Study of Biomarkers in HIV-1 Infected Individuals with Neurocognitive Disorders (HAND)

Mireia Llorà i Valls | Proposal of research project
Biotechnology bachelor degree, biosciences school, Autonomous University of Barcelona

BACKGROUND

HIV associated neurocognitive disorder (HAND) has become a common comorbidity in more than 30% patients infected by human immunodeficiency virus (HIV)-1, despite the presence of different antiretroviral treatments (ART).[1]

There are several clinical HAND syndromes depending on the severity of neurodegeneration: HIV associated dementia (HAD), mild neurocognitive disorder (MND) and asymptomatic neurocognitive impairment (ANI).[2]

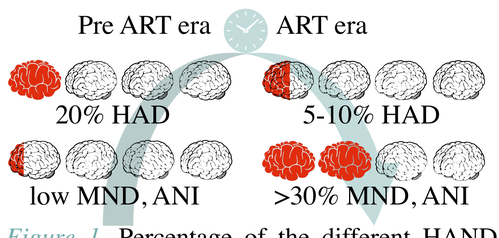


Figure 1. Percentage of the different HAND clinical syndromes before and after ART

HAND is associated with neuroinflammation and several biomarkers have been described throughout the last years to diagnose HAND, but a complete diagnostic analysis has not been found yet.[3]

HYPOTESIS

The levels of molecules present in blood and cerebrospinal fluid (CSF) are associated to chronic inflammation. They can be used as biomarkers to predict neurodegeneration in HIV-1 infected individuals in order to treat the patients earlier.

Furthermore, these biomarkers can be used to monitor treatments as nasal administration of anti-inflammatory drugs to improve symptoms of HAND.

OBJECTIVES

1. To find biomarkers associated with neurodegeneration in HIV-1 infected individuals
2. To find a transcriptomic profile associated with neurodegeneration in HIV-1 infected individuals
3. To test the panel of HAND biomarkers in mice treated with anti-inflammatory drugs administered intranasally

MATERIALS AND METHODS

OBJECTIVE 1

1 PEOPLE RECRUITMENT AND SAMPLE COLLECTION (9 MONTHS)

200 HIV+ ART, <20 copies/ml
- **65 HAND**
  20 HAD, 20 MND, 25 ANI
- **135 control**

200 HIV-
- **100 neurodegeneration**
- **100 control**

Inclusion criteria check

Informed consent delivery

Diagnosis of HAND (IHDS test)

HAND questionnaire (AAN and HNRC criteria)

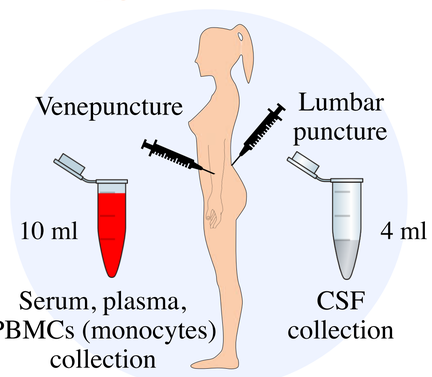


Figure 2. Blood and CSF extraction and collection

2 ANALYSIS OF BIOMARKERS (3 MONTHS)

Systemic biomarkers
HIV viral load
CD4 nadir
CD4 count
CD4/CD8 ratio

Inflammation biomarkers
IL-6 CRP
TNF-α IL-1ß
MCP-1

Monocyte/macrophage biomarkers
sCD14
sCD163

Neuronal injury biomarkers
NFL pNFG neopterin
glutamate/creatine ratio
NAA/creatine ratio

2 STATISTICAL ANALYSIS (3 MONTHS)

T student test
Wilcoxon rank sum test
Turkey test
p<0,05

Molecules used as biomarkers

Extreme phenotype substudy

Choice of transcriptomic profile samples

OBJECTIVE 2

1 RNA PREPARATION (3 MONTHS)

Monocytes
CSF
100 HIV+

Monocytes
CSF
100 HIV-

RNA isolation
Total RNA kit (Miltenyi Biotec) for monocytes
Exiqon Biofluids kit for CSF

