**	**SE**
WHO Stage				
2				
3	-0.013	0.090	-0.000	0.091
4	-0.069	0.105	-0.043	0.108
Baseline CD4				
<50				
50-99	0.119	0.094	0.115	0.094
100-149	0.046	0.094	0.041	0.095
150-199	0.119	0.099	0.111	0.101

Correlation between variables, e.g. fibrosis markers, was examined using R, the Pearson product-moment correlation coefficient.

Incidence rates were used to compare the incidence of outcomes over time. Survival analyses used Kaplan Meier failure estimate curves shown graphically and associations were examined using unadjusted and adjusted Cox proportional hazards models.

A significance level of $p < 0.05$ has been used throughout.

5 Baseline serology and virology

5.1 Introduction

The epidemiology of hepatitis B has been reviewed in chapter 1. Estimates of prevalence have varied widely in both Uganda and Zimbabwe. In this analysis we characterise the baseline hepatitis B and hepatitis C status of the participants in DART.

Aims

The aims were to determine, at entry to the trial, the proportion of participants and factors associated with:

1. evidence of exposure to HBV,
2. current HBV infection and HBeAg and HBV DNA status,
3. evidence of exposure to HCV.

5.2 Methods

5.2.1 Statistical methods

The prevalence of anti-HBc, HBsAg and HBeAg, the percentage with evidence of exposure and the percentage of those exposed who had evidence of having cleared HBV were shown using histograms stratified by study site, sex and age and the distribution of HBV DNA results shown by HBeAg status.

To examine associations between categorical variables (study site, sex, anti-HBc and HBsAg results, HBV DNA detection, anti-HCV result) we used chi-squared tests (or Fisher's exact test as appropriate due to small numbers). We used the chi-squared test for trend (Stata command ptrend) to examine associations with ordinal variables (age, baseline CD4 cell count and WHO stage of HIV disease).

Unadjusted (univariable) and adjusted (multivariable) logistic regression were used to determine factors that may influence the prevalence of anti-HBc, HBsAg and HBeAg positivity.

All analyses were performed using Stata version 12 (StataCorp LP, College Station, Texas, USA).

5.2.2 Serology and viral load testing

Current HBV status was classified according to the results of anti-HBc, HBsAg and anti-HBs testing as shown in Table 14. Anti-HBs results were classified as positive if the assay result was greater than 10 mIU/mL.

Table 14: Combinations of baseline HBV serology test results used to classify infection status

	Anti-HBc	HBsAg	Anti-HBs
Not exposed	Negative	Negative	Neg/Pos/Not tested
Resolved infection	Positive	Negative	Positive
Isolated anti-HBc	Positive	Negative	Negative
Infected	Any	Positive	Any

Some participants without detectable anti-HBc and/or with detectable HBsAg were tested for anti-HBs although this was not required in the algorithm.

5.3 Results

5.3.1 Patients tested

All 3,316 participants had baseline samples stored and available for HBV testing but due to insufficient sample not all tests mandated by the algorithm were performed. 3,311 were tested for anti-HBc and 3,315 for HBsAg. 1,505 of 1,521 with a positive anti-HBc result and a negative HBsAg result had a test for anti-HBs. Of 308 participants with a positive HBsAg result, 280 (90.9%) had tests for HBeAg and anti-HBe and 270 (87.7%) were tested for HBV DNA VL. Anti-HCV tests were performed on samples from 3,253 participants.

5.3.2 Test results

The results of individual tests are summarised in Table 15. All combinations of serology results are shown in Appendix 7.

Table 15: Baseline viral hepatitis tests

		Positive	
Test	N	n	%
HBsAg	3,315	308	9.3
Anti-HBc	3,311	1,774	53.6
Anti-HBs	1,865	1,004	53.8
HBeAg	325	107	32.9
Anti-HBe	325	145	44.6
HBV DNA VL	270	214[a]	79.3
Anti-HCV	3,253	77	2.4

a: detectable

5.3.2.1 HBsAg and anti-HBc results

308 (9.3%) and 1,774 (53.6%) participants tested positive for HBsAg and anti-HBc, respectively.

Patients with a positive result for HBsAg would be expected to also have a positive result for anti-HBc (except in rare cases of very recent infection). However 54 (17.6%) out of 307 patients with detectable HBsAg that had a test for anti-HBc were found to be anti-HBc seronegative.

5.3.2.2 Anti-HBs results

Of 1,521 participants with results positive for anti-HBc but negative for HBsAg, 1,505 (98.9%) were tested for anti-HBs and 962 (63.9%) results were positive, consistent with a resolved infection and natural immunity.

In addition, 360 patients with results other than positive anti-HBc and negative HBsAg had anti-HBs testing. Of these, 308 tested negative for both HBsAg and anti-HBc or tested negative for one and the other had not been tested; of these, 28 (9.1%) results were anti-HBs positive. 52 patients had a positive result for HBsAg (anti-HBc results negative in 6 and positive in 46) and were tested for anti-HBs; 14 (27%) had a positive anti-HBs result.

5.3.2.3 HBeAg and anti-HBe results

325 (280 HBsAg seropositive and 45 HBsAg seronegative) participants were tested for HBeAg and anti-HBe; 107 (32.9%) were HBeAg seropositive and 145 (44.6%) were anti-HBe seropositive. 7 (2.2%) had positive results on both tests and 80 (24.6%) on neither. Of the 280 HBsAg seropositive participants tested for HBeAg, 103 (36.8%) were HBeAg seropositive and 127 (45.4%) were anti-HBe seropositive, 56 (20.0%) were negative and 6 (2.1%) were positive on both tests.

5.3.2.4 HBV DNA VL results

270 (87.7%) of 308 HBsAg seropositive participants were tested for plasma HBV DNA VL at baseline; 56 (20.7%) had undetectable DNA, 30 (11.1%) had DNA detectable but below the level of quantification (BLQ) and 184 (68.1%) had a quantifiable level of DNA. In those with a quantifiable result the median was 7.0×10^5 IU/mL (IQR 2.0×10^3 to 2.0×10^8).

5.3.2.5 Anti-HCV results

77 (2.4%) of 3,253 participants were anti-HCV positive.

5.3.3 Exposure and current status

Overall, 1,829 (55.2%) of the 3,316 participants had evidence of exposure to HBV (positive HBsAg and/or positive anti-HBc results).

All but 21 (0.6%) of the 3,316 participants could be categorised as described in methods (section 5.2.2). Of those that could be categorised, 1,482 (45.0%) had not been exposed to HBV, 962 (29.2%) had evidence of having cleared the infection as defined by presence of anti-HBc and anti-HBs but without HBsAg, 308 (9.3%) had detectable HBsAg and 543 (16.5%) had isolated anti-HBc (defined as having test results positive for anti-HBc and negative for both HBsAg and anti-HBs), which could have represented cleared or "occult" infection (Table 16).

Table 16: Baseline HBV status - categories

	Total	
Category	**n**	**%**
Not exposed	1,482	45.0
Resolved infection	962	29.2
Isolated anti-HBc	543	16.5
Infected	308	9.3
Total	3,295	100.0

5.3.4 Unusual serological patterns

5.3.4.1 Positive HBsAg and anti-HBs

14 participants had positive results for HBsAg and anti-HBs. All 13 tested for anti-HCV were anti-HCV negative. 2 were anti-HBc negative; of these 2, HBV DNA was below the level of quantification in one and not detected in the other. Of the 12 who were anti-HBc positive, 10 were tested for HBeAg and anti-HBe; one was positive for HBeAg and 5 for anti-HBe. 11 of the 12 were tested for HBV DNA – in 7 HBV DNA was not detected, in 2 it was below the level of quantification and in 2 HBV DNA was detectable, one at a level of 532 IU/mL and the other at 2.6×10^7 IU/mL.

5.3.4.2 Positive HBsAg with negative anti-HBc

54 participants had positive HBsAg (all confirmed by neutralisation) but negative anti-HBc. 6 of these were also tested for anti-HBs; 4 results were negative and 2 were positive. 48 had tests for HBeAg and anti-HBe; 29 (60%) were negative for both HBeAg and anti-HBe, 1 (2%) was positive for both, 15 (31%) were HBeAg positive/anti-HBe negative and 3 (6%) were HBeAg negative/anti-HBe positive. 47 were tested for HBV DNA; in 23 (49%) HBV DNA was not detected, in 7 (15%) HBV DNA was below the level of quantification and in 17 (36%) HBV DNA was detectable and quantifiable, ranging from 54 IU/mL to the upper limit of quantification (1×10^9 IU/mL).

5.3.5 Predictors of baseline status

5.3.5.1 Anti-HBc

The prevalence of positive anti-HBc was similar in the three sites (Entebbe 51.4%, JCRC 53.2% and Harare 56.3%; p=0.09).

Male sex was associated with a higher prevalence of anti-HBc overall (59.6% *vs.* 50.3%), in Harare (65.7% *vs.* 49.4%) and in JCRC (58.2% *vs.* 51.0%) but in Entebbe, while males again had a higher prevalence of anti-HBc, the difference was not statistically significant (53.7% *vs.* 50.3%).

Older age was associated with increasing prevalence of anti-HBc in all three sites.

The relationship between age and anti-HBc positivity appeared to differ according to sex and in the three sites (Figure 13). In a logistic regression model examining the effect of site, age and sex on anti-HBc status, while interaction terms between site and age and between sex and age were not significant (p=0.28 and p=0.93 respectively) the interaction term between site and sex was (p=0.01).

Figure 13: Anti-HBc seroprevalence at baseline by age, sex and study site
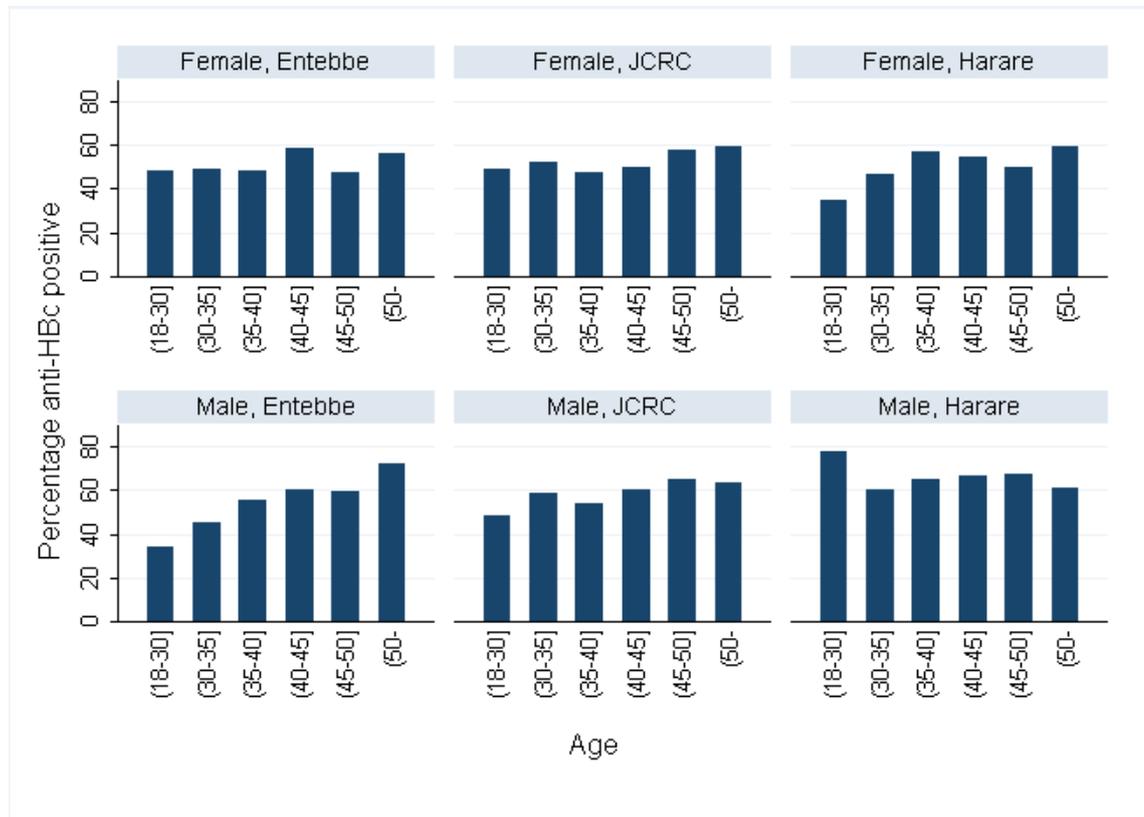
In multivariable logistic regression, anti-HBc positivity was higher in males and increased with age, but was not associated with site, stage, baseline CD4 or anti-HCV status (Table 17).

Table 17: Associations with anti-HBc status

	All	anti-HBc positive		OR	p	aOR	95% CI	p
	n	**n**	**%**					
All	3,311	1,774	53.6					
Site					0.09			0.26
Entebbe	1,019	524	51.4					
JCRC	1,297	690	53.2	1.07		1.08	0.91 to 1.27	
Harare	995	560	56.3	1.22		1.16	0.97 to 1.39	
Sex					<0.001			<0.001
Male	1,159	691	59.6					
Female	2,152	1,083	50.3	0.69		0.72	0.62 to 0.84	
Age group					<0.001			0.02
<30	531	249	46.9					
30-35	794	407	51.3	1.19		1.14	0.92 to 1.43	
35-40	848	454	53.5	1.30		1.21	0.97 to 1.50	
40-45	607	349	57.5	1.53		1.40	1.10 to 1.77	
45-50	312	180	57.7	1.54		1.39	1.04 to 1.85	
>50	219	135	61.6	1.82		1.63	1.17 to 2.26	
WHO Stage					0.54			0.96
2	673	364	54.1					
3	1,861	1,002	53.8	0.99		0.98	0.82 to 1.18	
4	777	408	52.5	0.94		0.97	0.78 to 1.20	
Baseline CD4					0.32			0.59
<50	1,107	576	52.0					
50-99	783	431	55.0	1.13		1.10	0.91 to 1.33	
100-149	759	403	53.1	1.04		1.06	0.87 to 1.28	
150-199	662	364	55.0	1.13		1.14	0.93 to 1.39	
Anti-HCV					0.96			0.89
Negative	3,171	1,700	53.6					
Positive	77	40	51.9	0.94		0.90	0.57 to 1.41	
Not done	63	34	54.0	1.01		0.96	0.58 to 1.60	

As mentioned above (section 5.3.4.2), in 54 participants HBsAg was detectable but anti-HBc was not, a phenomenon which has been linked to increasing immunosuppression (see discussion) as well as to acute infection or a false positive HBsAg. We observed a non-significant increasing trend in the proportion negative for anti-HBc at lower CD4 counts (Table 18).

Table 18: Anti-HBc status in those HBsAg seropositive, by baseline CD4

		Anti-HBc positive	
Baseline CD4	**N**	**n**	**%**
0-49	98	77	78.6
50-99	84	69	82.1
100-149	68	56	82.4
150-199	57	51	89.5
Total	307	253	82.4

Chi-squared test for trend p=0.11.

5.3.5.2 HBsAg

The prevalence of detectable HBsAg was three times higher in Harare (16.7%) than in the Ugandan sites, where the prevalence was similar (Entebbe 5.5%, JCRC 6.6%; Zimbabwe vs. Uganda p<0.001, Entebbe vs. JCRC p=0.29).

As stated above (section 5.3.2.1) we would expect anti-HBc results to be positive in all participants with CHB. In those anti-HBc positive, HBsAg was more prevalent in Harare (22.7%) than in Entebbe (8.8%) or JCRC (11.6%; p<0.001) but was no different between the 2 Ugandan sites (p=0.11). However HBsAg was also detected in 54 participants negative for anti-HBc (40 at Harare, 9 at Entebbe and 5 at JCRC).

Higher prevalence was associated with male sex (12.2% vs. 7.7%; p<0.001) but this was seen only at JCRC (10.6% vs. 4.7%; p<0.001) and not at Entebbe (5.7% vs. 5.4%; p=0.86) or Harare (18.8% vs. 15.3%; p=0.13).

There was no clear relationship between the prevalence of HBsAg and age (p=0.78) (Figure 14).

Figure 14: HBsAg seroprevalence at baseline by age, sex and study site
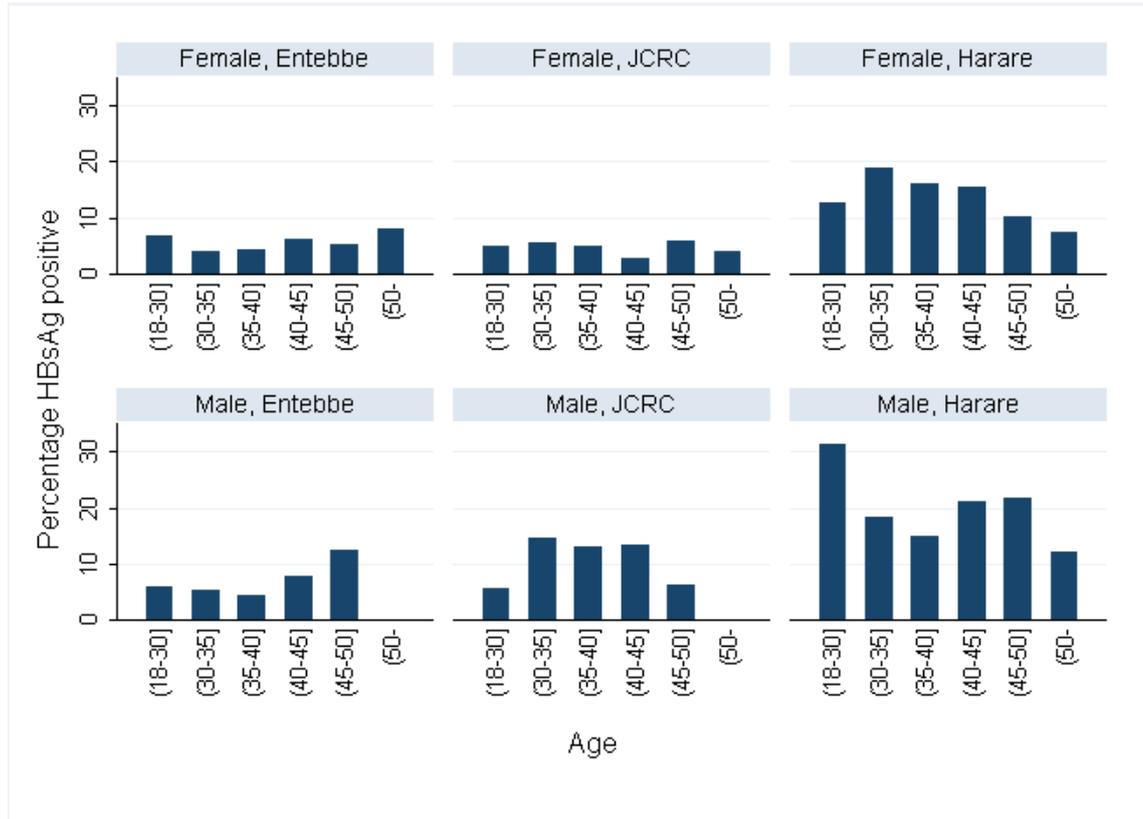
There was also no relationship between HBsAg status and WHO stage (p=0.85), baseline CD4 count (p=0.68) or anti-HCV status (p=0.64).

The median CD4 count at study entry was the same in those without detectable HBsAg (86 cells/mm3, IQR 31 to 140) and those with detectable HBsAg (87 cells/mm3, IQR 31.5 to 137.5; p=1.0).

In a logistic regression model, HBsAg status was associated with site (p<0.001) and sex (p=0.001) with higher prevalence of HBsAg in JCRC (aOR 1.22) and Harare (aOR 3.41) and lower prevalence in females (aOR 0.65). In adjusted analyses, HBsAg status was not associated with age (p=0.29), baseline WHO stage (p=0.99), baseline CD4 (p=0.66) or anti-HCV status (p=0.34) (Table 19).

Table 19: Predictors of HBsAg status

	All	HBsAg positive		OR	p	aOR	95% CI	p
	n	n	%					
All	3,314	308	9.3					
Site					<0.001			<0.001
Entebbe	1,020	56	5.5					
JCRC	1,297	85	6.6	1.21		1.22	0.86 to 1.74	
Harare	997	167	16.7	3.46		3.41	2.48 to 4.70	
Sex					<0.001			0.001
Male	1,160	141	12.2					
Female	2,155	167	7.7	0.61		0.65	0.50 to 0.83	
Age group					0.78			0.29
<30	532	46	8.6					
30-35	795	79	9.9	1.17		1.05	0.71 to 1.55	
35-40	848	77	9.1	1.06		0.89	0.60 to 1.32	
40-45	608	63	10.4	1.22		0.99	0.66 to 1.50	
45-50	313	31	9.9	1.16		0.91	0.55 to 1.49	
>50	219	12	5.5	0.61		0.48	0.25 to 0.95	
WHO Stage					0.85			0.99
2	672	61	9.1					
3	1,864	178	9.5	1.06		1.00	0.73 to 1.37	
4	779	69	8.9	0.97		1.02	0.69 to 1.49	
Baseline CD4					0.68			0.66
<50	1,109	99	8.9					
50-99	784	84	10.7	1.22		1.21	0.88 to 1.66	
100-149	759	68	9.0	1.00		1.06	0.76 to 1.48	
150-199	663	57	8.6	0.96		1.02	0.71 to 1.46	
HCV Ab					0.64			0.34
Negative	3,175	298	9.4					
Positive	77	5	6.5	0.67		0.70	0.28 to 1.77	
Not done	63	5	7.9	0.83		0.54	0.21 to 1.38	

5.3.5.3 HBeAg

Among HBsAg-positive participants, HBeAg positivity was higher at JCRC (46.3%) and Harare (34.7%) than at Entebbe (27.1%), although these differences did not reach statistical significance (p=0.07).

The proportion HBeAg positive was greater in males than females (44.0% *vs.* 31.0%; p=0.03). There was no relationship with age (p=0.17) (Figure 15).

Figure 15: HBeAg status by age, sex and study site
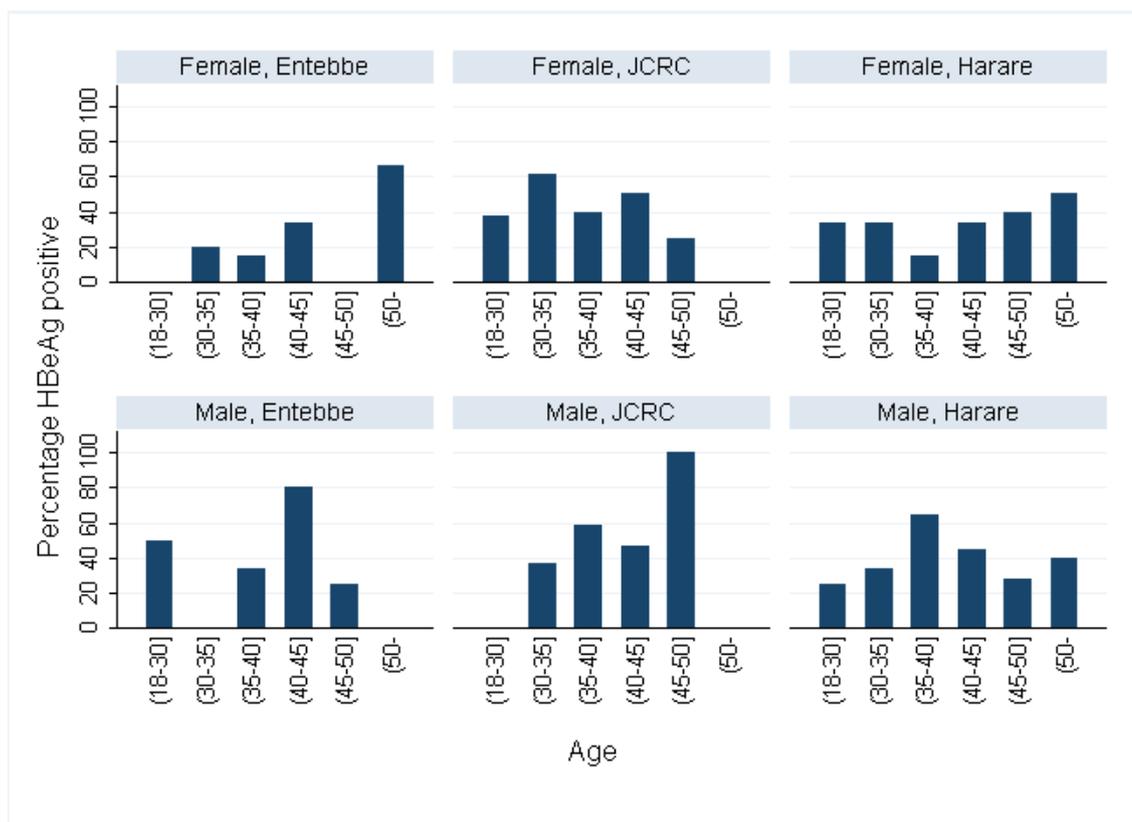
HBeAg prevalence increased with advancing HIV disease with rates of 24% in stage 2, 36% in stage 3 and 52% in stage 4 disease (p=0.001). The pattern by CD4 count was less clear. There was no association with anti-HCV (p=0.62).

In multivariable logistic regression there remained no association with site, sex, age or anti-HCV status and the relationship with baseline CD4 was no longer significant. However HBeAg positivity was more likely with advanced WHO stage of HIV disease (p=0.03) (Table 20). In a model including all variables apart from WHO stage, the aOR for baseline CD4 cell count did not materially change and remained non-significant (p=0.08).

Table 20: Predictors of HBeAg status

	All	HBeAg positive		OR	p	aOR	95% CI	p
	n	n	%					
All	280	103	36.8					
Site					0.07			0.35
Entebbe	48	13	27.1					
JCRC	82	38	46.3	2.33		1.55	0.67 to 3.62	
Harare	150	52	34.7	1.43		1.03	0.47 to 2.26	
Sex					0.03			0.12
Male	125	55	44.0					
Female	155	48	31.0	0.57		0.65	0.38 to 1.11	
Age group					0.17			0.68
<30	41	10	24.4					
30-35	70	26	37.1	1.83		1.71	0.69 to 4.25	
35-40	67	25	37.3	1.85		1.66	0.67 to 4.13	
40-45	61	27	44.3	2.46		2.19	0.87 to 5.53	
45-50	29	10	34.5	1.63		1.52	0.50 to 4.66	
>50	12	5	41.7	2.21		2.38	0.59 to 9.70	
WHO Stage					0.001			0.03
2	59	14	23.7					
3	159	57	35.8	1.80		1.67	0.80 to 3.48	
4	62	32	51.6	3.43		3.10	1.33 to 7.25	
Baseline CD4					0.05			0.10
<50	89	36	40.4					
50-99	74	35	47.3	1.32		1.57	0.81 to 3.06	
100-149	65	16	24.6	0.48		0.60	0.28 to 1.27	
150-199	52	16	30.8	0.65		0.97	0.44 to 2.15	
HCV Ab					0.62			0.32
Negative	276	102	37.0					
Positive	4	1	25.0	0.57		0.31	0.03 to 3.23	

5.3.5.4 *HBV DNA*

The proportion of those with a positive HBsAg result that had detectable HBV DNA was higher in JCRC (91.7%) and lower in Harare (72.3%) than in Entebbe (82.0%; p=0.003).

250 (92.6%) of those with HBV DNA results were tested for HBeAg; 83 (33.2%) were HBeAg positive. HBV DNA was detected in more HBeAg positive (96.4%) than HBeAg negative (70.1%) participants (p<0.001) and HBeAg positive patients had higher HBV DNA results (median 1.1 x10^8 *vs.* 2.9 x10^3, p<0.001) (Figure 16).

Figure 16: HBV DNA viral load in those quantifiable, by HBeAg status
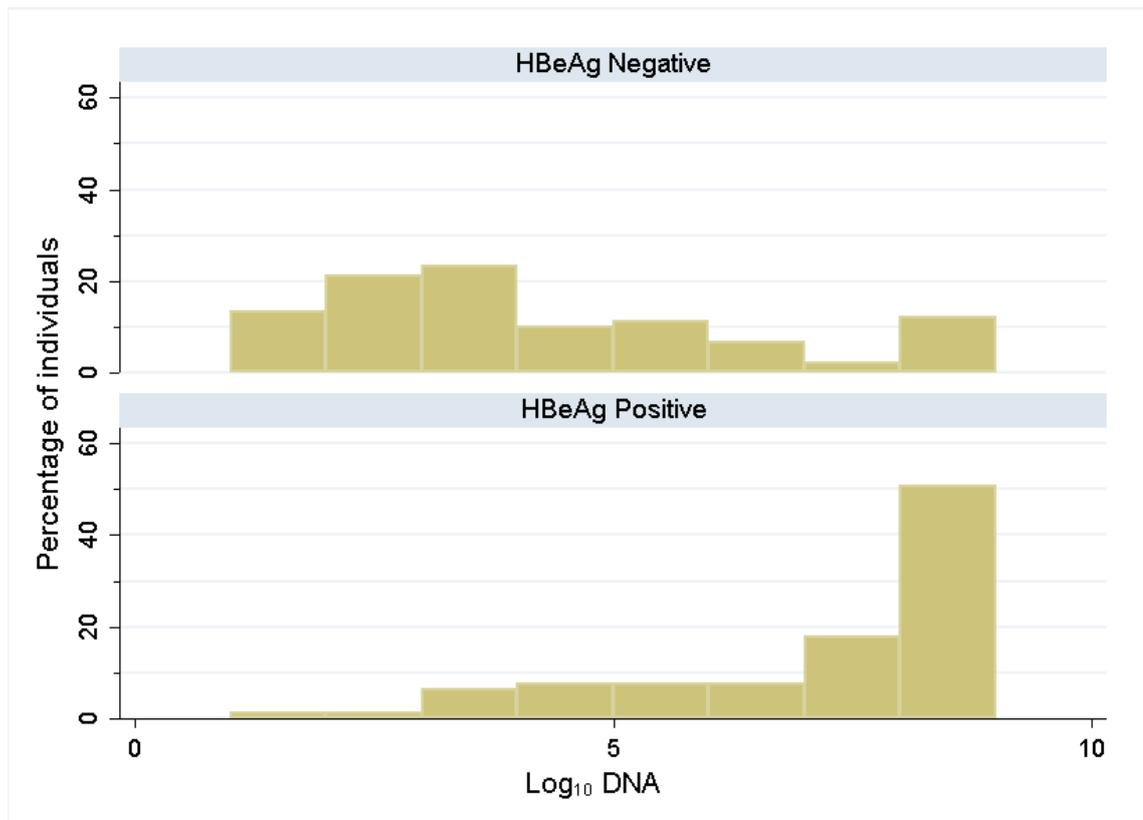
In a multivariable linear regression model limited to those with quantifiable HBV DNA, HBV DNA was associated with site (higher in JCRC) and HBeAg status but was not associated with sex, age, WHO stage or CD4 cell count (Table 21). HBV DNA was nearly 3 logs higher in those who tested HBeAg positive.

Table 21: Linear regression of log(HBV DNA)

	Unadjusted			Adjusted		
	Coeff.	95% CI	p	Coeff.	95% CI	p
Site						
Entebbe			0.01			0.004
JCRC	1.21	0.15 to 2.28		0.91	0.01 to 1.80	
Harare	0.04	-0.96 to 1.03		-0.25	-1.10 to 0.60	
Sex						
Male			0.81			0.62
Female	-0.09	-0.66 to 0.85		0.16	-0.47 to 0.78	
Age group						
<30			0.12			0.09
30-35	1.09	-0.19 to 2.37		1.36	0.31 to 2.41	
35-40	0.30	-1.01 to 1.62		0.67	-0.41 to 1.75	
40-45	0.51	-0.81 to 1.82		0.71	-0.35 to 1.78	
45-50	-0.76	-2.24 to 0.73		0.04	-1.15 to 1.23	
>50	0.51	-1.47 to 2.49		0.98	-0.57 to 2.53	
WHO Stage						
2			0.04			0.78
3	0.76	-0.23 to 1.74		0.06	-0.76 to 0.89	
4	1.52	0.36 to 2.68		-0.21	-1.20 to 0.78	
Baseline CD4						
<50			0.002			0.10
50-99	0.54	-0.40 to 1.49		0.41	-0.37 to 1.21	
100-149	-1.37	-2.38 to -0.36		-0.71	-1.59 to 0.16	
150-199	-0.74	-1.80 to -0.31		-0.19	-1.14 to 0.75	
HBeAg						
Negative			<0.001			<0.001
Positive	2.98	2.35 to 3.61		2.86	2.23 to 3.49	

Coeff: Coefficient.

5.3.5.5 *Exposure to HBV and resolution of infection*

Exposure to HBV (HBsAg and/or anti-HBc positive) was more prevalent in Harare (60.1%) than in Entebbe (52.4%) or JCRC (53.6%; p=0.001) and in males (61.4%) than females (51.8%; p<0.001). Exposure was more common with increasing age (p<0.001) (Figure 17).

Figure 17: Percentage with evidence of exposure to HBV by age, sex and site
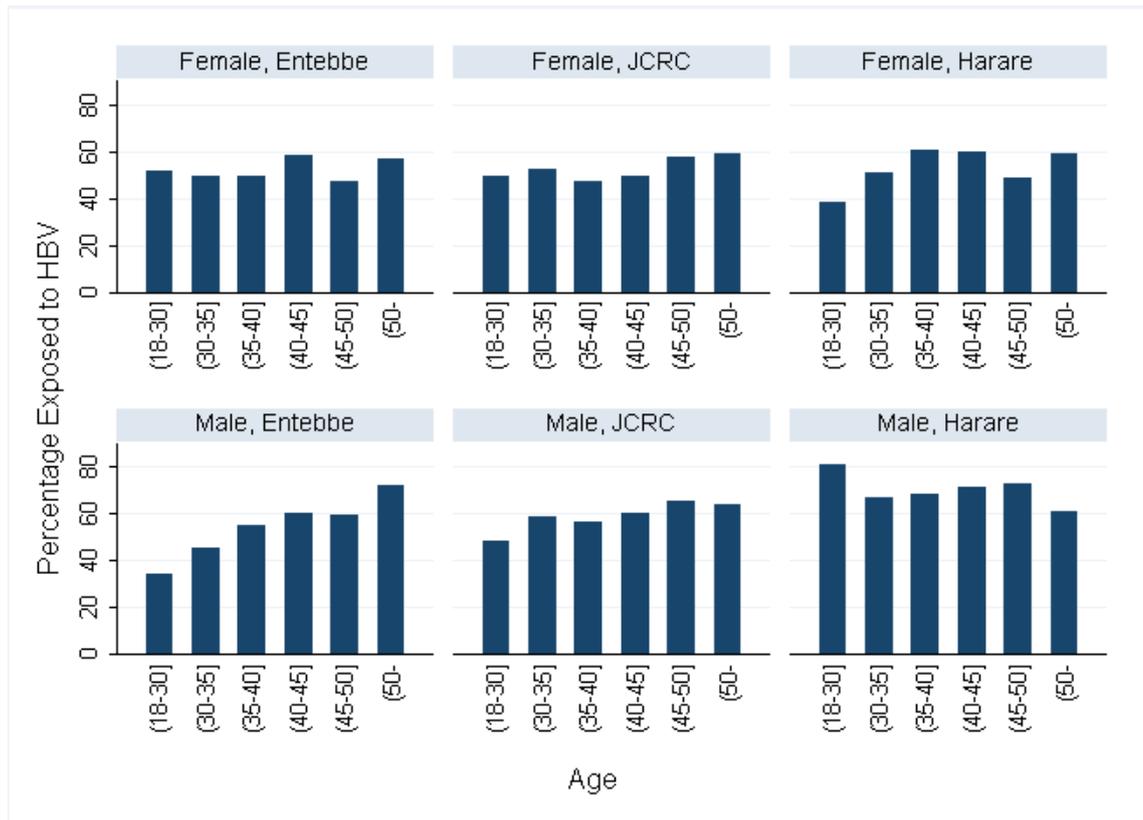
In multivariable logistic regression analysis, sex and site were associated with exposure (p<0.001 and p=0.006) while the association with age approached significance (p=0.06). Interaction terms for sex and age and for site and age were not significant (p=0.81 and p=0.24) whereas that for sex and site was (p=0.003).

The proportion of those exposed who had resolved HBV infection (defined as in section 5.2.2) was higher in JCRC (63.0%) and in Entebbe (50.4%) than in Harare (42.5%; p<0.001) and higher in females than in males (56.0% *vs.* 47.3%, p<0.001). There was no association with age (p=0.91). The proportion that had resolved HBV infection is shown in Figure 18 by site, sex and age.

Figure 18: Percentage of those HBV exposed who had cleared HBV by age, sex and site
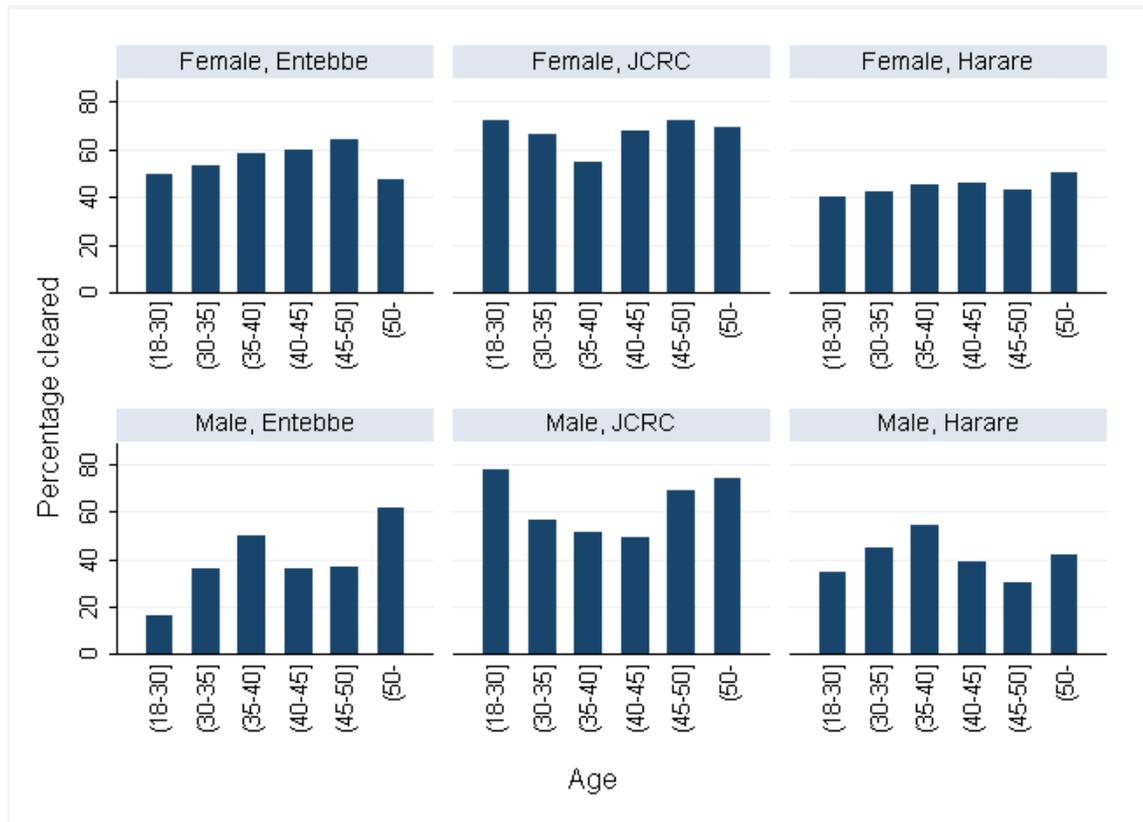
In logistic regression site and sex predicted resolution whereas age, WHO stage, baseline CD4 and anti-HCV status did not (Table 22). However the effects of sex and age appeared to vary between the sites (interaction factors between site and sex $p=0.15$, site and age $p=0.02$, sex and age $p=0.16$).

Table 22: Predictors of resolved HBV infection

	All	Resolved		aOR	95% CI	p
	n	n	%			
All	1,829	962	52.6			
Site						<0.001
Entebbe	534	269	50.4			
JCRC	695	438	63.0	1.71	1.35 to 2.16	
Harare	600	255	42.5	0.78	0.61 to 0.99	
Sex						0.005
Male	712	337	47.3			
Female	1,117	625	56.0	1.3	1.09 to 1.62	
Age group						0.71
<30	261	141	54.0			
30-35	423	222	52.5	0.98	0.72 to 1.35	
35-40	468	243	51.9	1.05	0.77 to 1.44	
40-45	359	181	50.4	0.97	0.70 to 1.35	
45-50	183	97	53.0	1.10	0.74 to 1.63	
>50	135	78	57.8	1.34	0.87 to 2.07	
WHO Stage						0.25
2						
3				0.86	0.68 to 1.10	
4				1.03	0.77 to 1.39	
Baseline CD4						0.28
<50						
50-99				0.83	0.65 to 1.07	
100-149				1.02	0.78 to 1.32	
150-199				0.83	0.63 to 1.09	
HCV Ab						0.76
Negative						
Positive				0.86	0.45 to 1.63	
Not done				0.71	0.40 to 1.63	

5.3.5.6   HCV serology results

The prevalence of anti-HCV was similar in the three sites (Entebbe 2.5%, JCRC 2.6% and Harare 2.0%; p=0.64).

There was no difference in prevalence of anti-HCV between those not exposed to HBV and those exposed (2.5% *vs*. 2.2%, p=0.56).

5.4 Discussion

5.4.1 Serology results compared with previously published data

5.4.1.1 HBV

We found that over half (55.2%) of DART participants had evidence of HBV exposure, defined as detectable HBsAg and/or anti-HBc. The probability of exposure was higher in males than in females and also higher in Zimbabwe than in Uganda, while rates in the two Ugandan sites were similar.

There was evidence of cleared HBV infection in 29.0% of participants, with detectable anti-HBc and anti-HBs but without detectable HBsAg; 9.3% had current infection with detectable HBsAg; 16.5% had isolated detectable anti-HBc with both HBsAg and anti-HBs negative; and 0.6% were unclassifiable due to missing results (i.e. tests not done due to insufficient samples). In those with evidence of having been exposed to HBV the percentages were: 53.2% cleared HBV; 16.8% HBsAg seropositive; 30.0% isolated anti-HBc. Previous studies have reported prevalence of HBsAg and/or anti-HBc rather than categories as defined above. We found the prevalence of anti-HBc in the three sites to be similar at 51.4 to 56.3%. These figures are consistent with rates found in previous studies, though data in HIV-positive patients are limited (chapter 1 Table 1 and Table 2). In an analysis adjusting for both age and sex, participants in Harare and at JCRC were more likely to be anti-HBc positive if male.

The systematic review of sub-Saharan HIV-positive adults quoted above found mean prevalence of HBsAg to be 15%, but with a very wide range from 3.9% to 70.3% [20]. Previous studies from both Uganda [48, 52-54] and Zimbabwe[31, 32] have also presented widely varying results, both between geographical areas and within the same location (chapter 1 Table 1 and Table 2). In this study 9.3% tested positive with the prevalence similar at the two Ugandan sites but considerably higher in Zimbabwe. In Harare and JCRC HBsAg was more prevalent in males. Our estimates lie within the ranges previously described in both Zimbabwe and Uganda. Although recent published data in HIV-positive patients in Kampala found HBsAg prevalence of 8.9% (95% CI 6.5 to 11.9%) at ART initiation [53] and of 18.0% (12.9 to 24.2%) in patients admitted to hospital [54], both of which are higher than the prevalence we found in Kampala (6.6%; 5.2 to 8.0%), the confidence levels overlap. Similarly, although two recent studies of patients in Harare found the prevalence in HIV-positive pregnant women to be 5.8% (1.2 to 15.9%) [31] and 11.0% (7.3 to 15.8%) [32], both of which are lower than the prevalence we found in Harare (16.6%; 14.3 to 19.1%), again our estimate is not significantly different.

