Published in final edited form as:

J Clin Virol. 2011 December ; 52(4): 328–332. doi:10.1016/j.jcv.2011.08.021.

Clinical Variables Identify Seronegative HCV Co-infection in HIV-Infected Individuals

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Abstract

Background—A substantial number of people living with HIV (PLWH) are co-infected with Hepatitis C Virus (HCV) but have a negative screening HCV antibody test (seronegative HCV infection, or SN-HCV).

Objective—To identify a concise set of clinical variables that could be used to improve case finding for SN-HCV co-infection among PLWH.

Study design—Two hundred HIV-infected participants of the CHARTER study were selected based on 7 clinical variables associated with HCV infection but were HCV seronegative. Data were analyzed using Fisher's exact tests, receiver-operating characteristic (ROC) curves, and logistic regression.

Results—Twenty-six (13%) participants had detectable HCV RNA. SN-HCV was associated with a history of IDU, elevated ALT and AST, low platelets, black ethnicity, and undetectable HIV RNA in plasma. Each of these clinical variables, except for abnormal AST, remained independently associated with SN-HCV in a multivariate logistic regression analysis. A composite risk score correctly identified SN-HCV with sensitivity up to 85% and specificity up to 88%.

Conclusions—In a substantial minority of PLWH, seronegative HCV viremia can be predicted by a small number of clinical variables. These findings, after validation in an unselected cohort, could help focus screening in those at highest risk.

Keywords

Seronegative HCV; HIV; co-infection; case finding

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competing interests: No conflict of interest.

ethical approval: This study was approved by the Institutional Review Board of the University of California San Diego (110089).

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1. background

Hepatitis C Virus (HCV) infection is common in people living with HIV (PLWH), particularly intravenous drug users (IDU), and substantially exceeds the prevalence in the general population.¹⁻⁴ In co-infected individuals, the outcomes of each disease are adversely impacted. Compared with people with HCV infection alone, PLWH have higher HCV viral loads⁵ and more rapid progression of liver fibrosis.^{6, 7} HCV co-infection, in turn, can accelerate HIV disease progression.^{8, 9} The effect on antiretroviral therapy (ART) in co-infected individuals is less clear. Findings range from a blunted early immune recovery and impaired late recovery in HCV genotype 3 infections¹⁰ to no effect on immunological or virological response to ART.^{4, 11} There is, however, a higher mortality and a higher incidence of adverse drug reactions such as rash and hepatotoxicity in co-infected individuals.^{11, 12} These important clinical implications have led to the recommendation to screen all HIV-infected patients for HCV.¹³

Although the HCV antibody test is adequate for screening HIV-uninfected individuals, its false negative rate is higher in PLWH. PLWH typically have chronic HCV infection with detectable HCV RNA in serum but some remain seronegative. Estimates of the prevalence of seronegative HCV infection (SN-HCV) have varied from 3-13%.¹⁴⁻¹⁷ Because detecting HCV RNA in serum is expensive, screening of all HIV-infected individuals may be impractical, particularly in resource-limited settings.

2. Objective

To identify a concise set of clinical variables that can improve case finding for SN-HCV co-infection among PLWH.

3. study design

3.1 Participants

CHARTER (CNS HIV AntiRetroviral Therapy Effects Research) is an observational cohort study funded by the National Institute of Mental Health. The study is conducted in six U.S. cities (Baltimore, Galveston, New York, St. Louis, San Diego, and Seattle) and has enrolled 1,555 volunteers. Institutional Review Boards at each site approved the study and all volunteers provided written informed consent.

In selecting the subjects, we first excluded all individuals seropositive for HCV (SP-HCV). Next, to increase the likelihood of capturing individuals with HCV infections, we chose 7 clinical variables that are known to be associated with it: IDU, AIDS diagnosis, absence of current ART use, or currently abnormal ALT (> 50 IU/mL), AST (> 50 IU/mL), platelets ($< 200,000/\text{mm}^3$), or albumin ($< 4\text{g/dL}$). These were selected because of their association with 1) HCV transmission (IDU), 2) severity of liver disease (abnormal AST, ALT, and platelets), 3) advanced HIV disease and reduced humoral immunity (AIDS diagnosis and absence of current ART use), or 4) both liver disease and HIV disease (low albumin). To ensure that we had adequate variability in these clinical variables, we summed the number of clinical variables in all HCV seronegative CHARTER subjects to derive a composite score ranging from 1-7. We then selected 200 cases to ensure reasonable representation across the range of the composite score. This was a convenience sample.

