Rise in ALT after HCV Treatment is a Highly Sensitive Screen for Treatment Failure

SEARCH-1

N=51

SOF DCV

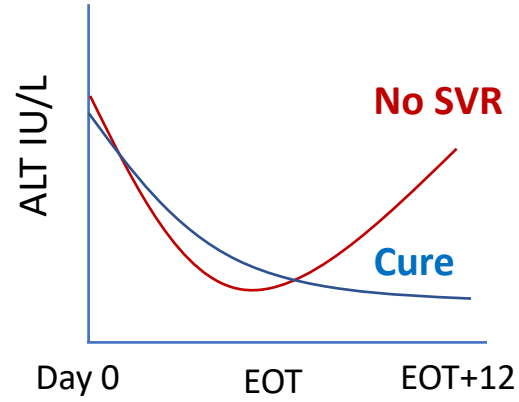
4-8 weeks
SVR 75%

STOPHCV-1

N=202

Ombitasvir
Paritaprevir
ritonavir

4-8 weeks
SVR 71%



WHY?

HCV RNA testing to confirm sustained virological response (SVR) after HCV treatment is technical and expensive, impeding simplification and decentralisation of HCV care.

We evaluated performance of change in levels of ALT and AST after end-of-treatment (EOT) for detecting treatment failure.

ANALYSIS 1

In a shortened treatment trial in Vietnam, we described how changes in ALT (ΔALT) and AST (ΔAST) from EOT to EOT+12 weeks compared in individuals who cured versus those that did not achieve SVR.

ANALYSIS 2

We then evaluated performance of ΔALT and ΔAST in a second, larger UK study population with differing demographics, comorbidities and genotypes, treated with different antivirals.

Participants in both studies had mild liver disease.

FINDINGS

Median ΔALT and ΔALT was higher in individuals who did not achieve SVR in both studies.

ΔALT was 100% sensitive (95% C.I. [93.7 – 100%] and 51% specific (42.4 - 59.7%) for detecting treatment failure, representing a highly sensitive, economical and practical screen for treatment failure in individuals with mild disease.

Rise in ALT after HCV treatment is a highly sensitive screen for treatment failure

Barnaby Flower*[1,2], Phuong Nguyen Thi Ngoc[1], Leanne McCabe[3], Chau Le Ngoc[1], Thu Vo Thi[1], Hang Vu Thi Kim[1], Thuan Dang Trong[1], Motiur Rahman[1,4], Guy Thwaites[1,4], Ann Sarah Walker[3,5,6], Le Manh Hung[7], Nguyen Van Vinh Chau[7], Graham S Cooke[ψ2], Jeremy Day[ψ1,4], on behalf of SEARCH and STOP-HCV investigators.

Corresponding author

[ψ] *Senior authors contributed equally*

AFFILIATIONS

1. Oxford University Clinical Research Unit (OUCRU), Ho Chi Minh City, Vietnam
2. Department of Infectious Disease, Imperial College London, UK
3. MRC Clinical Trials Unit at UCL, University College London, UK
4. Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, Oxford University, Oxford, United Kingdom
5. Nuffield Department of Medicine, University of Oxford, Oxford, UK
6. The National Institute for Health Research, Oxford Biomedical Research Centre, University of Oxford, Oxford, UK
7. Hospital for Tropical Diseases, Ho Chi Minh City

CORRESPONDING AUTHOR:

Barnaby Flower, Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam
bflower@oucru.org

KEY WORDS:

Hepatitis C, direct acting antivirals, ALT, SVR12, diagnostics

ABSTRACT

Background

Nucleic acid testing to confirm sustained virological response (SVR) after HCV therapy is technical, often expensive, and frequently unavailable where disease prevalence is highest. Alternative surrogate biomarkers merit evaluation.

Methods

In a short-treatment trial in Vietnam (SEARCH-1; n=52) we analysed how changes in alanine transaminase (ΔALT) and aspartate transaminase (ΔAST), from end of treatment (EOT) to EOT+12 weeks, related to SVR, defined as HCV RNA < lower limit of quantification 12 weeks after EOT. In a separate UK trial (STOPHCV1; n=202), we then tested the hypothesis that any elevation in ALT or AST between EOT and EOT12 is a sensitive screen for treatment failure.

Results

In SEARCH-1, among 48 individuals with data, 13 failed to achieve SVR. Median ΔALT and ΔAST were negative in cured patients but elevated when treatment failed [median ΔALT (IQR): -2 IU/L (-6, +2)] versus +17 IU/L (+7.5, +38) ($p< 0.001$). Amongst treatment failures, 12/13 had increase in ALT and 13/13 had increase in AST after EOT, compared with 12/35 in those cured. In STOPHCV1, 196/202 patients had evaluable data, of which 57 did not achieve SVR. A rise in ALT after EOT was 100% sensitive (95% C.I. [93.7 – 100%]) and 51% specific (42.4 - 59.7%) for detecting treatment failure. ΔAST >0 IU/L was 98.1% (89.9 - 99.9%) sensitive and 35.8% (27.3 - 45.1%) specific.

Interpretation

A rise in ALT or AST after HCV therapy is a highly sensitive screen for treatment failure in mild liver disease. This finding could reduce costs and complexity of managing HCV.

250 words

INTRODUCTION

WHO has called for a simplification of HCV care to improve access to treatment[1]. Pan-genotypic direct acting antivirals (DAAs) achieve cure rates of >95%, with cure defined by sustained virological response (SVR) on nucleic acid testing (NAT), 12-24 weeks after end of treatment (EOT). However, NAT is often expensive, particularly in resource-limited settings which shoulder the highest burdens of disease. In Vietnam, public sector NAT is priced at US$37-90[2] per test, and is not government-subsidised. NAT also involves technical expertise, requiring that samples are transported to specialised laboratories or tested with novel point of care platforms which are frequently unavailable. This impedes decentralisation of care. Alternative surrogate biomarkers merit evaluation.

