Simplified diagnostic monitoring for hepatitis C, in the new era of direct-acting antiviral treatment

Jennifer Cohn, Teri Roberts, Valerianna Amorosa, Maud Lemoine, and Andrew Hill

**Purpose of review**
Approximately 150–175 million people are infected with hepatitis C virus (HCV). Until very recently, the complexity, cost and poor efficacy and tolerability of pegylated interferon and ribavirin (PEG-RBV) treatment have hindered scale up in low-income and middle-income countries (L&MICs). Similarly, the diagnostic and monitoring algorithm associated with PEG-RBV has been expensive and complicated because of the poor efficacy and frequency of adverse drug effects of PEG-RBV therapy. This article provides an overview of the potential changes to the diagnosis and monitoring algorithm and describes key promising tools in the diagnostics pipeline.

**Recent findings**
Interferon-free direct-acting antiviral (DAA) therapy sets the stage to significantly simplify laboratory requirements and make the overall diagnostic package much less expensive. Diagnostic simplification and cost-reduction will be key to enable implementation of HCV screening and treatment in L&MICs.

**Summary**
There is the potential to introduce simplified monitoring for hepatitis C. Antigen testing could be used as a replacement for HCV RNA PCR tests, to establish active infection and then to prove cure after stopping DAA treatment. If new DAA treatments can be shown to be pan-genotypic, genotyping may no longer be required.

**Keywords**
access, diagnosis, hepatitis C virus, scale up, simplification

**INTRODUCTION**
Globally, approximately 150 million people are infected with hepatitis C virus (HCV), and it is estimated that up to 700 000 people die each year from HCV-related liver disease – predominantly from either liver cirrhosis or hepatocellular carcinoma [1,2]. Summary results are shown in Fig. 1 [2*], based on the recently published Global Burden of Disease report [2*]. Worldwide, viral hepatitis (from hepatitis B virus and HCV combined) is causing more deaths than HIV/AIDS.

There is a high burden of hepatitis C in low-income and middle-income countries (L&MICs), including resource-constrained settings such as India and Central Africa [3]. In these countries, the standard treatment is pegylated interferon and ribavirin (PEG-RBV). This is a complex treatment that involves significant toxicity, a relatively high cost and relatively poor efficacy and requires complex diagnostics and monitoring [4**]. The complexity and cost of currently required diagnostics serves as a major barrier preventing scale up and decentralization in low-resource contexts. However, the emergence of more effective and safe direct-acting antivirals (DAAs) [5**,6–8] offers the opportunity to significantly simplify the diagnostic and monitoring algorithm, thus decreasing costs and complexity and facilitating potential scale up in L&MICs.

*Division of Infectious Diseases, University of Pennsylvania, Medecins Sans Frontieres, Geneva, Switzerland, Division of Infectious Diseases, University of Pennsylvania, Philadelphia, Pennsylvania, USA, Department of Hepatology, St Mary’s Hospital, Imperial College, London, London and Department of Pharmacology and Therapeutics, Liverpool University, Liverpool, UK

Correspondence to Jennifer Cohn, MD, MPH, Division of Infectious Diseases, University of Pennsylvania, Rue de Lausanne, 78 1202 Geneva, Switzerland. E-mail: jennifer.cohn@genvea.msf.org

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KEY POINTS

- Currently, there is a complex set of diagnostic tests performed before, during and after treatment to cure hepatitis C. This includes genotyping, disease staging by fibroscan, HCV RNA PCR analysis and several other laboratory tests.

- New combinations of DAAs can cure hepatitis C infection in a high percentage of patients.

- It may be possible to simplify the set of diagnostic tests performed. Genotyping and disease staging may no longer be needed if treatments have pan-genotypic activity and work for people with and without liver cirrhosis.

- Hepatitis C core antigen testing is cheaper and less complex to perform than HCV RNA PCR analysis. The use of antigen testing to confirm chronic infection before treatment, and then confirm cure after treatment, could simplify diagnostic requirements.

DIAGNOSTIC TESTS FOR HEPATITIS C VIRUS

With improvements to diagnostic technologies, there is the opportunity to significantly simplify diagnosis and monitoring before and after DAA treatment. This simplification of the laboratory package could greatly improve the feasibility of treating HCV-infected individuals living in resource-limited settings.

The diagnostic package required for case detection and monitoring of treatment for HCV using PEG-based therapies is complex, expensive and largely prohibitive for L&MICs. Minimum laboratory tests performed before, during and after treatment for hepatitis C are summarized in Fig. 2.