3.2 Methods

Roche COBAS AmpliPrep/COBAS TaqMan HCV test was used for measuring HCV RNA in serum specimens that had been stored at -80°C . This test has a lower detection limit of approximately 10 IU/mL and a linear amplification range of HCV RNA from 43 to

69,000,000 IU/ml. HCV antibody test was done by LabCorp, San Diego using the Siemens ADVIA Centaur XP immunoassay.

3.3 Statistical analysis

Data were analyzed using Fisher's exact tests, receiver-operating characteristic (ROC) curves, and logistic regression. In addition to the 7 variables noted above, we considered ethnicity, age, blood CD4+ T-cell count, and detectable HIV RNA in plasma.

4. Results

The participant characteristics are shown in Table 1. HCV RNA was detected in blood from 26 (13%) of the 200 seronegative HCV participants. The levels ranged from 11 to 68,500,000 IU/mL with a median value of 2,100,000 (IQR 245,000-5,210,000 IU/mL). The distribution of HCV RNA is shown in Figure 1.

In univariate analyses, six clinical variables (4 original and 2 additional) were associated with detection of HCV RNA: IDU (57% vs. 30%, $p = 0.02$), abnormal ALT (> 50 IU/mL) (77% vs. 41%, $p = 0.001$), abnormal AST (> 50 IU/mL) (62% vs. 33%, $p = 0.008$), low platelets ($< 200,000/\text{mm}^3$) (69% vs. 38%, $p = 0.005$), and undetectable HIV RNA in plasma (< 50 copies/mL; 58% vs. 36%, $p = 0.05$). A sixth variable, ethnicity, showed a trend toward association with detection of HCV RNA in blood (black vs. other, (62% vs. 42%, $p = 0.09$). In multivariate logistic regression analysis, each of these covariates was associated with SN-HCV, except for abnormal AST. The odds ratios and confidence intervals are shown in Table 2. The p value for the deviance goodness of fit was less than 0.001. Raw prediction error for the model was 9.4%; cross-validated prediction error for the model was 10.8%. The c-statistic (estimated probability that the model assigns higher risk to those who develop HCV than to those who do not) for the logistic regression was 0.871 ($p < 0.001$).

We summed the clinical variables identified by univariate analyses into a composite score and evaluated it as a classifier of SN-HCV using ROC curve analysis (Figure 2). We also evaluated the sensitivity and specificity of two best cutpoints: 3 (less than 3 vs. 3 or more) and 4 (less than 4 vs. 4 or more). Using a cutpoint of 4 classified 37/200 individuals with SN-HCV (16/26 confirmed SN-HCV individuals and 21/174 individuals who did not have HCV). Using a cutpoint of 3 classified 86/200 individuals with SN-HCV (22/26 confirmed SN-HCV individuals and 64/174 who did not have HCV). As Table 3 shows, a cutpoint of 3 had a higher sensitivity (85%) whereas a cutpoint of 4 had a higher specificity (88%). Of the 26 SN-HCV participants, 10 had a follow-up visit after a mean of 11 months. Two of 10 (20%) became HCV seropositive and the remainder remained seronegative.

The prevalence of SN-HCV and SP-HCV varied substantially by site (Site (SN-HCV prevalence; SP-HCV prevalence): Baltimore (30%; 53%), New York (19%; 31%), Seattle (13%, 24%), Galveston (10%; 29%), San Diego (7%; 16%), and St. Louis (0%; 10%)). To examine the effect of local population prevalence of HCV on the relationship between SN-HCV and clinical factors, we used a logistic regression with two variables: sum of clinical factors and site SP-HCV prevalence. This identified that SN-HCV was associated with a larger number of clinical factors and was more common at sites that had greater proportions of participants with SP-HCV (Reduction in deviance = 44.8, $p < 0.0001$). Figure 3 illustrates this relationship.