Alanine aminotransferase (ALT) and aspartate transferase (AST) are non-specific markers of liver inflammation which are routinely tested before and after HCV treatment. Elevated pre-treatment ALT levels have been associated with slower virological response[3] and pre-treatment levels of both enzymes have been associated with failure to achieve SVR[4], though neither are reliably predictive. Both enzymes decline on therapy[5–7]and, in the pre-DAA era, a 'sustained biochemical response' was used as a surrogate for SVR[6,7]. Elevated ALT levels (greater than upper limit of normal) at EOT and EOT+12 weeks have also been associated with DAA treatment failure[8,9], but we found no published data evaluating how changes in ALT and AST after EOT relate to DAA outcomes or their sensitivity for detecting treatment failure.

Experimental treatment-shortening trials, which typically report cure rates <80%, provide an opportunity to compare biomarker responses in individuals who achieve SVR versus those that do not. In a treatment shortening study from Vietnam, we evaluated changes in liver enzymes after DAA therapy. We then analysed a larger UK study population to see if our findings were replicated.

METHODS

This diagnostic accuracy study was STARD compliant[10] (appendix table 1). Trial registrations and ethical permissions are provided with both published manuscripts[11,12].

In SEARCH-1 (Vietnam), genotype 1- or 6- infected adults with mild liver disease (FibroScan score ≤7.1kPa) received 4 or 8 weeks of sofosbuvir and daclatasvir (SOF/DCV) therapy according whether HCV RNA was below or above 500 IU/ml after two days treatment. HCV RNA was measured at regular intervals until end of follow up (EOT+12 weeks) or until treatment failure if it occurred first (appendix figure 1 & 3). Of 52 adults recruited, 34 received 4 weeks SOF/DCV, 17 received 8 weeks, and one

withdrew. SVR12 was achieved in 38/51 (75%). 13 (25%) experienced virological relapse (between 21 and 84 days after EOT) and commenced retreatment within 2 weeks.

ALT and AST were measured at baseline and at EOT in all participants, at start of retreatment in those with virological relapse, and at EOT+12 in those without evidence of treatment failure. We analysed change in ALT and AST from EOT to EOT+12 (ΔALT and ΔAST) in participants who cured, and from EOT to retreatment day zero (RTD0) in participants who failed treatment. Patients with ALT or AST greater than twice the upper limit of normal (>2xULN) at EOT were excluded on the basis that this would ordinarily prompt HCV RNA testing[13]. We calculated median ΔALT and ΔAST (and interquartile ranges) in patients according to whether their treatment was successful or unsuccessful, and used Wilcoxon's Rank Sum test to compare outcomes. We also compared enzyme levels at baseline, EOT and decline on treatment, and performed genotype-specific analysis. We evaluated sensitivity and specificity of any increase in ALT (ΔALT >0 IU/L) or any increase in AST (ΔAST>0 IU/L) compared to gold standard of HCV RNA >LLOQ at EOT+12 (or nearest available timepoint).

In a second study population we tested the hypothesis that any increase in ALT or AST between EOT and EOT+12 is a sensitive marker of treatment failure. STOPHCV1 (UK) was a randomised trial which assessed variable ultrashort-course treatment (4 - 8 weeks based on pre-treatment viral load) versus 8 weeks fixed-duration therapy with ombitasvir, paritaprevir, ritonavir +/- dasabuvir, +/- ribavirin (1:1) [14]. Of 199 individuals under follow up until EOT+12, SVR12 was achieved in 141 (71%), with 58 individuals experiencing virological rebound at or before this timepoint (appendix figure 2 & 4). ALT and AST were tested at baseline and EOT in all participants, and at start or retreatment or EOT+12 in those with or without evidence of virological rebound, respectively. We repeated the performance analysis of ΔALT and ΔAST used in SEARCH-1.

RESULTS

Patient characteristics are shown in appendix table 2. Both study populations had mild liver disease (Fibroscan scores ≤7.1kPa), and median ALT and AST levels within the normal range. Study populations differed in terms of genotypes, gender, ethnicity, HIV co-infection and intravenous drug use.

In SEARCH-1, ALT and AST data was available for 48 participants (figure 1) and data for treatment failures was from a median 10 weeks after EOT (IQR = 6, 10). Median ΔALT and ΔAST were negative in cured patients but elevated when treatment failed [median ΔALT (IQR): -2 IU/L (-6, +2)] versus +17 IU/L (+7.5, +38) ($p< 0.001$). Difference was significant in genotype 6 and non-6 infections (appendix table 3) but we found no evidence of difference between groups in ALT or AST levels at baseline, EOT

or in transaminase decline on therapy (appendix table 4). 12/13 and 13/13 patients who did not achieve SVR had an increase in ALT and AST between EOT and EOT+12, compared with 12/35 who cured. The one patient who did not have a rise in ALT accompanying virological rebound at EOT+12 (HCV RNA = 3390 IU/ml; ΔALT = -1 IU/L) had a clear rise in ALT at EOT+14 weeks (RTD0; HCV RNA = 125,032 IU/ml; ΔALT +46 IU/L).

In STOPHCV-1, 197 had evaluable ALT data and one patient was excluded on pre-specified grounds of having an ALT rise >2xULN at EOT. 139/196 (71%) achieved SVR and 57 (29%) did not. ALT data for treatment failures were from a median 10 weeks after EOT (IQR = 8, 13). An increase in ALT (ΔALT >0 IU/L) after EOT was 100% sensitive (95% C.I. 94 - 100) and 51% specific (95% C.I. 42 - 60) for detecting treatment failure. A total of 173 participants had AST data (87%). ΔAST >0 IU/L was 98% (89.9 – 100%) sensitive and 36% (27.3 – 45.1) specific. Only one patient did not have a corresponding rise in AST around time of treatment failure: virological rebound was detected at EOT+6 weeks, and ΔAST was 0 IU/L at EOT+8 weeks (RTD0). AUROCs demonstrated ΔALT and ΔAST are excellent markers for identifying treatment failure (appendix figures 5 & 6) with a negative predictive value exceeding 98% with standard rates of cure.

