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CLINICAL STUDY

# Improved detection of hepatitis C virus infection by transcription-mediated amplification technology in dialysis population

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## ABSTRACT

*Background*: Hepatitis C virus (HCV) infection remains common among patients undergoing maintenance dialysis and plays an adverse effect on survival in this population. Accurate detection of HCV viremia (HCV RNA) in dialysis patients requires a sensitive and specific diagnostic test. *Methods*: The Versant™ HCV RNA Qualitative Assay, based on transcription-mediated amplification (TMA) technique, was prospectively evaluated in 112 dialysis patients. Performance characteristics of the Versant HCV TMA Assay were evaluated in comparison to the Amplicor® 2.0 HCV test based on polymerase chain reaction (PCR) technique. In addition, anti-HCV serologic tests including third-generation enzyme immunoassay and Recombinant Immunoblot Assay were performed. *Results*: Of the 112 specimens tested, 29 were reactive by Versant HCV TMA Assay, yielding an overall prevalence of HCV viremia of 25.9%. The concordance between TMA and PCR techniques was excellent [91% (101/112)]. Eleven specimens (10%) were invalid or equivocal by PCR due to interference phenomena; all 11 specimens had valid TMA results (2 patients being TMA reactive and 9 nonreactive). Four specimens [3.6% (4/112)] that tested PCR-negative and HCV TMA nonreactive were anti-HCV seropositive, consistent with resolved HCV infection. In the group of seronegative samples, one was reactive by TMA Assay [1.25% (1/80)]. *Conclusions*: The HCV TMA technology seems a highly sensitive tool for detecting HCV RNA in the dialysis population, with no evidence of specimen interference. One EIA-negative but HCV-RNA-positive patient by Versant HCV TMA Assay was identified. Prospective clinical trials are under way to assess the clinical impact related to the use of HCV TMA technology in dialysis population.

**Keywords:** hepatitis C virus; HCV viremia; dialysis; transcription-mediated amplification technology

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## INTRODUCTION

Patients on maintenance dialysis continue to have a higher prevalence of hepatitis C virus (HCV) infection than the general population. The prevalence of anti-HCV seropositivity in patients undergoing regular dialysis in developed countries ranges between 7 and 40%.[1–4] A detrimental effect of HCV infection on survival in dialysis patients[5–8] and renal transplant recipients[9–14] has been repeatedly confirmed.

Serologic detection of antibody to HCV antigens by enzyme-linked immunoassay (ELISA) remains the initial diagnostic test for HCV in the dialysis population. The Centers for Disease Control and Prevention, Atlanta, Georgia, USA, currently recommend serial testing of alanine aminotransferase (ALT) levels and anti-HCV antibody to screen for HCV infection in patients undergoing maintenance hemodialysis (HD).[15] However, reliance on serum aminotransferase levels is not an appropriate strategy to screen for HCV, as levels are commonly depressed in chronic uratemia even if HCV infection is present.[16] In addition, ELISAs may not accurately reflect the true HCV prevalence in HD patients, as a number of anti-HCV-negative patients have evidence of viremia by

polymerase chain reaction (PCR) assays particularly with early versions of the serological tests.[17–20]

A new HCV RNA qualitative assay (Versant™ HCV RNA Qualitative Assay), which is based on transcription-mediated amplification (TMA) technology, appears to be very sensitive for detecting HCV RNA in human plasma and serum specimens but data on its use in the HD population is extremely limited.[21–23] The Versant™ HCV RNA Qualitative Assay is an in vitro diagnostic device that employs technology equivalent to Gen-Probe's other currently marketed amplified nucleic acid assays including the Procleix™ HIV-1/HCV Assay[24] and the Aptima™ Combo 2 Assay.[25] In effect, the Procleix™ HIV-1/HCV Assay is identical to the Versant HCV TMA Assay except for the intended use – the Versant™ HCV RNA Qualitative Assay is indicated for use in serum or plasma from patients suspected to be infected with replicative HCV infection.

