

Hepatitis C Virus Serotype-Specific Core and NS4 Antibodies in Injecting Drug Users Participating in the Amsterdam Cohort Studies

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In the present study, the RIBA HCV serotyping SIA was evaluated with a cohort of injecting drug users. Serotyping may be a rapid and cost-effective method of determining genotypes in cohort studies. In this study, hepatitis C virus (HCV) antibody-positive sera from a cohort of 331 chronically infected injecting drug users, of which 167 were coinfecting with human immunodeficiency virus (HIV), were serotyped by the RIBA HCV Serotyping SIA. Among the 331 specimens, serotype-specific antibodies were detected in 250 (sensitivity, 75.5%), in which serotype 1 was predominant (57.2%), followed by serotype 3 (26.8%). Among the 331 specimens, 164 were HIV negative, and serotype-specific antibodies were detected in 151 (sensitivity, 92.1%), in which serotype 1 was predominant (59.6%), followed by serotype 3 (33.8%). For a subset of 58 samples taken from 19 chronically infected HCV seroconverters with a mean follow-up of 5 years, serotypes were compared with genotypes, which were determined by a line probe assay (HCV LiPa) and by direct sequencing of the products obtained by nested PCR of the 5' untranslated region. Among the 58 samples with known genotypes, serotype-specific antibodies were detected in 38 (total sensitivity, 65.5%), with a specificity of 78.9%. Thirty of these serotypeable samples revealed a serotype that corresponded to the genotype in the 58 samples (total positive predictive value, 51.7%). Of the 58 samples, 23 were coinfecting with HIV, and when these were excluded, the total sensitivity increased to 76.5%, with a total specificity of 80.8% and a total positive predictive value of 61.8%. The serotyping assay showed a high total sensitivity (96.3%) for samples positive by HCV RIBA, version 3.0, with four bands. We conclude that the sensitivity of the RIBA HCV serotyping SIA is limited by the immunocompetence of the HCV-infected host. In general, samples from HIV-negative individuals containing genotype 1a had higher sensitivity, specificity, and concordance in the serotyping assay compared with genotyping, whereas samples containing genotype 3a were found to be more cross-reactive and untypeable. Therefore, the prevalence of genotypes other than genotype 1 could be underestimated if they are determined by serotyping, and improvements in specificity are recommended.

Since hepatitis C virus (HCV) was discovered and identified as the agent of parenterally transmitted non-A, non-B hepatitis (4), numerous isolates from different geographical origins have been cloned and sequenced. On the basis of the genetic variability of different strains, six HCV genotypes have been distinguished. Their prevalence differs, with 1a, 1b, 2a, 2b, and 3a being predominant in Western Europe and the United States and 1b, 2a, and 2b being predominant in Japan and Taiwan. HCV genotype 4 is most commonly found in the Middle East and Africa, genotype 5 is most commonly found in South Africa, and genotype 6 is most commonly found in Hong Kong (6, 13, 14). The six HCV genotypes have been associated with different histologic abnormalities in chronic hepatitis (6, 11, 16) and different responses to interferon therapy (1, 17). Several methods for HCV genotyping have been described, chiefly, reverse transcription-PCR of conserved regions of the HCV genome. The PCR products can subsequently be used in sequence analyses (3), in restriction fragment length polymorphism analysis (11), or in blot hybridization assays (line probe

assays [LiPas]) (15). These genotyping assays are complex and require proper handling and storage of specimens. Thus, PCR screening of large HCV-infected populations for the prevalence of genotypes is expensive and time-consuming, and serotyping may be a more rapid and cost-effective method of determining genotypes in cohort studies.

In the present study, a recently developed serotyping assay (Chiron RIBA HCV serotyping SIA) (5) was used to evaluate 331 samples from HCV-infected individuals confirmed to be positive for HCV by RIBA, version 3.0 (RIBA 3.0). A comparison was made between the sensitivity of the Chiron RIBA HCV serotyping SIA and the distribution of serotypes with different RIBA 3.0 band patterns, and serotypes were compared with genotypes by using 58 serial samples from a panel of 19 chronically infected HCV seroconverters.

