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# HCV seropositivity in sera of HIV-1, HTLV-1/-2 and *Trypanosoma cruzi* infected individuals from different areas of Brazil

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## Summary

Our aim was to estimate the prevalences of HCV in HIV-1, HTLV-1/-2 and *Trypanosoma cruzi* infected and non-infected individuals from different regions of Brazil. We investigated sera from Rio de Janeiro (Rio de Janeiro), Salvador (Bahia) and Virgem da Lapa (Minas Gerais) — a region of Brazil where Chagas disease is endemic — for anti-HCV antibodies.

For HIV-1 infected individuals we determined a HCV prevalence of 27.3% in Rio de Janeiro and 22.4% in Salvador. In the HIV negative population we measured a prevalence of about 4.4% and 7.1% respectively. From the HTLV Western Blot confirmed sera (18 in Rio de Janeiro and 7 in Salvador) were 66.7% and 71.4% infected with HCV. HIV-HTLV double infected sera showed by 84.6% and 80.0% superinfection with HCV.

None of the sera from the Chagas disease endemic region and the Anti-*T. cruzi* positive sera from Salvador showed any reaction by screening for anti-HCV antibodies.

Typing of some HCV positive sera showed a predominance of HCV type 1 and type 3.

***Key Words***

Brazil, Chagas, HCV, HIV, HTLV, PCR, Seroprevalence

# Introduction

Since the discovery of HCV [for review 19] fast progress has been made in the development of specific assays for diagnosis (although there is still a lack of sensitivity in some tests to detect all infected patients [6]) and screening purposes [5, 15, 25]. Most of the studies for epidemiology of HCV and its related transmission routes (blood transfusion blood products, hemodialysis, IVDU, sexual transmission) were carried out in Europe and USA [2, 15, 16, 20] but little is known about the epidemiology of HCV in South America. Only few data on HCV prevalences in Brazilian blood donors have been published until now [3, 49, 50]. In Brazil with its enormous geographic dimensions (larger as whole Western Europe), its demographic and cultural differences it is impossible to "define" a general population and to estimate one single prevalence for any disease in the country. There are high prevalences for HIV-1 and HTLV-1/-2 [1, 13 17, 18, 28, 41, 49] and, as far as known, a low prevalence for HIV-2 [21] in the regions of Rio de Janeiro and Salvador. Little is known about the prevalences of HCV in the "general populations" as well as in subpopulations such as HIV or HTLV infected individuals. We therefore wanted to estimate in a retrospective study the prevalences of HCV in HIV-1 and HTLV-1/-2 infected blood donors from different regions of Brazil and in *Trypanosoma cruzi* infected individuals [39]. We investigated sera from Rio de Janeiro, Salvador and Virgem da Lapa, by screening for HIV-1, HTLV-1/-2 and HCV. For confirming HCV reactive sera we used different ELISAs and two supplemental assays. Furthermore a few sera were typed by HCV-PCR.

# Materials and Methods

## *Sera*

The human sera from Brazilian blood donors ($n_{RJ+S}$ = 600) were collected between 1988-1991 from blood banks in the cities of Rio de Janeiro in the state of Rio de Janeiro ($n_{RJ}$ = 441) and Salvador in the state of Bahia ($n_S$ = 159), pre-selected for HIV-positive and -negative sera. Furthermore we investigated sera ($n_L$ = 235) from Virgem da Lapa in the state of Minas Gerais collected in 1992, a region were Chagas disease is endemic.

Tests for all serological markers were performed according to the manufacturer's protocols.

## *Hepatitis C Virus (HCV)*

Initially all sera were pre-screened with the Innotest HCV Ab II (N. V. Innogenetics S. A., Antwerpen, B). All reactive samples (borderline or exceeding the cut-off of the initial assay) were further tested with the Abbott HCV II EIA (Abbott Diagnostic Products GmbH, Wiesbaden, D), Enzygnost-Anti-HCV (Behringwerke AG, Marburg, D), Murex Anti-HCV (Murex Diagnostica GmbH, Burgwedel, D) and with the supplemental assay Inno-LIA HCV Ab (N. V. Innogenetics S. A). Sera reacting in one of the second step ELISAs were also tested with the Chiron HCV RIBA-2 (Chiron Corp., Raritan, NJ, USA). Sera were defined as HCV positive if at least all ELISAs gave positive results in combination with one positive supplemental assay (LIA or RIBA)

