ORIGINAL RESEARCH ARTICLE

# Prevalence of hepatitis C virus among users attending a voluntary testing centre in Rio Grande, southern Brazil: predictive factors and hepatitis C virus genotypes

**F N Germano** MSc*, **C A dos Santos** BSc*, **G Honscha** BSc†, **A Strasburg** BSc‡, **B Gabbi** BSc‡, **R A Mendoza-Sassi** MD PhD§, **E A Soares** MSc PhD**, **H N Seuánez** MD PhD**††, **M A Soares** MSc PhD**†† and **A M B Martínez** MSc PhD*

*Departamento de Patologia, Fundação Universidade Federal do Rio Grande, AV. General Osório S/N, Centro 96200-400 Rio Grande; †Laboratório Municipal de Análises Clínicas de Rio Grande, Rio Grande; ‡Faculdade de Enfermagem; §Departamento de Medicina Interna, Fundação Universidade Federal do Rio Grande, Rio Grande, RS; **Programa de Genética, Instituto Nacional de Câncer; ††Departamento de Genética, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil

**Summary:** We estimated the prevalence of hepatitis C (HCV) infection and associated risk factors in 750 individuals attending the Voluntary Counseling and Testing Center of Rio Grande (VCT/RG), in Southern Brazil, and identified viral genotypes. Demographic data and risk factors for HCV transmission were also collected and analysed. Anti-HCV antibody-positive individuals were tested for HCV-RNA and genotyped by sequencing the 5′ untranslated region of the viral genome. Prevalence estimates of anti-HCV and HCV-RNA were 6% and 5.5%, respectively. We identified genotypes 1 (67%), 2 (2%) and 3 (31%); the latter was more prevalent than in other regions of Brazil. Anti-HCV prevalence in VCT/RG users was similar to previous reports. Age, previous blood transfusion, sexual orientation and injecting drug use were independent predictors of HCV infection. The presence of multiple risk factors was also associated with a higher risk for HCV infection. HCV genotype was not associated with any variable analysed in this study.

**Keywords:** HCV, genotype, prevalence, risk factors, southern Brazil

## INTRODUCTION

Infection with hepatitis C virus (HCV) accounts for a high proportion of chronic liver disease throughout the world[1] and is recognized as the leading cause of liver transplantation.[2] HCV infection is mostly asymptomatic, although as many as 85% of infected individuals fail to clear the virus and become chronic carriers, with a high risk of progression to cirrhosis and hepatocellular carcinoma.[3] There are many recognized risk factors for HCV acquisition; it is spread mainly by direct contact with contaminated human blood in high-risk groups, including injecting drug users (IDU), recipients of unscreened blood, haemophiliacs, patients undergoing dialysis and individuals with multiple sex partners engaging in unprotected sex. HCV prevalence varies among different regions of the world or among different risk groups. The World Health Organization has reported a global estimate of 170 million individuals chronically infected with HCV, with three to four million people newly infected every year.[4] The epidemiology of HCV infection in Latin American countries has not been fully understood. The population prevalence of HCV in Brazil has not been well established, but several localized studies suggest that 0.5–3.6% of the population is infected.[5–7]

**Correspondence to:** M A Soares
**Emails:** masoares@biologia.ufrj.br; masoares@inca.gov.br

HCV variants display considerable sequence divergence and are classified into six major genotypes (1–6), with several subtypes.[8] HCV genotypes show different geographic distributions worldwide, with genotypes 1, 2 and 3 being responsible for more than 90% of infections in North and South America, Europe and Japan.[9] Determination of HCV genotype prior to treatment is indicated as it provides valuable data for prognosis and follow-up of infected patients. Accurate HCV genotyping is also important for predicting sustained response to antiviral therapy because patients infected with genotypes 1 and 4 are less likely to respond to interferon-ribavirin treatment than patients with genotypes 2 and 3.[10] Furthermore, identification of HCV genotypes, subtypes and isolates constitutes an essential tool for understanding viral evolution and epidemiology.[8] Nucleic acid sequencing and phylogenetic analysis of the 5′ untranslated region (5′UTR) of the HCV genome is the gold standard method for genotyping.[11]

Serological tests for viral and other infectious diseases in Brazil are carried out in voluntary counselling and testing (VCT) centres, with screening for anti-HIV, syphilis, HCV and hepatitis B (HBV) antibodies offered by the public health system within counselling and orientation programmes. The city of Rio Grande, in the state of Rio Grande do Sul (southern Brazil), comprises a harbour, a seaside resort and a university and has a shifting population with high rates of drug addiction and prostitution. In this study, we report the prevalence of HCV and risk factors for acquiring infection in individuals

attending the VCT Center of Rio Grande (VCT/RG). Additionally, we determined HCV genotypes by sequence analysis of the viral 5′UTR.

