Extending HIV Drug Resistance Testing to Low Levels of Plasma Viremia

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(See the Major Articles by Santoro et al on pages 1156–64 and Gonzalez-Serna et al on pages 1165–73.)

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Antiviral treatment that is not sufficient to completely suppress viral replication imposes the selective pressure that results in the emergence of drug-resistant viral escape mutants. Human immunodeficiency virus (HIV) drug resistance testing has become part of the standard management of patients, both those newly presenting to identify transmitted drug resistance and those in whom virus replication is not suppressed, which results in the emergence of acquired drug resistance [1–3].

We have known for a long time that escape mutations can emerge early after treatment failure, even with low levels of plasma HIV RNA [4, 5]. Guidelines from most clinical laboratories advised HIV drug resistance testing only on specimens with viral loads of at least 1000 copies HIV RNA/mL [1, 3]. This practice was based on the rationale that successful sequencing diminished in efficiency with diminishing levels of RNA, and that with smaller populations of HIV RNA molecules being tested, the results might not be representative of the variants in the circulation. Moreover, Food and Drug Administration (FDA)–approved HIV drug resistance genotyping platforms have specified application to specimens with >1000 copies HIV RNA/mL. Two companion papers in this issue of Clinical Infectious Diseases provide data from large clinical programs in British Columbia and central Italy to demonstrate that in fact the success rates for polymerase chain reaction (PCR) amplification to perform drug resistance testing in specimens from patients with 50–1000 copies HIV RNA/mL plasma are both reasonably efficient and clinically predictive [6, 7]. Success rates were >90% for specimens >250 copies HIV RNA/mL in one study and for specimens >200 copies in the other. For specimens with detectable viral loads below these levels, successful sequencing results were still near 75%. In addition, the paper by Santoro et al showed that similarly successful rates were obtained with non-B subtype infections.

Each of these studies provides a robust practical experience with data from a large number of subjects and plasma samples (6617 plasma samples with 50–1000 copies HIV RNA/mL were studied between the 2 studies) over periods of more than a decade, and they provide evidence for beneficial clinical outcomes as a result of their drug resistance testing at low levels of plasma HIV RNA. Gonzalez-Serna et al found that 8% of 212 treatment-naive patients had evidence of transmitted drug resistance. That study also showed that the detection of acquired drug resistance was predictive of subsequent treatment failure. Both studies showed a range of resistance mutations to different classes of antiretroviral drugs, and the benefits of using such results to guide the design of subsequent regimens is well established [8, 9].

The results are subject to several limitations, which the authors themselves acknowledge. One concern about either phenotypic or phenotypic drug resistance testing with low viral loads has been whether the results from the amplified RNA reflected the population of genetic variants in a representative manner. Reverse transcription of any RNA population followed by PCR amplification reflects only a minority of that population. When the population tested is small, for example in the hundreds, the amplified products may include a selected subset of the population that is not representative. In fact, in the Gonzalez-Serna et al study, the proportion of sequence reads without evidence of nucleotide reads with >1 base supports the likelihood of amplification of a single molecule. The concern thus remains about whether the resulting sequence reflects
an unrepresentative “founder effect”; however, Figure 3 in Gonzalez-Serna et al shows a substantial rate of resistance even in those samples from which only a single molecule was likely amplified, and even these results were predictive of treatment failure.

Another issue is that these 2 highly experienced and specialized laboratories used in-house methods, rather than the standard FDA-approved platforms that generate the majority of results in most developed countries. The British Columbia approach was to implement a second try on those samples that fail to generate results, initially by using alternative primers and a shorter amplicon. The value of this modification became progressively more important the lower the level of viremia in the specimen. The Italian approach was to concentrate RNA from a larger volume of plasma by centrifugation along with a nested amplification for those specimens negative on the first round. There, too, the proportion of positive results only after the nested PCR amplification progressively increased with lower levels of viremia. Nevertheless, both studies provide robust data showing the practical feasibility of these approaches for the clinical management of large numbers of patients. The results generated also proved useful to inform and improve clinical management. Moreover, results obtained earlier during treatment failure can help to guide modifications of regimens before additional resistance accumulates. These studies thus provide an impetus for investigators designing drug resistance assays and for laboratories providing diagnostic services to extend drug resistance assays to specimens with lower levels of viremia.

Implementing the conclusions from these 2 studies will expand the proportion of patients who will benefit from HIV drug resistance testing. In addition, by earlier detection of failure and resistance, interventions can be made before the continuing accumulation of resistance would inevitably occur. As we learn how better to manage failure, it is ironic, but not unwelcome, that with the availability of earlier treatment and with more effective and more tolerable drugs, the proportion of patients for whom treatment is unsuccessful is substantially diminishing in developed countries.

The increasing challenges regarding drug resistance are occurring in resource-limited settings, with the global rollout of antiretroviral therapy now reaching approximately 10 million people [10]. Substantial benefits on morbidity, mortality, and likely even transmission are resulting from this effort [10, 11]. Nevertheless, the reality of laboratory support in such settings is compromising some of the benefits of the rollout. Current antiretroviral management in resource-limited settings results in the much-delayed determination of treatment failure and the progressive accumulation of drug resistance mutations under the selective pressure of continuing drug treatment [12, 13]. This accumulation results in higher and broader antiretroviral resistance, thus diminishing effective treatment options [14]. These options are further restricted by second-line regimens that are much more limited than in resource-rich countries. There is an urgent need for practical viral load assays and drug resistance assays that will permit more prompt and informed detection and management of treatment failure to further improve the benefits of access to antiretrovirals in the settings where most infections occur.

Note

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References