RNA extraction for HCV-Typing with PCR-RFLP analysis was carried out with 200 µl of serum according to the method of Chomczynski [12]. The RNA was reverse transcribed in the 5'-noncoding region (5'-NCR) with the antisense primer 1 using Moloney murine leukemia virus reverse transcriptase according to the manufacturer's instruction (GIBCO, BRL Life Technologies Inc., Gaitherburg, MD, USA). Two rounds of PCR were carried out using Thermus aquaticus DNA polymerase (Promega, Madison, WI, USA). In the first round primers 1 and 2, in the second round primers 3 and 4 were used. In both rounds of PCR, amplification was done for 35 cycles (1 min 92°C, 1 min 54°C, 2 min 72°C; Table 1). For restriction fragment length polymorphism (RFLP) analysis the nested PCR fragments were digested with Ava II and Rsa I and visualized by electrophoresis in 3,5% agarose gels stained with ethidium bromide.

Different attempts have been made to classify the types of HCV by genome analysis [9, 10, 29, 32, 34, 35, 43, 45], in our study we are using the system proposed by Chan [10]. An overview of the HCV-type nomenclature is given in Stuyver et al. [45].

Accuracy : The ability of a test to classify samples correctly = (TP + TN) / (TP + FN+ TN + FP)

δ-value : $\overline{\lg(\text{Ratio})} / \sigma_{\lg(\text{Ratio})}$

Sensiticity : The ability of a test to classify positive samples as positive (diagnostic or clinical sensitivity) = TP / (TP + FN) × 100

Specificity : The ability of a test to classify negative samples as negative (diagnostic or clinical specificity) = TN / (TN + FP) × 100

Table 1: Oligonucleotide primers used in the RT-PCR. The nucleotide positions are according to Kato et al. [23]

| Primer | | Sequence (5'-3') | Position |
|---|---|---|---|
| 1. | antisense | GGTGCACGGTCTACGAGACC | 310-329 |
| 2. | sense | GGCGACACTCCRCCAT (R: A+G) | 6-21 |
| 3. | antisense | CGCAAGCACCCTATCAGGCAGT | 276-297 |
| 4. | sense | CACTCCCCTGTGAGGAACTACTGT | 26-49 |

### *Human Immunodeficiency Virus (HIV)*

All sera were screened with at least three different ELISAs: The Abbott Recombinant HIV-1 EIA (Abbott Laboratories do Brasil Ltda., São Paulo, BR), the Biochrom HIV-1/-2-ELISA (Biochrom KG, Berlin, D) and the Vironostika anti-HTLV-III (Organon-Teknika, São Paulo, BR). For confirmation of ELISA reactive samples the Biotech/DuPont HIV-1 Western-Blot kit (DuPont Nemours Deutschland GmbH, Bad Homburg, D) and the HIV Blot 2.2 (Diagnostic Biotechnology Ltd., Singapore) were used.

In our study HIV positivity (+) is defined either as a positive Western Blot with at least one band from each gene product Env, Gag and Pol (according to the guidelines of the American Red Cross) or a positive reaction in three different ELISAs. Sera with no bands in Western Blot or negative ELISAs are termed HIV negative (-); All other possibilities are called HIV indeterminate (±).

### *Human T-Lymphotropic Virus type 1 and 2 (HTLV-1/-2)*

All sera from Rio de Janeiro and Salvador were screened with the Biochrom HTLV-1/-2-ELISA (Biochrom KG). The ELISA reactive sera were confirmed with the HTLV Blot 2.3 (Diagnostic Biotechnology Ltd.). A Western Blot showing bands against Gag (p19 or p24) and Env (gp46 or rgp46-I/-II or rgp21) was classified as HTLV-1/-2 positive (according to the manufacturers instructions). Western Blots without any bands were classified as HTLV-1/-2 negative. Other reaction patterns were called indeterminate.

### *Trypanosoma cruzi*

In the present study, we also evaluated a panel of 235 sera obtained from chronic chagasic and healthy residents of Virgem da Lapa, Minas Gerais, Brazil. The chagasic patients have been part of a longitudinal study initiated in 1985 [38]. To asses the presence of a *T. cruzi* infection, all sera were thoroughly characterized by standard immunological tests (immunofluorescence and hemagglutination), ELISA using cloned *T. cruzi*-specific antigens [24], Xenodiagnosis and PCR [52].

