

# Prevalence of hepatitis C virus antibodies and genotypes in asymptomatic, first-time blood donors in Namibia

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Reported is the prevalence of hepatitis C virus (HCV) in Namibia as determined using a third-generation enzyme-linked immunosorbent assay (ELISA) on samples of blood collected from all asymptomatic, first-time blood donors between 1 February and 31 July 1997 ( $n = 1941$ ). The HCV seroprevalence was 0.9% (95% confidence interval (CI): 0.5–1.5%) and no associations were detected between a positive HCV serostatus and the person's sex, region of residence, or previous hepatitis B exposure or hepatitis B carrier status, as determined by hepatitis B surface antigen (HBsAg). The only significant association in a logistic regression model was an increase in HCV positivity with increasing age ( $P = 0.04$ ). Viral RNA was amplified from 2 out of 18 (11.1%) specimens that were ELISA positive. Genotyping of these specimens, by restriction fragment length polymorphism (RFLP), showed the presence of genotypes 5 and 1a. The positive predictive value of using HBsAg positivity as a surrogate screening marker for HCV in Namibian blood donors was poor (1.6%), with low sensitivity (16.7%) and specificity (89.3%), and detecting only 3 out of 18 serologically HCV-positive specimens. The results of this first study of the prevalence and epidemiology of HCV infection in Namibia suggest that donor blood should be screened for HCV by ELISA in order to prevent the transmission of hepatitis C virus.

**Keywords:** blood donors; enzyme-linked immunosorbent assay; hepatitis C antibodies, diagnosis; Namibia; polymerase chain reaction.

Voir page 970 le résumé en français. En la página 971 figura un resumen en español.

## Introduction

Viral hepatitis is a major global public health problem (1). The discovery of hepatitis C virus (HCV) in 1989 ended a period of intensive research aimed at finding the agent responsible for 80% of transfusion-associated ("non-A, non-B") hepatitis cases (2, 3). HCV is transmitted primarily through blood or blood products or contact with infected tissue (blood transfusion, intravenous immunoglobulins, intravenous drug abuse and tissue transplantation). Other routes of HCV transmission have also been implicated (sexual, vertical and household contacts), which may account for a proportion of the sporadic cases associated with this agent (4). HCV is not as

infectious as hepatitis B virus (HBV), but as many as 80% of infected individuals can become chronically infected and risk serious long-term sequelae, including cirrhosis, liver failure and hepatocellular carcinoma (5–7).

Current treatment of HCV infection is not highly effective (8), and at least 90% of patients who need treatment are unable to afford it (8). A globally applicable and fully protective HCV vaccine is not available and would be difficult to develop (9). Public health interventions, therefore, continue to be the only effective method of preventing HCV infection (1, 10). These include screening of blood and blood products, effective use of universal precautions and contraceptive barrier methods, destruction of disposable needles, adequate sterilization of reusable materials such as syringes, and promotion of health education on HCV infection and its prevention (11).

Any strategy to prevent HCV infection must be based on accurate data, including information about its incidence and prevalence. Such information is, at present, lacking, particularly in developing countries (10). Recent prevalence estimates by WHO suggest that 3% of the world's population (ca 170 million people) are currently infected with HCV (10) and are therefore at risk of developing liver cirrhosis and/or liver cancer. HCV is encountered worldwide, with a relatively high prevalence in Japan and the Mediterranean region, where 0.5–1.5% of blood donors are HCV antibody-positive (12). In northern Europe,

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USA and Canada the prevalence among blood donors is lower, between 0.01% and 0.05% (5, 12).

HCV prevalence data from southern Africa are incomplete, since no surveys have been published for Angola and Namibia. Countries in southern and central Africa show a range of HCV prevalences from >10% (United Republic of Tanzania) to <1% (Botswana, Zambia and Zimbabwe) (7). In South Africa, the prevalence of HCV infection varies widely in different geographical areas and among ethnic groups within regions, ranging from 0.41% to 3.84% in published studies on blood donors (13–15). Marked differences have also been shown in South Africa between urban and rural populations, with a higher prevalence in the former (1.7%) than in the latter (0.9%) (15).

The high HCV prevalences in these countries suggest that the infection may also be important in Namibia. Furthermore, hepatitis B virus (HBV) infection, which is thought to be transmitted in the same way as HCV, is endemic in Namibia. The HBV carrier rate, as determined by the prevalence of hepatitis B surface antigen (HBsAg), is 14.8% among Namibian blood donors (16) and can be as high as 17% among rural adult males (17), which suggests that the prevalence of HCV may also be high. Hepatocellular carcinoma, which is associated with both HBV and HCV infection, is the fourth most common cancer in Namibia (18). Although the magnitude of the interaction between HBV and HCV in hepatocellular carcinogenesis is uncertain (19), there appears to be a considerable increase in hepatocellular carcinoma for patients infected with both viruses (20). The Namibian Blood Transfusion Service (NBTS) currently has no policy or guidelines for mandatory screening of blood and blood products for HCV, although there is routine screening for HBsAg.