We did not find a relationship between CD4 count or stage of HIV disease and HBsAg prevalence, and therefore no evidence that immunosuppression resulted in either (i) reduction in the rate of loss of HBsAg over time or (ii) HBsAg reactivation. However, all participants had advanced HIV disease and low CD4 cell count at study entry. Thus we had no group with higher CD4 counts to compare with and it could therefore also be the case that all participants had CD4 count below the level at which these effects on HBV natural history appeared. A relationship between CD4 count and HBsAg status has been noted in one previous study in Africa, with causation suggested to be acting in the other direction, namely of HBV infection lowering CD4 cell count, but we found no difference in CD4 cell count at study entry between those with and without detectable HBsAg [87].

A previous study of mostly HIV-negative, HBsAg seropositive inpatients in Kampala found 27% HBeAg seropositive [54]. An earlier study, also of inpatients in the same hospital, found 6 (28.1%) of 23 HIV-positive and 3 (17.6%) of 17 HIV-negative patients to be HBeAg seropositive [48]. In Harare studies have found widely variable rates of HBeAg seropositivity, from 76.5% in jaundiced patients to 3.3% in pregnant women [30, 264]. A national survey found the overall rate to be 24.5% [29]. None of these three Zimbabwean studies tested for HIV. In HBV/HIV coinfected Zimbabwean patients recruited to a randomised controlled trial 54.2% (13 of 24) were HBeAg seropositive [32]. In the DART population, of those with detectable HBsAg 36.8% were HBeAg seropositive. The prevalence of a positive HBeAg result was higher in those with more advanced stage of HIV disease (in contrast to the lack of evidence for such a relationship between advanced disease and HBsAg status) which may have been due to immunosuppressed patients clearing HBeAg less often, or due to reactivation of HBeAg in previously seronegative patients as immunosuppression progressed. This relationship between HBeAg status and low CD4 count has been noted previously in a study in Nigeria [87].

543 participants, 16.4% of the study population and 30.0% of those with evidence of HBV exposure, had isolated anti-HBc. Similar rates have been found in Ugandan healthcare workers, both nationally (32.1%) [50] and in Kampala (35.4%) [55] and in HIV-positive patients elsewhere in sub-Saharan Africa, for example in South Africa (35.6%) [265] and in Côte d'Ivoire (42%) [266]. This pattern may be due to false positive anti-HBc test results, or be transient and occur during the resolution phase of acute HBV after the loss of HBsAg but before the appearance of anti-HBs. Persistent isolated anti-HBc may be due to occult HBV infection with negative HBsAg or due to loss of anti-HBs in patients who have cleared HBV, possibly related to immunosuppression. The testing algorithm used in this study was such that no further

testing was planned for individuals with isolated anti-HBc (section 4.2). In fact 10 such individuals did have a baseline sample tested for HBeAg and anti-HBe; 4 had detectable HBeAg and 7 had detectable anti-HBe, one testing positive for both. While we cannot rule out false positive results, this does suggest that all 10 had been infected with HBV. Repeat serology testing (to identify false positives and those with acute infection) and HBV DNA viral load testing (to identify occult infection) would help to determine more accurately the status of the 543 with isolated anti-HBc.

5.4.1.2 HCV

The overall prevalence of anti-HCV in sub-Saharan Africa has been estimated at 6.9%, though the range was wide, from 0% in studies from Botswana, Burkino Faso and Tanzania to 22.2% in Ethiopia [20]. There is very limited data on the prevalence of HCV exposure and/or infection in Uganda and Zimbabwe and reported rates are very low; only 0.6% of pregnant women in Kampala and 0.8% of those in rural Zimbabwe were shown to be positive [51, 267]. In the DART population we found the prevalence to be 2.4% and the prevalence was similar in the 3 sites.

5.4.2 Differences between study sites

The patterns of HBV test results differed between the sites, and even between Kampala and Entebbe which are only 35 km apart, despite the fact that the overall prevalence of anti-HBc was very similar. This variation is consistent with the wide variation seen in other prevalence studies. Populations geographically close to each other, or even living amongst each other, may carry what are in fact distinct endemic viruses. For example, it has previously been shown that different ethnic groups within Uganda may carry predominantly different types of HBV; Lwanga showed that amongst Bantu groups, 19 out of 24 (79%) individuals carried serotype adw whereas in non-Bantu ethnic groups, only 1 of 10 (10%) carried adw, all others in both groups carrying serotype ayw [268]. As noted in the introduction, it is believed that in sub-Saharan Africa hepatitis B is most often contracted in early childhood through horizontal routes of transmission. These include such culturally determined practices as scarification, which varies widely in prevalence, for example between 66% in Kisiizi and 14% in Kagando (both in Uganda, see Figure 2) [45]. Thus populations may have different prevalent infections and transmission patterns.

The age at which HBV is contracted is the strongest determinant of the probability that infection does not resolve and instead becomes chronic. It also affects the natural history of the infection, with those infected near birth showing a longer immunotolerant phase and having HBeAg present for longer, often until after the age of thirty. Those infected after 1 year of age but before 5 years having a short period of tolerance only

until early adulthood and those infected as adults having sometimes no immunotolerant phase at all [61, 269]. Sex also has an effect on the risk of chronicity with males being more likely to progress to chronic infection, both when infected in childhood and as adults [11-14].

If it were the case that all DART participants were infected at birth or as neonates, the expected pattern of results would show that, (1) the prevalence of exposure to HBV was constant over the age range of DART, (2) the fraction of exposed individuals that had cleared HBV (have evidence of exposure but negative HBsAg, possibly with anti-HBs) would be only a small minority by the age of 30 and would very gradually rise with age, and (3) the proportion HBeAg positive would be high in the 18-30 age group and decline until most had lost HBeAg after the age of 40. On the other hand, if it was the case that infection predominantly occurred through sexual exposure, the prevalence of exposure should rise through adulthood, though perhaps rapidly in early adulthood and more slowly in older age, depending on sexual behaviour. The large majority of those exposed should have cleared HBV infection and the proportion HBeAg positive should be lower. Thus examining the patterns of HBsAg, HBeAg and HBV DNA in those exposed to HBV may indicate the age at which HBV is acquired.

However the results show no clear pattern in any site. This may be due to a change in epidemiology over time, as a result of each of the three sites examined including diverse populations with different epidemiological patterns that cannot be distinguished once aggregated or due to the picture being complicated by HIV-associated immunosuppression leading to loss of antibody or reactivation of HBeAg or even HBsAg and HBV DNA.

We found an increase in the prevalence of anti-HBc with increasing age in all three sites which may indicate infection during adulthood but this may also be as a result of a cohort effect, with historically declining childhood infections (Figure 13).

The proportion HBeAg positive ranged from 27.1% in Entebbe to 46.3% at JCRC but the difference was not statistically significant. The proportion of those with HBsAg that had detectable HBV DNA was 72.3% in Harare, 82.0% in Entebbe and 91.7% at JCRC. This is similar to the 27 (79%) of 34 HBsAg-positive blood donors in Harare and the 50 (91%) of 55 HBsAg-positive inpatients in Kampala that were HBV DNA positive in previous studies [54, 270]. The variation between the sites is partly explained by the fact that those with positive HBeAg were more likely to have detectable HBV and the proportion with positive HBeAg was higher in JCRC (though this difference was not statistically significant). However even in those HBeAg negative, more had quantifiable HBV DNA VL in JCRC than in the other sites.

We also found differences in the relationship between sex and HBV exposure or anti-HBc prevalence in the three sites. This may also be as a result of differing patterns of transmission, either through gender-specific cultural practices (scarification) or through differential sexual transmission in adulthood.

Differences in HBsAg prevalence (chronic infection with HBV) despite very similar anti-HBc prevalence (HBV exposure) may also reflect genetic differences. It has been shown that the chance of clearing HBV is higher with certain single nucleotide polymorphisms in or near to the TNF-α gene in the MHC complex on chromosome 6. Clearance has been shown to be associated with TNF-α-863 CC and persistence with TNF-α-308 GG [271]. Genetic markers were not studied in this population.

5.4.3 Unusual patterns

HBsAg was detectable despite undetectable anti-HBc in 54 (1.6% of all study participants) and despite detectable anti-HBs in 14 (0.4%), including 12 (0.4%) in whom anti-HBc was also detectable. However these unusual patterns of serology are not novel and previous studies have found similar atypical patterns. In studies including both HIV-positive and negative individuals, the percentage of those with detectable HBsAg but undetectable anti-HBc has ranged widely, for example 3.7% in Thailand but 56.0% in Uganda, while the percentage of those with detectable HBsAg that also had detectable anti-HBs has also varied, for example 1.2% in Thailand but 32.5% in Angola [47, 272, 273]. One study from Uganda compared HIV-positive and negative patients and found a lower prevalence of undetectable anti-HBc (44.7% *vs.* 62.0%, p=0.01) but a higher prevalence of detectable anti-HBs (25.0% *vs.* 16.9%, p=0.16) in HIV-positive than in HIV-negative individuals, though the latter result did not reach significance [47].

5.4.3.1 Negative anti-HBc but positive HBsAg

In 1984 Trepo described patients with non-A, non-B (NANB) hepatitis who had HBsAg briefly detectable in serum without anti-HBc. In all these cases HBsAg disappeared within 2 weeks to 2 months. These were presumably acute HBV infections occurring in patients with other forms of hepatitis including HCV [274]. This pattern of HBV serology was also reported in cases of vertical transmission in Taiwan. Since the pattern of HBsAg without anti-HBc was not found in a previous epidemiological study in adults (also in Taiwan) it was postulated that anti-HBc would develop as the new-borns lost their natural immunotolerance [275]. As previously discussed, most HBV transmission in sub-Saharan Africa occurs in early childhood and so we expect that few of the participants with this serological pattern have acute infection. Follow-up serology would help to clarify this.

However anti-HBc-negativity has also been found in HBsAg seropositive children in Senegal where it has been suggested that infection is with a different type of HBV, named HBV_2 by the authors, which does not stimulate the usual immune response to HBV core antigen. However there has been no subsequent confirmation of this virus [276, 277].

The same pattern has also been reported in an individual with mutations including insertion resulting in a change in the location of core transcription initiation and a stop codon in the pre-core region, though in that case the lack of anti-HBc was postulated to be due to pre-existing HIV and immunosuppression [278].

It has been suggested that the relationship with immunosuppression may be dynamic in HIV-positive individuals, for example anti-HBc may be absent when the CD4 count is <50 and appear if CD4 rises in response to antiretroviral treatment [277] although successful treatment does not always result in development of anti-HBc [279]. In some cases anti-HBc may be present in small amounts due to other defects of the immune system, as described by Lazizi [280]. These patients may have high levels of viral replication with high HBV DNA and high levels of HBcAg circulating in the blood. The HBcAg then complexes with the little anti-HBc that is present and so tests that require uncomplexed anti-HBc will give a false negative result.

5.4.3.2 Positive anti-HBc, HBsAg and anti-HBs

The classical picture of evolution of HBV serology results states that HBsAg becomes detectable during acute infection and then persists, either for less than 6 months in cases of resolving acute hepatitis B infection or for greater than 6 months in cases of CHB, until seroconversion occurs at which point anti-HBs becomes detectable. However this picture of only one of either HBsAg or anti-HBs being detectable in the blood at any one time is overly simplistic.

Shulman was the first to detect both HBsAg and anti-HBs occurring together as immune complexes [281]. Trepo reported that the presence of both HBsAg and anti-HBs indicated a poor prognosis in patients with fulminant hepatitis [282].

Sometimes anti-HBs is directed at HBsAg from a different subtype of HBV than the one found circulating concurrently and thus both may exist together [283]. In some patients the antigenic 'a' determinant of HBsAg (the site of anti-HBs binding) has increased variability which may allow immune escape [284].

In patients who had HBsAg, anti-HBs and anti-HBc simultaneously detectable, Carman found a mutation in the gene for HBsAg (G145R) which affected the 'a' determinant. In

these cases anti-HBs was as a result of immunisation and so bound to a form of HBsAg without this mutation [175]. In the Thai study mentioned above patients with this pattern also had G145A [272].

A second mutation, consisting of a deletion after 21 amino acids of the S gene, was also found in a patient with positive HBsAg, anti-HBs and anti-HBc. This mutation caused a shift in reading frame and a stop codon which resulted in a severely truncated HBsAg in which the antigenic 'a' determinant was entirely missing. HBsAg occurs in three forms, encoded by the S gene alone, the S gene plus pre-S2 and the S gene plus pre-S2 and pre-S1. HBeAg and HBV DNA were detected in the serum and morphologically normal viral (Dane) 42nm particles and 22nm spheres were seen on electron microscopy, although there was an absence of 22nm filaments. It has been shown that such a truncated HBsAg should not have been able to form into intact viral particles. Also, since the open reading frames for the S gene and the P gene that encodes the viral polymerase overlap, this deletion and associated frame shift should have produced an ineffective enzyme incapable of effective viral replication. It was postulated that a minority population with a wild-type S gene probably accounted for HBsAg and polymerase production [285].

5.4.4 Limitations

One important caveat is that in general we assume that HBsAg is a marker of chronic infection with HBV. It is likely that a small proportion of individuals in fact have acute HBV. These cases could be identified by repeating HBsAg tests at least six months after study entry or by testing for anti-HBc IgM. Unfortunately neither was available in this study. It would be very interesting to repeat HBsAg tests at the end of follow-up and to derive an incidence rate in those susceptible since there are no published data on adult HBV incidence in HIV-positive individuals in sub-Saharan Africa.

A high proportion of DART participants (16.5%) had the HBV serological pattern of isolated anti-HBc. This result may represent a false positive, resolved and cleared infection or chronic infection with a very low rate of viral replication (occult HBV infection). For example in a previous study in Uganda, 14.6% of HIV-positive patients with negative HBsAg had detectable HBV DNA [54]. A positive HBV DNA result in an individual with isolated anti-HBc defines occult HBV infection. The clinical implications of occult HBV infection are unclear, but it is generally accepted that individuals with detectable plasma HBV DNA may be infectious and may also be at risk of HBV reactivation and inflammatory liver flares [286]. Thus clarifying the clinical situation of these individuals is important. Unfortunately, in this study, only patients with positive HBsAg were tested for HBV DNA. Alternatively repeating anti-HBc and anti-HBs tests

after some time on HAART or after HBV vaccination may show some patients to have resolved infection if anti-HBs which had been lost as a result of profound immunosuppression reappeared on immune reconstitution or after challenge with vaccine.

The data is insufficient to determine the age of acquisition of HBV in this cohort. Examining the age-specific HBV prevalence in those aged between birth and 18 years would be very informative, particularly if many or most infections occur between these ages. As the data in this study is cross-sectional it only gives us a snapshot of the distribution at one time. Any future attempt to clarify the epidemiological patterns may be limited by increasing HBV vaccination rates.

The different distributions of serological results may also be due to infections with different genotypes of HBV [61]. Unfortunately no genotypic sequence data is available in this study. Even if all HBV-infected participants carried the same genotype, sequence data would also allow phylogenetic analysis which may show to what extent patterns are due to independent networks of HBV infection.

Clearly the three sites include distinct populations with different patterns of HBV epidemiology. In this study we have attempted to discover some of the factors that are associated with HBV status.

5.4.5 Conclusion

We found that just over half the DART participants carried anti-HBc and just fewer than 10% carried HBsAg at study entry; both were more common in males than in females. Although the prevalence of anti-HBc was similar in the three sites, HBsAg prevalence was 3 times higher in Zimbabwe than in Uganda.

We were unable to deduce the patterns of transmission in the DART populations from the serological results available. Data that could better characterise these patterns could include results from those aged less than 18, from repeated tests in the same participants after time and from performing additional tests, for example HBV DNA assays on those with isolated anti-HBc.

An important conclusion is that HBV serological patterns in one population cannot be simply applied to other populations in sub-Saharan Africa or even other populations geographically close to each other. In so far as HBV prevalence may determine clinical policy, determination of local epidemiology is crucial.

6 Longitudinal analysis of plasma HBV DNA levels

6.1 Introduction

In a proportion of those infected with hepatitis B virus, infection leads to liver fibrosis, hepatocellular carcinoma, liver failure or death. The prognosis can be improved by treatment that reduces inflammation, fibrosis and the amount of HBV in the blood [149, 150]. Markers of treatment success include normalisation of liver transaminase activity levels, HBeAg to anti-HBe seroconversion, reduction in fibrosis and reduction in HBV DNA viral load in plasma [287]. These markers are associated with the improvement in prognosis although HBV remains in hepatocytes and is not entirely cleared from the body.

Current treatment approaches are either to inhibit virus replication directly or to enhance the immune response responsible for suppressing viral replication in most patients. Drugs for the treatment of HBV fall into two groups, interferons and nucleoside (or nucleotide) reverse transcriptase inhibitors. Interferon treatment is given for a limited duration, usually 12 months, and then stopped, with a successful outcome being such that hepatitis B remains either undetectable or at a low level (HBV DNA <2,000 IU/mL) in the patient's blood with normalisation of ALT, preferably with loss of HBeAg and seroconversion to anti-HBe if HBeAg positive at baseline and a persistently negative HBsAg, ideally with a positive anti-HBs [288]. These serological changes are prognostic markers of a sustained response.

Treatment with reverse transcriptase inhibitors can similarly result in HBeAg and HBsAg loss and anti-HBe and anti-HBs seroconversion, which if maintained may allow treatment for HBV to be stopped. However in individuals with HIV coinfection such seroconversions are less likely and if they do occur are also less likely to be durable on stopping treatment and so generally guidelines recommend that once treatment is started it should be continued indefinitely [125] although some guidelines state that treatment can be stopped in certain circumstances [289].

The probability of treatment success is lower in coinfected patients (see chapter 1) and, it is sometimes suggested, with advanced immunosuppression [110, 140, 287] although some studies examining the effect of reverse transcriptase inhibitors have found no association with baseline CD4 count [85, 139, 147, 290]. In DART all participants had advanced HIV disease with CD4 cell counts below 200 cells/mm^3.

Guidelines recommend treating individuals with more advanced HIV disease for both infections with a regimen that includes TDF and either 3TC or FTC, while those with a

need for HBV treatment but less advanced HIV disease could be treated with drugs that have no significant activity against HIV, such as interferon, ADV or telbivudine [123, 125, 133, 134, 287]. Recent guidelines recommend a CD4 cut-off of 500 cells/mm^3 for the initiation of HIV treatment in coinfected patients (and in the latest WHO guideline, all patients) [132-134]. Patients with a CD4 count greater than 500 cells/mm^3 with normal ALT, no fibrosis and HBV DNA viral load less than 2,000 IU/mL do not require treatment and should be monitored closely [125, 133, 287].

Previous published data have shown that regimes containing 3TC [136, 137], TDF [138-140], 3TC plus TDF [114, 139, 140, 144, 145] and FTC plus TDF [117, 140, 146] are able to suppress HBV in HIV-coinfected patients. There is evidence that virological suppression is more likely with 3TC plus TDF than with 3TC alone in patients on first line therapy [114, 139, 144, 147]. The suppressive effect of 3TC and TDF may be similar but rates of virological rebound lower when TDF is used [147].

All patients in DART initiated antiretroviral therapy with at least one drug active against HBV (3TC) and approximately three quarters with two active drugs (3TC and TDF). However, in coinfected patients treated with 3TC as the only HBV-active drug, resistance to 3TC emerges with continued use [291] and has been reported to occur in 60% after two years [292] and as much as 90% after four years [166]. However a recent small study in Thailand found resistance in only 20% at four years [169].

Resistance to TDF has not been definitively described. Two coinfected patients were found to have acquired the reverse transcriptase mutation A194T after approximately one year of treatment, one on TDF as the only HBV-active drug and one while taking TDF plus 3TC and this mutation was found to lower HBV susceptibility to TDF *in vitro* by a factor of about 10 [171]. It has also been shown that A194T reduces the viral replication rate but that this can be overcome by the acquisition of mutations in the precore/core region [172]. However in other studies patients with A194T responded well to TDF [250, 293] and the mutation was not found to confer TDF resistance *in vitro* [249, 294].This mutation has also been found in patients without a history of exposure to TDF [295, 296]. The mutation R192P has also been put forward as a possible TDF resistance mutation [297]. It is located close to A194 and the switch from arginine to proline induces a large kink in what is otherwise a straight section of the reverse transcriptase protein and it is suggested that this causes resistance via a change in protein conformation.

There is limited published data on long-term follow-up of HBV/HIV coinfected individuals on treatment (chapter 3). DART provides long term follow-up of a large cohort of well-characterised HIV/HBV coinfected participants, and thus provides an

excellent opportunity to examine the durability of virological suppression on therapy including 3TC with or without TDF.

In the meta-analysis reported in chapter 3, TDF treatment was associated with suppression of plasma HBV viral load below the level of detection at a rate of 57%, 79% and 86% after 1, 2 and 3 years respectively. The meta-analysis only included patients treated with TDF so the effect of 3TC without TDF could not be examined. There was no additional benefit to using 3TC in addition to TDF, or any effect of prior exposure to 3TC. .

Aims

1. To determine the proportion of participants with HBV VL quantifiable at baseline that achieved HBV virological suppression at 48 weeks on first line HBV-active treatment.

2. To examine any association between the proportion suppressed and specific drug treatment.

3. To examine associations between the proportion suppressed and baseline characteristics including WHO stage, CD4 count, HBeAg status and HBV VL.

4. In a subset of participants with HBV VL quantifiable at baseline, to examine viral load dynamics between 4 and 48 weeks.

5. To examine durability of suppression and the rate of virological rebound.

6. To confirm that patients with suppressed HBV VL at baseline remained so during treatment.

6.2 Methods

6.2.1 HBV DNA viral load testing

HBV DNA viral loads were tested as described in chapter 4 at baseline, week 48 and the latest sample available before any change in HBV-active treatment (the last sample if there had been no such change) provided this was at week 96 or later. For participants who underwent STI, the eligible period for this HBV VL study was terminated at the onset of the first STI. In addition, samples from weeks 4, 12 and 24 were tested in participants at JCRC only, as shown in Figure 12.

6.2.2 Statistical Methods

Analyses in this chapter grouped participants treated with ABC and NVP as the focus is on drugs with potent activity against HBV (section 4.1.5). In view of the testing strategy described above, all analyses are "on treatment" rather than "intention to treat".

To look for evidence of bias, those tested were compared with those eligible but not tested using Fisher's exact test for categorical characteristics (including sex, site, baseline HBeAg status, baseline HBV VL higher or lower than 10^7 IU/mL and use of TDF) and using the chi-squared test for trend (Stata command ptrend) with ordinal variables (age, baseline WHO stage of HIV disease and CD4 cell count group), stratified by baseline characteristics.

Three different definitions of viral suppression were examined: (A) undetectable viral load, (B) viral load below the limit of quantification (12 IU/mL) and (C) viral load <1,000 IU/mL, to enable comparisons with earlier literature. In quantitative analyses, viral load was examined on a log 10 scale.

In participants with quantifiable HBV VL at baseline, HBV VL was examined at 48 weeks and at the latest time point using graphical methods. Associations with the proportion with HBV VL below the level of quantification were examined using Fisher's exact test and the chi-squared test for trend as above. Logistic regression was used to examine the association of undetectable HBV VL at 48 weeks with both HBeAg status and high/low baseline HBV VL together.

HBV VL suppression was examined over the first 48 weeks on treatment in participants at JCRC in both aggregate form and individually. Kruskal-Wallis equality-of-populations rank test was used to compare HBV VL in those treated with or without TDF at time points during the first 48 weeks.

All viral load rebounds, including subsequent VL values to examine re-suppression, are shown graphically. Adherence data was reviewed and participant histories examained to see if prior breaks in treatment of less than 30 days may have predisposed to HBV VL rise or if rises were associated with flares in ALT. The association of treatment with the proportion with rebound after achieving undetectable HBV was examined using Fisher's exact test.

6.3 Results

6.3.1 Baseline

308 participants (9.3%) were HBsAg positive at baseline, of whom 270 (87.7%) had sufficient sample for measurement of HBV DNA VL. Results of HBV VL at baseline are presented in chapter 5 and in Figure 19 (below).

Figure 19: Flowchart of samples tested for HBV VL at baseline
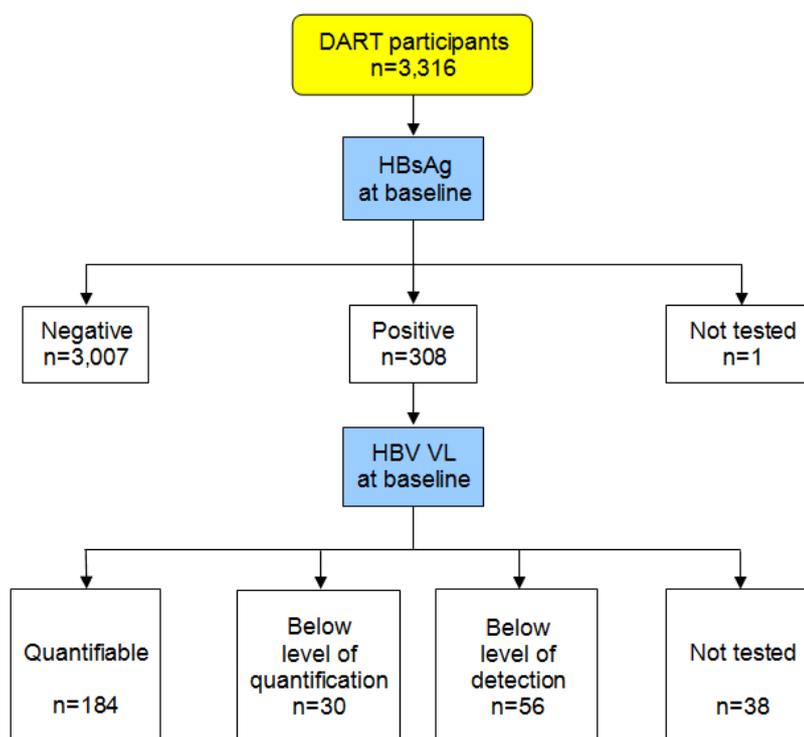
6.3.2 Outcomes of participants with quantifiable HBV VL at baseline

6.3.2.1 Samples tested

At baseline, 184 participants (67.5% of those tested) had a HBV VL result above the lower limit of quantification of whom 135 received a first line antiretroviral regimen that included TDF. Of these 184, 36 (19.6%) stopped, changed or interrupted either 3TC and/or TDF treatment before 48 weeks (Table 23). Of the remaining 148, 123 (83.1%) had HBV VL measured at 48 weeks.

Table 23: Participants with a change in treatment before week 48

n	Reason for treatment interruption / change before week 48
19	Died
4	STI at 24 weeks as part of the STI pilot study
3	Stopped due to adverse event
2	Switched NVP to TDF due to starting TB therapy
2	Switched NVP to TDF due to adverse event
2	Interrupted due to participant unable to attend
2	Stopped due to patient decision
1	Switched to second line therapy
1	Switched due to pregnancy

A further 26 participants died, switched or interrupted treatment between 48 and 96 weeks (Table 24).

Table 24: Participants who changed treatment between weeks 48 and 96

n	Reason for treatment interruption / change between weeks 48 and 96
2	Died
13	STI at 52 weeks as part of the STI study
4	STI at 76 weeks as part of the STI study
4	Switched to second line therapy
1	Switched NVP to TDF – reason not given
1	Interrupted due to participant unable to attend
1	Stopped due to patient decision

Of the remaining 122 participants, only 70 (57.4%) had a test at or after 96 weeks, primarily due to difficulties with assay procurement in Zimbabwe.

6.3.2.2 Length of follow-up

In this chapter, "follow-up" refers to the time from baseline until the last HBV VL result available. This may be significantly shorter than the time a participant was followed up in DART.

In those with quantifiable HBV VL at baseline, median follow-up was 48 weeks and total follow-up was 19,328 participant weeks, 14,299 of which were on TDF. Follow-up was 0 weeks for 42, 4 to 24 weeks for 14, 48 weeks for 58 and ranged from 96 to 276 weeks for 70 with 62 (45 on TDF) having at least 192 weeks and 41 (36 on TDF) at least 240 weeks of follow-up.

6.3.2.3 Baseline demographics

Baseline demographics, apart from site, did not significantly predict whether or not a participant had a HBV VL test at week 48 or end of treatment (Table 25). As stated above, in Harare more samples were found at 48 weeks but assays ran out before testing at the end of treatment could be completed.

Table 25: Baseline demographics of participants with quantifiable baseline HBV viral load testing/not testing at week 48 and at or after 96 weeks

		Week 48			At or after week 96		
		Tested		p	Tested		p
		n	%		n	%	
All		123	83.1		70	57.4	
Site	Entebbe	15	57.7	<0.001	14	70.0	0.005
	JCRC	36	81.8		30	73.2	
	Harare	72	92.3		26	42.6	
Sex	Male	58	79.5	0.28	36	55.4	0.72
	Female	65	86.7		34	59.6	
Age	18-30	25	89.3	0.42	14	65.0	0.57
	30-35	29	82.4		15	53.6	
	35-40	27	81.8		18	58.6	
	40-45	26	78.1		13	48.1	
	45-50	10	83.3		8	70.0	
	>50	6	83.3		2	40.0	
WHO Stage	2	23	79.3	1.00	15	60.0	0.32
	3	76	85.4		44	59.7	
	4	24	79.3		11	45.8	
Baseline CD4	<50	40	87.0	0.77	21	53.8	0.48
	50-99	30	72.5		19	52.9	
	100-149	28	87.5		17	63.0	
	150-199	25	86.2		13	60.0	
Monitoring strategy	LCM	66	88.0	0.13	36	61.0	0.47
	CDM	57	78.1		34	54.0	
Initial drug regimen	TDF	90	84.1	0.82	50	56.8	0.22
	ABC	11	78.6		10	47.6	
	NVP	22	81.5		10	76.9	

6.3.2.4 Virological suppression

HBV VL at 48 weeks

Figure 20 shows log(VL) at week 48 plotted against baseline log(VL) in 123 participants. Values "below the level of quantification" have been set to 12 IU/mL and values "below the level of detection" to 1 IU/mL for the purpose of illustration.

Figure 20: Log(HBV viral load) at week 48 against baseline
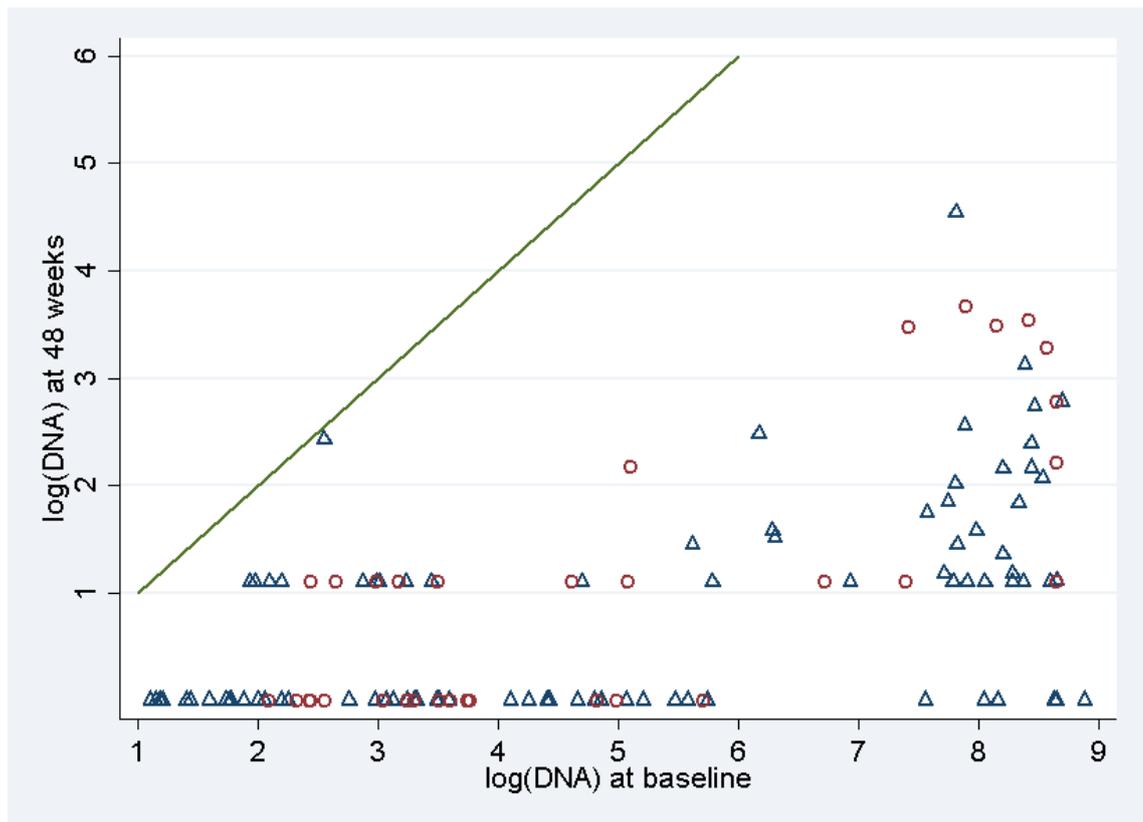
Participants treated with 3TC without TDF: red circles.
Participants treated with 3TC and TDF: blue triangles.

A single participant with baseline HBV VL above the limit of quantification failed to achieve a decline of at least 2 log in HBV VL (or to below the level of quantification) after 48 weeks on treatment. This participant had baseline HBV VL of 352 IU/mL, with a similar value at 48 weeks (265 IU/mL) within the range of assay variability. The participant was treated with TDF and reported good adherence.

Overall, 91 (74.0%) of 123 participants had a VL at week 48 that was either undetectable (n=63; 51.2%) or detectable below the level of quantification (n=28; 22.8%) and this was more likely in those with low baseline HBV VL and those who were HBeAg negative. In the 82 (66.7%) with baseline HBV VL below 10^7 IU/mL, VL at week 48 was below 12 IU/mL or undetectable in 76 (92.7%) compared to 15 (37%) in the 41 participants with baseline HBV VL above 10^7 IU/mL (p<0.001). Baseline HBeAg results were available in 116. HBV VL at week 48 was undetectable or below the level of quantification in 63 (93%) of 68 HBeAg negative participants compared with 22 (46%) of 48 HBeAg positive participants (p<0.001).

Using the other viral load cut-offs described in methods (undetectable and below 1,000 IU/mL), both negative HBeAg status and low baseline HBV VL remained predictive of having an undetectable HBV VL at 48 weeks (Table 26).

Table 26: Viral load suppression at 48 weeks by HBeAg and baseline HBV VL

| | | | Week 48 HBV viral load | | | | | | | | | |
| | | | A | | | B | | | C | | |
		N	n	%	P	n	%	p	N	%	p
All		123	63	51.2		91	74.0		116	94.3	
HBeAg	Negative	68	45	66.2	<0.001	63	92.6	<0.001	68	100.0	<0.001
	Positive	48	15	31.3		22	45.8		42	87.5	
BL VL (IU/mL)	<10^7	82	56	68.3	<0.001	76	92.7	<0.001	82	100.0	<0.001
	≥10^7	41	7	17.1		15	36.6		34	82.9	

VL: viral load. BL: baseline.
(A) undetectable viral load
(B) viral load below the limit of quantification (12 IU/mL)
(C) viral load <1,000 IU/mL

As described earlier, baseline HBV VL was higher in those with detectable HBeAg (chapter 5, Figure 16). In those with baseline HBV VL below 10^7 IU/mL, the proportion having an undetectable HBV VL at week 48 was similar between those HBeAg negative (42 of 60, 70%) and those HBeAg positive (11 of 17, 65%; p=0.77). In those with HBV VL above 10^7 IU/mL, the difference by HBeAg status was again not statistically significant (3 of 8, 38%, *vs.* 4 of 31, 13%; p=0.14).

There was no association between undetectable HBV VL at week 48 and baseline CD4 status (p=0.94), WHO stage (p=0.14) or initial drug regime; 48 (53%) of 90 participants treated with TDF and 3TC had undetectable HBV VL compared to 15 (45%) of 33 participants treated with 3TC alone (p=0.54).

In a multivariable logistic regression model including HBeAg status, baseline HBV VL, drug treatment, WHO stage and baseline CD4 group, baseline HBV VL remained significant (OR 0.12, p<0.001) but HBeAg status did not (OR 0.63, p=0.34). Drug treatmend had an adjusted OR of 1.75 but this was not significant (p=0.26) Advanced stage of HIV disease had an OR of 0.27 but again this was not significant (p=0.13) (Table 27).

Table 27: Predictors of viral load suppression at 48 weeks

	All	Undetectable VL		OR	p	aOR	95% CI	p
	n	n	%					
All	123	63	51.2					
HBeAg					0.001			0.59
Negative	68	45	66.2					
Positive	48	15	31.3	0.23		0.77	0.26 to 2.27	
Not done	7	3	42.9	0.38		0.40	0.06 to 2.57	
Baseline DNA					<0.001			<0.001
Low	82	56	68.3					
High	41	7	17.1	0.10		0.07	0.02 to 0.22	
Drug treatment					0.44			0.54
No TDF	33	15	45.5					
TDF	90	48	53.3	1.37		1.75	0.65 to 4.70	
WHO Stage					0.11			0.13
2	23	13	56.5					
3	76	42	55.3	0.95		0.92	0.29 to 2.90	
4	24	8	33.3	0.38		0.27	0.06 to 1.25	
Baseline CD4					0.94			0.27
<50	40	21	52.5					
50-99	30	15	50.0	0.90		0.89	0.26 to 3.04	
100-149	28	14	50.0	0.90		0.30	0.09 to 1.07	
150-199	25	13	52.0	0.98		0.52	0.15 to 1.86	

In those with baseline HBV VL $>10^7$ IU/mL, treatment with 3TC alone was associated with a smaller decline in VL at 48 weeks (-5.5 $\log_{10}$ IU/mL for 3TC alone *vs.* -6.5 $\log_{10}$ IU/mL for 3TC with TDF, p=0.01). While in participants with low baseline HBV VL there was no association of treatment with the proportion suppressed, in those with baseline HBV VL $>10^7$ IU/mL more suppressed when treated with TDF (Table 28).

Table 28: VL suppression at 48 weeks by baseline HBV VL and drug treatment

Baseline viral load	Drug treatment		Week 48 HBV viral load								
			A			B			C		
		N	n	%	p	n	%	p	n	%	p
All	3TC	33	15	45.5	0.54	25	75.8	1.00	28	84.8	0.02
	3TC+TDF	90	48	53.3		66	73.3		88	97.8	
$<10^7$ IU/mL	3TC	24	15	62.5	0.60	23	95.8	0.67	24	100.0	1.00
	3TC+TDF	58	41	70.7		53	91.4		58	100.0	
$\geq10^7$ IU/mL	3TC	9	0	0.0	0.32	2	22.2	0.45	4	44.4	0.003
	3TC+TDF	32	7	21.9		13	40.6		30	93.8	

(A) undetectable viral load
(B) viral load below the limit of quantification (12 IU/mL)
(C) viral load <1,000 IU/mL

Last evaluable HBV viral load

Samples were tested at a time at least 96 weeks after treatment initiation in 70 participants with quantifiable HBV VL at baseline, 65 of whom also had a test at 48 weeks. These samples were taken at times ranging from 96 to 276 weeks after treatment initiation, as described above. HBV VL was undetectable in 48 (69%), detectable below the level of quantification in 9 (13%) and quantifiable in 13 (19%).

There was no association between the proportion with undetectable HBV VL and HBeAg status at baseline (HBeAg positive 18 of 28, 64% *vs.* HBeAg negative 28 of 39, 72%; p=0.60) but there was an association with baseline HBV VL (baseline HBV VL $<10^7$ IU/mL 35 of 45, 78% *vs.* VL $\geq10^7$ IU/mL 13 of 25, 52%; p=0.03). There was no association with drug treatment (3TC alone 12 of 20, 60% *vs.* 3TC with TDF 36 of 50, 72%; p=0.40).

62 (89%) of 70 had HBV VL less than 1,000 IU/mL. There was an association between HBV VL below 1,000 IU/mL and treatment group with 15 (75%) of 20 treated with 3TC alone and 47 (94%) of 50 treated with 3TC with TDF achieving suppression below 1,000 IU/mL (p=0.04).

17 participants with quantifiable HBV VL at 48 weeks had a subsequent test after 96 weeks. Figure 21 shows an apparent effect of treatment. HBV VL increased in all 4 participants treated with 3TC alone compared with only 3 (23%) of 13 in those treated with TDF (p=0.02). All participants with rises in HBV VL after week 48 reported good

adherence; none reported having missed a dose of antiretroviral treatment in the month before the last measurement.

Figure 21: Evolution of HBV viral load in participants with quantifiable HBV VL at 48 weeks
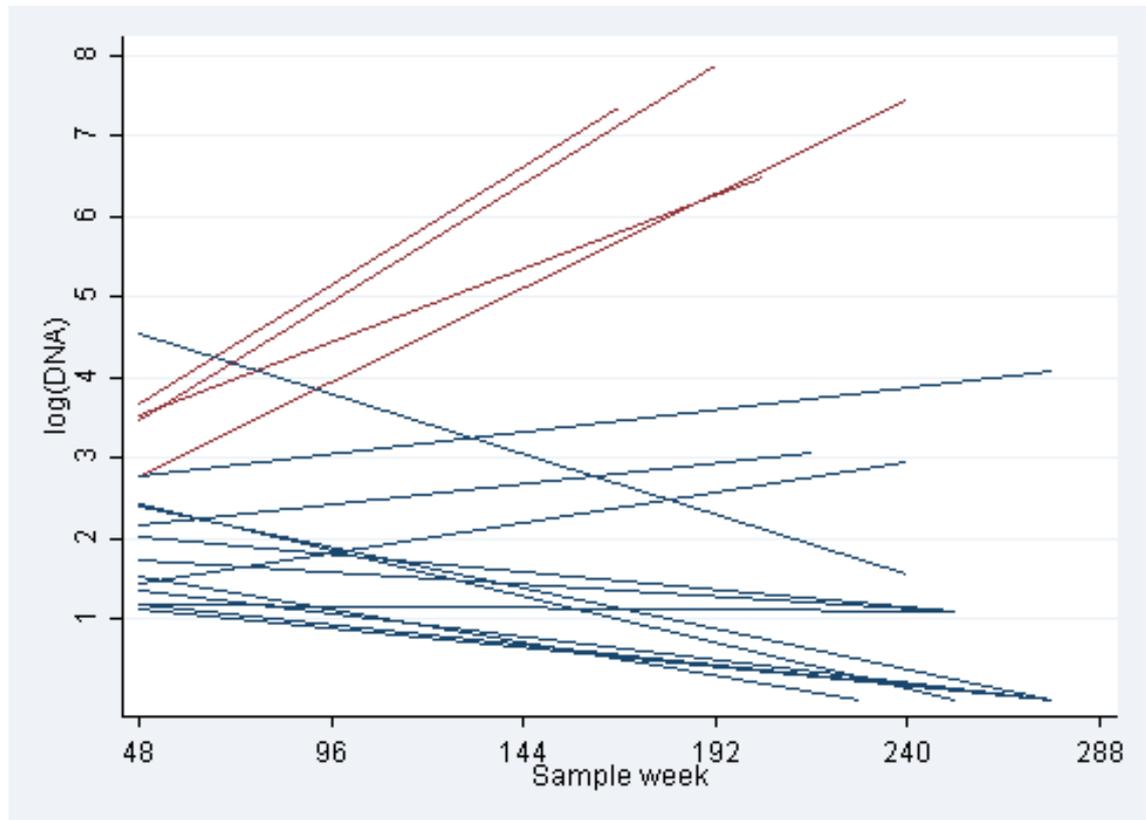

Participants treated with 3TC without TDF: red.
Participants treated with 3TC and TDF: blue.