The sera from Rio de Janeiro and Salvador were screened with an in-house Chagas ELISA of lysed *T. cruzi* antigen, kindly provided by the Instituto Nacional de Diagnostico e Investigación de la Enfermedad de Chagas Dr. Mario Fatala Chaben, Buenos Aires, Argentina.

### *Statistical analysis*

The δ-values for inter-test comparison were estimated by the method of Crofts and Maskill [14, 27]. For calculation of the accuracy, sensitivity and specificity the formularies given by Linnet [26] were used.

## Results

### *Rio de Janeiro*

We investigated a serum bank constituting of 441 sera from the city of Rio de Janeiro for the prevalence of HCV in HIV-1 infected and non-infected individuals. In this group the HIV-1 status of 290 sera was determined by HIV-1 Western Blots, and from additional 151 sera by the results from different ELISAs. In the population of HIV negative sera 4.4% were HCV positive and 0.4% indeterminate (Table 2). From the HIV-1 positive sera 27.3% were positive for anti-HCV antibodies, and from the HIV-1 indeterminate sera 5.5% were reactive in HCV tests (Figure 1).

The screening for HTLV-1/-2 revealed that 17% of the total number of sera from Rio de Janeiro were reactive in the HTLV-1/-2 ELISA assay, but only 4.1% were Western Blot positive, and 3% gave an indeterminate Western Blot result. 66.7% of the HTLV confirmed sera were also HCV positive. 84.6% of the HIV-HTLV double infected sera showed superinfections with HCV (Figure 1).

Out of the 441 sera from Rio de Janeiro 19 were suspicious, but not confirmed for Chagas disease. From these sera 2 were positive for HCV.

### *Salvador*

From the 159 sera of Salvador 135 had a HIV-1 Western Blot and 24 only different HIV-1 ELISAs. From the HIV-1 negative sera 7.1% were HCV positive, from the HIV-1 positive 22.4% were HCV reactive. No HCV

Table 2: Distribution of anti-HCV antibodies in individuals with and without infection of HIV-1 from Rio de Janeiro ($n_{RJ}$ = 411) and Salvador ($n_S$ = 159)

| | Rio de Janeiro | | | | | | Salvador | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| HIV | - | | ± | | + | | - | | ± | | + | |
| n | 229 | | 73 | | 139 | | 56 | | 27 | | 76 | |
| HCV - | 218 | 95,2% | 68 | 93,2% | 100 | 71,9% | 52 | 92,9% | 26 | 96,3% | 59 | 77,6% |
| HCV ± | 1 | 0,4% | 1 | 1,4% | 1 | 0,7% | 0 | 0,0% | 0 | 0,0% | 0 | 0,0% |
| HCV + | 10 | 4,4% | 4 | 5,5% | 38 | 27,3% | 4 | 7,1% | 1 | 3,7% | 17 | 22,4% |

indeterminate sera were detected in this group (Table 2 ).

Screening for HTLV-1/-2 resulted in 35 of 159 reactive sera (22.0%), but only seven (4.4%) could be confirmed by Western Blot. Five of these seven confirmed HTLV positive sera were positive for HCV (71.4%). 80.0% of the HIV-HTLV co-infected sera gave a positive reaction in the HCV test (Figure 1).

From the 159 sera from Salvador 26 were positive for Chagas disease, but no serum reacted in the HCV screening assay.

### *Virgem da Lapa*

None of the tested 235 sera had a detectable level of anti-HCV antibodies in the screening assay. The distribution of the human retroviruses in these sera is unknown.

### *Comparison of HCV-Tests*

From 835 ($n_\Sigma = n_{RJ} + ns + n_L$) tested sera 104 (12.2%) were borderline or reactive in the first screening assay. For the comparison of HCV tests, 14 sera negative for all investigated serological markers were used as internal negative controls (Table 3). From these 14 negative sera, all were negative in the other HCV-ELISAs, but one showed a weak reaction in the LIA (NS4 and Core 3). Another 14 sera which were repeatedly weakly reactive (ratio signal/cut off < 2.0) in the INNO HCV Ab II were negative in all other ELISAs and the supplemental assay LIA. The HCV positive sera can be divided into three groups: all tests positive, all ELISA positive - RIBA indeterminate and all ELISA positive - RIBA positive - LIA negative. A comparison of the LIA positive - RIBA indeterminate sera is shown in table 4. All RIBA indeterminate sera reacted with the core protein (C22-3), but none of these sera recognized the truncated NS4 protein (5-1-1), and only three sera showed a weak reaction with the complete NS4 protein (C100-3).

