

GB Virus C Infection: is there a Clinical Relevance for Patients Infected with the Human Immunodeficiency Virus?

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Abstract

In a search for new hepatitis viruses, two independent groups identified viruses named GB virus C and Hepatitis G Virus, which turned out to be different strains of the same agent. Despite its initial ascription to hepatitis viruses, this new virus does not cause any hepatitis. Thus, the term GB virus C is preferred. Soon after its discovery, a significantly better clinical course of HIV infection was demonstrated for patients coinfecting with the GB virus C in 1998. These results were confirmed in other further studies, but not in all of them. Importantly, studies not confirming a positive influence usually found a neutral effect. The data for most of the studies had been mainly collected in the time prior to the availability of highly effective antiretroviral therapy (HAART). Since HAART has become available, some authors have reported a beneficial effect while others have shown only a neutral influence of GB virus C on HIV, considering either survival or the degree of response to HAART. An explanation for these discrepancies may be that different GB virus C strains exhibit different replication capacities. Understanding the mechanisms by how GB virus C and HIV interact may help find new strategies to fight HIV infection. Some recent in vitro findings have added evidence on how GB virus C might interact with and inhibit HIV replication. In this review, we update the current knowledge on GB virus C, its role in HIV infection in the HAART era, and discuss the potential mechanisms of its beneficial effect. (AIDS Reviews 2005;7:3-12)

Key words

GBV-C. HGV. HIV. Coinfection. HAART. Hepatitis. Survival. Interferon.

Introduction

HIV infection is the second leading cause of death from infectious diseases worldwide with an estimated three million deaths annually among the 40 million infected persons. HIV infection mostly is acquired through routes that place people at risk for further viral infections. Additional infections usually either induce a more rapid course of HIV infection or run a more severe

course, especially once immune function deteriorates. For the hepatitis viruses hepatitis B virus (HBV) and hepatitis C virus (HCV) an accelerated course of disease has been shown in HIV positive patients leading to impaired survival in the pre-HAART era^{1,2}.

However, coinfections need not be detrimental in all circumstances. For instance, in liver transplantation it has been observed that patients coinfecting with multiple hepatitis-virus infections do better than those with single infections³.

An historical example is treatment of progressive paralysis in the tertiary stage of syphilis. About 30% of the patients achieved a complete remission and 60% a distinct remission of their neurological symptoms after infection with *Plasmodium vivax*, the pathogen that causes tertian malaria. The Austrian psychiatrist Walter Jauregg was honored with the Nobel Prize in physiology and medicine in 1927 for this form of unconventional therapy, which became the standard treatment for decades⁴.

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In the context of HIV infection, in 1998 some surprising data were published showing a significantly lower HIV viral load in one study⁵, and a better clinical course and a better survival of HIV-infected patients coinfecting with the GB Virus C (GBV-C)⁶.

The GB virus C

GBV-C was identified almost simultaneously by two different groups of researchers in the mid-1990s in humans with post-transfusion hepatitis, cryptogenic, or acute indeterminate hepatitis⁷⁻⁹. At first, two different names were given for the virus, but it soon became obvious that the two groups had isolated different strains of the same virus¹⁰.

GBV-C is a flavivirus with a single-stranded positive-orientated genome of approximately 9400 nucleotides in length. It is closely related to HCV, with an amino acid homology of approximately 30%¹¹. The phylogenetic tree shows that GBV-C strains are less genetically distant from each other than the HCV genotypes (Fig. 1). It further shows the relation to the other GBV viruses, GBV-A and GBV-B, as well as the pestivirus bovine viral diarrhoea virus (BVDV).

GBV-C and HCV are both flaviviruses but, besides many homologies between HCV and GBV-C, there are also interesting differences. In contrast to HCV, GBV-C only has a truncated core or even no core protein within the coding sequence¹².

The initial description of GBV-C led to an enormous eagerness to elucidate the association of GBV-C with any disease, resulting in some 100 publications. However, although GBV-C was originally identified in patients with documented hepatitis^{8,9}, subsequent studies were unable to prove any influence of GBV-C on chronic liver damage^{13,14}. Likewise, the course after liver transplantation is not altered by GBV-C infection^{15,16}. As there is no evidence that GBV-C does cause any hepatitis, we will hereafter refer in the text to GBV-C.

