Advances in molecular analysis in HTLV infection

Carolina Rosadas
MSc, PhD Student
Universidade Federal do Rio de Janeiro
Cerebrospinal Fluid Laboratory
HTLV: Introduction

- First Retrovirus described in humans
- **HTLV-1, HTLV-2, HTLV-3, HTLV-4**
- Transmission
  - IDUs
  - HIV/HTLV
- Prevalence

(A.Gessain and O.Cassar - 2012)
HTLV: clinical presentation

• Asymptomatic infection

• 1-5% disease
  • HAM/TSP
  • ATL
  • Other clinical presentation

HAM/TSP
Chronic progressive incapacitating neurologic disease

There is no treatment

HAM/TSP patient
HTLV morphology

Verdonck et al., 2007
HTLV replication cycle

Clonal expansion of Infected T-cell

Lairmore et al., 2012
HTLV proviral genome

Low mutation rate

Molecular analysis in HTLV infection

- Diagnosis
- Prognosis
- Epidemiology
- Pathogenesis
Diagnosis

**WHO (OSAME, 1990)**
- HAM/TSP Classical symptoms
- Abs in blood **and** CSF

**Castro-Costa (2006)**
- HAM/TSP Classical symptoms
- Abs in blood and CSF or PCR positive

Abs detection:
- ELISA: screening test
- WB: Confirmatory and typing test
- Indeterminate WB
Detection of proviral DNA

- Recent infection
- Passive transfer of Abs
- Indeterminate WB
- Abs in blood and not in CSF in symptomatic patients (HAM/TSP)
- Identification of HTLV types and subtypes
Human T-lymphotropic virus type 2 subtype b in a patient with chronic neurological disorder

Carolina Rosadas • Ana C. P. Vicente • Louise Zanella • Mauro J. Cabral-Castro • José M. Peralta • Marzia Fuccioni-Sohler
Nucleotide identity

HTLV-2 LTR and Tax region nucleotide identity in percentage between the patient isolate and different HTLV prototypes

<table>
<thead>
<tr>
<th>Versus patient isolate</th>
<th>Prototypes (% similarity)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>NRA (HTLV-2b)</td>
</tr>
<tr>
<td><strong>LTR region</strong></td>
<td>99%</td>
</tr>
<tr>
<td><strong>Tax gene</strong></td>
<td>99%</td>
</tr>
</tbody>
</table>
**LTR (623 nucleotides)**

- **HTLV-2b**
- **HTLV-2a**
- **HTLV-2c**
- **HTLV-2d**

![Diagram showing the classification of different HTLV-2 subtypes based on LTR (623 nucleotides) and their evolutionary relationships.](image-url)
Tax protein

ClustalW2
Prognosis

Determination of proviral load in PBMCs
Prognosis

Determination of proviral load in CSF

VALIDATION IS ESSENTIAL
Validation of a quantitative real-time PCR assay for HTLV-1 proviral load in peripheral blood mononuclear cells

Carolina Rosadas, Mauro Jorge Cabral-Castro, Ana Carolina Paulo Vicente, José Mauro Peralta, Marzia Puccioni-Sohler

Laboratório de Líquido Cefalorraquiano, Serviço de Patologia Clínica, Hospital Universitário Clementino Fraga Filho (HUCFF)/Programa de Pós Graduação em Doenças Infecciosas e Parasitárias, Faculdade de Medicina, Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil
Laboratório de Diagnóstico Imunológico e Molecular de Doenças Infecciosas e Parasitárias/Programa de Pós Graduação do Instituto de Microbiologia Paulo Góes, UFRJ, Rio de Janeiro, Brazil
Laboratório de Genética de Microorganismos, Instituto Oswald Cruz (IOC), Fundação Oswaldo Cruz (FIOCRUZ), Rio de Janeiro, Brazil
Escola de Medicina e Cirurgia, Universidade Federal do Estado do Rio de Janeiro (UNIRIO), Brazil
Validation of qPCR for HTLV-1 proviral load in PBMCs

