

# GB virus C replication in cerebrospinal fluid of HIV positive individuals

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**Background** GB virus C (GBV-C) is an orphan *Flavivirus* distantly related to hepatitis C virus (HCV). GBV-C infection in humans is common and its transmission is similar to both HCV and HIV, including sexual, parenteral and vertical transmission. The aim of this study was to investigate the presence and compartmentalization of GBV-C strains in plasma, PBMC and cerebrospinal fluid (CSF) of HIV positive patients in an attempt to identify GBV-C replication in CSF.

**Methods** This retrospective study involved 22 HIV positive patients who underwent a lumbar puncture for diagnostic purposes. To evaluate the prevalence of GBV-C infection in our Centre, a control group of 150 HIV positive patients was included in the study. GBV-C-RNA was searched for in paired plasma, CSF and PBMC by means of nested PCR for 5' UTR. GBV-C genotype was identified by direct sequencing and phylogenetical analysis. GBV-C population in plasma and CSF was characterized by analysis of at least 25 clones for each compartment. HIV load was measured by a quantitative PCR (Amplicor HIV Monitor).

**Results** GBV-C-RNA was detected in plasma from 37/150 (25%) control patients and in the PBMC from 3/34 (9%) patients with GBV-C detected in plasma.

GBV-C-RNA was identified in 5/22 (23%) plasma, in none of PBMC and in 2/5 (40%) CSF samples obtained from the 5 GBV-C positive patients in plasma. This data showed that the presence of GBV-C-RNA in plasma was similar in control group and study population. Albeit GBV-C-RNA was detected in 3 PBMC of control group and in none PBMC of the study population, the difference was not statistically significant. Direct sequencing of GBV-C 5'UTR detected in plasma revealed the presence of genotype 2 in three patients GBV-C-RNA negative in CSF.

Analysis of GBV-C population within the 5'UTR showed the presence of GBV-C genotype 2 in plasma and genotype 3 in CSF of one patient, whereas the other case had mixed infection in plasma (genotype 3/1) and genotype 3 alone in CSF.

Plasma HIV-RNA levels were significantly higher in patients with CSF positive for GBV-C than in those with GBV-C negative CSF (mean values 5.35 vs 4.39 Log copies/mL,  $p=0.027$ ). HIV viral load in CSF was similar in all patients (mean values 3.66 vs 3.14 Log copies/mL).

**Conclusion** These findings suggest the replication of GBV-C in CSF and the selection of genotype 3 in this compartment. The presence of discordant genotypes in paired plasma/CSF of one patient could be due to a past mixed infection in plasma.

## Background

GB virus C (GBV-C) is an orphan *Flavivirus* distantly related to hepatitis C virus (HCV). GBV-C infection in humans is common:

- ✓ 1-2% blood donors
- ✓ 15% HCV positive subjects
- ✓ 15-40% HIV-1 positive subjects

Route of transmission is similar to both HCV and HIV, including sexual, parenteral and vertical transmission.

## Aim of the study

To investigate the presence and compartmentalization of GBV-C strains in plasma, PBMC and cerebrospinal fluid (CSF) of HIV positive patients in an attempt to identify GBV-C replication in CSF.

## Patients

Extraepatic replication of GBV-C was searched in 65 biological specimens obtained from 22 HIV positive individuals:

- ✓ 22 plasma samples
- ✓ 21 PBMC samples
- ✓ 22 CSF sample

Clinical and virological data are shown in Table 1

A control group of 150 HIV positive patients was included in the study to evaluate the prevalence of GBV-C infection in our Centre (Division of Infectious Diseases, San Raffaele Scientific Institute)

Table 1.

Pts.	Age	Sex	Diagnosis	CD4 (n/ml)	HIV-RNA pI (Log copies/ml)	CSF Analysis	
						Cells (n/ml)	Proteins (mg/dl)
A	38	f	Sensory neuropathy	773	2,60	2	42
B	37	m	CMV encephalitis*	126	4,69	1	36
C	35	m	Neurotoxoplasmosis	218	5,42	18	64
D	42	f	Ischemic lesions	342	4,98	9	69
E	41	m	NHL	298	5,58	29	120
F	40	m	Neurotoxoplasmosis	25	5,11	NA	NA
G	36	m	Sensory neuropathy	292	4,45	3	48
H	34	m	HIV dementia	325	5,31	3	76
I	35	f	HIV dementia	218	2,68	6	86
J	42	m	Myelopathy	416	2,60	1	36
K	34	f	Ischemic lesions	NA	NA	11	43
L	31	m	HIV dementia	0	NA	3	73
M	38	m	Ischemic lesions	12	5,13	NA	NA
N	29	m	Polyradiculoneuropathy	243	4,18	1	40
O	35	f	NHL	346	2,60	1	59
P	39	m	None**	48	5,66	18	32
Q	38	m	Cryptococcosis	18	4,64	1	38
R	44	f	Neurotoxoplasmosis	57	2,60	1	48
S	37	m	Neurotoxoplasmosis	101	NA	NA	NA
T	36	m	Cryptococcosis	95	2,64	12	79
U	36	m	Neurotoxoplasmosis	80	5,04	2	26
V	31	m	PMI	170	5,61	2	36

