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Hepatitis C Resistance Testing: When, Why and How to Do it



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Hepatitis C virus (HCV) therapy has evolved dramatically in recent years with the development of highly effective and well-tolerated direct-acting antivirals (DAAs). Like other RNA viruses, HCV replicates at high levels and generates variants all the time, some of which, by chance, are less susceptible to the activity of specific DAA therapies. Although it was initially felt that DAA resistance would be a challenge, the extremely high cure rates in most patient populations have left many feeling that resistance is not a major issue. Fortunately, for most patient populations, this is true; resistance is not a big problem and as such, resistance testing is not necessary. However, for certain specific populations, resistance testing has utility. The presence of resistance at baseline may affect response to therapy and more importantly, simple adjustments can improve outcomes to levels similar to those achieved in patients without resistance. Fortunately, new therapies in development will likely make the need for resistance testing less and less relevant. In this review, the principles of resistance development are reviewed and the role of resistance testing in clinical practice is discussed, with specific recommendations on when to do testing, how to interpret the results and how to modify therapy appropriately based on the results.

Treatment for hepatitis C virus (HCV) infection has been revolutionized with the development of highly effective and extremely well tolerated direct-acting antivirals (DAAs). With these new therapies, cure rates well above 90% are now reliably achieved in almost all patient populations. With such

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remarkable success rates, patients, providers and payers have come to expect cure when a course of antiviral therapy is undertaken. With the cost of therapy and restrictions on retreatment, getting it right the first time, or certainly the second time, must be the priority for all clinicians. At least for the time being, maximizing the chance of success requires resistance testing in certain clinical scenarios. Fortunately, this is likely a temporary situation. New salvage regimens that work across all genotypes and against many resistant variants will likely significantly limit the need for resistance testing in the future, but until then, resistance testing has an important role.

Why is Resistance an Issue?

Like all RNA viruses, in a given patient, HCV circulates as a swarm of closely related but non-identical virions known as quasispecies. The virus has an error-prone polymerase that leads to approximately one error or substitution in the viral sequence with every round of replication. With upwards of 10^{12} new virions made per day, a virus with a substitution at every single site in the genome is generated every single day, just by chance.¹ Most of these substitutions will have a detrimental effect on the ability of the virus to replicate and they will thus be selected against and disappear from the viral population. However, some substitutions, just by chance, will affect how a given drug, or a class of drugs, inhibits HCV replication. During therapy, these substitutions provide a major survival advantage and virions containing them will outgrow any drug-susceptible viruses. DAAs do not create resistance, they just select for it.

Not all Resistance is Created Equal

If all resistance-associated substitutions are generated every day, it is perhaps surprising that DAA therapy works at all. A number of factors limit the effect of resistant variants (Table 1).^{2,3} The first is the genetic barrier to resistance. For some DAAs, a single point mutation will lead to high-level resistance. However for others, 2 or more substitutions are required. The more substitutions required, the greater the genetic barrier to resistance and the less likely resistance is to occur. In addition to the genetic barrier, the replicative fitness of the resistant variant is also relevant. If the substitution leading to drug resistance also markedly impairs the ability of the virus to replicate, variants with this substitution will replicate extremely poorly making them hard to detect and they will quickly be outgrown by wild-type virus in the absence of drug treatment.

The best example of this scenario is the substitution that leads to sofosbuvir resistance (S282T). This substitution in the viral polymerase prevents sofosbuvir from binding to HCV but it also almost completely incapacitates the virus' ability to replicate. As a result, even though variants with the S282T substitution are created as frequently as any other single substitution, they grow so poorly that they are effectively never found in patients before receiving sofosbuvir and remarkably have rarely even been identified and quickly disappear even in patients who have relapsed after a course of sofosbuvir treatment.^{4,5} It is the marked

fitness impairment of the S282T variant that has made sofosbuvir and any agents of this class (nucleotide polymerase inhibitors) 'special' in the sense that resistance is not really an issue and patients can be retreated with sofosbuvir or another member of this class (none yet approved) after failing a previous course of therapy with this agent.

