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The impact of wild-type reversion on transmitted resistance surveillance

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In 2008, Antiviral Therapy published the World Health Organization (WHO) recommendations for surveillance of transmitted resistance in countries scaling up antiretroviral treatment.[1] This work described a pragmatic approach to identifying recently infected patients which was subsequently applied in the TREAT Asia Study to Evaluate Resistance – Surveillance (TASER-S) study.[2] Briefly, patients were considered recently infected in TASER-S if they were 1) Less than 25 years of age and newly diagnosed with a confirmed HIV+ test; or 2) Had a confirmed HIV+ test with evidence of recent infection (ie. Positive BED assay or previous negative HIV test in the past year); or 3) Had an indeterminate or negative HIV test with detectable HIV RNA or positive p24 antigen. The mean duration of HIV infection at the time of resistance testing in the TASER-S cohort remains uncertain.

As many drug resistance mutations impair HIV fitness [3–6], reversion of transmitted mutations back to wild-type may confer a survival benefit for the virus in the absence of treatment. The rate of reversion may vary depending on the number of back mutations required, the relative fitness of the mutant and back-mutated viruses, the rate of viral turnover, and the presence of compensatory mutations.[7] The interpretation of population-based surveys of transmitted drug resistance, such as TASER-S, depends on understanding how long mutations are detectable after they are transmitted. Surveys provide an underestimation of the prevalence of mutations that are quickly replaced and this may be exaggerated by a long duration between HIV infection and resistance testing.

The TASER-S cohort includes 458 recently infected, antiretroviral-naïve patients who underwent HIV population sequencing between 2007–2010. Clinical research sites from Thailand (n=2), Hong Kong (n=1) and Philippines (n=1) participated in recruitment. Recently, we reported the overall prevalence of transmitted resistance in TASER-S was 6.1%; mutations associated with nucleoside reverse transcriptase inhibitor (NRTI), non-NRTI (NNRTI) and protease inhibitor (PI) resistance were present in 5.2%, 2.8% and 3.9% of the cohort, respectively. [8]

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Jain *et al* [9] utilized data on 75 patients from two prospective cohort studies (one recruited from San Francisco, USA [10], the other from Sao Paulo, Brazil [11]) to estimate the rate at which HIV drug resistance mutations are replaced after seroconversion in the absence of antiretroviral therapy. The cohorts enrolled between 1996–2009 (San Francisco) and 2002–2009 (Sao Paulo). Participants experienced seroconversion within the 12 months leading up to their baseline resistance test as determined by serial antibody testing [10] or the Serologic Testing Algorithm for Recent HIV Seroconversion (STARHS) assay.[11] Mutations were followed longitudinally and replacement rates compared between NNRTI mutations, M184V/I, thymidine analogue mutations (TAMs), T215 revertants, other NRTI mutations and PI mutations. M184V/I became undetectable very rapidly compared to other evaluated mutations with 100% of transmitted M184V/I undetectable within two years of seroconversion. The projected rates of replacement for other drug resistance mutation categories were not dissimilar to one another. For example, at one year after seroconversion the predicted replacement rates were: NNRTI – 4% (95%CI 1–15%), TAMs – 11% (4–26%), T215 revertants – 17% (5–50%), other NRTI – 2% (0–24%), and PI – 5% (2–13%).

Using the rates of mutation replacement reported by Jain *et al* [9] we evaluated how sensitive our reported prevalences of transmitted resistance in TASER-S were to time from seroconversion. We assumed mean duration of HIV infection prior to testing was six months, one, two, three or four years. Mutation frequencies were then projected based on the model's predicted rate of mutation decay. The prevalence for each mutation category was calculated as the number of mutations over the number of study participants although, for mutation categories other than M184V/I, participants may have contributed more than one mutation each. Results are shown in Table 1.