\*suspected chlamydia/HSV encephalitis  
\*\*Cryptococcosis Ag-positive serum  
NHL: non Hodgkin lymphoma  
PMI: progressive multifocal leukoencephalopathy  
NA: not available

## Methods

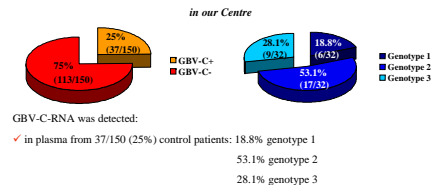
- ✓ GBV-C-RNA was searched for in paired plasma, CSF and PBMC by means of nested PCR for 5' untranslated region
- ✓ GBV-C genotype was identified by direct sequencing and phylogenetical analysis
- ✓ GBV-C population in plasma and CSF was characterized by analysis of at least 25 clones for each compartment
- ✓ HIV load was measured by a quantitative PCR (Amplicor HIV Monitor)

## Statistical analysis

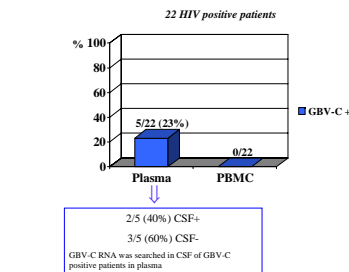
T-test was used to compare HIV-RNA levels among different groups

## Results

### Prevalence of GBV-C infection and genotype distribution in the 150 HIV patients recruited in our Centre



### Prevalence of GBV-C infection in different compartments (plasma, PBMC and CSF) of the 22 HIV positive patients

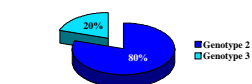


- ✓ Comparison of HIV-RNA levels between GBV-C positive and negative patients in plasma showed:

- ✓ Similar HIV plasma viremia (mean values 4.7 vs 4.2 Log copies/ml)
- ✓ Higher HIV-RNA levels in GBV-C positive patients in CSF than in negative ones (mean values 5.35 vs 4.39 Log copies/mL,  $p=0.027$ )

Comparison of HIV viral load in CSF between GBV-C positive and negative in this compartment was statistically different (mean values 3.66 vs 3.14 Log copies/mL)

### Direct sequencing of GBV-C 5' UTR detected in plasma



### GBV-C genotype in the 2 patients plasma and CSF positive

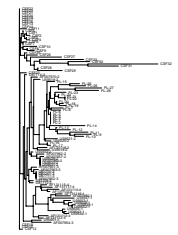
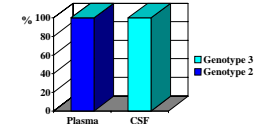
Patients	GBV-C genotype	
	Plasma	CSF
E	2	3
F	3	3

Analysis of GBV-C quasispecies by cloning within the 5'UTR showed the presence of GBV-C genotype 2 in plasma and genotype 3 in CSF of one patient (E), whereas the other case (patient F) had mixed infection in plasma (genotype 3/1) and genotype 3 alone in CSF.

### Patient E

PLASMA: 28/28 (100%) clones ⇨ genotype 2

CSF: 33/33 (100%) clones ⇨ genotype 3

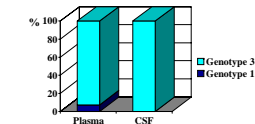


### Patient F

PLASMA: 26/28 (93%) clones ⇨ genotype 3

2/28 (7%) clones ⇨ genotype 1

CSF: 29/29 (100%) clones ⇨ genotype 3



## Summary

- ✓ GBV-C-RNA was identified in 5/22 (23%) plasma, in none of PBMC and in 2/5 (40%) CSF samples obtained from the 5 GBV-C positive patients in plasma. This data showed that the presence of GBV-C-RNA in plasma was similar in control group and study population (25% vs 23%)
  - ✓ GBV-C-RNA was detected in 3 PBMC of control group and in none PBMC of the study population, the difference was not statistically significant
  - ✓ In CSF, genotype 3 was dominant in both patients GBV-C positive in this compartment
  - ✓ In plasma, genotype 2 and 3 were dominant in patient E and F, respectively
- Additionally, Patient F showed a mixed infection in plasma (genotype 3 and 1)
- ✓ HIV viral load in plasma was associated with GBV-C detectability in CSF

## Conclusions

These data show:

- ✓ The active replication of GBV-C in CSF
- ✓ The selection of genotype 3 in this compartment

The presence of discordant genotypes in paired plasma and CSF of patient E could be the consequence of a past mixed infection in plasma with genotype 2 and 3