GBV-C is a lymphotropic virus, which replicates in CD4+ and CD8+ T- and B-lymphocytes, as well as in spleen and bone marrow cells, but probably not in hepatocytes^{17,18}. An *in vitro* amplification of GBV-C in peripheral blood mononuclear cell (PBMC) is possible in culture, but does not work with all isolates¹⁹.

The transmission of GBV-C is possible through the same routes as HIV or HCV infection. It seems that transmission of GBV-C occurs by sexual or vertical routes with a higher efficiency than HIV or HCV²⁰. In some patients with detectable GBV-C in blood, the virus can also be detected in semen and saliva²¹.

GBV-C is not known to cause any disease. Thus, it was decided not to test blood donors or blood products for GBV-C although, depending on the studies, 1 to 4% of blood donors have detectable levels of

GBV-C-RNA^{8,9,22} and transmission of GBV-C through blood transfusion has been documented²³.

The prevalence of the virus depends on the risk profile of the group investigated. It ranges from 14 to 37% in studies including mainly homosexual men^{24,25}, and 39 to 45% in subgroups of intravenous drug addicts^{26,27}. Interestingly, the prevalence of the GBV-C-RNA in non-risk cohorts starts declining at an age of 40 years²⁶.

The presence of GBV-C is usually determined by rt-PCR or bDNA assay²⁸. Past GBV-C contact can be determined by detection of an antibody against the envelope 2 region of GBV-C (anti-E2)^{29,30}. GBV-C-RNA and anti-E2 are almost exclusively present in the serum³¹. Still, it has become clear that some persons lose GBV-C-RNA without development of anti-E2³².

Furthermore, it has been determined that anti-E2 protects from GBV-C infection^{33,34}. This usually is also true for HIV infected patients³⁵, but some cases of reinfection or reactivation after loss of anti-E2 have been reported³⁶. For the purposes of analysis, it is important to differentiate patients with past exposure and detectable E2 antibodies from those who have lost GBV-C without developing antibodies, and those with ongoing GBV-C infection with detectable GBV-C-RNA.

GBV-C and survival of HIV-positive individuals

Data from the pre-HAART era

The initial studies about GBV-C in HIV patients have been done with the intention of showing that GBV-C coinfecting patients have a faster clinical progression and/or worse prognosis in relation to survival, similar to what has been observed with the other hepatitis viruses¹. Moreover, most of the studies indicate a positive influence of a GBV-C co-infection for HIV infected patients (Table 1).

Studies showing a positive influence of GBV-C coinfection

Surprisingly, CD4 counts usually tended to be higher in GBV-C positive patients, as reviewed in Tillmann and Manns, 2001³⁷. A Japanese study even demonstrated a significantly lower HIV viral load in GBV-C-RNA-positive patients compared to GBV-C-RNA-negatives, but only a trend for improved survival⁵. We were the first to publish a significantly better clinical course for HIV-infected patients with a GBV-C coinfection⁶.

Similar data have then been confirmed by French³⁸ and American colleagues³⁹. They have demonstrated a significantly improved survival, slower disease progression, slower increase of HIV viral load, and a slower drop of CD4 cells in HIV/GBV-C coinfecting patients compared to those without GBV-C-RNA in their sera.