The reported prevalence for M184V/I may differ substantially from the predicted true prevalence depending on mean time from seroconversion. Assuming a mean duration of six months between seroconversion and resistance testing, the true prevalence would be 3-fold greater than the reported prevalence (3.4% vs. 1.1%). With a mean duration of 12 months between seroconversion and testing the true prevalence would be over 30-fold greater than the estimated value (36.4% vs. 1.1%). As other mutation categories do not decay as rapidly, the projected rates of transmitted resistance are not greatly affected by time. At worst, if the mean duration between seroconversion and resistance testing was four years in TASER-S the reported rates of NNRTI, TAM, T215 revertant, other NRTI, and PI resistance would have dropped from 3.5%, 3.8%, 2.2%, 1.2%, 4.5% at seroconversion to 2.8%, 2.2%, 0.9%, 1.1% and 3.5%, respectively, at the time of testing.

A rapid rate of M184V/I decay has also been reported by Castro *et al* [7] in their analysis of transmitted drug resistance reversion in a cohort of patients with mostly chronic (infected for 18 months) or unknown duration of infection. If the mean period of HIV infection was greater than two years in TASER-S we may not have found any M184V/I virus. Such results have been reported following work on resistance in chronically infected, treatment-naïve patients.[12] Other resistance mutations are relatively insensitive to variation in the time from seroconversion to resistance testing although Castro *et al* [7] noted that there was considerable heterogeneity in the rate of reversion within the TAMs group, with T215F/Y and K70R being lost more rapidly than other TAMs. As TAMs were grouped together by

Jain *et al* [9], it is uncertain whether this is also true for patients recently infected with HIV. Nevertheless, it is reassuring that, with the exception of M184V/I, and possibly T215F/Y and K70R, the reported mutation prevalences in TASER-S are not likely to differ significantly from the true values.

Several limitations may distort the above extrapolation of Jain *et al* [9] to TASER-S. Population sequencing was used in the San Francisco, Sao Paulo and TASER-S cohorts. Replication of this work using more sensitive methods of genotypic resistance testing would reveal higher baseline resistance levels and possibly different rates of mutation replacement. Jain *et al* [9] used the WHO 2007 list for surveillance of transmitted resistance [13] to define and categorize mutations. We have used the WHO 2009 list [14]. Adjusting the model and categorization using the latest WHO list is unlikely to significantly alter the results reported above given that the differences between the 2007 and 2009 lists are not substantial. Both the model and TASER-S cohorts were mostly comprised of men who have sex with men and contained few participants exposed to HIV via intravenous drug use. However, differences in viral subtype distribution may be important given the frequency of typical and atypical drug resistance mutations varies among HIV subtypes.[15] Most (68.8%) TASER-S participants were infected with subtype CRF01_AE but unfortunately the dominant subtypes in the model cohorts were not reported. The model also assumed a steady state viral load of 40,000copies/ml and showed that the rate of mutation replacement was increased with increasing viral load. Longitudinal viral load data was not recorded in TASER-S however the median baseline viral load was >50,000copies/ml. This is higher than the median baseline viral loads for the San Francisco and Sao Paulo cohorts (12,710 and 34,050copies/mL, respectively) suggesting mutation replacement may have occurred more rapidly in TASER-S patients. Further, the model showed significant within person variation implying there are patient-level factors aside from viral load and mutation category that drive mutation loss. Jain et al (2011) did not investigate these further and it is therefore unknown if germane patient characteristics in the model cohort differed from those of TASER-S participants.

Despite the abovementioned limitations, we believe this extrapolation provides good evidence that the transmitted resistance prevalences reported for TASER-S are reasonably robust. Whilst our estimate of M184I/V prevalence is probably understated, estimates for NNRTI, TAMs, T215 revertant, other NRTI, and PI mutations are likely a good indication of the true extent of resistance at the time of seroconversion as measured by population sequencing. These findings should be taken into consideration when interpreting future transmitted resistance surveillance data.

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