JOURNAL OF CLINICAL MICROBIOLOGY, July 1993, p. 1764–1769
0095-1137/93/071764-06$02.00/0

Vol. 31, No. 7

Hepatitis C Markers in Hemodialysis Patients

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Received 4 January 1993/Accepted 23 March 1993

The prevalence of hepatitis C virus (HCV) infection among the patients of a hemodialysis unit in Taiwan was determined by an immunoblot and reverse transcriptase-polymerase chain reaction algorithm to be 58.8% (67 of 114 patients) after serological surveys with two advanced-generation enzyme-linked immunosorbent assays (ELISAs) for anti-HCV and a C 100-3 single-antigen test. The results of the second-generation ELISAs, the supplementary immunoblot test, and the test for HCV RNA were in good agreement with each other, from 86.0 to 98.2%. The first-generation C 100-3 test lacked the sensitivity of the four other systems. The two advanced-generation screening ELISAs for anti-HCV, a multiple-recombinant-antigen test, the Abbott second-generation ELISA, and a synthetic peptide multiple-antigen test, the UBI HCV EIA, provided reliable and virtually equivalent detection of potentially infected blood. Antibodies to capsid 1 and capsid 2 determinants of the Liatek immunoblot system were the most frequently detected reactivities to HCV in the HCV-infected hemodialysis patients. The percentage of HCV-infected patients with abnormal liver function (alanine aminotransferase level, greater than 100 IU/liter) was higher than that of the uninfected patients. The prevalence of HCV infection was correlated to the duration of hemodialysis treatment and the amount of blood transfused, and the most common transmission mode was thought to be patient-to-patient transmission through the dialysis equipment. Several means of reducing the frequency of transmission between hemodialysis patients are suggested.

Hepatitis C virus (HCV) has been recorded as the major cause of posttransfusion non-A, non-B hepatitis throughout the world (4, 12, 20, 22, 32, 33). Hemodialysis patients are at high risk for HCV infection, as was shown by serological surveys using the commercially available immunoassays (3, 5, 6, 9, 14, 15, 17, 26, 27, 29, 30, 36, 37). The prevalence of anti-HCV among hemodialysis patients may have been underestimated by the first-generation enzyme-linked immunosorbent assay (ELISA) for HCV which employed antibody to protein C 100-3 as the marker for HCV infection, and the serologic reactivities of these patients to structural and other nonstructural (NS) proteins of HCV were not characterized. Although the actual HCV antigen may not yet have been determined, the testing of HCV RNA is a useful technique for the detection of viremia (18). We have now reexamined the prevalence of HCV infection among the patients attending our hemodialysis facility in Taiwan with advanced-generation multiple-antigen immunoassays and a powerful technique for testing for HCV RNA. The frequency of markers for HCV infection was surveyed by using two ELISA kits based on multiple HCV antigens, by one first-generation ELISA, by Liatek HCV immunoblot for confirmation of anti-HCV antibodies, and by testing for HCV RNA by reverse transcriptase-polymerase chain reaction (RT-PCR). The frequency of HCV markers in this at-risk patient group was consistently and significantly higher when determined by the newer assays than by the C 100-3 test. The relationship of markers for HCV infection to disease and the possible routes of transmission are discussed.

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MATERIALS AND METHODS

The subjects consisted of 114 patients (65 male, 49 female) undergoing maintenance hemodialysis at Cathay General Hospital. The mean age of the patients was 52.9 ± 12.3 (range, 28 to 82) years. The mean duration of dialysis was 37.6 ± 24.3 (range, 1 to 144) months. Patient charts indicated that 103 patients received transfusions (2 to 181 U), no patient had a history of intravenous drug abuse, 5 patients had suffered from cardiac failure, 24 patients were positive for hepatitis B surface antigen (HBsAg), and 63 patients had elevated alanine aminotransferase (ALT) activity (>100 IU/liter). All of the HBsAg-positive patients were negative for hepatitis D virus (HDV) antigen, immunoglobulin M anti-HDV, and anti-HDV at the time of study. (Antibody markers for hepatitis B virus are not reported because they are commonly positive in Taiwan.)