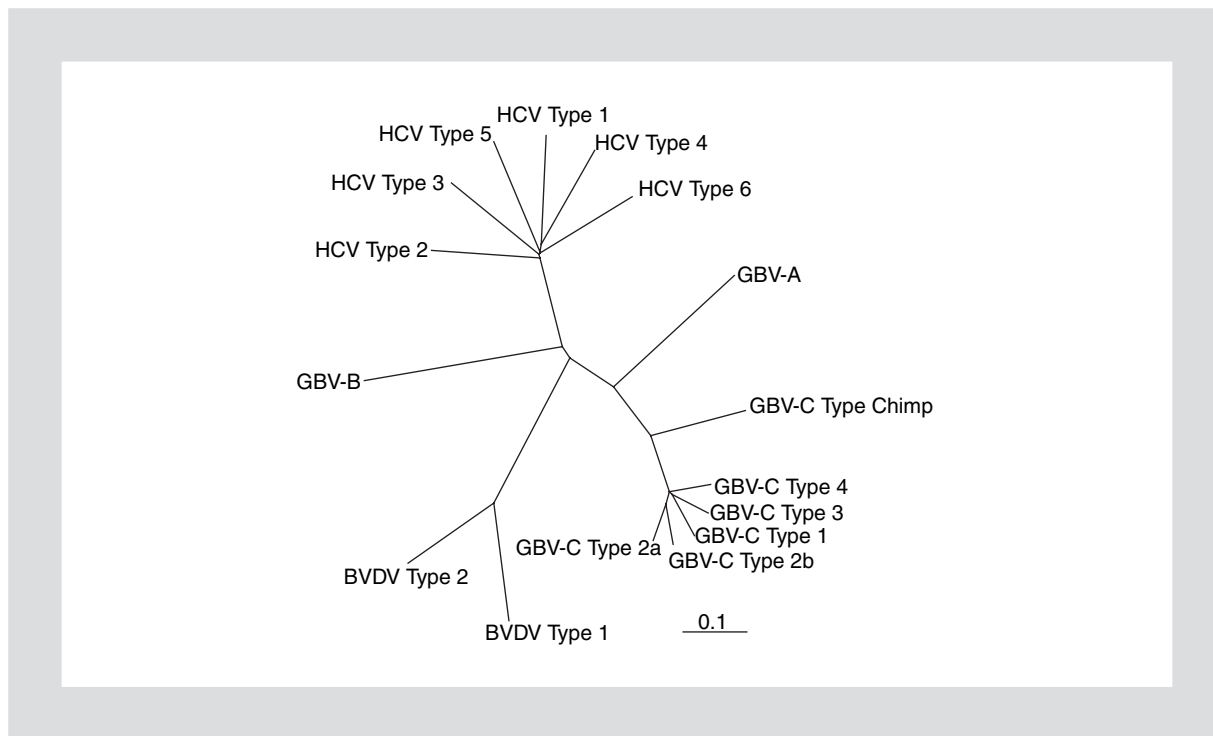


Figure 1. Phylogenetic tree of GBV A, B and C, a GBV-C isolate from a chimp, HCV, BVDV with different subtypes. The scale is 0.1 nucleotide substitutions per site. The tree was created using available sequences from GenBank and the software *clustalw* 1.81⁷¹ and *TreeView* 1.6.6⁷².

Still, these interesting findings were not able to draw much attention to this subject until two additional studies were published simultaneously in 2001 by ourselves and Xiang, et al., showing an even stronger significance in relation to improved survival in HIV/GBV-C coinfecting patients^{35,40}.

In 2004, Williams, et al.³² published a multicenter study with 271 homosexual men recruited shortly after HIV-seroconversion, of whom 39% tested positive for viral RNA and 46% were positive for the E2 protein 12 to 18 months after seroconversion. At this time there were no significant findings between the groups according to their GBV-C status. But, after five to six years follow-up, the patients without detectable GBV-C-RNA at any time were significantly more likely to die (95% CI 1.34-5.76; $p = 0.006$). The poorest prognosis, however, was actually determined for the patients who lost GBV-C-RNA ($p = 0.003$).

In addition, Nunnari, et al.⁵⁹ showed in their study longer cumulative AIDS-free survival rates at 24 and 48 months ($p = 0.02$) for HIV/GBV-C coinfecting patients.

Studies showing a neutral influence of GBV-C coinfection

There are some studies which are unable to find these beneficial effects of GBV-C coinfection. Birk, et al.⁴¹ investigated a cohort of 157 homosexual and bisexual

men with seroconversion dates at a median of about seven years. They did not find any significant difference between GBV-C-RNA-positive and -negative patients in the time to first CD4-lymphocyte count declining below 200 cells/ μ L ($p = 0.9$), the time to AIDS diagnosis ($p = 0.4$), or the time to death from AIDS ($p = 0.6$). The GBV-C status was not associated with any of the three outcome variables in multiple proportional hazard analyses, including age, risk behavior, zidovudine treatment, and PCP prophylaxis.

A study of Bjorkman, et al.⁴² in 2004 showed a significant minor prevalence of GBV-C for patients with AIDS at inclusion ($p = 0.008$). In contrast to the study of Williams, et al.³², this study did not find any significant results at the follow-up visit about four years later between the groups without detectable GBV-C-RNA and detectable RNA at all times. Sabin, et al.⁴³ also showed in their study no improved survival, but they studied GBV-C-RNA and anti-E2 positive patients as one group. This appears inadequate, as the influence of ongoing GBV-C infection is likely different from past infection with GBV-C.