Baseline HBeAg results were available for 16 of the 17 shown in Figure 21; 1 (33%) of 3 HBeAg negative and 5 (38%) of 13 HBeAg positive participants had undetectable HBV VL at the last result (p=1.0). There was also no association between final HBV VL and baseline HBV VL (p=0.52).

Of 53 participants with either VL below the level of quantification (n=18) or undetectable VL (n=35) at 48 weeks, only 5 had quantifiable VL at their last measurement (Figure 22 and Figure 23). HBV VL at or after 96 weeks was quantifiable in 2 treated with 3TC without TDF and 3 treated with both 3TC and TDF. Viral load when quantifiable was generally low (HBV DNA less than 10,000 IU/mL).

Figure 22: Subsequent HBV VL in those with HBV VL detectable below the level of quantification at 48 weeks
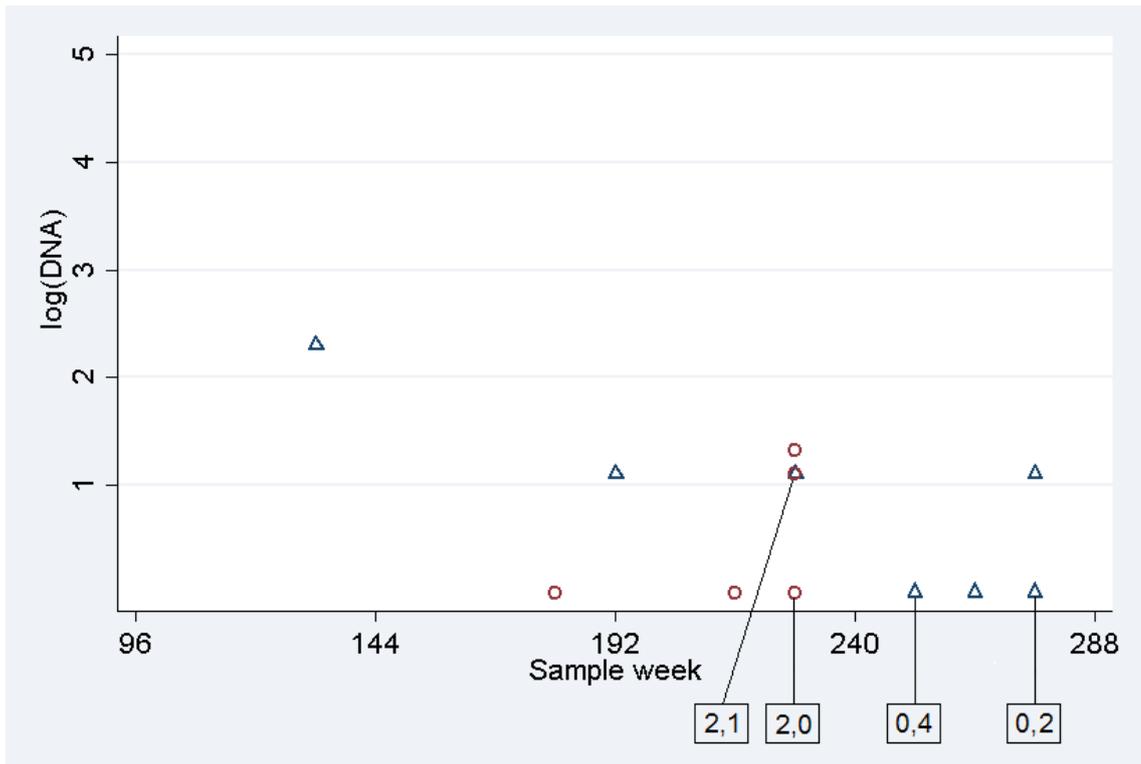
See below for legend.

Figure 23: Subsequent HBV VL in those with undetectable HBV VL at 48 weeks
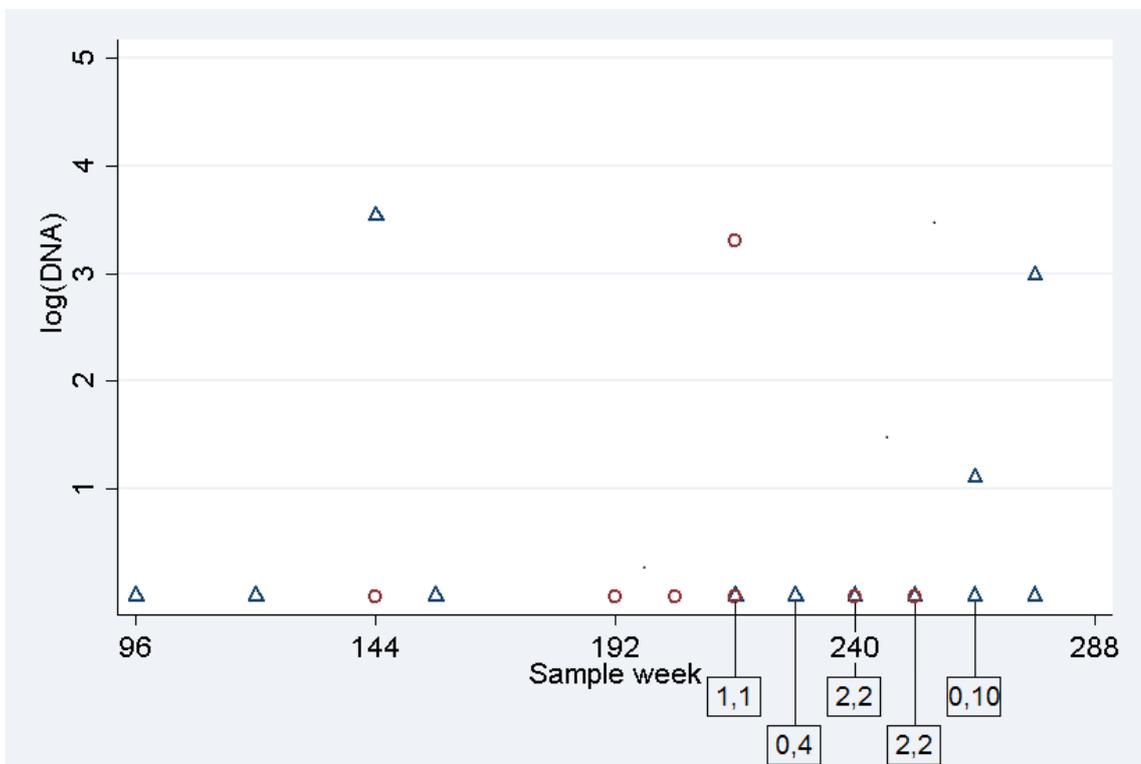
Participants treated with 3TC without TDF: red circles.
Participants treated with 3TC and TDF: blue triangles.
Overlapping markers: N,N indicates number of participants treated with 3TC alone, 3TC with TDF.

Thus, the proportion of those with undetectable HBV VL at 48 weeks that maintained suppression at or after 96 weeks was 89%, with only 9% having a quantifiable HBV VL (Table 29).

Table 29: Subsequent HBV VL in those with HBV VL undetectable or detectable below the level of quantification at 48 weeks

HBV VL at 48 weeks	HBV VL at or after 96 weeks						
	Undetectable		Detectable BLQ		Quantifiable		Total
	N	%	N	%	N	%	N
BLQ	11	61.1	5	27.8	2	11.1	18
Undetectable	31	88.6	1	2.9	3	8.6	35

As with the analysis at week 48, there was no association between baseline CD4 cell count or WHO stage and detectable HBV VL at or after week 96 (p=0.80 and p=0.34).

HBV VL during the first 48 weeks of treatment

Participants at JCRC with quantifiable HBV VL at baseline underwent additional testing during the first 48 weeks on treatment (Table 28).

Table 30: HBV VL tests over first 48 weeks (JCRC only)

Week	Not died/ switched	Tested	
		N	%
0	59	59	100
4	58	36	62.1
12	54	34	63.0
24	48	35	72.9
48	44	36	81.8

Mean log(HBV VL) fell from 5.9 at baseline to 1.4 at week 48. Again 12 IU/mL was used for values "below the level of quantification" and 1 IU/mL for values "below the level of detection". There was no difference in the pattern of decline of HBV VL between those treated with 3TC alone and those treated with 3TC with TDF (Figure 24).

Figure 24: Mean log(HBV VL) over the first 48 weeks
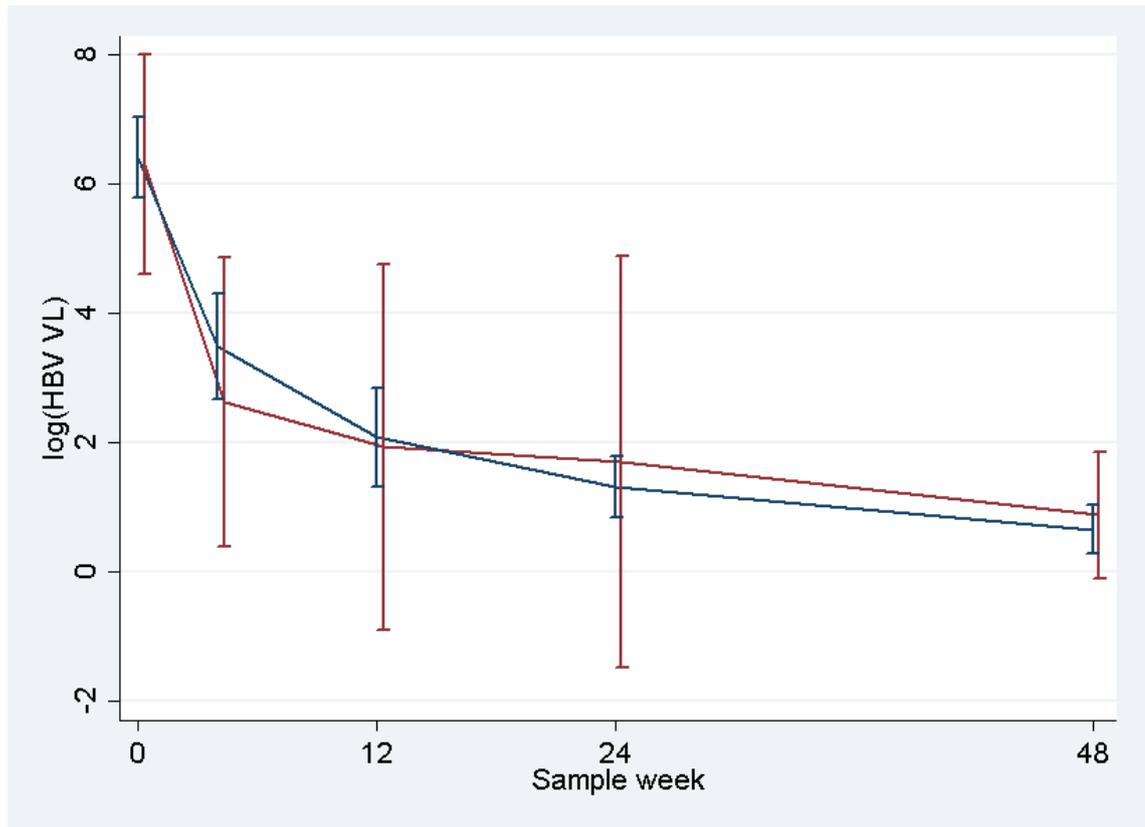
Participants treated with 3TC without TDF: red.
Participants treated with 3TC and TDF: blue.

The proportion with undetectable HBV VL increased with time: 11%, 38%, 40% and 61% at 4, 12, 24 and 48 weeks respectively (Table 31). Again, there was no evidence that this was affected by treatment. Overall, 31 (53%) participants achieved an undetectable HBV VL at some time during the first 48 weeks.

Table 31: HBV VL results over first 48 weeks (JCRC only)

| | 3TC without TDF | | | 3TC with TDF | | | All | | | |
| | | Undetectable | | | Undetectable | | | Undetectable | | |
Week	N	n	%	N	N	%	N			p
0	11			48			59			
4	6	1	16.7	30	3	10.0	36	4	11.1	0.54
12	7	4	57.1	27	9	33.3	34	13	38.2	0.39
24	6	3	50.0	29	11	37.9	35	14	40.0	0.66
48	9	5	55.6	27	17	63.0	36	22	61.1	0.71
Any	11	7	63.6	48	21	43.8	59	31	52.5	0.32

6.3.2.5 Virological rebound on treatment

25 participants had an increase in HBV VL while on HBV-active treatment (Figure 25). Of these, 15 were treated with 3TC and TDF and 10 were treated with 3TC alone. 15 were HBeAg negative and 10 HBeAg positive at baseline. 23 participants had a single rise in HBV VL (1 at each time point unless stated) occurring at weeks 4 (n=2), 12 (n=3), 24 (n=3), 48 (n=4), 132, 168, 192, 204, 216 (n=2), 240 (n=2), 264 and 276 (n=2); 2 participants had 2 rises each at weeks 4 and 48 and at weeks 24 and 144.

Figure 25: HBV VL over time in participants experiencing a rise in HBV VL
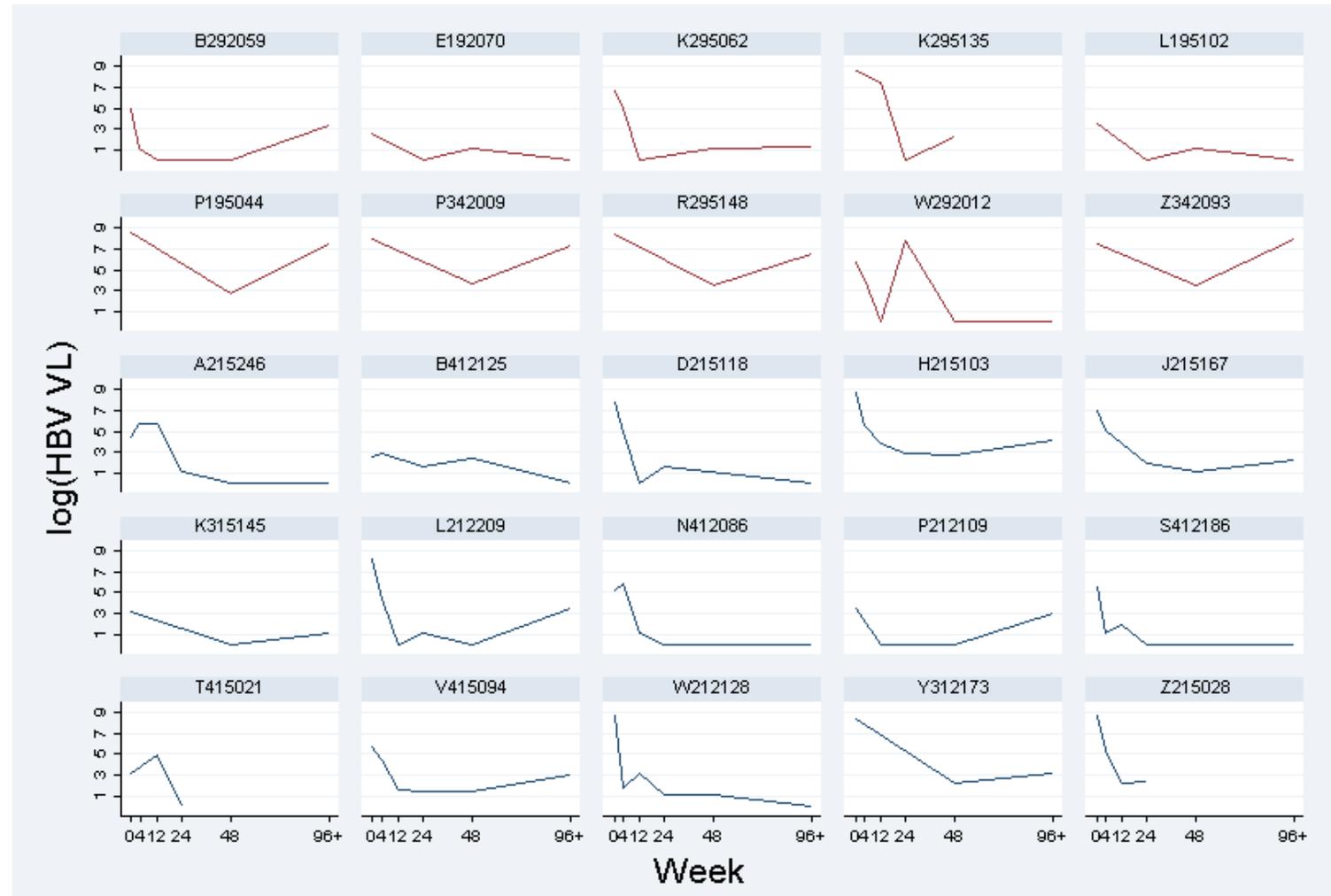
Participants treated with 3TC without TDF: red. Participants treated with 3TC and TDF: blue.
All results at or after 96 weeks shown at 96 weeks for ease of comparison.

A rise by a factor of ten (1 log) is usually taken as the definition of virological rebound or breakthrough; 6 rises were only from HBV VL being undetectable to being below the level of quantification, 6 were by less than 1 log, 7 by between 1 and 2 log, 2 between 2 and 3, 1 between 3 and 4, 2 between 4 and 5 and 1 by over 6 log.

4 (17%) of 23 had rebound at 48 weeks after having previously achieved full suppression. A further 4 (11%) of 35 had undetectable HBV at 48 weeks with rebound later during follow-up which was to at least 192 weeks after initiation in 30 (22 treated with TDF) and to at least 240 weeks in 21 (17 treated with TDF). Treatment was associated with rebound at 48 weeks (TDF: 0 of 14, 0% *vs.* 3TC: 4 of 9, 44.4%; p=0.01) but not after (3 of 26, 11.5% *vs.* 1 of 9, 11.1%; p=1.0).

As discussed in the methods, treatment interruptions of 30 days or more were interpreted as a change in treatment. However some participants had breaks in treatment of less than 30 days which could conceivably result in viral load rebound. Of the participants with a rise in HBV VL, 7 had a break followed by a rise. These are shown in Table 32.

Table 32: Rises in HBV VL coming immediately after treatment interruptions of less than 30 days

Duration of interruption (days)	Week			HBV VL	
	Test before	Interruption start	Test after	Before	After
7, 6, 6, 8	48	124, 204, 228, 252	264	UD	BLQ
5	24	4	48	UD	BLQ
7	12	5	24	UD	BLQ
4	48	256	276	UD	954
5	0	168	252	UD	BLQ
14	0	8	12	1,352	71,512
14	48	199	216	146	1,190

Eleven (37.9%) of the 29 participants with a rise in HBV VL subsequently suppressed such that the last HBV VL measured was undetectable, and a further 6 had a final HBV VL that was detectable but below the level of quantification.

Only 1 rise in HBV VL was associated with a rise in ALT to above the ULN. This was grade 3 (i.e. >5x ULN [181]) and resolved on treatment. The participant had HBV VL of 4.4 x10^8 IU/mL at baseline, which fell to 53 IU/mL at week 4. Baseline ALT was 34 IU/L and at week 4 was 33 IU/L but rose to 222 IU/L at week 12 before falling to 36 IU/L at week 24 and remaining below 36 IU/L until the end of follow-up. HBV VL rose to 1,400 IU/mL at week 12 and fell to detectable below the level of quantification at week 24. The final HBV was undetectable at week 276.

6.3.3 Participants with baseline HBV below the level of quantification

A test was performed at the end of follow-up in 31 (82%) of 38 participants with baseline HBV VL below the level of quantification and in 20 (36%) of 56 with undetectable baseline HBV VL. 46 (90%) had undetectable HBV VL and the remaining 5 (10%) had HBV VL detectable below the level of quantification. No participants had quantifiable HBV VL. Of those with undetectable HBV VL at baseline, 1 had a result that was detectable below the level of quantification at 252 weeks. This participant was treated with TDF and reported very poor adherence, only taking between 20 and 50% of prescribed doses within the preceding 3 months.

6.3.4 Participants with baseline detectable HBV VL

In section 6.3.2 above, the analysis of HBV VL suppression was restricted to those with quantifiable HBV VL at baseline. However many published studies examine HBV VL suppression in all participants with detectable HBV VL at baseline, i.e. including both those with quantifiable, and those with detectable HBV VL but below the level of quantification, for example many of those studies included in the meta-analysis (chapter 3). The analysis was therefore repeated with this wider entry criterion.

As stated above, in the DART study 214 participants had detectable HBV VL at baseline: of these 143 (66.8%) had a test at 48 weeks. 81 (56.6%) had undetectable HBV VL at 48 weeks. The probability of undetectable HBV VL was associated with baseline HBV VL less than or greater than 10^7 IU/mL (72.6% *vs.* 17.1%, p<0.001) and baseline HBeAg status (72.4% *vs.* 30.6%, p<0.001) though in a logistic model, while baseline HBV VL remained significant (OR 8.7, p<0.001), HBeAg status was no longer significant (OR 2.1, p=0.10). Treatment with TDF was not associated with the probability of undetectable HBV VL (p=0.45) whereas HBV VL <1,000 IU/mL was more likely in those treated with TDF (79.8% *vs.* 59.0%, p=0.02).

55 (69.6%) of 79 with a test at or after 96 weeks had undetectable HBV VL which was not associated with HBeAg status at baseline (p=0.31) or use of TDF (p=0.79) whereas an undetectable HBV VL was more likely in those with low HBV VL at baseline (78% *vs.* 52%, p=0.03).

6.4 Discussion

This study provides a significant addition to the published data on HBV suppression in HIV/HBV coinfected patients treated with TDF, as seen in the meta-analysis in chapter 3. Previous studies included in the meta-analysis reported a total of 550 patients with each study reporting a maximum of 78 patients (Table 7). Collectively, while 516 were tested at 1 year, only 90 were tested at 4 years and 48 at 5 years (Table 8).

Considering those with HBV VL above 48 IU/mL at baseline we found that, after 48 weeks of treatment, HBV VL was undetectable in 15 (45%) of 33 participants treated with a first-line regimen containing 3TC without TDF and in 48 (53%) of 90 participants treated with 3TC and TDF (p=0.54). The proportion of those treated with TDF achieving undetectable HBV VL at 48 weeks is similar to the 110 (63.2%) of 174 individuals with detectable HBV VL at baseline included in the meta-analysis (p=0.15). When comparing with other studies, assay cut-off is important since when using a higher cut-off samples with low levels of HBV will be reported as undetectable. Cut-off was lower in the DART study than in most of the studies included in the meta-analysis. For example, of the studies in the meta-analysis, the two that gave data on the largest number of participants at 1 year included 24 and 28 participants and found 63% and 43% fully suppressed, respectively [139, 230]. In these studies the cut-offs used were about 200 IU/mL (about 1,000 copies/mL) and 20 IU/mL respectively. Many of those in DART with detectable HBV VL at 48 weeks had low level viraemia; using a cut-off of 1,000 IU/mL, the proportion suppressed on treatment with TDF was 98%.

There was no association between use of TDF and the proportion suppressed at 48 weeks (45% *vs.* 53%, p=0.54) or at the end of follow-up (60% *vs.* 72%, p=0.40). We thus found no evidence that treatment with TDF and 3TC is more likely to suppress HBV than treatment with 3TC alone. There was also no evidence of TDF resulting in quicker suppression with a similar proportion of each group achieving an undetectable HBV VL at each time point during the first 48 weeks.

Only 2 previous studies directly compare the HBV response to treatment with 3TC *vs.* 3TC with TDF in HIV coinfected patients. Study 903 enrolled naive patients in Western Europe, North America and Australia with HBV DNA >10^6 copies/mL and randomised to treatment with EFV plus 3TC and either D4T or TDF [144]. At week 48, HBV viral suppression (HBV DNA <1,000 copies/mL) was achieved by 1 (17%) of 6 treated with 3TC and D4T and 4 (80%) of 5 treated with 3TC and TDF (p=0.08). Mean DNA declined by 3.0 log *vs.* 4.7 log, respectively (p=0.06). In TICO, 36 naive patients in Thailand were randomised to receive EFV plus one of: AZT with 3TC (n=13), AZT with TDF (n=12) or 3TC with TDF (n=11) [114, 147]. Median change in HBV DNA was 4.07

log, 4.57 log and 4.73 log, respectively (p=0.70). 6 (46%) of 13, 9 (75%) of 12 and 7 (64%) of 11 suppressed HBV DNA to <170 copies/mL at week 48 (p=0.65). Using a cut-off of 1,000 copies/mL the proportions suppressed were 46%, 92% and 91% (p=0.013). The subject who failed to suppress to <1,000 copies/mL on 3TC and TDF and 1 of those on TDF alone had been lost to follow-up while the other on TDF alone had a fall followed by a rise in HBV DNA at the same time as a rebound in HIV VL. Poor adherence or malabsorption were suggested. There was no difference in HBV viral dynamics by treatment over 48 weeks. These results are comparable to those in DART. Although we found a higher rate of suppression in those treated with TDF this was only significant when using 1,000 IU/mL as the cut-off.

One important question in clinical practice, in those regions where HBV VL monitoring is available, is what to do when HBV VL does not suppress after a period on treatment; should treatment be continued or switched? In this study the number of participants who failed to suppress at 48 weeks and had a subsequent test is low (4 on 3TC and 7 on 3TC and TDF) and so conclusions must be drawn with caution. The data suggests that if a patient is treated with 3TC as the only active drug and fails to suppress at 48 weeks then continuing the same treatment will not lead to suppression. For patients treated with 3TC and TDF, 10 (77%) had HBV VL that continued to decline on continuing the same treatment and only 3 (23%) had a rise in HBV VL. In view of the lack of prior demonstration of HBV resistance to TDF and of evidence for another strategy it may be argued that continuing 3TC and TDF is reasonable.

An undetectable HBV VL is a surrogate marker for treatment success. When managing patients we are in fact concerned about disease progression and complications. Since HBV-related disease tends to occur over many years as liver fibrosis progresses, it may be that durability of suppression (i.e. whether a successful suppression of HBV is maintained over time or whether rebound in VL occurs, whether through development of antiretroviral resistance or by another mechanism) is more important than achievement of undetectable VL. It may be the case that a brief period of undetectable VL followed by rebound may not inhibit development of liver-related morbidity and mortality. Of those with fully suppressed HBV replication (undetectable VL) at 48 weeks and a subsequent test follow-up was of at least 192 weeks in 30 and 240 weeks in 21. Even more surprisingly than the similarity of the response to 3TC treatment with and without TDF, we found that suppression of HBV was as durable when treatment was with 3TC as the only HBV-active drug as with TDF and 3TC used in combination (p=1.0). Almost 90% of those treated with 3TC alone and with an undetectable VL at 48 weeks maintained this through until the end of treatment. This is surprising as high rates of HBV resistance development have previously been described in coinfected

individuals treated with 3TC alone. For example, Benhamou found only 9% of coinfected patients treated 3TC alone had fully suppressed HBV at 4 years [166]. Our results are more in keeping with the much lower rate of resistance seen in a more recent study from Thailand [169].

Rebound, when it occurred, was very rarely associated with a flare in ALT. Only one participant had a rise in ALT above the ULN at the same time as a rise in HBV VL and this resolved on treatment. Subsequent HBV VL was undetectable. This rise in ALT occurred early after treatment initiation (at 12 weeks) and so may have been related to adverse drug reaction rather than being as a result of HBV rebound.

Similarly to previous data (chapter 3, Figure 8), we found that suppression at 48 weeks was more likely in those HBeAg negative at baseline (66% *vs.* 31%, p<0.001), but that the proportion with suppressed HBV was similar later (72% *vs.* 64%, p=0.60). This may be largely explained by the fact that baseline HBV VL tends to be much higher in HBeAg positive individuals (chapter 5, Figure 16). Few participants had negative HBeAg with high baseline HBV VL (n=8) or positive HBeAg with low baseline HBV VL (n=17). Although the OR for the effect of HBeAg status on HBV VL suppression when controlling for baseline HBV VL was 1.58, this may be due to chance (p=0.34) and the observed effect of HBeAg driven by HBV VL.

As all participants received antiretroviral combinations that included 3TC, we are unable to assess outcomes when TDF is given as monotherapy. A consideration in comparing participants treated with and without TDF is that while this was a prospective study, allocation to treatment with TDF was not randomised and so any apparent difference in response may be subject to selection bias.

As described earlier, the design of the hepatitis substudy was such that analyses are "on treatment" rather than "intention to treat". Thus we could postulate that participants with more advanced disease would either die or switch due to treatment failure and that this would bias the population tested at times after initiation, giving an inaccurately high degree of treatment success. However, we found no evidence of baseline characteristics (apart from site) being associated with the chance of being included or not (untested) at 48 weeks or after. The association with site was due to the order in which testing was performed at each location and to the availability of stored samples and assays.

In the DART study all participants had advanced HIV disease with CD4 cell count less than 200 cells/mm^3 and results may not be applicable to patients started on treatment with less advanced immunosuppression, as guidelines recommend.

6.4.1 Conclusion

Approximately half of the participants achieved an undetectable HBV VL at 48 weeks and two-thirds by the end of treatment and there was no statistically significant difference between those treated with or without TDF. Baseline HBeAg status and HBV VL were predictive of the probability of achieving an undetectable HBV VL, although the association with HBeAg was at least partly explained by higher HBV VL in those testing HBeAg positive.

Once achieved, suppression of HBV VL to undetectable levels was maintained to the end of treatment in the vast majority of participants and again 3TC performed as well as 3TC plus TDF. These results do not support the consensus that treatment of HBV/HIV coinfected patients with 3TC as the only HBV-active drug is inadequate for reasons of poor durability. Participants in whom HBV VL was fully suppressed after 48 weeks on 3TC alone were as likely to have remained suppressed to the end of the study as those treated with TDF, while those on 3TC alone that failed to supress at 48 weeks all had an increase in HBV VL over time.

We found no evidence of HBV VL rebound being associated with a rise in ALT. Thus monitoring of ALT is unlikely to be useful in predicting HBV virological treatment failure.

7 Liver inflammation and fibrosis

7.1 Introduction

The DART trial provides a useful opportunity to examine the effect of HBV on the liver in HIV-positive individuals and to determine the incidence and consequences of liver injury associated with initiating, continuing and interrupting highly active antiretroviral therapy (HAART) in HBV/HIV coinfected participants.

A liver inflammatory flare is an acute worsening of liver disease and is marked by a rise in transaminases (ALT and AST). Studies have found that up to half of all HBV-coinfected patients starting HAART experience a liver flare, but interestingly data from Africa indicate much lower rates (Table 3). The aetiology of flares occurring on initiation of HAART is unclear and may be due to adverse drug reactions to components of HAART, to improvement in immune status (IRD) or even as a response to HBV viral suppression. Liver flares may also occur in other situations, of note (i) on HBV rebound, whether due to virological breakthrough or treatment interruption, (ii) as a result of reaction to other drugs, particularly drugs used to treat TB and (iii) on HBeAg seroconversion.

In patients coinfected with HBV and HIV the risk of liver damage on commencing HAART has been shown to be higher than in those with HIV alone [85, 178, 179, 187, 191, 193] although some studies have not found this to be the case [182, 190, 195]. However previous estimates of the rate of significant liver damage have varied and studies have been limited by the use of different case definitions and low numbers of patients, particularly in Africa.

Aims

1. To determine the baseline liver status of participants in the DART study and examine associations with other characteristics.
2. To determine the change in ALT and rate of liver inflammatory flares:
   a. on first-line HAART,
   b. on switching to second-line ART with change in HBV treatment and
   c. during Structured Treatment Interruption cycles.

7.2 Methods

7.2.1 Participants and samples

It is of note that although baseline ALT over five times the ULN was an exclusion criteria in DART, two participants with ALT greater than five times the local ULN were enrolled into DART; one had baseline ALT of 198 (ULN 37) and the other had baseline ALT of 332 (ULN 44).

Participants were excluded from all analyses if HBsAg was not tested. According to the protocol, ALT was tested at baseline, at weeks 4 and 12, and every 12 weeks thereafter. As with all tests, results were returned to the clinician if the patient was in the LCM arm. Results in the CDM arm were returned only if they had been requested for a clinical reason or if there was grade 4 toxicity (ALT >10 x ULN). Samples from 17 participants were also taken at 2 weeks. Over and above testing once at weeks 0, 4, 12 and 12-weekly thereafter, 2,885 extra tests were done in a total of 1,213 participants. Since we were interested in ALT as a marker for the occurrence of inflammation, all available results were included. In the DART structured treatment interruption (STI) substudy ALT was measured 8 weeks after treatment changes. In participants with more than one result at the time of an analysis, the highest was used. Week 2 results (which in no cases were materially higher than the corresponding week 4 result) were dropped except for 4 participants with no week 4 result, when the week 2 result was used as the week 4 result in the analyses. Data were censored at the first change in treatment combination, excluding breaks of less than 4 weeks and switches from AZT to stavudine (D4T).

The effect of stopping TDF was examined in all participants with ALT results after a switch from a TDF-containing regimen to second line HAART that contained neither TDF nor 3TC.

Most single, isolated high ALT values are likely to have been the result of data entry errors. We therefore deleted observations if ALT was five times greater than the preceding and following ALT result, except if (i) AST measured at the same time was greater than 1.5 times the ULN, or (ii) a participant had more than one such isolated high ALT value. A total of 36 (0.05%) of 70,066 ALT results were dropped following these rules.

7.2.2 Definition of a flare

The three laboratories used different ULN for ALT; JCRC used 40 IU/L, Harare used 44 IU/L and Entebbe used 37 IU/L for females and 55 IU/L for males. Where ULN is referred to in analyses it is the local ULN that was used for participants at each site.

Previous studies of flares in HBV/HIV coinfection have used a variety of definitions of a flare (Table 3). For simplicity a single value for males and females across all three sites of 200 IU/L, with a rise of at least 100 IU/L from baseline, was used to define a flare. For the analysis of flares after a switch to second line HAART "baseline" was time of switch. A flare was considered to have resolved if ALT decreased to less than 40 IU/L.

7.2.3 Fibrosis scoring methods

Of the components of serum fibrosis markers that have been used, those available in all DART participants are platelets, ALT, bilirubin, white blood cell count and age. In published studies these five have been used as markers alone. Age and platelets have also been used in combination as the Age and Platelets Index (API).

Of these six serum fibrosis markers that could be used for all DART participants, none have been validated in HBV/HIV coinfection or in HIV monoinfection and in HBV monoinfection API performs the best. 15 different assessments with AUROCs ranging from 0.68 to 0.93 have been published.

API can range from 0 to 10 and is calculated by adding together a point score for each of age and platelets as shown in Table 33. A score of 6 is taken as a marker of significant fibrosis [298].

Table 33: Calculation of Age and Platelets Score

Age (years)	Points		Platelets (x10^9 / L)	Points
<30	0		>225	0
30-40	1		200-225	1
40-50	2		175-200	2
50-60	3		150-175	3
60-70	4		125-150	4
>70	5		<125	5

AST was also tested in DART participants in Uganda. Using AST with the markers above several more serum fibrosis markers are calculable, of which AST, AST/ALT ratio, FIB-4 and APRI have been validated in HBV positive patients. Three of these were evaluated in HBV/HIV coinfected patients and, of these 3, FIB-4 was consistently the most discriminating (Table 5).

FIB-4 was calculated as described by Sterling [299]:

Equation 1: Calculation of FIB-4

$$\text{FIB-4} = \frac{\text{age} \times \text{AST}}{\text{platelets} \times \sqrt{\text{ALT}}}$$

Age in years, AST and ALT in IU/L and platelets in 10^9/L.

The cut-off commonly used for FIB-4 to determine advanced fibrosis (Ishak score 4-6) is 3.25 [299].

7.2.4 Statistical Methods

The analysis of baseline ALT used graphical and descriptive methods and the Kruskal-Wallis equality-of-populations rank test to compare median values by HBsAg status and other predictors. The Chi-squared test or Fisher's exact test were used to compare the distribution of categorical variables, as appropriate. Baseline ALT distribution was positively skewed. Box-Cox transformation indicated that the logarithm of baseline ALT had a distribution that was close to normal and so this was used in subsequent analyses. Unadjusted and adjusted (i.e. univariable and multivariable) linear regression were used to determine which baseline factors were associated with baseline ALT.

Distribution of baseline platelet count was shown graphically. Correlation of platelet count with age and platelets index (API) and with FIB-4 was calculated. In the case of FIB-4, the score was transformed to achieve a more normal distribution by taking the logarithm. The proportion of participants with significant fibrosis used published cut-offs and was examined by HBsAg status using Chi-squared tests [298, 299].

The rate and predictors of ALT flares on first-line treatment were examined using incidence rates, survival analysis and Cox regression. Only the first flare, if any, for each participant was included.

To examine those who stopped TDF and 3TC during a switch to second line HAART mean ALT was plotted for one year before and one year after switch and flares examined using survival analysis.

In the STI substudy, baseline characteristics were examined using Fisher's exact test or Kruskal-Wallis equality-of-populations rank test as appropriate. The rate of flare was examined by study arm and by HBsAg status using Fisher's exact test.

7.3 Results

7.3.1 Participants

Of 3,316 participants in DART, 3,315 had baseline HBsAg testing. 326 (9.8%) were on TB therapy at baseline and 400 (12.1%) started TB therapy during follow-up. Of the 3,315 participants with an HBsAg result, 2,468 (74.4%) were started on TDF, 300 (9.0%) on ABC and 547 (16.5%) on NVP. BMI was determined in 3,282 of whom 605 (18.4%) were underweight (BMI<18.5) and 116 (3.5%) were obese (BMI>30).

7.3.2 ALT at baseline

The median ALT at baseline was 25 IU/L (IQR 18-36) (Table 34). It was marginally higher in Harare, in males, in those HBsAg seropositive and in those on TB treatment.

Table 34: Baseline ALT (IU/L)

| | n | Mean | | Median | IQR | p |
		Arithmetic	Geometric			
All	3315	30.9	25.8	25	18 to 36	
Site						<0.001
Entebbe	1020	28.3	23.2	23	15 to 35	
JCRC	1297	31.0	26.0	25	17 to 37	
Harare	998	33.2	28.5	26	20 to 37	
Sex						<0.001
Male	1160	34.7	29.1	28	20 to 40	
Female	2155	28.8	24.1	23	17 to 34	
BMI						0.06
<18.5	605	33.3	27.0	26	18 to 40	
18.5 to 30	2561	30.3	25.4	25	18 to 36	
>30	116	29.7	26.2	27	19 to 34	
HBsAg						<0.001
Negative	3007	30.4	25.4	25	17 to 36	
Positive	308	35.5	30.2	28	21 to 40	
TB treatment						<0.001
No	2989	30.5	25.4	24	18 to 36	
Yes	326	34.6	29.0	29	20 to 41	

ALT at baseline was above the local ULN in significantly more participants testing HBsAg positive than HBsAg negative (22.4% *vs.* 16.8%, p=0.01).

The distribution of ALT at baseline is shown in Figure 26, Figure 27 and Figure 28.

Figure 26: ALT distribution at baseline – All
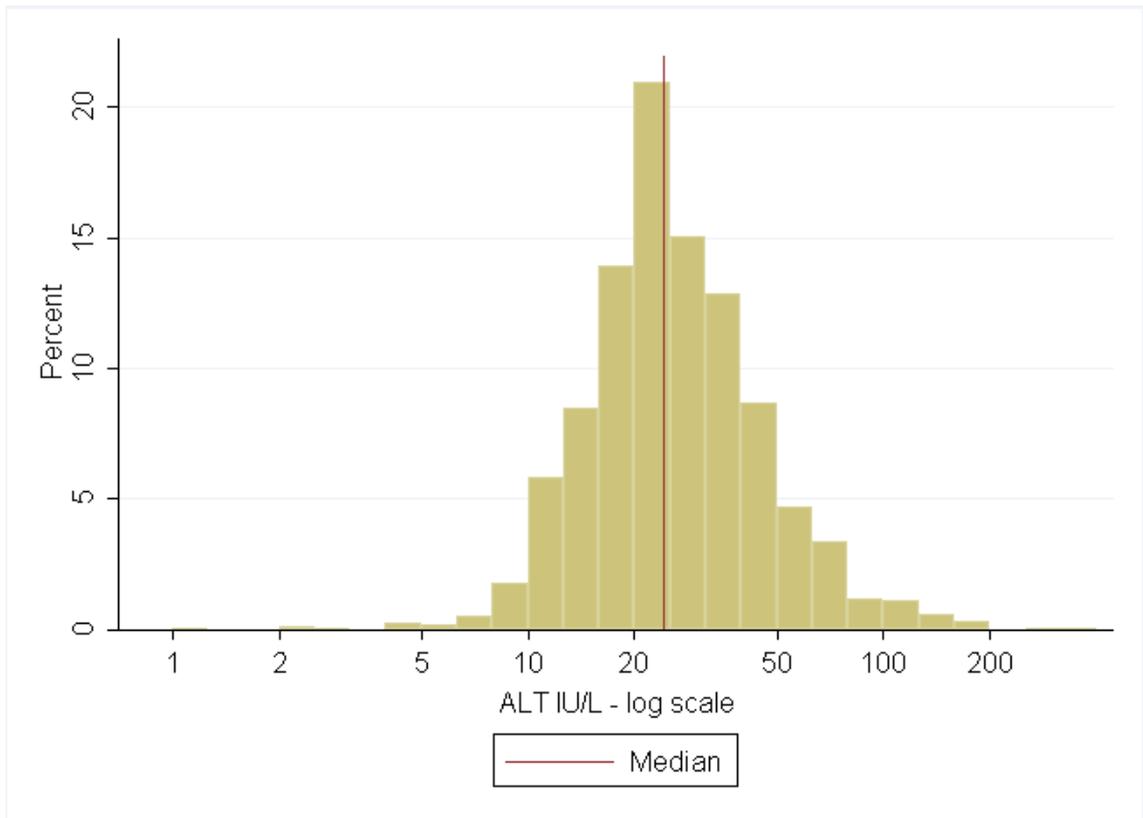
Figure 27: ALT distribution at baseline – HBsAg seronegative
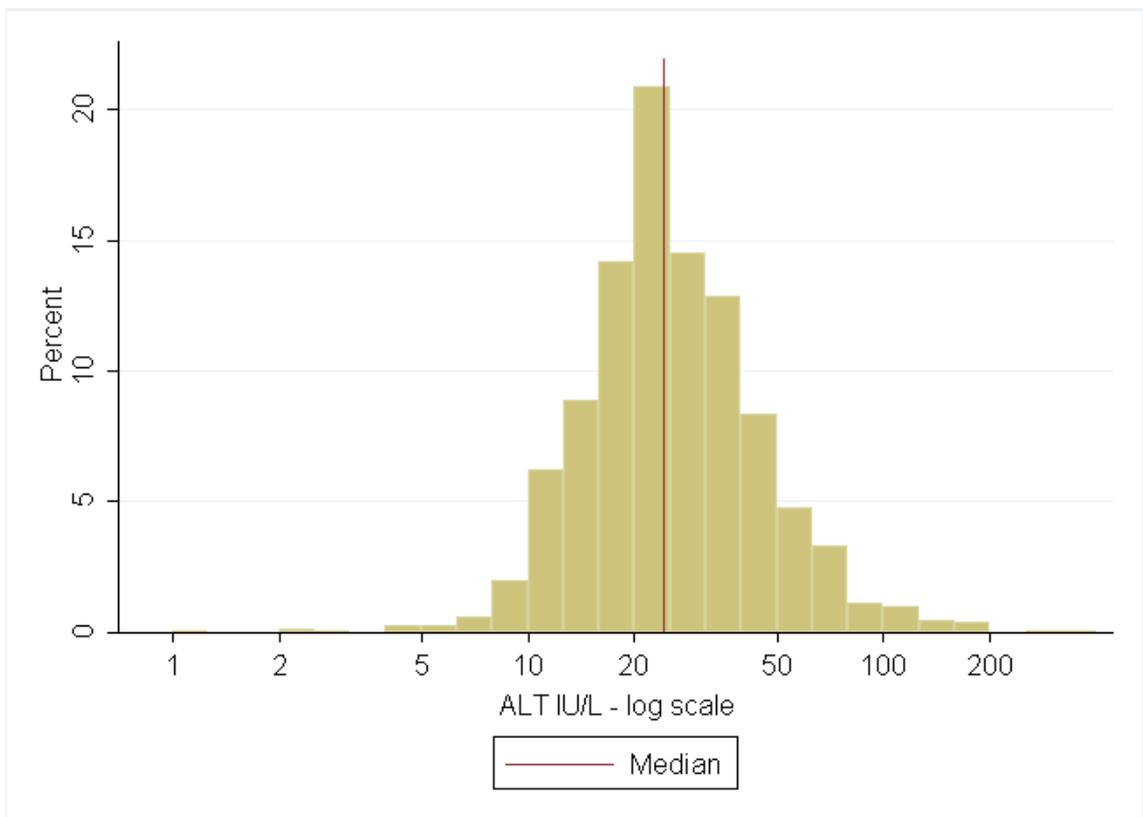
Figure 28: ALT distribution at baseline – HBsAg seropositive
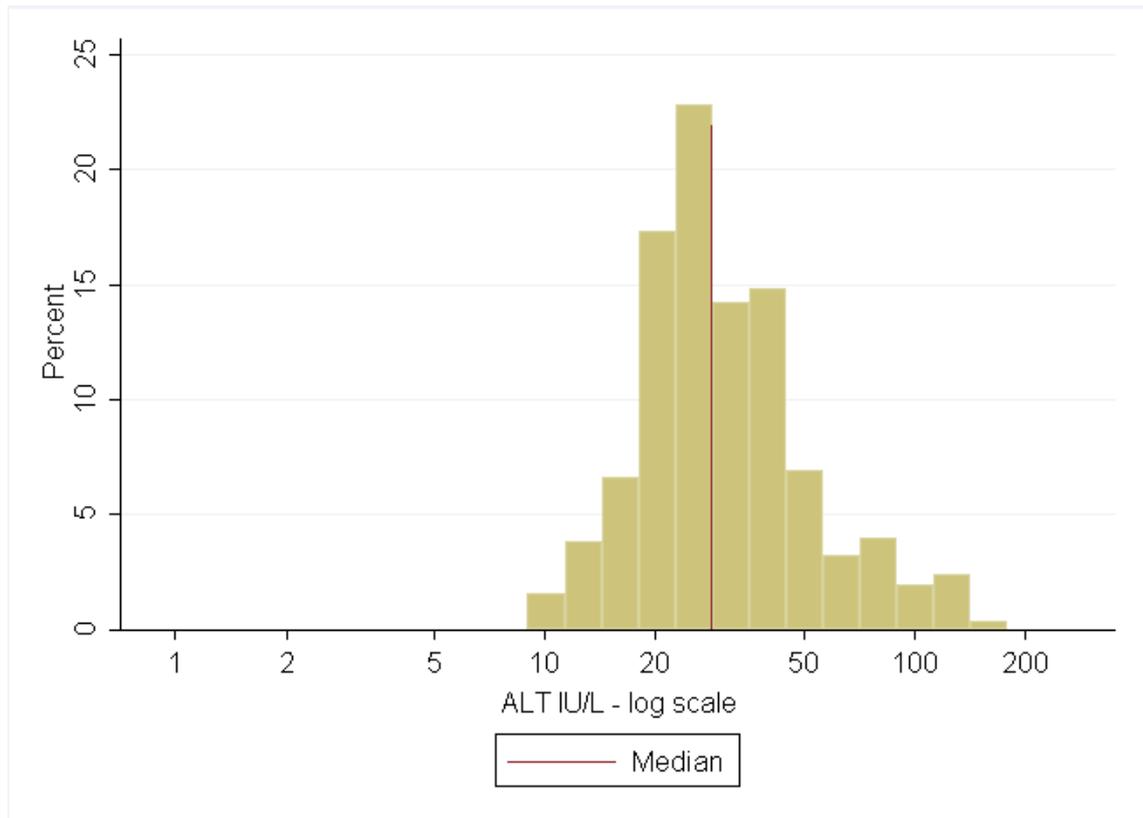
In unadjusted and adjusted linear regression analysis, higher baseline ALT was associated with site, male sex, younger age, low baseline CD4, WHO stage 4 disease, positive HBsAg status, baseline platelet count and TB treatment (Table 35). BMI was not associated with baseline ALT (p=0.10) and was excluded in the adjusted analysis in order to include those participants without a BMI measurement.