For detection of the HCV markers, five different tests were used. Anti-HCV was measured by three ELISA kits: a first-generation ELISA (ELISA A1) based on the C 100-3 antigen (20) (Abbott Laboratories, North Chicago, Ill.), a recombinant second-generation ELISA (ELISA A2) based on the combination of proteins HC 31 (encompassing, more or less, C 100-3 and C33c HCV epitopes) and HC 34 (core protein) (1) (Abbott HCV second generation; Abbott Laboratories), and a synthetic second-generation ELISA (ELISA B) based on synthetic peptides for capsid and NS proteins (16) (United Biomedical, Inc., Hauppauge, N.Y.). Anti-HCV reactivity was expressed by the ratio of optical density of individual tests to the cutoff value (signal/co), and results were interpreted as described in the manufacturer's instructions. Supplementary tests for anti-HCV were performed with Liatek HCV immunoblot strips (Innogenetics-Organon) (8). Antigens representing NS4, NS5, and four capsids (C1 to C4) had been coated as discrete lines on each strip along with

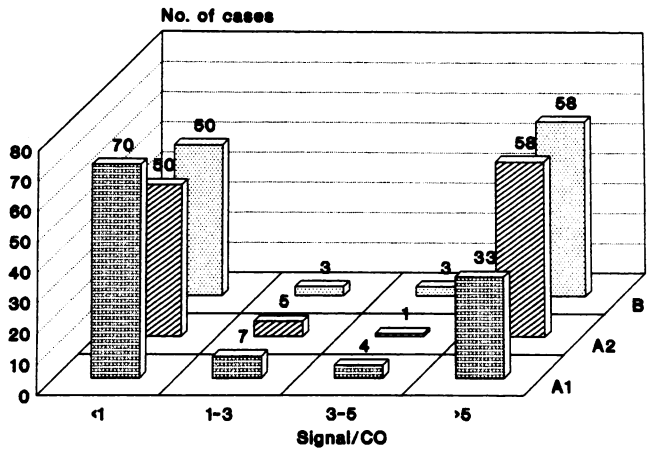

FIG. 1. Distribution of signal/co ratio values of the 114 subjects by the ELISAs A1, A2, and B. Details of the ELISAs can be found in Materials and Methods.

four control lines (human immunoglobulin G). The sequence of amino acids was IIPDREVLYREFDEMEE?SQ in NS4, ETWKKPDYEPPVVHG????? in NS5, PQRKTKRNTNRR PQDVKEPG in C1, RNTNRRPQDVKFPGGGQIVG in C2, and TRKTSERSQPPGR??????? in C4, respectively. The results were interpreted by the instructions provided by the manufacturer and expressed as negative (no reactive bands), indeterminate (having only one band less than a 2-plus reactive band), and positive (having two or more reactive bands or one band greater than a 2-plus reactive band).

The HCV RNA was extracted from serum by a method described previously (10). The first-strand cDNA of the highly conserved 5'-end noncoding region (28) was synthesized by RT with an antisense primer (CCGGTCGTCCTG GCAATTCCGG) and amplified by PCR with the same antisense primer and another sense primer (GGCGACACT CCACCATAGATCAC). The PCR product was detected by hybridization with an end-labelled oligonucleotide as the internal probe (GAGGAACTAATGTCTTCACGC). All oligonucleotides were synthesized by an automated synthesizer (Applied Biosystems, Inc.). The conditions for 35 thermal cycles were 1 min at 94°C, 2 min at 42°C, and 2 min at 72°C, respectively. Precautions such as the use of a laminar-flow hood for all operations, the separation of pre- and post-PCR rooms, and the utilization of positive-displacement pipette tips were taken to prevent contamination.

For the convenience of dividing the study subjects into two groups, patients with either a positive supplementary test for anti-HCV or a positive result for HCV RNA were scored as HCV infected; the remaining subjects were classified as uninfected patients. Clinical data on HCV-infected patients and uninfected patients were compared by Mann-Whitney, Fisher's exact probability, and chi-square tests. A P value of less than 0.05 was considered statistically significant.

