HCV, CMV viral load—treatment, testing, timing

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April 2013—Treatment for hepatitis C virus infection turned a corner in 2011 when direct-acting antiviral inhibitors were approved and combined with dual therapy—pegylated interferon and ribavirin. Cure rates shot up from about 45 percent to 70 to 75 percent. With antivirals that are even more potent in late-stage clinical trials now, “Use of oral antiviral therapy without interferon appears to be a real possibility for the near future,” said Mitchell L. Shiffman, MD, director of the Liver Institute of Virginia at Bon Secours Health System in Richmond and Newport News, in an Association for Molecular Pathology session at the 2012 annual meeting, where he spoke about viral load monitoring for HCV.

In the same session, Aneesh K. Mehta, MD, assistant professor of medicine and assistant director of transplant diseases at Emory University School of Medicine, addressed viral load monitoring for cytomegalovirus. Which viral load value predicts disease, how long to treat, and how often to test are the important questions clinicians and laboratorians face for both HCV and CMV.

Others weigh in too. “We have seen the development of [HCV] diagnostics, therapies, and monitoring tests,” says David Hillyard, MD, professor of pathology at the University of Utah School of Medicine and director of molecular infectious disease testing at ARUP Laboratories. “Now we have the development of very advanced therapeutics. For a disease that threatens various dire consequences for 4 million Americans, to potentially be able to diagnose them and cure those who are able to come into the health care system, even though these are expensive treatments, is an amazing clinical story.”

For CMV, “Introduction of molecular testing certainly improved things relative to culture,” Rodney C. Arcenas, PhD, D(ABMM), says. “We were waiting up to 48 hours for a culture result.” Turnaround time is far more rapid now. “There have been some instances where we have been able to diagnose neonatal CMV infection much more quickly than with culture,” Dr. Arcenas says. “We are trying to convert entirely to PCR and convince our clinicians not to ask for culture to decrease send-out costs.

“We do CMV viral load monitoring on our pediatric cardiac and adult stem-cell transplant patients,” says Dr. Arcenas, clinical scientist-microbiology/molecular pathology for Consultants of South Broward in the Memorial Healthcare System, Hollywood, Fla.

Early detection and treatment of HCV infection with one of the new direct-acting agents against the virus’ serine protease, telaprevir or boceprevir, along with PegIfn and ribavirin, produces sustained virological response, or SVR, rates of 70 percent to 75 percent (Jacobson IM, et al. *N Engl J Med*. 2011;364:2405–2416; Zeuzem S, et al. *N Engl J Med*. 2011;364:2417–2428; Poordad F, et al. *N Engl J Med*. 2011;364:1195–1206; Bacon B, et al. *N Engl J Med*. 2011;364:1207–1217). (SVR is defined as undetectable HCV RNA 24 weeks after cessation of therapy.)

Dr. Shiffman

Attaining a sustained virological response equates to cure—the five-year recurrence rate after SVR is less than one percent (Swain MG, et al. *Gastroenterol*. 2010;139:1593–1601). Patients who attained SVR had a 50 percent to 70 percent reduction in five-year mortality (Backus L, et al. *Clin Gastroenterol Hepatol*. 2011;9:509–516) and significant reductions in hepatocellular carcinoma and need for liver transplantation. Dr. Shiffman showed in his

AMP presentation that even liver histology improves on followup biopsy.

Response-guided therapy relates duration of therapy to rate of reduction in viremia. For example, patients who achieve a rapid virologic response on telaprevir triple therapy—HCV RNA undetectable within four weeks of starting the oral antiviral agent—are treated for 24 weeks. Patients who become HCV RNA undetectable more than four weeks after initiating treatment with an oral antiviral agent plus peginterferon and ribavirin require 48 weeks of treatment. Similar criteria apply for boceprevir.

Hard stop rules are defined in the FDA-recommended algorithms. For telaprevir, treatment is terminated if HCV RNA is not less than 1,000 IU/mL at week four or week 12 or is not undetectable at week 24. For boceprevir, the criteria are less than 100 IU/mL at week 12 and undetectable at week 24. “If viral load is greater than those values at those times, we stop giving drugs,” Dr. Shiffman says.