Two studies evaluated cohorts solely consisting of women. One consisting of 101 HIV-1 (28 GBV-C positive and 73 GBV-C negative) and 159 HIV-2 (30 GBV-C positive and 129 GBV-C negative) infected women did not find any role of GBV-C on the course of HIV disease after more than five years median follow-up⁴⁴. Likewise, recent data were reported by Williams, et al.⁴⁵ concern-

Table 1. Studies on GBV-C and long term prognosis and survival. Data from pre-HAART era

Study	N	Follow up in years	Prevalence (%)		Patients cohort	Results
			GBV-C RNA	GBV-C anti-E2		
A. Positive influence						
Lefrere ³⁸	95	8.5	24	19	Mainly formerly blood donors	GBV+ slower progression in CDC status (p = 0.005), lower mortality (p = 0.004), cumulative survival (p = 0.01), lower HIV viral load (p = 0.03), higher CD4 count (p = 0.008)
Yea ³⁹	131	12	17	31	Men with haemophilia	AIDS incidence in these men was 3.9 per 100 person-years compared with 5.9 per 100 person-years in HGV-negative patients (difference, 1.9 per 100 person-years [CI, 20.3 to 4.2])
Tillmann ³⁵	197	4	17	57	22% women, 53% homosexual, 30% ivdu	Higher CD4 cell count (p = 0.01), higher CD8 cell count (p = 0.003), slower progression to AIDS (p < 0.001), longer survival (p = 0.02)
Xiang ⁴⁰	362	4	40		12% women, 16% ivdu	Improved survival (p < 0.001), higher CD4 cell count (p = 0.07)
Williams ³²	271	5-6	39	46	Homosexual men	Improved survival (p = 0.006)
Nunnari ⁵⁹	80	8	21		35% women	Higher CD4 (p = 0.041), lower HIV-RNA level (p = 0.033), longer AIDS free survival (p = 0.02) IL-2 and IL-12 decreased (p = 0.005 and p = 0.01), IL-4 and IL-10 increased (p = 0.01 and p = 0.004)
B. Neutral influence						
Birk ⁴¹	157	7	23		Homo and bisexual men, some ivdu	Progress AIDS (p = 0.4), CD4 decline (p = 0.9), death from AIDS (p = 0.6)
Bjorkman ⁴²	230	4.3	27	30	11% women, 65% homosexual, 15% heterosexual, 8% ivdu	All-cause mortality (p = 0.12), HIV-related mortality (p = 0.18), development of AIDS (p = 0.84), GBV-C RNA less prevalent in patients with AIDS at inclusion (p = 0.008)
Sabin ⁴³						GBV-C RNA+ & AntiE2+ were combined to one group
Kaye ⁴⁴	101 (HIV 1) 159 (HIV 2)	5	28 (HIV-1) 19 (HIV-2)		African women only	No association concerning CD4, HIV-1 or HIV-2 or mortality
Williams ⁴⁵	352	6.5	38		Women only	No association with viral load or mortality (p = 0.29)
C. Negative influence						
Van de Bij ⁴⁶	326	8	42	41	Homosexual men only	1-2 years after HIV sero-conversion GBV-C positive patients showed worse results: less CD4, (p = 0.04), CD4-cell counts below 200/ μ l (HR 1.37, 95% CI, 1.21-2.1), AIDS (HR, 1.37, 95% CI, 1.02-1.81), death (HR, 1.44, 95% CI, 1.06-1.96) 8 years later, persistently GBV-C RNA positive patients showed a lower mortality (HR 0.52, CI 95%, 0.32-0.85)
D. Loss Of GBV-C						
Van de Bij ⁴⁶	326	8	42	41	See above	Higher risk of: decreasing CD4 cells < 200 μ l (HR 3.26, 95%, 2.20-4.80), SI conversion 2.50 (1.43-4.36), AIDS 2.91 (1.93-4.40), death 3.26 (2.31-4.59), progression from AIDS to death 1.69 (1.18-2.41)
Williams ⁴⁵	271	5-6	38		See above	Highest mortality for the patients with loss of GBV-C RNA (HR 5.87; p = 0.003)
Bjorkman ⁴²	230	4.3	27	30	See above	Patients loss of GBV-C RNA without E2 sero-conversion compared to all other included patients: increased all-cause mortality (p = 0.018)