RESULTS

Figure 1 represents the distribution of the signal/co ratios of the 114 subjects by the three ELISAs. The ratios of 70 subjects by ELISA A1, 50 subjects by ELISA A2, and 50 subjects by ELISA B were less than 1.0 (negative for anti-HCV). Of the subjects with positive determinations for anti-HCV, 7 subjects by ELISA A1, 5 subjects by ELISA A2, and 3 subjects by ELISA B had signal/co values of between 1.0 and 3.0; 4 subjects by ELISA A1, 1 subject by ELISA A2, and 3 subjects by ELISA B had signal/co values of between 3.0 and 5.0; and 33 subjects by ELISA A1, 58 subjects by ELISA A2, and 58 subjects by ELISA B had signal/co values of >5.0.

Results for all five HCV test systems are compared in Table 1. Forty-nine patients were negative for anti-HCV in all three ELISAs. The immunoblot supplementary test indicated that 46 of the 49 nonreactive patients were negative and 3 were indeterminate. Forty-seven of the seronegative subjects were negative for HCV RNA, and the remaining two were positive for viremia by the presence of HCV RNA. Forty-three subjects were positive in all three ELISAs; and all 43 were confirmed to be positive by immunoblot. RT-PCR for HCV RNA revealed that 39 of the 43 seropositive subjects had viremia. Of the 20 subjects positive in both ELISA A2 and ELISA B, anti-HCV positivity was confirmed for 17 by immunoblot and 18 subjects were proven to be positive for HCV RNA. One subject was positive in only ELISA A1 and ELISA B. Another serum sample was positive in ELISA A2 only. The seropositivity of these two patients was not confirmed (immunoblot results were negative), but they were positive for HCV RNA. The prevalence of HCV markers in the five systems was 38.6% in ELISA A1, 56.1% in ELISA A2, 56.1% in ELISA B, 52.6% in the supplementary test, and 53.5% in the test for HCV RNA,

TABLE 1. Results of five tests of HCV markers[a]

ELISA results	No. of subjects	Immunoblot test results									RT-PCR for HCV RNA	
		Total no. positive	No. positive for antibodies to[b]:						No. indeterminate	No. negative	No. positive	No. negative
			NS4	NS5	C1	C2	C3	C4				
Negative for A1, A2, and B	49								3	46	2	47
Positive for:												
A1, A2, and B	43	43	35	18	30	28	18	12			39	4
A2 and B only	20	17	1	6	14	15	9	7	3		18	2
A1 and B only	1									1	1	
A2 only	1									1	1	

[a] The prevalence of HCV markers in the five tests was as follows: ELISA A1, 38.6%; ELISA A2, 56.1%; ELISA A3, 56.1%; immunoblot, 52.6%; RT-PCR for HCV RNA, 53.5%.

[b] The frequency of anti-HCV antibodies in the patients with positive immunoblots was as follows: NS4, 60%; NS5, 40%; C1, 73.3%; C2, 71.7%; C3, 45%; C4, 31.7%.

TABLE 2. Test-by-test correlation among the 5 systems[a]

	ELISA A1		ELISA A2		ELISA B		Immunoblot		
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Indeterminate	Negative
ELISA A2									
Positive	43	21							
Negative	1	49							
	(80.7)[b]								
ELISA B									
Positive	44	20	63	1					
Negative	0	50	1	49					
	(82.4)		(98.2)						
Immunoblot									
Positive	43	17	60	0	60	0			
Indeterminate	0	6	3	3	3	3			
Negative	1	47	1	47	1	47			
	(78.9)		(93.8)		(93.8)				
HCV RNA									
Positive	40	21	58	3	58	3	54	3	4
Negative	4	49	6	47	6	47	6	3	44
	(78.1)		(92.1)		(92.1)			(86.0)	

[a] The number of patients with positive negative, and indeterminate results are shown.
[b] The values in parentheses indicate percentages of agreement.

individually. In the 60 patients with reactive immunoblots, 73.3% had detectable antibodies (Ab) to C1, 71.7% had Ab to C2, 60.0% had Ab to NS4, 45.0% had Ab to C3, 40.0% had Ab to NS5, and 31.7% had Ab to C4, respectively.