## METHODS

### Subjects and samples

This study included VCT/RG users tested for anti-HCV between March and August 2007. Eligibility criteria included age over 17 years and agreement to answer a standardized questionnaire based on the one developed by Metzger *et al.*[12] and later adapted to the Brazilian Portuguese language by Pechansky *et al.*[13] It contains variables on sociodemographic characteristics as well as questions on risk behaviours and practices relevant to sexually transmitted infections (STIs) including HCV. Participation in the study was voluntary and confidentiality was guaranteed.

Informed consent was obtained from a total of 750 individuals and these participants were interviewed. Venous blood samples were collected into ethylenediaminetetraacetic acid tubes and HCV serology was carried out by enzyme immunoassay (EIA) at the VCT laboratory. Plasma was aliquoted and sent to the Laboratory of Molecular Biology of the Fundação Universidade Federal de Rio Grande (FURG) and stored at $-70^{\circ}$C. EIA-positive samples ($n = 45$) were thawed and analysed for HCV-RNA. Finally, HCV-RNA-positive samples were sent to the Division of Genetics of the Instituto Nacional de Câncer for sequencing. This project was approved by the FURG Ethics Committee under number 5744/6.13.

### Viral RNA extraction, reverse transcriptase polymerase chain reaction and DNA sequencing

Viral RNA was extracted from 140 μL of plasma with the QIAamp® Viral RNA extraction kit (Qiagen, Chatsworth, CA, USA) and precipitated with 100% isopropanol, Dextran T500® (1 μg/μL) and 70% ethanol at $-20^{\circ}$C. Viral RNA was resuspended in a final volume of 10 μL of diethylpyrocarbonate-treated water with 300 ng of random primers (Invitrogen, Carlsbad, CA, USA) and incubated for ten minutes at 70°C. Reverse transcription was carried out with 200 U of Moloney Murine Leukemia Virus reverse transcriptase (M-MLV RT; Invitrogen), 0.1 mol/L DTT, 25 U of RNaseOUT® (Invitrogen) and 0.5 mmol/L of each dideoxynucleotide for 90 minutes at 37°C.

Nested polymerase chain reaction (PCR) assays were performed with primers PTC1 (5′-CGTTAGTATGAGTGTCG TGC-3′) and NCR2 (5′-ATACTCGAGGTGCACGGTCTACGA GACCT-3′) in the first round, and with PTC3 (5′-GTGTCGTG CAGCCTCCAGG-3′) and NCR4 (5′-CACTCTCGAGCACCC TATCAGGCAGT-3′) in the second round, as previously described for reactions and cycling conditions.[14] PCR products of approximately 225 bp were further purified with Illustra™ GFX™ PCR DNA and Gel Band Purification (GE HealthCare, São Paulo, Brazil), and subsequently labelled with the ABI Prism® BigDye™ Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. Primers used in sequencing reactions were the same as in the second PCR round. Samples were run in an ABI PRISM® 377 automated DNA Sequencer (Applied Biosystems).

Sequence data were assembled and edited manually with SeqMan and EditSeq programs of the DNAStar Package (LaserGene Inc, Madison, WI, USA). Consensus sequences were aligned and compared with representative sequences of HCV genotypes retrieved from GenBank. HCV genotypes were assigned with the BioEdit Sequence Alignment Editor.[15] HCV genotype and subtypes were also analysed by BLAST and the NCBI HCV genotyping tool. All HCV sequences were deposited in GenBank (accession numbers pending).