DISCUSSION

In mild liver disease, an increase in ALT or AST >0 IU/L within median 10 weeks after EOT is highly sensitive for detecting treatment failure. This represents important proof of concept that could have a major impact in reducing treatment costs and decentralising care. ALT testing is cheap ($2-5 in Vietnam), does not entail additional visits or investigations, and can be performed in most laboratories. Novel point of care ALT tests from finger prick specimens could negate the need for a lab entirely[15]. Assuming a PPV of 46% (figure 1), a NPV of 100%, and a cure rate of 95%, this screening strategy would reduce NAT by 51%. In Vietnam this translates to a saving of US$18-46,000 per thousand patients treated (equivalent to approximately 36-90 courses of DAA therapy). This strategy could be used alongside emerging point-of-care HCV RNA platforms to facilitate treatment at primary health facilities or harm reduction sites[1].

The main strength of our study is that our findings were replicated in two independent populations, with differing demographics, genotypes and antivirals. A higher median ΔALT and ΔAST after EOT in individuals not achieving SVR was observed in the UK study population in which alcohol abuse, illicit substance abuse and HIV co-infection were more commonly reported (Appendix table 2). A major limitation is that all participants had mild liver disease and were treated with short-duration therapy.

Our findings should be tested in the context of cirrhosis (where transaminase dynamics are altered and consequences of a false negative result may be more serious) as well as with standard treatment durations with other WHO-approved antiviral regimens. In addition, timing of ALT/AST testing was earlier than EOT12 in most patients who failed to achieve SVR (median 10 weeks after EOT (IQR 8,13). Despite these limitations, our data indicate that an increase in ALT or AST >0 IU/L after EOT is a highly sensitive marker of treatment failure, with potential to reduce costs and complexity of HCV care.

1418 words

Authors Contributions

BF designed the study, conducted the analysis, and wrote the manuscript. PNTN conducted the analysis. LM curated and verified the data and assisted the analysis. CLN, TVT, HVTK, TDT, MR oversaw data collection and review of manuscript. GT, ASW, LMH, NVVC, JD provided study oversight. GC and ASW designed the clinical trials. GC and JD provided study oversight and assisted with writing and review of the manuscript. All authors have reviewed and approved the final manuscript.

Funding:

This work was supported by the Medical Research Council (grant MR/P025064/1) and The Global Challenges Research Fund (Wellcome Trust Grant 206/296/Z/17/Z). GC is supported in part by the NIHR Biomedical Research Centre of Imperial College NHS Trust and an NIHR Professorship. BF received a travel grant to attend AASLD conference in Washington DC from Gilead Sciences in 2017. ASW is a NIHR Senior Investigators. LM and ASW are supported by core support from the Medical Research Council UK to the MRC Clinical Trials Unit [MC_UU_00004/03]. The views expressed are those of the author(s) and not those of the NIHR or the Department of Health and Social Care.

Conflict of Interests

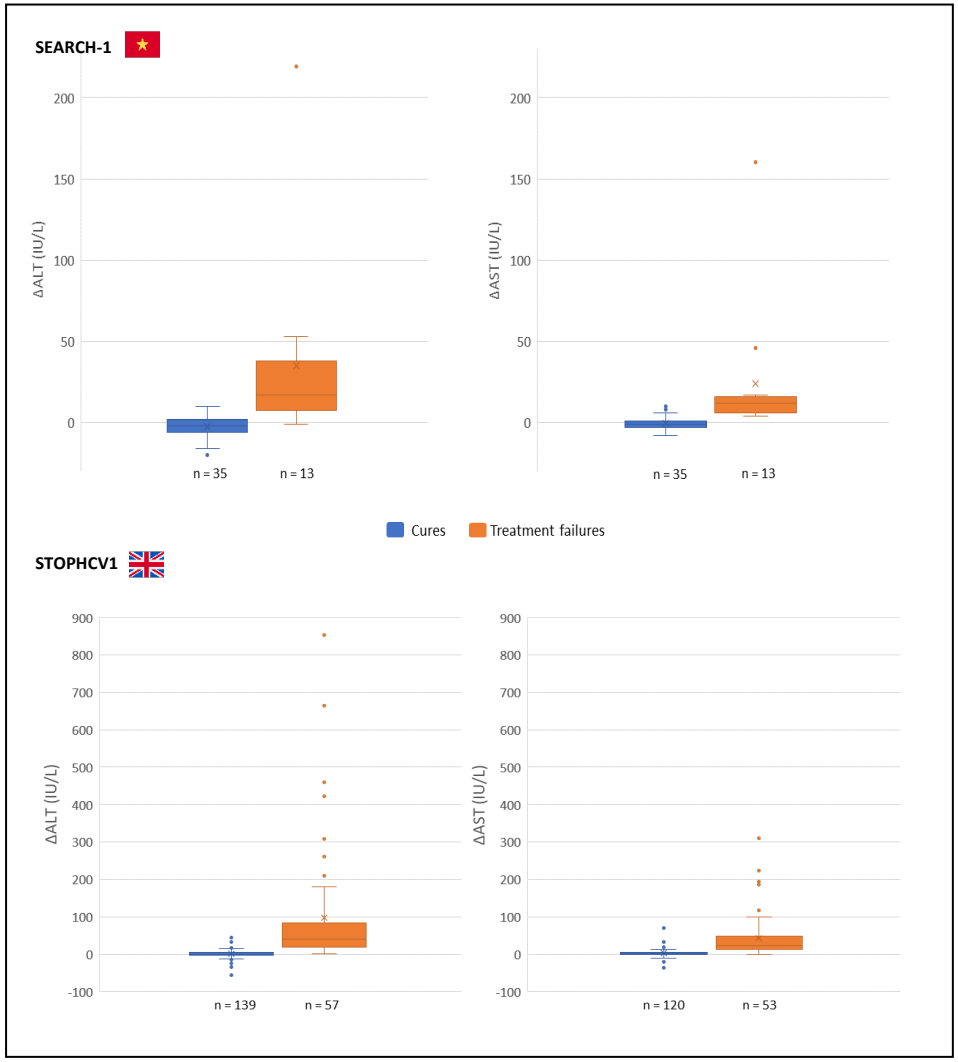
No authors report conflicts of interest relating to this work.

Acknowledgements

We would like to thank the patients of the Hospital for Tropical Disease, Ho Chi Minh City for their participation in SEARCH-1 and the NHS patients who participated in STOPHCV1 across multiple sites in the UK. We are also grateful to the hard-working staff in both trials for their diligent collection of data.