A test-by-test correlation of the systems is shown by the comparison in Table 2. The degree of agreement was 78.1% between ELISA A1 and the test for HCV RNA, 78.9% between ELISA A1 and immunoblot, 80.7% between ELISA A1 and ELISA A2, 82.4% between ELISA A1 and ELISA B, 86.0% between the test for HCV RNA and immunoblot, 92.1% between ELISA A2 and the test for HCV RNA and between ELISA B and the test for HCV RNA, 93.8% between ELISA A2 and immunoblot and between ELISA B and immunoblot, and 98.2% between ELISA A2 and ELISA B.

There were six indeterminate results on the confirmatory test (Table 3). The reactive band of the three subjects with positive results in ELISA A2, ELISA B, and the test for HCV RNA was located on C1 or C2, whereas that of the other three subjects with a negative result in the three ELISAs and the test for HCV RNA was located on NS4 and NS5.

All six subjects with a positive result in the immunoblot test but a negative result for HCV RNA (Table 4) had ALT activity of over 100 IU/liter at some time, and four of them were positive in all three ELISAs, while the remaining two were positive by ELISA A2 and B. All of the ELISA-positive subjects had signal/co values higher than 5.0. On the other hand, four subjects were confirmed to be negative for detectable anti-HCV by immunoblot but were positive on the PCR test for HCV RNA. Only one of these four subjects had ever displayed elevated ALT activity (119 IU/liter). Two of the four subjects were negative in all three ELISAs. One subject was positive in ELISA A1 (signal/co, 1.38) and ELISA B (signal/co, 1.99); the other subject was positive in ELISA A2 only (signal/co, 4.46).

TABLE 3. Results of six subjects with indeterminate immunoblots

Subject	Results on: ELISA A1	ELISA A2	ELISA B	Test for HCV RNA	Reactive band
1	−	+	+	+	C1 (+)
2	−	+	+	+	C1 (trace), C2 (trace)
3	−	+	+	+	C1 (trace), C2 (+)
4	−	−	−	−	NS4 (+)
5	−	−	−	−	NS5 (+)
6	−	−	−	−	NS4 (trace), NS5 (trace)

Sixty-seven patients (58.8% of the study subjects) were either seropositive by immunoblot or positive for the presence of HCV RNA by PCR and considered to be infected with HCV. The other 47 subjects were classified as uninfected patients. As shown in Table 5, the sex, age, and HBs antigenemia of the group had no association with the prevalence of HCV infection. However, in the HCV-infected patient group, the duration of dialysis treatment was longer (medium duration, 45 versus 23 months; $P < 0.001$), the amount of blood transfused was greater (medium amount, 24 versus 10 U, $P < 0.001$), and the percentage of subjects with ALT activity greater than 100 IU/liter (74.6 versus 27.7%; $P < 0.001$) was significantly higher than that in the uninfected patient group.

The relationships between duration of dialysis treatment and prevalence of HCV infection and between amount of blood transfused and prevalence of HCV infection are presented in Tables 6 and 7. All of the data showed that the prevalence paralleled the length of dialysis and the amount of blood transfused.

DISCUSSION

The results of the two multiple-antigen anti-HCV ELISAs, the HCV immunoblot supplementary test, and the RT-PCR test for HCV RNA were in agreement for prevalence of HCV infection among the hemodialysis patients of the clinic

TABLE 4. Results of 10 subjects with discordant data in test for HCV RNA and immunoblot