The magnitude of the changes were similar for site (up to 19.5% change), sex (15.5%), age (18.1%) and CD4 cell count (16.6%) and these were all larger than the effect of HBsAg positivity (12.6%). The magnitude of the effects of WHO stage, baseline platelet count and TB treatment were less (up to 9.8%).

Table 35: Linear regression analysis of predictors of baseline log(ALT)

	Factor	n	Unadjusted % difference in ALT	Unadjusted 95% CI	Unadjusted p	Adjusted % difference in ALT	Adjusted 95% CI	Adjusted p
Site	Entebbe	1020			<0.001			<0.001
	JCRC	1297	12.0	6.9 to 17.4		9.9	4.8 to 15.1	
	Harare	998	22.8	16.8 to 29.2		19.5	13.6 to 25.6	
Sex	Male	1160			<0.001			<0.001
	Female	2155	-17.3	-20.6 to -13.8		-15.5	-18.9 to -11.9	
Age group	<30	532			<0.001			<0.001
	30-35	795	-5.3	-11.1 to 0.9		-7.2	-12.8 to -1.3	
	35-40	848	-5.1	-10.8 to 1.1		-7.8	-13.3 to -2.0	
	40-45	608	-6.4	-12.5 to 0.1		-10.5	-16.2 to -4.4	
	45-50	313	-15.7	-22.3 to -8.6		-18.1	-24.4 to -11.4	
	>50	219	-10.7	-18.5 to -2.2		-13.7	-21.0 to -5.6	
BMI	Per kg/m2	3282	-0.4	-0.9 to 0.1	0.10			
WHO Stage	Stage 2	672			<0.001			0.01
	Stage 3	1864	9.7	4.2 to 15.4		3.7	-1.5 to 9.0	
	Stage 4	779	16.6	9.8 to 23.8		8.1	1.8 to 14.9	
Baseline CD4	<50	1109			<0.001			<0.001
	50-99	784	-13.1	-17.6 to -8.4		-11.9	-16.4 to -7.2	
	100-149	759	-16.7	-21.0 to -12.2		-13.3	-17.8 to -8.6	
	150-199	663	-20.9	-25.1 to -16.4		-16.6	-21.1 to -11.8	
HBsAg	Negative	3007			<0.001			0.001
	Positive	308	19.2	11.4 to 27.6		12.6	5.3 to 20.4	
Anti-HCV	Negative	3175			0.11			0.44
	Positive	77	3.8	-9.0 to 18.3		4.7	-7.8 to 18.9	
	Not done	63	16.2	0.6 to 34.4		8.1	-6.1 to 24.5	
Baseline log(platelet count)		3315	-15.0	-23.0 to -6.1	0.001	-9.8	-18.1 to -0.5	0.04
TB treatment	No	2989			<0.001			0.006
	Yes	326	13.8	6.5 to 21.6		9.5	2.6 to 16.9	

7.3.3 Markers of liver fibrosis at baseline

Platelets results at baseline are available for all DART participants. The median platelet count was 204 and the interquartile range was 157 to 260 $x10^9$ /L. Median platelet count was higher in Entebbe and lower in Harare (p<0.001) (Table 36).

Table 36: Distribution of platelet count by site

	N	Median	IQR
All	3,315	204	157 to 260
Entebbe	1,020	214	157 to 273.5
JCRC	1,297	204	158 to 259
Harare	998	195	154 to 245

Platelet count declines with advanced liver fibrosis. In this population, 720 (21.7%) had a platelet count at baseline below the lower limit of normal (150 $x10^9$ /L). The proportion with a low platelet count at baseline did not vary between the sites (p=0.70) (Figure 29). Low platelet count was associated with HBsAg status, with 26.6% of HBsAg seropositive participants having a count below 150 $x10^9$ /L compared to 21.2% of HBsAg seronegative participants (p=0.03).

Figure 29: Distribution of platelet count at baseline by site
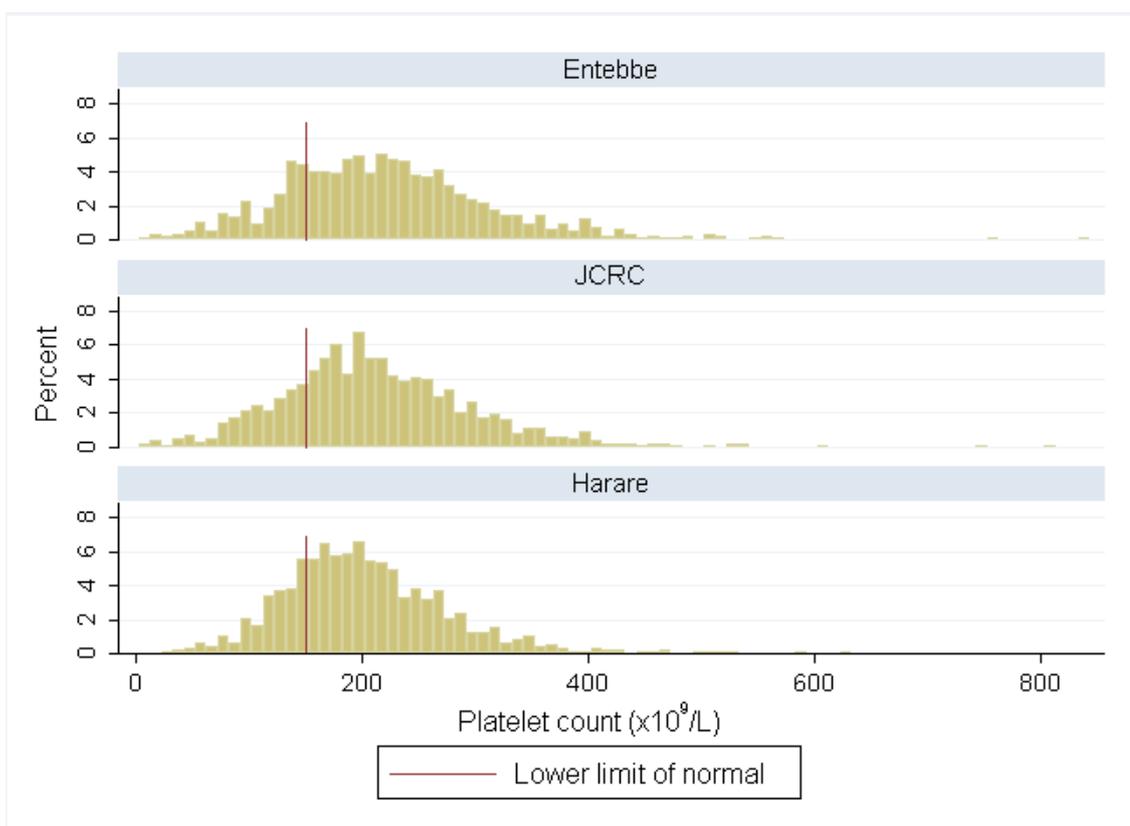
The median API was 3 and the interquartile range was 1 to 5. For 510 (15.4%) of the 3,316 participants API was greater than 6 consistent with significant fibrosis. The

proportion with advanced fibrosis by API was higher in those HBsAg seropositive than in those HBsAg seronegative (19.5% *vs*. 15.0%, p=0.04). In participants from Uganda, 344 (14.8%) of 2,317 had an API greater than 6. AST results at baseline were available for the 2,317 participants in Uganda. The median FIB-4 in these 2,317 was 1.28 (IQR 0.90 to 1.87). In 180 (7.8%) FIB-4 was greater than or equal to 3.25, indicating advanced fibrosis and again the proportion was higher in those HBsAg seropositive (12.8% *vs*. 7.4%, p=0.02).

Platelet count, API and FIB-4 (Ugandan participants only) were correlated (platelets and API; R=0.80, p<0.001: platelets and FIB-4; r=0.81, p<0.001: API and FIB-4; R=0.78, p<0.001). Of 180 with severe fibrosis determined by FIB-4, 151 (83.9%) had severe fibrosis by API. However of 344 Ugandan participants with severe fibrosis by API, only 151 (43.9%) had severe fibrosis using FIB-4.

Since the markers were correlated and since age was also included separately in all analyses, baseline platelet count was chosen as the most appropriate marker of fibrosis in all subsequent analyses. Baseline ALT was weakly correlated with baseline platelet count (R=0.06, p=0.001) (Figure 30).

Figure 30: ALT *vs*. platelet count at baseline
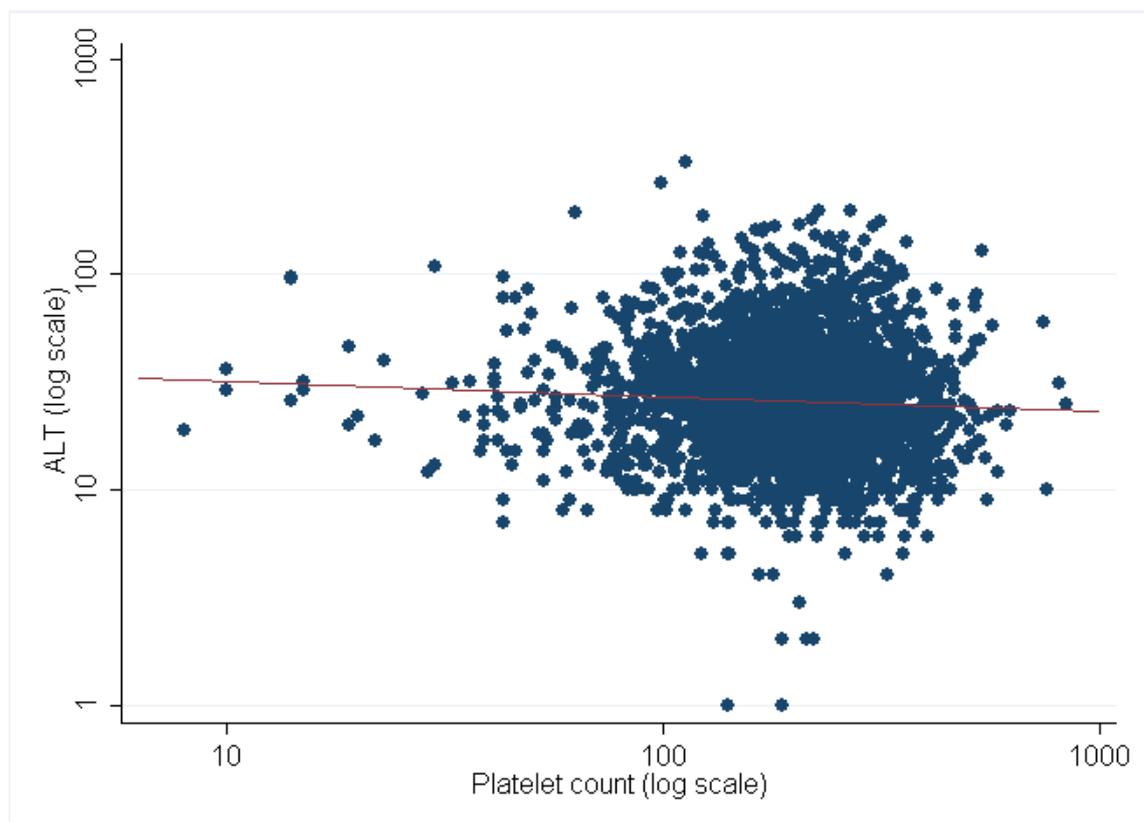
7.3.4 ALT results on first-line therapy

The number of participants with ALT results available declined from 3,315 at baseline to 2,625 at 48 weeks, 1,624 at 192 weeks and 1,062 at 240 weeks, as shown in Table 37.

Table 37: Availability of ALT results on first-line treatment

Week	0	48	96	144	192	240	288
Total available	3,315	2,625	2,044	1,832	1,624	1,062	47
% complete	100	98.9	98.4	98.8	98.5	97.3	97.9

The first participant was randomised in January 2003 but all participants randomised before January 2004 received TDF. All participants were followed until the end of 2008 and thus maximal follow-up was longer for TDF (2,129 days) than for ABC (1,764 days) or NVP (1,765 days).

The decline in the number of available ALT results over the course of the study was not due to missing data but rather to later study entry or change in drug treatment (and, to a lesser extent, deaths).

7.3.5 Change in ALT on first-line treatment

The mean ALT increased by 5 IU/L from baseline to 4 weeks and then declined to below the baseline value, reaching 2 IU/L below baseline by 24 weeks and 4 IU/mL below baseline by 48 weeks (Figure 31).

This was not driven purely by a few large values. Median ALT also increased at 4 weeks.

Figure 31: Mean change in ALT from baseline over 240 weeks (with 95% confidence intervals)
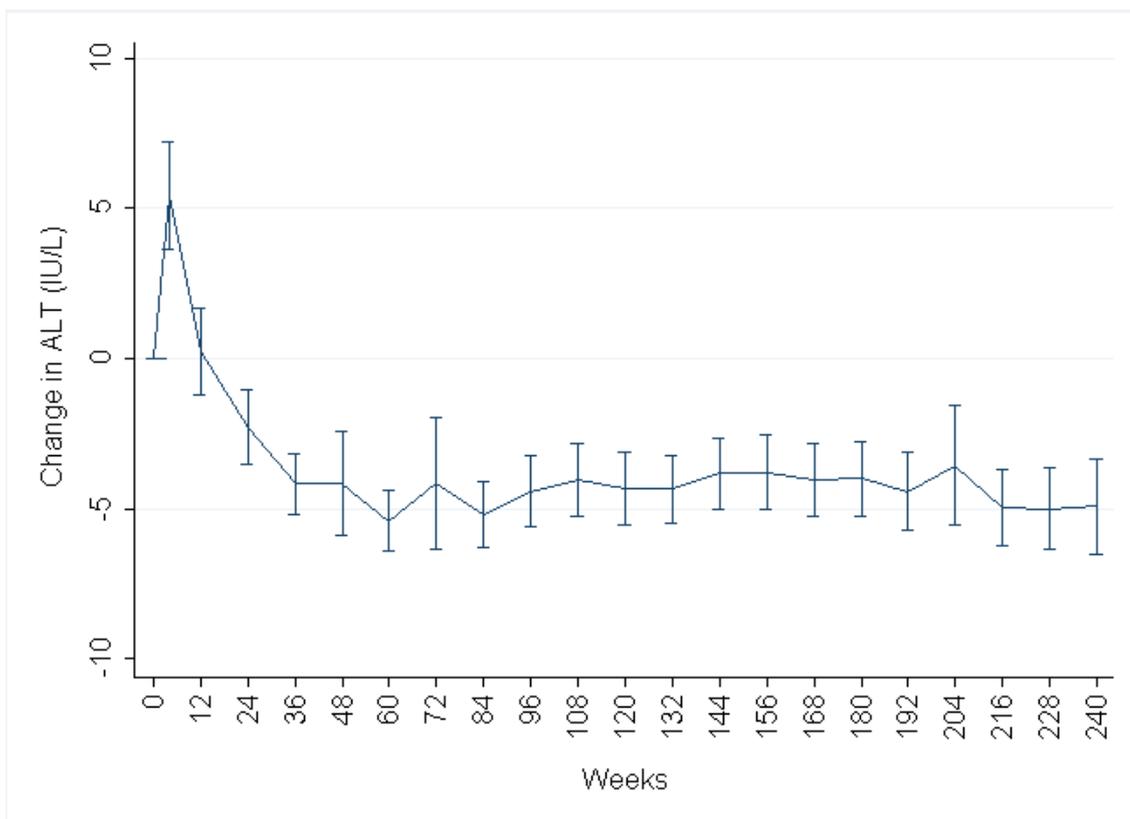
Although mean ALT was higher at week 4 than at baseline, the majority of participants not only had a normal ALT at baseline (using the local upper limits of normal) but also had an ALT less than 50 at baseline and throughout the first 4 weeks (Table 38); this was also true of those HBsAg seropositive (Table 39). In each 50 IU/L band few (347, 10.5%) had an ALT in a higher band within the first 4 weeks of treatment than at baseline.

Table 38: Peak ALT after baseline and within 4 weeks

	Peak ALT after baseline within 4 weeks					
Baseline ALT	**0-49**	**50-99**	**100-149**	**150-199**	**≥200**	**Total**
0-49	2,608	246	29	12	20	2,915
50-99	179	115	24	7	4	329
100-149	25	14	12	2	3	56
150-199	6	2	3	2	0	13
≥200	2	0	0	0	0	2
Total	2,820	377	68	23	27	3,315

Table 39: Peak ALT after baseline and within 4 weeks in HBsAg seropositive participants

Baseline ALT	Peak ALT after baseline within 4 weeks					Total
	0-49	50-99	100-149	150-199	≥200	
0-49	223	28	2	0	7	260
50-99	18	14	3	0	1	36
100-149	4	5	2	0	1	12
150-199	0	0	0	0	0	0
≥200	0	0	0	0	0	0
Total	245	47	7	0	9	308

The majority of participants (1,789, 54.0%) had normal ALT throughout follow-up. Only 275 (8.3%) had at least one grade 1 (>2.5x ULN) ALT rise during follow-up and only 26 (0.8%) had an ALT rise of grade 4 (10x ULN [181]).

Factors affecting change in ALT on first-line treatment
The change in ALT is shown stratified by HBsAg status in Figure 32, by drug in Figure 33, and then by drug limited to those HBsAg seropositive in Figure 34. The rise at week 4 was more pronounced in those treated with NVP than with TDF or ABC (Figure 33) and in those HBsAg seropositive than those HBsAg negative (Figure 32). Late in follow-up there appears to be a greater relative decrease in ALT for those HBsAg seropositive. There is an early decline in ALT in those treated with ABC compared with those treated with TDF or NVP but this is not sustained (Figure 33) and this is not seen in those HBsAg seropositive (Figure 34).

Figure 32: Mean change in ALT from baseline over 240 weeks by HBsAg status
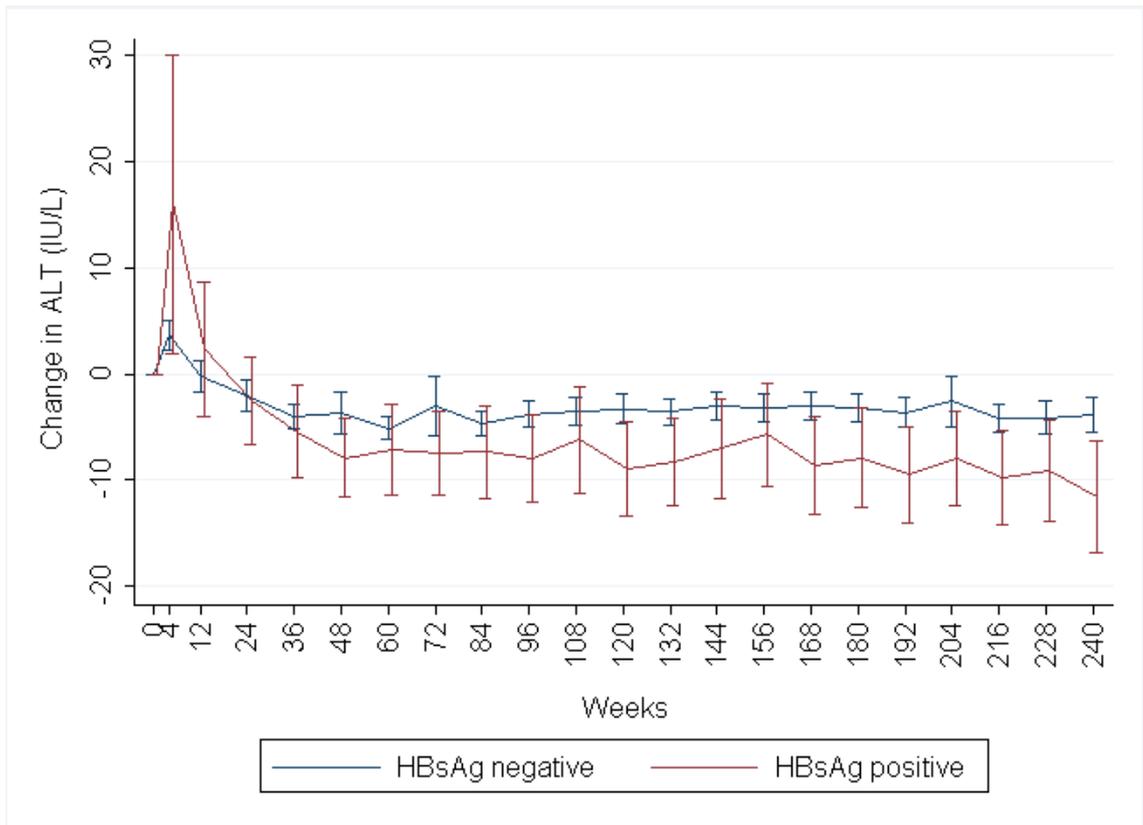
Figure 33: Mean change in ALT from baseline over 240 weeks by drug
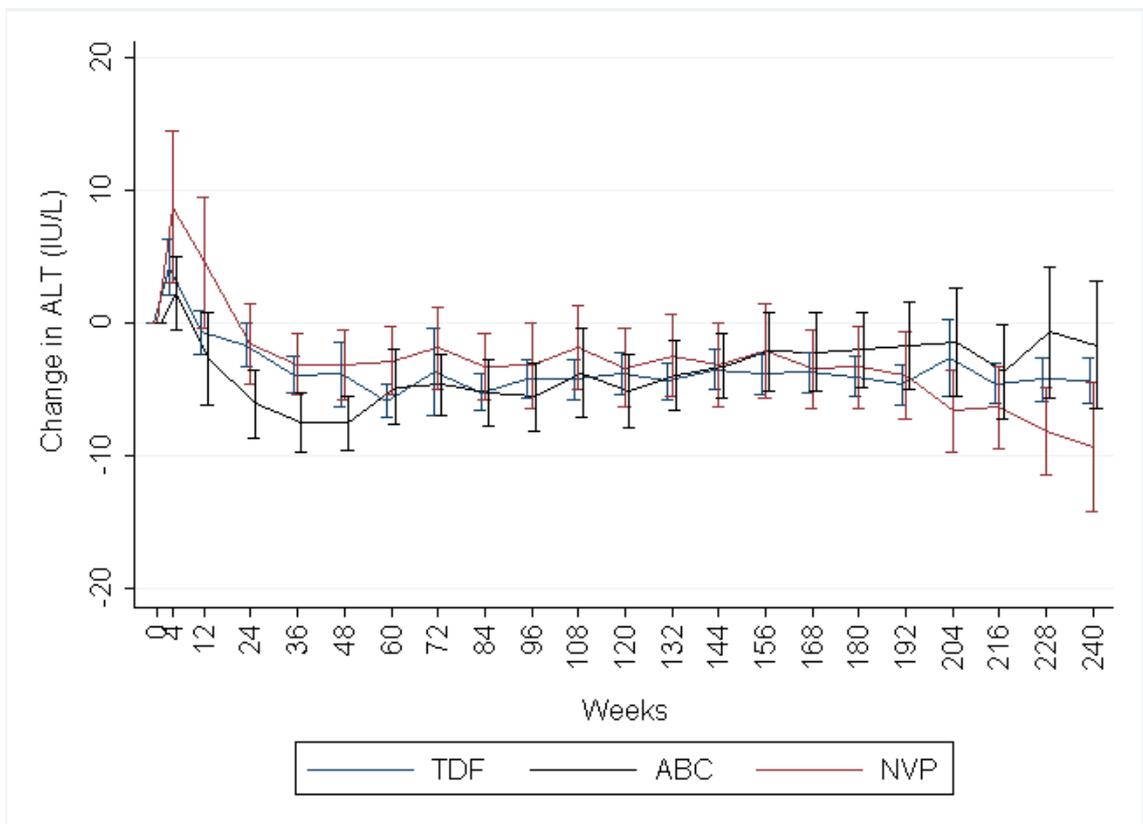
Figure 34: Mean change in ALT from baseline over 240 weeks by drug in those HBsAg seropositive only
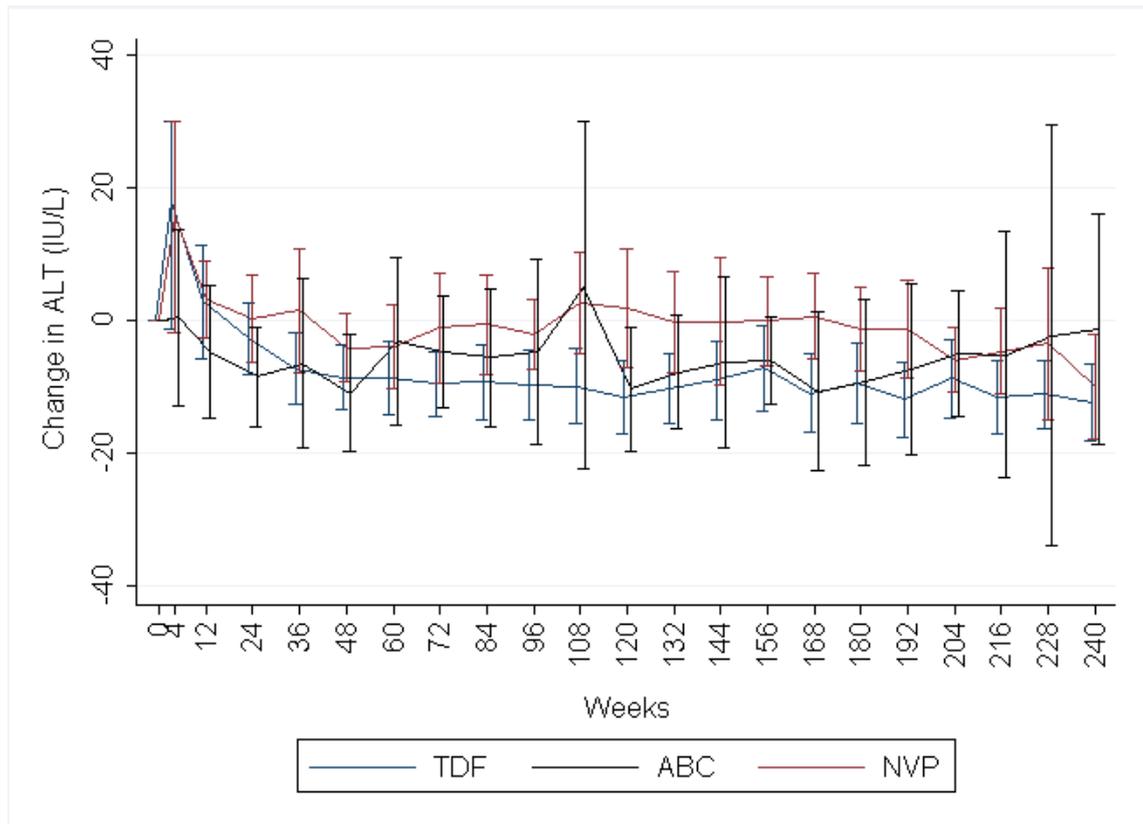
The decline in mean ALT over time in those HBsAg seropositive could have been due to participants with higher ALT dropping out of the analysis by switching to second line antiretroviral therapy. However the decline was also apparent in an intention to treat analysis which included all ALT results, even after a switch from first-line antiretroviral treatment (Figure 35).

Figure 35: Mean change in ALT from baseline over 240 weeks by HBsAg status (including results after switch off first-line treatment)
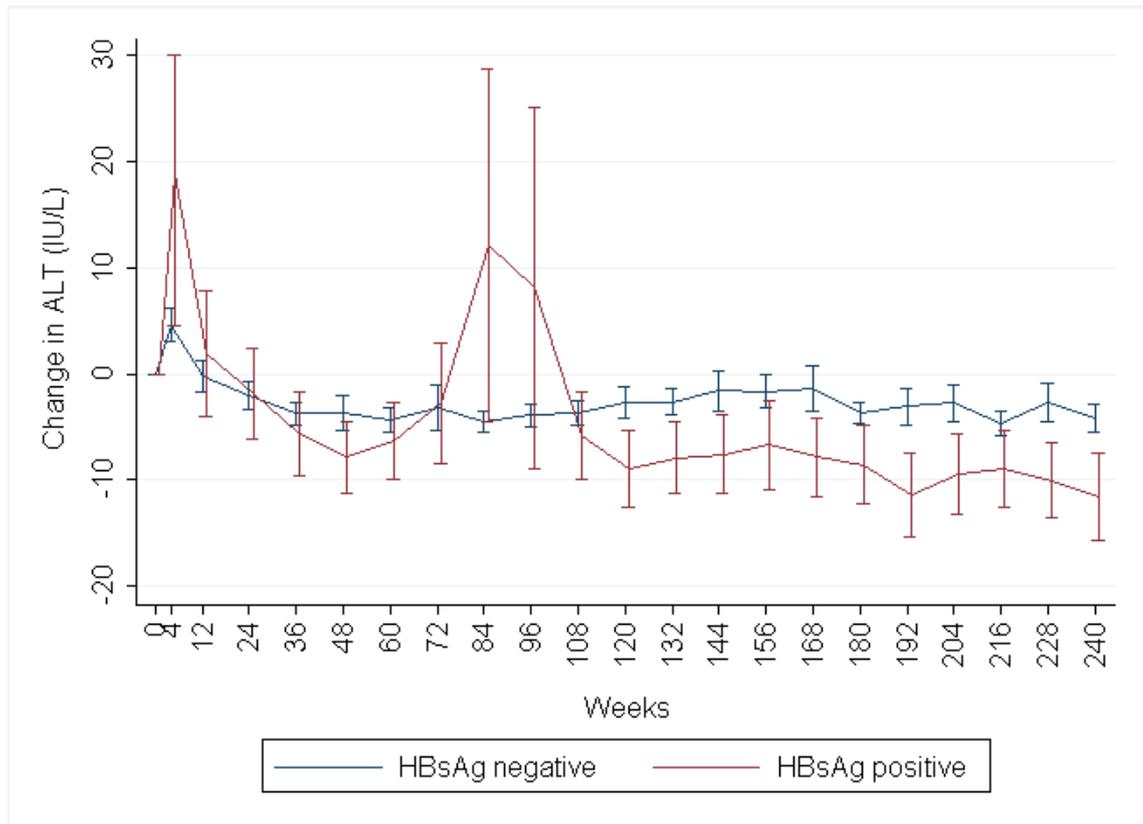
A pronounced increase in mean ALT at weeks 84 and 96 in those HBsAg seropositive is entirely driven by a single participant who developed cryptococcal disease and switched to lopinavir, RTV and NVP at week 76. ALT rose from 23 IU/L at week 72 to 1,348 IU/L at week 84 and 1,632 IU/L at week 96. Antiretroviral drugs were stopped and ALT fell to 12 IU/L at week 108. ALT remained normal after antiretroviral medication was restarted with a regimen of lopinavir, RTV and EFV. In comparison, the median change in ALT did not show a rise at weeks 84 and 96, with the rise in ALT in a single participant having no effect on the median change (Figure 36).

Figure 36: Median change in ALT from baseline over 240 weeks by HBsAg status
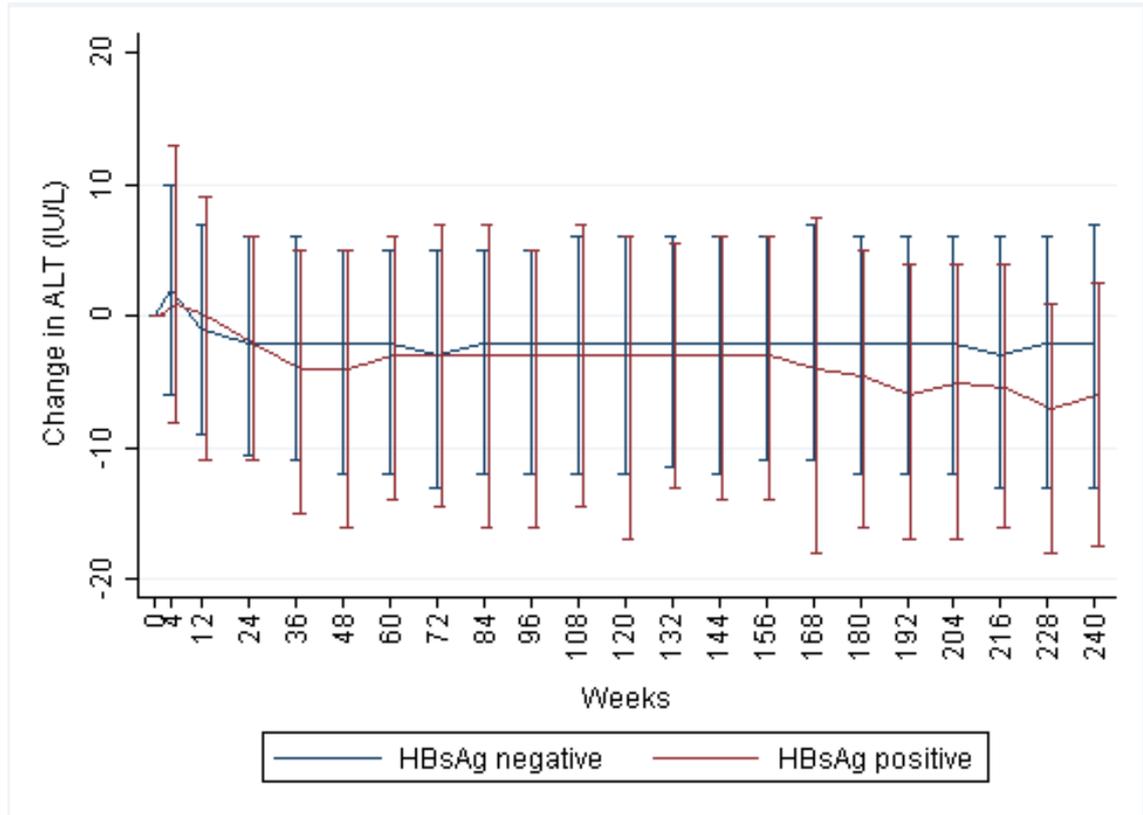
Graph shows median change in ALT. Bars show interquartile range.

7.3.6 Flares on first-line treatment

The analysis of flares was restricted to those participants with results of all variables used in the regression models; 33 participants were excluded as they had no baseline BMI result available and 5 as they had no follow-up. Using the definition of flare described above (section 7.2.2), 80 (2.4%) of 3,277 had a flare with 26 occurring by week 4, 13 between weeks 4 and 12, 9 between weeks 12 and 24, 6 between weeks 24 and 48 and 26 after week 48.

The incidence of flares is clearly much higher early after treatment initiation and was relatively stable after 24 weeks.

Figure 37: Cumulative incidence of ALT flares - all participants
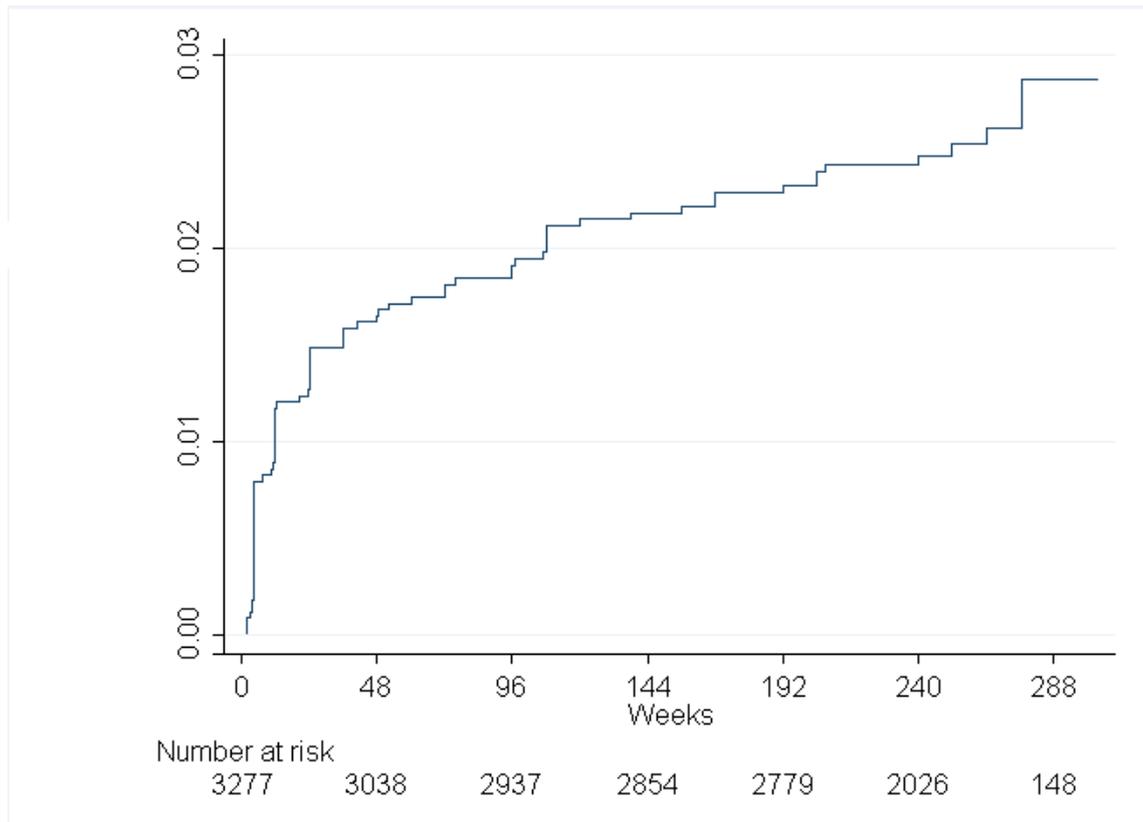
Factors associated with flares

In unadjusted Cox regressions of flare, advanced WHO stage, HBsAg positivity and higher baseline ALT were associated with an increased risk while site, sex, age, BMI, baseline CD4 count, anti-HCV status, lower baseline platelet count, monitoring strategy and drug regimen were not (Table 40).

In the adjusted model, WHO stage, HBsAg positivity and baseline ALT remained significant (Table 41). Site and drug regimen were also significant with a higher risk of flare in Entebbe and a lower risk in Harare and with use of NVP associated with higher and ABC with lower risk of flare than TDF. The HR for log of baseline ALT was 5.0 meaning the risk of flare was 5.0 times higher per 10 fold increase in baseline ALT.