### Statistical analyses

We estimated the mean and standard deviation of continuous variables and the proportion of categorical variables. HCV prevalence and 95% confidence intervals (CIs) were estimated. Bivariate analysis was performed by calculating the prevalence ratios and CIs of several factors and outcomes. In order to adjust for potential confounders, a multivariate analysis was performed using Poisson regression and prevalence ratios (PRs). This model was constructed with all factors studied herein using a hierarchical model with two levels.[16] In the first, more distal level we included the sociodemographic factors, and in the second more proximal level the risk factors associated with HCV transmission. Variables with $P \leq 0.05$ were retained for analysis in the next level and adjusted according to risk factors. A Wald test was performed to analyse significant associations. A linear trend test was performed with ordinal variables. For categorical variables without order, a Wald heterogeneity test was carried out. In all statistical analyses, $P \leq 0.05$ was adopted in a double/two-tailed test. Bivariate associations between HCV genotype and sociodemographic and behavioural variables were also investigated in the HCV-positive group. Genotype 2 was excluded from this analysis because this group consisted of only one patient. Statistical analyses included calculation of the 95% CI of genotypes 1 and 3, as well as a $\chi^2$ test for evaluating differences between genotype and variables. Analysis of age was carried out with the Student's *t*-test for unequal variances. Estimates of $P \leq 0.05$ in a two-tailed test were considered significant. All analyses were performed in Stata v.9.2.[17]

## RESULTS

The sample of 750 individuals comprised 56.4% women and the mean age was 35.7 years. With respect to education, 13% were illiterate and 44.6% had not completed secondary education. Sociodemographic characteristics of participants are shown in Table 1. Most of those interviewed reported at least one potential risk factor for HCV transmission.

Indeterminate serological results were found in 1.5% of individuals who were also HCV-RNA negative. Anti-HCV EIA revealed that 45 (6%; 95% CI = 4.3–7.7%) were repeatedly reactive (Table 1), prevalence being slightly higher in men (6.7%) than in women (5.4%). We found 23% of anti-HCV-positive participants to also be anti-hepatitis B core (HBc) EIA reactive, indicating previous infection with HBV, and 2% were HCV-HIV co-infected. The mean age of HCV-positive participants was 48.5 years (95% CI = 44.1–52.9), which was significantly higher than the mean age of HCV-negative individuals ($P = 0.001$). Detection of HCV-RNA in plasma confirmed infection in 42 (93%) individuals, giving an active HCV prevalence of 5.5% (95% CI = 3.9–7.3).

Table 1 **Sociodemographic profile of participants seen at VCT/RG**

| Characteristic | *N* (750) | Percentage |
|---|---|---|
| **HCV** | | |
| Positive | 45 | 6.0 |
| Negative | 705 | 94.0 |
| **Gender** | | |
| Female | 423 | 56.4 |
| Male | 327 | 43.6 |
| **Years of education** | | |
| <4 | 97 | 12.9 |
| 4–7 | 231 | 30.8 |
| 8–11 | 338 | 45.1 |
| ≥12 | 84 | 11.2 |
| **Blood transfusion** | | |
| Yes | 65 | 8.7 |
| No | 685 | 91.3 |
| **Previous surgery** | | |
| Yes | 274 | 36.5 |
| No | 476 | 63.5 |
| **Tattoos and/or piercing** | | |
| Yes | 183 | 24.4 |
| No | 567 | 75.6 |
| **Cocaine inhalation** | | |
| Yes | 119 | 15.9 |
| No | 631 | 84.1 |
| **Sexual orientation** | | |
| Homo/bisexual | 55 | 7.3 |
| Heterosexual | 695 | 92.7 |
| **Number of sexual partners*** | | |
| ≤1 | 443 | 59.1 |
| 2 | 160 | 21.3 |
| ≥3 | 147 | 19.6 |
| **Injecting drug use** | | |
| Yes | 29 | 3.9 |
| No | 721 | 96.1 |
| **Needle/syringe sharing** | | |
| Yes | 17 | 2.3 |
| No | 733 | 97.7 |
| **Previous STIs** | | |
| Yes | 58 | 7.7 |
| No | 692 | 92.3 |
| **Anti-HBc** | | |
| Positive | 11 | 1.5 |
| Negative | 749 | 98.5 |
| **Anti-HIV** | | |
| Positive | 3 | 0.4 |
| Negative | 747 | 99.6 |

VCT/RG = Voluntary Counseling and Testing Center of Rio Grande; HCV= hepatitis C virus; STI = sexually transmitted infection; HBc = hepatitis B core
*In the last year

Table 2 shows the crude and adjusted PRs for HCV infection according to sociodemographic characteristics. Multivariate analysis revealed a PR for blood transfusion of 2.09 ($P = 0.03$); participants reporting homo/bisexual relationships showed a higher PR (2.17; $P = 0.04$) than heterosexuals; and the PR for IDU was 3.6 ($P = 0.006$). Increasing age was also correlated with HCV acquisition ($P = 0.001$).