The present study was carried out because there is currently no available estimate of the burden of HCV infection and no information on the circulating HCV genotypes in Namibia. Such information would be important for use in the design of future HCV vaccines for use in southern Africa and for the formulation of appropriate treatment protocols for individuals chronically infected with the virus.

## Materials and methods

### Study design and population

New blood donors in Namibia are recruited among groups and individuals using standard methods (16). The fixed age range for blood donors is 16–60 years; however, the majority of first-time donors are recruited from secondary schools and centres for tertiary education and are in the age group 16–35 years. The ratio of male to female donors is approximately 2:3. New blood donors must respond to a general health questionnaire and undergo an examination by an attending nurse to determine their

blood pressure, body weight, haemoglobin level, pulse rate and general physical status.

All voluntary, asymptomatic, first-time blood donors in Namibia were recruited into the study between 1 February and 31 July 1997 ( $n = 1941$ ). First-time donors were used because they represent a population that has not yet been screened for other parenterally transmitted infections such as HBV. New donors were included in the study only if they gave written, informed consent for their participation. No donors refused to participate. The donors were recruited from four “regions” — Windhoek, north-west, north-east, and central/south Namibia. The division of the country in this way was based on the location of blood collection points for the Namibian Blood Transfusion Service (NBTS) and does not correspond to district boundaries.

### Sample collection

All specimens of blood were collected by experienced NBTS staff during routine blood donation activities. The specimens were allowed to clot, and the serum was separated and divided into two halves. For each donor one half was retained by the NBTS for HBsAg, HIV and syphilis serology testing. The other half was aliquoted into coded tubes and sent at 4 °C by overnight courier to the National Institute for Virology (NIV), South Africa. The specimens were then stored at the NIV at –20 °C until tested in May 1998. Demographic information (age and date of birth, sex, and current address of the blood donor) on each coded specimen was also noted whenever available.

### Laboratory testing

**Serology.** The Murex anti-HCV (Version III) (Murex Diagnostics Laboratories, United Kingdom) third-generation, enzyme-linked immunosorbent assay (ELISA) was used to screen all the samples for antibodies to HCV. According to the manufacturers, this test has an improved sensitivity (99%) and specificity (99.9%), compared with earlier generation tests since it includes the core and the following nonstructural (NS) genome regions of HCV — NS3, NS4 and NS5. Testing was automated allowing a rapid and standardized analysis of the specimens, which were tested over a period of 10 consecutive days. The standard testing protocol, as specified by the manufacturer, was adhered to at all times.

Screening of each specimen for HBsAg was carried out at the NBTS on the aliquot retained in Namibia, using the Murex HBsAg GE 13 ELISA test (Murex Diagnostics Laboratories, United Kingdom). All the HBsAg-positive specimens identified in this way were then tested for other HBV markers at the NIV in order to establish whether these donors were chronic carriers or recently infected individuals in the recovery phase. Further screening of HBsAg-positive cases included tests for hepatitis B virus envelope antigen (HBeAg), hepatitis B envelope antibody (HBeAb), and hepatitis B core antibody IgG

and IgM (anti-HBc IgG and anti-HBc IgM, respectively). All these markers were tested using the appropriate radioimmunoassay kit (Abbott Laboratories, United Kingdom). Specimens found to be HBsAg-negative by the NBTS were only tested for one additional marker — hepatitis B core antibody IgG (anti-HBc IgG), to determine past exposure to HBV.

**Genotyping.** Extraction of HCV RNA, using 140 µl serum in Eppendorf tubes and the Qiagen Viral RNA kit (Qiagen, Hilden, Germany), was carried out on all specimens that were positive for HCV antibodies by ELISA. A combined reverse-transcription polymerase chain reaction (RT-PCR) was then carried out on each extract using 0.2-ml thin-walled tubes (reaction volume, 50 µl). The RT-PCR mixture contained final buffer concentrations of 10 mmol/l Tris-HCl (pH 8.3); 50 mmol/l potassium chloride and 1.5 mmol/l magnesium chloride (27). The other constituents of the reaction mixture were 0.2 mmol/l of each deoxynucleoside triphosphate (Boehringer Mannheim, Ingelheim, Germany); 2.5 units of Taq polymerase (Boehringer Mannheim); 28 units of ribonuclease inhibitor (Boehringer Mannheim); 5 units of avian myeloblastic reverse transcriptase (Boehringer Mannheim); and 20 picomoles of each outer primer.

RT-PCR was performed at 43 °C for 45 min, followed immediately by 35 cycles at 94 °C for 45 s, 50 °C for 45 s, and 72 °C for 60 s. A final extension at 72 °C for 10 min was also included. All of these steps were carried out in a commercially available cycler (Perkin-Elmer Geneamp 2400, Perkin-Elmer Corporation, Connecticut, USA) These conditions for cycling are modifications of a previously published method (22). For the second round of amplification, a 1-µl aliquot from the first reaction was transferred to 99 µl of PCR mix 2, containing the same concentration of reagents as the first round, but excluding ribonuclease inhibitor and avian myeloblastic reverse transcriptase. The conditions for the second round of the PCR were 30 cycles at 94 °C for 45 s, 50 °C for 45 s, and 72 °C for 60 s. All primers used were adapted from published sequences derived from the highly conserved 5'-noncoding region (21, 23, 24).