Figure 1: Performance analysis of ΔALT and ΔAST from EOT to EOT+12 in cures and treatment failures in SEARCH-1 and STOPHCV1

		SEARCH-1	STOPHCV1
ALT	ALT data	48	196
	Cures	35	139
	Treatment failures	13	57
	Median ΔALT (IQR) Cures	-2 IU/L (-6, +2)	0 IU/L (-2, +5)
	Median ΔALT (IQR) Failure	+17 IU/L (+8, +38)	+41 IU/L (+20, +85)
	Sensitivity (95% C.I.)	92% (64 – 99.8)	100% (93.7, 100%)
	Specificity (95% C.I.)	66% (48 – 81%)	51.1% (42.4 - 59.7%)
	PPV (95% C.I.)	50% (29 – 71%)	46% (37 – 55%)
	NPV (95% C.I.)	96% (79 - 100)	100% (95 – 100%)
	AUROC (95% C.I.)	0.95 (0.87-1.00)	0.96 (0.94 -0.99)
AST	AST data	48	173
	Cures	35	120
	Treatment failures	13	53
	Median ΔAST (IQR) Cures	-1 IU/L (-3, +1)	+2 IU/L (-1, +5)
	Median ΔAST (IQR) Failure	+12 IU/L (+6, +16)	+23 IU/L (+13, +49)
	Sensitivity (95% C.I.)	100% (75 – 100%)	98.1% (89.9, 99.9%)
	Specificity (95% C.I.)	66% (48 – 81%)	35.8% (27.3, 45.1%)
	PPV (95% C.I.)	52% (31 -72%)	40% (32 – 49%)
	NPV (95% C.I.)	100% (85 – 100%)	98% (88 – 100%)
	AUROC (95% C.I.)	0.96 (0.91 - 1.00)	0.92 (0.88 - 0.96)



REFERENCES

1. WHO. *Updated Recommendations on Simplified Service Delivery and Diagnostics for Hepatitis c Infection*.; 2022. https://www.who.int/publications/i/item/9789240052697.

2. Clinton Health Access Initiative Market Memo 2022. Hepatitis C Market Report. 2022;15(1):2020-2023.

3. Etzion O, Dahari H, Yardeni D, et al. Response guided therapy for reducing duration of direct acting antivirals in chronic hepatitis C infected patients: a Pilot study. *Sci Rep*. 2020;10(1):17820. doi:10.1038/s41598-020-74568-x

4. Xie Z, Deng K, Xia Y, et al. Efficacy and safety of direct-acting antiviral therapies and baseline predictors for treatment outcomes in hepatitis C patients: A multicenter, real-world study in Guangdong, China. *J Med Virol*. 2022;94(9):4459-4469. doi:https://doi.org/10.1002/jmv.27851

5. Huynh T, Zhang J, Hu K-Q. Hepatitis C Virus Clearance by Direct-acting Antiviral Results in Rapid Resolution of Hepatocytic Injury as Indicated by Both Alanine Aminotransferase and Aspartate Aminotransferase Normalization. *J Clin Transl Hepatol*. 2018;6(3):258-263. doi:10.14218/JCTH.2018.00014

6. McHutchison JG, Gordon SC, Schiff ER, et al. Interferon Alfa-2b Alone or in Combination with Ribavirin as Initial Treatment for Chronic Hepatitis C. *N Engl J Med*. 1998;339(21):1485-1492. doi:10.1056/NEJM199811193392101

7. Reichard O, Andersson J, Schvarcz R, Weiland O. Ribavirin treatment for chronic hepatitis C. *Lancet*. 1991;337(8749):1058-1061. doi:10.1016/0140-6736(91)91707-2

8. Naravadi VVR, Gonzalez B, Cotler S, Loy V. Normalization of Alanine Aminotransferase Levels at the End of Treatment with Direct-Acting Antiviral Therapy for Chronic Hepatitis C Infection Is Associated with Sustained Virological Response: 803. *Off J Am Coll Gastroenterol | ACG*. 2016;111. https://journals.lww.com/ajg/Fulltext/2016/10001/Normalization_of_Alanine_Aminotransferase_Levels.803.aspx.

9. Tacke F, Boeker KHW, Klinker H, et al. Baseline risk factors determine lack of biochemical response after SVR in chronic hepatitis C patients treated with DAAs. *Liver Int*. 2020;40(3):539-548. doi:https://doi.org/10.1111/liv.14186

10. Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig L, LijmerJG Moher D, Rennie D, de Vet HCW, Kressel HY, Rifai N, Golub RM, Altman DG, Hooft L, Korevaar DA, Cohen JF F the SG. *STARD 2015: An Updated List of Essential Items for Reporting Diagnostic Accuracy Studies.*; 2015.

11. Flower B, Hung LM, Mccabe L, et al. Efficacy of ultrashort, response guided sofosbuvir and daclatasvir therapy for Hepatitis C: a single arm, mechanistic, pilot study. *medRxiv*. January 2022:2022.08.15.22278752. doi:10.1101/2022.08.15.22278752

12. Cooke GS, Pett S ML et al. Strategic treatment optimization for HCV (STOPHCV1): a randomised controlled trial of ultrashort duration therapy for chronic hepatitis C [version 2; peer review: 2 approved]. *Wellcome Open Res*. 2021;6:93. https://doi.org/10.12688/wellcomeopenres.16594.2.

13. AASLD-IDSA. HCV Guidance: Recommendations for Testing, Managing, and Treating Hepatitis C. 2021. https://www.hcvguidelines.org/sites/default/files/full-guidance-pdf/AASLD-IDSA_HCVGuidance_October_05_2021.pdf.

14. Cooke GS, Pett S ML et al. Strategic treatment optimization for HCV (STOPHCV1): a randomised controlled trial of ultrashort duration therapy for chronic hepatitis C. *[version 1; peer Rev 1 Approv 1 Approv with Reserv Wellcome Open Res /wellcomeopenres165941)*. 2021;6:93. doi:https://doi.org/10.12688

15. Howell J, Van H, Pham M, et al. Validation of novel point-of-care test for alanine aminotransferase measurement: A pilot cohort study. *Clin Liver Dis*. 2022;20(4):136-139. doi:https://doi.org/10.1002/cld.1261

Appendix

Rise in ALT after HCV treatment is a highly sensitive screen for treatment failure; Flower et al.

