Poster #0601

HCV antibody avidity-based method to estimate population-level incidence

Denali Boon[1], Veronica Bruce [2], Eshan U. Patel[1], Jeffrey Quinn[1], Aylur K. Srikrishnan[3], Shanmugam Saravanan[3], Syed Iqbal , Pachamuthu Balakrishnan[3], David L. Thomas[1], Thomas C. Quinn[4], Andrea Cox[1], Kimberly Page[2], Sunil S. Solomon[1], Shruti H. Mehta[1], Oliver Laeyendecker[4]

[1]Johns Hopkins University, Baltimore, MD, USA, [2]University of New Mexico, Albuquerque, NM, USA, [3]YR Gaitonde Center for AIDS Research and Education, Chennai, India, [4]National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA

Correspondence to:
Denali Boon
Postdoctoral Fellow
Johns Hopkins University
855 North Wolfe Street
Baltimore, MD 21205
Phone: (202)-631-7452
email: denali@jhu.edu

Background

- Accurate HCV incidence estimates are critically important to monitor epidemics, and to evaluate interventions.
- Traditionally, HCV incidence is estimated using long-term follow-up of persons at risk for infection, which is expensive, logistically difficult, resource intensive, time consuming, and subject to its own biases. Previous avidity-based approaches to estimate cross-sectional HCV incidence have been limited by their high cost and differential results between genotypes.
- Here, we modify and evaluate the inexpensive Genedia 3.0 HCV ELISA Assay using well-characterized samples from prospective cohort studies of persons who inject drugs (PWID) for the cross-sectional estimation of HCV incidence.

Methods

Study Design and Participants

- 1,840 HCV seropositive samples with HCV RNA ≥500 IU/mL collected from 875 individuals enrolled in 5 prospective cohort studies of PWID were used (Table 1).
- For 299 samples collected from 116 individuals with an observed HCV seroconversion (SC) event during follow-up, the date of SC was estimated. The date of SC for samples collected from HCV antibody positive individuals at enrollment could not be estimated, but samples were only selected from visits ≥2 years after enrollment.

Modified Genedia Assay

- Samples were incubated overnight (12-18 hours) in duplicate on a Genedia 3.0 HCV EIA plate (10 µL specimen in 200 µL sample diluent) on a plate shaker (500 rpm) at 22-25°C. The plate was washed 1x with 200 µL 1X wash buffer (WB). One well of each sample was treated with 300 µL of 11M urea to dissociate antigen-antibody complexes, while the second well was treated with 300 µL of 1X WB for 5 minutes at 22-25°C. The plate was washed 1x with 200 µL 1X WB. The dissociation step was repeated 2x. Following the third dissociation step the plate was washed 6x with 300 µL 1X WB. The remainder of the assay was performed per manufacturer's protocol.

Statistical Analysis

- Avidity Index (AI) = $\frac{\text{OD of Urea treated well}}{\text{OD of WB treated well}} \times 100\%$
- Mean duration of recent infection (MDRI) = the average time spent recently-infected using samples collected < a specified time cutoff (T = 2 years).
- False recent ratio (FRR) = proportion of long-term infected individuals > the time cutoff (>T) misclassified as 'recent'.

Methods cont.

- Factors associated with falsely appearing recent (AI <40%) among samples collected ≥2 years post-HCV SC were determined using modified Poisson regression incorporating generalized estimating equations and robust standard errors.
- The sample size needed to estimate HCV incidence using an AI cutoff of 40% with a relative standard error (RSE) of 30%, as guided by the WHO/UNAIDS Working Group on Cross-Sectional Incidence Assays was determined across a range of HCV incidence and HCV and HIV prevalence. For all precision calculations, the RSE of the MDRI and FRR were set to 14% and 33%, respectively.