The majority of individuals (59.1%) reported exposure to one or to none of the risk factors, while approximately 5% reported exposure to four risk factors. Multivariate analysis indicated that the relative risk of HCV infection increased with the number of risk factors ($P = 0.007$; Table 3).

HCV genotyping was carried out in 39 (87%) of the HCV-RNA-positive individuals, with genotypes 1 (26/39; 67%), 2 (1/39; 2%) and 3 (12/39; 31%) identified. Among genotype 1 isolates, 7 were assigned to subtype 1a, 16 to subtype 1b and 3 to subtype 1c. The genotype 2 isolate belonged to subtype 2b, while all 12 genotype 3 isolates were of subtype 3a.

The association of characteristics of HCV-positive individuals with the HCV genotypes was tested excluding genotype 2 ($n = 1$). Bivariate analyses did not show a statistical association among HCV genotypes and sociodemographic characteristics (data not shown). The mean age was similar in participants with genotypes 1 and 3. The association between HCV genotype and number of risk factors for HCV transmission was not significant ($P = 0.4$; data not shown).

## DISCUSSION

This study included individuals tested for anti-HCV antibodies at VCT/RG, the only public centre in the city of Rio Grande performing serological screening for STIs and HCV. According to a previous study, approximately 60% of VCT/RG attendees were women, with a mean age of 31 years and whose educational status varied from incomplete primary education (30.1%) to incomplete secondary education (45.7%).[18] Our study confirmed these estimates.

Possible limitations of this study include the potential underestimation of self-reported risk factors for HCV infection by interviewees, due to the stigmatization of certain risk behaviours and the illicit nature of drug use. Another important limitation relates to the generalization of data presented herein. The current study was performed at a voluntary testing centre, and therefore its conclusions can only be extended to similar settings and not to the general population. Finally, due to the study's cross-sectional design, no causal inference can be established.

Bearing in mind these limitations, we found an anti-HCV prevalence rate of 6% with an HCV-RNA prevalence of 5.5%. There are few published surveys on the prevalence of HCV infection in Brazil, as cases are not always reported to health authorities. The vast majority of data has been provided from analysis of specific populations or from risk groups with specific behaviours. Data from blood banks indicate that HCV infection varies among different Brazilian regions, 0.62% in the North, 0.55% in the Northeast, 0.28% in the Center-West, 0.43% in the Southeast and 0.46% in the South,[19] but few studies of VCT users have been reported. The HCV prevalence found in this study was similar to an earlier report carried out in VCT/RG (8.6%)[20] and to another study of VCT users from Fortaleza, in the State of Ceará (6.3%).[21] The higher anti-HCV prevalence of VCT attendees might result from heightened risk awareness or medical indication.

It is important to emphasize that several risk factors predispose to HCV infection, and that multiple risk factors may be present in an individual.[22] In our study, the risk for HCV infection increased significantly with the number of reported risk factors, probably because individuals at risk had been exposed repetitively over a long period time. Our study revealed that approximately 23% of anti-HCV-positive participants had evidence of previous infection with HBV, underlining common transmission risks for both infections, although previous VCT/RG data have shown that HBsAg prevalence is only 0.56%.[20] The low anti-HBc prevalence seen in our study is probably accounted for by the low prevalence of HBV infection in southern Brazil, contrary to the higher rates in the North and Northeast.[19]

In this study, age, blood transfusion, drug injection and sexual orientation were significantly associated with HCV