Appendix Table 1: STARD Checklist for reporting diagnostic accuracy studies

Section & Topic	No	Item	Reported on page #
TITLE OR ABSTRACT			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	Title. page 1
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	Abstract, page 2
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	Introduction, p3
	4	Study objectives and hypotheses	
METHODS			
Study design	**5**	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	Methods, p3-4
Participants	**6**	Eligibility criteria	Methods, p3-4
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	Methods, p3-4
	8	Where and when potentially eligible participants were identified (setting, location and dates)	Methods, p3-4, published trial papers
	9	Whether participants formed a consecutive, random or convenience series	Methods, p4, published trial papers
Test methods	**10a**	Index test, in sufficient detail to allow replication	Methods, p4, published trial papers
	10b	Reference standard, in sufficient detail to allow replication	Methods, p4, published trial papers
	11	Rationale for choosing the reference standard (if alternatives exist)	Methods p4
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory	Methods p4
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory	Methods, p4
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test	Methods, Appendix figures 1 & 2
	13b	Whether clinical information and index test results were available to the assessors of the reference standard	Methods, p4, Appendix figures 3 & 4
Analysis	**14**	Methods for estimating or comparing measures of diagnostic accuracy	Methods, p4
	15	How indeterminate index test or reference standard results were handled	N/A
	16	How missing data on the index test and reference standard were handled	Methods, Appendix figures 1 & 2
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	Methods, Appendix figures 1 & 2

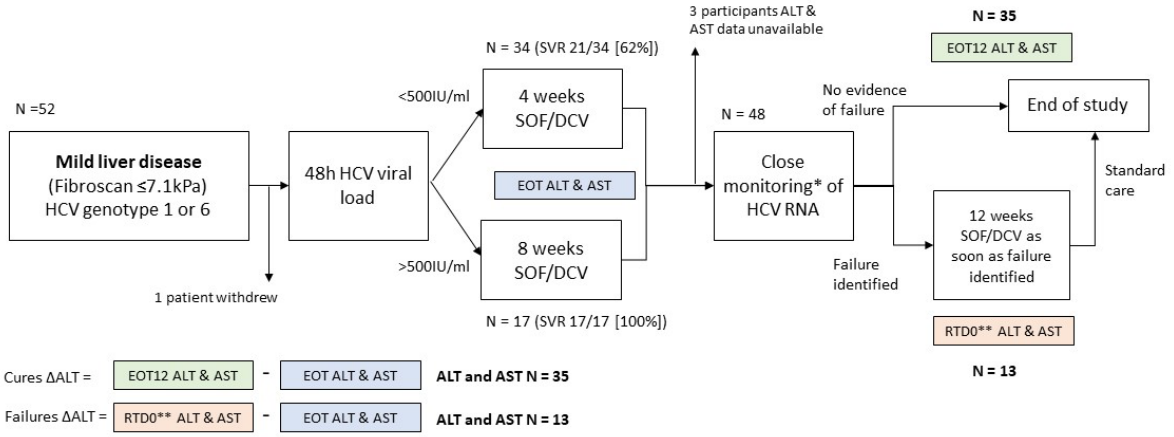
	18	Intended sample size and how it was determined	Published trial papers
RESULTS			
Participants	**19**	Flow of participants, using a diagram	Appendix figures 3 & 4
	20	Baseline demographic and clinical characteristics of participants	Appendix table 1
	21a	Distribution of severity of disease in those with the target condition	Appendix table 1
	21b	Distribution of alternative diagnoses in those without the target condition	Appendix table 1
	22	Time interval and any clinical interventions between index test and reference standard	Appendix figures 1 & 2
Test results	**23**	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	Appendix figures 3 & 4
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	Figure 1
	25	Any adverse events from performing the index test or the reference standard	N/A
DISCUSSION			
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	Discussion p6
	27	Implications for practice, including the intended use and clinical role of the index test	Discussion, p6
OTHER INFORMATION			
	28	Registration number and name of registry	In original papers
	29	Where the full study protocol can be accessed	Both study potocols available with published papers
	30	Sources of funding and other support; role of funders	Acknowledgements

Appendix Table 2: participant characteristics at enrolment in SEARCH-1 (Vietnam) and STOPHCV1 (UK)

	SEARCH-1 (VN)	STOPHCV1 (UK)
Total participants	52	202
Age (years)	49.5 (39.5, 59.0)	45.5 (37.5, 53.0)
Female at birth	29 (56%)	62 (31%)
Weight	55.4 (51.5, 64.9)	74.0 (66.0, 84.6)
BMI (kg/m^2)	23.3 (20.8, 25.1)	24.9 (22.2, 27.2)
White ethnicity	0 (0%)	176 (87%)
Vietnamese Asian	52 (100%)	
Enrolment HCV viral load (IU/ml) (n=199 in STOPHCV-1)	1,932,775 (618, 11,200,000)	741,946 (249,097,1872136)
HCV genotype/subgenotype:		
1a	11 (21%)	166 (82%)
1b	12 (23%)	34 (17%)
2	1 (2%)	0 (0%)
3	0 (0%)	2 (1%)
4	1 (2%)	0 (0%)
6	27 (53%)	0 (0%)
HIV coinfected	0 (HIV excluded)	68 (34%)
Fibroscan result (kPa)	6.0 (5.0, 6.6)	4.9 (4.2, 5.8)
ALT (IU/L)	39 (26, 66)	52 (34, 87)
AST (IU/L) (n=189 in STOPHCV-1)	32 (25, 47)	38 (30, 57)
Current/recent alcoholism/alcohol abuse	4 (8%)	13 (6%)
Current/recent illicit substance abuse	4 (8%)	64 (32%)
Treated with sofosbuvir + daclatasvir	52 (100%)	-
Treated with paritaprevir + ombitasvir + dasabuvir	-	198 (98%)
Treated with paritaprevir + ombitasvir	-	2 (1%)
Treated with glecaprevir + pibrentasvir	-	2 (1%)
Withdrew or lost to follow up before EOT	1	3
ALT data not available	3	2
AST data not available	3	22
ALT or AST >2xULN at EOT warranting exclusion	0	1
Total number with ΔALT analysed	48 (92%)	196 (97%)*
Total number with ΔAST analysed	48 (92%)	173 (86%)
Timing of RTD0 in treatment failures (weeks from EOT)	10 (6,10)	11 (8, 13)