5. Discussion

Our main findings regarding SN-HCV among PLWH were: 1) we identified its association with a relatively sparse number of clinical factors (history of IDU, elevated ALT, low

platelets, black race, and undetectable HIV RNA); 2) we present a clinically useful method of summing clinical factors that may simplify identification of SN-HCV; 3) we found that SN-HCV prevalence varied by site prevalence of HCV; and 4) we present a model that takes into account both the number of risk factors and site SP-HCV prevalence in predicting SN-HCV.

IDU is a well-recognized risk factor for HCV infection among HIV-infected individuals.^{2,3} Our study confirms the earlier reported association between a history of IDU and SN-HCV.^{14,16} However, in contrast to our findings, George et al reported that SN-HCV infection was more common from mucosal or sexual compared to parenteral exposure.¹⁷ Elevated serum ALT levels indicate hepatocellular damage.¹⁸ Similar to our study, Chamie et al reported an association between elevated ALT levels and SN-HCV viremia in their cohort¹⁴ but no link was found in several other studies.¹⁵⁻¹⁷ Thrombocytopenia is associated with SP-HCV and with HIV infection.¹⁹ We believe that this is the first study to find an association between SN-HCV and thrombocytopenia among PLWH. We also found higher rates of SN-HCV in Blacks than in other races and this may be related to a higher HCV prevalence in Black race (3.2%) as seen in the NHANES III cohort.¹

People who have had advanced immunosuppression may have persistently abnormal humoral immunity, resulting in the absence of antibodies to pathogens such as HCV. Support for this hypothesis comes from prior reports that people with SN-HCV had lower CD4+ T-cell counts than those with SP-HCV infection.^{14,15,17} CD4+ T-cell count nadirs in CHARTER are self-reported and may be susceptible to recall bias. To overcome this, we also included other measures that might reflect a history of advanced immunosuppression: current (measured) CD4+ T-cell count, AIDS diagnosis, current antiretroviral use, and HIV RNA levels in blood, an indicator of antiretroviral use. However, like Hall et al¹⁶, we did not find an association between immunosuppression or use of antiretroviral drugs and SN-HCV, although we did find an association with undetectable HIV RNA levels in plasma. The explanation for this finding may be the relatively advanced immunosuppression of our group. While most subjects achieved some degree of immune recovery (CD4+ T-cell counts at the assessment visit exceeded the nadir CD4+ T-cell count by a mean of 232 cells/ μ L), the selected subgroup was more likely than the CHARTER cohort as a whole to have worse immunosuppression, whether estimated by nadir CD4+ T-cell counts, current CD4+ T-cell count, or AIDS diagnosis. In fact, we saw a trend in which subjects who had undetectable plasma HIV RNA levels had slightly lower nadir CD+ T-cell counts (mean 122 vs. 154/ μ L, $p = 0.07$). Thus, in this analysis, an undetectable HIV RNA level in plasma may be a surrogate for a history of more advanced immunosuppression and persistently impaired antibody production. Antiretrovirals themselves are not known to suppress HCV viremia or antibody production.

To optimize the clinical relevance of our model, we compared the number of clinical variables to rates of SN-HCV. A threshold of 3 clinical variables detected most of the SN-HCV cases but had a higher false negative rate that would increase the cost of screening with an HCV RNA assay. On the other hand, a threshold of 4 clinical variables missed some SN-HCV cases but would result in lowering screening costs. The cost of under-diagnosing SN-HCV infection could result in higher ultimate costs because of progressive liver disease in the undiagnosed individuals and perpetuation of transmission. The predictive ability of the model was further improved by including HCV prevalence at each site, suggesting that the optimal threshold for testing seronegative PLWH for HCV viral load should be tailored based on HCV prevalence in the population.