Discordant results	Peak ALT (IU/liter)	ELISA					
		A1		A2		B	
		Test result	Signal/co	Test result	Signal/co	Test result	Signal/co
HCV RNA (−), immunoblot (+)	225	−		+	>5.0	+	>5.0
	205	+	>5.0	+	>5.0	+	>5.0
	477	+	>5.0	+	>5.0	+	>5.0
	204	+	>5.0	+	>5.0	+	>5.0
	1,432	−		+	>5.0	+	>5.0
	117	+	>5.0	+	>5.0	+	>5.0
HCV RNA (+), immunoblot (−)	<100	+	1.38	−		+	1.99
	119	−		−		−	
	<100	−		+	4.46	−	
	<100	−		−		−	

in Taipei. The frequency of seropositive samples from the patients by either of the advanced-generation ELISAs was 56.1%, and by immunoblot alone it was 52.6%. The frequency of samples positive for HCV RNA was 53.5%. By using our test algorithm for HCV infection of either positivity for anti-HCV by Liatek immunoblot or positivity for HCV RNA by RT-PCR test, 67 of the 114 patients were infected for an overall prevalence of 58.8% of the patients. In contrast, the prevalence of anti-HCV was only 38.6% with the first-generation single-antigen ELISA. Of the 64 patients reactive with the multiple-antigen ELISAs, 58 (90.6%) had signal/co ratios greater than 5.0, while only 75% (33 of 44) of the serum samples reactive with the single-antigen ELISA showed ratio values greater than 5.0 ($P < 0.05$). The agreement (78.9%) between the first-generation ELISA and the immunoblot used in our study for confirmation of anti-HCV (8) was significantly lower than the 93.8% correlation between the second-generation ELISAs and the immunoblot test ($P < 0.05$). The agreement (78.1%) between the first-generation ELISA and the test for HCV RNA was also significantly lower than that (92.1%) between the second-generation ELISAs and the test for HCV-RNA ($P < 0.05$). Therefore, the higher sensitivity of the second-generation immunoassays among the hemodialysis patients did not seem to be a false-positive reaction. These data substantiate that the second-generation ELISAs offer the same improved sensitivity of detection of anti-HCV over the earlier C 100-3 immunoassays as has been demonstrated for other patient populations at high and low risk of infection (1, 16, 19, 21, 23, 25, 34).

HCV capsid antibodies were found to be detected more frequently in chronic HCV infection, to appear earlier in acute HCV infection, and to remain positive for a longer time than C 100-3 (NS4) antibodies (2, 25). HCV capsid antibodies also appeared to be extremely specific in distinguishing false from true positive for HCV infection (11). Our data also show that hemodialysis patients have antibodies to both the structural and NS proteins of HCV and that antibodies to the Liatek C1 and C2 determinants from HCV capsid protein occur more frequently than do antibodies to C3, C4, NS4, and NS5. Of six patients with indeterminate results on immunoblot, three with reactivity for C1 and C2 were positive for HCV RNA and on both multiple-antigen ELISAs, while the three with reactivity to NS4 or NS5 were negative for HCV RNA and negative on all three ELISAs. These observations are most consistent with cross-reactivity of other antibodies to immunoblot bands NS4 and NS5 being the cause of the discrepancies among immunoblot indeterminates and with samples reactive to antigens C1 or C2 being considered positive for anti-HCV.

In 10 patients whose immunoblot and RT-PCR results were discrepant, 4 were positive for HCV RNA and negative on the immunoblot test. One of these four immunoblot-negative patients displayed a mild elevation of ALT activity

TABLE 5. Comparison of clinical data of infected and uninfected patients

Patient Characteristic	Patients		*P* value
	Infected with HCV	Uninfected	
No. of patients	67	47	
Male/female	39/28	26/21	NS[a]
Median age (yr)	50	53	NS
Age range (yr)	28–52	29–77	
Median duration of dialysis (mo)	45	23	<0.001
Range of duration of dialysis (mo)	2–144	1–77	
Never transfused/transfused	5/62	6/41	NS
Median amt of blood transfused (units)	24	10	<0.001
Range in amt of blood transfused (units)	0–149	0–181	
No. of cardiac failures	3	2	NS
No. HBsAg positive	14	10	NS
No. (%) with ALT ever >100 IU/liter	50 (74.6) (13)[b]	13 (27.7) (5)[b]	<0.001

[a] NS, nonsignificant.
[b] Second value in parentheses indicates the number of HBsAg-positive patients.