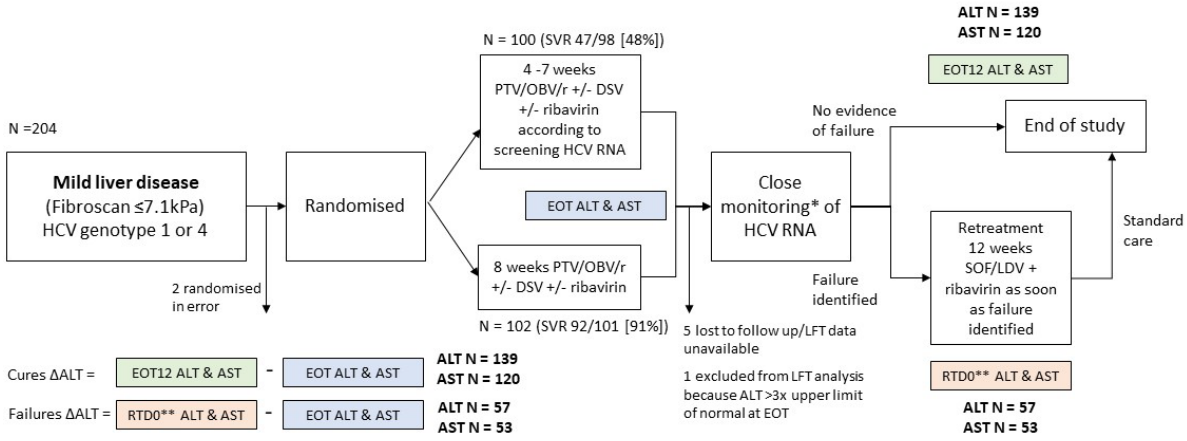
*Note: showing n (%) for categorical factors, or median (IQR) for continuous factors. Missing data indicated by denominators in the row label. *3/135 individuals had EOT24 ALT data but no EOT12 ALT data.*

Appendix figure 1: SEARCH-1 flow diagram



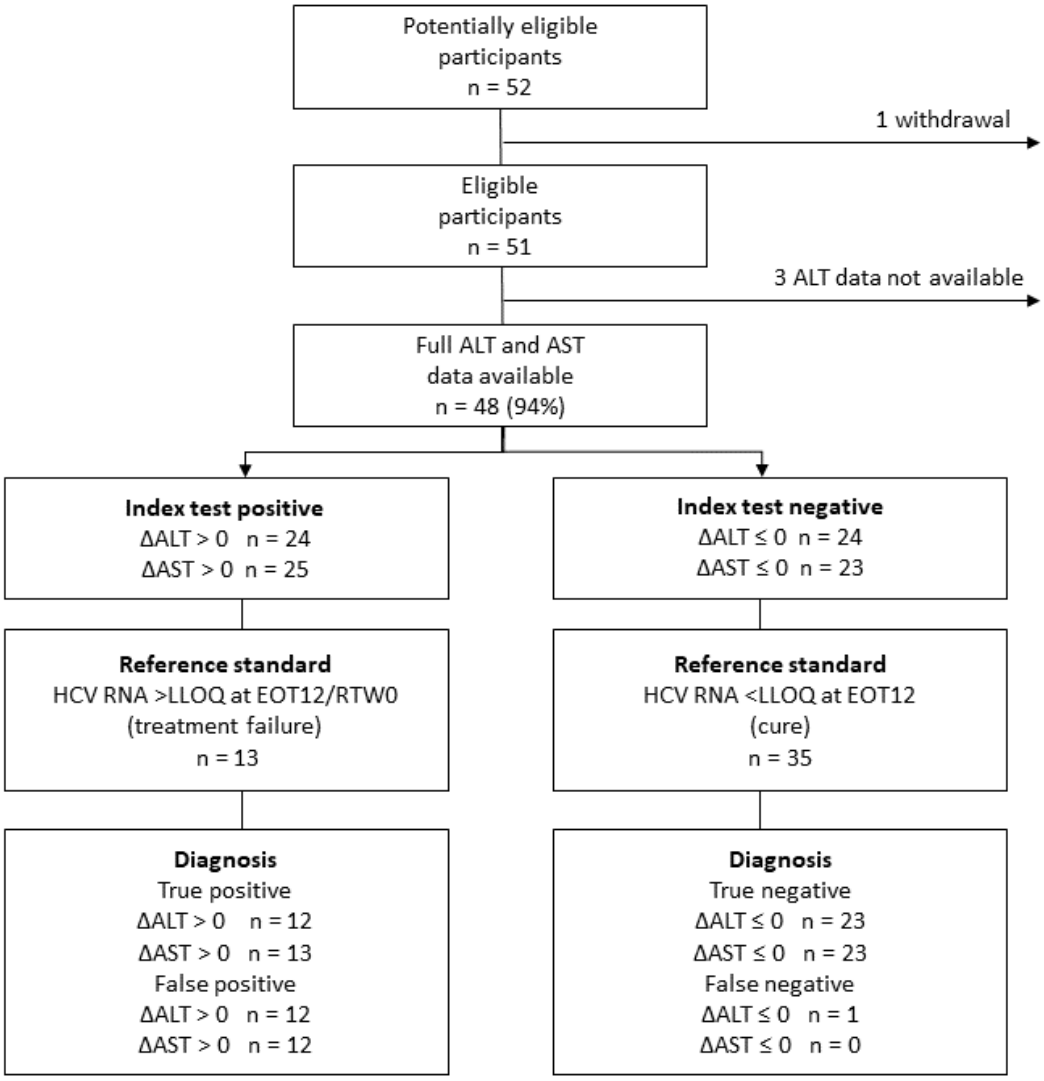
Of 52 adults enrolled, 34 received 4 weeks SOF/DCV, 17 received 8 weeks and one withdrew. SVR12 was achieved in 21/34 (62%) treated for 4 weeks, and 17/17 (100%) treated for 8 weeks, equating to 38 cures and 13 treatment failures overall. LFT data were available for 48 participants (35 cures and 13 treatment failures). ΔALT was calculated as change in ALT from EOT to EOT+12 weeks in those without evidence of treatment failure during EOT monitoring (cures; n= 35), and from EOT to retreatment day 0 (RTD0) in those experiencing virological rebound during EOT monitoring (n=13). Timing of RTD0 lay between EOT+6weeks and EOT+14weeks in the 13 participants failing treatment.