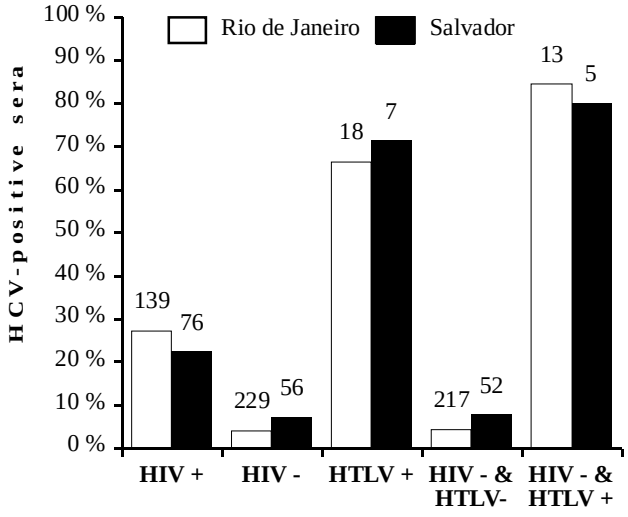


Figure 1: HCV seropositivity in different populations. For every column the total number of individuals is indicated. It should be noted that in the columns “HTLV+” and “HIV+ & HTLV+” the total number of individuals is very low.

According to our results described above we calculated a specificity of 98%, a sensitivity of 100% and an accuracy of 98% for our screening assay. The δ-values (Figure 2), which can be used as a numeric parameter for inter-test comparisons are estimated to -2.0 for the negative population and +5.7 for the confirmed HCV population.

### *PCR*

We tested 23 HCV antibody positive sera from Rio de Janeiro and 5 sera from Salvador by PCR (Table 5). Furthermore, 17 PCR positive sera out of these 28 sera were typed. 65% (11/17) belonged to type 1 and 29% (5/17) to type 3. One serum gave an indeterminate result, being either type 1 or type 2.

## Discussion

Our retrospective study revealed that the prevalences

Table 3: Results of the different HCV assays. All 835 sera were screened with the Innotest HCV Ab. All reactive sera were tested a second time with the same ELISA and with the anti-HCV ELISAs from Abbott, Behring and Murex. Supplemental assays (LIA & RIBA) were used for "confirmation" of ELISA positive sera. (n.d. = not done)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Innotest HCV Ab | - | + only 1st test | + | - | + | + | + | + | + | + |
| Abbott, Behring, Murex | - | - | - | - | +, 2- | ± | + | + | + | 2+, 1- |
| LIA | - | - | - | + | ± | - | + | + | - | + |
| RIBA | n. d. | - | - | n. d. | - | n. d. | + | ± | + | ± |
| ∑ (n=118) | 14 | 12 | 13 | 1 | 1 | 3 | 59 | 13 | 1 | 1 |
| Classification | HCV - | | | HCV ± | | | HCV + | | | |

of HCV in different Brazilian groups are highly varying. The overall prevalence of HCV in the sera from the regions of Rio de Janeiro and Salvador was between 4% and 7%. These sera were collected before general screening of blood donors for HCV was introduced in Brazil. More recent investigations with sera from Rio de Janeiro still revealed a prevalence of 2.7% [49] and 2.9% [37]. High prevalences have also been reported from Kiribati (4.8%) and Zaire (6.4%) [46]. In contrast to the results obtained with the sera collected in the cities, we were not able to detect HCV infections in the rural area of Virgem da Lapa, a region were Chagas disease is endemic. These findings could be explained by the traditional life style of these people (e.g. no IVDUs, no open homosexuality). Also, in the group of Chagas disease positive individuals from Rio de Janeiro and Salvador the incidence for HCV is very low. Although the number of these sera is small, it could be possible that these people were born in an endemic area and moved to the cities without changing their traditional life style. These results show clearly that it is not possible to estimate one single prevalence for any disease for Brazil as a whole.

Brazil is also a country with a growing problem of HIV and HTLV infections [1, 18, 21,28, 31]. We therefore tried to estimate the prevalence of HCV in HIV-1 and HTLV-1/-2 infected individuals and in HIV-HTLV double infections. In our study groups from Rio de Janeiro and Salvador 22% and 27% of the HIV infected individuals (Hayashi [20] reported 7% in HIV-infected individuals) and 66% to 71% of the HTLV-1/-2 infected individuals were co-infected with HCV. About 80% of the HIV-HTLV infected patients were superinfected with HCV. The accumulation of double and triple infections is not surprising because these viruses are using almost the same transmission routes (blood and blood products, needle exchanging) and replicate in lymphoid cells [4, 22, 30, 42, 47, 48]. Because our sera were collected in a period where irregular donation practice [e.g. payment] could not be totally excluded there is a probability that our serum bank contains also sera from drug addicts (IVDUs) who have high prevalences of multiple virus infections [40].