TABLE 6. Relationship between duration of dialysis and prevalence of HCV infection

Duration of dialysis (mo)	Prevalence		Amt of blood transfused (U)	
	No. of HCV-infected patients/ total no.	%	Range	Median
<12	6/17	35.3	0–80	4
13–24	11/24	45.8	0–128	10
25–36	12/20	60.0	18–86	28
37–48	7/11	63.6	0–181	24
49–60	13/17	76.5	0–72	36
>61	18/25	72.0	0–175	62

(119 IU/liter), two of the four (including the case with mild liver dysfunction) had no detectable anti-HCV, and the other two had low- or intermediate-level signal/co reactivities on ELISAs. These four patients may have been in the early phase of infection because, prior to the onset of hepatitis, antibodies to HCV are at a low concentration and HCV RNA is the only reliable diagnostic marker (13). In a follow-up study, we found that three of the four patients were immunoblot positive 3, 7, and 10 months later, respectively (the remaining patient expired 1 month later). The above explanation can be confirmed by this finding. In the six individuals who were immunoblot positive and negative for HCV RNA, all had high anti-HCV reactivity and had shown abnormal liver function at some period. These six patients appear to be at a stage in which HCV infection has been resolved, at which time anti-HCV remains while virions have been cleared (13).

The 58.8% prevalence of HCV infection in our hemodialysis patients is 36 times higher than the 1.6% prevalence in voluntary blood donors in Taiwan (31). It is clear from this study and others (5, 6) that HCV infection is the major risk factor for liver disease in hemodialysis patients. The prevalence (24 of 114 patients; 21.0%) of HBsAg in the study population is not higher than that of the general population of Taiwan, 19.5% (35), and all of the HBsAg-positive subjects were negative for markers of HDV infection. Therefore, the route of transmission in hemodialysis patients needs to become known. The duration of the course of dialysis treatments and amounts of blood transfused in the HCV-infected group were approximately double those of the 47 uninfected patients (Table 5), and these two risk factors paralleled the prevalence of HCV infection (Tables 6 and 7). However, because prevalence of HCV in our patients who have never been transfused is high (5 of 11; 45.5%), patient-to-patient transmission due to the diffusion of HCV or its persistence in equipment is more common than transfusion as the route of transmission (5, 7, 9, 24). Practical ways to reduce HCV infection in hemodialysis patients is to replace blood transfusion with erythropoietin for treatment of uremic anemia, to treat materials in hemodialysis areas as potentially infectious (6, 24), and to treat HCV-positive patients on hemodialysis in isolation (7, 9, 24).

TABLE 7. Relationship between amount of blood transfused and prevalence of HCV infection

Amt of blood transfused (units)	Prevalence		Duration of dialysis (mo)	
	No. of HCV-infected patients/ total no.	%	Range	Median
0	5/11	45.5	1–144	13
1–10	15/29	51.7	2–84	19
11–20	13/22	59.1	2–65	26
21–40	11/17	64.7	19–72	34
>41	23/35	65.7	7–77	57

This study confirms that HCV infection is a serious problem in our hemodialysis unit. The 67 of 114 patients (58.8%) with serological markers for HCV infection is consistent with the 50 who have histories of abnormal liver function. In addition, the two advanced-generation screening ELISAs for anti-HCV, a multiple-recombinant-antigen test, the Abbott second-generation ELISA, and a synthetic peptide multiple-antigen test, the UBI HCV EIA, provide reliable and virtually equivalent detection of potentially infected blood.

ACKNOWLEDGMENTS

This work was supported by research grants from Academia Sinica and Cathay General Hospital.

We thank Kun-Yen Huang, Wu-Tse Liu, and Barbara Hosein for reading our manuscript and for their helpful suggestions.