Appendix figure 2: STOPHCV1 flow diagram



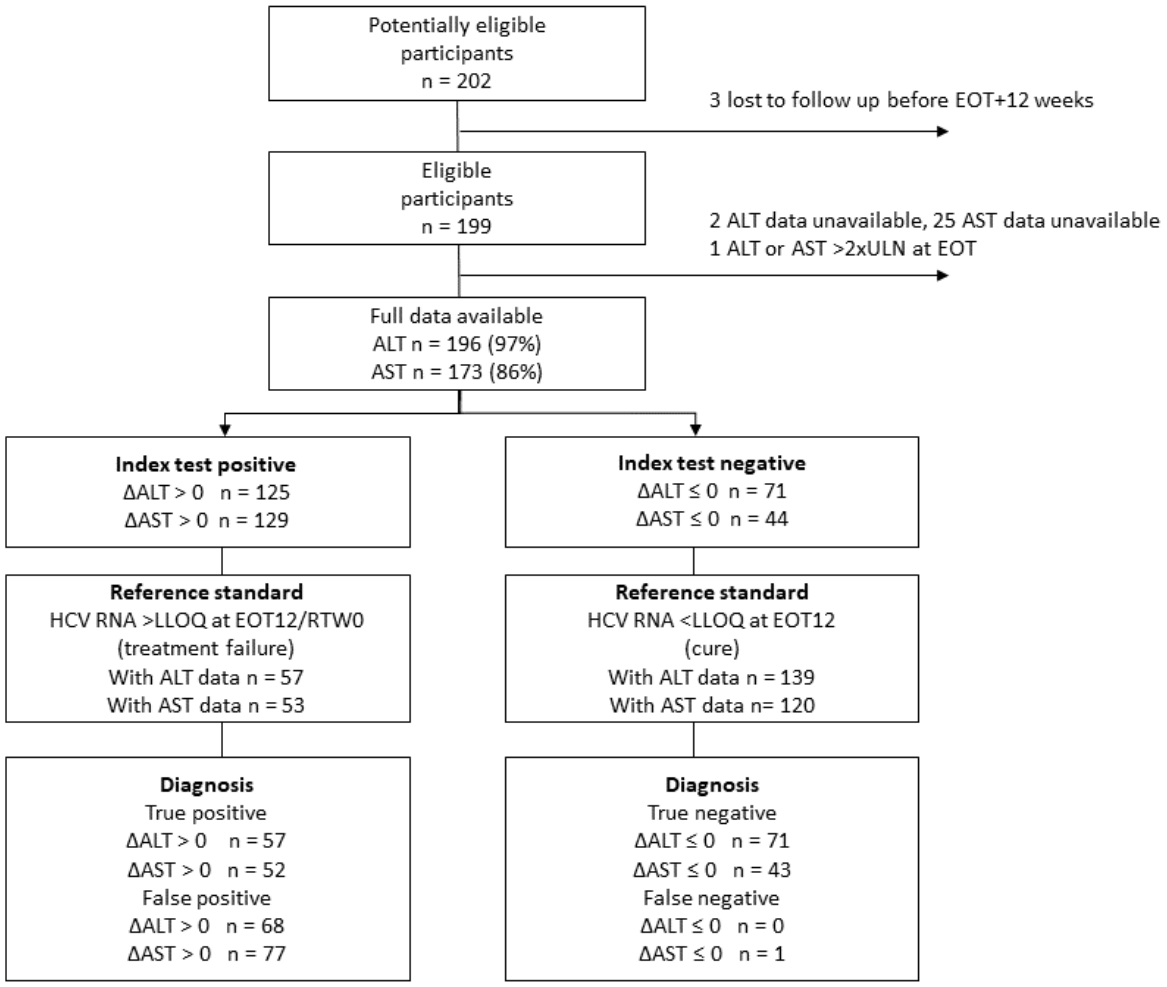
204 participants were enrolled. Two individuals were randomised in error, leaving 202 participants. 100 were randomised to receive variable ultrashort-course treatment with ombitasvir(OBV)/paritaprevir(PTV)/ritonavir(r) +/- dasabuvir(DSV) (49 with ribavirin and 51 without ribavirin), and 102 were randomised to receive 8 weeks fixed-dose therapy with the same antivirals (51 with ribavirin and 51 without ribavirin). Three individuals were lost to follow up and one experienced an increase in ALT on treatment >2xULN so was excluded from this analysis. Of the remaining 196 participants, 139 achieved SVR12 and 57 experience virological rebound during EOT follow up, commencing retreatment with sofosbuvir and ledipasvir as soon as possible. ΔALT was calculated as change in ALT from EOT to EOT+12 weeks in those without evidence of treatment failure during EOT monitoring (cures; n= 139), and from EOT to RTD0 in those with virological rebound (treatment failure; n=57). Timing of RTD0 lay between EOT+7weeks and EOT+42weeks in the 57 participants failing treatment.

Appendix figure 3: STARD diagram for flow of participants through SEARCH-1



LLOQ – lower limit of quantification; EOT12 – end of treatment +12 weeks; RTW0 – retreatment week 0

Appendix figure 4: STARD diagram for flow of participants through STOPHCV1



LLOQ – lower limit of quantification; EOT12 – end of treatment +12 weeks; RTW0 – retreatment week 0

Appendix table 3: Comparison of baseline and EOT ALT and AST in cures and those experiencing virological rebound in SEARCH-1 and STOPHCV1.

