M. tuberculosis. Additional studies with all four drugs should be performed to confirm our results as well as to determine the critical concentration of drugs required, especially those of streptomycin and rifampin. If an automated system incorporating MGIT technology is made available, it may further enhance the performance.

References

1. Bloch AB, Gauthen G, Onorato I, et al. Nationwide survey of drug resistant-tuberculosis in the United States. JAMA 1994; 271: 665–71.
2. Frieden TR, Sterling T, Pablos-Mendez A, Kilburn JO, Cauthen GM, Dooley SW. The emergence of drug-resistant tuberculosis in New York City. N Engl J Med 1993; 328: 521–6.
3. Raviglione MC, Snider DE Jr, Kochi A. Global epidemiology of tuberculosis. Morbidity and mortality of a worldwide epidemic. JAMA 1995; 273: 220–6.
4. National Committee for Clinical Laboratory Standards. Antimycobacterial susceptibility testing for *Mycobacterium tuberculosis*; tentative standard. NCCLS Document M24-T. Wayne, Pennsylvania: NCCLS, 1995.
5. Doern GV. Diagnostic mycobacteriology: where are today? J Clin Microbiol 1996; 34: 1873–6.
6. Pfyffer GE, Welscher HM, Kissling P, et al. Growth Indicator Tube (MGIT) with radiometric and solid culture for recovery of acid-fast bacilli. J Clin Microbiol 1997; 35: 364–8.
7. Reisner BS, Gatson AM, Woods GL. Evaluation of Mycobacteria Growth Indicator Tubes for susceptibility testing of *Mycobacterium tuberculosis* to isoniazid and rifampin. Diagn Microbiol Infect Dis 1995; 22: 325–9.
8. Canetti G, Rist N, Grosset J. Mesure de la sensibilité du bacille tuberculeux aux drogues antibacillaires par la methode des proportions: methodologie, criteres de resistance, resultats, interpretations. Rev Tuberc Pneumol 1963; 27: 263–72.
9. Laszlo A, Rahman M, Raviglione M, Bustreo F, WHO/IUATLD Network of Supranational Reference Laboratories. Quality assurance programme for drug susceptibility testing of *Mycobacterium tuberculosis* in the WHO/IUATLD Supranational Laboratory Network: first round of proficiency testing. Int J Tuberc Lung Dis 1997; 1: 231–8.
10. Gonzalez N, Torres MJ, Palomares JC, Aznar J. Detection of isoniazid resistant *M. tuberculosis* strains by single strand conformation polymorphism analysis. Clin Microbiol Infect 1997; 3 (suppl 2): 268.
11. Gonzalez N, Torres MJ, Aznar J, Palomares JC. Characterization of mutations in the *rpoB* gene in rifampin-resistant *M. tuberculosis* strains. Clin Microbiol Infect 1997; 3 (suppl 2): 268.
12. Bergmann JS, Woods GL. Reliablility of Mycobacteria Growth Indicator Tube for testing susceptibility of *Mycobacterium tuberculosis* to ethambutol and streptomycin. J Clin Microbiol 1997; 35: 3325–7.
13. Kodsi SE, Walter SB, Stitt DT, Hanna BA. Rapid detection of MDRTB from culture using a novel susceptibility system [abstract C-115]. In: Program and abstracts of the 94th General Meeting of the American Society for Microbiology. Washington, DC: ASM, 1994: 510.
14. Palaci M, Ueki SYM, Sato DN, da Silva Telles MA, Curcio M, Matheus Silva EA. Evaluation of Mycobacteria Growth Indicator Tube for recovery and drug susceptibility testing of *Mycobacterium tuberculosis* isolates from respiratory specimens. J Clin Microbiol 1996; 34: 762–4.

Hepatitis C virus RNA (HCV RNA) and viral types in dialysis patients in Dakar, Senegal

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Patients undergoing renal dialysis are at increased risk of hepatitis C virus (HCV) infection, depending on the duration of treatment, extended exposure to transfusion and possible nosocomial transmission. The reported [1] incidence of HCV infection in dialysis units in southern Europe, Japan and the USA ranges from 10% to 30%, while in northern Europe the incidence is lower, ranging from 1% to 9%.

Information about HCV infections in the general African population is scant, with reported prevalence rates varying greatly in different countries, from more than 10% in the pygmy population in Cameroon to 0–1.5% in South Africa. Socio-economic factors as well as cultural tradition (such as cosmetic tattooing) are likely to be relevant to the observed variation. To our knowledge, no data are available for sub-Saharan Africa

on HCV infection in dialysis patients. The only report from South Africa describes a prevalence of 21% of HCV antibody (Ab) reactive patients confirmed with RIBA second generation among 103 subjects [2]. No data on HCV RNA and viral types were provided.