PCR products were detected in the following way. Both sets of PCR products underwent electrophoresis in 2% Nusieve GTG (FMC Bioproducts, Rockland, USA)/1% ultra pure agarose (Gibco BRL, Life Technologies, Paisley, United Kingdom), in TBE buffer (134 mmol/l Tris hydrochloric acid, pH 10.0; 68 mmol/l boric acid; 2.5 mmol ethylenediaminetetraacetic acid). The gel contained 0.5 µg/ml ethidium bromide and the products were viewed under ultraviolet light. The size marker used was Boehringer Mannheim DNA Marker V. The expected sizes of the PCR products were 296 and 252 base pairs for the outer and inner reactions, respectively.

Genotype determination was carried out using an established methodology (25). Briefly, digestion by restriction endonucleases was performed for 2 h

on 10 µl PCR product in the presence of 5 units each of (a) *RsaI* and *HaeIII*, (b) *MvaI* and *HinfI*, (c) *SrfI*, and (d) *MvuI* (all supplied by Boehringer Mannheim, Ingelheim, Germany). The digestion products were separated on 12% polyacrylamide gels run in TBE buffer. Following electrophoresis, the gel was stained in 0.5 µg/ml ethidium bromide solution and viewed under ultraviolet light. Electrophoretic types, as previously defined (25), were used to determine the genotype from the results of restriction fragment length polymorphism studies.

### Statistical analysis

Prevalences of HCV and HBV markers and 95% confidence intervals (CI) were calculated by each donor's region of residence, sex and age group. Univariate associations between variables were carried out using  $\chi^2$  tests. A logistic regression model was used to determine the significance of associations between age, sex, region and HCV positivity by adding a separate term for each stratification factor using SAS statistical software. The sensitivity, specificity, and positive and negative predictive values for HBsAg as a surrogate marker of HCV were calculated using standard formulae (26).

### Results

The characteristics of the study population and statistical tests of association for each demographic variable are shown in Table 1. The study sample ( $n = 1941$ ) comprised 42% males and 58% females. The seroprevalence of HCV was 0.9% (1.6% in males and 0.4% in females) and there was no significant difference in HCV seroprevalence between the sexes after adjustment for age and place of residence. The distribution of ages for the 1129 donors who had valid information on their age or date of birth (such data were not available for 812 donors) was skewed towards the youngest age group of 16–24 years (89.5%), because most new donors were recruited from universities and schools. The other three age groups (25–29 years, 30–34 years and  $\geq 35$  years) each contained 5.8%, 2.3% and 2.3% of first-time donors, respectively. The seroprevalence of HCV in the four age groups covered by the study was 0.2% (16–24 years group), 1.5% (25–29 years), 3.8% (30–34 years) and 0% (0/26) ( $\geq 35$  years); and a significant trend was found in relation to age after adjustment for place of residence and sex ( $P = 0.042$ ).

The majority of donors were from the north-west/west region (36.5%), followed by the central/south region (24.9%), Windhoek (21.7%) and the north-east/east (16.9%). Current addresses were not known for 1.4% of the sample. No significant association was demonstrated between the current place of residence and HCV positivity in either univariate ( $P = 0.824$ ) or multivariate testing ( $P = 0.922$ ).

The HBV carrier status was assessed using a single marker, HBsAg, in 1733/1941 subjects (data

Table 1. Description of the first-time Namibian blood donors tested, with the statistical significance of association between demographic variables and hepatitis C virus (HCV) positivity

Variable	No. of subjects	No. HCV positive	95% CI <sup>a</sup>	<i>P</i> trend <sup>b</sup>
<b>Sex</b>				
Male	816 (42.0) <sup>c</sup>	13 (1.6) <sup>c</sup>	0.89–2.78	0.578
Female	1125 (58.0)	5 (0.4)	0.16–1.09	
Total	1941 (100.0)	18 (0.9)	0.5–1.50	
<b>Age (years)</b>				
16–24	1011 (89.5)	2 (0.2)	0.03–0.79	0.042
25–29	66 (5.8)	1 (1.5)	0.08–9.27	
30–34	26 (2.3)	1 (3.8)	0.20–21.58	
≥35	26 (2.3)	0		
Unknown	812	14 (1.7)	0.98–2.95	
<b>Region</b>				
Windhoek	416 (21.7)	4 (0.9)	0.31–2.62	0.922
Central + south	476 (24.9)	3 (0.6)	0.16–1.99	
North-west + west	698 (36.5)	7 (1.0)	0.44–2.06	
North-east + east	323 (16.9)	4 (1.2)	0.39–3.36	
Unknown	28	0		
<b>Hepatitis B virus carrier status</b>				
HBsAg <sup>d</sup> positive	192 (11.1)	3 (1.5)	0.40–4.87	0.293
HBsAg negative	1541 (88.9)	15 (0.9)	0.57–1.64	
Unknown	208	0		
<b>Hepatitis B virus exposure<sup>e</sup></b>				
Positive	629 (69.9)	6 (0.9)	0.39–2.17	0.849
Negative	271 (30.1)	2 (0.7)	0.13–2.93	
Unknown	1041	10 (1.0)	0.49–1.82	

<sup>a</sup> 95% confidence interval.