Table 40: Unadjusted analysis of factors associated with flare (Cox regression)

		N	Flare	Hazard Ratio	95% CI	p
Site	Entebbe	991	32	1		0.15
	JCRC	1289	28	0.67	0.40 to 1.11	
	Harare	997	20	0.61	0.35 to 1.07	
Sex	Male	1146	34	1		0.14
	Female	2131	46	0.72	0.46 to 1.12	
Age	18-30	525	14	1		0.25
	30-35	785	22	1.06	0.54 to 2.06	
	35-40	836	22	0.98	0.50 to 1.92	
	40-45	604	11	0.68	0.31 to 1.50	
	45-50	310	7	0.83	0.34 to 2.06	
	>50	217	4	0.68	0.22 to 2.06	
BMI kg/m2	Per unit	3277		0.97	0.91 to 1.03	0.26
WHO Stage	Stage 2	660	9	1		0.003
	Stage 3	1841	42	1.72	0.84 to 3.53	
	Stage 4	776	29	2.88	1.36 to 6.08	
Baseline CD4	<50	1096	31	1		0.36
	50-99	774	16	0.70	0.38 to 1.28	
	100-149	752	19	0.86	0.48 to 1.52	
	150-199	655	14	0.72	0.38 to 1.35	
HBsAg	Negative	2971	61	1		<0.001
	Positive	306	19	3.20	1.91 to 5.35	
Anti-HCV	Negative	3137	77	1		0.90
	Positive	77	2	1.04	0.26 to 4.23	
	Not done	63	1	0.63	0.09 to 4.52	
Baseline log(ALT) IU/mL	Per log	3277	80	6.20	2.78 to 13.82	<0.001
Baseline log(PLT)	Per log	3277	80	0.47	0.17 to 1.26	0.13
Monitoring strategy	LCM	1633	39	1		0.81
	CDM	1644	41	1.05	0.68 to 1.64	
Drug	TDF	2433	59	1		0.11
	ABC	298	3	0.44	0.14 to 1.40	
	NVP	546	18	1.46	0.86 to 2.48	
TB treatment	No	2702	65	1		0.71
	Yes	575	15	1.11	0.63 to 1.95	

Table 41: Multivariable (adjusted) analysis of factors associated with flare (Cox regression)

		Adjusted Hazard Ratio	95% CI	p
Site	Entebbe	1		0.004
	JCRC	0.54	0.32 to 0.92	
	Harare	0.38	0.21 to 0.69	
Sex	Male	1		0.45
	Female	0.83	0.51 to 1.35	
Age	18-30	1		0.47
	30-35	1.12	0.57 to 2.22	
	35-40	0.99	0.50 to 1.96	
	40-45	0.74	0.33 to 1.66	
	45-50	0.98	0.39 to 2.50	
	>50	0.82	0.26 to 2.53	
BMI kg/m2	Per unit	1.01	0.94 to 1.08	0.83
WHO Stage	Stage 2	1		0.005
	Stage 3	1.78	0.85 to 3.75	
	Stage 4	2.90	1.32 to 6.41	
Baseline CD4	<50	1		0.80
	50-99	0.85	0.46 to 1.57	
	100-149	1.09	0.60 to 1.97	
	150-199	1.03	0.53 to 2.01	
HBsAg	Negative	1		<0.001
	Positive	3.44	2.00 to 5.92	
Anti-HCV	Negative	1		0.97
	Positive	1.01	0.25 to 4.13	
	Not done	0.78	0.11 to 5.74	
Baseline log(ALT) IU/mL	Per log	5.04	2.17 to 11.72	<0.001
Baseline log(PLT) IU/mL	Per log	0.61	0.22 to 1.64	0.32
Monitoring strategy	LCM	1		0.97
	CDM	1.01	0.65 to 1.57	
Drug	TDF	1		0.05
	ABC	0.43	0.13 to 1.41	
	NVP	1.69	0.98 to 2.92	
TB treatment	No	1		0.70
	Yes	0.89	0.50 to 1.60	

HBsAg seropositive participants had 3.4 times the risk of a flare compared to those without HBsAg. The effect of HBsAg positivity increasing the risk of flare was apparent very early after treatment initiation (Figure 38).

Figure 38: Kaplan-Meier failure estimate - by HBsAg status
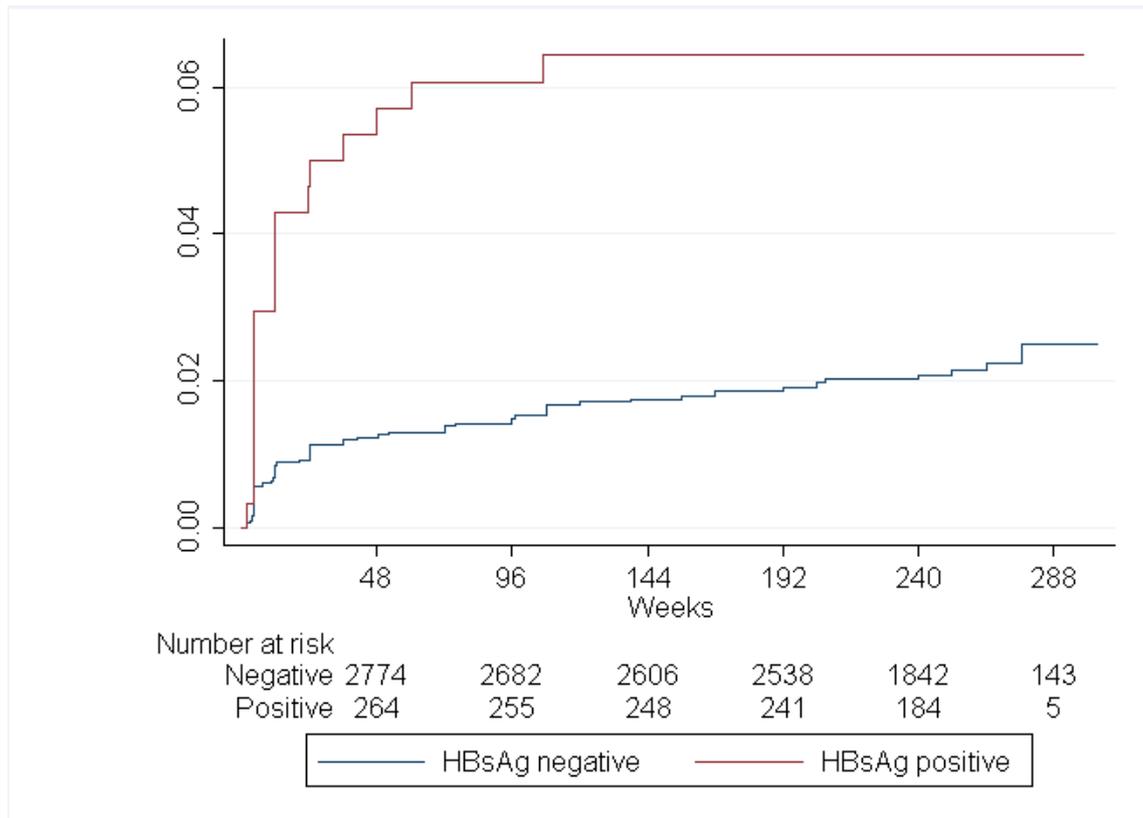
The effect of drug was also apparent within the first months after starting treatment and maintained throughout follow-up (Figure 39). Flares were more common in those treated with NVP than in those treated with TDF, and were twice as common with TDF as with ABC, though the confidence intervals included 1 (Table 41).

Figure 39: Kaplan-Meier failure estimate – by drug treatment
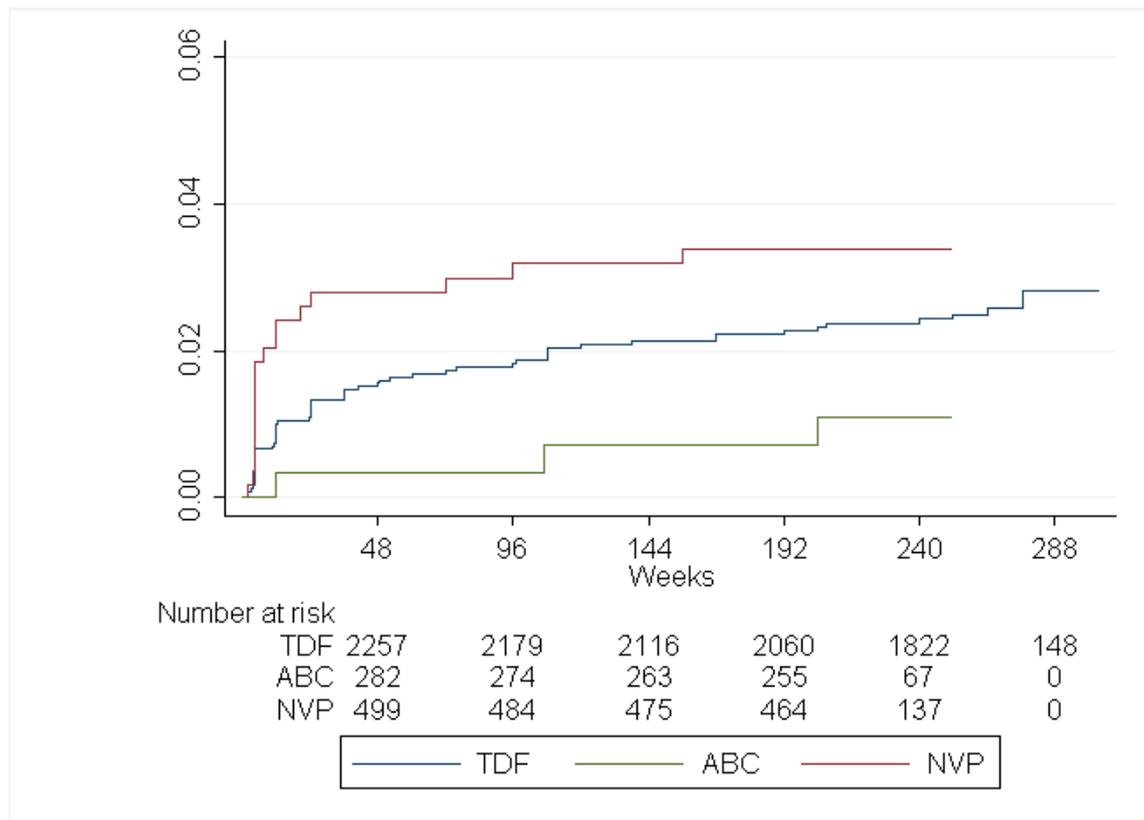
The risk of flare was higher in Entebbe than at the other two sites and was more than twice as high as in Harare.

HBeAg

The effect of HBeAg was examined in the subset of participants with detectable HBsAg. The unadjusted HR for HBeAg was 1.38 (95% CI 0.55 to 3.50; p=0.49) and the aHR was 1.27 (95% CI 0.42 to 3.83; p=0.67).

Figure 40: Kaplan-Meier failure estimate in those HBsAg seropositive - by HBeAg status
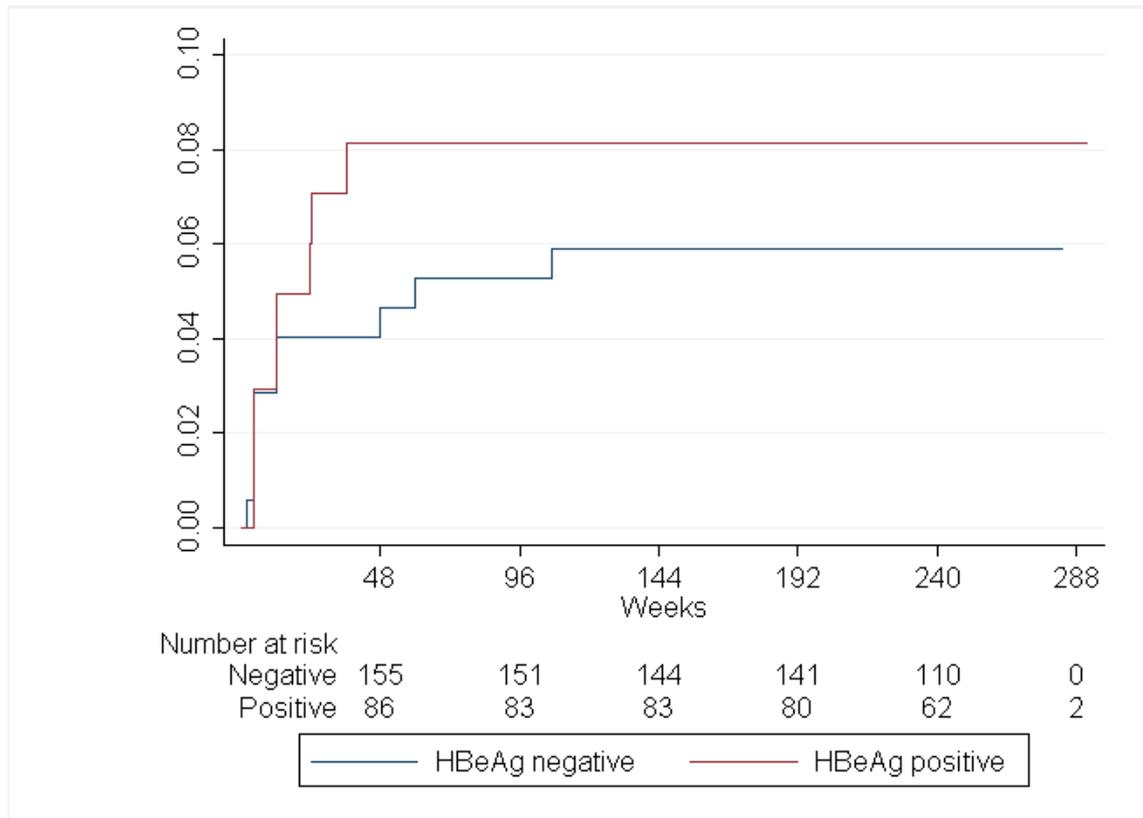
Flare incidence rate

The incidence of flare over the whole of follow-up on first-line therapy was 0.56 (95% CI: 0.45 to 0.70) per 100 person years. The incidence rate was higher in those HBsAg seropositive at 1.55 (95% CI: 0.99 to 2.44) with an IRR of 3.3 (95% CI: 1.86 to 5.60; p<0.001).

The incidence was higher in those treated with NVP (0.84, 95% CI: 0.53 to 1.33) than in those treated with TDF (0.54, 95% CI: 0.42 to 0.70) or ABC (0.25, 95% CI: 0.08 to 0.79) but these differences were not statistically significant. However, of note, the effect of HBV infection on the rate of flare differed with drug regimen used. The IRR for HBsAg seropositive *vs*. HBsAg seronegative participants was 4.1 (95% CI: 2.1 to 7.3; p<0.001) in those treated with TDF, 6.2 (95% CI: 0.1 to 119.1; p=0.11) in those treated with ABC but only 1.1 (95% CI: 0.1 to 4.6; p=0.43) in those treated with NVP. Thus the association with HBsAg appeared to be only statistically significant in those on TDF.

To examine whether the effect of HBsAg-status was different in participants according drug used, we performed a Cox regression with interaction factor between HBsAg and drug. This test indicated that there was no interaction (p=0.24).

The incidence of flare declined over time. For the first 24 weeks on ART, the incidence was 3.22 (95% CI: 2.42 to 4.28) flares per 100 person years, while over the rest of follow-up it was 0.31 (95% CI: 0.22 to 0.42) flares per 100 person years. In the latter case the IRR for HBsAg seropositive *vs.* seronegative was 1.57 (95% CI: 0.48 to 4.03), consistent with there being no association with HBsAg status once treatment has been given for 24 weeks.

Clinical course after flare
Of the 80 participants with a flare on first-line antiretroviral therapy, 15 (19%) died. 6 of these deaths occurred within a week of the flare and one other after 15 days while the other 8 deaths were between 3 months and 4 years later.

One patient had an ALT of 399 IU/L that was then normal on the next sample (taken 12 weeks later) and remained normal for all other samples until death 19 months later. The cause of death was given as "traumatic". One patient died of disseminated TB 2 years and 8 months after the first flare with normal ALT results in between although ALT rose again on the last sample taken before death, one week after starting TB therapy. Three others also had normal ALT after flare while the other 10 participants all had abnormal ALT results from the time of flare until their death (although in one case ALT declined to 41 IU/L on the day they died having been persistently abnormal since the flare 15 weeks earlier). Liver failure was given as a cause of death for 5 of the 10 (the one who died after 15 weeks and 4 who died at the time of flare). For the other 5 with persistently abnormal ALT until death, 1 died of pancreatitis, 1 of "HIV-related CNS disease" and 1 of cryptococcal meningitis, and in 2 the cause of death was uncertain (Table 42).

Table 42: Cause of death after flare on first-line therapy

Patient	HBsAg	Cause of death	Time from flare to death (days)	ALT returned to normal before death
1	Neg	Liver failure	1	No
2	Neg	Liver failure	2	No
3	Neg	Septicaemia / acute hepatitis	2	No
4	Neg	Liver failure	3	No
5	Neg	HIV-related CNS disease	3	No
6	Pos	Uncertain	3	No
7	Neg	Cryptococcal meningitis	15	No
8	Neg	Septicaemia / liver failure	104	No
9	Neg	Pancreatitis	148	No
10	Pos	Uncertain	241	No
11	Neg	Traumatic	583	Yes
12	Neg	Sepsis	873	Yes
13	Neg	Disseminated TB	991	Yes
14	Neg	Alcohol related	1216	Yes
15	Neg	Uncertain	1325	Yes

After a flare, ALT returned to normal in most participants, being subsequently normal at some point in 65 (81.3%). In 44 ALT was normal within 12 weeks, and in another 18 within 48 weeks. Of the 15 without a normal ALT after the flare, 10 died before the end of follow-up.

Most flares did not appear to result in any change in therapy. 25 (31.3%) of the 80 were followed by a change or interruption in treatment within 12 weeks. Of these 25, 13 were in the LCM arm and 12 in the CDM arm of DART. In 18 of the 25, the reason for the change in therapy was given as "hepatotoxicity", "raised LFTs" or, in 1 case, "cirrhosis". In these 18, median time to a change in treatment was 5 days (IQR 3.25 to 20.75 days). 8 participants stopped their therapy but then restarted the same regimen after a median of 21.5 days (IQR 12.25 to 32 days, range 7 to 43 days). 8 switched NVP to TDF, 1 switched TDF to NVP and 1 switched NVP to EFV.

7.3.7 Flares on switch off TDF to second line therapy

402 participants switched from a regimen that included TDF and 3TC to a regimen that included neither and had ALT test results on the second regimen. Baseline demographics (i.e. at entry to DART) for these patients are shown in Table 43. Switching to second line occurred when indicated by clinical progression or, in the LCM arm only, by low CD4 cell count [259]. Switching was more frequent in the LCM arm from 2 to 3 years after treatment initiation but similar before and after that period.

Table 43: Baseline demographics of those switching off tenofovir to second line

		HBsAg negative		HBsAg positive		p
		n	%	n	%	
All		363		39		
Site	Entebbe	99	27.3	6	15.4	0.189
	JCRC	139	38.3	15	38.5	
	Harare	125	34.4	18	46.2	
Sex	Male	157	43.3	19	48.7	0.611
	Female	206	56.7	20	51.3	
Age	18-30	61	16.8	8	20.5	0.065
	30-35	88	24.2	12	30.8	
	35-40	92	25.3	6	15.4	
	40-45	64	17.6	12	30.8	
	45-50	27	7.4	1	2.6	
	>50	31	8.5	0	0.0	
WHO Stage	Stage 2	51	14.0	6	15.4	0.859
	Stage 3	220	60.6	22	56.4	
	Stage 4	92	25.3	11	28.2	
Baseline CD4	<50	209	57.6	20	51.3	0.771
	50-99	86	23.7	10	25.6	
	100-149	47	12.9	6	15.4	
	150-199	21	5.8	3	7.7	
Monitoring strategy	LCM	199	54.8	23	59.0	0.735
	CDM	164	45.2	16	41.0	
Anti-HCV	Negative	8	2.2	2	5.1	0.381
	Positive	20	5.5	1	2.6	
	Not done	335	92.3	36	92.3	

Mean ALT at the time of switch was 33.6 IU/L and there was no difference by HBsAg status (p=0.47). After switch, mean ALT declined in those HBsAg negative to 26.1 IU/L at 12 weeks while in those HBsAg seropositive mean ALT rose dramatically to 82.0 IU/L at 12 weeks, returning to 33.1 IU/L at 24 weeks after switch (Figure 41). The large increase at week 12 in HBsAg seropositive participants was driven by 3 participants with ALT over 200 IU/L. Of these 3, in 1 ALT continued to rise after another 12 weeks but treatment was then switched again and ALT fell to normal 12 weeks later, while in the other two ALT fell after the rise 12 weeks after switch but both patients died while ALT was still abnormal (both 36 weeks after switch). The mean ALT in the other 35 participants with detectable HBsAg was 34.6 IU/L at switch and 34.5 IU/L at week 12.

Figure 41: Mean ALT before and after switch to second line
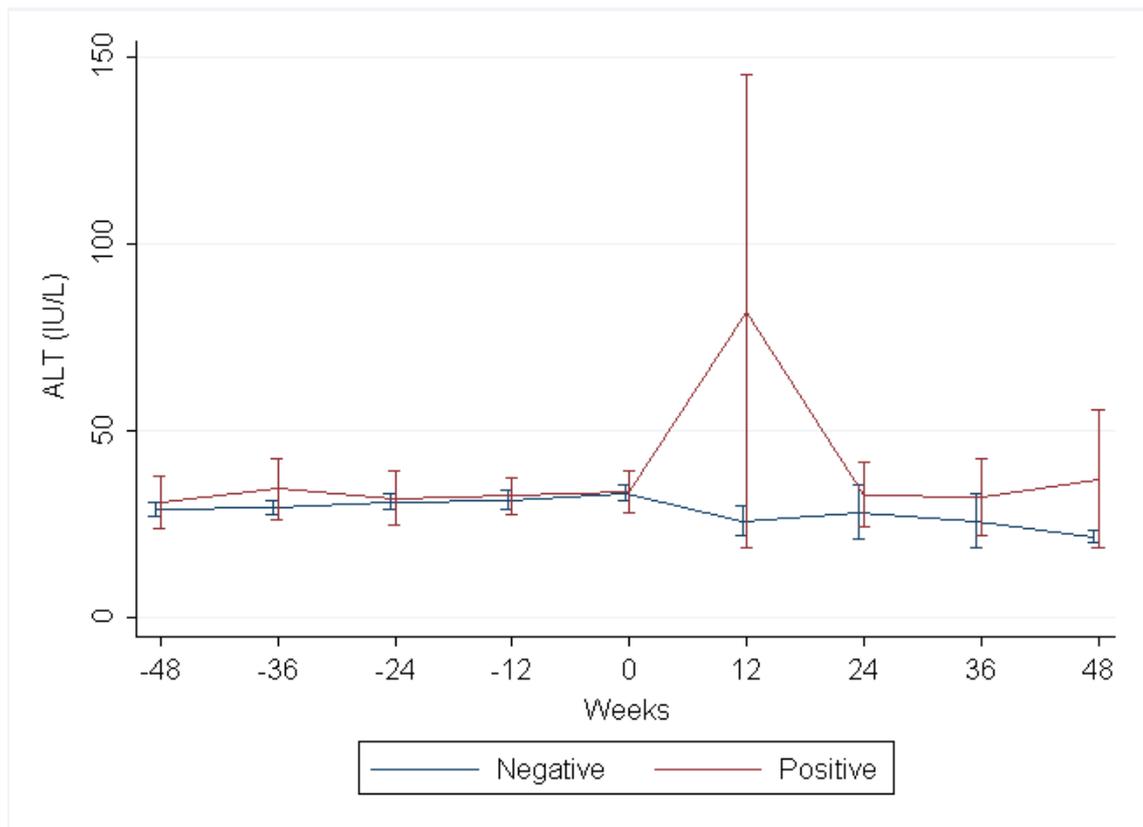
Using the definition of flare of an ALT ≥200 IU/L with a rise of at least 100 IU/L from baseline (time of switch), 12 (3.0%) participants had a flare on switching to second-line ART including 4 (10.3%) of those with detectable HBsAg, 1 of whom had detectable HBeAg. 3 of these flares in HBsAg seropositive participants were at 12 weeks (described above) while the other was at 48 weeks after switch. The Kaplan Meier failure estimate is shown in (Figure 42). In Cox regression analysis the HR for flare of HBsAg positivity was 4.45 (p=0.02).

Figure 42: Kaplan Meier failure estimate of risk of flare by HBsAg status
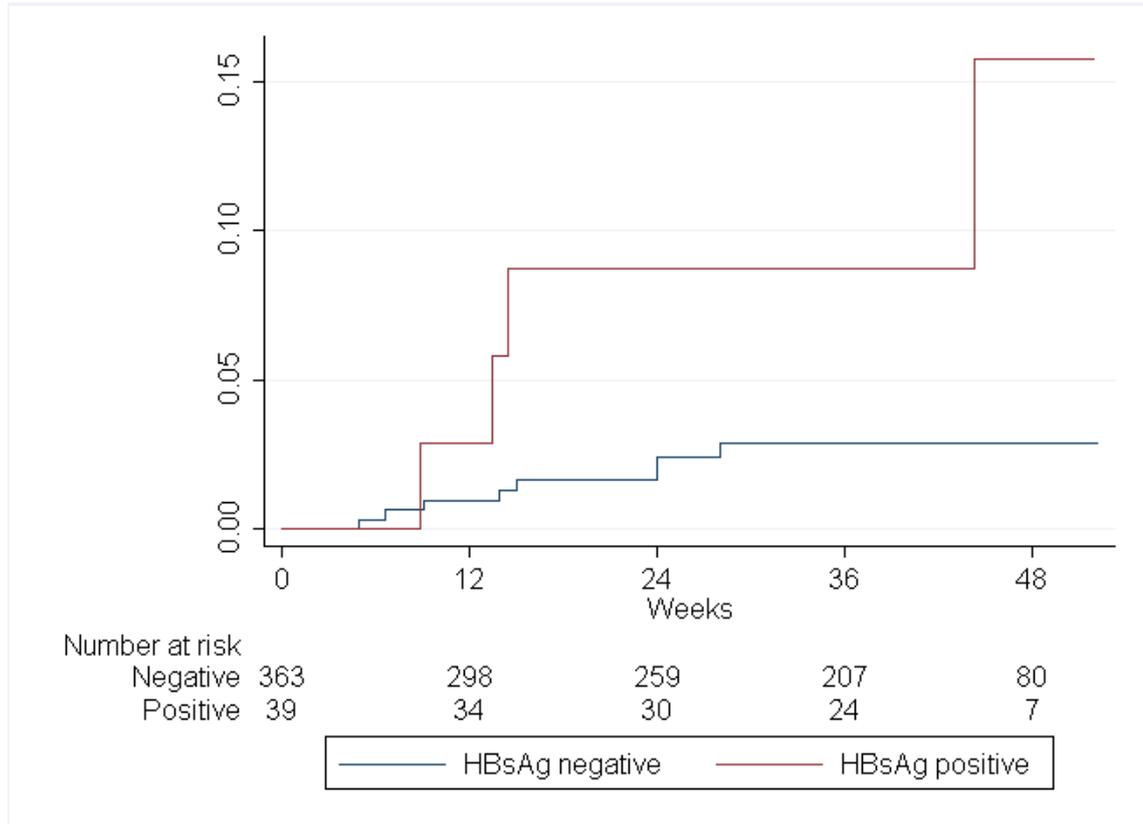
There was no difference in the risk of flare by monitoring strategy arm (unadjusted HR 1.4, p=0.53).

Of the 8 HBsAg negative participants with a flare, 4 died; 2 the day after flare, 1 10 days later and one 60 days later. For 2 the cause of death was recorded as liver failure. In the HBsAg seropositive participants with flare 3 out of 4 died; one after 47 days, 1 after 241 and 1 after 248. Liver failure was recorded as the cause of death in 2 (Table 44).

Table 44: Timing and cause of death in those with flare after switch

HBsAg	Time of death after flare (days)	Cause of death
Negative	1	Pancreatitis
Negative	1	Liver failure
Negative	10	Liver failure
Negative	60	Pneumonia / pulmonary hypertension
Positive	47	Liver failure
Positive	241	Uncertain
Positive	248	Liver failure

In participants who switched to second line therapy and did not experience an ALT flare, 33 (8.5%) of 390 died before the end of follow-up, which was less than the proportion of those with flare that died (p<0.001).

7.3.8 Flares during and after Structured Treatment Interruption

A total of 813 participants were randomised in the STI substudy. Baseline demographics are shown in Table 45. HBsAg was positive in 68 (8.4%) of whom 41 and 27 were randomised to CT and STI respectively.

Table 45: Baseline demographics in the Structured Treatment Interruption substudy

		CT		STI	
		n	%	n	%
All		405		408	
Site	Entebbe	164	40.5	167	40.9
	JCRC	111	27.4	109	26.7
	Harare	130	32.1	132	32.4
Sex	Male	106	26.2	112	27.5
	Female	299	73.8	296	72.5
Age	18-30	69	17.0	74	18.1
	30-35	102	25.2	100	24.5
	35-40	93	23.0	106	26.0
	40-45	74	18.3	64	15.7
	45-50	43	10.6	32	7.8
	>50	24	5.9	32	7.8
WHO Stage	Stage 2	93	23.0	103	25.2
	Stage 3	238	58.8	219	53.7
	Stage 4	74	18.3	86	21.1
Baseline CD4	<50	58	14.3	57	14.0
	50-99	83	20.5	73	17.9
	100-149	113	27.9	114	27.9
	150-199	151	37.3	164	40.2
HBsAg	Negative	364	89.9	381	93.4
	Positive	41	10.1	27	6.6
Drug	TDF	264	65.2	257	63.0
	ABC	53	13.1	36	8.8
	NVP	88	21.7	115	28.2
Monitoring strategy	LCM	208	51.4	207	50.7
	CDM	197	48.6	201	49.3
Anti-HCV	Negative	393	97.0	392	96.1
	Positive	8	2.0	8	2.0
	ND	4	1.0	8	2.0

One participant was randomised to CT but the wrong allocation was sent to the clinicians and she underwent 3 interruptions. 4 in the STI arm had clinical reasons for not stopping only identified after randomisation. The numbers of participants that underwent each of 0, 1, 2, 3 or 4 structured treatment interruption cycles through to early termination of the study by the Data Monitoring Committee in March 2006 are shown in Table 46.

Table 46: Number of Structured Treatment Interruptions by randomisation arm

Structured treatment interruptions	CT n	STI n
0	404	4
1	0	86
2	0	148
3	1	139
4	0	31

Flares occurred in 7 (0.9%) participants, 4 in the STI arm (3 HBsAg seronegative and 1 HBsAg seropositive) and 3 in the CT arm (2 HBsAg seronegative and 1 HBsAg seropositive); 1 HBsAg seropositive participant in the STI arm had two flares (Table 47).

Only a single HBsAg seropositive patient in the STI arm (on AZT/3TC/TDF – see Figure 43) experienced a liver flare (two episodes, both during "on" cycles). In the CT arm there was also a single HBsAg seropositive participant with a flare (on AZT/3TC/NVP – see Figure 44).

Figure 43: ALT – HBsAg seropositive with flare in STI arm

Figure 44: ALT – HBsAg seropositive with flare in CT arm
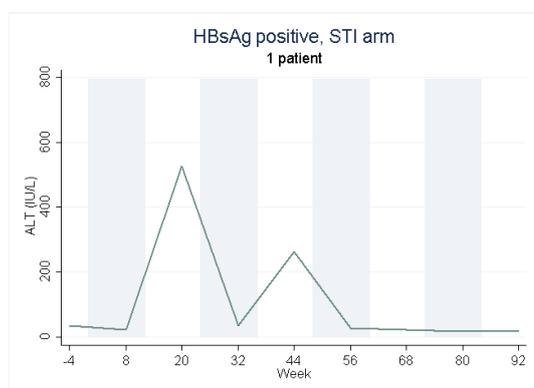
Off-treatment periods shown shaded
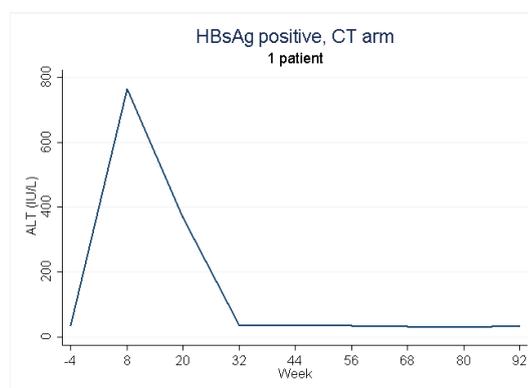
The rate of flare in HBsAg seropositive participants in the STI arm (1 out of 27, 3.7%) was no higher than the frequency observed in the CT arm (1 out of 41, 2.4%, p=1.0). Taking both arms together, there was a trend towards flares being less common in those HBsAg negative (5 out of 745, 0.7% *vs*. 2 out of 68, 3%, p=0.08).

Monitoring strategy arm had no effect on the rate of flare, either overall (LCM 0.5% *vs*. CDM 1.3%, p=0.28) or in those in the STI arm (0.5% *vs*. 1.5%, p=0.37).

Table 47: Flares during the Structured Treatment Interruption substudy

Arm	HBsAg	HBeAg positive %	n	Third drug			Participants with flare		
				TDF	ABC	NVP	n	%	95% CI
CT	Negative	-	364	239	51	74	2	0.5	0.1 to 2.0
	Positive	34.2	41	25	2	14	1	2.4	0.1 to 12.9
STI	Negative	-	381	242	34	105	3	0.5	0.1 to 1.9
	Positive	20.0	27	15	2	10	1	3.7	0.1 to 19.0

One participant with a flare during the STI substudy died during follow-up; this participant was HBsAg negative, in the CDM arm of DART and the STI arm of the substudy and underwent one STI without event from weeks 0 to 12. However a second STI was stopped after 1 week when the substudy was terminated by the Data Monitoring Committee. 1 year later non-Hodgkins lymphoma (NHL) was diagnosed, TB treatment started and AZT/3TC/NVP changed to AZT/3TC/EFV. ALT flared 2 weeks later and ART was stopped. Death from NHL occurred two months after flare with ALT remaining abnormal until death. Thus death was unlikely to have been related to STI.

There were no deaths during or within 72 weeks of the end of the STI study among HBsAg seropositive participants.

7.4 Discussion

While HBsAg status is associated with higher baseline ALT and with increased risk of flare these effects are generally of little clinical significance.

7.4.1 Liver status at baseline

Baseline ALT was higher in males as was expected. Surprisingly ALT fell with age until 50 years and then rose slightly which is the inverse of what has been described in healthy subjects [300, 301]. It was higher in those with more advanced WHO stage of HIV disease and in those with lower CD4 count. Baseline ALT was higher in those HBsAg seropositive, but the magnitude of this effect was similar to that of several other factors, including sex, age, and CD4 count. ALT was lower than the local ULN in most (83.2%) participants, including those who were HBsAg positive (77.6%).

Results of the baseline platelet count and the two serum fibrosis markers were highly correlated but, when used to categorise those with severe fibrosis, use of API allocated approximately twice as many to the F3/4 group as FIB-4. These correlations are unsurprising, not only because the scores attempt to measure the same thing, but also because both composite methods include the platelet count, with fibrosis score increasing as platelet count decreases.

Low platelet count and severe fibrosis determined by both API and FIB-4 were weakly associated with HBsAg status. The proportion with significant fibrosis determined by API was similar to the 17% found in a Ugandan study which measured fibrosis using TE [56]. That study found the prevalence in HBsAg seropositive individuals to be 2 times higher whereas we found the difference to be only one third. Unfortunately they did not also report platelet count or any serum fibrosis marker. There are no studies examining the association of TE and serum fibrosis markers and only two comparing biopsy with serum markers in HIV-HBV coinfected patients. The studies that utilised biopsy did not compare serum markers that rely only upon widely used blood tests such as ALT and platelet count [216, 217]. Although TE is portable it remains expensive and rarely available. Further studies comparing TE and/or biopsy with serum markers would be valuable and allow the further assessment of the predictive value of such fibrosis markers.

7.4.2 Change in ALT on first-line treatment

Mean ALT rose slightly at 4 weeks after HAART initiation before falling to below baseline. This increase has only rarely been noted in previous studies of first-line HAART [198]. There were apparent trends towards the rise in ALT early in treatment being higher in those treated with NVP and in those HBsAg seropositive who were

treated with TDF, but no significant interaction was found between HBsAg status and drug treatment.

It could be postulated that a small change like this represents an increase in the degree of liver inflammation after starting HAART. However the clinical significance of any such small change is unclear. Taking the mean change in ALT averages across those with no change, those with small and clinically insignificant changes and those with large changes indicating severe inflammation and hepatocyte damage. Table 38 may give a more useful picture, in that it shows that the majority of participants do not have a clinically significant increase in ALT and the majority of those with raised ALT at baseline subsequently have a decline (though of course this may simply be a regression to the mean). A similar decline in the proportion of both HBsAg seropositive and seronegative patients with abnormal ALT was seen over the first few months of HAART in a study in Gambia [88]. Of note, in that study there was a transient but marked increase in the proportion of those with occult HBV who had an abnormal ALT at 1 month after initiation. Another study, from Ghana, also found a reduction in the proportion with raised transaminases after treatment initiation [96].

The decline in ALT was greater in those treated with ABC during the first year of follow-up but there was no difference later. Why this should be is unclear. The number of participants treated with ABC was low and perhaps the confidence intervals unrealistically narrow since many comparisons are made in these graphs. The decline in ALT gradually increased in those HBsAg seropositive and continued to diverge from those HBsAg seronegative. This could have been due to sicker patients with higher ALT dropping out (switching off first-line) over time. However an intention to treat analysis, in which ALT results were included including after a switch to second line antiretroviral therapy, showed a very similar pattern.

7.4.3 Flares on first-line antiretroviral therapy

This is the largest study to determine the incidence of liver flares in HBV/HIV coinfected individuals. We found flares on starting first-line HAART occurred at a similar rate to previous studies in populations in sub-Saharan Africa, though substantially lower than previously reported in resource-rich countries. Why this difference between rates should exist is unclear.

The overall proportion of patients experiencing a flare is a rather crude measure in the light of widely varying length of follow-up in different studies. We found the incidence rate was ten times higher in the first 24 weeks after starting HAART than subsequently but that flares continued to occur for the rest of follow-up.

As has been reported in previous studies, flares were more common in those with higher baseline ALT. This is unsurprising since those with pre-existing liver inflammation are likely to be at higher risk of further damage. Flares were also more common in those HBsAg seropositive, in those treated with NVP and in those with WHO stage 4 HIV disease. Flares were less common in Harare than in the Ugandan sites but again the reason for this is unclear. We did not find an association between baseline platelet count (a marker of advanced liver disease) and the risk of flare.

In most of those who experienced a flare on first line treatment the ALT subsequently returned to normal. Mortality was low and in those that died only one third were known to have died of liver disease. This is similar to previously published data. In a Malawian study of 300 participants with advanced HIV, 7% of whom were coinfected with HBV, all flares on starting antiretroviral treatment resolved without clinical incident [195]. In another study which enrolled 5,832 participants (hepatitis status unknown) in Kenya and Mozambique the investigators found no increase in mortality in the 124 (2.4%) with flare on starting HAART [302].

The aetiology of the flares in DART participants is unknown. The higher rate in those treated with NVP suggests drug reaction to be responsible for some at least. We did not find that HBV coinfection significantly increased the sensitivity to NVP-related liver flare. In future, NVP may be a less frequent cause of flare since its use is declining worldwide as alternatives become more widely available and as guidelines recommend starting HAART at higher CD4 counts, when NVP is contraindicated due to an even higher risk of hepatotoxicity [303]. Detectable HBsAg appeared to be associated with an increased risk of flare only in the first 24 weeks after starting HAART, suggesting that once on stable HBV-active therapy, flares are not related to HBV. If flares on treatment-initiation are due to IRIS then in future the incidence of flare may also decline in frequency as participants are started at higher CD4 counts.

In view of the fact that the majority of flares in this population were without serious clinical sequelae and resolved spontaneously, routine lab monitoring of ALT in order to detect flares may not be of significant benefit, as suggested by Moore [195] and Chu [302]. The DART trial did report better outcomes in those who had routine monitoring but this is likely driven by other monitoring tests done (i.e. FBC, CD4/CD8, bilirubin, urea and creatinine) [259].

7.4.4 Flares on switch off TDF to second line therapy

A switch to second line therapy from a regimen that contains TDF and 3TC to one that contains neither allows examination of the effect of stopping therapy for HBV while continuing therapy for HIV.

A flare in ALT occurred in 10.3% (4/39) of HBsAg seropositive but only 2.2% (8/363) of HBsAg seronegative participants (HR 4.45) during second-line therapy. In the HBsAg seronegative group there was no significant change in ALT at or after the time of switch. However in HBsAg seropositive participants the mean ALT was 34.3 IU/L at switch but 73.2 IU/L 12 weeks later. This change was in fact driven by 3 with a flare at 12 weeks while in the remaining 36 participants there was no change in mean ALT.

A high proportion of those with a flare on switch died, both in the HBsAg seronegative and seropositive groups. Liver failure was commonly recorded as cause of death regardless of HBsAg status though allocation of cause of death may have been subject to bias, in that liver disease could have been decided upon as a probable cause of death as a result of the high ALT.

There are limited data previously published on stopping HBV-active treatment while maintaining HAART. HAART was continued in approximately half of 147 3TC interruptions in 109 participants in the Swiss Cohort Study [116]. Follow-up was limited to 6 months, during which time 29% of interruptions led to liver enzyme elevation, 5.4% to flare and 1 (0.7%) to death from liver failure. Flares occurred a median of 5 to 6 weeks after discontinuation of HBV-active treatment. No association was found with age, sex, CD4 count or whether ART was continued when 3TC stopped. Discontinuation of 3TC was also identified as a risk factor for flare in a cohort in Amsterdam, with 22% having a grade 4 rise in liver enzymes (10x ULN), though it was unclear how many had stopped all antiretroviral treatment at the same time as 3TC [192]. These studies concluded that HBV-active treatment, once started, should be continued. Our data supports this, since although we found death was common after flare in both those with and without detectable HBsAg, the rate of flare was higher in those coinfected with HBV.

7.4.5 Flares during and after Structured Treatment Interruption

The SMART study showed that treatment interruption in HAART leads to an increased risk of opportunistic infection or death although earlier, smaller studies had not shown such increased risks [159, 304, 305]. In SMART, coinfection with viral hepatitis (mostly HCV) was found to increase the risk of death but no data has been reported on liver enzyme changes [97].

In DART, flares were rare during the STI substudy and there was no significant difference between the rate of flare in the two arms. As in the first-line antiretroviral therapy analysis, there was a higher rate of flare in those HBsAg seropositive, though this difference was not statistically significant in the STI substudy. There was only one death in a participant with a flare during the STI substudy. This occurred over a year

later and there was no suggestion that liver disease or STI played any contribution. Thus, brief treatment interruptions of 12 weeks duration were not associated with an increase in liver flares in coinfected participants. The higher risk of death in those with flare after switch than in those with flare after STI is likely to be due to the fact that those undergoing switch were often doing so because their clinical situation was deteriorating while those undergoing STI were a selected group that were doing well on treatment. The risk of death in those switching who did not have a flare was lower but still considerable at 8.5%.

In contrast, a trial of CD4-guided STI that recruited participants in Thailand, Switzerland and Australia (STACCATO) included 6 HBV/HIV coinfected patients who underwent structured treatment interruptions [146]. Of these 6, 5 had a rise in ALT and 1 had a flare. The authors stated that ALT in those without flare rose but this was to a maximum of only 43 IU/L. Again the authors concluded that HBV-active treatment should not be interrupted in HIV/HBV-coinfected patients.

One potential cause of liver enzyme elevation is HBeAg to anti-HBe seroconversion which can occur in association with treatment interruption [116, 146]. Unfortunately we have hepatitis B serology only at entry to DART.

7.4.6 Limitations

In DART, ALT was measured every 3 months with additional tests as requested by clinicians. We found that the majority of flares on first-line therapy were without clinical sequelae, as were flares during the STI substudy, while flares on a switch to second line therapy were frequently followed by death. Over one third of DART participants had extra ALT testing performed and these tests are likely to have been performed if participants were sick. Thus the probability of finding a flare may be increased in those sick purely through the increase in testing frequency. As such, it may be that participants who were at increased risk of dying through other pathology may have been at increased risk of having a flare detected. Brief asymptomatic flares may have been missed, reducing the apparent incidence of flare in those who were well and introducing bias to any association found between flare and death.

It is well recognised that flares can also occur at the time of HBeAg seroconversion. Unfortunately we do not have HBV serology results for time points after study entry. For a full investigation of flares in HBV/HIV coinfected individuals this would also be interesting to examine.

Despite DART being a large cohort with a moderately high rate of HIV-HBV coinfection, there is still a need to include more individuals to examine factors associated with rare

events, such as coinfected patients experiencing flare after switch to second-line therapy.

7.4.7 Conclusions

The effects on liver inflammation and fibrosis of HBV coinfection in HIV infected individuals are limited in public health significance, but include an increase baseline ALT, increases in the rise in ALT and the risk of flare on first-line HAART and an increase in the risk of flare on stopping HBV-active treatment. HBV does not significantly increase the risk of flare in participants on stable HAART after the initial six months and in DART did not increase the risk of flare during 12 week treatment interruptions.