ing a cohort of 352 HIV-infected women, 38% of whom had detectable GBV-C-RNA in serum at the first visit. The HIV viral load did not differ between GBV-C positive and negative individuals. Interestingly, the presence of GBV-C was not predicted by education, number of sexual partners, or current or past history of injection drug use. After a median follow-up of 6.5 years there were no significant differences, according to the GBV-C status, in the percentage of women who initiated HAART, or the death rate. The acquisition rate of GBV-C-RNA was 3.1 per 100 persons per year, and this was slightly higher than the control group of HIV-negative women. The clearance rate of 6.3 per 100 person-years was not different by HIV status.

Studies showing a negative influence of GBV-C coinfection

A recently published study from Van der Bij, et al.⁴⁶ showed a slightly higher risk to progress to CD4-cell counts below 200/ μ L (HR 1.37, 95% CI 1.21-2.1), AIDS (HR 1.37, 95% CI 1.02-1.81), or death (HR 1.44, 95% CI 1.06-1.96) for patients who tested positive for GBV-C-RNA in their first sample in 1984-1985. The results remained significant after adjustment for several criteria but, after an adjustment for CD4 cell counts, the hazard ratio became balanced towards one. However, in the follow-up tests about eight years later, they found a better prognosis with decreased mortality for patients who were persistently GBV-C-RNA positive (HR 0.52, 95% CI 0.32-0.85). Other criteria which were evaluated were not significant in this group.

Loss of GBV-C-RNA

Interestingly, all studies finding a subgroup losing GBV-C-RNA showed a correlation between the loss of detectable GBV-C-RNA in the patients' blood and a considerably worse prognosis for HIV infection of these patients. In the study of Van der Bij, et al.⁴⁶ these patients had a higher risk of decreasing CD4 cells below 200 μ L (HR 3.26, 95% CI 2.20-4.80), SI conversion 2.50 (1.43-4.36), AIDS 2.91 (1.93-4.40), death 3.26 (2.31-4.59), and progression from AIDS to death 1.69 (1.18-2.41). All these parameters, with the exception of progression from AIDS to death, remained significant even after adjusting for age at HIV seroconversion, HAART, CCR5 genotype, CD4 cell count, and HIV viral load. In the study from Williams, et al.³² which has been previously discussed, the poorest prognosis was also associated with the loss of GBV-C-RNA (relative hazard for death 5.87; $p = 0.003$).

The prognosis for these patients appears to become even worse if the loss of GBV-C-RNA occurs without development of E2 antibodies in serum. This subgroup

of patients who cleared the virus without anti-E2 serum conversion showed a significantly increased all-cause mortality ($p = 0.018$), HIV related mortality ($p = 0.007$), and AIDS incidence ($p = < 0.001$) compared to all other included patients in the study of Bjorkman⁴².

GBV-C and response to HAART

Considering a lower HIV viral load in some clinical and *in vitro* studies, the presence of GBV-C might also be associated with a better response to HAART (Table 2).

GBV-C and a better response to HAART

Voirin, et al.⁴⁷ studied HIV patients on HAART coinfecting with both HCV and GBV-C, coinfecting with either virus, or infected with HIV only. Only the patients coinfecting with both HCV and GBV-C experienced a constant CD4 increase during four years of HAART, whereas the control groups had only a median increase over two years ($p = 0.03$). Interestingly, there was no difference in response to HAART between the patients who were only coinfecting with either GBV-C or HCV.

Rodriguez, et al.⁴⁸ showed, in a study of 146 HIV patients, significantly higher baseline CD8 cell counts ($p = 0.048$), higher baseline CD4 cell counts ($p = 0.043$), and lower plasma HIV-RNA levels ($p = 0.05$). After four years, the group was able to show a more frequent complete response to HAART for individuals coinfecting with GBV-C, and a greater increase in median CD4 cell count. These findings were independent of baseline CD4 cell count and plasma HIV load. The median HIV-RNA level decrease was only marginally greater in these patients.