The aim of this study was to compare the diagnostic performance of HCV TMA technology with PCR-based technique among patients on maintenance HD. In addition, we aimed to determine the prevalence of HCV infection by Versant™ HCV RNA Qualitative Assay in this clinical setting.

## MATERIAL AND METHODS

### Subject enrollment

A total of 112 male and female patients were enrolled from a HD unit at the University of Miami School of Medicine, Florida, USA. Of the 112 subjects enrolled in this study, none were withdrawn due to protocol non-compliance, insufficient specimen volume, or loss of a specimen. All subjects included in the study were on or had been on dialysis at the time of the study. Subjects were not on anti-HCV therapy at the time of enrollment. The study was approved by the Institutional Review Board at the University of Miami. All subjects had given written informed consent to participate in the study.

### Demographic and clinical information

Case report forms were used to record: (1) subject demographic information; (2) a history of HCV infection or liver disease reflected in abnormal liver histopathology or elevated ALT; (3) if symptoms related to hepatitis C were present at the time of enrollment; (4) if a subject was infected with HIV; (5) if a subject was infected with another hepatitis virus such as hepatitis A (HAV), hepatitis B (HBV), or hepatitis D (HDV); (6) risk factors for HCV in addition to HD, such as blood product transfusion, past or current intravenous (IV) drug use, multiple sex partners, a history of a sexually transmitted disease (STD), or occupational exposure (i.e., needlestick accident). Chronic HCV infection was established by at least 6 months of either anti-HCV and/or reactivity with HCV RNA nucleic amplification tests, or with HCV-associated liver histopathology. In the absence of this information, a subject's HCV status was classified as unknown.

A case report form was also used to collect liver histology results, if available. Liver histology results indicating evidence of liver damage were recorded as follows: (1) cirrhosis, (2) fibrosis, (3) hepatocellular carcinoma, or (4) other liver histopathology. These data were used to classify subjects into specific study populations for data analysis as described below. Liver histology may not have represented disease activity at the time of specimen collection, as the time between histological examination of the liver and HCV RNA testing varied.

### Specimens

Two 10 mL tubes of whole blood without anticoagulant were collected and processed within 4 hours of collection. Processing included centrifugation followed by aliquoting 0.8 mL of serum into each of six cryogenic polypropylene vials. Vials were labeled for each intended test including two vials for the Versant™ HCV RNA Qualitative Assay, one vial for serology testing (i.e., enzyme immunoassay (EIA) and recombinant immunoblot assay (RIBA)), and one vial each for ALT, PCR, and HCV Genotyping. Specimens for the HCV TMA technology were maintained at –20°C or below, under monitored conditions until a sufficient number were collected to constitute a 'batch' for testing (i.e., less than 100 specimens). Specimens for the reference tests were collected, transported, and processed according to standard operating procedures.

### Reference methods

Every specimen was tested for the presence of anti-HCV using FDA-licensed serologic assay such as Hepatitis C Virus Encoded Antigen Ortho® HCV Version 3.0 ELISA Test (Ortho-Clinical Diagnostics, Inc., Raritan, NJ, USA). If the latter was reactive, supplemental testing was performed with another serologic assay, Chiron RIBA HCV 3.0 SIA (Chiron Corporation, Emeryville, CA, USA). Routine testing for determining ALT concentrations, Alanine Aminotransferase Synchron CX® Systems Chemistry Information (Beckman Coulter, Inc., Brea, CA, USA), was used.

Specimens were also tested by a reverse transcription PCR assay that detects the presence of HCV RNA using the Roche Amplicor® Hepatitis C Virus (HCV PCR, qualitative assay) Test, which has been approved for marketing with an analytical claim of 100 IU/mL.