<sup>b</sup> Two-sided multivariate *P* value.

<sup>c</sup> Figures in parentheses are percentages.

<sup>d</sup> Hepatitis B surface antigen.

<sup>e</sup> Hepatitis B surface and/or core antibody positive.

were missing for 10.7%); 11.1% of these individuals were positive and therefore classified as chronic HBV carriers. There was no significant association between HBsAg and HCV positivity in a multivariate analysis ( $P = 0.293$ ). The results of using HBsAg as a surrogate marker of HCV among first-time Namibian blood donors are shown in Table 2 (26). The sensitivity of HBsAg in detecting HCV was 16.7% (95% CI: 4.4–42.3) and specificity 89.3% (95% CI: 87.7–90.7); positive predictive value was 1.6% (95% CI: 0.4–5.1), and negative predictive value, 99.0% (95% CI: 98.3–99.4). In this scenario, HBsAg screening of blood donors would only effectively detect 3 out of 18 HCV-positive specimens, thereby allowing 15 infected donors (83%) to enter the blood donor pool.

Determination of any previous exposure to HBV included all individuals who were positive for hepatitis B surface antibodies and/or core antibodies, either anti-HBc IgG or IgM. Exposure data were missing for 1041 individuals. Of those individuals

tested for these HBV exposure markers, 69.9% (95% CI: 57.4–63.4) had previously been exposed to HBV and 30.1% (95% CI: 27.2–33.2) had not been exposed. Previous HBV exposure was again not significantly associated with HCV positivity in a multivariate analysis ( $P = 0.849$ ).

HCV genetic material was amplified from 2 out of 18 (11.1%) ELISA-positive specimens after repeated attempts to extract RNA from these specimens. Genotyping yielded two different genotypes, type 5 and 1a. The characteristics of these donors are as follows. The first donor (genotype 1a) was a male from the north-east/east region in the age group 16–24 years, and had previously been exposed to HBV infection but was currently not a chronic carrier of this infection. The second donor (genotype 5) was a female from the north-west/west area of Namibia and had also been previously exposed to HBV, but was not a carrier; the age of this individual was not available.

## Discussion

The 0.9% HCV seroprevalence found in this study in Namibia is comparable to the range of prevalences already published for other Southern African populations: 1.0–2.4% (1) and 0.41–3.84% (13, 14). However, since our subjects were young blood donors — about 50% were aged 16–24 years — they were not representative of the whole community in Namibia. Moreover, certain individuals were excluded because they are not allowed to donate blood, e.g. those aged <15 years or >60 years as well as persons showing high-risk behaviour or with a past history of jaundice. Community-based studies of HCV prevalence should therefore be carried out to obtain more accurate age-specific and overall estimates of HCV infection in Namibia.

No association was found between being HCV seropositive and the individuals' place of residence, previous HBV exposure, or HBV carrier status. Although a significant association was found between a person's sex and HCV positivity in the univariate analysis, this association was lost in the logistic regression model when all other variables were controlled for. The lifetime risk of HCV infection is generally considered to be the same for both sexes if all other risk factors and confounders are taken into account (4). The only significant association of HCV positivity, which was found in both the univariate and multivariate analyses, was with age group. This relationship of HCV positivity to age has been demonstrated also in other studies (4, 5, 12).

The urban–rural differences in HCV prevalence, which were identified in the South African studies (14, 15), were not obvious in our Namibian sample. Our classification of the study sample into regional groups was perhaps too crude because it was based on the current address of each participant. Also, blood donors in Namibia are recruited only in

the areas where the regional blood transfusion centres are located, and these places depend on the distance from Windhoek, where the main offices of the NBTs are situated. As a result, the population in remote rural areas of Namibia was under-represented in our study.

The majority of HCV-positive donors (15 out of 18) were not chronically infected with HBV; the remaining three were infected with both viruses. Previous studies to determine the relationship between these two viral hepatitis agents showed that circulating anti-HCV was found more often in HBsAg-negative than in HBsAg-positive patients with hepatocellular carcinoma (6, 27). This inverse relationship between HCV and HBV seropositivity has been demonstrated by *in vitro* work, which indicates that the HCV core protein can suppress HBV expression and encapsulation, thus decreasing HBV replication in a co-infected cellular model (28). Thus, co-infection of HBV carriers with HCV may suppress HBV replication, rather than act synergistically to increase the relative risk for chronic liver disease and hepatocellular carcinoma. However, there are no clinical data at present to support this hypothesis.

