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Prevalence of false-positive hepatitis C antibody results, National Health and Nutrition Examination Study (NHANES) 2007–2012

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Keywords

hepatitis C; false-positive; RIBA; anti-HCV; prevalence; predictive value positive

Background

Screening for HCV infection is recommended in the US for groups considered to be at risk for infection or with a higher prevalence of HCV infection than the general population [1]. Testing is recommended in certain situations for asymptomatic persons in the general population, such as baseline and follow-up testing of healthcare providers after a needlestick exposure or persons notified to receive bloodborne pathogen testing after a potential exposure resulting from an identified healthcare infection control breach. From 1998–2014 well over 100,000 persons received such notifications [2].

Current recommended screening consists of a test for hepatitis C antibodies (anti-HCV) followed by a test for HCV RNA if antibody positive [3]. Persons testing HCV RNA positive should be referred for care and treatment; most HCV infections can now be cured with the availability of highly effective anti-viral drugs. Persons testing anti-HCV positive but HCV RNA negative (in the absence of recent or ongoing behavioral risks for HCV acquisition) are considered to be negative for current infection without need for further

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follow-up. Anti-HCV positive/HCV RNA negative results may indicate either a past resolved HCV infection (approximately 20% of all acute infections spontaneously resolve [4]), or a false-positive screening test. The predictive value positive of laboratory tests is typically much reduced in populations with prevalence <10% [5].

Objectives

We sought to determine the prevalence of true vs false-positive anti-HCV screening tests among participants in the 2007–2012 National Health and Nutrition Examination Survey (NHANES), a nationally representative sample of the US non-institutionalized civilian population [6].

Study Design

NHANES survey methods have been described elsewhere [7]; during 2007–2012 an initial screening anti-HCV test was performed on all NHANES participants aged 6 years or older [6]. Testing for anti-HCV in serum or plasma was conducted using anti-HCV chemiluminescent assay (CIA) on VITROS automated immunodiagnostic platform (Ortho Clinical Diagnostics, Raritan, NJ). Signal to cut-off ratios (S/CO) ≥1.0 were considered to be anti-HCV reactive. Reactive specimens were tested using a confirmatory recombinant immunoblot assay (RIBA) (Chiron RIBA HCV 3.0 Strip SIA), an *in vitro* qualitative immunoassay for the detection of anti-HCV. Serum samples that were confirmed positive or indeterminate for RIBA were further tested for HCV RNA using the qualitative COBAS AMPLICOR HCV Test, version 2.0 (Roche Diagnostics, Indianapolis, Indiana) with lower limit of detection of 50 IU/mL, on the COBAS AMPLICOR Analyzer (Roche Diagnostics).

Case definitions

Persons testing anti-HCV reactive and RIBA positive were considered to have had a ‘true-positive’ anti-HCV test. Those testing anti-HCV reactive and RIBA indeterminate were categorized as having unknown anti-HCV status, although indeterminate results are commonly associated with false-positive anti-HCV tests [7]. Persons testing anti-HCV reactive and RIBA negative were considered to have had a ‘false-positive’ anti-HCV screening test.

Persons with a ‘true-positive’ anti-HCV test and a negative HCV RNA test were considered to have had past resolved infection. For those testing RIBA-indeterminate with a negative HCV RNA test result we were unable to differentiate between past resolved infection and a false-positive anti-HCV result.

All persons with a positive test for HCV RNA were considered to have current HCV infection; all those with a negative test for HCV RNA were considered to be currently uninfected.

Results

Of 22,359 NHANES participants tested for anti-HCV by CIA during 2007–2012, 479 (2.1%) were anti-HCV reactive. Of these, 477 (99.6%) had a confirmatory RIBA test and were included in further analysis.

RIBA testing results

RIBA positive cases—Of the total 477 participants who were anti-HCV reactive and received RIBA testing, 323 (67.7%) were positive and considered true-positive cases. Specimens from 45 (13.9%) participants who were RIBA positive could not be tested for HCV RNA due to insufficient sample volume and were excluded from further analysis; the remaining 278 (86.1%) were tested. Among these patients, 216 (77.7%) were HCV RNA positive indicating current HCV infection, and 62 (22.3%) were RNA negative indicating past resolved infection. (Figure)

RIBA indeterminate cases—Of the total 477 anti-HCV positive participants with RIBA testing, 49 (10.3%) were indeterminate. (Figure) Specimens from 8 (16.3%) participants who were indeterminate could not be tested for HCV RNA due to insufficient sample volume and were excluded from further analysis; the remaining 41 (83.7%) were tested. Among these cases, 2 (4.9%) were HCV RNA positive indicating current HCV infection, and 39 (95.1%) were RNA negative indicating either false-positive anti-HCV results or past clearance of infection. (Figure)

RIBA negative cases—Of the total 477 anti-HCV positive participants with RIBA testing, 105 (22%) were negative, indicating a false-positive anti-HCV result. These specimens were not tested further for HCV RNA and were considered to be HCV RNA negative [7].