Table 2 **Crude (cPR) and adjusted (aPR) prevalence ratios for HCV infection according to sociodemographic characteristics ($n = 45$)**

| Variable | Prevalence | cPR | 95% CI | *P* | aPR | 95% CI | *P** |
|---|---|---|---|---|---|---|---|
| Age (years)† | – | 1.05 | 1.03–1.06 | 0.001 | 1.05 | – | 0.001 |
| **Gender** | | | | | | | |
| Female | 5.44 | 1.23 | 0.70–2.18 | 0.5 | 1.29 | 0.72–2.28 | 0.3 |
| Male | 6.73 | 1.00 | – | – | 1.00 | – | – |
| **Years of education** | | | | | | | |
| <4 | 10.31 | 1.00 | – | 0.03‡ | 1.00 | – | 0.9§ |
| 4–7 | 6.93 | 0.67 | 0.32–1.43 | – | 1.05 | 0.48–2.27 | – |
| 8–11 | 4.82 | 0.48 | 0.22–0.99 | – | 0.97 | 0.40–2.32 | – |
| ≥12 | 3.57 | 0.35 | 0.98–1.22 | – | 0.91 | 0.25–3.43 | – |
| **Blood transfusion** † | | | | | | | |
| Yes | 17.20 | 3.56 | 1.89–6.70 | 0.001 | 2.09 | 1.06–4.11 | 0.3 |
| No | 4.88 | 1.00 | – | – | 1.00 | – | – |
| **Surgery events** | | | | | | | |
| Yes | 8.03 | 1.71 | 0.96–3.03 | 0.06 | 1.07 | 0.60–1.95 | 0.8 |
| No | 4.69 | 1.00 | – | – | 1.00 | – | – |
| **Tattoos and/or piercing** | | | | | | | |
| Yes | 4.37 | 0.68 | 0.32–1.43 | 0.3 | 0.68 | 0.29–1.57 | 0.3 |
| No | 6.43 | 1.00 | – | – | 1.00 | – | – |
| **Cocaine inhalation** | | | | | | | |
| Yes | 8.40 | 1.54 | 0.79–3.05 | 0.2 | 2.08 | 0.86–5.06 | 0.1 |
| No | 5.42 | 1.00 | – | – | 1.00 | – | – |
| **Sexual orientation** † | | | | | | | |
| Homo/bisexual | 12.73 | 2.39 | 1.12–5.10 | 0.02 | 2.17 | 1.01–4.64 | 0.04 |
| Heterosexual | 5.33 | 1.00 | – | – | – | – | – |
| **No. of sexual partners in last year** † | | | | | | | |
| ≤2 | – | 1.00 | – | – | 1.00 | – | – |
| ≥3 | – | 0.99 | 0.55–1.77 | 0.9 | 1.06 | 0.82–1.38 | 0.6 |
| **Injecting drug use** † | | | | | | | |
| Yes | 17.30 | 3.17 | 1.35–7.45 | 0.008 | 3.60 | 1.44–9.00 | 0.006 |
| No | 5.40 | 1.00 | – | – | 1.00 | – | – |
| **Needle/syringe sharing** | | | | | | | |
| Yes | 17.65 | 3.14 | 1.07–9.15 | 0.03 | 1.81 | 0.71–4.57 | 0.2 |
| No | 5.64 | 1.00 | – | – | – | – | – |
| **STDs** | | | | | | | |
| Yes | 8.62 | 1.44 | 0.61–3.38 | 0.4 | 1.5 | 0.68–3.39 | 0.3 |
| No | 5.69 | 1.00 | – | – | – | – | – |

HCV = hepatitis C virus; STD = sexually transmitted disease
*Wald test
†Final model
‡Linear trend test
§Heterogeneity test

infection, the majority of VCT/RG users being represented by young adults and women. According to a previous study, 6.5% of VCT/RG users exhibited an HIV-associated risk behaviour (injecting drug use, sex work, *etc.*), while 12.5% had between five and 50 sexual partners/year.[18]

Several geographic and ethnic differences in the patterns of HCV infection, as well as temporal differences, have been reported.[23] Studies conducted in the USA showed that most infections occurred in adults of 30–50 years of age.[24] Conversely, the age-specific prevalence of HCV infection increased steadily with age in Turkey, Spain and Japan.[23] The mean age of the anti-HCV-positive individuals studied herein (48.5 years) was coincident with a previous report indicating an age-specific prevalence between 30 and 60 years in Brazil.[25] Some risk factors and demographic characteristics might be interrelated, as is the case of individuals exposed to blood transfusions who are generally older than those exposed to injected drugs and unprotected sex.[19]