Fitness of resistant variants varies markedly by DAA class. Variants resistant to non-structural 5a (NS5A) inhibitors are highly fit and can effectively compete with wild-type virus. This has two important consequences. Firstly, variants resistant to these agents are more frequently found at baseline, even in patients who have never been treated before, and secondly, if they emerge after a failed attempt at treatment, they will persist long-term, affecting options for future therapy. The other classes of DAAs fall in between the extremes of the highly fit NS5A inhibitor-resistant variants and the extremely unfit sofosbuvir-resistant variants. Variants resistant to non-nucleotide protease inhibitors (NNI) are generally fit and more similar to NS5A-resistant variants, while polymerase inhibitor-(PI)-resistant variants are relatively unfit and are thus uncommon at baseline and do not persist long-term when they emerge after treatment.⁶

Beyond genetic barrier and fitness, the degree and the prevalence of resistance also affect the impact of detected variants. Certain substitutions can lead to high-level resistance, making the virus 100 or even 1000-fold less susceptible, while others may only lead to 2 to 5-fold reduced susceptibility. In addition, resistant variants may be very rare within the quasispecies population or very frequent. Most studies suggest that unless a variant is present in at least 15% of the quasispecies population, it is unlikely to be clinically significant.⁷ It is important to be aware of these details because the impact of resistance can easily be manipulated through selective reporting.

Terminology

There has been some debate in the literature about the preferred term to describe resistance. The original term proposed was resistance-associated variant or RAV. However, the appropriateness of this term has been challenged because a variant is either resistant or it is not (i.e. it replicates better than wild-type virus in the presence of drug or it does not). It is the substitution in the sequence that is associated with resistance and as such, the term resistance-associated substitution

or RAS has largely supplanted RAV. Other terms including resistance-associated polymorphism (RAP) or resistance-associated mutation (RAM) have also been proposed.³ This is largely a semantic argument and for clinical purposes, the terms are interchangeable.

When is Resistance Testing Useful?

In order for any test to be clinically useful, outcomes should differ based on the result. If the outcome is the same whether the test is positive or negative, there is no point in doing the test. For most clinical scenarios, baseline HCV resistance testing is not useful because it has no meaningful impact on management. In such cases, it is best not to do the testing, as it only leads to confusion.

In fact, based on data available to date, baseline resistance testing is only relevant for certain patients with genotype 1a infection and for patients with genotype 3 infection and cirrhosis (Table 2). For all other patients, existing data do not support resistance testing.

Elbasvir/Grazoprevir

The importance of detection of substitutions associated with elbasvir-resistance in patients receiving elbasvir/grazoprevir in different contexts highlights why the details matter. For example, the SVR12 rate in patients with genotype 1b infection treated with elbasvir/grazoprevir is 98% in those with baseline substitutions associated with elbasvir resistance compared to 100% in those without any substitutions.⁸ Clearly, patients with genotype 1b respond very well to this regimen irrespective of the presence of elbasvir resistance and as such, no testing is warranted. In contrast, in those with genotype 1a infection treated with the same regimen, SVR12 rates drop to 58% in those with elbasvir-specific resistance-associated substitutions compared to 98% in those without baseline resistance. Clearly there is a large impact of resistance in genotype 1a and thus baseline testing is warranted. Furthermore, simple alterations in the treatment regimen can overcome the effect of resistance. Although the numbers are small, available data suggest that with extension of therapy from 12 to 16 weeks and the addition of ribavirin, SVR12 rates increased from 53% to 100% in genotype 1a patients with elbasvir-resistance at baseline.⁹

Sofosbuvir-Ledipasvir

Data regarding the importance of baseline resistance

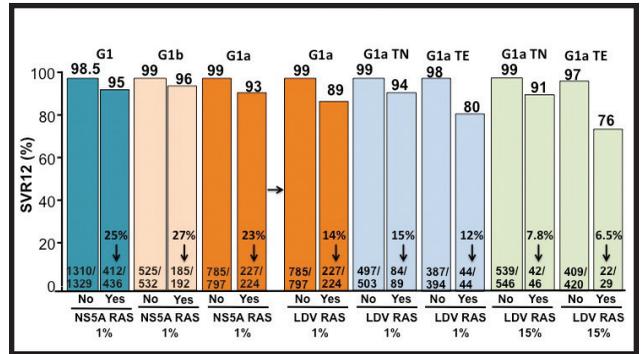


Figure 1. Effect of Resistance Based on the Specific Population Studied

Data on 1765 patients from Phase 2 and 3 trials of sofosbuvir/ledipasvir are shown. For each bar, the prevalence of the resistance-associated substitutions is shown with an arrow and the effect of having a resistance-associated substitution (yes or no) on SVR is shown in the context of the type of resistance-associated substitution (class-specific vs drug-specific), the prevalence (1% vs 15%) and the population (1a vs 1b, treatment-experienced vs treatment-naïve, cirrhosis vs non-cirrhotic), which may significantly affect the perceived impact of detected resistance.