The cost savings of our selection criteria can be significant but will depend on the clinical variables cutpoint used and the prevalence of SN-HCV in the population tested. For

example, in our cohort of 200 HIV+ individuals, the cost of screening everyone by HCV RNA would be \$25,000 (\$125 per PCR assay). Using a cutpoint of 4 clinical variables to test selected individuals would result in a much lower cost of \$4,500 (80% saving). This approach would capture 16 people with SN-HCV in the 37 people that we test but will miss 10 people with SN-HCV in the 163 people not tested. A cutpoint of 3 would increase the sensitivity identifying 22 people with SN-HCV but also increase the cost to \$10,750 (57% saving).

The analysis has important limitations. First, the selection of the subgroup based on clinical variables associated with HCV limits generalizability of the findings. This selection approach was used intentionally to determine if it would improve the efficiency of case finding compared with historical reports. Second, our quantitative assay to measure HCV RNA in serum we may have missed some SN-HCV people with low viremia. There are methods to improve the sensitivity of HCV PCR and include ultracentrifugation of serum samples²⁰ and use of whole blood¹⁷ or liver tissue specimens²¹ but these methods are either not commercially available or use specimens that are not readily accessible. Third, 16 of the 26 SN-HCV individuals had no follow-up. As a result, we cannot confidently exclude acute infection with subsequent seroconversion as an explanation for SN-HCV in all subjects. However, the likelihood of this is low as shown by a longitudinal study of early HCV infections in PLWH of whom 60% became seropositive at 3 months and 95% at 1 year.²² We found that only 20% of the individuals who did have follow-up seroconverted over nearly a year of follow-up. Also, there was no significant difference in the characteristics of the subjects with or without follow-up.

In conclusion, we found that a combination of clinical risk factors identified those at a higher risk of HCV viremia among HCV seronegative PLWH. Given the characteristics of our cohort, our findings may be most relevant to patients who have had advanced immunosuppression and have achieved some degree of immune recovery in response to ART. However, if validated in a less selected cohort, this approach could provide a simple screening method to identify patients who might benefit from further HCV assessment.

Acknowledgments

We would like to thank all CHARTER participants for their invaluable contribution.

funding: CHARTER is funded by the National Institutes of Health (N01 MH22005 (CHARTER; PI: Igor Grant). Ajay R. Bharti is supported by National Institute of Mental Health grants 1K23MH085512-01A2 and R25 MH81482, and the REACH (Research and Education in HIV/AIDS for Resource-Poor Countries) Initiative of Tibotec.

References

1. Alter MJ, Kruszon-Moran D, Nainan OV, et al. The prevalence of hepatitis C virus infection in the United States, 1988 through 1994. *N Engl J Med.* Aug 19; 1999 341(8):556–562. [PubMed: 10451460]
2. Brau N, Bini EJ, Shahidi A, et al. Prevalence of hepatitis C and coinfection with HIV among United States veterans in the New York City metropolitan area. *Am J Gastroenterol.* Aug; 2002 97(8): 2071–2078. [PubMed: 12190179]
3. Sherman KE, Rouster SD, Chung RT, Rajicic N. Hepatitis C Virus prevalence among patients infected with Human Immunodeficiency Virus: a cross-sectional analysis of the US adult AIDS Clinical Trials Group. *Clin Infect Dis.* Mar 15; 2002 34(6):831–837. [PubMed: 11833007]
4. Turner J, Bansi L, Gilson R, et al. The prevalence of hepatitis C virus (HCV) infection in HIV-positive individuals in the UK - trends in HCV testing and the impact of HCV on HIV treatment outcomes. *Journal of viral hepatitis.* Aug; 2010 17(1):569–577. [PubMed: 19840365]

