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Sensitivity of second-generation enzyme immunoassay for detection of hepatitis C virus infection among oncology patients

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Abstract

Background: The second-generation hepatitis C virus (HCV) enzyme immunoassay (EIA 2), an antibody-detection test, has high sensitivity and is one of the recommended screening tests for detecting HCV infection in the United States. However, its sensitivity among oncology patients is unknown.

Objective: Assess the EIA 2 sensitivity among a group of oncology patients at a Nebraska clinic where an HCV outbreak occurred during 2000–2001 using nucleic acid testing (NAT) and recombinant immunoblot assay (RIBA) as the gold standards.

Study design: Serum specimens were collected from patients 16 months after transmission had stopped. We tested the specimens using EIA 2 (Abbott HCV EIA 2.0), a NAT assay based on transcription-mediated amplification (TMA) (Gen-Probe TMA assay) and RIBA (Chiron RIBA® HCV 3.0 SIA). HCV infection was defined as a positive RIBA or TMA test in an oncology patient. Alanine aminotransferase (ALT) levels were determined in EIA 2-negative/TMA-positive samples.

Results: A total of 264 samples were included in the study. We identified 92 HCV infections, 76 of which were Abbott EIA 2 positive. Abbott EIA 2 sensitivity was 83% (76/92), lower than that reported among healthy adults (90%) ($p = 0.01$) and poor sensitivity was associated with receipt of chemotherapy during the outbreak period ($p = 0.02$). Only 1 (6%) of the 16 EIA 2-negative cases had elevated ALT.

Conclusions: In this study, EIA 2 sensitivity among oncology patients was lower than that previously reported among immunocompetent persons. Impaired antibody production related to cancer and/or chemotherapy might explain the reduced sensitivity. These findings indicate that, when assessing HCV status in oncology patients, a NAT test should be routinely considered in addition to EIA.

Keywords: Hepatitis C virus; Sensitivity; Immunosuppression; Oncology; Chemotherapy

1. Introduction

Enzyme immunoassays (EIAs), both second- and third-generations, are antibody-detection tests recommended for diagnosing hepatitis C virus (HCV) infection in the United States (Alter et al., 2003). Since these tests depend on antibody levels at or above test detection limits, sensitivity among persons with low antibody levels because of recent infection or immunosupression may be decreased. In addition, antibody tests do not distinguish active infection, characterized by the presence of HCV ribonucleic acid (RNA) detected by nucleic acid testing (NAT), from resolved infection (Erensoy, 2001). Because of the possibility of EIA–false-positive results, more specific tests (e.g., recombinant immunoblot assay [RIBA] and NAT) are recommended to

Abbreviations: ALT, alanine aminotransferase; CIA, chemiluminescence immunoassay; EIA, enzyme immunoassay; HCV, hepatitis C virus; NAT, nucleic acid testing; PCR, polymerase chain reaction; RIBA, recombinant immunoblot assay; TMA, transcription-mediated amplification

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confirm positive results. EIA-negative results are considered final and no additional tests are recommended unless recent infection is suspected or in immunosuppressed persons when other evidence of HCV infection (e.g., elevated alanine aminotransferase [ALT] level) exists (Alter et al., 2003).

Previous studies have shown poor EIA sensitivity among persons undergoing hemodialysis and transplant recipients, because of the immunosuppression associated with these conditions (Feucht et al., 1995; Schneeberger et al., 1998; Pawlotsky, 1999). For these patients, HCV infection diagnosis can be made using a combination of NAT and EIA. NAT includes qualitative and quantitative reverse transcriptase polymerase chain reaction (PCR) and the newly developed transcription-mediated amplification (TMA) assay (Giachetti et al., 2002; Gorrin et al., 2003). Despite some reports of HCV EIA poor performance in oncology patients, its sensitivity in this group of persons remains unknown (Paydas et al., 2003). In this study, we evaluated the sensitivity of a second-generation EIA (EIA 2) to detect HCV infection in oncology patients.