Table 3 **Crude (cPR) and adjusted (aPR) prevalence ratios for HCV infection according to the number of risk factors for HCV* ($n = 750$)**

| | cPR | 95% CI | *P* | aPR | 95% CI | *P* |
|---|---|---|---|---|---|---|
| **No. of risk factors** | | | | | | |
| 1 | 1.00 | – | – | 1.00 | – | – |
| 2 | 2.65 | 1.33–5.24 | 0.005 | 2.73 | 1.37–5.44 | 0.004 |
| 3 | 2.93 | 01.32–6.49 | 0.008 | 3.61 | 1.64–7.94 | 0.001 |
| 4 | 2.72 | 0.83–8.91 | 0.09 | 3.71 | 1.24–11.11 | 0.01 |
| 5 | 5.80 | 0.93–35.92 | 0.05 | 7.92 | 1.78–35.24 | 0.007 |

HCV = hepatitis C virus
*Adjusted for age, sex and education level

Several risk factors related to behavioural and cultural issues are involved in HCV infection apart from blood transfusions carried out before 1992.[26] These include sharing needles and syringes among IDU, exposure to multiple sex partners, tattooing and piercings, HIV-positive individuals, haemodialysis patients, haemophiliacs and prison inmates. Injecting drug use has been the predominant mode of transmission in many developed countries.[27,28] In Brazil, studies showed that HCV prevalence rate among IDU ranged from 53% to 84%,[29,30] although a recent report showed a drastic decline in the city of Rio de Janeiro,[31] probably resulting from behavioural

changes in recent years. The anti-HCV prevalence among VCT users who reported to be IDU (17.3%) was similar to this latest report, but this estimate might be higher in an IDU population.

HCV infection has also been associated with other types of exposures such as cocaine inhalation and blood contact resulting from frequent razor sharing.[22] Although sexual practices appear to play a minor role in HCV transmission, an association between HCV infection and sexual orientation (homo/bisexual) was observed in the VCT/RG group. This was probably because a large number of homosexuals and bisexuals had multiple sexual partners, being more frequently exposed to STIs,[32] with a high risk of acquiring and transmitting HCV sexually. Another aspect that may contribute to HCV transmission in these populations is anal sex, which favours HIV and other STI transmission by blood contact during sexual intercourse.[33]

Serological and virological tests are essential for diagnosing and managing HCV infection, particularly when selecting treatment and assessing virological response to antiviral therapy.[34] Different markers can be detected: anti-HCV by EIAs and HCV-RNA by molecular testing, crucial when diagnosing acute and chronic hepatitis C.[10] The presence of HCV-RNA in the absence of anti-HCV antibodies is strongly indicative of acute HCV infection, eventually confirmed by anti-HCV seroconversion. Chronic hepatitis C is defined when both anti-HCV antibodies and HCV-RNA are present.[10,34] Some participants in this study, with positive anti-HCV and negative HCV–RNA, should be tested on a second occasion since HCV-RNA can be temporarily undetectable due to a transient and partial control of viraemia before chronic infection is established.[35] The presence of anti-HCV and absence of HCV-RNA is generally observed in individuals who have recovered from a past HCV infection, but this cannot be distinguished from a false-positive EIA result, the exact prevalence of which is unknown.[34]

Clinical laboratories usually perform HCV genotyping in view of its relevance for predicting response to treatment.[36] The 5′UTR has been used historically as a target for viral genotyping, being one of the best characterized viral genomic regions, and for which there are several commercially available test kits.[37] Although 5′UTR is sufficiently variable for identifying HCV genotypes, it cannot always accurately characterize HCV subtypes,[36–39] although subtyping is apparently irrelevant to clinical outcome.[11] The genotype distribution was similar to those shown previously in Brazil, with a preponderance of genotypes 1 and 3.[14,40] Several studies have reported that genotype 3 has a higher prevalence in the South of Brazil, showing that it is common in this region.[14,41]

In summary, this study showed that HCV prevalence in VCT/RG attendees was similar to those reported in previous studies and that the presence of multiple risk factors was associated with increased HCV infection. With respect to molecular diagnosis, HCV 5′UTR was useful for HCV genotyping. These data are valuable to public health and should contribute to the general HCV molecular epidemiology in Brazil and other countries of South America with which the state of Rio Grande do Sul shares borders and intense commercial relations.