MATERIALS AND METHODS

Participants. Injecting drug users (IDUs) were recruited from a cohort of drug users who live in Amsterdam, The Netherlands, and who have been participating since December 1985 in the Amsterdam Cohort Studies on human immunodeficiency virus (HIV) and AIDS (18). In March 1996, we selected 358 individuals who had been monitored for at least 3 years and who had been seen at least seven times. Among these 358 subjects, 19 HCV seroconverters with a mean follow-up of 5 years were identified as described elsewhere (2). All serum and plasma samples were initially stored at 4°C, then frozen at -20°C within 24 h of collection and handling and ultimately stored at -70°C.

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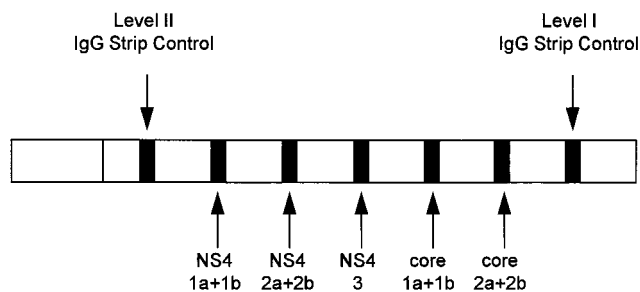


FIG. 1. Diagram of the Chiron RIBA HCV serotyping SIA strip.

Serological data. Sera were tested for the presence of antibodies to HCV by a third-generation enzyme immunoassay (EIA 3.0; Abbott Laboratories, Chicago, Ill.). All specimens positive by EIA 3.0 were confirmed by the third-generation strip immunoblot assay (RIBA; Chiron Corporation, Emeryville, Calif.). HCV seropositivity is confirmed only when at least two bands show reactivities of 1+ or more. Antibodies to HIV-1 were determined by a commercial EIA (Abbott Laboratories), and the results were confirmed by Western blotting (Diagnostic Biotechnology, Herent, Belgium). All serological assays were performed according to the instructions in the manufacturer's manual.

Serotyping analysis. The serotypes in RIBA-confirmed HCV-positive samples were determined by the Chiron RIBA HCV serotyping SIA 3.0. This assay is designed to detect type-specific antibodies against core and NS4-derived epitopes (5), using eight synthetic HCV-encoded peptides immobilized as bands on the test strips, with two lanes of high-level and low-level immunoglobulin G (IgG) as controls. Interpretation of the test results is based on comparison of the intensity of each peptide band with the intensities of both IgG internal control bands. All eight synthetic HCV-encoded peptides except for the serotype 1 core peptide are highly serotype specific; the serotype 1 core peptide shows significant cross-reactivity with serotype 3 samples. Five peptides are derived from serotype-specific sequences of the NS4 region of the genome, and three peptides are derived from the core regions of different HCV isolates. Synthetic peptides from consensus sequences from the NS4 region are present in band 1 (representing genotype 1a and 1b epitopes), band 2 (representing genotype 2a and 2b epitopes), and band 3 (representing genotype 3 epitopes). Band 4 contains a peptide derived from the consensus sequence of the core regions of genotypes 1a and 1b, while band 5 contains a peptide from the consensus sequence from the core regions of HCV genotypes 2a and 2b. The peptides are coated individually or in combinations of two peptides in each band on a strip (Fig. 1). The HCV serotype is determined primarily by a reactivity of 1+ or greater to one of the NS4 serotype-specific HCV peptide bands. HCV core peptide reactivity of 1+ or greater is used only in the absence of reactivity to NS4. If the greatest core peptide reactivity is to band 4, the serotype is either 1 or 3 due to cross-reactivity between the core regions of types 1 and 3. If the reactivity to band 5 (specific for antibodies to the core regions of genotypes 2a and 2b) is the highest and NS4 reactivity is absent, the serotype is 2. This assay is designed to discriminate between infections with HCV serotypes 1, 2, and 3, to determine seroprevalence, and to aid in the diagnosis. The test was performed according to the instructions in the manufacturer's manual.

Genotyping analysis. The genotypes in the 19 HCV seroconverters were determined either by the HCV LiPa protocol (Innogenetics, Ghent, Belgium) (15), according to the instructions in the manufacturer's manual, or by direct sequencing of the products obtained by nested PCR of the 5' untranslated region as described previously (2).