2. Methods

2.1. Subject selection

We invited oncology patients from a hematology/oncology clinic in eastern Nebraska to take part in this study. During March 2000–June 2001, an HCV outbreak occurred at this clinic due to cross-contamination of shared saline bags (Centers for Disease Control and Prevention, 2003; Macedo de Oliveira et al., 2005). Variables potentially associated with antibody-detection tests performance such as sex, age, type of cancer and receipt of chemotherapy during the outbreak period were collected from clinic medical records.

2.2. Sample collection

Blood was collected in October 2002, 16 months after the end of the outbreak, which is beyond the seronegative window period for HCV EIA. After blood collection in 7 mL SST Vacutainers®, serum was separated by centrifugation within 4 h. We aliquoted the serum in 2 mL tubes and stored at −20 °C until processing. All specimen handling and testing were performed in accordance with manufacturer's guidelines, as described below.

2.3. Serological testing

HCV serological testing was done using EIA 2 (Abbott HCV EIA 2.0, Abbott Laboratories, Abbott Park, Illinois). This assay consists of a microtiter plate with viral antigens embedded in the wells. Antibodies present in patients' sera adhere to the well and anti-immunoglobulins containing a colorimetric marker are added to allow antibody detection (Carithers et al., 2000). In samples with two Abbott EIA 2-positive repeats, HCV infection was confirmed using qualitative PCR (Cobas Amplicor™ HCV Test, v2.0; Roche Molecular Diagnostic Systems, Branchburg, New Jersey) or, in case of qualitative PCR-negative samples, RIBA (Chiron RIBA® HCV 3.0 SIA, Chiron Corporation, Emeryville, California).

2.4. TMA testing

We tested all samples using TMA (Gen-Probe Incorporated, San Diego, California). This assay involves three steps within a single tube: sample preparation with target capture, HCV RNA target amplification by TMA and detection of the amplification products by hybridization. For verification purposes, an internal control is added to each reaction (Giachetti et al., 2002).

2.5. Abbott EIA 2-negative/TMA-positive samples

We further tested the Abbott EIA 2-negative/TMA-positive specimens using enhanced chemiluminescence immunoassay (CIA) (Vitros® Anti-HCV Assay, Ortho-Clinical Diagnostics, Raritan, New Jersey). This test is one of the third-generation HCV immunoassays, which have reported sensitivity of at least 97% (Colin et al., 2001). For these samples, we confirmed the TMA results using quantitative PCR (Cobas Amplicor™ HCV Monitor Test, v2.0; Roche Molecular Diagnostic Systems, Branchburg, New Jersey) and determined serum ALT levels (Roche Diagnostics Corporation, Indianapolis, Indiana).

2.6. Case definition

HCV infection was defined as a positive RIBA or TMA test in an oncology patient.

2.7. Statistical analysis

We used the z-test to compare Abbott EIA 2 sensitivity with previous reports in the literature. Abbott EIA 2 sensitivity was also stratified by sex, age, type of cancer and use of chemotherapy during the outbreak period and was evaluated using the Fisher exact and the chi square tests, as appropriate.

3. Results

3.1. Subject demographics

Of the 472 patients seen at the clinic during the outbreak period, 269 (57%) oncology patients agreed to be tested. Five of those were excluded from analysis: three with previous HCV treatment, one with insufficient material for complete testing and one with indeterminate laboratory results. Among the 264 patients included in the analysis, the mean age was 67 years and 183 (69%) were females. The most common cancer