HCV RNA testing results

HCV RNA negative cases—By combining the 105 RIBA negative cases considered HCV RNA negative, the 62 RIBA positive cases that tested HCV RNA negative, and the 39 RIBA indeterminate cases that tested HCV RNA negative, we determined that a total of 206 participants had a negative HCV RNA status. Among these HCV RNA negative participants, 62 (30.1%) were RIBA positive (resolved infections), 105 (51.0%) were RIBA negative (false-positive anti-HCV), and 39 (18.9%) were RIBA indeterminate.

HCV RNA positive cases—By combining the 216 RIBA positive cases that tested HCV RNA positive and the 2 RIBA indeterminate cases that tested HCV RNA positive, we determined that a total of 218 participants had a positive HCV RNA status. Among these HCV RNA positive participants, almost all 216 (99.1%) had confirmed as RIBA positive; only 2 (1.0%) were indeterminate.

In sum, among the 424 anti-HCV positive participants with known HCV RNA status, 218 (51.4%) were HCV RNA positive indicating current infection and 206 (48.9%) were HCV RNA negative indicating no current infection. (Figure)

Anti-HCV signal to cut-off ratio (S/CO)

Among 332 participants with S/CO >8.0 (range 8.16–49): HCV RNA status could be ascertained for 288 (87%) and among these 217 (75%) were positive indicating current infection; overall RIBA was positive for 311 (93.7%), indeterminate for 2.4%, and negative for 3.9%. In contrast, among 147 with S/CO ≥ 1.0 but <8 (range 1.0–7.9): HCV RNA status could be ascertained for 136 (93%) and was positive indicating current infection for one (0.7%); overall RIBA was positive for 8.3%, 28.3% indeterminate for 28.3%, and negative for 63.4%.

Discussion

Screening persons in a population with low prevalence of a disease leads to many false-positives that may have health, economic and psychological impacts on patients and providers. In this sample with 1% HCV infection prevalence, the predictive value positive of the test [5] was lower than in higher prevalence populations: about seven of every ten screening-reactive anti-HCV results (68%) were true-positives and two of ten (22%) false-positives. One in 10 (10%) was “indeterminate,” of which most were also likely false-positive [7]. Resulting from these false positive tests, the overall prevalence of chronic infection among those testing anti-HCV screening reactive was much lower (51%) than would be expected due to disease clearance alone. However, among the subset of RIBA confirmed true-anti-HCV positive cases, about eight in ten were HCV RNA positive indicating current infection with two in ten HCV RNA negative indicating past resolved infection, mirroring the expected clearance rate of HCV infection [4]. In contrast, among the subset of persons who were anti-HCV positive but tested HCV RNA negative, more than half were found to be anti-HCV false-positive and thus never exposed to HCV.

This study was conducted prior to the widespread availability of direct-acting antiviral therapy, when only a small proportion of patients (5–6%) had been diagnosed and experienced cure [11]; thus few if any of the HCV RNA negative results among RIBA positive cases were likely to be due to clearance of virus after successful therapy. These findings are not generalizable to populations with elevated prevalence of HCV infection such as injection drug users, for whom prevalence is typically well above 10% and the rate of anti-HCV false-positivity is substantially lower, with HCV RNA prevalence among persons testing anti-HCV reactive generally >70% [9,10].

The confirmatory anti-HCV assay, RIBA, is no longer available in the US. Further, concordance between confirmation and S/CO was not perfect, and with the availability and frequent use of an FDA-approved rapid test for anti-HCV use of these thresholds for determining the true or false positivity of screening assay results is no longer relevant. False-positive antibody assays may occur with great frequency, emphasizing the need for “reflex” HCV RNA testing to ascertain current infection status. In accordance with current guidelines [3] *all* screening anti-HCV positive tests including rapid tests, regardless of anti-HCV S/CO or other factors, should be followed by an HCV RNA test in order to confirm whether the patient has current infection so that infected persons can be referred to care and treatment to avoid the significant morbidity and mortality associated with chronic HCV infection.