In Senegal, Sarr et al [3] presented data on the seroprevalence of HCV in 43 patients suffering from histologically confirmed chronic hepatitis C. In their series, reactivity rate was 14% versus 2% in the control group. Interestingly, indicating a possible synergistic action of hepatitis B virus (HBV) and HCV, double positivity was found only in chronic hepatitis C patients. In this study, a striking proportion of patients (41.9%) tested negative for both HBV and HCV. The authors suggested an etiologic role for other carcinogenic factors: environmental, chemical or viral.

Mbaye et al [1] investigated patients with chronic hepatitis (two subjects), cirrhosis (24 subjects) and hepatocellular carcinoma (41 subjects) admitted to the Hôpital Principal in Dakar. They confirmed the high circulation of HBV in Senegal (with 74.5% of the patients being HBV carriers and 20% among the controls) and the likely relevance of this virus in chronic liver diseases. Moreover, in 12 patients a double infection with hepatitis delta virus (HDV) was noticed. Unfortunately, no data on HCV were reported [4].

In our experience [4], anti-HCV Ab were found by third-generation ELISA in only five of 172 subjects belonging to the rural population of two villages in Senegal, and two of them were confirmed by supplementary test (RIBA 3.0—Ortho Diagnostics System, Raritan, New Jersey, USA). These subjects proved to be viremic by reverse transcription— polymerase chain reaction (RT-PCR). These data, obtained in a similar population with overlapping diseases in the same country, differ from those reported by Sarr et al [3] and by Coursaget et al [5] demonstrating a much lower incidence of HCV (1.4% versus 14% and 4.1%, respectively). These discrepancies, partly attributable to the better performance of the third-generation assay, require further studies to provide a satisfactory explanation.

To understand HCV prevalence better in Senegal, we decided to investigate the distribution of this virus in all Senegalese patients on maintenance hemodialysis by investigating their antibody status.

Twenty-two patients (21 males and one female) with kidney diseases (nephroangiosclerosis, 12; rapidly progressive glomerulonephritis, 4; indeterminate, 4; nephrotic syndrome, 1; chronic glomerulonephritis, 1) belonging to the upper middle class of the urban society of Dakar were enrolled in the present study.

The clinical features of the patients are shown in Table 1. Liver biopsy was not performed for ethical and medical reasons. The patients had a mean age of 47 years (38–65), their serum alanine aminotransferase (ALT) level, (mean and range of values) was 30 (11–60) IU/L, and their serum aspartate aminotransferase (AST) level (mean and range of values) was 30 (4–100) IU/L.

Time on hemodialysis did not appear to be a major risk factor for seroconversion or active viral replication. Dialysis treatment had a mean duration of 3.5 (range 1–6) years. The majority of subjects (18/22) had normal AST and ALT levels, except for four patients, two of whom were HCV RNA positive.

The patients were tested for HBsAg and only two (9%) were positive, whereas all sera (100%) were anti-HBc IgG positive and 10 of them were also anti-HBs positive. All sera, in 1-mL aliquots, were stored frozen at −80°C until use.

Screening for anti-HCV antibodies was performed by a third-generation enzyme immunoadsorbent assay (EIA 3.0—Ortho Diagnostics System).

Positive samples were confirmed by a second-generation recombinant immunoblot assay (RIBA II —Ortho Diagnostics System) according to the manufacturer's instructions.

In positive samples, evidence of viremia was sought by RT-PCR for HCV RNA (HCV Amplicor, Roche, Basel, Switzerland).

The RNA in all positive serum samples was typed by INNO-LIPA II assay (Innogenetics, NV, Antwerp, Belgium). The test allows the determination of up to six HCV genotypes and their subtypes. It is based on variations found in the 5′ untranslated regions (5′ UTR) of the different HCV genotypes. Biotin-labeled amplified products are hybridized to probes, on a strip, which gives a perfect sequence match at 50°C, with high specificity. The biotin group is incorporated by employing a 5′-biotinylated primer during amplification. After hybridization, streptavidin labeled with alkaline phosphatase is added and binds to any biotinylated hybrid previously formed. Following incubation with BCIP/NBT chromogen, a purple–brown color will develop on positive bands only when there is a perfect match. The reactivity of an amplified fragment with one or more bands on the strips allows recognition of different HCV genotypes.

Among the 22 patients examined, who were attending the two hemodialysis units in Dakar (Hôpital Le Dantec and Hôpital Principal), HCV Ab reactivity was found in 11 cases.

All results positive in the screening test were confirmed by RIBA II.

HCV RNA was detected in six of the 11 patients, a percentage positivity very close to that found by us in northern Italy. Dual infection (with HBV and HCV) was detected in one subject who was positive for HBsAg and HCV-RNA.