Statistical analysis. The Student *t* test was used for comparison of the prevalence of serotypes between different groups. A *P* value of <0.05 was considered significant.

Definitions. The definitions of sensitivity and positive predictive value are based on those in the American College of Physicians series on medical writing and communication (9).

(i) **Sensitivity.** Sensitivity is the ability of the serotyping assay to identify certain serotypes in individuals who are infected with HCV (number of serotypeable samples/total number of RIBA 3.0-positive samples).

(ii) **Specificity.** According to the American College of Physicians series on medical writing and communication, the specificity of a certain method gives the proportion of true negatives. We only tested by the serotyping assay samples confirmed to be positive for HCV by RIBA 3.0; therefore, we defined the specificity as the ability of the serotyping assay to identify the correct serotype in individuals whose sera were reactive by the serotyping assay compared with the known genotype (number of correct serotypeable samples/total number of serotypeable samples).

(iii) **Positive predictive value.** Positive predictive value is the ability of the serotyping assay to identify the correct serotype in individuals compared to the

total number of samples genotyped (number of samples with correct serotypes/total number of samples whose genotypes are known).

RESULTS

Identification of serotypes in specimens confirmed to be positive by RIBA 3.0. A total of 345 EIA 3.0-seropositive samples from chronically infected IDUs were obtained and analyzed by RIBA 3.0. The samples were considered to be confirmed to be positive by RIBA 3.0 if at least two bands showed a reactivity of 1+ or more. The results for nine samples were found to be indeterminate by RIBA 3.0, and the results for the other 336 samples could be confirmed to be positive by RIBA 3.0. We tested 331 of these 336 samples by the serotyping assay and found that the serotypes in 81 (24.5%) samples were untypeable. Type-specific antibodies were detected in the remaining 250 (75.5%) of the 331 samples and had the following distributions. Serotype 1 was found in 143 (57.2%) of the 250 serotype-positive samples, followed by serotype 3 in 67 (26.8%) samples and serotype 2 in 18 (7.2%) samples. A total of 22 (8.8%) samples showed cross-reactivity between serotypes 1 and 3.

Identification of serotypes in specimens confirmed to be positive by RIBA 3.0 with respect to HIV status. Among the 331 samples tested, 164 were HIV negative and 167 were HIV positive. Type-specific antibodies were detected in 151 (92.1%) samples from the HIV-negative individuals. The distribution was as follows. Serotype 1 was found in 90 (59.6%) of the 151 type-specific-antibody-positive samples, followed by serotype 3 in 51 (33.8%) samples and serotype 2 in 8 (5.3%). Only 2 (1.3%) samples showed cross-reactivity between serotypes 1 and 3. As expected, type-specific antibodies were detected in a lower number of the samples from the 167 HIV-positive individuals. Type-specific antibodies were detected in 99 (59.3%) of the 167 HIV-coinfected samples and had the following distribution. Serotype 1 was found in 53 (53.5%) samples, followed by serotype 3 in 16 (16.2%) samples and serotype 2 in 10 (10.1%) samples. A large proportion of samples ($n = 20$ [20.2%]) showed cross-reactivity between serotypes 1 and 3.

Correlation between serotype and genotype. Fifty-eight serial samples taken from 19 HCV seroconverters were both serotyped and genotyped (Table 1). Type-specific antibodies for types 1, 3, and 1 or 3 were found in 38 of 58 samples, revealing a sensitivity of 65.5%. Overall, 30 type-specific antibodies were found in the 38 typeable samples, showing a specificity of 78.9%. The overall positive predictive value of the serotyping assay was 51.7%. After separation of the 58 samples according to whether they were infected with genotype 1 or 3, the sensitivity, specificity, and positive predictive value of the serotyping assay were determined. For serum samples infected with genotype 1, the serotyping assay revealed a sensitivity of 63.2%, a specificity of 91.7%, and a positive predictive value of 57.9%. On the other hand, for serum samples infected with genotype 3, the serotyping assay showed a sensitivity of 68.4%, a specificity of 61.5%, and a positive predictive value of 42.1% (Table 2). Excluding all samples from HIV-seropositive individuals, we evaluated 34 samples which revealed a different result regarding sensitivity, specificity, and positive predictive value. Type-specific antibodies were found in 26 of the 34 samples, revealing a sensitivity of 76.5%. Overall, 21 type-specific antibodies were found in the 26 typeable samples, showing a specificity of 80.8%. The overall positive predictive value of the serotyping assay was 61.8%. For serum samples from these HIV-negative individuals infected with HCV genotype 1, the serotyping assay revealed a sensitivity of 86.7%, a specificity of 100%, and a positive predictive value of 86.7%