### Versant™ HCV RNA Qualitative Assay

Qualitative assessment of HCV RNA was performed by Versant™ HCV RNA Qualitative Assay according

to the manufacturer's instructions. The Versant™ HCV RNA Qualitative Assay has three main steps that have been reported in detail elsewhere[26–28]: sample preparation, target amplification by means of TMA, and amplicon detection by means of hybridization protection assay (HPA). In sample preparation, RNA is isolated from plasma or serum specimens using the target capture system. TMA technology[29] is used to amplify conserved regions within the 5′-UTR of the hepatitis C viral genome. TMA technology utilizes Moloney murine leukemia virus (MMLV) reverse transcriptase (RT) and T7 RNA polymerase to generate multiple copies of the nucleic acid. Assay performance is monitored by means of an internal nucleic acid control that is added to each specimen with the Target Capture Reagent.

The validity of a sample was determined using the ratio of the analyte signal to the analyte cutoff (Analyte S/CO) and the value of the internal control (IC) signal relative to the IC cutoff. For positive calibrators or samples that are reactive for analyte, the IC signal was not used to validate the result.

The sample result was valid and considered nonreactive when the sample had an analyte signal less than the analyte cutoff (i.e., Analyte S/CO<1) and an IC signal greater than or equal to the IC cutoff. The sample result was invalid when the sample had an analyte signal less than the analyte cutoff (i.e., Analyte S/CO<1) and the IC signal was less than the IC cutoff. The sample result was considered reactive when the sample had an analyte signal greater than or equal to the analyte cutoff (i.e., Analyte S/CO≥1) and the IC signal was less than or equal to 475,000 RLU.

### Clinical specimen protocol

In this study, the specimens were prospectively collected at the start of each HD procedure. Blood was taken before being connected to HD machine and initiating dialysis. Clinical performance characteristics of the Versant HCV TMA Assay were evaluated in comparison to the HCV PCR assay.

## RESULTS

### Demographic and clinical information

The information on the demographic and clinical characteristics of patients included in the study group is reported in Tables 1 and 2.

### Data analysis

Of the 112 results by HCV TMA technology, 100% were valid on initial testing. Of the 112 HCV PCR assay results, 9 (8.0%) were repeatedly invalid and 2 repeatedly equivocal (1.8%). Of the 112 EIA results, all were valid on initial testing. Of the 31 RIBA test results, 1 (3.2%) was indeterminate. Of the 7 HCV TMA Assay runs initiated, all (100%) were valid. No information on HCV genotypes was available.

As listed in Table 3, 70 specimens (62.5%) were not reactive with HCV TMA technique; also, they were negative by EIA and PCR. Twenty-six (23.2%) HCV TMA reactive specimens were also reactive for

TABLE 1. Subject demographics.

| Demographic | Category | Value |
|---|---|---|
| Total, *n* | | 112 |
| Gender, *n* (%) | Female | 40 (35.7) |
| | Male | 72 (64.3) |
| Age, years | Mean (±SD) | 54.8 (13.2) |
| | Range | 25–85 |
| Ethnic origin, *n* (%) | Asian/Pacific Islander | 3 (2.7) |
| | Black, Hispanic | 2 (1.8) |
| | Black, Non-Hispanic | 69 (61.6) |
| | Native American, Alaskan | 0 |
| | White, Hispanic | 38 (33.9) |
| | White, Non-Hispanic | 0 |

TABLE 2. Subject clinical information.

| Parameter | Category | Value |
|---|---|---|
| Medical history of liver disease[a] or HCV antibody positive, *n* (%) | Yes | 40 (35.7) |
| | No | 72 (64.3) |
| Symptom status, *n* (%) | Symptomatic | 12 (10.7) |
| | Asymptomatic | 100 (89.3) |
| Type of HCV, *n* (%) | Chronic | 31 (27.7) |
| | Unknown | 81 (72.3) |
| Infected with HIV, *n* (%) | Yes | 13 (11.6) |
| | No or unknown | 99 (88.4) |
| Infected with other hepatitis virus, *n* (%) | Yes | 6 (5.4) |
| | No | 105 (93.8) |
| | Unknown | 1 (0.9) |
| Frequency of risk factors for HCV infection, *n* (%) | Blood transfusion | 58 (51.8) |
| | Past or current IV drug use | 14 (12.5) |
| | Multiple sex partners | 6 (5.4) |
| | History of STD | 15 (13.4) |
| | Occupational exposure | 0 |
| | Dialysis | 112 (100) |

[a]Liver disease is characterized by cirrhosis, fibrosis, hepatocellular carcinoma, or other liver histopathology, or current or prior elevated ALT.