Viral nucleic acid was detected by PCR in only 2 out of 18 (11.1%) HCV-seropositive donors, i.e. in 0.10% (2/1941) of all donors. This poor yield is similar to the findings in two other studies — one where 0.35% of British blood donors had antibodies but only 5% of those with antibodies had viraemia (29), and a study of blood donors from the Western Cape Province, South Africa, where 0.41% were positive for antibodies but virus was detected in only 0.056% (13). With the improved sensitivity and specificity of third-generation ELISA tests for HCV, it is expected that on average at least 70–80% of ELISA-positive specimens will be positive by PCR (30, 31). The poor predictive value of serology for viraemia in our investigation may be partly due to the low prevalence of HCV in the population we studied. Also, the pattern of reactivity to specific epitopes in the test system may differ depending on the genotype present in the population being screened (32), particularly with the use of synthetic peptides in the ELISA test system. Assuming that the sensitivity and specificity provided by the ELISA test manufacturer were correct, and that the specimens found positive in the test were not false positives, the following problems need to be addressed. First, the PCR sensitivity may have been too low, or the specimens were unsuitable or had extremely low levels of viral RNA. The published literature and our experience with this PCR suggest that the first possibility is unlikely. However, the specimens may not have been appropriately stored for preservation of the viral nucleic acid, since they were kept at –20 °C from their arrival at the NIV to the time of testing (approximately 10–13 months later). Some donors may have retained reactivity to HCV antibodies after clearing the virus on recovery (33), or some may have had a mild disease with no viraemia at the time of the

Table 2. Relationship between hepatitis C virus (HCV) and hepatitis B surface antigen (HBsAg) positivity indicating the sensitivity, specificity, and positive and negative predictive values (PV) of HBsAg as a screening test for HCV

	HCV +ve	HCV –ve	Total
HBsAg +ve	3	180	183
HBsAg –ve	15	1503	1518
<b>Total</b>	<b>18</b>	<b>1683</b>	<b>1701</b>
Sensitivity (%)	16.7	(4.4–42.3) <sup>a</sup>	
Specificity (%)	89.3	(87.7–90.7) <sup>a</sup>	
Positive PV (%)	1.6	(0.4–5.1) <sup>a</sup>	
Negative PV(%)	99.0	(98.3–99.4) <sup>a</sup>	

<sup>a</sup> 95% confidence interval.

serological determination (34). It has been suggested that HCV viraemia may exist transiently and at very low levels in asymptomatic individuals, establishing them as low-level chronic healthy carriers (35) who may not have had detectable quantities of HCV nucleic acid.

Relatively few data exist on the genotypic characteristics of HCV in developing countries, particularly in sub-Saharan Africa (1, 7). Although the presence of novel sequences has previously been reported in sub-Saharan Africa (36), the correlates with severity of disease, pathology and potential vaccine development are currently unknown. The genotype results on the two Namibian donors, from whom viral genetic material was obtained (genotypes 1a and 5), suggest similar circulating genotypes in Namibia as in South Africa (37, 38). The low yield of genetic material for genotyping may also have been attributable to the less than ideal storage of these specimens. Further study involving groups at high risk for HCV infection, from whom HCV genetic material may be obtained more easily because of higher viral loads, will be needed for clear characterization of the genotypes in Namibia.

The high cost of anti-HCV screening of blood donors has certainly delayed its introduction in developing countries. The costs involved include the cost of the HCV ELISA tests as well as that resulting from having to discard donor blood due to false-positive results in the initial screening test. These false-positive units are usually not subject to confirmatory testing (recombinant immunoblot assays or PCR), which requires a high level of laboratory expertise and is too expensive to be carried out routinely within blood transfusion services in developing countries (39). Before the development of specific test systems for HCV, surrogate markers for post-transfusion hepatitis — elevated aminotransferase levels and hepatitis B virus anti-core antibody (anti-HBc) — were initially used in the USA and Europe to screen blood donor populations for non-A, non-B hepatitis. These surrogate markers reduced the incidence of post-transfusion hepatitis by 30–50% (40). In an attempt to decrease the cost of HCV screening in Namibian blood donors, we

examined the possibility of using HBsAg, which is currently routinely used on all donors to exclude HBV infection, as a surrogate marker for HCV infection in this population. Our results indicate that this marker exhibits poor sensitivity (16.7%), specificity (89.3%), and positive predictive value (1.6%) in detecting HCV-positive specimens. Thus, in the present study, if only HBsAg screening had been used, it would have allowed 15 out of the 18 ELISA HCV-positive samples to enter the blood donor pool. However, screening blood donors for human immunodeficiency virus (HIV) infection and other sexually transmitted diseases may exclude some new HCV-infected donors and this should be the subject of further research.

Certain modifications of the HCV ELISA screening systems have been proposed in order to decrease the cost of testing and make tests affordable by developing countries. These modifications include pooling of samples (31), testing only first-time donors with repeat anti-HCV testing on a rotational basis on every third or fifth donation (39), and using three consecutive ELISAs of different sensitivity and specificity beginning with the most sensitive and ending with the most specific test to confirm the result (5, 12).