NS5A – non-structural 5a; LDV – ledipasvir; 1% or 15% – threshold of detection of resistance-associated substitutions; TN – treatment-naïve; TE – treatment experienced (with peginterferon + ribavirin)

on outcomes with sofosbuvir/ledipasvir therapy are very illustrative of how the details matter for correct interpretation. Initial reports claimed that the presence of baseline resistance-associated substitutions had no effect on the response to sofosbuvir/ledipasvir in clinical trials. This conclusion was based on the observation that among genotype 1 patients, 25% had baseline substitutions associated with NS5A resistance, however the SVR12 rate was 95% in this group of patients, similar to the 98.5% in those without baseline resistance. However, as illustrated in Figure 1, the importance of baseline resistance increases markedly as relevant sub-populations are considered and low-level or low-frequency variants that dilute the apparent effect are removed from consideration. Similar to elbasvir/grazoprevir, with sofosbuvir/ledipasvir, resistance has minimal or no effect on genotype 1b meaning that resistance should be reported by subtype, not for genotype 1 as a whole. In addition, only ledipasvir-specific substitutions and not all NS5A substitutions, and only those with at least 15% prevalence in the quasiespecies population, should be reported. Among treatment-experienced patients with genotype 1a

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infection, only 6.5% harboured ledipasvir-specific resistance-associated substitutions at the 15% threshold, but only 76% of these patients achieved SVR12, compared to 98% among those without detectable resistance[10]. Based on a careful review of the data, baseline resistance testing is warranted in patients who previously failed interferon-based therapy and have genotype 1a infection with a plan to add ribavirin in those in whom resistance is found to be present. It may also be helpful in treatment-naïve patients with cirrhosis who will receive this regimen, as the difference in outcome is smaller but the consequences of failure are potentially greater.

Other Regimens for Genotype 1

With only 2 virological failures out of 624 patients in the ASTRAL 1 study of sofosbuvir/velpatasvir, resistance does not appear to have an effect and testing is not required before using this regimen for patients with genotype 1.¹¹

Data suggest that resistance-associated substitutions may affect outcomes with paritaprevir/r/ombitasvir plus dasabuvir, but again only in patients with genotype 1a infection. However, for genotype 1a infection, ribavirin is recommended with this regimen for all patients and with this approach, SVR12 rates do not differ by the presence of resistance-associated substitutions. Whether a population with genotype 1a infection with no baseline resistance who does not require ribavirin could be identified, remains to be seen.¹²

A frequent polymorphism at position 80 (Q80K) is associated with treatment-failure with simeprevir. The effect was quite significant when simeprevir was combined with peginterferon and ribavirin. When simeprevir was combined with sofosbuvir, the effect of Q80K was only notable in patients with cirrhosis. As such, patients with cirrhosis and genotype 1a infection scheduled to receive this regimen should have Q80K testing done and if positive, should consider an alternative therapy.⁷

Genotype 3 Cirrhosis

The preferred therapy for patients with genotype 3 infection and cirrhosis is sofosbuvir/velpatasvir for 12 weeks. Although the results with this regimen are much improved over previous options, in patients with cirrhosis, SVR rates were 91%, compared to 97% in this without cirrhosis.¹³ Closer inspection revealed that SVR

rates were down to 88% in patients with baseline NS5A inhibitor resistance-associated substitutions compared to 97% in those without these baseline substitutions. A similar effect was not seen in the sofosbuvir/velpatasvir arm of the recent POLARIS 3 study, with no obvious explanation for the differences between the two studies. Currently, international guidelines recommend NS5A inhibitor resistance testing for patients with genotype 3 and cirrhosis.^{7,14} If resistance is found, the addition of ribavirin is recommended based on extrapolation from the ASTRAL 4 study of patients with decompensated cirrhosis in whom those who received ribavirin had the highest SVR rates.¹⁵