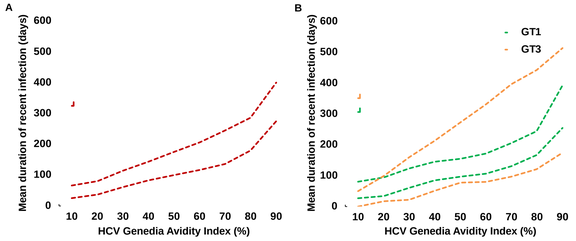
Results

Table 1. Characteristics of study population

Cohort	BBAASH		UFO		ALIVE		CHHEERS		MIDACS	
	Indiv. (%)	Samp. (%)	Indiv. (%)	Samp. (%)	Indiv. (%)	Samp. (%)	Indiv. (%)	Samp. (%)	Indiv. (%)	Samp. (%)
Follow-up frequency	monthly		quarterly		semiannually		semiannually		semiannually	
Total n	48	212	73	165	396	541	161	564	197	358
Duration of HCV infection (n)*										
<2 years	47	162	69	137	0	0	0	0	0	0
2+ years	25	50	15	28	396	541	161	564	197	358
Gender										
Female	39.5	33.9	31.5	21.2	27.3	26.2	0	0	0	0
Male	60.5	66.1	68.5	78.8	72.7	73.8	100	100	100	100
Race/Ethnicity										
Black	10.4	8.0	1.4	0.6	89.1	91.3				
Non-Black	89.6	92.0	98.6	99.4	10.9	8.7				
Tamilian							93.8	94.2	97.5	97.5
Non-Tamilian							6.2	5.8	2.5	2.5
Median Age	25		26		46		44		39	
HCV Genotype										
1	81.3	84.9	50.7	52.7	34.4	46.2	13	13.7	12.2	10.6
2	8.3	6.1	9.6	7.9	1.5	2.2	0	0	0	0
3	8.3	7.1	31.5	32.7	0.3	0.4	72.0	71.1	83.8	86
4	0	0	1.4	1.8	0	0	0.6	1.4	0	0
6	0	0	0	0	0	0	3.1	3.5	0.5	0.3
Unknown	2.1	1.9	6.8	4.9	63.9	51.2	11.2	10.3	3.6	3.1
HIV positive	2.1	0.5	1.4	0.6	30.1	27.9	31.7	43.1	24.4	15.4
Median CD4+ T-cell count (cells/µL) among HIV+ (IQR)	N/A		N/A		272 (148-423)		368 (279-498)		475 (351-606)	

All values shown are percentages unless otherwise indicated
*Some individuals contributed samples > and < 2 years post seroconversion
Abbreviations: BBAASH, Baltimore-Before and after Acute Study of Hepatitis; ALIVE, AIDS Linked to the IntraVenous Experience; CHHEERS, Chennai HIV, Hepatitis C and EEral Study; MIDACS, Madras Injection Drug User and AIDS Cohort Study; Indiv., individuals; Samp, samples; IQR, interquartile range

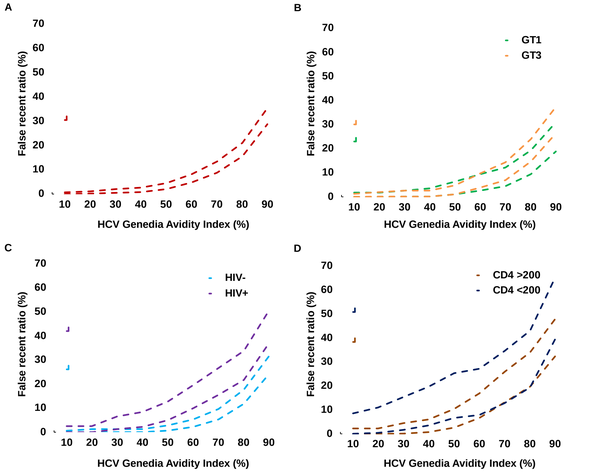
Figure 1. Mean duration of recent infection of Genedia-Avidity by AI



Mean duration of recent infection (MDRI) in days is shown by avidity index (AI) cutoffs in increments of 10%. MDRI (solid line) and 95% CI (dashed lines) for 306 samples from 116 individuals is shown in Panel A. The MDRI for 210 genotype (GT) 1 samples from 73 individuals (green), and 60 GT 3 samples from 26 individuals (orange) is shown in Panel B.