Despite its limitations, this study is the first to describe the prevalence of HCV infection in previously unscreened, asymptomatic, first-time Namibian blood donors. The results suggest that since HCV infection is a potentially important health problem in Namibia, the introduction of routine screening of blood donors for HCV may be required. The limited evidence gathered so far suggests that the

different cost-saving methodologies of HCV testing of donors should be investigated in order to implement, ideally in the near future, universal screening of blood donors for HCV. ■

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Free and informed written consent was obtained from all the participants in this study. Clearance was given by the Committee for Research on Human Subjects and Ethics at the University of the Witwatersrand, Johannesburg, South Africa (protocol number M980334), and the human experimentation guidelines specified by this Committee were followed in the conduct of the clinical research. Permission to carry out the study was also obtained from the Ministry of Health in Namibia and the Namibian Blood Transfusion Service.

The views and opinions expressed in this work are those of the authors and do not necessarily reflect those of the Department of Health in South Africa or Namibia, the National Institute for Virology, or the Director of the National Institute for Virology.

### Résumé

#### Prévalence des anticorps dirigés contre le virus de l'hépatite C et génotypes du VHC chez des nouveaux donneurs de sang asymptomatiques en Namibie

Cette étude avait pour but de déterminer la séroprévalence des anticorps dirigés contre le virus de l'hépatite C (VHC) chez des nouveaux donneurs de sang asymptomatiques, en utilisant un titrage immuno-enzymatique (ELISA) de troisième génération vendu dans le commerce. Les sujets recrutés dans l'étude se situaient principalement dans la tranche d'âge des 16 à 35 ans. Nous n'avions pas de données sur la prévalence de cette infection en Namibie et actuellement les dons de sang n'y sont pas soumis au dépistage du VHC. Nous avons également examiné la relation entre la positivité pour le VHC et des paramètres tels que l'âge du donneur, le sexe, le lieu de résidence, le portage du virus de l'hépatite B (VHB) ou une exposition passée au VHB.

Globalement, la séroprévalence du VHC était de 0,9% (IC 95% : 0,5-1,5%), et aucune association n'a été observée entre la positivité pour le VHC et le sexe du donneur, la région de résidence et l'exposition passée au VHB ou l'état de porteur du VHB, ces derniers paramètres étant déterminés par la présence de l'antigène de surface de l'hépatite B (HBsAg). Une tendance à l'augmentation de la positivité pour le VHC avec l'âge ( $p = 0,034$ ) a été

notée, après ajustement multivarié sur le sexe et le lieu de résidence.

Nous avons aussi identifié les génotypes du VHC circulant en Namibie afin de rassembler les informations nécessaires pour l'ensemble de l'Afrique australe, ce qui permettrait une approche rationnelle du traitement par l'interféron et d'assurer que les génotypes circulant en Namibie, s'ils sont différents des autres, seront inscrits dans les programmes futurs de mise au point de vaccins anti-VHC destinés à l'Afrique australe. Nous avons pu procéder à l'amplification de l'ARN sur 2 échantillons sur 18 positifs en ELISA (soit 11,1%). L'analyse du polymorphisme de longueur des fragments de restriction (RFLP) pratiquée sur ces deux échantillons — provenant de donneurs du nord de la Namibie — a montré la présence des génotypes 5 et 1a. Ces résultats médiocres obtenus avec la PCR sont dus aux mauvaises conditions de stockage des échantillons, à la présence de génotypes rares, à des taux très faibles d'acide nucléique viral circulant chez les porteurs chroniques, et à une faible sensibilité de la PCR elle-même.

Compte tenu des difficultés financières auxquelles doivent faire face les services de transfusion sanguine dans les pays en développement, nous avons étudié la possibilité d'utiliser le dépistage de l'HBsAg, actuellement pratiqué en routine pour exclure les dons de sang positifs pour le VHB, en tant que marqueur de remplacement pour le VHC. La valeur prédictive positive de la positivité pour l'HBsAg comme marqueur de remplacement pour le VHC chez les donneurs de sang namibiens était très basse (1,6%), avec une faible sensibilité (16,7%) et une faible spécificité (89,3%), puisque ce marqueur ne permettait de retrouver que 3 donneurs parmi les 18 dont la sérologie VHC était

positive. D'après nos observations sur la prévalence du VHC et étant donné l'incapacité du dépistage de routine du VHB à détecter une infection par le VHC, nous recommandons d'envisager diverses méthodes de réduction des coûts afin de pouvoir mettre en œuvre un dépistage de routine du VHC dans toutes les unités de sang et de produits sanguins en Namibie.

Cette étude décrit pour la première fois la prévalence de l'infection à VHC en Namibie. Nous suggérons de réaliser des études en communauté pour obtenir des estimations de la prévalence du VHC qui soient représentatives de tous les groupes d'âges et déterminer quels sont les génotypes circulants.