Table 1 Characteristics and virologic results of dialysis patients investigated at Hôpital Le Dantec and Hôpital Principal, Dakar, Senegal

	n	Mean	Range
No. tested	22		
Gender (male/female)	20/1		
Age (years)		46.8	38–65
AST (IU/L)		30.0	11–60
ALT (IU/L)		30.2	4–100
Pathology			
Nephroangiosclerosis	12 (54.6%)		
Rapidly progressive glomerulonephritis	4 (18.2%)		
Indeterminate nephropathy	4 (18.2%)		
Nephrotic syndrome	1 (4.5%)		
Chronic glomerulonephritis	1 (4.5%)		
Duration of dialysis (years)		3.5	1–6
Virologic results			
Ab anti-HCV positive	11 (50.0%)		
RIBA positive	11 (50.0%)		
HCV-RNA positive	6 (27.3%)		
Genotype HCV 2ac	3 (13.6%)		
Genotype HCV, unclassified	3 (13.6%)		

AST, aspartate aminotransferase; ALT, alanine aminotransferase.

The predominant genotype was 2ac. It was not possible to differentiate between the two subtypes with the commercially available method used. It was of special interest that typing was impossible, in the remaining three cases, suggesting the circulation in Senegal of new subtypes of known types, or hitherto unknown additional types. The virologic results are shown in Table 1.

To our knowledge this is the first report on HCV RNA detection and typing in the sera of all the patients on dialysis in an African country. In the two units examined, the epidemiology as measured by the percentage of patients infected (HCV Ab reactive) and the percentage with HCV viremia does not appear to differ significantly from what has been observed in European hospitals.

In this population, biochemical liver functions (ALT and AST) appear to be in the normal ranges. The use of these surrogate markers, which are certainly technically simpler and less costly to perform, appears unsuitable to detect viral replication and for liver disease activity.

The molecular epidemiologic data are of substantial interest. In this group of patients, type 2 is predominant; the pattern of types is completely different from that in Europe and Japan, where types 1a and 1b prevail. Additionally, a difference can be noted with the detection of types 4c and 5a in South Africa or type 4c (B)—4f (B), previously found in Zaire [6] and Gabon [7]. The presence of non-typeable strains is intriguing, and the possibility of new types circulating in Senegal as has been described in other regions of Africa, such as Gabon and Nigeria [8,9], makes sequencing of the HCV genome worth attempting.

Acknowledgments

This paper has been previously presented as a poster entitled 'HCV RNA e tipi virali in pazienti dializzati a Dakar, Senegal' at XXVI Congresso Nazionale Associazione Microbiologi Clinici italiani (AMCLI), Naples, 4–7 November 1997.

References

1. Mbaye PS, Renaudineau Y, Diallo A, et al. Maladies hepatiques chroniques et virus des hepatites B, C at D à l'Hôpital Principal de Dakar. Abstract Proc Quarantenaire Soc. médicale d'Afrique Noir de langue française Dakar 1996.
2. Cassidy MJ, Jankelson D, Becker M, Dunne T, Walzl G, Moosa MR. Prevalence of antibodies to hepatitis C virus at two hamodialysis units in South Africa. South Afric Med J 1995; 10: 996–8.
3. Sarr A, Sow AM, Diallo A, Cisse KA, Mendez V. Seroprevalence comparées du VHC et VHB au cours de l'hépatome. Abstract Proc. Quarantenaire Soc. médicale d'Afrique Noir de langue française Dakar, 1996.
4. Botta GA, Raphenon G. HCV in Africa: a review of the present knowledge. Alpe Adria Microbiol J 1998; 8(1): 1–19.
5. Coursaget P, Leboulleux D, Le Cann P, Bao O, Coll-Seck AM. Hepatitis C virus infection in cirrhosis and primary hepatocellular carcinoma in Senegal. Trans R Soc Trop Med Hyg 1992; 86: 552–3.
6. Bukh J, Purcell RH, Miller RH. Sequence analysis of the core gene of 14 hepatitis C virus genotypes. Proc Natl Acad Sci USA 1993; 91: 8239–43.
7. Stuyver L, Vanarnhem W, Wyseur A, Hernandez F, Delaporte E, Maertens G. Classification of hepatitis C viruses based on phylogenetic analysis of the envelope 1 and nonstructural 5b regions and identification of five additional subtypes. Proc Natl Acad Sci USA 1994; 91: 10134–8.
8. Mellor J, Holmes EC, Jarvis LM, Yap PL, Simmonds P, The International HCV Collaborative Study Group. Investigation of the pattern of hepatitis C virus sequence diversity in different geographical regions: implications for virus classification. J Gen Virol 1995; 76: 2493–507.
9. Jadoul M, van Ypersele de Strihou C. Viral hepatitis in dialysis patients. Forum 1994; 4(1): 36–41.