DATA	INDEX	TIMEPOINT	Cures		Treatment Failure		P value*
			N	Median (IQR)	N	Median (IQR)	
SEARCH DATA	ALT	Baseline	35	48 (24 - 76)	13	36 (24 - 41)	0.275
		EOT	35	13 (11 - 22)	13	13 (10 - 15.5)	0.464
		Baseline to EOT	35	-28 (-49, -11)	13	-19 (-28.5, -15)	0.437
	AST	Baseline	35	33 (25 - 47)	13	28 (24.5 - 41)	0.472
		EOT	35	18 (15 - 20)	13	19 (14.5 - 21)	0.981
		Baseline to EOT	35	-14 (-27, -8)	13	-10 (-20.5, -7.5)	0.430
STOP DATA	ALT	Baseline	139	55 (31 - 88)	57	50 (34 - 90)	0.885
		EOT	139	18 (13 - 23)	57	17 (14 - 21.5)	0.712
		Baseline to EOT	139	-35 (-67, -18)	57	-32 (-58, -20)	0.828
	AST	Baseline	117	39 (31 - 58)	51	39 (29 - 55)	0.674
		EOT	117	20 (17 - 24)	51	20 (17 - 26)	0.794
		Baseline to EOT	117	-19 (-38.5, -9)	51	-19 (-31,-10)	0.898

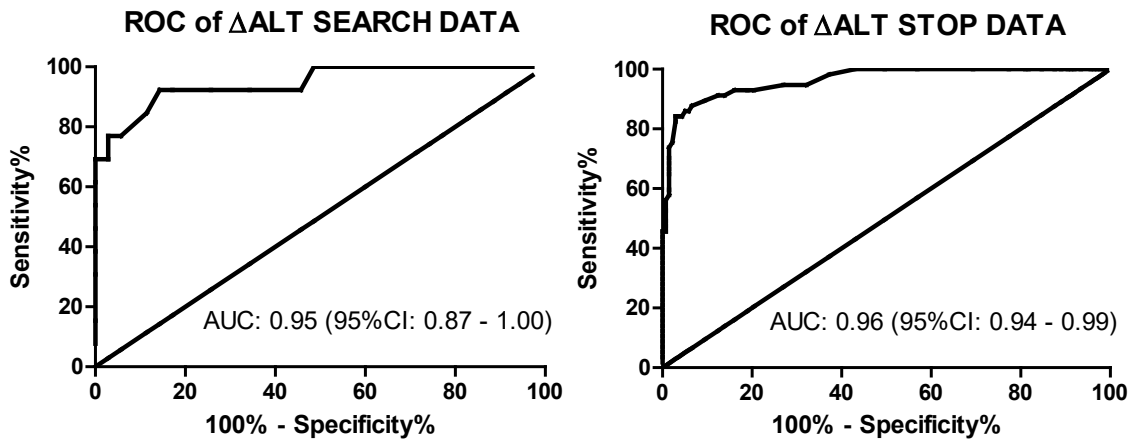
** Analyses performed using Wilcoxon rank-sum test*

Appendix table 4: Performance analysis with regards to infecting HCV genotype in SEARCH-1 and STOPHCV1

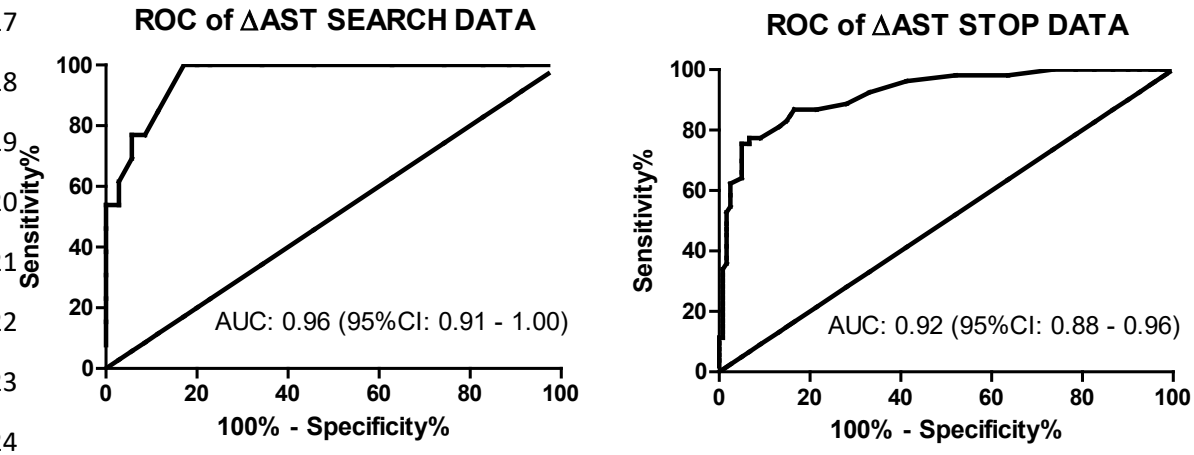
DATA	Genotype	Group	n	ΔALT	*p* *	n	ΔAST	*p**
SEARCH1	non-G6	Cure	17	0 (-3 - 2)	< 0.001	17	-1 (-2 - 1.5)	< 0.001
		Fail	7	+22 (+12 - +38)		7	+12 (5 - 14)	
	G6	Cure	18	-2.5 (-7.25 - +1.25)	< 0.001	18	-1 (-4 - 1.8)	< 0.001
		Fail	6	+12.5 (+5.5 - +95)		6	+12 (7 - 74.5)	
STOPHCV1	1a	Cure	108	+1 (-2 - +5)	< 0.001	91	+2 (-1 - 6)	< 0.001
		Fail	52	+42 (+20 - +124)		48	+23 (10 - 54)	
	1b	Cure	27	-1 (-5 - 3)	< 0.001	26	+1 (0 - 2.3)	< 0.001
		Fail	7	41 (19 - 60)		7	+22 (14 - 30)	

**Analyses performed using Wilcoxon rank-sum test*

Appendix figure 5: Receiver operator curves for ΔALT in SEARCH1 and STOPHCV1



Appendix figure 6: Receiver operator curves for ΔAST in SEARCH1 and STOPHCV1



END OF APPENDIX