Pretreatment tests typically include HCV serology, viral load and genotype [8]. In addition, because PEG-RBV is associated with relatively frequent toxicity and, especially for genotypes 1 and 4, only results in sustained virologic response in 50–70% of patients, guidelines may suggest delaying initiation of PEG-RBV until significant fibrosis is demonstrated and the clinical benefit of treatment is clear. Thus, a staging test to assess degree of fibrosis is often performed. Noninvasive markers of fibrosis, in particular transient elastography (fibroscan), are now very useful to stage liver fibrosis and avoid expensive and invasive liver biopsy [9]. In high-income countries, biomarkers with prognostic value for the success of interferon-based therapy, such as interleukin-28B (IL-28B), have also been recommended in difficult to treat genotypes 1 and 4 [10]. Due to the toxic side-effects of interferon-α, and teratogenicity of ribavirin, monthly laboratory-based monitoring is recommended. Complete blood count, alanine transaminase (ALT), creatinine, thyroid stimulating hormone and, in women of reproductive age, pregnancy testing must be monitored regularly during therapy. There are typically 5–6 HCV viral load measurements while on therapy [11]. Alpha-fetoprotein and ultrasonography are also used to exclude hepatocellular carcinoma.

Taken together, the complexity and cost of this diagnostic package represents yet another barrier to HCV treatment and care in L&MICs. First, many of these laboratory tests, such as HCV viral load, genotype and IL-28B, are expensive, and require

![Figure 1. Worldwide deaths from HCV, HBV, HIV, tuberculosis, and malaria in 2013. (Based on data from reference [2]).](image-url)
suitable laboratory infrastructure and trained techni-
cians, and thus are not routinely available, even at
hospital level. Although both rapid-care and point-
of-care diagnostic serological tests are available, no
test meets all affordable, sensitive, specific, user-
friendly, rapid and robust, equipment-free and
delivered criteria of being practical, accurate, low-
cost and manufactured under Good Manufacturing
Practice (GMP) guidelines, and it is not known how
reliable these tests are in HIV-coinfected patients
[12]. In many African settings and HIV-HCV coin-
fected patients, false-positive and false-negative
results are common [13–15]. The current test pre-
ferred by Médecins Sans Frontières for its perfor-
mance characteristics and GMP certification is the
OraQuick HCV Rapid antibody test (Orasure, Penn-
sylvania, USA) – but at over 10 euros per test for
general purchase, this test is also about 10 times the
price of other serology tests and represents a signifi-
cant barrier to its increased use.

Furthermore, the staging diagnostics required are
not ideally suited to decentralized care and low-
income settings. Transient elastography is expensive
and the technology required is rarely available out-
side large cities. Thus, people living in peripheral
areas are excluded from screening and care of viral
hepatitis. Thus, its optimal use is in a referral center,
which may be difficult to get to for patients without
resources and little access to efficient transportation.
Simple nonpatented biomarkers (e.g. AST to platelet
ratio index or Fibrosis-4) that require the measure-
ments of only platelets and transaminases have
poor sensitivity and specificity particularly in HIV-
infected individuals, and none of them have been
validated in African patients who frequently experi-
ence thrombocytopenia [16,17]. Finally, the overall
cost of this diagnostic package may be prohibitive-
ly high for many L&MICs.

### FIGURE 2. Current diagnostic tests used for monitoring of
treatment for HCV. HCV, hepatitis C virus.

<table>
<thead>
<tr>
<th>Diagnostic tests during DAA treatment: current system</th>
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<tbody>
<tr>
<td>Fibroscan</td>
</tr>
<tr>
<td>Genotype</td>
</tr>
<tr>
<td>IL2BB*</td>
</tr>
<tr>
<td>AFP</td>
</tr>
<tr>
<td>Pregnancy test</td>
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<tr>
<td>HCV RNA PCR</td>
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<tr>
<td>Full blood count</td>
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<tr>
<td>Clinical chemistry</td>
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</tbody>
</table>

Baseline Week 4 Week 12 EDT SVR12 SVR24

1IL-2BB testing for genotype 1 and 4 patients

### POTENTIAL FOR SIMPLIFIED DIAGNOSTICS DURING DIRECT-ACTING ANTVIRAL TREATMENT

All-oral, interferon-free DAA therapies may signifi-
cantly reduce the need for complex diagnostic test-
ing. New diagnostic tools are being developed that
are better suited for use in resource-poor settings.
Potentially pan-genotypic combinations may elimi-
nate the need for genotyping. As phase II and III
trials are showing therapy efficacy up to 87–100%
for treatment-naive patients [4••], staging may
become unnecessary as the risk-benefit ratio of
therapy now bends much more toward benefit for
the vast majority of patients, regardless of liver
fibrosis stage [18]. This is particularly true for
HCV-HIV coinfected patients who tend to progress
more frequently and more rapidly than those who
are HCV monoinfected [19]. If staging is still neces-
sary for monoinfected patients, where resources to
provide treatment are constrained, new simple and
affordable indirect blood tests are needed.

DAA treatment with fewer associated adverse
events will also require less laboratory monitoring.
It is possible that only regular monitoring of ALT,
aspartate aminotransferase, platelets, hemoglobin
and creatinine will be required during therapy,
and pregnancy testing, if ribavirin is still used. As
response-driven treatment algorithms will be a
thing of the past with new DAA regimens, the
number of virological tests can be reduced to an
initial confirmatory virological test and a post treat-
ment virological test to prove cure [i.e. 12 weeks
posttreatment sustained virologic response
(SVR12)]. The marked reduction in the number of
tests required will also dramatically drop the full
cost of the diagnostic package (Fig. 3).