TABLE 1. Comparison of genotype with serotype as measured in serial samples from 19 HCV seroconverters

IDU	Genotype ^a	Serotype ^b	HIV status ^c
0073	1a (3), 1a (8), 1 (56)	S1, S1, S1	sc (46)
0146	3a (2), 3a (9)	S13, S13	neg
0245	1a (22), 1a (36)	UTY, UTY	pos
1020	1a (26), 1a (33), 1a (44), 1a (59)	S1, S1, UTY, UTY	pos
1083	1a (12), 1a (16), 1a (70), 1a (92)	S1, S1, UTY, UTY	pos
1085	1a (2)	S1	neg
1102	1a (5), 1a (23), 1a (73)	S1, S1, S1	neg
1114	1a (3), 1a (83)	UTY, S1	neg
1123	3a (2), 3a (26), 3a (57), 3a (73)	UTY, S3, S3, S3	neg
1213	3a (5), 3a (9), 3a (17), 1a (30), 1a (35)	UTY, UTY, UTY, S1, S1	sc (24)
1217	1a (2), 1a (18), 1a (30), 1 (41)	S1, S1, UTY, UTY	sc (2)
3006	1 (2), 1 (19), 3a (34)	UTY, S1, S1	neg
3009	3a (2), 1 (103)	S3, S1	neg
3059	3a (6), 3a (26), 3a (43)	S3, S3, S3	neg
5044	1b (2), 1b (23), 1b (46), 1b (75)	S1, S1, S13, S13	sc (17)
6048	1a (3), 1a (13), 1a (39), 1a (80)	UTY, UTY, UTY, UTY	pos
7030	3a (20), 3a (44)	UTY, S3	neg
7041	3a (4), 1a (29)	UTY, S1	neg
7095	1a + 3a (2), 1a (26), 3a (57), 3a (61)	S1, S1, S1, S1	sc (57)

^a Boldface indicates HIV-coinfected samples. Values in parentheses are times (in months) after HCV seroconversion.

^b S13, serotype 1 or 3; S1, serotype 1; S3, serotype 3; UTY, untypeable.

^c sc, seroconversion for HIV; neg, negative; pos, positive; values in parentheses are times (in months) before HIV seroconversion.

(Table 3). Of the 26 serotypeable samples, 5 (19.2%) revealed a serotype different from the genotype. Strikingly, four were found to be genotype 3. Of these, two samples contained a single genotype 3, which was identified as serotype 1 or 3. On the other hand, in two samples the genotype was completely different from the serotype: both were identified as serotype 1 but both contained genotype 3. One of these was infected with both genotypes 1 and 3, although it was identified as serotype 1. In total, eight samples remained untypeable. Of these, 2 of the 15 (13.3%) samples infected with genotype 1 failed to raise type-specific antibodies to NS4, whereas 6 of the 19 (31.6%) samples infected with genotype 3 failed to raise type-specific antibodies because NS4 was absent from 2 samples and both the core and NS4 epitopes were absent from the other four samples (data not shown).

TABLE 2. Comparison of serotyping and genotyping results for serial samples from 19 HCV seroconverters

Genotype (no. of samples)	No. of samples with the following serotype:				Sensitivity ^a	Specificity ^b	PPV ^c
	1	3	1 or 3	UTY ^d			
1 (38)	22	2	14	24/38 (63.2)	22/24 (91.7)	22/38 (57.9)	
3 (19)	3	8	6	13/19 (68.4)	8/13 (61.5)	8/19 (42.1)	
1 + 3 (1)	1						
Total (58)	26	8	4	38/58 (65.5)	30/38 (78.9)	30/58 (51.7)	

^a Sensitivity is depicted as number of serotypeable samples/total number of samples tested (percent).

^b Specificity is depicted as number of correct serotypes/total number of serotypeable samples (percent).