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TABLE 3. Summary of results.

| HCV TMA assay | EIA | RIBA | HCV PCR | *n* (%) |
|---|---|---|---|---|
| Nonreactive | Nonreactive[a] | N/A | NEG | 70 (62.5) |
| Nonreactive | Reactive[b] | POS | NEG | 4 (3.6) |
| Reactive | Reactive | POS | POS | 26 (23.2) |
| Reactive | Reactive | IND | POS | 1 (0.8) |
| Nonreactive | Nonreactive | N/A | Invalid | 9 (8.0) |
| Reactive | Nonreactive | N/A | Invalid | 1 (0.8) |
| Reactive | Reactive | POS | Equivocal | 1 (0.8) |

Notes: N/A, not applicable; POS, positive; NEG, negative; IND, indeterminate.
[a]Initial negative or two negatives out of three EIA tests.
[b]Repeat reactive.

HCV PCR. Similarly, one specimen (0.9%) that tested HCV TMA reactive was reactive for EIA and HCV PCR, but the RIBA was indeterminate. Four specimens (3.6%), who tested negative by HCV TMA and PCR techniques, were positive for anti-HCV serology, consistent with resolved HCV infection. In the subset of 81 EIA-negative patients, there was one (1/81 = 1.2%) patient with detectable HCV RNA in serum by TMA assay (PCR giving equivocal results).

Eleven specimens (10%) were invalid or equivocal by PCR due to interference phenomena; all 11 specimens had valid TMA results. As reported in Table 3, the concordance between TMA and PCR techniques was excellent [91% (101/112)]. The prevalence of HCV infection by serologic investigation was 27.7% (31/112); the patient being EIA-positive/RIBA indeterminate was considered as positive. The prevalence of detectable HCV viremia in serum by Versant HCV TMA Assay was 25.8% (29/112).

HCV prevalence based on Versant™ HCV RNA Qualitative Assay results for patients with anti-HCV, ALT, and liver histopathology is presented in Table 4.

TABLE 4. ALT and liver histopathology results in patients with detectable HCV RNA by Versant™ HCV RNA Qualitative Assay.

| | *n* | HCV TMA assay reactive | Prevalence (%) |
|---|---|---|---|
| Anti-HCV positive | 31 | 27 | 87.1 |
| Elevated ALT | 5 | 4 | 80 |
| Normal ALT | 26 | 23 | 88.5 |
| Histopathology, yes | 1 | 1 | 100 |
| Histopathology, no | 0 | 0 | 0 |
| Histopathology, unknown | 30 | 26 | 86.7 |
| Elevated ALT or histopathology | 5 | 4 | 80 |

Of the 112 total subjects, 31 (27.7%) were anti-HCV seropositive, the prevalence of active HCV infection by HCV TMA technique for subjects with a positive anti-HCV antibody was 87.1%. Of these individuals with anti-HCV, 5 (16.1%) had documentation of current or prior elevation of serum ALT. Furthermore, 1 (3.2%) had a history of liver disease as determined by liver histology. When combined, 5 (16.1%) of the 31 subjects with anti-HCV had an elevated ALT and/or abnormal liver histopathology.