Quantitative PCR assays for HCV RNA are central in these algorithms. “An HCV RNA assay that both quantifies and discriminates unquantifiable from undetectable is essential,” Dr. Shiffman says. “An assay that reports less than 25 IU/mL without qualifying if HCV RNA is detectable or undetectable should not be utilized. A value of less than 25 IU/mL detectable is not undetectable and the patient is not eligible for a shorter duration of treatment.”

A few years ago SNPs were discovered that affect triple therapy by modulating the impact of PegIfn. Patients who have the CC haplotype of the IL28B SNP have a high rate of rapid response, 80 percent, while those with the TT version have a very low rate, 30 to 50 percent. Tests are available to assess the IL28B haplotype, one of which Dr. Shiffman’s program uses. “If the patient has an unfavorable allele, we counsel them that the chance for a rapid response is much lower. If the patient has several poor prognostic factors such as previous failure to respond to peginterferon and ribavirin, cirrhosis, and an unfavorable IL28B allele, we probably will not offer that patient retreatment. The risk for complications is not worth the risk and we offer them the option of liver transplantation.”

Dr. Shiffman showed a list of 16 anti-HCV drugs now in phase two or three trials that target the protease, polymerase, or other viral proteins. Early data from some of these drugs used in combination with ribavirin and each other but without PegIfn are impressive. Not only are cure rates above 90 percent, but resistance would be expected to be greatly diminished, since dual viral proteins are being targeted, as with HAART for HIV. “In two or three years,” he said, “some of these direct-acting agents will end clinical trials and we will then know whether they can truly produce high cure rates when used without PegIfn.” Eliminating PegIfn is a goal, he says, because of its many side effects and how difficult it can be for patients to take it over an extended period.

In a session Dr. Hillyard presented at last year’s AACC annual meeting, he focused on molecular testing in the HCV management process. “Diagnosis of HCV has always been based on serological screening,” he said. “However, serologies need to be confirmed.” For some time confirmation has relied on RIBA, when signal/cutoff in the serological assay was low, or PCR, if it was high. Since the supply of reagents for RIBA has been unreliable, and may now have ceased, Dr. Hillyard says, “The confirmatory test going forward will be PCR.” In addition to confirming serological results, quantitative PCR tests provide a baseline viral load for therapeutic monitoring. Although qualitative HCV tests are still available, “it is difficult to see a good clinical role for them at this time,” he says.

Dr. Hillyard

Viral load measurement is integral to all HCV treatment and “one of the best examples of how laboratory testing can both improve patient care and help manage total health care costs,” he says. HCV quantitative testing is the

major determinant of length of therapy using established rules that prescribe extension or abandonment of therapy. With the approval of protease inhibitors telaprevir and boceprevir in May 2011, controversies arose regarding sensitivity and cutoffs for different viral load tests. “Clinical trials for these drugs had been conducted using a test with manual extraction that was not in widespread use in clinical laboratories,” Dr. Hillyard says. Although its quantitative performance at cutoffs of 100 and 1,000 IU per mL is equivalent to that of other tests, he says, its cutoff for limit of quantification (LOQ) of 25 IU/mL is different from that of other assays used to perform most clinical testing. Sometimes lost in the discussion, he says, is that commonly used tests have very similar limits of detection (LOD) for HCV genotype 1 virus (about 7 IU/mL). Therapeutic guidelines for these drugs include decision points based on whether virus is detected or not at this level of sensitivity.

“An important concept for clinicians and laboratorians to understand,” he says, “is that a report of less than a given limit of quantification is not equivalent to a report of virus undetected.” This situation was further complicated by the fact that some labs using tests in which LOD does not equal LOQ were not reporting detection status for samples below LOQ. “This experience has been an education to many about the small but significant intrinsic variability of molecular results, especially at levels approaching the statistical limits of detection,” Dr. Hillyard says.

Genotyping HCV infections has always been critical. “And with the advent of telaprevir and boceprevir therapies, which are approved only to treat HCV type 1, there is the new indication of genotyping as a qualifier for therapy,” he says. The importance of viral subtyping is a continuing controversy. “Type 1a infections are somewhat more difficult to treat, and new data with the protease inhibitors also reveals an easier pathway to resistance for type 1a virus.” The significance of this for managing patients remains to be elucidated, although accumulating data from clinical trials with newer direct-acting antivirals also reveal differences in treatment outcome between these two subtypes.