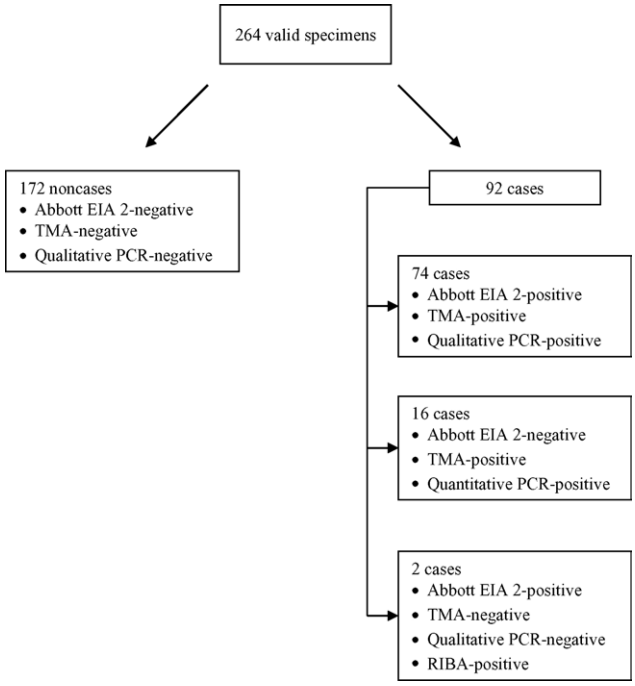

Fig. 1. Laboratory results of valid specimens included in the analysis ($n = 264$), Nebraska 2000–2001.

diagnosis was breast cancer (114 [43%] patients), followed by colon cancer (55 [21%] patients) and leukemia/lymphoma (37 [14%] patients). One hundred and two (39%) patients received chemotherapy during the outbreak period.

3.2. HCV test results

We identified 92 (35%) cases among the 264 valid samples. Seventy-four cases were positive by Abbott EIA 2, qualitative PCR and TMA, and 16 cases were Abbott EIA 2-negative and TMA-positive (Fig. 1). Additionally, two of the 92 cases were EIA 2-positive, TMA-negative and qualitative PCR-negative. In these two cases, we confirmed the EIA 2-positive result by RIBA.

3.3. Abbott EIA 2-negative/TMA-positive samples

To exclude the possibility of TMA false positivity, the 16 Abbott EIA 2-negative/TMA-positive samples were retested with quantitative PCR and all yielded positive results. Four (25%) of these samples were CIA-positive. We determined ALT levels in these 16 specimens, and 15 (94%) were within the normal range (<31 IU/mL).

3.4. Abbott EIA 2 sensitivity

In this study, Abbott EIA 2 sensitivity was 83% (76/92), lower than that in previous studies (90%), which were based on immunocompetent persons ($p = 0.01$) (Whyte and Beal, 1995). All 16 Abbott EIA 2–false-negative results were seen among patients who received chemotherapy during the outbreak period. The difference in Abbott EIA 2 sensitivity between patients receiving and not receiving chemotherapy during the outbreak period (78% versus 100%, respectively) was statistically significant ($p = 0.02$) (Table 1). Abbott EIA 2 sensitivity did not statistically differ when stratified by sex, age, or type of cancer.

4. Discussion

Abbott EIA 2 sensitivity was estimated at 83% among oncology patients from a Nebraska clinic where an HCV outbreak occurred (Centers for Disease Control and Prevention, 2003; Macedo de Oliveira et al., 2005). The 16 patients with Abbott EIA 2–false-negative results had HCV infection demonstrated by NAT. Previous studies have shown lower sensitivity of HCV EIA in persons immunosuppres-

Table 1
Abbott EIA 2 sensitivity stratified by age, sex, type of cancer and use of chemotherapy, Nebraska 2000–2001

Risk factor	Number of Abbott EIA 2-positive results	Number of cases	Sensitivity (%)	*p*-Value
Age				0.65
<61 yr.	28	33	85	
61–74 yr.	29	34	85	
>74 yr.	19	25	76	
Sex				0.09
Female	43	56	77	
Male	33	36	92	
Type of cancer				0.12
Breast cancer	24	29	83	
Colon cancer	20	27	74	
Leukemia/lymphoma	13	17	76	
Other cancer	19	19	100	
Chemotherapy				0.02
Yes	56	72	78	
No	20	20	100	

Yr. = years.

sion due to hemodialysis and organ transplantation, cases of acute HCV infection and infants born to HCV-infected mothers (Bukh et al., 1993; Soffredini et al., 2004). However, few studies have assessed the sensitivity of HCV immunoassays among patients with cancer or receiving chemotherapy.