### HEPATITIS C ANTIGEN TESTS AVAILABLE

Although currently, quantitative HCV RNA, or viral
load, testing is used more commonly (to measure
response to interferon-based treatment, which
requires a log drop calculation from baseline), given
that new DAA regimens will only require the con-
firmation of chronic HCV infection and the confirm-
amation of cure, qualitative virological tests may be
used. These have the added benefits of potentially
being cheaper and simpler to develop and perform.

Advances in laboratory technology may also
provide diagnostics better adapted to resource-lim-
lited environments. Antigen-based tests, such as
those measuring HCV core antigen (cAg), could be
used to replace the more costly molecular tests [20].
However, the only commercially available, fully
automated HCV core antigen assay is the Abbott
ARCHITECT HCV cAg assay, which is still complex
and laboratory-based. Optimizing tests for core HCV antigen would require the platform to be significantly simplified and made more affordable. The only known pipeline point-of-care test for core antigen is being developed by Daktari and will likely be available in 2017. The battery-operated, polyvalent, cassette-based test will only require 50 μl of fingerprick blood and deliver a result in 30 min, with a limit of detection equivalent to a viral load of 1000 IU/ml. Although difficult to calculate at this point, the test is likely to cost US$10–20, with volume-based decreases possible (Daktari Diagnostics, personal communication, 11 August 2014). Such a test could be used in a decentralized clinic and be flexible enough to serve a range of diagnostic needs such as CD4 and HIV viral load.

HEPATITIS C VIRUS ANTIGEN TESTING AS A REPLACEMENT FOR HEPATITIS C VIRUS RNA

Importantly, an HCV antigen test that is sufficiently sensitive, specific and low-cost can be used as a one-step screening and diagnostic test rather than the two-step serology and viral load algorithm currently used. As pipeline HCV antigen tests have detection thresholds that correspond to viral loads of 1000–2000 IU/ml, it is important to determine if this level of sensitivity will be sufficient for determining SVR12. There is already evidence from several studies that existing HCV antigen tests have sufficient sensitivity and specificity to replace viral load in HCV diagnosis and proof of cure. A systematic review is currently underway to more fully assess this question. In small convenience samples of 65 patients who experienced virologic failure after becoming undetectable on either PEG-RBV or PEG-RBV and a DAA (boceprevir or sofosbuvir) and had a measure of viral load less than 175 days after completing treatment (SVR24 + 7 days), only three (4.6%) patients had a viral load (VL) that was detectable but below the limit of detection for the antigen prototypes (<2000 IU/ml). An additional three (4.6%) patients had a VL that was detectable, but below the limit of detection for the antigen prototypes, but the VL was taken before 77 days after the end of therapy (SVR12–7 days). Additional field validation studies and estimates of cost–effectiveness of the one-step versus two-step algorithm are needed before antigen testing becomes the standard of care.

Evidence also points to the fact that dried blood spots, a simple method of drying a drop of blood on filter paper for the stable storage of samples at ambient temperature, may be used to more easily transport samples to a central laboratory for virological testing [21,22]. Finally, some point-of-care test manufacturers are currently developing simpler tests for HCV viral load [23**].

A recent survey of diagnostic testing in Africa showed that a significant percentage of people who are anti-HCV antibody positive have undetectable levels of virus when retested using HCV RNA PCR [24*]. If the antibody tests are so unreliable, it may be wiser to test initially for virus using a cheap, point-of-care antigen test. This would then remove the need for two stages of diagnostic testing to establish a clear diagnosis of hepatitis C infection, with the advantage of reducing the time to treatment initiation and potentially avoiding losing people from care.

CONCLUSION

Taken together, oral therapies in combination with new diagnostic tools will lead to a revolution in laboratory requirements for care of HCV and may lead to cost reductions, allowing a diagnostic and treatment monitoring package to cost as little as US$171–360 [4**]. Although the complexity and cost of HCV diagnosis and management should not be used as a reason not to treat patients in clinical need of therapy, it does represent a barrier to the rapid and successful scale up of HCV treatment in L&MICs. Leveraging the strong efficacy and tolerability of DAA-based all-oral therapies will help break down the diagnostic and monitoring barriers. Of course, realizing the health benefits of innovations in HCV care is predicated on ensuring access to those in need globally and this will depend significantly on the price of both drugs and diagnostics. If the financial costs of diagnostics and drugs are too great, HCV may become a disease that is readily curable among those with adequate financial resources and remain a scourge to those without them. In situations where access to treatment is limited, it could be beneficial to include staging tests and only treat those with the most severe disease. However, given that there is a benefit to
achieving SVR for patients free of cirrhosis [18], universal treatment should be recommended where economically feasible.

The implementation of low-cost diagnostic, treatment and monitoring packages in L&MICs could be a model for HCV care the world over and move HCV one more step toward global control.

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Conflicts of interest

The authors acknowledge no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

■ of special interest

□ of outstanding interest


Very detailed analysis of causes of death worldwide, showing high death rates from viral hepatitis.


Analysis showing the potential to mass produce DAAs for low costs.