A few attempts have been made to show the distribution of HCV types around the world [7, 36]. Typing some of our HCV positive sera revealed a predominance of HCV type 1, which is widely distributed around the world, and type 3, which occurs in Europe and the pacific region. Since we have typed only a few sera from Brazil our results should be confirmed by others. Further studies should also include subtyping of HCV types. In the tropical region of Africa HCV type 4 was described [7]. More detailed studies including the North-East of Brazil with its predominantly black population could show whether there are similarities between this region and Africa.

With our results the screening assay had a specificity

Table 4: Reaction patterns of the RIBA indeterminate sera in comparison with the LIA (n = 14).

| | | RIBA | | | LIA | |
|---|---|---|---|---|---|---|
| Core | | C22-3 | 14 | 100 % | 14 | 100 % |
| NS3 | | C33c | 7 | 50 % | - | - |
| NS4 | { | 5-1-1 | 0 | 0 % | 2 | 14 % |
| | { | C100-3 | 3 | 21 % | | |
| NS5 | | - | - | - | 7 | 50 % |

Table 5: Results of the HCV-PCR and the HCV-typing from 28 HCV positive sera.

| | Rio de Janeiro | Salvador | ∑ |
|---|---|---|---|
| PCR - | 10 | 1 | 11 |
| Typ 1 | 8 | 3 | 11 |
| Typ 2 | 1* | 0 | 1 |
| Typ 3 | 4 | 1 | 5 |
| ∑ | 23 | 5 | 28 |

* For one serum it was not possible to classify it without doubt to type 1 or type 2

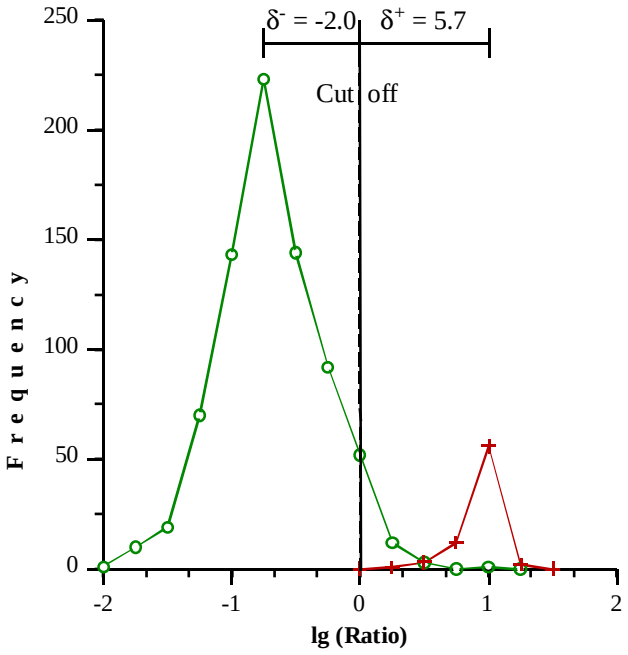


Figure 2: Frequency distributions of the HCV negative (-) and positive (+) populations based on the logarithm of the ratio (signal/cut off).

of 98%, a sensitivity of 100% and an accuracy of 98% . A rate of 1.5% (13/854) repeatedly false positive reactions (Table 3) in our screening assay showed the importance of supplemental assays also for the diagnosis of HCV. The possibility of cross-reactions with other flaviviruses like Dengue and Yellow Fever Virus resulting in false-positive reactions in HCV tests can be excluded by the findings of Yoshida et al. [53]. Until now, there are no other confirmation assays available which could improve the possibilities of HCV diagnosis and epidemiology [5, 6, 25]. Also, with PCR as a confirming assay a reasonable number of HCV infected individuals will remain PCR negative but positive in HCV serology although they show all clinical signs of the disease. But with screening assays alone the prevalence of HCV in a population will be overestimated [3, 8, 20].

# Disclaimer

The views expressed in this report are those of the authors and are not necessarily also held by the institute. Use of trade names is for identification only and does not imply endorsement by the INCQS.

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