Souza, et al.⁴⁹ recently collected data from 175 patients on initiating HAART, of whom 24% had detectable GBV-C-RNA. These patients showed a significantly better virological response to HAART ($p = 0.009$), even after adjustment for age, ART group, and baseline CD4 cell count. In addition, the CD4 counts at week 48 were significantly higher for GBV-C coinfecting patients.

Similarly, Antonucci, et al.⁵⁰ tested the response to HAART depending on the GBV-C status in 400 HIV-infected patients, of whom 117 (29.3%) had detectable GBV-C-RNA. While the probability of achieving initial virological success or CD4+ response after initiating HAART did not differ between groups, after more than three years of follow-up the patients who were GBV-C viremic had a significantly lower risk of HIV rebound than those who were GBV-C negative.

GBV-C and a neutral response to HAART

Similar to survival in relation to GBV-C status, studies concerning response to HAART are also conflicting, with some studies showing no benefit.

Table 2. Studies in relation of GBV-C and its role during HAART

Study	N	Follow up in years	Prevalence (%)		Patients cohort	Results
			GBV-C RNA	GBV-C anti-E2		
A. Better response to HAART						
Voirin ⁴⁷	105	4	32		14% women, 22% heterosexual, 48% homosexual, 9% ivdu	CD4 increase for 4 years ($p = 0.03$) compared to only 2 years
Rodriguez ⁴⁸	146	1	21	23	14,6% women,	Higher baseline CD8 cell counts ($p = 0,048$), CD4 cell counts ($p = 0.043$) and lower plasma HIV RNA levels ($p = 0.05$) after four years follow up: more frequent complete response to HAART ($p = 0.036$), greater increase in median CD4 cell count ($p = 0.05$)
Nunnari ⁵⁹	80	8	21		35% women	After 4 years of uninterrupted HAART: HIV-1 viral load below 400 copies/ml ($p = 0.03$) CD4 cell counts > 350 cells/nl ($p = 0.01$) and less treatment failures ($p = 0.01$). Better response to HAART ($p = 0.009$)
Souza ⁴⁹	175	1			30% women, 41% homosexual, 19% parenteral transmission	
Antonucci ⁵⁰	400	3-4	29		24,5% women, 61% ivdu	No influence of GBV-C on HAART in the first 3 years of follow up after 3 years: lower risk of HIV rebound ($p = 0.03$)
B. Neutral response to HAART						
Brumme ⁵¹	441	3	20		15% women, 24% ivdu	Lower HIV load at baseline ($p = 0.0004$), but neutral influence on: time to virological success (HR, 0.98, 95% CI, 0.75-1.27), time to virological failure (HR, 1.10, 95% CI, 0.74-1.65), time to immunological failure (HR, 1.09, 95% CI, 0.73-1.63), no correlation between detection of GBV-C RNA and mutations in the human chemokine receptors CCR5 and CXCR4, or HIV viral tropism ($p > 0.1$)
Tillmann ⁵²	258	1	34		31% heterosexual, 53% homosexual men, 10% ivdu, 3% haemophilic	Baseline CD4 cell count, and HIV-1 viral load was not different according to GBV-C status immunological response defined as time to an increase of ≥ 100 CD4 cells (HR: 0.9 (0.7, 1.3), virological response at week 48 (HR 1.1, 95% CI: 0.6, 1.9), time to development of grade 3 or 4 adverse events or clinical progression

In a study from Brumme, et al.⁵¹, 441 individuals initiating antiretroviral therapy between June 1996 and August 1998 were evaluated for GBV-C-RNA, and 90 (20.4%) of them were positive. These patients showed a significantly lower HIV load at baseline ($p = 0.0004$). But, furthermore, the GBV-C-RNA positive and negative individuals did not differ with respect to time to virological success (HR 0.98, 95% CI 0.75-1.27), time to virological failure (HR 1.10, 95% CI 0.74-1.65), nor time to immunological failure (RR 1.09, 95% CI 0.73-1.63). There was also no correlation between detection of

GBV-C-RNA and mutations in the human chemokine receptors CCR5 and CXCR4, or HIV viral tropism as predicted by the HIV envelope sequence ($p > 0.1$). In this study the median follow up was 40 months.