RNA quantification
NanoDrop and Qubit fluorometer

2 SEQUENCING (3 MONTHS)

Library generation[4]
- NEB-based directional RNA-Seq (Illumina)
- RT-PCR amplification

cDNA library

Next generation sequencing
- HigSeq2000 Illumina sequencer
- Bowtie software (FastQ format)

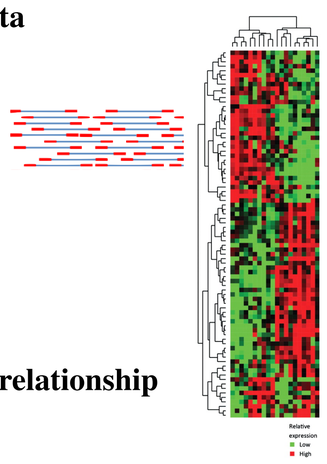
3 DATA ANALYSIS (3 MONTHS)

Monocyte and CSF RNA-seq data

Reads alignment
· Spliced Transcripts Alignment to Reference (STAR, Illumina)
· Trim Galore software

Differential expression
· Normalitzation of raw data
· Bioconductor software package
· p<0,05

Neurodegeneration pathways relationship
· DAVID and STRING database



4 VALIDATION OF RNA-SEQ DATA (3 MONTHS)

qPCR of significant results found in RNA-seq

OBJECTIVE 3

1 SCID MICE ORDER (3 MONTHS)

Ethics Committe approval
20 SCID mice (immunosupressive model)

2 HIV-1 MICE INFECTION

HIV-1 monocyte-derived macrophages infection

Biosafety level 3+ laboratory (P3+)

Intracerebral injections of HIV-1 monocyte-derived macrophages in 16 mice

16/20 HIV+ (HAND model) SCID mice

4/20 HIV- (no HAND model) SCID mice

3 TREATMENT (9 MONTHS)

Intranasal
BB-882 **4 mice**
BB-1101 **HIV+**
PBS **4 mice**
HIV+

Intraperitoneal and subcutaneous
BB-1101
BB-882
4 mice HIV+
PBS
4 mice
HIV+

Initiation of treatment — month 2 — month 5 — End of treatment

Figure 3. Study design and number of times that biomarkers will be analysed

Treatment monitoring with panel of HAND biomarkers

Panel of HAND biomarkers tested

Analyse the effectiveness of each treatment used

Data analysis

EXPECTED RESULTS

1. Some of the molecules analysed may be used as HAND biomarkers, considering that they are significantly more expressed in HIV+ individuals with HAND than in patients with no neurodegeneration.
2. There may be some CSF and monocytes RNAs in HIV+ patients with HAND symptoms differentially expressed in comparison to HIV+ patients without these symptoms.
3. The panel of biomarkers may be used to monitor drug treatments in mice. Furthermore, BB-882 and BB-1101 anti-inflammatory treatments provided through intranasal route may have better results in SCID mice when compared to the previous studies.[5]

DIFFUSION PLAN

- 3 or more publications in high-impact journals of this area
- Results presentation at some relevant conferences, e.g. the Conference on Retroviruses and Opportunistic Infections (CROI)
- Dissemination of results to HIV seminars
- Dissemination of results to patients

REFERENCES

1. González-Scarano, F. & Martín-García, J. The neuropathogenesis of AIDS. *Nat. Rev. Immunol.* **5,** 69–81 (2005).
2. Antinori, A. *et al.* Updated research nosology for HIV-associated neurocognitive disorders. *Neurology* **69,** 1789–1799 (2015).
3. Mcguire, J. L. *et al.* Central and peripheral markers of neurodegeneration and monocyte activation in HIV-associated neurocognitive disorders. *J. Neurovirology* 439–448 (2015). doi:10.1007/s13365-015-0333-3
4. Hossein-Nezhad, A. *et al.* Transcriptomic Profiling of Extracellular RNAs Present in Cerebrospinal Fluid Identifies Differentially Expressed Transcripts in Parkinson's Disease. *J. Parkinsons. Dis.* **6,** 109–117 (2016).
5. Persidsky, Y. *et al.* Reduction in glial immunity and neuropathology by a PAF antagonist and an MMP and TNFα inhibitor in SCID mice with HIV-1 encephalitis. *J. Neuroimmunol.* **114,** 57–68 (2001).

UAB Universitat Autònoma de Barcelona