#### ACKNOWLEDGEMENTS

We are indebted to the staff from the Voluntary Counseling and Testing Center of Rio Grande (VCT/RG) and from the Rio Grande City Health Office for participating in the study by providing testing and counselling to the patients herein studied. Work was supported by the Brazilian Ministry of Health (CSV 107/06) to MAS and by the Brazilian Ministry of Education (CAPES 199/052) to AMBM.

#### REFERENCES

1 Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. *Lancet Infect Dis* 2005;**5**:558–67
2 Moghaddam SM, Alavian SM, Kermani NA. Hepatitis C and renal transplantation: a review on historical aspects and current issues. *Rev Med Virol* 2008;**18**:375–86
3 NIH Consensus Statement on Management of Hepatitis C: 2002. *NIH Consens State Sci Statements* 2002;**19**:1–46
4 Sy T, Jamal MM. Epidemiology of hepatitis C virus (HCV) infection. *Int J Med Sci* 2006;**3**:41–6
5 Aquino JA, Pegado KA, Barros LP, Machado LF. Seroprevalence of hepatitis B virus and hepatitis C virus infections among individuals in the State of Para. *Rev Soc Bras Med Trop* 2008;**41**:334–7
6 Fagundes GD, Bonazza V, Ceretta LB, Back AJ, Bettiol J. Detection of the Hepatitis C virus in a population of adults. *Rev Lat Am Enfermagem* 2008;**16**:396–400
7 Nascimento MC, Mayaud P, Sabino EC, Torres KL, Franceschi S. Prevalence of hepatitis B and C serological markers among first-time blood donors in Brazil: a multi-center serosurvey. *J Med Virol* 2008;**80**:53–7
8 Kuiken C, Simmonds P. Nomenclature and numbering of the hepatitis C virus. *Methods Mol Biol* 2009;**510**:33–53
9 Dehesa-Violante M, Nunez-Nateras R. Epidemiology of hepatitis virus B and C. *Arch Med Res* 2007;**38**:606–11
10 Pawlotsky JM. Use and interpretation of virological tests for hepatitis C. *Hepatology* 2002;**36**:S65–73
11 Hnatyszyn HJ. Chronic hepatitis C and genotyping: the clinical significance of determining HCV genotypes. *Antivir Ther* 2005;**10**:1–11
12 Metzger D, Dephilippis D, Druley P. The impact of HIV testing on risk for AIDS behaviors. In: Harris LS, ed. *Problems of Drug Dependence*. Washington DC: National Institute on Drug Abuse, 1992:297–8
13 Pechansky F, Metzger D, Hirakata V. Adaptação e validação de um questionário sobre comportamento de risco para AIDS em usuários de drogas em Porto Alegre. *Rev Bras Psiquiatr* 2002;**24**:130–6
14 Campiotto S, Pinho JR, Carrilho FJ, *et al.* Geographic distribution of hepatitis C virus genotypes in Brazil. *Braz J Med Biol Res* 2005;**38**:41–9
15 Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* 1999;**41**:95–8
16 Victora CH, Fuchs S, Olinto S MT. The role of conceptual frameworks in epidemiological analysis: a hierarchical approach. *Int J Epidemiol* 1997;**26**:224–7
17 STATA. Stata Statistical Software: Release 9.2. College Station, TX: Stata Corp LP, 2006
18 Germano FN, da Silva TM, Mendoza-Sassi R, Martinez AM. High prevalence of users who did not return to the testing and counseling center (TCC) for knowing their serological status: Rio Grande, RS, Brazil. *Cien Saude Colet* 2008;**13**:1033–40
19 Ministério da Sańde Brasileiro. Hepatites Virais. Secretaria de Vigilância em Saúde. Brasília, 2005
20 Moraes E, Honscha G, Silva PEA, *et al.* Estudo sorológico das hepatites B e C na cidade do Rio Grande no período 2002–2005. *Braz J Infect Dis* 2005;**9**:S43
21 Mal A, Aar S, Mar D. Hepatites B e C em usuários do Centro de Testagem e Aconselhamento (CTA) de Fortaleza-Ceará. *DST – J Bras Doenças Sex Transm* 2006;**18**:161–7
22 Yee LJ, Weiss HL, Langner RG, *et al.* Risk factors for acquisition of hepatitis C virus infection: a case series and potential implications for disease surveillance. *BMC Infect Dis* 2001;**1**:8
23 Wasley A, Alter MJ. Epidemiology of hepatitis C: geographic differences and temporal trends. *Semin Liver Dis* 2000;**20**:1–16
24 Brown RS Jr., Gaglio PJ. Scope of worldwide hepatitis C problem. *Liver Transpl* 2003;**9**:S10–3
25 Brandao AB, Fuchs SC. Risk factors for hepatitis C virus infection among blood donors in southern Brazil: a case-control study. *BMC Gastroenterol* 2002;**2**:18
26 Khaja MN, Madhavi C, Thippavazzula R, *et al.* High prevalence of hepatitis C virus infection and genotype distribution among general population, blood donors and risk groups. *Infect Genet Evol* 2006;**6**:198–204