The present study indicates that EIA 2 sensitivity in oncology patients is lower than that reported among immunocompetent persons (Whyte and Beal, 1995; Abdel-Hamid et al., 2002). A more sensitive immunoassay, CIA, was positive in 4 (25%) of the 16 EIA 2–false-negative cases. EIA 2-positive samples ($n = 76$) were not tested by CIA, but assuming that all these cases were also CIA-positive, we can estimate a maximum CIA sensitivity of 87% (80/92). This performance is below the sensitivity of third-generation HCV immunoassays among immunocompetent persons and HIV-infected patients (>99%) (Abdel-Hamid et al., 2002; Thio et al., 2000). One limitation inherent in the analysis of HCV test sensitivity is the lack of a consensus regarding the gold standard for HCV infection (Pawlotsky, 1999). In this investigation, we used a combination of serological (RIBA) and NAT (TMA) assays as the gold standards, a strategy adopted in previous studies (Janot et al., 1994; Colin et al., 2001).

Decreased EIA 2 sensitivity was associated with receipt of chemotherapy. We were unable to distinguish the relative contributions of cancer and chemotherapy to decreased EIA 2 sensitivity because all false-negative results were seen among patients undergoing chemotherapy and few patients without chemotherapy exposure ($n = 20$) were available for comparison. Future studies documenting HCV-antibody response among oncology patients are needed. Sex, age and type of cancer were not significantly associated with EIA 2 performance; however, our study might have lacked the power to find such association if it existed.

Cancer and its treatment may cause cellular and humoral immune deficiencies, but the impact on HCV EIA performance is unknown. Previous reports have shown reduced EIA sensitivity for diagnosing adenovirus infection among bone marrow transplant recipients (Raboni et al., 2003). Additionally, other studies have shown decreased vaccine-induced antibody levels after chemotherapy and recommended patient revaccination (Reinhardt et al., 2003). It follows that among oncology patients, cancer- and/or chemotherapy-induced immunosupression might have contributed to low levels of HCV antibodies and the reduced EIA sensitivity seen in our study.

Three generations of HCV immunoassays have been developed to detect circulating HCV antibodies. The first-generation HCV EIA was developed in 1989 but had poor sensitivity and specificity (Baath et al., 1992). The second- and third-generation assays improved sensitivity to 90% and 97%, respectively, and are equally recommended as screening tests in the United States (Whyte and Beal, 1995; Abdel-Hamid et al., 2002; Colin et al., 2001). According to current guidelines, EIA-negative results are considered final, except when recent infection is suspected or in immunosuppressed persons with a high index of suspicion for HCV infection, such as abnormal ALT levels (Alter et al., 2003). In this study, 15 (94%) of the 16 Abbott EIA 2–false-negative cases had ALT levels within the normal range and would have gone undiagnosed if NAT had not been performed. We recognize that, based on previously published reports, clinicians already employ a combination of EIA and NAT testing to assess HCV status in oncology patients (Paydas et al., 2003). However, because of the limited number of study subjects, these reports could not offer definitive conclusions about EIA sensitivity and appropriate testing algorithms within this patient population.

In summary, our study suggests that EIA sensitivity among oncology patients is low, which limits the role of this assay as a screening test. The ultimate importance of HCV testing is to identify patients with active infection, i.e. circulating HCV RNA, and provide treatment. However, exclusive use of NAT is also suboptimal, because it will not identify patients with resolved HCV infection (EIA-positive and NAT-negative patients), who should be identified, regularly monitored and treated if their disease recrudesces (Lauer et al., 2001). Therefore, both EIA and NAT should be routinely performed in all oncology patients when assessing HCV status.

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