Similarly, we did not find any influence of GBV-C when we investigated the role of GBV-C within the 48-week prospective, randomized, MaxCmin1 trial comparing the safety and efficacy of two different HAART regimens⁵². The serum of 258 patients at week 12 of the trial was analyzed for the presence of GBV-C-RNA⁵³ and 34% of the patients were GBV-C RNA-positive. Neither

the baseline CD4 cell count nor the HIV-1 viral load was different according to GBV-C status. GBV-C-RNA was neither associated with immunological response defined as time to an increase ≥ 100 CD4 cells (RH 0.9, 95% CI 0.7-1.3), nor with virological response (hazard ratio of virological failure at week 48 for coinfecting *versus* uninfected: 1.1 (95% CI 0.6-1.9), nor with time to development of grade 3 or 4 adverse events, nor with clinical progression. Among those infected with GBV-C, outcome was not different according to GBV-C-RNA levels.

Possible mechanisms of interactions between GBV-C and HIV

There are several possible mechanisms whereby GBV-C may interact with HIV. In our initial study cohort, we were able to rule out CCR5 polymorphism as being responsible for the observed better survival⁵⁴. However, Nattermann, et al.⁵⁵ demonstrated that the GBV-C E2-protein is able to bind to the CD81 receptor on T-lymphocytes, followed by a dose-dependant release of RANTES, and a consecutive downregulation of CCR5 on the CD4 and CD8 cells' surface ($p < 0.01$). These results could be confirmed for PBMC by Xiang, et al.⁵⁶. The latter had further been able to demonstrate downregulation for the CXCR4 receptor. They incubated PBMC with GBV-C clinical isolates or an infectious laboratory clone of GBV-C prior to HIV infection, and demonstrated lower replication of the HIV strains that used either CCR5 or CXCR4 as their coreceptor compared to mock-infected cells as negative control. This inhibition was related to the dose and timing of the GBV-C infection of the cells. Expressions of mRNA for RANTES, MIP-1 α , MIP-1 β , all being ligands to the CXCR5 receptor, and SDF-1, being a ligand to the CXCR4 receptor, and the secretion of these chemokines into the supernatants were higher in the GBV-C-infected cells compared to the negative control cells. These effects could be suppressed by adding antibodies for the ligands of the CCR5 and CXCR4 receptors to the medium.

New data show that the E2 protein seems to also be able to bind to some cells without the CD81 receptor on the cell surface⁵⁷. Additional to this, there are probably also effects independent from the presence of the E2 protein, as it was shown that the NS5A region may suppresses HIV replication⁵⁸.

Immune modulating effects of GBV-C coinfection in HIV patients probably also exist. In a study from Nunari, et al.⁵⁹, 80 asymptomatic HIV-1 seropositive patients were tested for GBV-C-RNA level. The 63 GBV-C-negative individuals showed a significant decrease in IL-2 and IL-12 levels in serum ($p = 0.005$ and $p = 0.01$, respectively) and a significantly increased IL-4 and IL-10 ($p = 0.01$ and $p = 0.004$, respectively). These

changes in the interleukin levels could not be observed in the 17 patients seropositive for GBV-C. It can be speculated that GBV-C coinfection helps to maintain an intact T-helper cytokine profile.

Further, there were very recent *in vitro* data showing GBV-C specific T-cell responses⁶⁰.

GBV-C virus infection and the response to interferon alpha

Several studies have documented that chronic GBV-C infection is sensitive to treatment with standard interferon (IFN)- α in immunocompetent hosts⁶¹. In most patients, standard IFN- α treatment leads to a decline of detectable GBV-C-RNA. The patients who totally lost detectable GBV-C-RNA showed a relapse rate of up to 53%, and a sustained response in about 17%. Similarly, GBV-C clearance was observed in 45% of HIV-positive patients after 24 weeks of IFN- α 2a therapy, with sustained clearance noted in 28%⁶². New data concerning the sensitivity of GBV-C to pegylated interferons in combination with ribavirin are still awaited.

Effects of different GBV-C genotypes and different GBV-C strains

Little is known about the influence of different GBV-C strains on the interaction with HIV. This may be a source for the sometimes-conflicting data in the studies. To estimate the influence of genotypes, a study was performed by Muerhoff, et al.⁶³. GBV-C genotype was identified in 33 HIV-infected patients. Two patients were infected with genotype 1, 12 with type 2a, and 19 patients with type 2b. CD4 cell counts tended to be lower in patients with GBV-C genotype 2a than those with genotype 2b ($p = 0.054$). However, further studies are needed to estimate the influence of all six known genotypes.