Flares were usually without clinical importance although they were frequently followed by death when they occurred after a switch to second line.

These analyses support the recommendations from DART and elsewhere that routine ALT monitoring is not of benefit, even in HBV/HIV coinfected individuals, but demonstrate that HBV status should be determined before stopping HBV-active treatment and such treatment should be continued in coinfected patients.

8 CD4 count changes and clinical disease progression

8.1 Introduction

In HIV-positive patients with access to antiretroviral medication liver disease is one of the leading non-AIDS causes of death [62, 66, 67]. Liver-related and all-cause mortality are higher in HBV coinfected patients [82, 83, 118]. Most studies examining the effects of HBV coinfection on HIV outcomes have not shown any effect on CD4 rise on ART, on progression to AIDS or on HIV-related death (reviewed in chapter 1). However published data is mixed, with some studies showing deleterious effects on all three outcomes.

HIV management guidelines, including those from the UK, the USA and the WHO, recommend the use of two drugs with anti-HBV activity in HBV/HIV-coinfected patients who require treatment for HIV [123, 133, 134]. It is unknown whether this strategy abrogates the excess mortality associated with HBV-coinfection documented in some earlier studies.

The DART population provides a valuable opportunity to examine the effect of HBV infection on the clinical and immunological responses to HIV treatment.

Aims

1. To examine the association between HBsAg status at treatment initiation and change in CD4 cell count over time
2. To determine the predictors of clinical progression to new WHO stage 4 event and death
3. To examine causes of death and determine the proportion of deaths due to liver-related causes

8.2 Methods

8.2.1 Analyses

Baseline CD4 was defined as the last measurement before treatment initiation.

The examination of mean CD4 at 12 week intervals was stratified by baseline HBsAg status. The analysis was truncated at 288 weeks as very few HBsAg seropositive individuals had data beyond that point.

Clinical outcomes were examined using two endpoints, the first being a composite one of new WHO stage 4 disease or death and the second being limited to death.

All deaths were reviewed by an Endpoint Review Committee (ERC) which allocated a cause of death where possible. Causes of death were then reviewed by hand to determine which were liver-related. In two cases the cause of death was then questioned and in one case was re-allocated to a liver cause.

8.2.2 Statistical Methods

The effect of HBsAg status on CD4 cell response was examined using graphs and confidence intervals and assessed by a global test accounting for within-patient correlation (Stata regress command with option cluster()).

Two clinical endpoints of new WHO stage 4 disease or death and death alone were examined using survival analysis methods, including regression and and Kaplan-Meier plots, truncated at 288 weeks due to small numbers. Analyses were stratified by HBsAg status and adjusted for initial drug regimen, age, sex, country, baseline WHO stage, CD4 count, HCV antibody status, liver fibrosis (estimated using baseline platelet count or FIB-4) and monitoring strategy. Platelet count was available for all participants but AST results were not available for participants in Harare and so FIB-4 could only be calculated on participants at Ugandan centres. To determine the effect of HBsAg in each drug and monitoring group models were rerun with an interaction factor.

The associations between the two end points and HBV DNA and HBeAg status were also examined using survival analysis, but restricted to participants who were HBsAg seropositive. In the analysis using HBV DNA, a cut off of HBV DNA greater or less than 2,000 IU/mL was used since this is used to inform treatment decisions in published guidelines [131].

Median ALT and CD4 count at death were compared using the Kruskal-Wallis equality-of-populations rank test.

8.3 Results

3,315 of 3,316 randomised patients who were tested for HBsAg were included in this analysis. 308 (9.3%) were HBsAg seropositive.

8.3.1 Change in CD4

The number of participants in this analysis declined with time from HAART initiation (Figure 45). Only 9% (including only 14 HBsAg seropositive participants) had samples after 288 weeks and 3% after 300 weeks. The analysis of change in CD4 was thus limited to the first 288 weeks. Overall, mean CD4 was 88 cells/mm^3 at baseline and 12 weeks after HAART initiation rose to 181 cells/mm^3. At 48 weeks it had risen to 222 cells/mm^3. However, mean CD4 count and change in CD4 count from baseline did not differ by baseline HBsAg status as shown in Figure 45 (p=0.51) and Figure 44 (p=0.61).

Figure 45: Mean CD4 count after treatment initiation
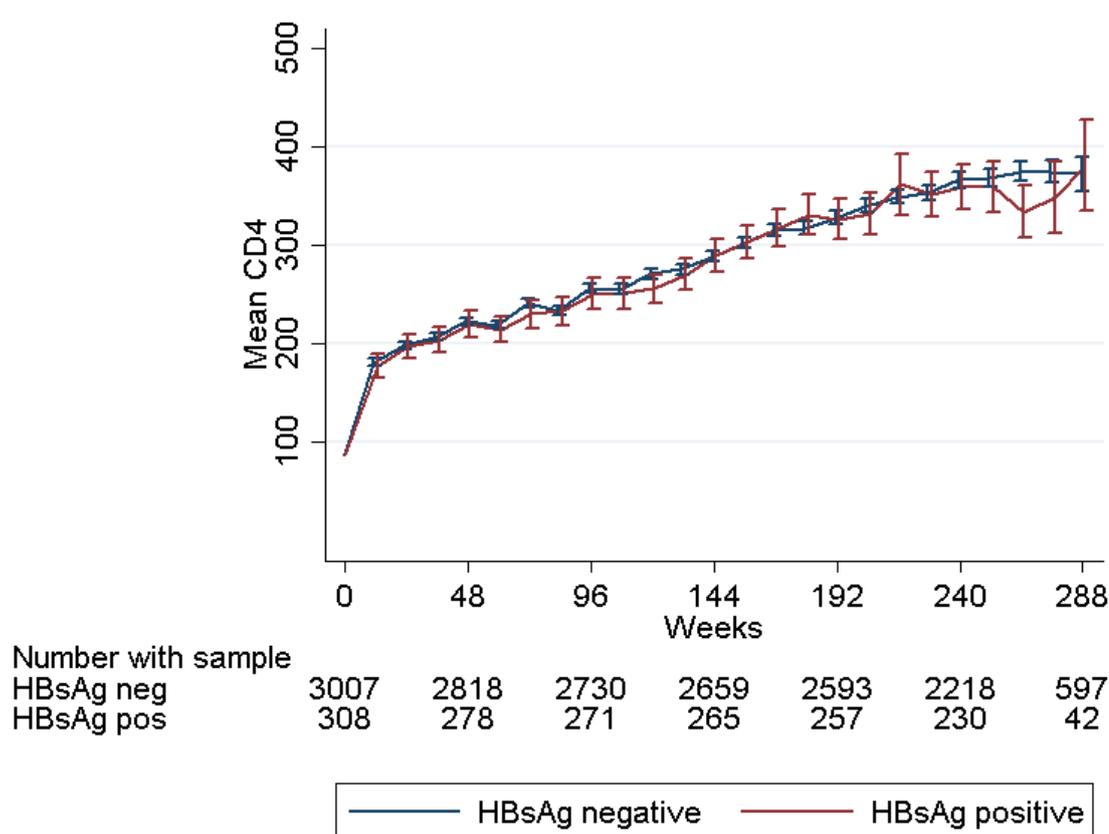
Figure 46: Change in CD4 count after treatment initiation

Number with sample							
HBsAg neg	3007	2818	2730	2659	2593	2218	597
HBsAg pos	308	278	271	265	257	230	42

HBsAg negative —— HBsAg positive ——
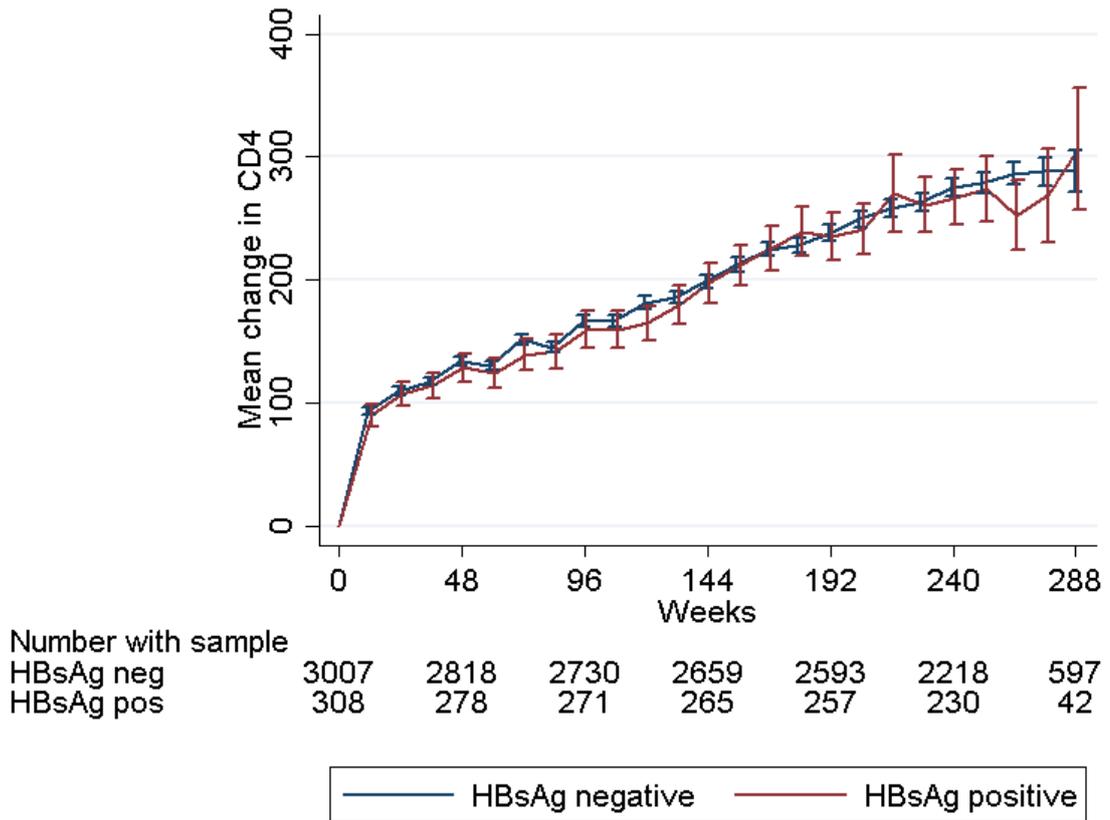
8.3.2 Progression to WHO stage 4 event or death

823 (24.8%) of 3,315 patients (731 HBsAg seronegative, 92 HBsAg seropositive) either had a new WHO stage 4 event or died over a median follow-up of 4.9 years (IQR 3.6 to 5.4 years).

Figure 47 shows the cumulative probability (1 minus the survival probability) of a new WHO stage 4 event or death following HAART initiation; Figure 46 shows the same curves limited to the first 48 weeks of follow-up.

Figure 47: Cumulative probability of WHO stage 4 event or death by HBsAg status
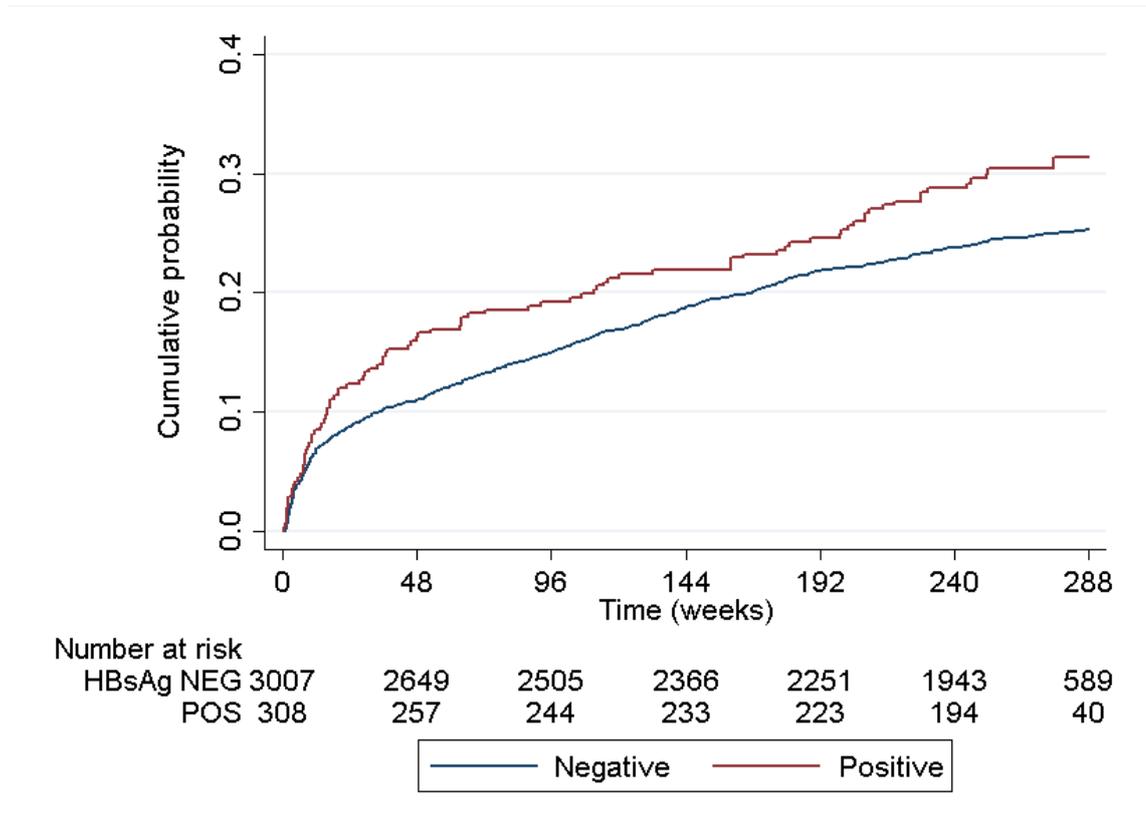
The curves diverge between 8 and 24 weeks and appear to remain approximately parallel thereafter.

Figure 48: Cumulative probability of WHO stage 4 event or death by HBsAg status (first 48 weeks)
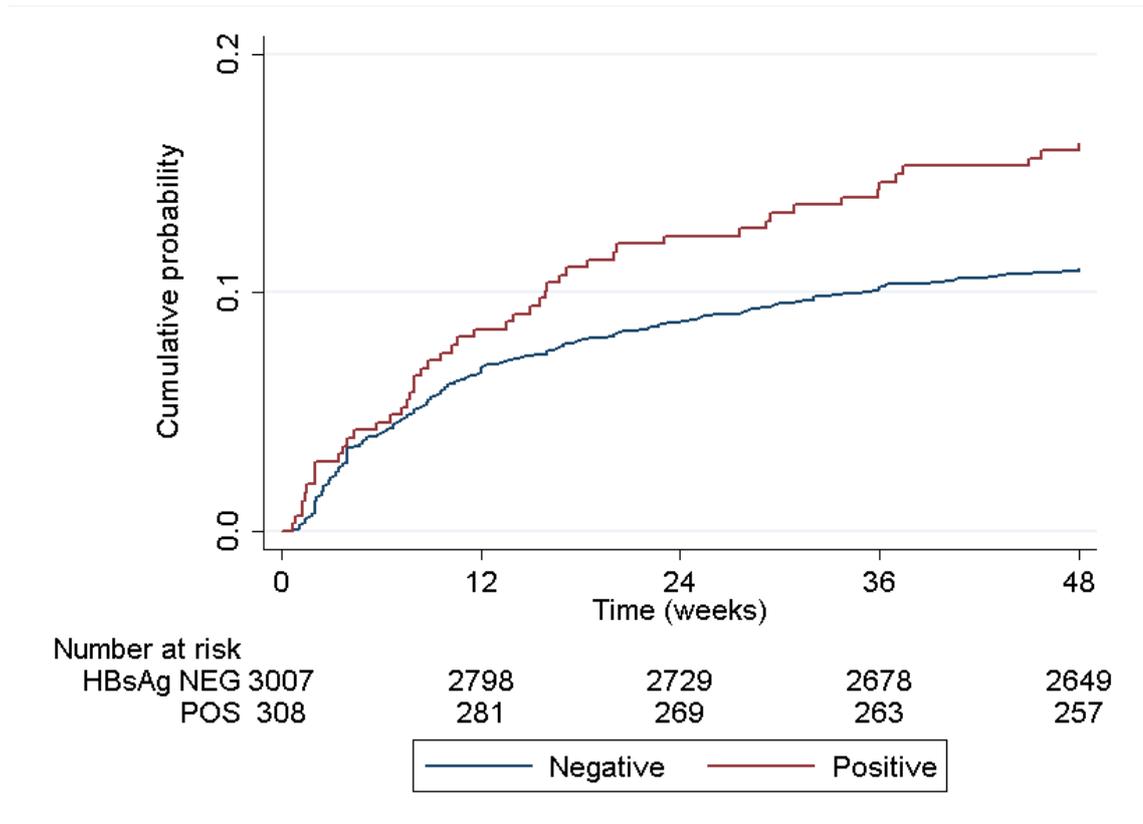
The IRR and aHR for the effect of HBsAg positivity on death over the initial 48 weeks after HAART initiation are shown in Table 48. The aHR was calculated using a Cox model adjusted for sex, age, site, drug regimen, monitoring strategy and baseline CD4. The IRR was significantly different from 1 during the first 12 weeks but not between 12 and 48 weeks. The aHR was greater than 1, though this was only statistically significant during weeks 12 to 24 and weeks 36 to 48 (Figure 49).

Table 48: Incidence rate and adjusted hazard ratios for HBsAg on WHO stage 4 or death over the initial 48 weeks

Weeks after treatment initiation	IRR	95% CI	P	aHR	95% CI	p
0 to 12	1.30	1.04 to 1.62	0.02	1.21	0.80 to 1.84	0.37
12 to 24	1.29	0.98 to 1.66	0.06	1.99	1.05 to 3.77	0.03
24 to 36	1.20	0.88 to 1.59	0.22	1.81	0.80 to 4.10	0.15
36 to 48	1.17	0.85 to 1.58	0.31	2.69	1.08 to 6.72	0.03

Incidence rates were calculated using participant-weeks.

Figure 49: Adjusted hazard ratio for HBsAg on WHO stage 4 or death over the intial 48 weeks
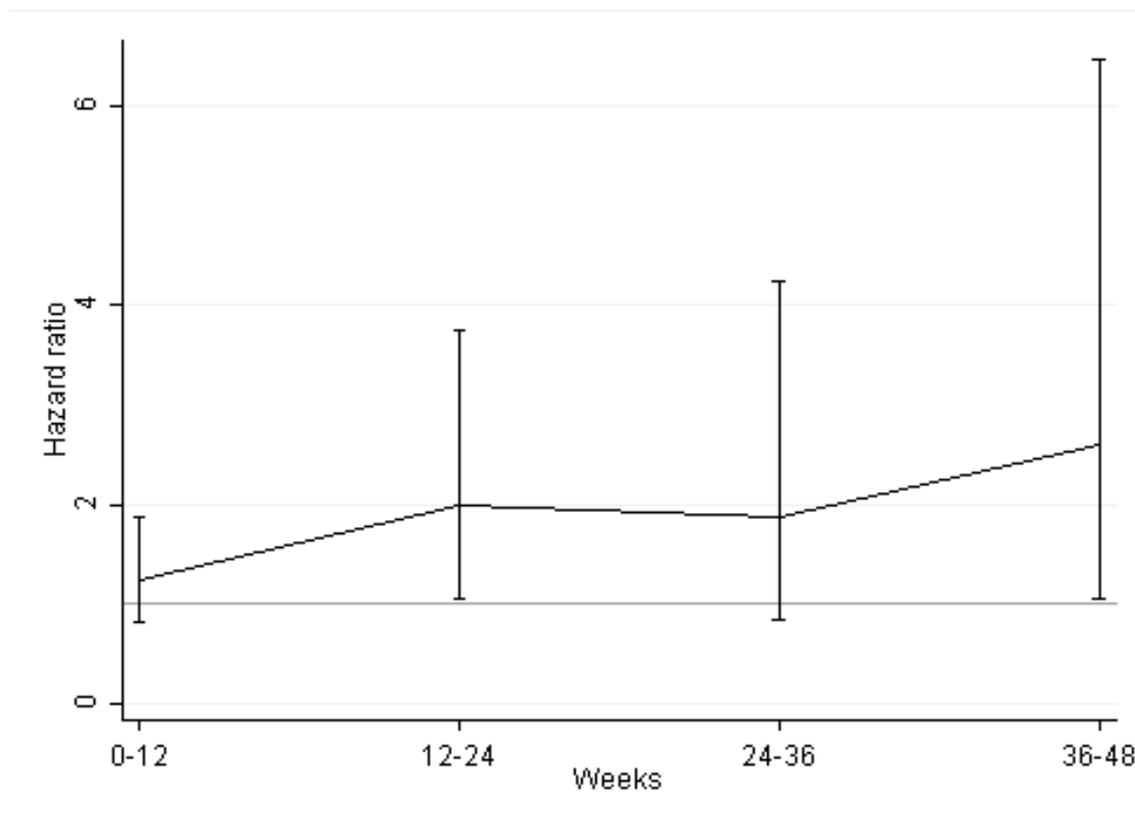
To attempt to further determine the timing of the effect of HBsAg on the incidence of a new WHO Stage 4 event or death we examined a plot of the hazard function (not shown) but this did not provide any further information.

Table 49 shows the IRR and aHR for the effect of HBsAg positivity on death over the whole of follow-up. Both the IRR and HR were significantly different from 1 during the first 48 weeks and between 192 and 240 weeks.

Table 49: Incidence rate and adjusted hazard ratios for HBsAg on WHO stage 4 or death over the whole of follow-up

Weeks after treatment initiation	IRR	95% CI	P	aHR	95% CI	p
0 to 48	1.53	1.11 to 2.06	0.008	1.54	1.13 to 2.11	0.007
48 to 96	0.85	0.40 to 1.61	0.64	0.95	0.49 to 1.84	0.88
96 to 144	0.72	0.30 to 1.47	0.38	0.61	0.28 to 1.33	0.21
144 to 192	0.90	0.38 to 1.85	0.82	0.81	0.38 to 1.69	0.57
192 to 240	2.21	1.08 to 4.18	0.02	2.00	1.02 to 3.91	0.04
240 to 288	1.81	0.55 to 4.76	0.24	1.76	0.66 to 4.70	0.26

Incidence rates were calculated using participant-weeks.

Figure 50: Adjusted hazard ratio for HBsAg on WHO stage 4 or death over the whole of follow-up
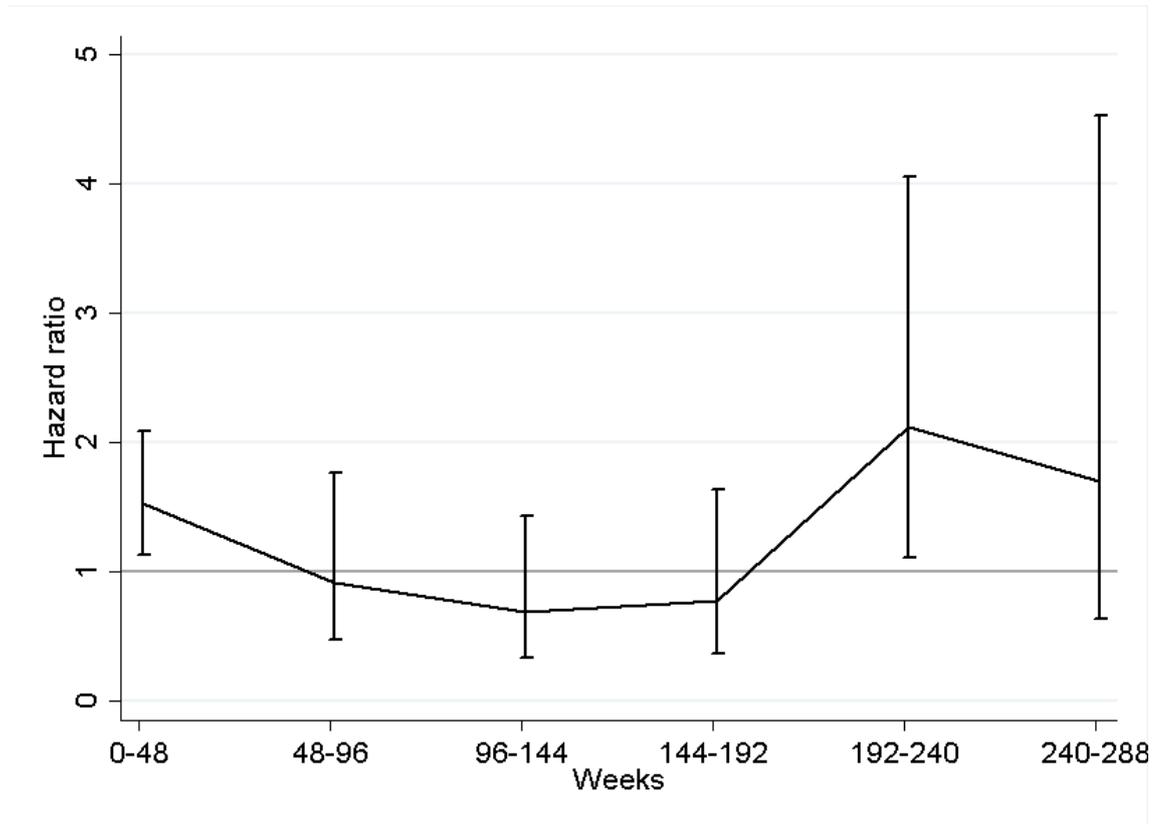
In an adjusted Cox regression model, HBsAg-positivity was associated with a 30% (adjusted; 95% CI 4 to 62%; p=0.02) increased risk of a new stage 4 event or death (Table 50). Age, site, anti-HCV status, baseline ALT, baseline platelet count and drug treatment regimen were not associated with new WHO stage 4 disease or death while sex, prior WHO stage 3 or 4 disease, lower baseline CD4 and study arm (clinical monitoring only) were associated with an increased risk. There was no association with liver fibrosis measured by FIB-4 in a model restricted to participants in Uganda (data not shown).

Table 50: Factors associated with risk of new WHO stage 4 or death in a Cox regression model

		N	Events n	Events %	Adjusted HR	95% CI	p
Site	Entebbe	1,020	229	22.5			0.81
	JCRC	1,297	337	26.0	1.06	0.89 to 1.25	
	Harare	998	257	25.8	1.05	0.87 to 1.27	
Sex	Male	1,160	323	27.8			0.05
	Female	2,155	500	23.2	0.86	0.75 to 1.00	
Age group	18-30	532	136	25.6			0.63
	30-35	795	203	25.5	0.99	0.79 to 1.23	
	35-40	848	219	25.8	1.03	0.83 to 1.28	
	40-45	608	143	23.5	0.90	0.71 to 1.15	
	45-50	313	64	20.4	0.84	0.62 to 1.14	
	>50	219	58	26.5	1.07	0.78 to 1.47	
WHO Stage	Stage 2	672	104	15.5			<0.001
	Stage 3	1,864	485	26.0	1.56	1.26 to 1.94	
	Stage 4	779	234	30.0	1.67	1.31 to 2.12	
Baseline CD4	<50	1,109	396	35.7			<0.001
	50-99	784	186	23.7	0.64	0.54 to 0.76	
	100-149	759	139	18.3	0.49	0.40 to 0.60	
	150-199	759	102	13.4	0.42	0.34 to 0.52	
HBsAg	Negative	3,007	731	24.3			0.02
	Positive	308	92	29.9	1.30	1.04 to 1.62	
Anti-HCV	Negative	3,175	786	24.8			0.95
	Positive	77	19	24.7	1.04	0.66 to 1.64	
	Not done	63	18	28.6	1.07	0.67 to 1.72	
Log ALT		3,315	823	24.8	0.97	0.69 to 1.36	0.85
Log Platelets		3,315	823	24.8	1.42	0.99 to 2.05	0.06
Drug	TDF	2,468	652	26.4			0.08
	ABC	300	54	18.0	0.76	0.57 to 1.01	
	NVP	547	117	21.4	0.87	0.71 to 1.06	
Monitoring	LCM	1,656	359	21.7			<0.001
	CDM	1,659	464	28.0	1.29	1.12 to 1.48	

The incidence of a new WHO stage 4 event or death was 5.8 per 100 patient years in those HBsAg negative and 7.6 per 100 patient years in those HBsAg seropositive (p=0.02). The IRR was such that those HBsAg seropositive had a higher incidence of new WHO stage 4 event or death than those HBsAg seronegative in those treated with TDF (IRR 1.47, 95% CI 1.15 to 1.87) but a not in those treated with ABC (IRR 0.95, 95% CI 0.25 to 2.58) or NVP (0.75, 95% CI 0.35 to 1.43) though the difference in IRR did not reach statistical significance (p=0.12) (Table 51).

Table 51: Incidence of new WHO stage 4 event or death by HBsAg status and drug treatment

		Drug			Non-TDF *vs.* TDF	
		TDF	ABC	NVP	IRR	95% CI
HBsAg	Neg	6.0	4.5	5.7	0.88	0.73 to 1.05
	Pos	8.8	4.3	4.3	0.48	0.25 to 0.86
HBsAg pos *vs.* neg	IRR	1.47	0.95	0.75		
	95% CI	1.15 to 1.87	0.25 to 2.58	0.35 to 1.43		

Incidence rates are shown per 100 participant years.

Participants in the LCM arm of DART had a higher incidence of an event if HBsAg seropositive (IRR 1.46, 95% CI 1.04 to 2.00) but in the CDM arm the association with HBsAg was not statistically significant (IRR 1.19, 95% CI 0.86 to 1.61) (Table 52) and again the difference in IRR was not significant (p=0.27).

Table 52: Incidence of new WHO stage 4 event or death by HBsAg status and monitoring strategy

		Monitoring		CDM *vs.* LCM	
		LCM	CDM	IRR	95% CI
HBsAg	Neg	4.9	6.7	1.36	1.17 to 1.58
	Pos	7.2	8.0	1.11	0.72 to 1.71
HBsAg pos *vs.* neg	IRR	1.46	1.19		
	95% CI	1.04 to 2.00	0.86 to 1.61		

Incidence rates are shown per 100 participant years.

In participants with detectable HBsAg, there was no significant difference in risk of new WHO stage 4 event or death by HBV DNA result (p=0.51, adjusted analysis) (Figure 51).

Figure 51: Cumulative probability of new WHO stage 4 event or death by baseline HBV DNA status
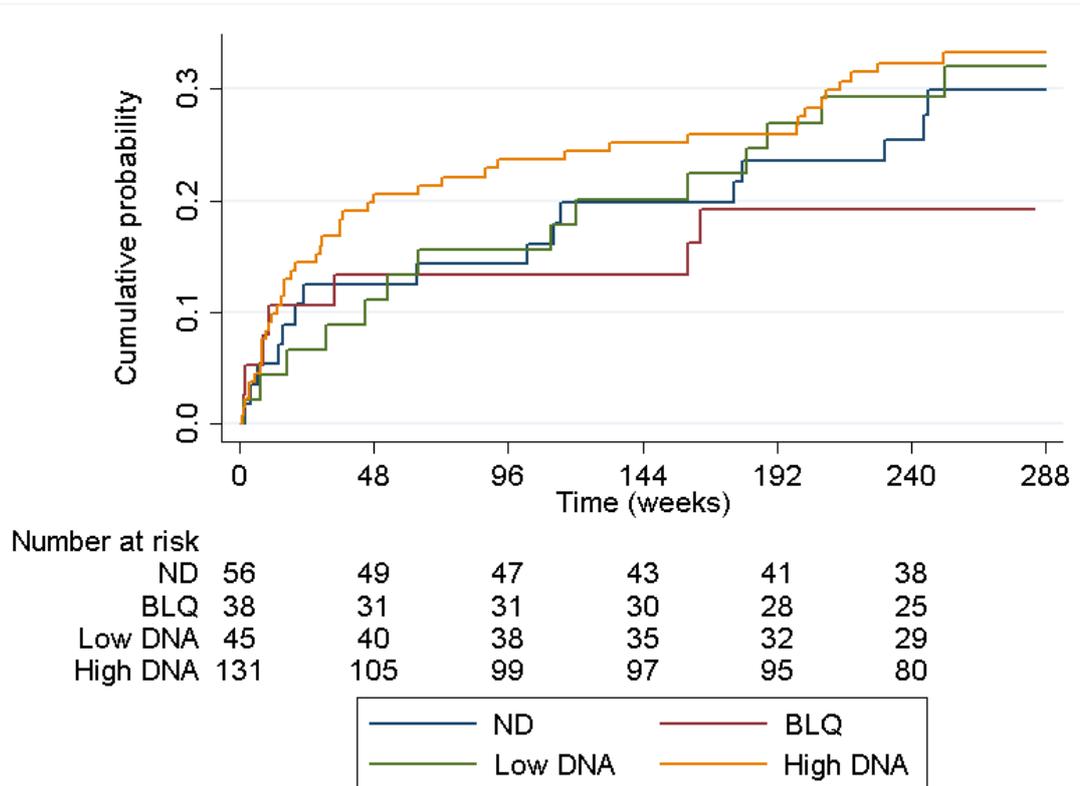
ND: HBV DNA not detected. BLQ: HBV DNA detected below the level of quantification. Low DNA: HBV DNA <2,000 IU/mL. High DNA: HBV DNA >2,000 IU/mL.

Similarly there was no difference in risk by HBeAg status (p=0.89, adjusted analysis) (Figure 52).

Figure 52: Cumulative probability of new WHO stage 4 event or death by baseline HBeAg status
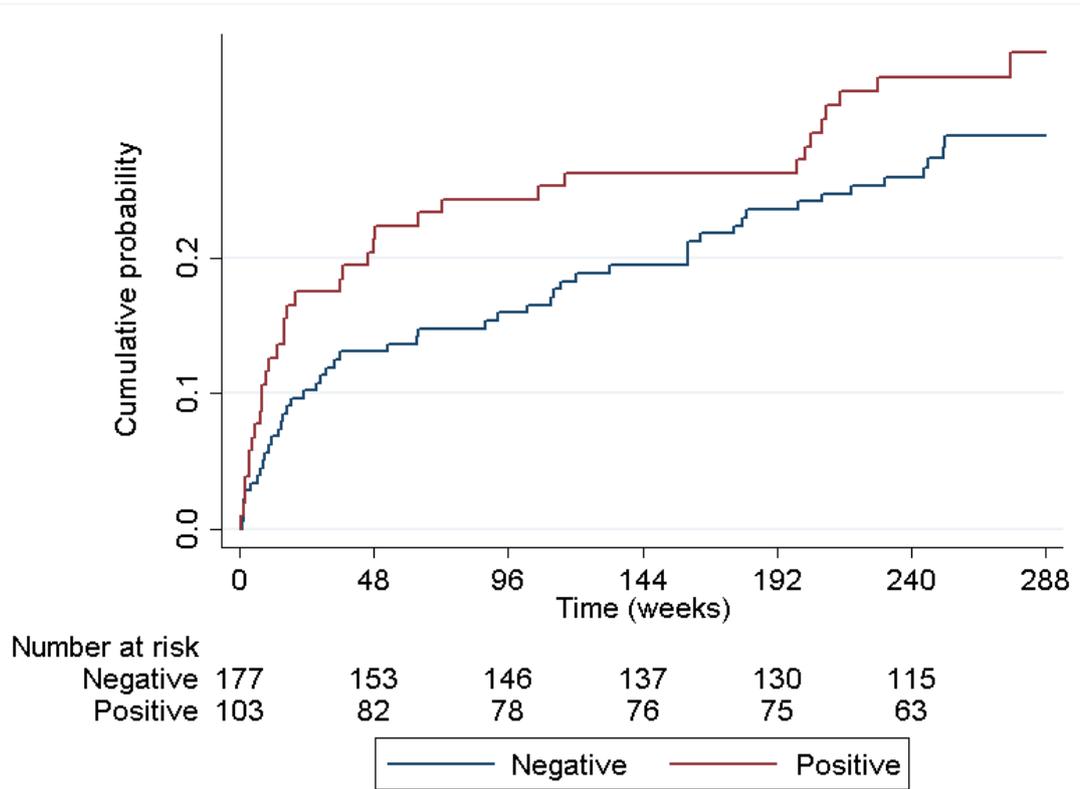
8.3.3 Death

387 (11.7%) of 3,315 patients (337 HBsAg seronegative, 50 HBsAg seropositive) died over a median follow-up of 5.1 years (IQR 4.6 to 5.5 years).

The cumulative probability curves are similar to those in the previous analysis, in that most of the difference in mortality between those HBsAg seropositive and seronegative occurred within the first year (Figure 53).

Figure 53: Cumulative probability of death by HBsAg status
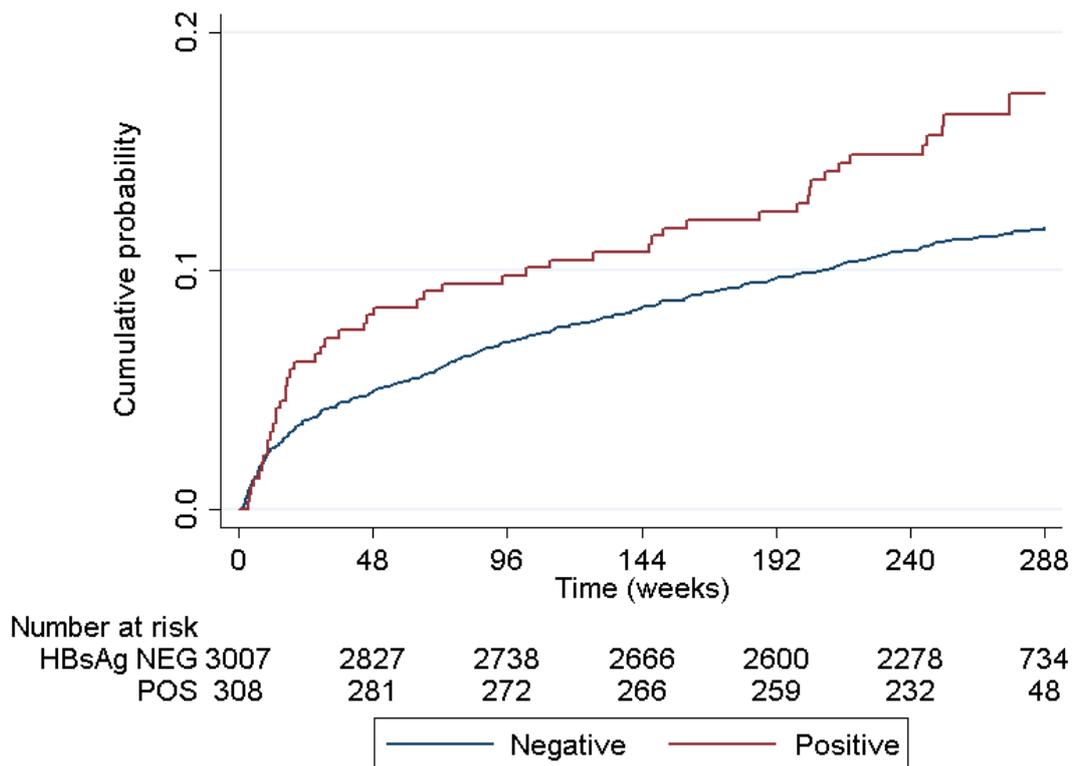
The risk of death appeared unaffected by HBsAg status during the first 12 weeks of follow-up but diverged sharply between 12 and 24 weeks (Figure 54).

Figure 54: Cumulative probability of death by HBsAg status (first 48 weeks)
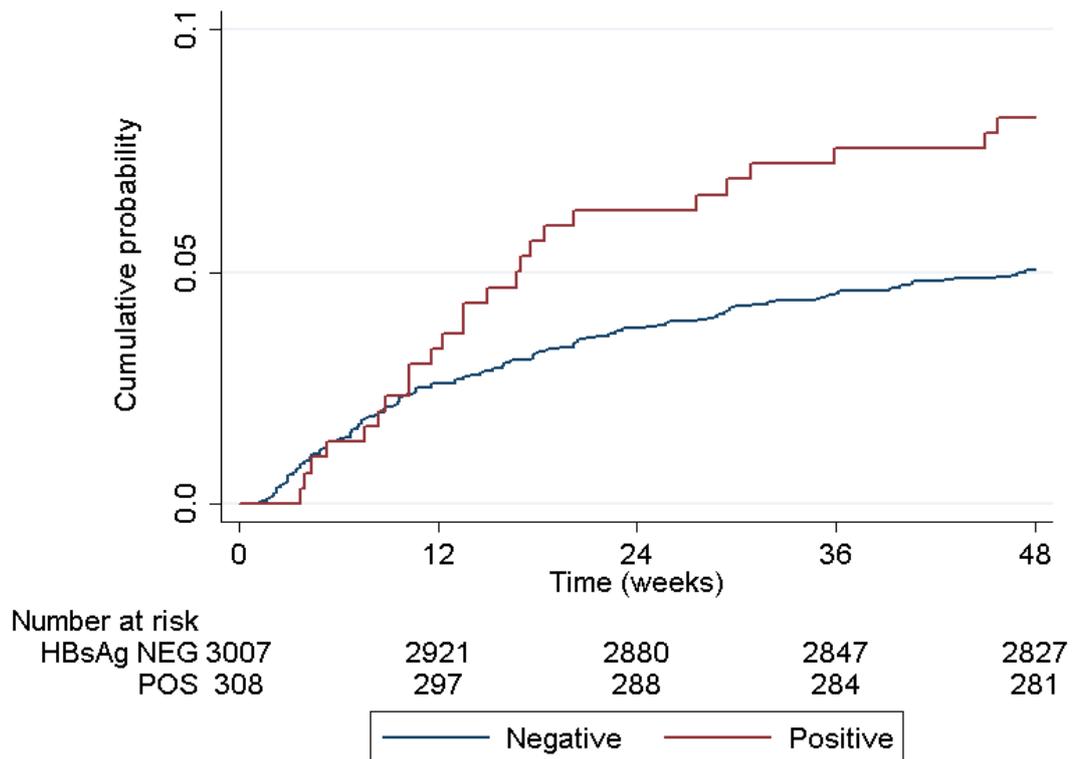
Overall 71 (18.3%) out of 387 deaths occurred between 8 and 24 weeks (inclusive) after treatment initiation. By HBsAg status, 14 (28%) out of 50 deaths in HBsAg seropositive participants and 57 (16.9%) out of 337 deaths in HBsAg seronegative participants were during this period.

The IRR and aHR for HBsAg on mortality are shown over the first 48 weeks in Table 53 and over the whole of follow-up in Table 54. [Fig] and Figure 56 show the aHR for the initial 48 weeks and for the whole of follow-up respectively. In the analysis examining the whole of follow-up, the only period during which the hazard was different by HBsAg status was from 0 to 48 weeks. Within the first 48 weeks, the only period with a statistically significant effect was between 12 and 24 weeks. As in the analysis of new Stage 4 event or death, we examined a plot of the hazard curve over time to try to determine the timing of the effect of HBsAg on mortality but this did not provide any further information.

Table 53: Incidence rate and adjusted hazard ratios for HBsAg on death over the initial 48 weeks

Weeks after treatment initiation	IRR	95% CI	P	aHR	95% CI	p
0 to 12	1.51	1.10 to 2.04	0.01	1.33	0.68 to 2.62	0.41
12 to 24	1.56	1.09 to 2.19	0.01	3.00	1.40 to 6.42	0.01
24 to 36	1.40	0.93 to 2.04	0.09	2.46	0.82 to 7.36	0.11
36 to 48	1.35	0.87 to 2.03	0.15	1.20	0.27 to 5.37	0.82

Incidence rates were calculated using participant-weeks.