## Resumen

### Prevalencia de anticuerpos contra el virus de la hepatitis C y genotipos del VHC en donantes de sangre noveles asintomáticos en Namibia

El objetivo de este estudio consistió en determinar la seroprevalencia de los anticuerpos contra el virus de la hepatitis C (VHC) en donantes de sangre noveles asintomáticos en Namibia, utilizando para ello una prueba comercial de inmunosorción enzimática (ELISA) de tercera generación. Las personas analizadas pertenecían principalmente al grupo de edad de 16-35 años. No se disponía de información sobre la prevalencia de esta infección en Namibia, país donde actualmente la sangre procedente de donantes no se somete a cribado para el VHC. También examinamos la relación entre la condición de VHC-positivo y el sexo del donante, su edad, su región de residencia y el estado de portador del virus de la hepatitis B o su exposición previa a la hepatitis B.

La seroprevalencia general del VHC fue de un 0,9% (IC95%: 0,5%-1,5%), sin ninguna asociación entre la seropositividad VHC y el sexo, la región de residencia y la exposición previa a la hepatitis B o el estado de portador del virus de ésta, determinado mediante el análisis del antígeno de superficie (HBsAg). Se observó cierta tendencia al aumento de la seropositividad VHC con el aumento de la edad ( $P = 0,034$ ) tras un ajuste multivariable para el sexo y el lugar de residencia.

También identificamos los genotipos del VHC circulantes en Namibia, a fin de reunir la información necesaria para toda la región de África meridional, lo que permitiría enfocar racionalmente la terapia con interferón y asegurar que los genotipos de Namibia, en caso de ser diferentes, se incluyan en los programas de diseño de vacunas anti-VHC que se lleven a cabo en el futuro en África meridional. Se obtuvo ARN vírico por amplificación a partir de 2 de las 18 muestras (11,1%) ELISA-positivas. La genotipificación de estas muestras en

función del polimorfismo de la longitud de los fragmentos de restricción (RFLP) mostró la presencia de los genotipos 5 y 1a. Las dos muestras procedían de donantes del norte de Namibia. Entre las razones del bajo rendimiento de la RCP cabe citar las deficientes condiciones de almacenamiento de las muestras, el carácter inhabitual de los genotipos, el bajo nivel de portadores crónicos, la baja concentración de ácidos nucleicos víricos circulantes y la escasa sensibilidad a la RCP.

Conocedores de las limitaciones financieras afrontadas por los servicios de transfusión en los países en desarrollo, investigamos el posible uso del HBsAg, empleado corrientemente de forma sistemática para descartar las unidades de sangre VHB-positivas, como un posible marcador indirecto del VHC. El valor predictivo positivo de la presencia de HBsAg como marcador indirecto de tamizaje del VHC entre los donantes de sangre de Namibia fue escaso (1,6%), con una sensibilidad (16,7%) y especificidad (89,3%) bajas, que sólo permitió detectar 3 de las 18 muestras VHC-positivas en la serología. Basándonos en nuestros datos de prevalencia y en la ineficacia de las pruebas sistemáticas de detección del VHB como medio de detección del VHC, recomendamos que se investiguen varios métodos de ahorro de costos para poner en práctica un cribado sistemático del VHC en toda la sangre y los productos sanguíneos en Namibia.

En este estudio se describe por primera vez la prevalencia de la infección por el VHC en Namibia. Proponemos que se lleven a cabo estudios comunitarios para calcular prevalencias del VHC que sean representativas de todos los grupos de edad de la comunidad y para describir los genotipos del VHC circulantes.

## References

1. Hepatitis C: global prevalence. *Weekly epidemiological record*, 1997, **72** (46): 341-344.
2. Choo Q-L et al. Isolation of a cDNA clone derived from a non-A, non-B hepatitis genome. *Science*, 1989, **244**: 359-362.
3. Kuo G et al. An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. *Science*, 1989, **244**: 362-364.