^c PPV, positive predictive value, which is depicted as number of correct samples with serotypes/total number of samples with known genotypes (percent).

^d UTY, untypeable.

TABLE 3. Comparison of serotyping and genotyping results for serial samples from 15 HCV seroconverters without HIV infection

Genotype (no. of samples)	No. of samples with the following serotype:				Sensitivity ^a	Specificity ^b	PPV ^c
	1	3	1 or 3	UTY ^d			
1 (15)	13		2		13/15 (86.7)	13/13 (100)	13/15 (86.7)
3 (18)	2	8	2	6	12/18 (66.7)	8/12 (66.7)	8/18 (44.4)
1+3 (1)	1						
Total (34)	16	8	2	8	26/34 (76.5)	21/26 (80.8)	21/34 (61.8)

^a Sensitivity is depicted as number of serotypeable samples/total number of samples tested (percent).

^b Specificity is depicted as number of correct serotypes/total number of serotypeable samples (percent).

^c PPV, positive predictive value, which is depicted as number of samples with correct serotypes/total number of samples with known genotypes).

^d UTY, untypeable.

Serotyping of specimens with different RIBA patterns. Table 4 shows the distributions of HCV serotypes with different RIBA HCV patterns. Serum samples with different RIBA 3.0 patterns were randomly selected and were compared with the serotypes found in the same sample by RIBA HCV serotyping SIA. Among 54 serum samples positive by RIBA 3.0 with four bands, 52 (96.3%) were successfully typed. Of these samples, the majority, 36 (66.7%), were found to be serotype 1, followed by 9 (16.7%) serotype 3 samples, 4 (7.4%) serotype 2 samples, and finally, 3 (5.6%) serotype 1 or 3 samples. Only 2 (3.7%) of the 54 samples were untypeable. Among the 35 samples positive by RIBA 3.0 with three bands, 22 (62.9%) were successfully typed. Of these samples, the majority, 13 (37.1%), were found to be serotype 1, followed by 4 (11.4%), 2 (5.7%), and 3 (8.6%) samples of serotypes 3, 2, and 1 or 3, respectively. A total of 13 (37.1%) samples were untypeable. Only 6 (35.3%) samples positive by RIBA 3.0 with two bands were found to be typeable. The distribution of serotypes was approximately equal, with 2 (11.8%) being serotype 1, 2 (11.8%) being serotype 2, 2 (11.8%) being serotype 3, and 1 (5.9%) being serotype 1 or 3. A total of 11 (64.7%) samples were untypeable. The same trend was found among the 58 samples with known HCV genotypes (Table 5).

DISCUSSION

Several methods for the genotyping of HCV have been described, and each has advantages and disadvantages. Reverse

TABLE 4. HCV serotype distribution for specimens with different RIBA banding patterns

RIBA 3.0 banding pattern (no. of samples)	No. of specimens with the following serotype (% sensitivity) ^a :					
	1	2	3	1 or 3	Total ^b	UTY ^c
Four bands positive (54)	36 (66.7)	4 (7.4)	9 (16.7)	3 (5.6)	52 (96.3)	2 (3.7)
Three bands positive (35)	13 (37.1)	2 (5.7)	4 (11.4)	3 (8.6)	22 (62.9)	13 (37.1)
Two bands positive (17)	2 (11.8)	2 (11.8)	2 (11.8)	1 (5.9)	6 (35.3)	11 (64.7)

^a Distribution of serotypes and sensitivity in comparison with the RIBA 3.0 pattern.

^b Total number of serotypes and sensitivity in comparison with the RIBA 3.0 pattern.

^c UTY, untypeable.

TABLE 5. HCV serotype distribution for specimens with concordant HCV genotypes with different RIBA 3.0 banding patterns

RIBA 3.0 banding pattern (no. of samples)	No. of specimens with the following serotype (% sensitivity) ^a :				
	1	3	1 or 3	Total ^b	UTY ^c
Four bands positive (12)	12 (100)	0 (0)	0 (0)	12 (100)	0 (0)
Three bands positive (27)	14 (51.9)	5 (17.2)	1 (3.7)	20 (74.3)	7 (25.9)
Two bands positive (19)	1 (5.3)	2 (12.5)	0 (0)	3 (15.8)	16 (84.2)

^a Distribution of serotypes and sensitivity (%) in comparison with the RIBA 3.0 pattern.