REFERENCES

1. **Aach, R. D., C. E. Stevens, F. B. Hollinger, et al.** 1991. Hepatitis C virus infection in post-transfusion hepatitis, an analysis with first- and second-generation assays. N. Engl. J. Med. **325:**1325–1329.
2. **Abe, K., G. Inchauspe, T. Shikata, and A. M. Prince.** 1991. Three different patterns of hepatitis C virus infection in chimpanzees. Hepatology **15:**690–695.
3. **Alonso, M. C., D. Novoa, R. Romero, V. Arcocha, and D. Sanchez-Guisande.** 1991. Antibodies to hepatitis C virus in patients on hemodialysis. Nephron **57:**247. (Letter.)
4. **Alter, H. J., R. H. Purcell, J. W. Shih, et al.** 1989. Detection of antibodies to hepatitis C virus in prospectively followed transfusion recipients with acute and chronic non-A, non-B hepatitis. N. Engl. J. Med. **321:**1494–1500.
5. **Anonymous.** 1990. Hepatitis C virus antibodies in hemodialysis patients. Lancet **335:**1409–1410. (Letter.)
6. **Anonymous.** 1990. Anti-HCV positive patients in dialysis units? Lancet **336:**243–244. (Letter.)
7. **Arici, C., G. P. Gregis, D. Marchesi, G. Mingardi, G. Mecca, and P. Bellavita.** 1990. Effectiveness of a preventive program for non-A, non-B hepatitis in a large dialysis unit. Nephrol. Dial. Transplant. **5:**902–903. (Letter.)
8. **Boudart, D., J. C. Lucas, C. Adjou, and J. Y. Muller.** 1992. HCV confirmatory testing of blood donors. Lancet **339:**372–373. (Letter.)
9. **Calabrese, G., G. Vagelli, R. Guaschino, and M. Gonella.** 1991. Transmission of anti-HCV within the household of hemodialysis patients. Lancet **338:**1466. (Letter.)
10. **Chomczynski, P., and N. Sacchi.** 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. **162:**156–159.
11. **Claeys, H., A. Volckaerts, H. D. Beenhouwer, and C. Vermylen.** 1992. Association hepatitis C virus carrier state with the occurrence of hepatitis C core antibodies. J. Med. Virol. **36:**259–264.
12. **Esteban, J. I., R. Esteban, L. Viladomic, et al.** 1989. Hepatitis C virus antibodies among risk group in Spain. Lancet **ii:**294–296.
13. **Farci, P., H. J. Alter, D. Wong, et al.** 1991. A long-term study of hepatitis C virus replication in non-A, non-B hepatitis. N. Engl. J. Med. **325:**98–104.
14. **Gilli, P., M. Moretti, S. Soffritti, et al.** 1990. Non-A, non-B hepatitis and anti-HCV antibodies in dialysis patients. Int. J. Artif. Organs **13:**737–741.
15. **Ha, S. K., J. H. Park, W. C. Choi, et al.** 1991. Hepatitis C infection in hemodialysis units. Korean J. Intern. Med. **5:**83–86.
16. **Hosein, B., C. T. Fang, M. A. Popovsky, J. Ye, M. Zhang, and**