4. **MacDonald M, Crofts N, Kaldor J.** Transmission of hepatitis C virus: rates, routes and cofactors. *Epidemiological reviews*, 1996, **18** (2): 137.
5. **Sharara AI, Hunt CM, Hamilton JD.** Hepatitis C. *Annals of internal medicine*, 1996, **125**: 658–668.
6. **Tanaka K et al.** Hepatitis C and B in the etiology of hepatocellular carcinoma in the Japanese population. *Cancer research*, 1991, **51**: 2842–2847.
7. **Hopf U et al.** Long-term follow up of post-transfusion and sporadic chronic hepatitis non-A, non-B and frequency of circulating antibodies to hepatitis C virus antibodies (HCV). *Journal of hepatology*, 1990, **10**: 69–76.
8. **Bruix J et al.** Prevalence of antibodies to hepatitis C virus in Spanish patients with hepatocellular carcinoma and hepatic cirrhosis. *Lancet*, 1989, **2**: 1004–1006.
9. **Choo Q-L et al.** Vaccination of chimpanzees against infection by the hepatitis C virus. *Proceedings of the National Academy of Sciences of the USA*, 1994, **91**: 1294–1298.
10. Hepatitis C. *Weekly epidemiological record*, 1997, **72**(10): 65–72.
11. **Lavanchy D.** The threat to public health of hepatitis C. *Research in virology*, 1997, **148**: 143–145.
12. **Van der Poel CL, Cuypers HT, Reesnik HW.** Hepatitis C virus six years on. *Lancet*, 1994, **344**: 1475–1479.
13. **Tucker TJ et al.** Hepatitis C virus infection in volunteer blood donors from the Western Cape — comparison of screening tests and PCR. *South African medical journal*, 1997, **87**: 603–605.
14. **Ellis LA et al.** Prevalence of hepatitis C virus in South Africa: detection of anti-HCV in recent and stored serum. *Journal of medical virology*, 1990, **32**: 249–251.
15. **Abdool Karim SS, Tait DR.** Hepatitis C virus infection in urban and rural Natal/Kwazulu. *South African medical journal*, 1993, **83**: 191–193.
16. **Seidel K et al.** *The prevalence of serological markers for hepatitis B amongst first-time blood donors in Namibia — Baseline studies for immunisation.* Namibian Blood Transfusion Service and Department of Community Health, University of the Witwatersrand, 1997 (unpublished document).
17. **Botha JF et al.** Hepatitis B virus carrier state in black children in Ovamboland: role of perinatal and horizontal transmission. *Lancet*, 1984, **1**: 1210.
18. *Cancer deaths 1996–1997.* Namibian Cancer Registry, unpublished data, 1996, 1997.
19. **Tsukuma H et al.** Risk factors for hepatocellular carcinoma among patients with chronic liver disease. *New England journal of medicine*, 1993, **328**: 1797–1801.
20. **Chuang WL et al.** The role of hepatitis B and C viruses in hepatocellular carcinoma in a hepatitis B endemic area. *Cancer*, 1992, **69**: 2052–2054.
21. **Garson JA et al.** Enhanced detection by PCR of hepatitis C virus RNA. *Lancet*, 1990, **336**: 878–879.
22. **Quan CM et al.** High-performance liquid chromatography to assess the effect of serum storage conditions on the detection of hepatitis C virus by the polymerase chain reaction. *Journal of virological methods*, 1993, **43**: 299–307.
23. **Okamoto H et al.** Detection of hepatitis C virus RNA by a twostage polymerase chain reaction with two pairs of primers. *Journal of virological methods*, 1990, **43**: 299.
24. **Widell A et al.** Hepatitis C virus RNA in blood donor sera detected by the polymerase chain reaction: Comparison with supplementary hepatitis C virus antibody assays. *Journal of medical virology*, 1991, **23**: 458–462.
25. **Davidson F et al.** Variation in the hepatitis C virus 5' non-coding region: implications for secondary structure. *Journal of general virology*, 1995, **67**: 498–504.
26. **Galen RS, Gambino SR.** *Beyond normality: the predictive value and efficiency of medical diagnosis.* London, Churchill Livingstone, 1991.
27. **Saito I et al.** Hepatitis C virus infection is associated with the development of hepatocellular carcinoma. *Proceedings of the National Academy of Sciences of the USA*, 1990, **87**: 6547–6549.
28. **Shih CM et al.** Suppression of hepatitis B virus expression and replication by hepatitis C virus core protein in HuH7 cells. *Journal of virology*, 1993, **67**: 5823–5832.
29. **Mutimer DJ et al.** Hepatitis C virus infection in the asymptomatic British blood donor. *Journal of viral hepatitis*, 1995, **2**: 47–53.
30. **Courouce AM et al.** Significance of NS3 and NS5 antigens in screening for HCV antibody. *Lancet*, 1994, **343**: 853–854.
31. **Busch MP et al.** Re-instatement of donors who test false-positive in second-generation hepatitis C virus enzyme immunoassays should wait availability of licensed third-generation tests. *Transfusion*, 1994, **34**: 130–134.
32. **Garcia Z et al.** Evaluation of a pooling system for routine HCV screening of blood donors to lower the cost burden on blood banks in countries under development. *Journal of medical virology*, 1996, **49**: 218–222.
33. **Fong TL et al.** Clinical significance of hepatitis C viral RNA and its correlation to antibodies to structural HCV antigens in anti-HCV reactive patients with normal liver tests. *Journal of medical virology*, 1996, **49**: 253–258.
34. **Kolho E et al.** Clinical significance of hepatitis C antibodies in blood donors. *Journal of medical virology*, 1994, **43**: 129–134.
35. **Caporaso N et al.** HCV infection in symptom-free patients. *Research in virology*, 1997, **148**: 123–125.
36. **Oni AO, Harrison TJ.** Genotypes of hepatitis C virus in Nigeria. *Journal of medical virology*, 1996, **49**: 178–186.
37. **Smuts H, Kannemeyer J.** Genotyping of hepatitis C virus in South Africa. *Journal of clinical microbiology*, 1995, **33**: 1679–1681.
38. **Sithebe NP, Aspinall S, Smuts H.** Molecular epidemiology of hepatitis C at Ga-Rankuwa Hospital. *South African medical journal*, 1996, **12**: 1543–1545.
39. **Bird AR, Gibbs BJ.** Blood transfusion and hepatitis viruses. *South African medical journal*, 1994, **8**: 570–572.
40. **Donahue JG et al.** The declining risk of post-transfusion hepatitis C virus infection. *New England journal of medicine*, 1992, **327**: 369–372.