^b Total number of serotypes and sensitivity in comparison with the RIBA 3.0 pattern.

^c UTY, untypeable.

transcription-PCR and direct sequencing of HCV are considered to be definitive and the "gold standard," but they are impractical for routine use and the screening of large populations. Therefore, serotyping may be a more rapid, convenient, and cost-effective alternative for the screening of HCV genotypes in large populations of HCV-infected individuals.

In the present study, the RIBA HCV serotyping SIA was used to screen a cohort of 331 HCV-infected individuals, and the ability of the assay to detect type-specific antibodies to HCV types 1, 2, and 3 was evaluated. The serotypes of a relatively high proportion of the HCV isolates infecting anti-HCV-positive individuals (75.5%) could be determined, although the proportion was less than that in other studies (5, 10). Among the 250 HCV specimens that were serotyped, we found that the prevalence of serotype 1 was comparable to the prevalence found in a previous study by the same serotyping assay performed with a cohort of blood donors. However, findings differed slightly as to the distribution of serotypes 2 and 3, perhaps due to the difference in study populations (5, 12). A total of 81 (24.5%) samples were untypeable due to either the lack of antibody responses to the core and NS4 epitopes, the presence of genotype-specific antibodies not recognized by this serotyping assay, or immunosuppression caused by coinfection with HIV. Serotyping assays provide an indirect typing method based on the type-specific antibody production of the host. These assays are dependent on the immunocompetence of the infected host, and it is well known that HIV causes immunodeficiency. To find a potential explanation for the large number of untypeable samples, the whole cohort was separated with respect to HIV status. As expected, the majority of the untypeable samples in the whole cohort (68 of 81; 84.0%) were associated with HIV coinfection, with antibody responses to both core and NS4 lacking in 54 individuals. However, the other 14 had antibody responses to both the core and the NS4 antigens. Among the 13 untypeable samples from HIV-negative individuals, only 1 individual lacked antibody responses to both the core and the NS4. The other 12 untypeable samples had antibody responses to both the core and the NS4 (6 individuals) or to either the core (2 individuals) or NS4 (4 individuals). Thus, samples from a total of 26 (32.1%) HCV-infected individuals with antibody responses to the core and/or NS4 were untypeable regardless of their HIV status, probably implying that they were infected with HCV genotypes other than genotype 1, 2, or 3.

Our analysis of the correlation between serotypes and genotypes in 58 serial samples from 19 HCV seroconverters found that the serotyping assay had a relatively poor overall sensitivity and positive predictive value. The main problem was the number of untypeable samples (20 [34.5%]), of which 12 were

coinfected with HIV. The same analysis performed with serial samples from HIV-negative individuals revealed a slightly better overall result, and the sensitivity and the positive predictive value of the serotyping assay for samples from HIV-negative individuals were much higher, with the values being comparable to those found in other studies (5, 10). Of interest were the differences in sensitivity, specificity, and positive predictive value of the serotyping assay for samples from HIV-negative individuals infected with genotype 1 or 3. For all samples infected with genotype 1, the sensitivity, specificity, and positive predictive value of the serotyping assay were higher than the values for samples infected with serotype 3. A possible explanation for this finding might be found in the two samples harboring genotype 3a (samples from IDUs 3006 and 7095), which showed cross-reactivity with serotype 1. In IDU 3006, the initial HCV infection was caused by a genotype 1 isolate and serotype 1-specific antibodies were detected. The patterns of detection of HCV RNA fluctuated for this individual, but the subject remained EIA 3.0 positive during follow-up. After a period in which HCV RNA was not detected by PCR, reinfection (or coinfection) with genotype 3a occurred, and it may be that the serotype 3-specific antibodies were not detectable or were not raised at all in this sample. On the other hand, because of the indirect nature of serotyping, it may detect antibodies from a past and resolved infection. Interestingly, in individual 7095 the initial HCV infection was caused by both genotypes 1a and 3a, and on the basis of genotyping by the LiPa method, both genotypes were presumed to be present at an almost 1:1 ratio. However, only serotype 1-specific antibodies were detected, suggesting that this serotyping assay is more sensitive for the detection of genotype 1 or that antibody responses to genotype 1 are stronger than the responses to the other genotypes. Even after genotype 3a was later found in individual 7095, only serotype 1-specific antibodies were detectable. This intriguing phenomenon may be explained by the so-called clonal dominance and deceptive imprinting, as has been described for HIV (7, 8). HCV is known for its ability to produce a diverse spectrum of genotypes and variants, and this probably leads to the high rate of chronicity of HCV infections. Primary antibody responses to the genotype (or variant) causing the initial infections may also become clonally dominant in HCV infections, and recognition of antibodies to closely related HCV variants or genotypes is probably limited or suppressed. In total, among the samples from HIV-negative individuals, eight samples remained untypeable, and all these samples contained genotypes 1 ($n = 2$) and 3 ($n = 6$), genotypes which were supposed to be recognized in the serotyping assay. These data suggest that in some cases, the absence or presence of anti-core or anti-NS4 antibody is not always indicative or predictive for serotyping.