Results

Figure 2. False recent ratio of HCV Genedia-Avidity by AI



False recent ratio (FRR) is shown by avidity index (AI) cutoffs in increments of 10%. FRR (solid line) and 95% confidence interval (dashed lines) for 1541 samples from 794 individuals is shown in Panel A. FRR for 426 genotype (GT) 1 samples from 208 individuals (green), and 723 genotype 3 samples from 291 individuals (orange) is shown in Panel B. FRR for 1091 HIV-uninfected samples from 578 individuals (light blue), and 449 HIV-infected samples from 218 individuals (purple) is shown in Panel C. FRR for 274 HIV-infected samples from 163 individuals with a CD4 T-cell count >200 (brown), and 76 HIV-infected samples from 63 individuals with a CD4 T-cell count <200 (navy) is shown in Panel D.

Table 2. Factors associated with falsely appearing recent

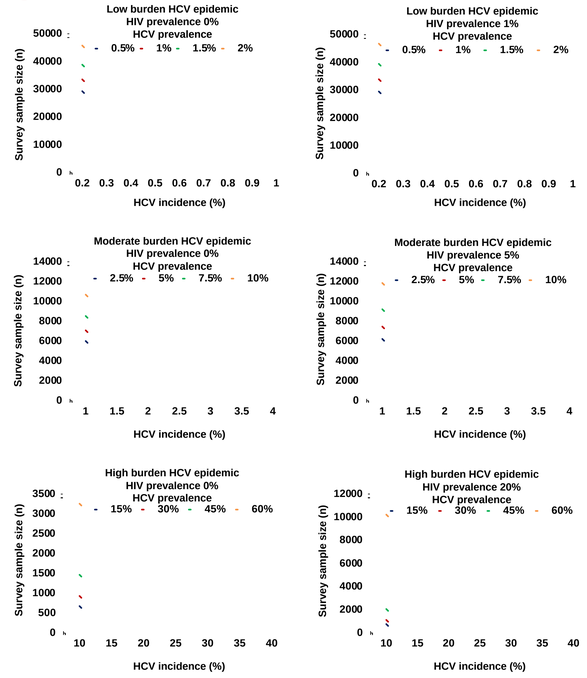
Characteristic	(n/N)	Univariable PR [95% CI]	Univariable P value	Multivariable PR [95% CI]	Multivariable P value
Gender					
Male	(19/1383)	(ref)			
Female	(3/158)	1.35 (0.35, 5.31)	0.663		
HCV Genotype					
1	(4/426)	(ref)		(ref)	
Other (2, 3, 4, 6)	(9/769)	1.52 (0.07, 34.41)	0.791	0.54 (0.02, 14.47)	0.712
HIV Status					
Negative	(5/1091)	(ref)		(ref)	
Positive CD4+ T-cell count ≥ 200 cells/µL	(6/274)	5.18 (1.49, 17.94)	0.01	3.05 (0.58, 15.90)	0.187
Positive CD4+ T-cell count < 200 cells/µL	(8/76)	21.27 (6.17, 73.37)	<0.001	22.0 (6.28, 77.01)	<0.001
Total	22/1541				

Prevalence ratios (PR) and 95% confidence intervals (CI) were calculated from modified Poisson regression models using generalized estimating equations to account for the clustering of samples within subjects, an exchangeable correlation structure, and a robust variance estimator to indicate associations with a Genedia-Avidity Index <40% among samples collected after two years post-HCV seroconversion. These data are indicative of misclassification of samples collected after two years post-HCV seroconversion as 'recent' infection. All models adjusted for cohort.

- Using an AI of <40% had a MDRI of 113 days (95%CI:84-146) (Figure 1), and FRR of 0.4% (95%CI:0.0-1.2) and 4.6% (95%CI:2.2-8.3) among HIV negative and positive individuals, respectively, and did not differ between HCV genotypes 1 and 3 (Figure 2).
- In hypothetical scenarios of high-risk settings, a sample size of <1000 individuals was necessary to reliably estimate HCV incidence with an RSE of <30% (Figure 3).

Results cont.

Figure 3. Precision to estimate HCV incidence



Sample sizes represent the total number of HCV seropositive and seronegative individuals required in a single cross-sectional survey to achieve an incidence estimate with a relative standard error (RSE) of 30%. HCV and HIV seroprevalence was varied to represent different epidemic states. The RSE for the mean duration of recent infection and false recent ratio was 14%, and 33%, respectively.

Conclusions

- This inexpensive cross-sectional approach can estimate HCV incidence for the most common genotypes. This tool can serve as a valuable resource for program and policy planners seeking to monitor and reduce the burden of HCV.

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