C. Y. Wang. 1991. Improved serodiagnosis of hepatitis C virus infection with synthetic peptide antigen from capsid protein. Proc. Natl. Acad. Sci. USA **88:**3647–3651.
17. **Jeffers, L. J., G. O. Perez, M. D. De Medina, et al.** 1990. Hepatitis C infection in two urban hemodialysis units. Kidney Int. **38:**320–322.
18. **Kaneko, S., M. Unoura, K. Kobayashi, K. Kuno, S. Murakami, and N. Hattori.** 1990. Detection of serum hepatitis C virus RNA. Lancet **335:**976. (Letter.)
19. **Kudesia, G., S. Chapman, M. Makris, and F. E. Preston.** 1992. Need for second-generation anti-HCV testing in hemophilia. Lancet **339:**501–502. (Letter.)
20. **Kuo, G., G. L. Choo, H. J. Alter, et al.** 1989. An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. Science **244:**362–364.
21. **Lazizi, Y., E. Elfassi, and J. Pillot.** 1992. Detection of hepatitis C virus sequences in sera with controversial serology by nested polymerase chain reaction. J. Clin. Microbiol. **30:**931–934.
22. **Lee, S. D., Y. T. Tsai, S. J. Hwang, et al.** 1991. A prospective study of posttransfusion non-A, non-B (type C) hepatitis following cardiovascular surgery in Taiwan. J. Med. Virol. **33:**188–192.
23. **Lenzi, M., P. J. Johnson, I. G. Mcfarlane, et al.** 1991. Antibodies to hepatitis C virus in autoimmune liver disease: evidence for geographical heterogeneity. Lancet **338:**277–280.
24. **Marchesi, D., C. Arici, E. Poletti, G. Mingardi, E. Minola, and G. Mecca.** 1988. Outbreak of non-A, non-B hepatitis in centre haemodialysis patients: a retrospective analysis. Nephrol. Dial. Transplant. **3:**795–799.
25. **McHutchison, J. G., J. L. Person, S. Govindarajan, et al.** 1992. Improved detection of hepatitis C virus antibodies in high risk populations. Hepatology **15:**19–25.
26. **Moroni, G. A., P. Cori, F. Marelli, et al.** 1991. Indirect evidence for transfusion role in conditioning hepatitis C virus prevalence among dialysis patients. Nephron **57:**371–372. (Letter.)
27. **Oguchi, H., M. Terashima, S. Tokunaga, et al.** 1990. Prevalence of anti-HCV in patients on long-term hemodialysis. Nippon Jinzo Gakkai Shi **32:**313–317.
28. **Okamoto, H., S. Okada, Y. Sugiyama, et al.** 1990. Detection of hepatitis C virus RNA by a two-stage polymerase chain reaction with two pairs of primers deduced from the 5′-nonconding region. Jpn. J. Exp. Med. **60:**215–222.
29. **Ponz, E., J. M. Campistol, M. Bruguera, et al.** 1991. Hepatitis C virus infection among kidney transplant recipients. Kidney Int. **40:**748–751.
30. **Roger, S. D., A. Cunningham, E. Crewe, and D. Harris.** 1991. Hepatitis C virus infection in hemodialysis patients. Aust. N.Z. J. Med. **21:**22–24.
31. **Tsai Lin, S. J., C. J. Chen, Y. S. Hsieh, and C. S. Yang.** 1991. Seroprevalence of anti-HCV among voluntary blood donors in Taiwan. Chin. J. Microbiol. Immunol. **24:**61–70.
32. **Vander Poel, C. L., H. W. Reesink, P. N. Lelie, et al.** 1989. Anti-hepatitis C antibodies and non-A, non-B post-transfusion hepatitis in the Netherlands. Lancet **ii:**297–298.
33. **Wang, J. T., T. H. Wang, J. T. Lin, J. C. Sheu, J. L. Sung, and D. S. Chen.** 1990. Hepatitis C virus in a prospective study of posttransfusion non-A, non-B hepatitis in Taiwan. J. Med. Virol. **32:**83–86.
34. **Weiner, A. J., G. Kuo, D. W. Bradley, et al.** 1990. Detection of hepatitis C viral sequences in non-A, non-B hepatitis. Lancet **335:**1–3.
35. **Wu, J. S., C. H. Chen, Y. H. Chiang, et al.** 1980. Hepatitis B virus infection in Taiwan with reference to anti-HBc versus HBs Ag and anti-HBs. J. Formosan Med. Assoc. **79:**760–767.
36. **Yoshida, C. T., C. Takahashi, A. C. Gaspar, H. G. Schatzmayr, and F. Ruzany.** 1992. Hepatitis C virus in chronic hemodialysis patients with non-A, non-B hepatitis. Nephron **60:**150–153.
37. **Zeldis, J. B., T. A. Depner, I. K. Kuramoto, R. G. Gish, and P. V. Holland.** 1990. The prevalence of hepatitis C virus antibodies among hemodialysis patients. Ann. Intern. Med. **112:**958–960.