5. Thomas DL, Shih JW, Alter HJ, et al. Effect of human immunodeficiency virus on hepatitis C virus infection among injecting drug users. *J Infect Dis.* Oct; 1996 174(4):690–695. [PubMed: 8843204]
6. Benhamou Y, Bochet M, Di Martino V, et al. Liver fibrosis progression in human immunodeficiency virus and hepatitis C virus coinfecting patients. The Multivirc Group. *Hepatology.* Oct; 1999 30(4):1054–1058. [PubMed: 10498659]
7. Soto B, Sanchez-Quijano A, Rodrigo L, et al. Human immunodeficiency virus infection modifies the natural history of chronic parenterally-acquired hepatitis C with an unusually rapid progression to cirrhosis. *J Hepatol.* Jan; 1997 26(1):1–5. [PubMed: 9147999]
8. Backus LI, Phillips BR, Boothroyd DB, et al. Effects of hepatitis C virus coinfection on survival in veterans with HIV treated with highly active antiretroviral therapy. *Journal of acquired immune deficiency syndromes (1999).* Aug 15; 2005 39(5):613–619. [PubMed: 16044016]
9. Sabin CA, Telfer P, Phillips AN, Bhagani S, Lee CA. The association between hepatitis C virus genotype and human immunodeficiency virus disease progression in a cohort of hemophilic men. *J Infect Dis.* Jan; 1997 175(1):164–168. [PubMed: 8985212]
10. Seminari E, Tinelli C, Ravasi G, et al. Hepatitis C infection on immune recovery in HIV-positive patients on successful HAART: the role of genotype 3. *Curr HIV Res.* Apr 1; 8(3):186–193. [PubMed: 20163342]
11. Fuping G, Wei L, Yang H, et al. Impact of Hepatitis C Virus Coinfection on HAART in HIV-Infected Individuals: Multicentric Observation Cohort. *Journal of acquired immune deficiency syndromes (1999).* Apr 26; 2010 54(2):137–142. [PubMed: 20431395]
12. Grebely J, Raffa JD, Lai C, et al. Impact of hepatitis C virus infection on all-cause and liver-related mortality in a large community-based cohort of inner city residents. *Journal of viral hepatitis.* Jan; 2011 18(1):32–41. [PubMed: 20196806]
13. USPHS/IDSA. 2001 USPHS/IDSA guidelines for the prevention of opportunistic infections in persons infected with human immunodeficiency virus. *HIV Clin Trials.* Nov-Dec; 2001 2(6):493–554. [PubMed: 11742438]
14. Chamie G, Bonacini M, Bangsberg DR, et al. Factors associated with seronegative chronic hepatitis C virus infection in HIV infection. *Clin Infect Dis.* Feb 15; 2007 44(4):577–583. [PubMed: 17243063]
15. Bonacini M, Lin HJ, Hollinger FB. Effect of coexisting HIV-1 infection on the diagnosis and evaluation of hepatitis C virus. *Journal of acquired immune deficiency syndromes (1999).* Apr 1; 2001 26(4):340–344. [PubMed: 11317075]
16. Hall CS, Charlebois ED, Hahn JA, Moss AR, Bangsberg DR. Hepatitis C virus infection in San Francisco's HIV-infected urban poor. *J Gen Intern Med.* Apr; 2004 19(4):357–365. [PubMed: 15061745]
17. George SL, Gebhardt J, Klinzman D, et al. Hepatitis C virus viremia in HIV-infected individuals with negative HCV antibody tests. *Journal of acquired immune deficiency syndromes (1999).* Oct 1; 2002 31(2):154–162. [PubMed: 12394793]
18. Pratt DS, Kaplan MM. Evaluation of abnormal liver-enzyme results in asymptomatic patients. *N Engl J Med.* Apr 27; 2000 342(17):1266–1271. [PubMed: 10781624]
19. Streiff MB, Mehta S, Thomas DL. Peripheral blood count abnormalities among patients with hepatitis C in the United States. *Hepatology.* Apr; 2002 35(4):947–952. [PubMed: 11915043]
20. Bartolome J, Lopez-Alcorocho JM, Castillo I, et al. Ultracentrifugation of serum samples allows detection of hepatitis C virus RNA in patients with occult hepatitis C. *Journal of virology.* Jul; 2007 81(14):7710–7715. [PubMed: 17475654]
21. Castillo I, Pardo M, Bartolome J, et al. Occult hepatitis C virus infection in patients in whom the etiology of persistently abnormal results of liver-function tests is unknown. *J Infect Dis.* Jan 1; 2004 189(1):7–14. [PubMed: 14702147]
22. Thomson EC, Nastouli E, Main J, et al. Delayed anti-HCV antibody response in HIV-positive men acutely infected with HCV. *AIDS (London, England).* Jan 2; 2009 23(1):89–93.