Table 54: Incidence rate and adjusted hazard ratios over time for HBsAg on death

Weeks after treatment initiation	IRR	95% CI	p	aHR	95% CI	p
0 to 48	1.68	1.05 to 2.57	0.02	1.78	1.15 to 2.77	0.01
48 to 96	0.81	0.25 to 2.00	0.70	0.89	0.35 to 2.26	0.81
96 to 144	0.72	0.14 to 2.25	0.63	0.77	0.23 to 2.52	0.66
144 to 192	1.36	0.42 to 3.46	0.51	1.29	0.50 to 3.36	0.60
192 to 240	2.20	0.82 to 5.08	0.08	1.93	0.82 to 4.54	0.13
240 to 288	2.76	0.80 to 7.72	0.07	2.30	0.82 to 6.39	0.11

Incidence rates were calculated using participant-weeks.

Figure 55: Adjusted hazard ratio for HBsAg on death over the initial 48 weeks
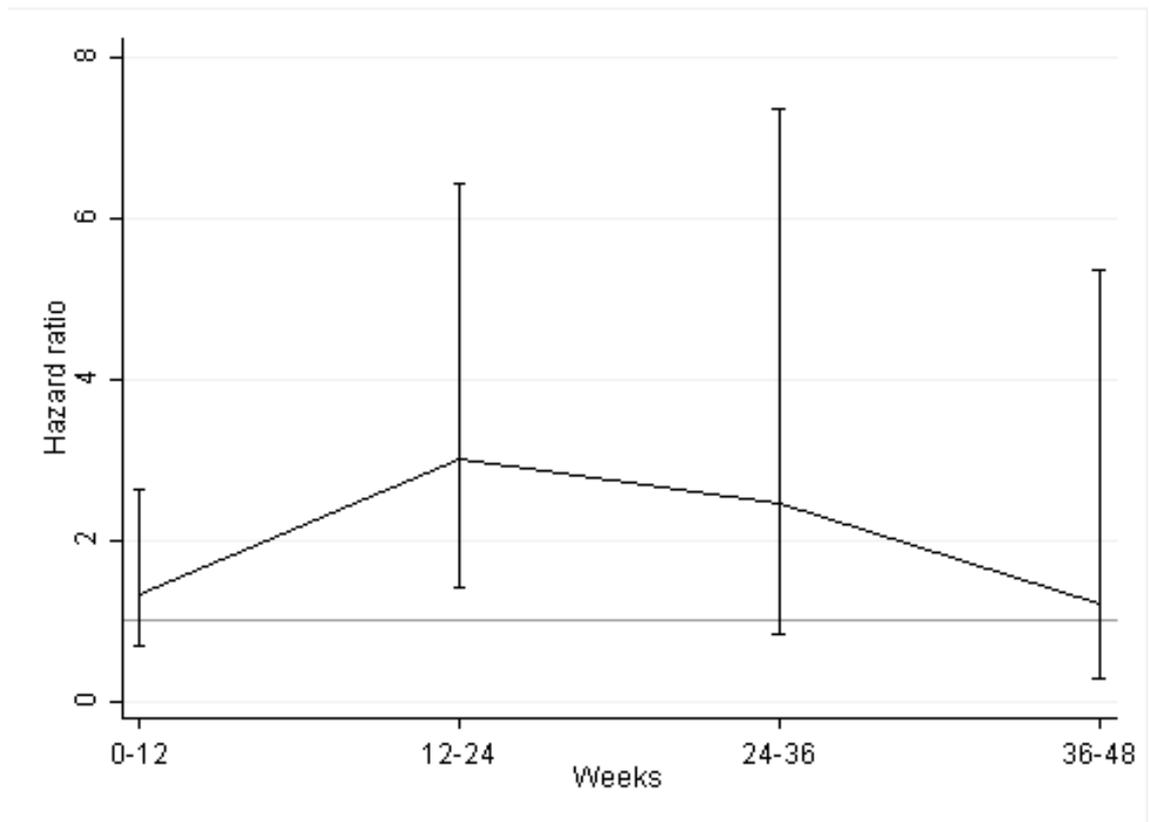
Figure 56: Adjusted hazard ratio for HBsAg on death over the whole of follow-up
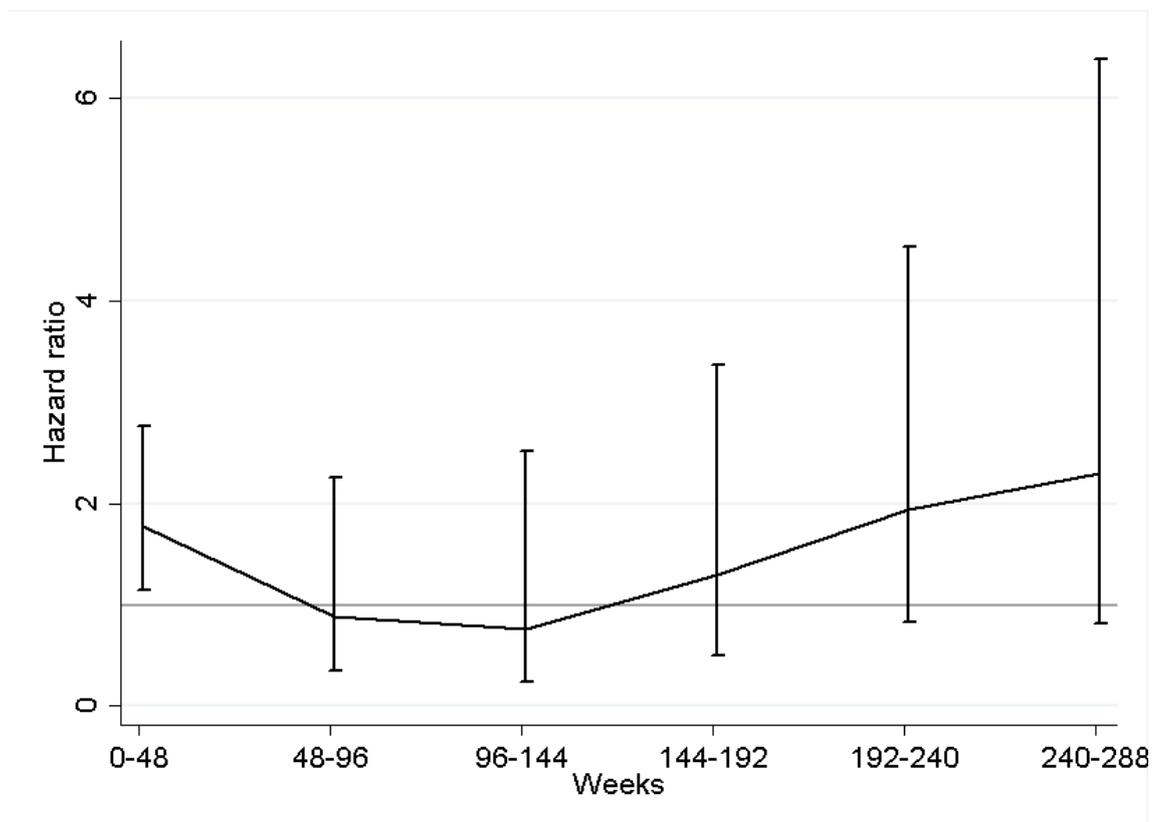
Over the whole of follow-up, HBsAg-positivity was associated with a 51% (95% CI 11 to 105%; p=0.009) higher mortality in an adjusted Cox regression analysis (Table 55).

Sex, age, site, anti-HCV status, baseline ALT, baseline platelet count and drug treatment regimen were not associated with death while WHO stage 3 or 4 disease, lower baseline CD4 and study arm (clinical monitoring only) were associated with an increased risk. Restricting to participants in Uganda, FIB-4 also did not have an effect (data not shown).

Table 55: Factors associated with risk of death in a Cox regression model

			Deaths		Adjusted		
		N	n	%	HR	95% CI	p
Site	Entebbe	1,020	111	10.9			0.79
	JCRC	1,297	164	12.6	1.01	0.79 to 1.30	
	Harare	998	112	11.2	0.93	0.70 to 1.23	
Sex	Male	1,160	155	13.4			0.29
	Female	2,155	232	10.8	0.89	0.72 to 1.10	
Age group	18-30	532	54	10.2			0.38
	30-35	795	104	13.1	1.30	0.93 to 1.80	
	35-40	848	95	11.2	1.16	0.83 to 1.63	
	40-45	608	78	12.8	1.33	0.93 to 1.90	
	45-50	313	28	8.9	0.98	0.62 to 1.57	
	>50	219	28	12.8	1.44	0.91 to 2.30	
WHO Stage	Stage 2	672	44	6.5			0.002
	Stage 3	1,864	222	11.9	1.60	1.15 to 2.22	
	Stage 4	779	121	15.5	1.92	1.34 to 2.75	
Baseline CD4	<50	1,109	194	17.5			<0.001
	50-99	784	87	11.1	0.65	0.50 to 0.83	
	100-149	759	65	8.6	0.52	0.39 to 0.69	
	150-199	663	41	6.2	0.39	0.28 to 0.55	
HBsAg	Negative	3,007	337	11.2			0.009
	Positive	308	50	16.2	1.51	1.11 to 2.05	
HCV Ab	Negative	3,175	376	11.8			0.35
	Positive	77	7	9.1	0.76	0.36 to 1.60	
	Not done	63	4	6.3	0.53	0.20 to 1.43	
Log ALT		3,315	387	11.7	1.25	0.77 to 2.02	0.37
Log Platelets		3,315	387	11.7	1.43	0.85 to 2.41	0.18
Drug	TDF	2,468	310	12.6			0.40
	ABC	300	28	9.3	0.89	0.60 to 1.32	
	NVP	547	49	9.0	0.82	0.60 to 1.12	
Monitoring	LCM	1,655	166	10.0			0.007
	CDM	1,659	221	13.3	1.32	1.08 to 1.61	

The incidence of death in those HBsAg seropositive was 3.6 per 100 patient years compared to 2.4 per 100 patient years in those HBsAg seronegative (p=0.009). The IRR for HBsAg seropositive *vs.* seronegative did not differ by initial antiretroviral drug regimen (p=0.99) (Table 56).

Table 56: Incidence of death by HBsAg status and drug treatment

		Drug treatment			Non-TDF *vs.* TDF	
		TDF	ABC	NVP	IRR	95% CI
HBsAg	Negative	2.5	2.1	2.0	0.81	0.61 to 1.06
	Positive	3.9	3.1	2.9	0.77	0.34 to 1.56
	IRR	1.53	1.47	1.45		
	95% CI	1.07 to 2.14	0.29 to 4.83	0.55 to 3.25		

Incidence rates are shown per 100 participant years.

In participants in the LCM arm, the incidence of death was 2.0 deaths per 100 patient years in those HBsAg seronegative but 3.5 deaths per 100 patient years in those HBsAg seropositive while in the CDM arm the respective incidence rates were 2.8 deaths per 100 patient years for HBsAg seronegative and 3.8 deaths per 100 patient years for HBsAg seropositive participants. However, while there was a trend towards monitoring being effective (i.e. a lower rate of death in the LCM arm) in HBsAg seronegative participants, the difference between the IRR in each arm did not reach statistical significance (p=0.27) (Table 57).

Table 57: Incidence of death by HBsAg status and monitoring strategy

		Monitoring		LCM *vs.* CDM	
		LCM	CDM	IRR	95% CI
HBsAg	Negative	2.0	2.8	1.41	1.13 to 1.76
	Positive	3.5	3.8	1.07	0.59 to 1.93
	IRR	1.76	1.34		
	95% CI	1.10 to 2.71	0.84 to 2.03		

Incidence rates are shown per 100 participant years.

Restricting the analysis to those with detectable HBsAg, there was a trend towards higher risk of death in those with HBV DNA greater than 2,000 IU/mL (Figure 57).

Figure 57: Cumulative probability of death by baseline HBV DNA status
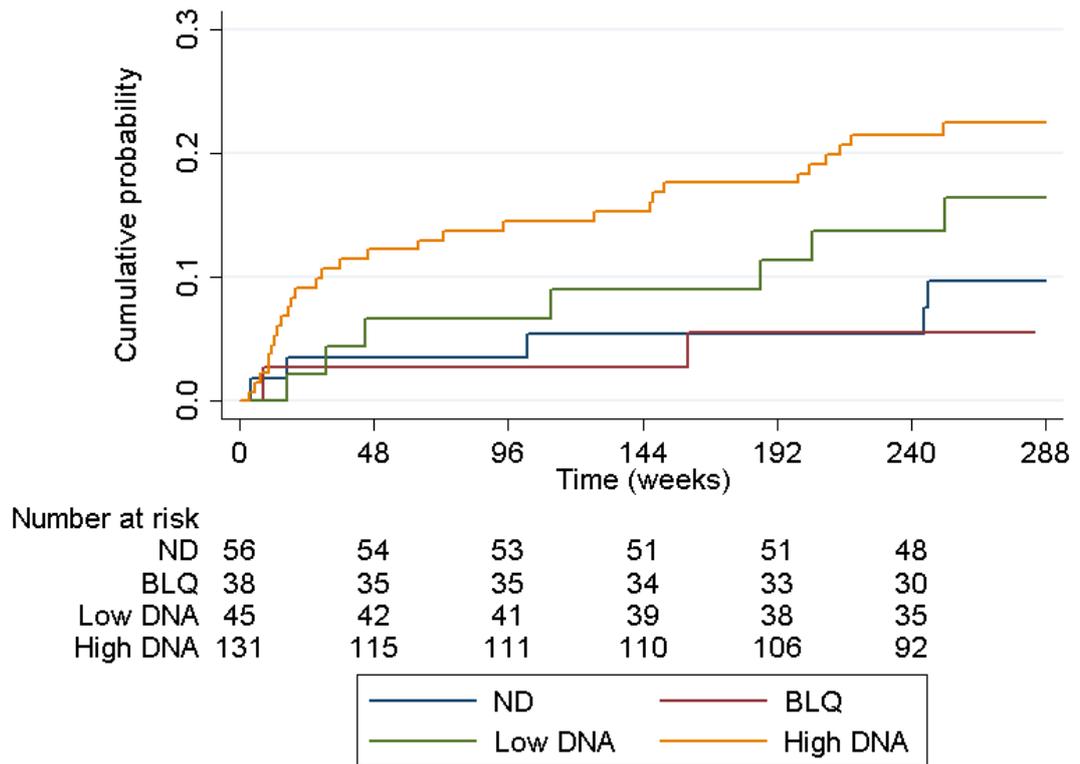
ND: HBV DNA not detected. BLQ: HBV DNA detected below the level of quantification. Low DNA: HBV DNA <2,000 IU/mL. High DNA: HBV DNA >2.000 IU/mL.

However, in a Cox regression model controlled for age, sex, drug, site, monitoring, baseline CD4, WHO stage, liver fibrosis (using API) and anti-HCV (not shown), the trend was not significant, (p=0.15).

Similarly there was a trend to higher risk of death in those with detectable HBeAg (Figure 58). However this was not significant in an unadjusted Cox model (HR 1.4; p=0.29) and the effect disappeared in an adjusted model that also included age, sex, drug, site, monitoring, baseline CD4, WHO stage, API and anti-HCV (HR 1.0; p=0.98).

Figure 58: Cumulative probability of death by baseline HBeAg status
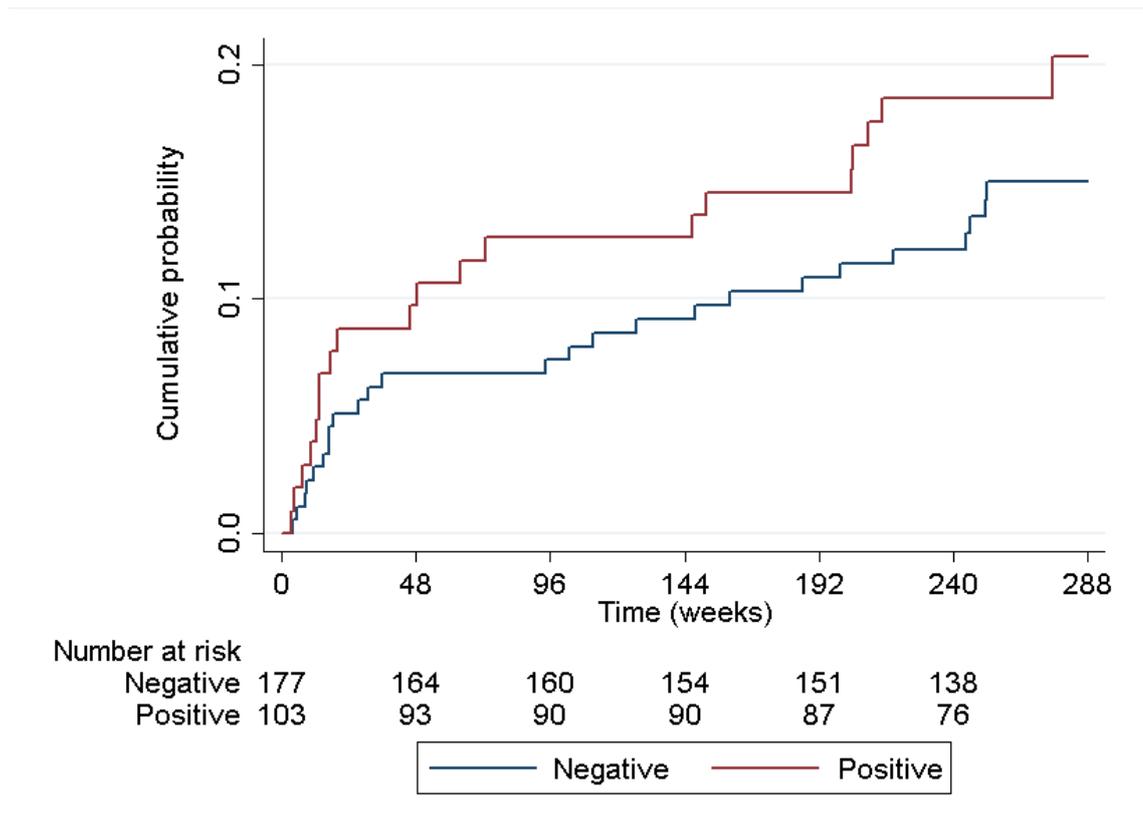
8.3.3.1 Cause of death

Deaths were classified as definitely/probably due to HIV, definitely/probably due to drug and a third group not known/uncertain/unlikely to be due to HIV or drug (which included participants given codes uncertain HIV/drug, unlikely HIV/drug, not known/insufficient information, uncertain HIV/not drug and uncertain drug/not HIV). The distribution of deaths did not differ between HBsAg seropositive and seronegative participants (p=0.14) (Table 58).

Table 58: Classification of death

	HBsAg positive		HBsAg negative		p
Category	n	%	n	%	
Definitely/probably HIV	16	32.0	162	48.1	0.14
Definitely/probably drug	4	8.0	15	4.5	
Uncertain	30	60.0	160	47.5	
Total	50	100.0	337	100.0	

A cause of death could be assigned in 270 (80.1%) HBsAg seronegative and 39 (78.0%) HBsAg seropositive participants. Primary or secondary causes of death included liver-related events in 12 (4.4%) and 3 (8%) respectively (p=0.42) (Table 59).

Table 59: Liver-related causes of death by HBsAg status

Cause of death	HBsAg negative	HBsAg positive	Total
Liver failure	10	2	12
Sepsis/liver failure	2		2
Hepatocellular carcinoma		1	1
Total	12	3	15

Of the 15 liver-related deaths, using the classification in Table 58, the deaths of 3 participants were reported as "Definitely/probably drug" related (all HBsAg negative, two with "Liver failure" and one with "Sepsis"). The other 12 were reported as "Uncertain".

As stated above, the additional risk of death associated with HBsAg was statistically significant only between 0 and 48 weeks and from Figure 54 it appears this effect mostly occurred between 8 and 24 weeks after treatment initiation. Of those who died between 8 and 24 weeks after starting HAART, cause of death was recorded for 13 HBsAg seropositive and 47 HBsAg seronegative participants. Of these, 2 HBsAg negative participants died of liver failure. The HBsAg seropositive participants who died during this period died of: neurological disease (1 meningitis, 1 epilepsy, 1 transverse myelitis, 1 indeterminate), infections (1 cryptosporidium, 3 of sepsis, 1 miliary TB), cancer (1 solid tumour, 1 CNS lymphoma), 1 anaemia and 1 of COPD.

There was no significant difference between the last ALT count before death in those HBsAg seropositive compared with those HBsAg seronegative (Table 60) or both with baseline ALT (p=0.59) (Figure 59).

Table 60: Last ALT before death by HBsAg status

HBsAg	Median	IQR	Range	p
Negative	26	15 to 42	3 to 1802	0.59
Positive	24	15 to 41	4 to 1615	

Figure 59: Distribution of ALT at baseline and before death in HBsAg seronegative and seropositive participants
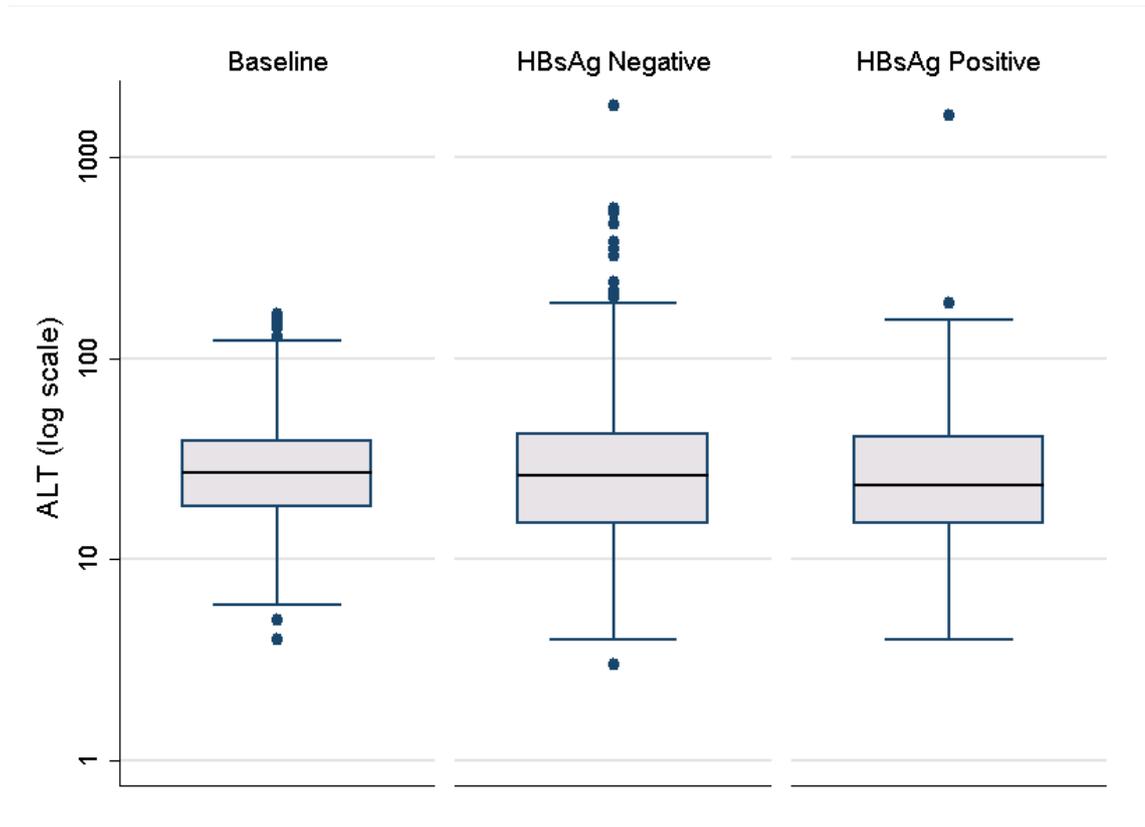
In those who died in the first 24 weeks after treatment initiation, the majority had normal ALT and very few had an ALT greater than 5 times the ULN (Table 61).

Table 61: Last ALT before death in those who died within 24 weeks of treatment initiation

HBsAg	N	ALT > ULN			ALT > 5x ULN		
		N	%	P	N	%	P
Negative	112	26	23.2	0.77	2	1.8	0.35
Positive	19	5	26.3		1	5.3	

There was also no difference between the last CD4 count before death in those HBsAg seropositive compared with those HBsAg negative (Table 62).

Table 62: Last CD4 count before death by HBsAg status

| HBsAg | HBsAg positive | | | |
	Median	IQR	Range	p
Negative	86	22 to 169	1 to 830	0.66
Positive	87	33 to 235	1 to 399	

8.4 Discussion

8.4.1 CD4 count at baseline and over time

We found no difference in baseline CD4 count between those positive or negative on testing for HBsAg. There was also no difference in the rise in CD4 after treatment initiation. This latter finding is in agreement with most previously published data [74, 75, 79, 80, 82, 84-86]. However a recently published study in Tanzania found lower CD4 counts at baseline (median 101 $vs.$ 116 cells/mm^3) and smaller rises in CD4 at 6 (71 $vs.$ 77 cells/mm^3, p=0.46) and 12 months (143 $vs.$ 158 cells/mm^3, p=0.05) of follow-up in those HBsAg seropositive [199]. It was a large study with 1,079 coinfected and 16,460 HIV-monoinfected patients starting ART. The authors stated that their study was the first to show such effects of HBsAg status on CD4 count on ART and suggested that previous studies may have included too few patients and that the result is real, perhaps due to coinfection leading to CD4 cell death via either activation or splenic sequestration. Indeed some of the earlier studies did show a non-significant higher CD4 rise in those HBsAg seronegative [74, 80, 84, 85] but in one that included 178 HBV coinfected and 2,781 HIV monoinfected patients there was no difference [86]. Another recent study (from Ghana) also found an association between a positive HBsAg status and lower CD4 rise, with HBsAg-positive patients having a 2 cells/mm^3 smaller increase per month over 36 months [96]. This study included 143 coinfected and 228 monoinfected patients. Of particular note that study found HBsAg was associated with higher baseline CD4 count (133 $vs.$ 119 cells/mm^3, p=0.08). It may be that differences in baseline and rise in CD4 exist but that DART was not powered to detect them. However it appears that if any such associations are real the differences are small and perhaps not clinically important.

8.4.2 Clinical progression

In this study, all patients were treated with at least one drug with activity against HBV and 75% with dual therapy, i.e. 3TC alone or with TDF. Despite this, HBsAg positivity was associated with an increased rate of progression to death and to the combined end-point of WHO stage 4 HIV disease and death. Most of this excess risk appeared to occur within the first year after treatment initiation. In fact the risks were similar very early after treatment initiation, then diverged for a period of weeks and then were again similar, but with a suggestion of a difference between 192 and 240 weeks. An explanation of the lack of difference early on could be that sicker individuals with HBV coinfection were excluded from study entry. In fact there were no deaths of HBsAg seropositive individuals for almost a month after initiation. After about one year HBV will be suppressed in the majority of coinfected participants (chapters 3 and 6) and there may have been some improvement in liver fibrosis [306] and so perhaps the

effect of HBV minimised. The apparent difference later in follow-up may be related to participants changing to second line therapy. However the analysis of the changing hazard over time is based on only 29 deaths in HBsAg seropositive individuals during the first year and so should be interpreted with caution.

As discussed by Walker, the risk of death was low immediately after entry to DART and rose to a maximum between 30 and 50 days after study entry [307]. This was likely to be because patients at high risk of imminent death were excluded. Other possible causes suggested for an initial increase in the risk of death after HAART initiation included deaths due to drug toxicity or IRD or a delay in HAART having an effect. It is impossible to determine to what extent these play a role. Drug toxicity has been reported to cause death in the presence of liver flare. However few deaths in DART were reported as being due to liver disease and we did not find evidence of a rise in ALT before death. IRD has been reported against HBV [308] and the timing of the rise in risk of death does fit with a process occurring during immune reconstitution, in that IRD most often occurs during the first 90 days of treatment [309]. There is no indisputable definition of IRD but one commonly used includes: (i) onset after antiretroviral initiation or change, (ii) a rise in CD4 cell count by at least 50 cells/mm^3 or by a factor of 2 or a decrease in HIV RNA by at least 0.5 log copies/mL, (iii) an inflammatory process and (iv) lack of an explanatory infectious cause of the signs and symptoms observed [310]. In trying to explain the incidence of death after treatment initiation in DART we are limited in that all participants recently started treatment, HIV RNA data is not available and we have limited information on infectious conditions. However we can likely exclude IRD against HBV in hepatocytes since so few of those who died in the first 24 weeks had raised ALT at death and there was no difference in ALT by HBsAg status. CD4 count was measured in DART but it has been suggested that CD4 rise may not be relevant in IRD since restoration of immune function may not be directly related to CD4 cell count in plasma [311]. Studies of IRD related to other conditions such as TB or cryptococcal disease have attempted to identify other factors in blood that may be related, such as IL-6, IFN-γ, TNF-α and others [312]. Crane found CXCL-10 and soluble CD30 to be associated with liver inflammatory flares after starting antiretroviral treatment [202] and suggested that such flares are due to IRD [313]. Andrade examined biomarkers of inflammation in HBV/HIV coinfected patients and identified increased D-dimer, IL-6 and soluble CD14 as associated with mortality while increased I-FABP was associated with a lower risk of death [314]. The same study identified increased ALT and IL-10 at baseline as associated with a liver flare in the first 4 months on ART and increased CCL26 associated with a lower risk of flare. Testing for such immune markers has not been performed on the DART population. Liver

biopsy would also be very useful to determine whether drug toxicity or IRD were involved in deaths but DART participants did not have liver biopsies [310].

Despite the higher risk of death in those with HBsAg there was no evidence of an increase in death due to liver disease. This is surprising in light of the greatly increased rate of liver-related death demonstrated in the Multicentre AIDS Cohort Study (MACS) [118]. However there are significant differences between the MACS and DART populations. HIV-positive patients in the MACS study included individuals on and off HAART and with higher CD4 counts and the whole of the MACS study period examined was prior to the introduction of TDF. In addition, our lack of finding an increase may be related to low numbers; there were 61 liver-related deaths in the MACS study but only 15 in the current one.

We did not find an association between baseline ALT and mortality or between baseline platelet count (which may be reduced in advanced liver disease) and mortality. This is in contrast to previous findings, for example in the Veterans Aging Cohort Study both ALT [315] and FIB-4 (which includes ALT and platelet count) [316] were associated with all-cause mortality. In addition we did not find a significant increase in ALT before death compared to baseline, which also suggests that few deaths were related to liver inflammation.

However this study, in common with many others including the Tanzanian study referenced above [199], suffered from a lack of clarity in cause of death, in that in one fifth of deaths no cause of death was determined. This limits the validity of our findings. However there are inherent problems in cause of death analyses of HIV populations, as reviewed by Justice [317]. HIV has many effects, not only immunosuppression, but also including immune activation and inflammation, anaemia and thrombocytopaenia and interactions with infections such as hepatitis B. Other factors involved in risk of death include age, sex, smoking, alcohol, other comorbidity and drug toxicity and these may well not act independently of one another and of HIV-related factors. The added complication that many deaths occur away from health facilities make it even harder to record accurate causes of death. Thus, Justice argued for not performing cause of death analyses but rather analysing all-cause mortality.

As noted in chapter 1, there is a risk that any association found between coinfection and mortality may in fact be due to confounding factors, as in the SMART study, in which although the risk of non-AIDS death was higher in those with HBV or HCV coinfection, such deaths were very rarely due to liver disease with the three most commonly stated causes being "unknown", substance abuse and non-AIDS malignancy [97]. This may help explain why in the present study the rate of death was

higher in coinfected participants and yet the majority of deaths do not appear to be as a result of liver disease. This would be in keeping with the fact that we found that treatment with TDF did not eliminate this difference in risk. This is again in contrast with the Tanzanian study mentioned above, in which they found that HBV coinfection was associated with an increased risk of death only in those not treated with TDF [199].

There was also no increase in risk of death in those treated with NVP (in all participants or in those coinfected with HBV) despite NVP being a well-recognised cause of liver toxicity and indeed being associated with an increased risk of flare in the current study.

8.4.3 Benefits of routine laboratory monitoring

The overall risk of death was reduced in the LCM arm, as described previously [259]. However we found no evidence that routine 3-monthly laboratory monitoring reduced the effect of HBV infection on mortality. In fact we found a trend towards laboratory monitoring reducing the risk of death only in those who were HBsAg negative. This contradicts the conclusions of the authors of the Tanzanian study who suggested that "HIV/HBV co-infected individuals clearly need to be monitored more closely" [199]. Monitoring can only be of benefit to individuals if clinical decisions are made as a result of such monitoring. Any benefit of monitoring that could be detected in DART may be partially obscured since grade 4 abnormal results were returned to clinicians regardless of study arm (LCM or CDM). However only 26 (0.8%) DART participants had a grade 4 rise in ALT during follow-up (as described in chapter 6) and the majority of participants (54.0%) had normal ALT throughout follow-up while only 8.3% had a rise of ALT of greater than Grade 1 (2.5x ULN [181]). Of 80 participants with a flare (chapter 6), in only 25 (31%) were these flares followed by a change in treatment (18 of which were reported as being made due to liver disease) and 8 of these changes were interruptions after which the same regimen was restarted after a break of between 7 and 43 days. Thus even when the result of a monitoring test was abnormal the test rarely resulted in a change in management.

8.4.4 Conclusion

HBV coinfection was associated with an increased risk of death in HIV-positive individuals on HAART. The reason for this increase remains unclear and may not be related to liver disease or immunosuppression.

The use of TDF did not eliminate the increase in risk of death associated with HBV infection and so this analysis did not find evidence to support the use of more than one drug against HBV in coinfected patients, as recommended in guidelines [123].

We did not find evidence to support the use of routine monitoring of ALT.

9 Conclusions

9.1 Epidemiology

Of the 3,316 participants of the DART study, 1,829 (55.2%) had evidence of exposure to HBV and 308 (9.3%) were seropositive for HBsAg at study entry. Exposure was more common in Zimbabwe than in Uganda, in males than in females and with increasing age. HBsAg seropositivity was also more common in Zimbabwe and in males but age had no effect, either on the prevalence of HBsAg seropositivity in the whole population, or on the probability of having evidence of having cleared an infection if exposed. Of those with a positive HBsAg test result, 36.8% tested positive for HBeAg and 79.3% had detectable HBV DNA. HBeAg test results were more often positive in those with advanced HIV disease. As expected, DNA was more likely to be detected in plasma in participants with detectable HBeAg than in those without HBeAg and when detected to be at a higher level. Unlike HBeAg test results, DNA test results were not associated with stage of HIV disease.

These results are in line with previously published prevalence of anti-HBc and HBsAg in Uganda and Zimbabwe (chapter 1, Tables 1 and 2). Data on the prevalence of HBeAg seropositivity in HBV/HIV coinfected individuals in Uganda and Zimbabwe is limited to 23 individuals in Uganda and 24 in Zimbabwe where the rates found were 28% and 54% respectively [32, 48]. In a systematic review of viral hepatitis serology in sub-Saharan Africa, the prevalence of HBeAg in HBV/HIV coinfected individuals in 13 studies was 17.1% (82/480) [20]. This review included the Ugandan study mentioned above [48].

However, put together with previous data, the results underline the fact that there are differences in HBV epidemiology not only between countries in sub-Saharan Africa but even between areas quite close together in the same country. These differences include variation in the proportion exposed and also in the outcome from exposure. Thus, in order for providers of HIV care to consider the importance of HBV coinfection, local epidemiological data is required since assumptions made about coinfection prevalence by extrapolating from data from elsewhere may well be inaccurate.

9.2 Suppression

A systematic review and meta-analysis of all available data describing HBV DNA suppression in HIV infected patients treated with TDF showed that 57.4% had fully suppressed HBV DNA at 1 year and the proportion suppressed increased over time to 85.6% at three years. Suppression of HBV DNA to undetectable levels was more common at 1 year in those HBeAg seronegative, but at 2 and 3 years there was no

difference by HBeAg status. Suppression was durable, with very few patients experiencing breakthrough on treatment. There was no evidence that the proportion achieving suppression is increased when TDF is used with 3TC or FTC compared with when TDF is the only drug with activity against HBV. There was also no evidence that prior exposure to 3TC and/or FTC is associated with a lower probability of suppression when treated with TDF.

DART included patients treated with 3TC as the only drug active against HBV. In the DART population we found similar results to those from the meta-analysis; half (51.2%) of those with quantifiable baseline HBV VL suppressed to undetectable after 48 weeks of treatment and two-thirds (68.6%) suppressed by the end of follow-up with no difference in suppression at 48 weeks between those treated with 3TC alone or with 3TC in combination with TDF. Positive HBeAg status and baseline HBV VL >10^7 IU/mL were associated with failing to achieve an undetectable HBV VL. Maintenance of suppression once achieved was as likely in those treated with 3TC alone as in those treated with 3TC and TDF. In contrast to this lack of difference by treatment group, those treated with TDF who had not fully suppressed at one year were likely to go on to do so on continued treatment but none of those treated with 3TC alone who had failed to suppress at one year did so later.

9.3 Flares

Liver transaminase flares on starting ART were more common in HBsAg seropositive participants (HR 3.4) though in those treated with NVP, HBV coinfection was not associated with increased risk. 7 of 80 with a flare on first line ART died within a month of the flare. Only 1 of these 7 was HBsAg seropositive. Most flares on first line ART did not result in any clinical change and resolved spontaneously.

Detectable HBsAg was also a predictor of flare on a switch to a second line ART regimen that did not contain TDF or 3TC (HR 4.5). Death was very common after such flares (50% of HBsAg seronegative and 75% of HBsAg seropositive participants, p=0.58) and liver failure a commonly cited cause although these results should be interpreted with caution as the absolute numbers were very low (3 deaths from 4 flares in HBV coinfected participants and 4 from 8 in HIV monoinfected participants) and deaths occurred up to 8 months after the onset of the flare.

However HBsAg seropositivity was not a predictor of a flare when participants underwent 12-week structured treatment interruption and flares that did occur in such interruptions were not associated with death.

Laboratory monitoring strategy was not associated with a better clinical outcome in any of these scenarios. However any benefit of monitoring may have been obscured since results were returned to clinicians when grade 4 abnormalities were found.

These somewhat contradictory results may be explained by the fact that (i) at study entry participants had advanced HIV disease and therefore a high risk of death, (ii) participants switching to second line may have done so as a result of a failure of first line therapy and thus be a population with a higher risk of death and (iii) subjects in the structured treatment interruption substudy were a selected group who had responded to treatment and thus were at a lower risk of death.

9.4 Clinical Outcome

There was no difference in CD4 count by HBsAg status, either at baseline or on ART. However HBsAg seropositive participants had a higher cumulative probability of reaching the two endpoints examined (progression to new WHO stage 4 event or death and progression to death) after treatment initiation. The difference between those HBsAg seropositive and those HBsAg seronegative appeared to occur within the first year of treatment, possibly after a lag of 2 months. The increased progression to new stage 4 event or death in those HBsAg seropositive was not significantly reduced in the laboratory monitoring strategy arm. However in HBsAg negative participants there was a reduced incidence in the LCM arm compared to the CDM arm. In HBsAg seropositive participants there was no reduction in progression in those treated with TDF compared to those treated with ABC or NVP. In fact the incidence of new WHO stage 4 disease or death was higher in the TDF group. However treatment allocation was not randomised or blind so this result may be a result of bias.

Although all-cause mortality was higher, few deaths were recorded as due to liver disease and there was no evidence of more frequent liver inflammation at death (as measured by ALT) in those HBsAg seropositive compared to negative. In patients with advanced liver fibrosis the liver may be unable to produce enough ALT to have a high value and thus ALT may be misleading as a marker of inflammation. However platelet count at death was rarely very low, which suggests that few of the participants had advanced liver fibrosis or cirrhosis.

9.5 Limitations

The primary aim of this hepatitis substudy was to examine HBV virological suppression in coinfected participants of the DART study. One limitation of describing this suppression is that, as a result of time and resource constraints, in the majority of individuals we have HBV viral load measurements only at baseline, at week 48 and at

the end of first line therapy. In participants who had a detectable HBV VL at 48 weeks and an undetectable VL at the end of first-line therapy, HBV VL measurements at intervening points would allow us to better determine the time over which suppression occurs.

In addition to showing the proportion of HBV/HIV coinfected individuals that achieved HBV virological suppression on ART we have also attempted to answer other questions relevant to the management of coinfection such as the effect of HBV-active treatment on progression. A better understanding would be gained if we were able to compare with a control group who received ART which did not have anti-HBV activity. However DART was a randomised controlled trial of monitoring strategy in which all participants received treatment with 3TC. We have compared participants treated with and without TDF although treatment allocation was not randomised. The study was performed in the absence of HBV testing and in a period when TDF was not routine. The latest guidelines for antiretroviral treatment from the World Health Organization state that TDF (with 3TC or FTC) should be included in first line regimens unless there are contraindications while AZT (with 3TC) is given as an alternative and ABC or D4T can be used in special circumstances [132].

One possible reason for failing to achieve virological suppression is poor adherence to therapy. DART participants had adherence assessed by a healthcare worker every 4 weeks through the study. In the majority of cases of poor HBV VL response to treatment there was no association with poor adherence. However it may also have been informative to examine blood levels of ART in those who did not achieve an undetectable HBV viral load to estimate how many participants were exposed to sub-therapeutic levels of drugs, whether through poor adherence or through drug interaction or malabsorption.

Another explanation for virological failure is drug resistance. Mutations in the reverse transcriptase of HBV giving rise to resistance to 3TC are well recognised and characterised. On the contrary, mutations giving rise to resistance to TDF have not been consistently demonstrated. HBV resistance testing of baseline and on-treatment samples from participants in whom HBV DNA was detectable on treatment could demonstrate the contributions of both pre-existing and emergent viral resistance. Since the DART cohort contains a large number of HBV-coinfected participants treated with TDF and follow-up is long it would be a good population in which to look for the emergence of TDF resistance mutations. However there were only 10 participants treated with TDF that had HBV VL greater than 1,000 IU/mL after more than 48 weeks on treatment and so even in this large study, the absolute number of participants in

which to look for such resistance mutations is low. Sequencing will be performed but this represents further work beyond the scope of this thesis.

In addition to not being able to compare patients treated with HBV-active and HBV-inactive ART, our study would benefit from an HIV-negative control group. This would allow us to compare outcomes by HIV status. The published literature on HBV from resource-poor areas is more extensive in HIV-negative or HIV-untested than HIV-positive populations. The degree to which this literature is directly relevant to HIV coinfected populations is unclear. The DART population all had advanced HIV disease with CD4 counts below 200 and so the relevance of our findings to individuals with higher CD4 count, including those with very high CD4 count who thus do not need immediate treatment, is also unclear.

The outcomes we hope to avoid in HBV-infected individuals are liver failure and death. These become more likely as liver fibrosis progresses. In examining the issue of progression of liver disease this study would have greatly benefitted from a well-validated measure of liver fibrosis. Paired liver biopsies have been used to determine fibrosis progression and biopsy is the gold standard measurement of liver fibrosis. However transient elastography (TE, FibroScan®) is being increasingly used in resource-poor areas as well as resource-rich ones. For example, in a study of 59 HBV-monoinfected patients in Burkina Faso, TE has been shown to perform very well against biopsy (AUROC 0.87 to distinguish F0-1 from F2-4) [318]. However a study of 117 HBV-monoinfected patients in Indonesia found TE did not perform any better than APRI (AUROCs: TE 0.72 *vs*. APRI 0.80 for F0-1/F2-4 and TE 0.87 *vs*. APRI 0.86 for F0-2/F3-4) [319]. Unfortunately, in DART ALT was tested routinely in all patients but AST was not. Thus we are unable to calculate APRI for all participants.