Another interesting aspect of different GBV-C strains is their differential capacity to replicate in lymphocytes, as shown by George, et al.⁶⁴, who demonstrated dramatic differences in both the ability of clinical GBV-C isolates to persistently replicate, and in the yield of GBV-C-RNA in cells grown without PHA/IL-2 stimulation using PBMC-based culture methods. They found 16 unique amino acid polymorphisms in the detected GBV-C isolates. Fourteen of these polymorphisms were located in the coding regions for nonstructural proteins associated with interferon resistance and RNA replication. Similar findings were recently reported from Knauer, et al, showing that different GBV-C strains show different abilities to inhibit HIV replication *in vitro* due to different expression of the chemokines RANTES and MIP-1 α in relation to GBV-C replication⁶⁵.

GBV-C virus coinfection and impact on the patient's quality of life

GBV-C is most closely related to HCV, which has been linked to impaired quality of life even in the absence of significant liver disease. However, in contrast to HCV, GBV-C infection was associated with a significantly better quality of life for GBV-C positive *versus* GBV-C negative HIV-positive patients using the "HIV-SELT" and the "EQ-5D" assessment questionnaires⁶⁶.

Probability of vertical HIV transmission according to GBV-C coinfection

Weintrop, et al.⁶⁷ tested 186 HIV-positive pregnant women for GBV-C to determine whether this coinfection is associated with a lower incidence of vertical transmission of HIV. However, neither active nor prior GBV-C infection was associated with a lower rate of HIV acquisition among infants. This finding was confirmed in other studies⁶⁸⁻⁷⁰.

Concluding remarks

Extensive studies have been unable to link GBV-C to any disease; thus this virus should be regarded as a harmless virus. In the context of HIV infection, there are several studies favoring a beneficial effect of GBV-C on the course of HIV infection, but this could not, however, be universally demonstrated. An explanation for these different findings might be a role of different GBV-C genotypes⁶³; however, more data are required to evaluate this further. Another reason for these differences might be the various replication capacity of different GBV-C strains, which has repeatedly been observed *in vitro*, but not yet been analyzed in relation to outcome of HIV-infection in patients^{64,65}.

Still, there seem to be some common themes when all studies are considered. Losing GBV-C, especially if GBV-C is lost without anti-E2 development, leads to an accelerated course of HIV-infection^{32,42,46}. This point should be considered when interferon therapy is planned in an HIV/HCV plus GBV-C coinfecting patient, as losing GBV-C due to IFN might accelerate the subsequent course of HIV. Although there are currently no data to support this, we would encourage the setting up of a database to evaluate this, or inclusion of such information into existing databases, so that this can be evaluated further.

Furthermore, another common finding from the different studies was that being positive after some time of HIV infection correlates with a better outcome of HIV infection. Even in the study by Van der Bej, et al.⁴⁶, who actually showed a worse prognosis for those patients who were GBV-C-RNA positive at study entry, in follow-

up eight years later, survival was slightly better for the GBV-C coinfecting patients. After adjustment for CD4 counts, this correlation, however, became insignificant.

Assuming a bilateral interaction between GBV-C and HIV, one could speculate that a very aggressive HIV strain with a high viral load would lead to downregulation of GBV-C, potentially below the level of detection. As we observed a gain of GBV-C in the serum in one patient with < 10 CD4 cells/ μ L, we do not believe that losing GBV-C is merely a consequence of decreasing CD4 cells.

It could be that the stage at which GBV-C is present is important, similar to the experience with HDV coinfection in hepatitis B. Usually the HDV coinfection is associated with a more pronounced course of hepatitis, leading to faster development of liver cirrhosis than hepatitis B alone. However, after liver transplantation and in the presence of immunosuppression, the course of hepatitis D coinfection is more benign than with hepatitis B mono-infection.

Finally, a strong argument for a role of GBV-C in HIV-infection comes from *in vitro* studies, which indicate a reduction of HIV replication by GBV-C coinfection. Still more work needs to be done to understand the role of GBV-C in HIV-infection. Future studies analyzing the role of GBV-C in HIV should also address the replication capacity of GBV-C *in vitro*, and the subsequent clinical course of patients in relation to GBV-C replication capacity and GBV-C genotypes, as this might discriminate patients with a positive or neutral influence of GBV-C.

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