## DISCUSSION

Molecular-based assays that detect replicative (or viremic) HCV infection are based on qualitative HCV RNA detection or on HCV RNA quantitation. Qualitative detection assays are based on the principle of target amplification (conventional or real-time PCR), or TMA. All commercially available assays have equal sensitivity for the detection of all HCV genotypes. Quantitative assays are based either on target amplification techniques (conventional or real-time PCR) or on signal amplification techniques (branched DNA). Branched DNA and most quantitative conventional-PCR assays have detection limits higher than those of qualitative assays. Recent studies in immunocompetent populations have found that the TMA assay is an ultrasensitive technique for HCV RNA detection and that HCV RNA can be detected by TMA in samples that are HCV-RNA-negative by standard PCR assays.[30–32]

The high sensitivity shown by TMA technology makes it very appropriate for testing of HCV infection in patients undergoing maintenance dialysis where the viral load is commonly low. Unfortunately, the information available in the literature on this point is very small. Only a few studies with a small number of patients have been published. Two surveys reported on the presence of anti-HCV-negative patients on regular dialysis with detectable HCV RNA in serum by TMA technique; their frequency ranging between 7.8% and 15.8% by second[23] or third[22] generation ELISA testing. The authors suggested that HCV RNA qualitative assay based on TMA technique improves the accuracy for detecting HCV infection in this population.[22,23] Only one study exists on the comparison between TMA and PCR techniques in assessing HCV viremia in dialysis population[21]; Khan and coworkers found three patients being positive by TMA but not by PCR in their subset of EIA-negative patients.[21] An improved sensitivity by TMA over PCR technology was suggested. However, the study was biased by the fact that only 'in house' PCR was performed (low detection limit around 150 IU/mL), and a limited number of patients was enrolled.

Our cross-sectional study on 112 patients on maintenance HD showed an excellent (91%) concordance between HCV TMA (by Versant™ HCV RNA Qualitative Assay) and PCR (by Roche Amplicor Hepatitis C Virus Test) techniques. In addition, there was one (1.2%) patient with detectable HCV RNA by TMA assay in the subgroup of EIA-negative patients. Presence of HCV viremia in the absence of anti-HCV antibody may be indicative of a negative window period in the early stages of HCV infection, or inability to produce anti-HCV antibody as patients on maintenance dialysis have blunted antibody response due to immunosuppression.[33] Alternatively, these results could have been attributable to false positive results from laboratory or handling error. Additional testing on follow-up samples is required to clarify this point.

The comparison between HCV TMA and PCR techniques in the current study gave no evidence of specimen interference with Versant HCV TMA Assay. Eleven patients tested negative by EIA with invalid ($n = 9$) or equivocal ($n = 2$) results by PCR due to interference phenomena; they had valid TMA results. It is possible that many of these PCR assays were adversely affected by the presence of heparin, a substance commonly used during HD and frequently present in blood samples from patients on regular HD.[34]

Our current study is limited by several reasons. First, the sample size was rather small. Second, false positive results by HCV TMA technique, although a possibility would be an unlikely explanation given the previously reported specificity of this assay.[35,36] Finally, we used conventional PCR, which is provided with higher detection limits compared to real-time PCR. The classical techniques for genome quantification are now progressively being replaced in many laboratories by real-time PCR, which has lower limits of detection of the order of 10–15 IU/mL, and it can be fully automated.[37]

A comparison of labor and cost requirements of TMA assay versus PCR analysis is beyond this paper. Information on this point is not abundant in the medical literature; a preliminary analysis,[38] performed on a small group of non-uratemic individuals, showed that TMA assay is easy to perform, requires minimal instrument maintenance, and has labor costs lower than PCR technique. However, the reagent cost of PCR analysis is significantly lower than that of the TMA assay.

In conclusion, this cross-sectional survey highlighted an excellent concordance between TMA and PCR technology among patients on long-term dialysis. No evidence of specimen interference with Versant HCV TMA Assay was noted. The rate of anti-HCV-negative patients having detectable HCV RNA by TMA technique was around 1%. Additional prospective surveys are in progress to compare HCV TMA versus PCR technology for diagnosis of active HCV infection in dialysis population.

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