As Dr. Shiffman demonstrated, IL28B genotype is a robust pretreatment predictor of outcome. Dr. Hillyard noted that when the test first became available, there were mixed opinions about its role in patient care. The 2011 American Association for the Study of Liver Diseases guidelines concluded that data were lacking to justify use of IL28B to determine choice or length of treatment. At this time, Dr. Hillyard points out, “whether a patient has a favorable or unfavorable allele, their candidacy [for drug treatment] would be the same.”

On the other hand, whether to treat is a complicated decision, and the use of IL28B genotyping is evolving. “For a patient who has failed treatment or who may be a borderline candidate for treatment due to factors like state of liver decompensation, IL28B genotyping might help in the decision to treat, especially given the many emerging therapeutic options that are around the corner,” Dr. Hillyard says. IL28B testing is being included in clinical trials of new drugs, he adds, and it may well be integral to future testing algorithms.

CMV infection can present with a spectrum of disease in those with a compromised immune system, Dr. Mehta said in the AMP session. In HIV-positive patients, it may manifest as retinitis, polyradiculopathy, dementia, colitis, or cholangitis. In immunosuppressed transplant recipients, it can cause “CMV syndrome”—unexplained fever lasting more than 48 hours, malaise, and a decreased neutrophil count. It can also cause CMV tissue disease: pneumonitis, hepatitis, colitis, or encephalitis. In either situation, the risk of allograft rejection rises, as does the risk of bacterial or fungal infections or EBV post-transplant lymphoproliferative disorder (PTLD) (Fishman *J. N Engl J Med*. 2007;357:2601–2614).

Transplant programs employ a variety of strategies to prevent CMV infection. Universal prophylaxis requires giving antivirus therapy to all at-risk patients in the early post-transplant period. In preemptive therapy, patients are monitored at regular intervals—for instance weekly—and a rising viral load is treated to prevent symptomatic disease. “Resistance has been observed with both strategies,” Dr. Mehta said, later adding in an interview: “There are no large studies on this question so far, although some are ongoing. Choosing a strategy is part of the art of medicine and local culture.”

Dr. Mehta

In the Emory transplant programs, clinicians employ what Dr. Mehta calls “a mixed bag of strategies,” which differ by organ and physician culture. Recipients of a donor-positive liver are monitored weekly for three months, then biweekly for three months. Universal prophylaxis is used for three or six months in heart, lung, and kidney transplants.

For diagnosis of CMV, PCR is the most widely used method, Dr. Mehta says. He uses an in-house PCR based on commercial primers and probes. Quantitative PCR has become widespread at transplant centers, he notes, though correlation of absolute value to disease state is difficult. Only one group has attempted to establish an ROC curve relating viral load to development of disease (Humar A, et al. *Transplantation*. 1999;68:1305–1311). In this study, viral load greater than 2,000 copies/mL had 91 percent sensitivity and 75 percent specificity for predicting clinical illness, with a positive predictive value of 50 percent and a negative predictive value of 99.6 percent. “In reality, there is considerably more heterogeneity in transplant patients than in that clinical trial,” Dr. Mehta says. Cutoffs should be different for different organs, in his view: The lower the immunosuppressive regimen, the higher the treatment threshold. “Different organ transplants receive different degrees of immunosuppression,” Dr. Mehta said. His laboratory has converted to the WHO standard for expressing viral load. In this system, the 2,000 copies/mL in the Humar study equals about 500 IU/mL, which is what they use as a rough cutoff.

Kinetics of increase of CMV DNA is also important. Initial viral load detected and rate of viral load increase are independent risk factors for disease, Dr. Mehta said. “The combination of initial viral load and rate of change can identify patients at imminent risk of CMV disease.” During therapy, viral load should be monitored every one to two weeks; an undetectable viral load is the goal of treatment. In one study, all 16 non-relapsing patients had an undetectable viral load after therapy (Sia IG, et al. *JID*. 2000;181:717–720).