27 Alter MJ. Epidemiology of hepatitis C virus infection. *World J Gastroenterol* 2007;**13**:2436–41
28 Esteban JI, Sauleda S, Quer J. The changing epidemiology of hepatitis C virus infection in Europe. *J Hepatol* 2008;**48**:148–62
29 Caiaffa WT, Proietti FA, Carneiro-Proietti AB, *et al.* The dynamics of the human immunodeficiency virus epidemics in the south of Brazil: increasing role of injection drug users. *Clin Infect Dis* 2003;**37** (Suppl. 5):S376–81
30 Marchesini AM, Pra-Baldi ZP, Mesquita F, Bueno R, Buchalla CM. Hepatitis B and C among injecting drug users living with HIV in Sao Paulo, Brazil. *Rev Saude Publ* 2007;**41**(Suppl. 2):57–63
31 Oliveira ML, Yoshida CF, Telles PR, *et al.* Trends in HCV prevalence, risk factors and distribution of viral genotypes in injecting drug users: findings from two cross-sectional studies. *Epidemiol Infect* 2009;**137**:970–9
32 de Andrade SM, Tamaki EM, Vinha JM, *et al.* Vulnerability of men who have sex with men in the context of AIDS. *Cad Saude Publica* 2007;**23**:479–82
33 Carret ML, Fassa AG, da Silveira DS, Bertoldi AD, Hallal PC. Sexually transmitted diseases symptoms in adults: prevalence and risk factors. *Rev Saude Publ* 2004;**38**:76–84
34 Chevaliez S, Pawlotsky JM. Hepatitis C virus serologic and virologic tests and clinical diagnosis of HCV-related liver disease. *Int J Med Sci* 2006;**3**:35–40
35 Lavillette D, Morice Y, Germanidis G, *et al.* Human serum facilitates hepatitis C virus infection, and neutralizing responses inversely correlate with viral replication kinetics at the acute phase of hepatitis C virus infection. *J Virol* 2005;**79**:6023–34
36 Sandres-Saune K, Deny P, Pasquier C, *et al.* Determining hepatitis C genotype by analyzing the sequence of the NS5b region. *J Virol Methods* 2003;**109**:187–93
37 Cantaloube JF, Laperche S, Gallian P, *et al.* Analysis of the 5( noncoding region versus the NS5b region in genotyping hepatitis C virus isolates from blood donors in France. *J Clin Microbiol* 2006;**44**:2051–6
38 Hraber PT, Fischer W, Bruno WJ, Leitner T, Kuiken C. Comparative analysis of hepatitis C virus phylogenies from coding and non-coding regions: the 5(untranslated region (UTR) fails to classify subtypes. *Virol J* 2006;**3**:103
39 Tamalet C, Colson P, Tissot-Dupont H, *et al.* Genomic and phylogenetic analysis of hepatitis C virus isolates: a survey of 535 strains circulating in southern France. *J Med Virol* 2003;**71**:391–8
40 Vigani AG, Pavan MH, Tozzo R, *et al.* Comparative study of patients with chronic hepatitis C virus infection due to genotypes 1 and 3 referred for treatment in southeast Brazil. *BMC Infect Dis* 2008;**8**:164
41 Krug LP, Lunge VR, Ikuta N, *et al.* Hepatitis C virus genotypes in Southern Brazil. *Braz J Med Biol Res* 1996;**29**:1629–32

*(Accepted 8 April 2009)*