Although this study benefitted from long follow-up of up to 5.8 years this may not be long enough to observe some benefits of treatment. Recent data from a study in HIV-negative patients showed that a reduction in risk of HCC only became apparent 3.3 years after initiation of TDF [320]. Thus longer follow-up may show increasing differences between those treated effectively and those not. However this study used a risk score equation developed and validated in Asian individuals (from Taiwan, Hong Kong and South Korea) infected with HBV without HIV coinfection, untreated for HBV and followed for a median of 12.0 years [321]. The authors cautioned against applying the model to HIV coinfected individuals and thus a model developed and validated in a sub-Saharan African coinfected population would be useful. The model in HBV monoinfection was developed using a cohort of over 3,500 and validated with a further 1,500 individuals. P-values derived from the Cox model were small (generally <0.001)

so it may be possible to develop a model in a smaller cohort but the DART HBV/HIV coinfected cohort is only one tenth the size. Of more importance, the risk equation development cohort included 131 and the validation cohort 111 who developed HCC while in DART only 1 death was recorded as being due to HCC. The on-treatment cohort included 641 patients followed for 6 years and 13 developed HCC [320]. If we accept the observed rate of HCC in DART to be accurate any reduction in HCC rate will be of minimal benefit at best.

HBV immunisation was introduced into childhood schedules in Zimbabwe in 2000 and in Uganda in 2002 and coverage is estimated at 95% and 78% respectively [37, 38]. Thus 4 years from now the first cohort immunised at birth will become young adults. This will clearly have dramatic effects on the disease burden due to HBV, but HBV coinfection will continue to be a major problem for decades to come for those already infected. There is no data on HBV incidence in sub-Saharan Africa and so it is hard to predict whether immunisation of HIV-positive adults (as recommended in UK guidelines [125]) would have a significant effect. Retesting HBV serology at the end of follow-up in those of the DART population who were negative for all HBV markers at baseline would allow an estimate of HBV incidence to be made, as well as an indication of the probability of HBV clearance or chronicity in this population. However the incidence of HBV can be expected to be low in the DART population, since a recent study showed a reduced incidence of 0.14/100py in HBV-susceptible HIV-positive MSM treated with TDF and 1.36/100py in those treated with 3TC/FTC, compared with an incidence of 2.85/100py in those not treated with HBV-active drugs [322].

9.6 Change in clinical practice

The vast majority of HBV/HIV coinfected patients starting antiviral treatment now will start a triple therapy ART combination that includes TDF and either 3TC or FTC, in line with treatment guidelines [124, 132, 134]. We found no reduction in clinical progression in those HBsAg seropositive participants treated with TDF compared to those treated with ABC or NVP (each given in combination with AZT and 3TC), which might have been expected if TDF was more effective against HBV. Thus, although HBsAg seropositive participants were more likely to progress and die than HBsAg negative participants it is not clear that knowledge of HBsAg status, in order to ensure treatment of HBV, offers any benefit.

We found evidence that HBV coinfection increased the risk of flare on starting therapy but these flares were without clinical consequence and thus knowledge of HBsAg status would not affect outcome. Similarly knowledge of HBsAg status would not affect outcome in structured treatment interruptions undergone by participants with good CD4

count on stable ART. However, in participants switching to second line, HBsAg positivity was associated with a high risk of flare, and these flares were frequently associated with death.

These results imply that, as far as liver transaminase flares are concerned, there is no clinical benefit in knowing HBsAg status on HBV/HIV dual-active treatment initiation or in stable participants with good CD4 response to ART undergoing STI but that it may be very important to determine HBsAg status before stopping HBV-active ART. These results support WHO guidelines which state that HBsAg testing at HIV diagnosis and before switching antiretroviral regimen at treatment failure is "desirable (if feasible)" [132]. The WHO guideline suggests that, when switching off TDF, patients could be treated with an "alternative drug for hepatitis B treatment (such as entecavir)".

A large proportion of individuals achieved HBV virological suppression regardless of treatment regimen and those treated with 3TC alone were as likely to remain suppressed as those treated with 3TC and TDF. However, in those that had not achieved an undetectable HBV VL at one year, treatment with TDF was likely to result in suppression later whereas treatment with 3TC never did. Thus a treatment strategy could be to treat with 3TC and test HBV VL at 48 weeks. Those that have detectable HBV VL should then switch to TDF while those that have achieved complete suppression can continue on 3TC as the only HBV-active treatment. The results of the meta-analysis show that suppression on TDF is not affected by prior 3TC exposure. However this strategy would rely on HBV VL monitoring which is unavailable in many resource-poor areas, including much of Africa. A cheap and reliable test that could distinguish between detectable and undetectable HBV VL could be enough to inform this strategy. New WHO guidelines advise that HIV VL testing should be used wherever possible but state that less than 20% of those on antiretroviral therapy in Africa have HIV VL monitoring [132, 323]. Recent cohort evidence from resource-limited settings has supported the benefit of moving to HIV VL monitoring [324]. Point-of-care tests for HIV VL are in development and such technological advances should facilitate the development of similar tests for HBV VL. HIV VL point-of-care tests may provide only a binary result (with a cut-off of perhaps 1000 IU/mL) which, in the case of HBV, could be useful in monitoring those with suppressed virus but not useful in determining the trend of HBV VL in those who have yet to suppress.

In the absence of HBV VL testing, ALT could be used to monitor patients for HBV VL rebound on treatment. However we did not find rises in ALT associated with virological rebound in the participants in the DART study, suggesting that this strategy is not effective.

While clinical progression (to new WHO stage 4 disease or death) was higher in those with detectable HBsAg, it appears that in HBsAg-positive participants, routine monitoring (including testing ALT) does not affect clinical outcome. Overall, the DART study found there was a benefit from routine monitoring, although the benefit was small when comparing both groups with an untreated historical cohort and outcomes for both groups were comparable with those achieved in resource-rich areas [259]. It may be that routine monitoring does not need to include ALT. WHO guidelines suggest ALT testing only when patients are treated with NVP, and then only in certain populations at increased risk of liver flare [132].

The results of this DART hepatitis substudy suggest that treating with 3TC as the only HBV-active drug may be a reasonable strategy but that in these patients detection of virological failure at one year would indicate a need to switch to a more potent regimen. However both the substudy and the meta-analysis suggest that if patients are treated with TDF, patients who have not yet achieved full suppression of HBV VL at one year can reasonably be continued on the same regimen with an expectation of suppression occurring later. Thus it may be that testing of HBV VL in patients on TDF would not give any benefit in determining clinical practice.

In summary, the following conclusions can be made of particular relevance to clinical practice:

(1) HBV prevalence may vary widely between populations even in close geographical proximity.

(2) Knowledge of HBV status infrequently influences clinical decision making.

(3) Knowledge of HBV status is important if patients are to stop HBV-active treatment.

(4) Clinicians should consider whether to continue HBV-active treatment in patients with detectable HBsAg when such drugs are no longer indicated for treatment of HIV.

(5) HBV VL testing is of limited benefit in patients treated with TDF.

(6) HBV VL testing may be useful to determine the need for treatment switch if first-line HBV treatment is with 3TC alone.

(7) Routine ALT testing is of limited use in guiding management.

10 References

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Appendix 1 – Systematic review search strings

ISI Web of Science

Topic=((TS=hepatitis OR TS=hbv) AND (TS=hiv OR TS=human immunodeficiency virus OR TS=AIDS OR TS=acquired immunodeficiency syndrome OR TS=acquired immune deficiency syndrome) AND (TS=tenofovir OR TS=TDF OR TS=truvada OR TS=viread))

Timespan=All Years. Databases=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH.

EMBASE & MEDLINE

((exp HUMAN IMMUNODEFICIENCY VIRUS/) OR (hiv.ti,ab) OR (exp ACQUIRED IMMUNE DEFICIENCY SYNDROME/) OR (aids.ti,ab))

AND

((exp HEPATITIS/) OR (hbv.ti,ab) OR (hepatitis.ti,ab))

AND

((exp TENOFOVIR/) OR (exp TENOFOVIR DISOPROXIL/) OR (tenofovir.ti,ab) OR (tdf.ti,ab) OR

(viread.ti,ab) OR (truvada.ti,ab))

[Limit to: Human and English Language]

Appendix 2 – Meta-analysis – Regression Stata code

The following Stata command produced the estimates in the "overall" columns in Table 10.

```
xi: xtmelogit u i.prior i.con i.studytype || study: , or
```

u   0 if not suppressed

              1 if suppressed

prior   0 if not previously exposed to 3TC/FTC

              1 if previously exposed to 3TC/FTC

con   0 if treated with TDF without concomitant 3TC/FTC

              1 if treated with TDF with concomitant 3TC/FTC

studytype 1 if randomised controlled trial

              2 if prospective cohort study

              3 if retrospective cohort study

study   numbers 1-23 for the 23 studies included (Table 7)

Estimates of effects within strata were obtained by selecting appropriate cases e.g.

```
xi: xtmelogit u i.prior i.studytype if con==0 || study: , or
```

Appendix 3 – Results of meta-analysis – forest plots

Figure 60: Meta-analysis – forest plots of study arms at year 1
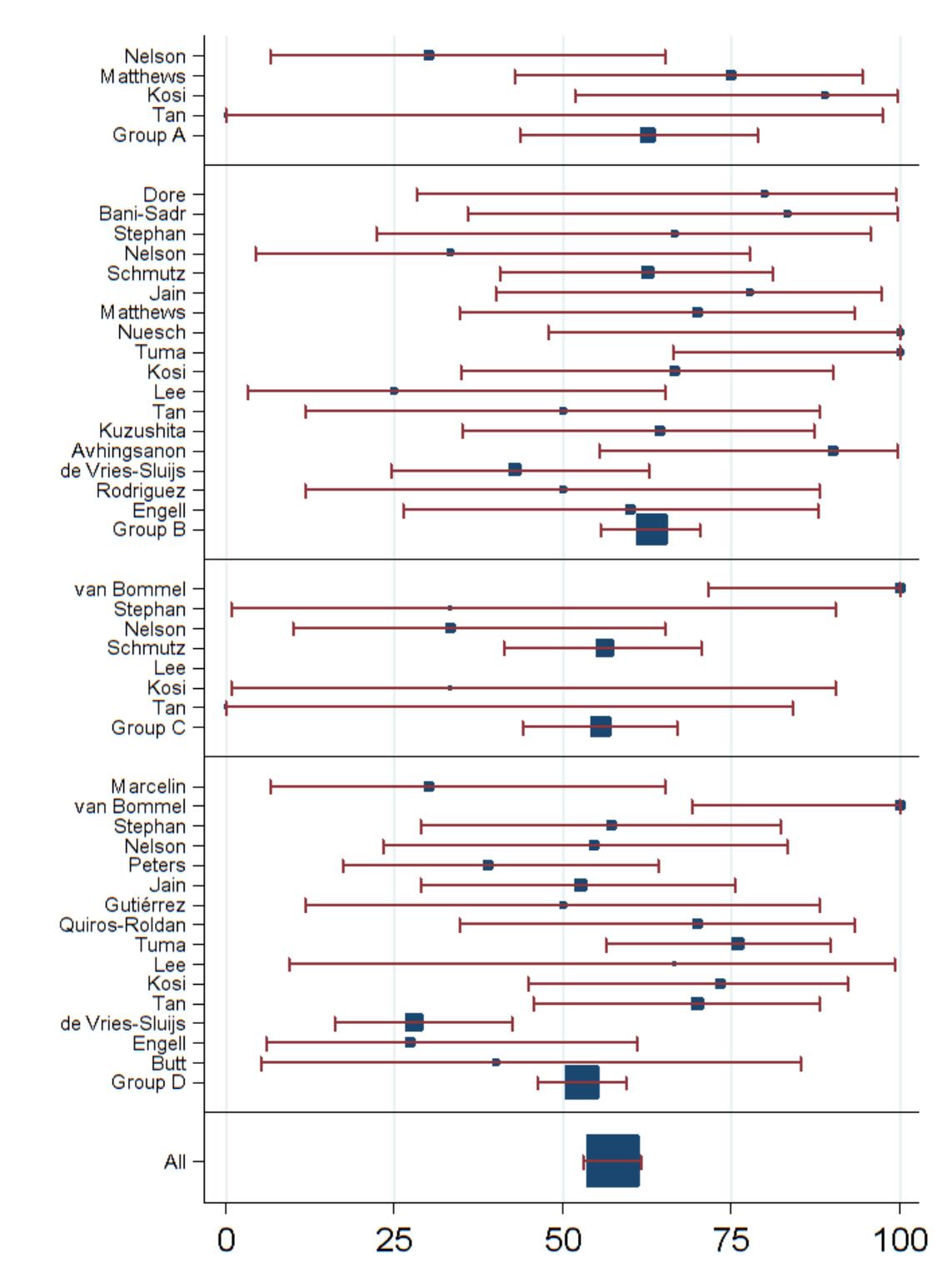
Figure 61: Meta-analysis – forest plots of study arms at year 2
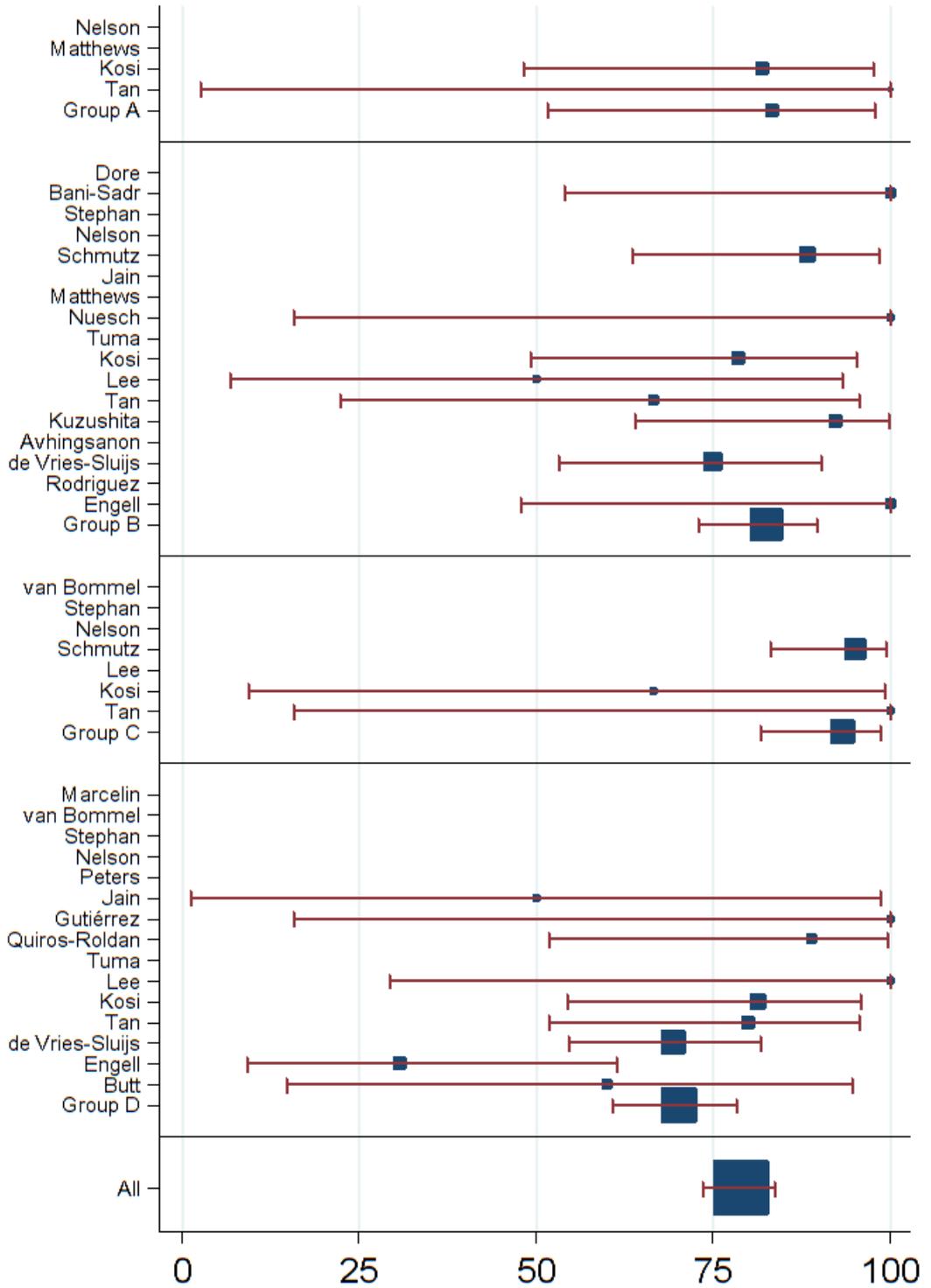
Figure 62: Meta-analysis – forest plots of study arms at year 3
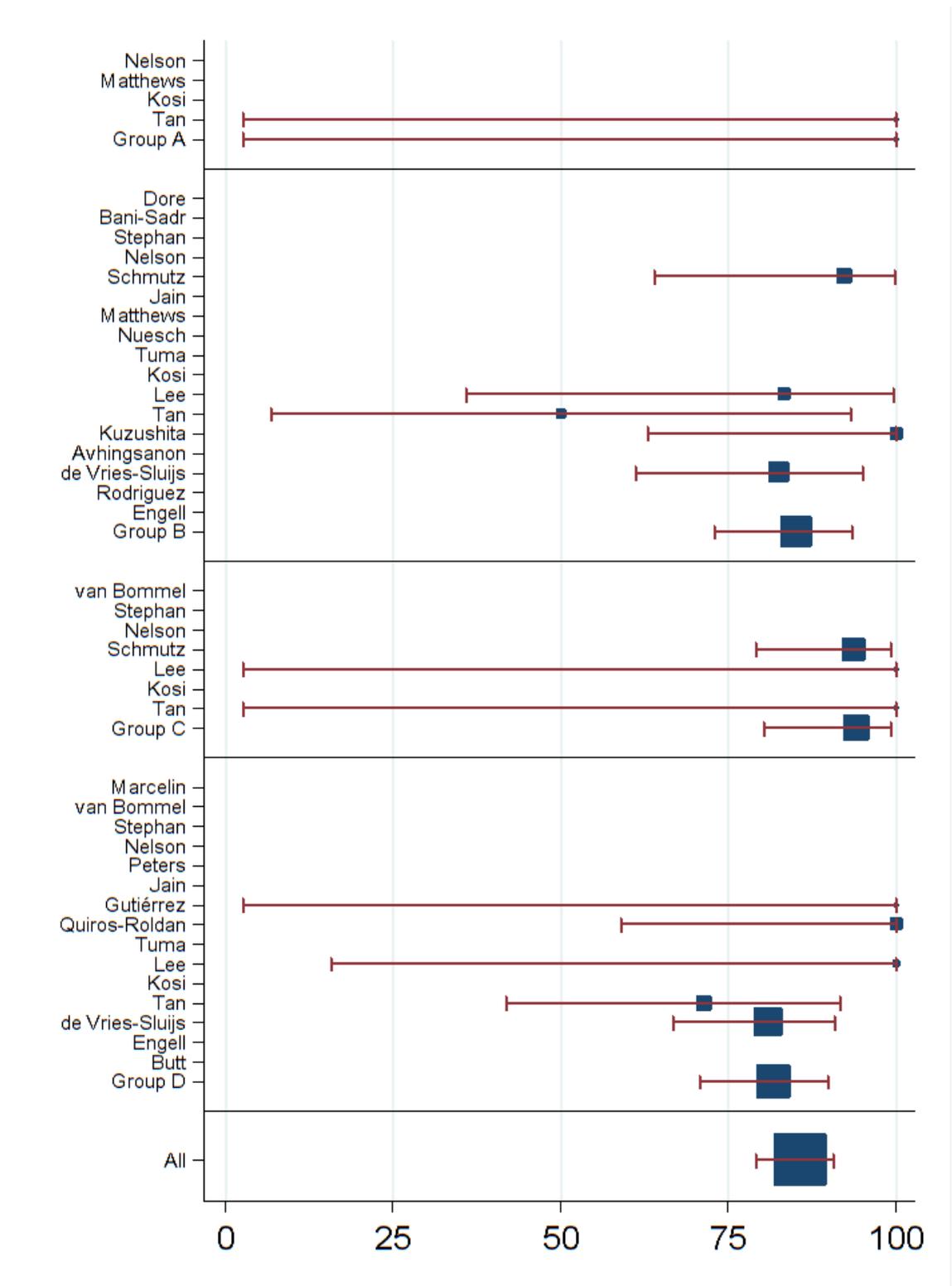
Appendix 4 – Meta-analysis – Forest plot Stata code

```
* HBV / HIV resistance studies
*    repeating each year
*****************************

version 10.1
clear
clear all
set more off
set mem 100m
cap log close

set scheme s2mono , permanently

global drive H
global data_dir     "$drive:\Documents\Thesis\Co-infection
                     Review\Resistance\Stata\data"
global log_dir      "$drive:\Documents\Thesis\Co-infection
                     Review\Resistance\Stata\logs"
sysdir set PERSONAL "$drive:\Programs\Stata10\ado\personal"

cd "$log_dir"
datestamp_h
log using "`r(datestamp)'_HBVHIV_meta_09_forest.log" , replace

* Import from data directory
***************************
cd  "$data_dir"
insheet using "Table.2.1.csv" // NB includes group and overall totals
rename firstauthor author
drop if author==""

*keep prior-n1
gen prior=(prior3tc=="Yes")
gen con  =(with3tc =="Yes")
drop source year prior3tc with3tc

gen studyarm=_n
label define sarm               ///
      1 "Nelson"                ///
      2 "Matthews"             ///
      3 "Kosi"                  ///
      4 "Tan"                   ///
      5 "Group A"               ///
      6 " "                     ///
      7 " "                     ///
      8 "Dore"                  ///
      9 "Bani-Sadr"             ///
     10 "Stephan"               ///
     11 "Nelson"                ///
     12 "Schmutz"               ///
     13 "Jain"                  ///
     14 "Matthews"             ///
     15 "Nuesch"                ///
     16 "Tuma"                  ///
     17 "Kosi"                  ///
     18 "Lee"                   ///
     19 "Tan"                   ///
     20 "Kuzushita"             ///
     21 "Avhingsanon"           ///
     22 "de Vries-Sluijs"       ///
     23 "Rodriguez"             ///
     24 "Engell"                ///
     25 "Group B"               ///
     26 " "                     ///
     27 " "                     ///
     28 "van Bommel"            ///
     29 "Stephan"               ///
     30 "Nelson"                ///
     31 "Schmutz"               ///
```

```
      32 "Lee"                  ///
      33 "Kosi"                 ///
      34 "Tan"                  ///
      35 "Group C"              ///
      36 " "                    ///
      37 " "                    ///
      38 "Marcelin"             ///
      39 "van Bommel"           ///
      40 "Stephan"              ///
      41 "Nelson"               ///
      42 "Peters"               ///
      43 "Jain"                 ///
      44 "Gutiérrez"            ///
      45 "Quiros-Roldan"        ///
      46 "Tuma"                 ///
      47 "Lee"                  ///
      48 "Kosi"                 ///
      49 "Tan"                  ///
      50 "de Vries-Sluijs"      ///
      51 "Engell"               ///
      52 "Butt"                 ///
      53 "Group D"              ///
      54 " "                    ///
      55 " "                    ///
      56 " "                    ///
      57 "All"                  ///
      58 " "
label values studyarm sarm

cd "$log_dir"

* Make confidence intervals for each studyarm
*   and get standard errors
*********************************************
foreach x of numlist 1 2 3 4 5 6 7 {
      gen p`x'=s`x'/n`x'
      gen l`x'=.
      gen u`x'=.
      gen se`x'=.

      foreach y of num 1/58 {
        if n`x'[`y']!=. { di n`x'[`y'] " " s`x'[`y']
                qui cii n`x'[`y'] s`x'[`y'] , exact
                qui replace l`x'=r(lb) if _n==`y'
                qui replace u`x'=r(ub) if _n==`y'
                qui replace se`x'=r(se) if _n==`y'
          }
        }
}

list s1 n1 p1 l1 u1 s2 n2 p2 l2 u2 s3 n3 p3 l3 u3 if  ///
      author=="A" | author=="B" | author=="C" |       ///
      author=="D" | author=="All", noobs

* Draw forest plots
*******************
foreach x of numlist 1 2 3 4 5 6 7 {
      twoway (scatter p`x' studyarm [fweight = n`x'] ,        ///
          msize(vsmall) msymbol(square) )                     ///
          (rcap l`x' u`x' studyarm),                          ///
          ylabel(0 "0" 0.25 "25" 0.5 "50" 0.75 "75" 1 "100")  ///
          yscale(alt)                                         ///
          legend(off) ytitle("") xtitle("")                   ///
          xlabel(#58, labsize(vsmall) angle(vertical)         ///
          valuelabel )                                        ///
          xline(6.5 26.5 36.5 54.5, lcolor(black) lwidth(thin))  ///
          graphregion(fcolor(white) lcolor(white))
      graph export forest_y`x'.png , replace
}

cap log close
```

Appendix 5 – Results of meta-analysis – Funnel plots

Figure 63: Funnel plots of standard error against proportion undetectable at one year – by analysis group

Group A

Group C

Group B

Group D
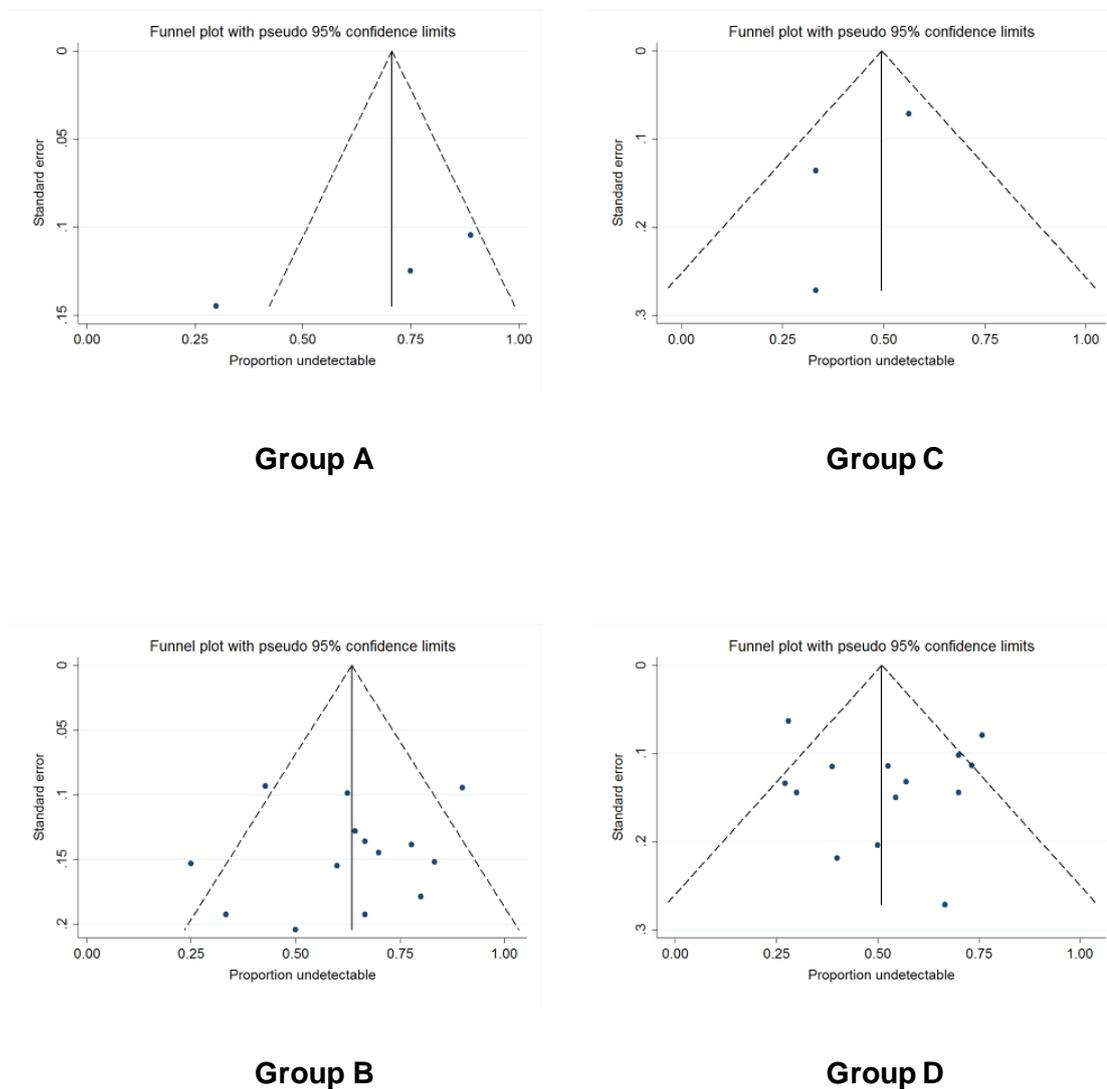
Appendix 6 – HBsAg screening tests and repeat tests

All 3,316 patients had a screening test for HBsAg; 610 (18.4%) were positive with the proportion positive being much higher at Entebbe (26.6%) and Harare (23.4%) than at JCRC (8.1%). Of these 610 with a positive HBsAg screening test, 605 had a confirmation test; 2 at Harare and 3 at JCRC did not. The proportion of those who had a positive screening test who subsequently had a positive HBsAg confirmation test was much lower at Entebbe (30.6%) than at either Harare (71.6%) or JCRC (81.0%).

On subsequent testing, 35 of the 83 participants at Entebbe with a confirmed positive HBsAg result had either negative results for all other HBV markers (anti-HBc, anti-HBs, HBeAg, anti-HBe and HBV DNA), had positive results for both anti-HBc and anti-HBs with negative results for HBeAg and HBV DNA or, in one case, had positive anti-HBs but negative anti-HBc, HBeAg, anti-HBe and HBV DNA. When considered in the light of the suspiciously low confirmation rate in Entebbe, it was suspected that these could have been falsely positive HBsAg. Samples were sent to JCRC for repeat HBsAg testing; 27 tested negative and 8 tested positive. Those testing negative were reclassified as HBsAg in all subsequent analyses [Table 63].

Of the 56 participants at Entebbe with a final positive HBsAg result, 8 had had HBsAg retested and found to be positive at JCRC, 40 had a positive result for HBV DNA, 7 had positive anti-HBc without positive anti-HBs and 1 had positive HBsAg and negative anti-HBc but insufficient sample remaining for any other testing. Thus the final classification of Entebbe participants as HBsAg seropositive is believed to be reliable.

Table 63: Patterns of results in Entebbe patients subsequently retested for HBsAg at JCRC

HBsAg	anti-HBc	anti-HBs	HBeAg	anti-HBe	HBV DNA	n	HBsAg retest	
							Pos	Neg
Pos	Neg		Neg	Neg	Neg	14	2	12
Pos	Neg	Neg	Neg	Neg	Neg	8	2	6
Pos	Neg	Pos	Neg	Neg	Neg	1	0	1
Pos	Pos	Pos	Neg	Neg	Neg	6	2	4
Pos	Pos	Pos	Neg	Pos	Neg	6	2	4

Pos: positive. Neg: negative.

The three participants at JCRC all had detectable anti-HBc at baseline and were treated as HBsAg seropositive in all subsequent analyses.

Of the two participants in Harare without a confirmation test, one was anti-HBc seropositive and one anti-HBc seronegative. The one that was anti-HBc seropositive was also tested for HBV DNA at baseline and this was detectable at a level of 976 IU/mL. This participant was then considered HBsAg seropositive while the other was excluded.

Appendix 7 – HBV serology results combinations

Anti-HBc	HBsAg	Anti-HBs	HBeAg	Anti-HBe	Frequency
	NEG	NEG			3
	NEG	POS			1
	POS		NEG	POS	1
NEG		NEG			1
NEG	NEG				1166
NEG	NEG		NEG	NEG	12
NEG	NEG		NEG	POS	1
NEG	NEG	NEG			270
NEG	NEG	NEG	NEG	NEG	6
NEG	NEG	POS			26
NEG	NEG	POS	NEG	NEG	1
NEG	POS				5
NEG	POS		NEG	NEG	25
NEG	POS		NEG	POS	2
NEG	POS		POS	NEG	15
NEG	POS		POS	POS	1
NEG	POS	NEG	NEG	NEG	3
NEG	POS	NEG	NEG	POS	1
NEG	POS	POS			1
NEG	POS	POS	NEG	NEG	1
POS	NEG				13
POS	NEG		NEG	POS	3
POS	NEG	NEG			533
POS	NEG	NEG	NEG	POS	6
POS	NEG	NEG	POS	NEG	3
POS	NEG	NEG	POS	POS	1
POS	NEG	POS			950
POS	NEG	POS	NEG	NEG	5
POS	NEG	POS	NEG	POS	7
POS	POS				19
POS	POS		NEG	NEG	19
POS	POS		NEG	POS	94
POS	POS		POS	NEG	71
POS	POS		POS	POS	4
POS	POS	NEG			1
POS	POS	NEG	NEG	NEG	4
POS	POS	NEG	NEG	POS	18
POS	POS	NEG	POS	NEG	10
POS	POS	NEG	POS	POS	1
POS	POS	POS			2
POS	POS	POS	NEG	NEG	4
POS	POS	POS	NEG	POS	5
POS	POS	POS	POS	NEG	1
Total					**3316**

Appendix 8 – My role

I am responsible for the work that makes up this thesis.

I carried out a review of the literature around HBV/HIV coinfection and wrote the background chapter, with a view to setting out the context of the research.

In conjunction with my supervisors, I decided upon the analyses to be performed. I wrote the necessary Stata code to tidy the data and run the analyses.

The meta-analysis arose from an attempt to review the published data on the suppression of HBV in HIV-infected patients treated with tenofovir. I decided upon the end point and treatment categories and extracted what data I could. Many studies presented data which did not quite fit the categories and so I contacted lead authors asking for further data. Some of this data came in the form I requested, fitting the analysis; some came in form of spreadsheets of results which I then had to convert into usable Stata files. I wrote the Stata code and performed the analyses and wrote the draft of the paper which I then circulated to the other authors and I then rewrote it incorporating their comments. I formatted and submitted it for publication.

For the DART analyses, I first coordinated the testing of the samples. This involved
1. Planning testing, ordering assays and organising their delivery.
2. Communicating with laboratories to monitor progress.
3. Receiving results in the form of excel spreadsheets, tidying and converting them into Stata format.
4. Organising quality control provided by UKNEQAS.

I presented partial results at meetings.

I wrote the thesis and edited it after discussion with my supervisors.

The PhD was funded by a Clinical Research Training Fellowship from the Medical Research Council, which paid my salary, fees and costs for the initial three years, extended to 4 by UCL (assay costs were funded by Gilead). Since my thesis was primarily one of analysis and had no lab-based component I felt I should study to understand how and why statistical tests are applied and how they work. To do this I attended lectures and practicals of 10 modules taken from the undergraduate statistics degree course. As I was registered for the MPhil/PhD I was unable to register for a statistics degree or diploma and so I did not sit the exams.

Through being a member of the research department I also had the opportunity to be involved in other interesting studies, notably the analysis of HBV in UK CHIC and the analysis of HCV in the Gay Mens Sexual Health Survey.

H. Price, D. Dunn, T. Zachary, T. Vudriko, M. Chirara, C. Kityo, P. Munderi, J. Hakim, C. Gilks, D. Pillay, R. Gilson, DART Virology Group

Low risk of alanine aminotransferase (ALT) flares in HBV/HIV coinfected patients starting HAART in the DART Study

7th International Workshop on HIV & Hepatitis Co-infection, 2011, Milan, Italy

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Potent HBV therapy and availability of laboratory monitoring fail to abrogate increased risk of death in HBV-HIV-co-infected individuals in the DART study
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Potent HBV therapy and availability of laboratory monitoring fail to abrogate increased risk of death in HBV-HIV-co-infected individuals in the DART study

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Background: WHO guidelines (2010) recommend the use of two drugs with anti-HBV activity in HIV-HBV-coinfected patients. It is unknown whether this strategy abrogates the excess mortality associated with HBV-coinfection documented in earlier studies.

Methods: DART was a large randomised trial of clinically-driven monitoring (CDM) versus laboratory and clinical monitoring (LCM) [including LFTs] for HIV infection in Zimbabwe/Uganda (regimen was not randomised). Patients had symptomatic disease and baseline CD4 < 200 cells/mm3. Baseline samples were retrospectively tested for HBV markers. The prevalence of HBsAg between demographic groups was compared using Fisher's exact test. Cox regression analysis was used to relate mortality to baseline HBsAg-status, adjusting for initial drug regimen (fitted as stratification factor), age, sex, country, baseline WHO stage, CD4 count and HCV antibody status and monitoring strategy.

Results: Of 3181 eligible patients (65% female), 258 (8.1%) were confirmed HBsAg+ve. In combination with zidovudine/lamivudine, 2366 (74%), 293 (9%), 522 (16%) initiated therapy with tenofovir, abacavir, and nevirapine respectively. HBsAg status was strongly associated with sex and country.

363 patients (321 HBsAg-ve, 42 HBsAg+ve) died over a median follow-up of 4.9 years. HBsAg-positivity was associated with a 51% (95% CI 9-110%; P=0.01) higher mortality, with the difference occurring within the first year.

There was no evidence that the effect of HBsAg-positivity was modified by initial regimen (tenofovir +43%, abacavir +26%, nevirapine +121%; P=0.61) or by monitoring strategy (CDM +27%, LCM +86%; P=0.33). Similar but weaker trends were observed when the endpoint was defined as progression to death or WHO-4 disease. Changes in CD4 count were unrelated to HBsAg-status.

Cause of death was known in 233 (73%) HBsAg negative and 31 (74%) HBsAg positive patients. On death forms, the liver was mentioned in 8 (3.4%) and 2 (6.5%) respectively (p=0.33).

Baseline demographics and percentage HBsAg positive

	All		HBsAg+ve		p
	n	Group %	n	+ve %	
All	3,181		258	8.1	
Sex					<0.001
Male	1,113	35.0	125	11.2	
Female	2,068	65.0	133	6.4	
Age group					0.43
18-30	506	15.9	38	7.5	
30-35	758	23.8	66	8.7	
35-40	813	25.6	61	7.5	
40-45	585	18.4	54	9.2	
45-50	307	9.7	28	9.1	
>50	212	6.7	11	5.2	
Country					<0.001
Uganda	2,260	71.0	128	5.7	
Zimbabwe	921	29.0	130	14.1	
WHO Stage					0.68
2	640	20.1	47	7.3	
3	1,786	56.1	151	8.5	
4	755	23.7	60	7.8	
Baseline CD4					0.25
<50	1,065	33.5	82	7.7	
50-99	749	23.5	74	9.9	
100-149	727	22.9	56	7.7	
150-199	640	20.1	46	7.2	
HCV Ab					0.84
Negative	3,105	97.6	253	8.1	
Positive	75	2.4	5	6.7	
Drug					0.72
TDF	2,366	74.4	196	8.3	
ABC	293	9.2	20	6.8	
NVP	522	16.4	42	8	
Monitoring					0.80
LCM	1,587	49.9	131	8.3	
CDM	1,594	50.1	127	8.0	

Survival by baseline HBsAg status

aHR 1.51

p = 0.01

Number at risk
HBsAg NEG 2923 | 2733 | 2645 | 2571 | 2492 | 1109
POS 258 | 233 | 225 | 218 | 209 | 77

HBsAg status — NEG — POS

Factors associated with risk of death

	aHR	95% CI	p
HBsAg			0.01
Negative	1		
Positive	1.51	1.09 - 2.11	
Sex			0.15
Male	1		
Female	0.85	0.69 - 1.06	
Age			0.34
18-30	1		
30-35	1.23	0.87 - 1.72	
35-40	1.03	0.73 - 1.46	
40-45	1.31	0.92 - 1.88	
45-50	0.99	0.62 - 1.58	
>50	1.43	0.90 - 2.29	
Country			0.42
Uganda	1		
Zimbabwe	0.90	0.71 - 1.15	
WHO Stage			<0.01
2	1		
3	1.59	1.13 - 2.23	
4	1.95	1.35 - 2.80	
Baseline CD4			<0.001
0-49	1		
50-99	0.61	0.49 - 0.80	
100-149	0.50	0.38 - 0.68	
150-199	0.36	0.25 - 0.52	
HCV Ab			0.56
Negative	1		
Positive	0.80	0.37 - 1.70	

Hazard ratio of HBsAg on risk of death, stratified by initial drug regimen or monitoring strategy

	HR	95% CI	P
Drug			0.61
TDF	1.43	0.99 - 2.07	
ABC	1.26	0.29 - 5.39	
NVP	2.21	0.98 - 4.99	
Monitoring			0.33
LCM	1.86	1.23 - 2.82	
CDM	1.27	0.81 - 2.00	

Conclusions: Patients with chronic HBV infection experienced significantly higher mortality despite the fact that all patients received one HBV-active drug (lamivudine) and three-quarters received two drugs (lamivudine and tenofovir) with potent HBV-activity. 3-monthly laboratory monitoring, including LFTs, did not impact on this excess mortality.

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Brief antiretroviral interruptions do not increase the risk of liver flares in HBV/HIV coinfected individuals on dual-active treatment

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Suppression of HBV by tenofovir in HBV/HIV coinfected patients: a systematic review and meta-analysis.
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Suppression of HBV by Tenofovir in HBV/HIV Coinfected Patients: A Systematic Review and Meta-Analysis

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Abstract

Background: Hepatitis B coinfection is common in HIV-positive individuals and as antiretroviral therapy has made death due to AIDS less common, hepatitis has become increasingly important. Several drugs are available to treat hepatitis B. The most potent and the one with the lowest risk of resistance appears to be tenofovir (TDF). However there are several questions that remain unanswered regarding the use of TDF, including the proportion of patients that achieves suppression of HBV viral load and over what time, whether suppression is durable and whether prior treatment with other HBV-active drugs such as lamivudine, compromises the efficacy of TDF due to possible selection of resistant HBV strains.

Methods: A systematic review and meta-analysis following PRISMA guidelines and using multilevel mixed effects logistic regression, stratified by prior and/or concomitant use of lamivudine and/or emtricitabine.

Results: Data was available from 23 studies including 550 HBV/HIV coinfected patients treated with TDF. Follow up was for up to seven years but to ensure sufficient power the data analyses were limited to three years. The overall proportion achieving suppression of HBV replication was 57.4%, 79.0% and 85.6% at one, two and three years, respectively. No effect of prior or concomitant 3TC/FTC was shown. Virological rebound on TDF treatment was rare.

Interpretation: TDF suppresses HBV to undetectable levels in the majority of HBV/HIV coinfected patients with the proportion fully suppressed continuing to increase during continuous treatment. Prior treatment with 3TC/FTC does not compromise efficacy of TDF treatment. The use of combination treatment with 3TC/FTC offers no significant benefit over TDF alone.

Introduction

Approximately 10% of people infected with HIV are coinfected with hepatitis B virus (HBV). Among populations with access to antiretroviral therapy (ART), in whom serious opportunistic infections have become a rare event, liver diseases including HBV infection represent a major cause of morbidity and mortality. [1] Since the life-cycles of HIV and HBV both utilise a reverse transcriptase enzyme, some drugs that inhibit reverse transcriptase have activity against both viruses. Guidelines now recommend tenofovir (TDF) in combination with lamivudine (3TC) or emtricitabine (FTC) as first-line therapy for patients with HIV/HBV coinfection. [2] Many studies have reported on the effect of TDF, either with or without 3TC or FTC, in treatment-naïve or experienced patients, however many studies are small and with relatively short follow-up.

It is uncertain what proportion of patients achieves suppression of HBV DNA (viral load) and whether those in whom suppression is not seen after one year may achieve HBV suppression later. It is also unclear to what extent, if at all, those with complete suppression may relapse despite continued treatment, e.g. in case of development of resistance mutations. Finally, it remains uncertain whether sequential treatment, for example with 3TC initially and TDF later, compromises the chance of successful treatment with TDF.

A recent meta-analysis examined all randomised controlled trials of treatment for HBV but excluded patients with HIV coinfection and only compared responses at 12 months. [3]