<table>
<thead>
<tr>
<th></th>
<th>Actin (HTLV-1)</th>
<th>Tax (HTLV-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actin-F</td>
<td>CACATCGTGGCCCATCTACGA</td>
<td>ACAAAGTTAACCATGCTTATTATCAGC</td>
</tr>
<tr>
<td>Actin-R</td>
<td>CTCAGTGAGGATCTTCATGAGGTAGT</td>
<td>ACACGTAGACTGGGTATCCGAA</td>
</tr>
<tr>
<td>Probe</td>
<td>FAM-ATGCCCTCCCCCATGCCATCCTGCGT-TAMRA</td>
<td>FAM-TTCCCAAGGTTTGGACAGAGTCTTCT-TAMRA</td>
</tr>
</tbody>
</table>

Number of HTLV-I (pX) copies/100 cels = (number of copies of pX)/(number of copies of β-actin/2) x 100 (Nagai et al., 1998)
Validation of qPCR for HTLV-1 proviral load in PBMCs

- Sensibility and specificity
- Limit of detection
- Intra- and inter-assay variability
Validation of qPCR for HTLV-1 proviral load in PBMCs

All seropositive samples (ELISA) presented gene amplification (both genes: actin e pX)

Negative samples (ELISA):
- Not amplify pX gene
- Amplification of actin

Figure

Validation of qPCR for HTLV-1 proviral load in PBMCs

- Limit of detection: 1 copy/rxn.
- qPCR efficiency, slope and $r^2$: 98.58%, -3.298 e 0.993 respectively.

*Fig. 4. Amplification plot of the sample containing one copy of pXgene from HTLV-1.*
Validation of qPCR for HTLV-1 proviral load in PBMCs
Validation of qPCR for HTLV-1 proviral load in PBMCs
Validation of qPCR for HTLV-1 proviral load in PBMCs

Table 2
Reproducibility experiments: intra- and inter-assay reproducibility of TARL-2 cells DNA standard dilutions analyzed in triplicate on four different assays.

<table>
<thead>
<tr>
<th>CT Mean</th>
<th>Intra-assay variability</th>
<th>Inter-assay</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Experiment 1</td>
<td>Experiment 2</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>CV (%)</td>
</tr>
<tr>
<td>pX</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50,000</td>
<td>22.7</td>
<td>0.6</td>
</tr>
<tr>
<td>500</td>
<td>26.0</td>
<td>0.2</td>
</tr>
<tr>
<td>50</td>
<td>33.0</td>
<td>0.6</td>
</tr>
<tr>
<td>5</td>
<td>36.3</td>
<td>1.7</td>
</tr>
<tr>
<td>β-actin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>66,600</td>
<td>23.4</td>
<td>0.1</td>
</tr>
<tr>
<td>13,320</td>
<td>24.3</td>
<td>0.1</td>
</tr>
<tr>
<td>2664</td>
<td>25.0</td>
<td>0.2</td>
</tr>
<tr>
<td>532</td>
<td>25.6</td>
<td>0.1</td>
</tr>
<tr>
<td>106.6</td>
<td>26.8</td>
<td>0.1</td>
</tr>
</tbody>
</table>

**Experiment 1.**
Same sample 15 times

**Experiment 2.**
Same sample 15 times

**Intra-assay:** CV = 0.4%
**Inter-assay:** CV = 2%
Validation of qPCR for HTLV-1 proviral load in PBMCs

The assay can reliably quantify HTLV-1 proviral load.

PVL of patients (mean ± SD = 36.3 ± 40.2; SE: 9.5) was higher than in the non-HAM/TSP group (mean ± SD = 6.9 ± 8.8; SE: 2.9; p < 0.005).
Epidemiology

Subtypes

Associated with geographic distribution

No association with clinical progression

Gessain & Mahieux, 2012
Epidemiology

HTLV-1 subtypes in Rio de Janeiro, Brazil
Pathogenesis

G29→S

↑ p12

Immune evasion of HTLV-1 infected cells
- ↓ MHCI expression
- ICAMI e ICAMII

Infected T-cell proliferation:
- ↑ STAT activation,
- ↑ cytoplasmatic calcium → activation of NFAT

Bind to IL-2 promoter, ↑ IL-2 expression
Important remarks

- Importance of HTLV-1 screening

- Molecular assays can be used for different purposes in HTLV infection

- Prior validation is essential before the implementation in laboratorial routine
Acknowledgments

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Laboratório de Diagnóstico imunológico e molecular de doenças infecciosas e parasitárias
José Mauro Peralta, MD, PhD

Laboratório de Genética Molecular de Microorganismos
Ana Carolina Paulo Vicente, PhD
Louise Zanella, MSc, PhD Student

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CAROLROSADAS@GMAIL.COM