“CMV resistance remains a major problem in all forms of transplantation,” Dr. Mehta said. “Fortunately, it is not that frequent,” he added later, even though anti-CMV drugs are used as monotherapy. A typical patient in whom to suspect resistance is one who had an initial good response to therapy but then starts to exhibit an increasing viral load. To detect resistance, phenotypic (plaque reduction) assays are available, but genotypic (sequencing) assays are more commonly used. Dr. Mehta sends out samples for genotypic testing. To interpret sequence results, an understanding of the genetics of resistance is necessary (Lurain NS, Chou *S. Clin Microbiol Rev*. 2010;23:689–712).

Testing cell-mediated immunity may help to select patients who no longer need monotherapy (Kumar D, et al. *Am J Transplant*. 2009;9:1214–1222). “The data don’t give us a clear indication yet” for how useful such tests will be, Dr. Mehta says, but there is high clinical interest and ongoing research.

Dr. Arcenas, in an interview, said one of the caveats in laboratory testing for CMV is to distinguish true active infection from latent virus. “On the anatomic pathology side, we can suspect CMV lung infection from an immuno-stain on tissue, and try to correlate it with a bronchoalveolar lavage sample, then do PCR.” Sometimes there is a question of whether CMV is the primary infection. “In that case we can do studies to rule out other viral causes,” he says. “We may tell our doctors that the test is detecting CMV DNA but we can’t tell whether it is an active or latent infection.”

Sequential monitoring for viral load is important to detect true increases. “Sometimes we get low level ‘blips,’” Dr. Arcenas says. “The next week virus may be undetectable. So we need to watch it over time.”

Dr. Arcenas

Since many laboratories, including Dr. Arcenas', have developed their own in-house PCR assay, the WHO international standard is valuable. "Not all LDTs [lab-developed tests] are equal," he says. Having a standard allows patients to get testing at another laboratory and still get the benefit of serial test results. He is now making the conversion from copies/mL to reporting out IU/mL.

Quantitative PCR is preferable for monitoring viral load in plasma, in Dr. Arcenas' view, since undetectable viremia is the crucial factor. "With other sample types," he says, "qualitative PCR is acceptable as long as the results support the clinical diagnosis." These would include urine, as well as white blood cells. "It is kind of up in the air as to what a viral load means in lymphocytes," he says. "And just the presence of the CMV in retinitis is important."

Laboratorians should be ready to address the question of possible resistance. "We had a couple of cases where the doctor called us because the viral load wasn't going down or wasn't negative," Dr. Arcenas says. "They asked, 'Is my patient developing resistance against ganciclovir?' We don't do resistance testing in-house. We send out." When a physician feels that a patient isn't responding as they should, Dr. Arcenas, in consultation with the clinician, may recommend changing therapy or sending out for resistance testing.

"There is no FDA-approved test for detecting resistance in CMV," Dr. Hillyard said in an interview. "Most often resistance testing is performed by Sanger sequencing following amplification of the two genes where resistance can occur." These are *UL97*, a phosphotransferase gene with associated ganciclovir resistance mutations, and *UL54*, the viral DNA polymerase gene, with mutations conferring resistance to ganciclovir, cidofovir, and foscarnet.

Dr. Hillyard enumerated several challenges in resistance testing for CMV. "From the analytical perspective, patients failing therapy sometimes have relatively low levels of CMV," he says. "So we need to have reasonably sensitive resistance assays. Also, minor populations of resistant virus sometimes need to be characterized against a background of wild-type virus." Sanger sequencing struggles when resistant virus is less than 20 percent of the major population, he says. "We can use pyrosequencing to get down to the five percent level, but other issues make its use problematic."

One of the most critical problems, Dr. Hillyard says, is distinguishing between polymorphisms and resistance variants. A growing number of mutations in *UL54* and *UL97* genes are being associated with resistance. However, new polymorphisms not associated with significant resistance are also showing up. "An exciting way forward in this field is the use of genetic recombineering technology, in which specific DNA base changes can be quickly introduced into well-characterized drug-sensitive strains. This provides a much clearer assignment of which mutations cause resistance and which are irrelevant polymorphisms." Dr. Hillyard sees a big need for well-curated databases of CMV resistance mutations that have been validated in this fashion.

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