Figure 1.
Distribution of HCV RNA among individuals with seronegative HCV.

Figure 2.

Receiver-operating characteristic curve comparing summed clinical variables to occurrence of SN-HCV.

Figure 3.

Relationship of SN-HCV to site and number of clinical variables.

The utility of clinical variable numbers in distinguishing SN-HCV depends in part on the underlying population prevalence. Thus, at a site with low prevalence (“A”), the actual likelihood of SN-HCV remains relatively low even when multiple clinical variables (up to 4) are present. By contrast, at a site with high overall prevalence (“B”), the likelihood of SN-HCV increases steeply with each additional clinical variable.

Table 1
Demographic and disease characteristics of the CHARTER cohort and the subgroup assessed in this analysis.

	CHARTER (N = 1,355)	Current Analysis (N = 200)	p value	HCV Infection Status		p value
				Ab-/RNA+ (N = 26)	Ab-/RNA- (N = 174)	
Median age, y (+SD)	43±8	44 ± 8	0.6	46 ± 8.5	43 ± 8.3	0.16
Gender (Number (%) men)	1022 (75%)	170 (85%)	0.003	24 (92%)	146 (84%)	0.38
Race/ethnicity (%)			0.04			0.09
Black	673 (50%)	88 (44%)		16 (62%)	72 (42%)	
White	527 (39%)	85 (42%)		6 (23%)	79 (45%)	
Other	156 (11%)	27 (14%)		4 (16%)	23 (13%)	
CD4+ cell count						
Current (Median, (IQR), /mL)	427 (276, 609)	312 (132, 555)	<0.001	309 (149, 510)	312 (132, 558)	0.9
Nadir (Median, (IQR), /mL)	185 (54, 311)	88 (20, 219)	<0.001	87 (22, 203)	87 (20, 236)	0.65
Proportion below 200/ μ L	14%	33%	<0.001	31%	33%	0.79
HIV RNA in Plasma (% < 50 c/mL)	41%	39%	0.64	58%	36%	0.05
AIDS (Proportion)	61%	78%	<0.001	85%	77%	0.46
Taking ART (Proportion)	70%	76%	0.08	85%	75%	0.46
Reported history of IDU (Proportion)	19%	31%	0.3	57%	30%	0.02
AST > 50 IU/mL (Proportion)	18%	36%	<0.001	62%	33%	0.008
ALT > 50 IU/mL (Proportion)	23%	46%	<0.001	77%	41%	0.001
Platelets < 200K/ μ L (Proportion)	30%	42%	<0.001	69%	38%	0.005
Albumin < 4 g/dL (Proportion)	27%	46%	<0.001	54%	45%	0.53

Table 2

Logistic regression model simultaneously assessing clinical variables related to seronegative HCV infections.

Characteristics	OR	95% CI
History of IDU	6.95	2.1, 22.9
ALT > 50 mg/dL	5.49	1.5, 20
Platelets < 200k	4.15	1.3, 13
Detectable plasma HIV viral load	0.25	0.08-0.77
Black race	4.04	1.3, 12.5

Table 3

Summed clinical variables: Performance of cutpoints of 3 or 4 clinical variables in identifying seronegative HCV participants.

Cutpoint	Sensitivity	Specificity	Accuracy	Total Misclassified
3	0.85 (0.65, 0.96)	0.63 (0.56, 0.70)	0.66 (0.59, 0.72)	0.34 (0.28, 0.41)
4	0.62 (0.41, 0.8)	0.88 (0.82, 0.92)	0.84 (0.79, 0.89)	0.16 (0.11, 0.21)