As expected, the reactivity in the serotyping assay was highly dependent on the number of positive bands found by RIBA 3.0. Significantly more samples positive by RIBA 3.0 with four bands than samples positive by RIBA 3.0 with three bands ($P < 0.01$) or two bands ($P < 0.001$) were positive by serotyping. Remarkably, the prevalence of serotype 1 was higher in samples with four positive bands than in those with fewer positive bands. A large proportion of samples positive by RIBA 3.0 with four bands were serotype 1 (66.7%), whereas smaller proportions were serotypes 2 and 3 (13%; $P < 0.001$). A decrease in reactivity by RIBA 3.0 showed a markedly decreased prevalence of serotype 1 ($P < 0.01$), whereas the prevalence of serotypes 2 and 3 was higher for samples positive by RIBA 3.0 with two bands. Thus, serotypes 2 and 3 seem to be less dependent on RIBA 3.0 band reactivity, a finding also suggested in other studies (5, 10). To determine whether these

findings were real with respect to the HCV genotype, the same analysis was done with a much smaller population of 58 samples with known HCV genotypes, and the same trend was observed. A possible explanation is that RIBA 3.0 is based on HCV genotype 1a antigens, and therefore, the absence of reactivity to particular bands by RIBA 3.0 might be associated with infections other than those caused by type 1 due to the heterogeneity in particular regions of HCV included in the assay.

In conclusion, the sensitivity of the assay is limited by the immunocompetence of the infected host. In general, samples from HIV-negative individuals that contained genotype 1a showed a higher sensitivity, specificity, and concordance in the serotyping assay, whereas samples containing genotype 3a were found to be more distinct in that they were cross-reactive and untypeable. Therefore, the prevalence of genotypes other than genotype 1 could be underestimated if the genotype is determined by serotyping. Although PCR remains the gold standard because of its higher sensitivity and specificity, the present study indicates that the RIBA HCV serotyping SIA could be useful for the screening of large numbers of samples from immunocompetent HCV-infected individuals and providing epidemiological data, but improvements in specificity are recommended.