Resistance Testing After Treatment Failure

The AASLD guidelines recommend resistance testing in all patients prior to retreatment after a failed course of DAAs.⁷ Importantly they also caution that the strategies to overcome resistance with current regimens have not been validated in the retreatment setting. However, numerous ‘salvage’ regimens that have shown proven efficacy for retreatment are in late-stage development.¹⁶ Although the detailed resistance data from these salvage studies have not yet been made publically available, the very high SVR rates despite a very high frequency of detectable resistance-associated substitutions at baseline suggest that most patients will not need baseline resistance testing prior to their use. However, it will be important to scrutinize the data carefully to avoid drawing incorrect conclusions about the value of resistance testing, as was originally done with sofosbuvir/ledipasvir. If specific substitutions are shown to be associated with failure, testing may be warranted. Fortunately, retreatment of HCV is rarely an emergency. As such, most patients would likely be better to wait for one of the coming therapies than to be retreated with one of the existing approved regimens.

The Counter-Argument to Testing

Some argue that baseline resistance testing is not necessary even for the specific populations mentioned because the number of patients with baseline resistance-associated substitutions who will not respond represents a relatively small percentage of the overall population and testing may be a barrier to treatment access or uptake. Other arguments against testing include cost, limited access in some regions and hard to interpret reports.³ While the effect may not be huge at the population level, for the individual patient, the

Table 1. Factors Affecting Impact of Resistance

Factor	Definition	Impact
Genetic Barrier to Resistance	Number of substitutions required for resistance	Genotype 1b has a higher barrier to resistance for protease inhibitors, NS5A inhibitors and non-nucleotide polymerase inhibitors
Variant Fitness	The ability of the resistant variant to replicate and compete with wild-type virus	Sofosbuvir-resistant variant has extremely low fitness and thus is not found at baseline and disappears after treatment if selected for NS5A inhibitor-resistant variants are very fit and thus are present at baseline and persist if selected during treatment
Degree of Resistance	Fold-shift in the EC50 of a drug compared to wild-type virus	Substitutions with small EC50 changes generally have less clinical impact EC50 are agent-specific, not class-specific so drug-specific substitutions should be reported
Prevalence of Resistance	The percent of all quasispecies that carry the resistance-associated substitution	Substitutions found above 15% of the population (or by population sequencing) are likely to be of clinical relevance

Table 2. Current Situations for which Resistance Testing is Recommended

Genotype/ Subtype	Regimen	Specific Population for Testing	Management if Resistance Found
Genotype 1a	Elbasvir/ grazoprevir	All G1a	Extend therapy to 16 weeks and add ribavirin
	Sofosbuvir/ ledipasvir	Peginterferon-experienced Naïve cirrhotic	Add ribavirin
	Sofosbuvir + simeprevir	Cirrhotic	If Q80K present, use alternative regimen
Genotype 3	Sofosbuvir/ velpatasvir	Cirrhotic	Add ribavirin
All Genotypes	All regimens	Previous DAA failures	Wait for new ‘salvage’ regimens in most cases

presence of baseline resistance may have a major effect on the chance of SVR. The cost of resistance testing is generally fairly low, particularly if centralized at a reference center. Given the high cost of the therapies, even infrequent improvements in treatment approach or a small absolute effect on SVR would still pay for the cost of most, if not all testing.

Ideally resistance reports should be improved. Currently most HCV resistance reports follow the

approach used for antibiotic and/or HIV resistance. However, unlike in other disease areas, DAAs for HCV cannot be easily mixed and matched. HCV regimens are studied as combinations and particularly with the increasing use of fixed dose combination pills; it is difficult, if not impossible, for example, to use a protease inhibitor from one company with the NS5A inhibitor from another. As such, resistance reports should give guidance on how to use an overall regimen in the

context of the resistance profile observed e.g. extend to 16 weeks and add ribavirin for a detected elbasvir resistance-associated substitution. Improved reports would make it much easier for clinicians with limited virological experience to enter the HCV field and would reduce the reluctance of experienced clinicians to order resistance testing.

CONCLUSION

Resistance has added a layer of complexity to HCV management. If properly interpreted, baseline resistance data can add significant clinical value for specific patient populations. Hopefully standardized reporting in the literature will more clearly define the importance of resistance in studies and improved clinical reports will make resistance testing easier to use clinically. Fortunately, this is likely a temporary situation. Future regimens are unlikely to require baseline resistance testing and may not even require testing before retreatment. Until these new regimens arrive, resistance testing has a relatively small but potentially important role. ■

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