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Abbreviations

anti-HCV	HCV antibody test
NHANES	National Health and Nutrition Examination Study
RIBA	recombinant immunoblot assay
S/CO	signal-to-cutoff ratio

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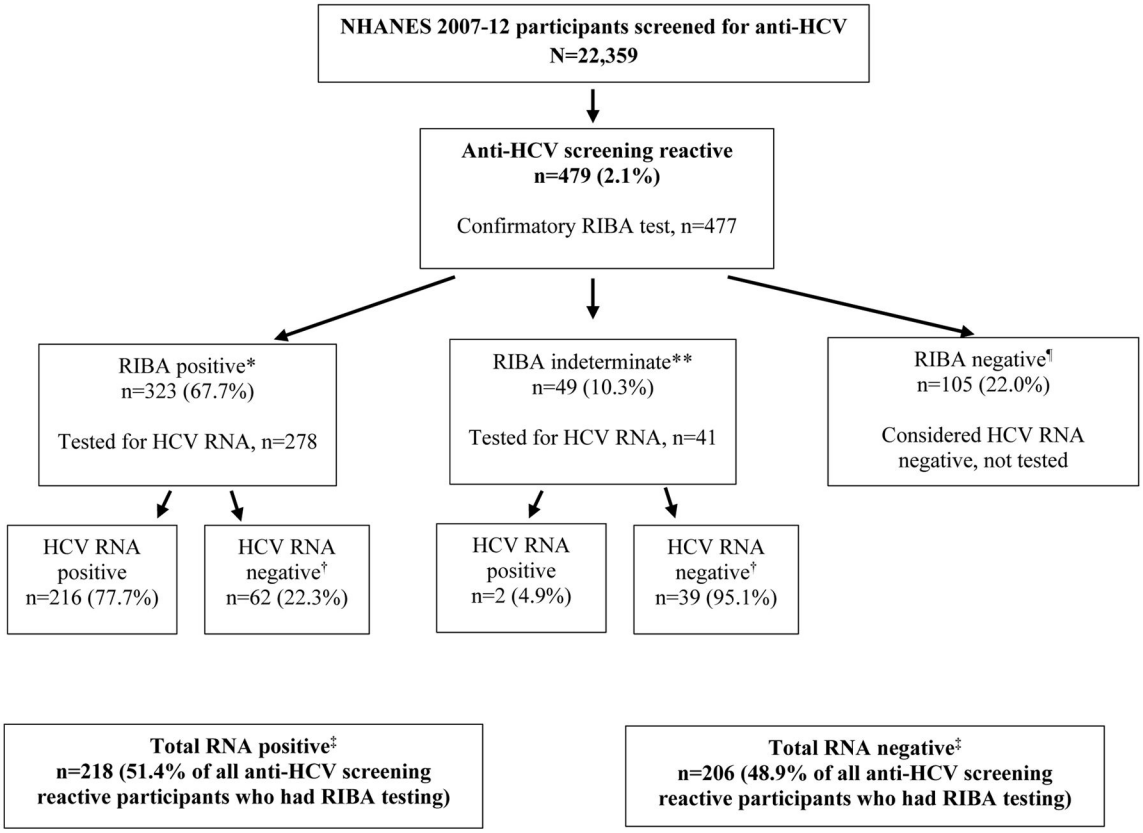

Figure.

Testing flow diagram.

*Persons testing anti-HCV screening reactive and RIBA positive were considered to have had a ‘true-positive’ anti-HCV test.

**Persons testing anti-HCV screening reactive and RIBA indeterminate were of unknown anti-HCV confirmatory status.

¶ Persons testing anti-HCV screening reactive and RIBA negative were considered to have had a ‘false-positive’ anti-HCV test and to be HCV RNA negative[13].

† The 62 persons with a ‘true-positive’ anti-HCV test and a negative HCV RNA test were considered to have had past resolved infection. For the 39 RIBA-indeterminate persons with a negative HCV RNA test we were unable to differentiate between past resolved infection and a false-positive anti-HCV result with no prior infection.

‡ All persons with a positive test for HCV RNA were considered to have active chronic infection; all those with a negative test for HCV RNA were considered to be currently uninfected.