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REFERENCES

- Aiyama, T., K. Yoshioka, H. Hirofuji, A. Kusakabe, M. Yamada, K. Tanaka, and S. Kakumu. 1994. Changes in serum hepatitis C virus RNA titer and response to interferon therapy in patients with chronic hepatitis C. *Dig. Dis. Sci.* **39**:2244-2249.
- Beld, M., M. Penning, M. McMorro, J. Gorgels, A. van de Hoek, and J. Goudsmit. 1998. Different hepatitis C virus (HCV) RNA load profiles following seroconversion among injecting drug users without correlation with HCV genotype and serum alanine aminotransferase levels. *J. Clin. Microbiol.* **36**:872-877.
- Bukh, J., R. H. Purcell, and R. H. Miller. 1992. Sequence analysis of the 5' noncoding region of hepatitis C virus. *Proc. Natl. Acad. Sci. USA* **89**:4942-4946.
- Choo, Q. L., G. Kuo, A. J. Weiner, L. R. Oberby, D. W. Bradley, and M. Houghton. 1989. Isolation of cDNA derived from a blood-borne non-A, non-B hepatitis genome. *Science* **244**:359-362.
- Dixit, V., S. Quan, P. Martin, M. Brezina, R. DiNello, K. Sra, J. Y. N. Lau, D. Chien, J. Kolberg, A. Tagger, G. Davis, A. Polito, and G. Gitnick. 1995. Evaluation of a novel serotyping system for hepatitis C virus: strong correlation with standard genotyping methodologies. *J. Clin. Microbiol.* **33**:2978-2983.
- Dusheiko, G., H. Schmilovitz-Weiss, D. Brown, F. McOmish, P. Yap, S. Sherlock, N. McIntyre, and P. Simmonds. 1994. Hepatitis C virus genotypes: an investigation of type-specific differences in geographic origin and disease. *Hepatology* **19**:13-18.
- Köhler, H., J. Goudsmit, and P. Nara. 1992. Clonal distance: cause for a limited and failing immune response to HIV-1 infection and vaccination. *J. Acquired Immune Defic. Syndr.* **5**:1158-1168.
- Köhler, H., S. Müller, and P. Nara. 1994. Deceptive imprinting in the immune response against HIV-1. *Immunol. Today* **15**:455-501.
- Lang, T. A., and M. Secic. 1997. Determining the presence or absence of disease, p. 147-169. *In* E. J. Huth (ed.), *How to report statistics in medicine*. BMJ Publishing Group, Philadelphia, Pa.
- León, P., J. A. López, C. Elola, S. Quan, and J. M. Echevarria. 1997. Typing of hepatitis C virus antibody with specific peptides in seropositive blood donors and comparison with genotyping of viral RNA. *Vox Sang.* **72**:71-75.
- McOmish, F., S. W. Chan, B. C. Dow, J. Gillon, W. D. Frame, R. J. Crawford, P. L. Yap, E. A. Follett, and P. Simmonds. 1993. Detection of three types of hepatitis C virus in blood donors: investigation of type-specific differences in serologic reactivity and rate of alanine aminotransferase abnormalities. *Transfusion* **33**:7-13.
- Pawlotsky, J. M., L. Tsakiris, F. Roudot-Thoraval, C. Pellet, L. Stuyver, J. Duval, and D. Dhumeaux. 1995. Relationship between hepatitis C virus genotypes and sources of infection in patients with chronic hepatitis C. *J. Infect. Dis.* **171**:1607-1610.
- Simmonds, P., E. C. Holmes, T. A. Cha, S. W. Chan, F. McOmish, B. Irvine, E. Beall, P. L. Yap, J. Kolberg, and M. S. Urdea. 1993. Classification of hepatitis C virus into six major genotypes and a series of subtypes by phylogenetic analysis of the NS-5 region. *J. Gen. Virol.* **74**:2391-2399.
- Simmonds, P., F. McOmish, P. L. Yap, S. W. Chan, C. K. Lin, G. Dusheiko, A. A. Saeed, and E. C. Holmes. 1993. Sequence variability in the 5' non-coding region of hepatitis C virus: identification of a new virus type and restrictions on sequence diversity. *J. Gen. Virol.* **74**:661-668.
- Stuyver, L., R. Rossau, A. Wyseur, M. Duhamel, B. Vanderborcht, H. Van Heuverswyn, and G. Maertens. 1993. Typing of hepatitis C virus isolates and characterization of new subtypes using a line probe assay. *J. Gen. Virol.* **74**:1093-1102.
- Takada, N., S. Takase, N. Enomoto, A. Takada, and T. Date. 1992. Clinical backgrounds of the patients having different types of hepatitis C virus genomes. *J. Hepatol.* **14**:35-40.
- Tsubota, A., K. Chayama, K. Ikeda, A. Yasuji, I. Koida, S. Saitoh, M. Hashimoto, S. Iwasaki, M. Kobayashi, and K. Hiromitsu. 1994. Factors predictive of response to interferon- α therapy in hepatitis C virus infection. *Hepatology* **19**:1088-1094.
- van den Hoek, J. A. R., R. A. Coutinho, H. J. van Haastrecht, A. W. van Zadelhoff, and J. Goudsmit. 1988. Prevalence and risk factors of HIV infections among drug users and drug-using prostitutes in Amsterdam. *AIDS* **2**:55-60.