Commentary

Recent HIV infection testing algorithms

The history of HIV/AIDS spreads over nearly four decades since 1981 and has seen major landmarks. The emergence of antiretroviral drugs changed the nature of HIV/AIDS from a fatal disease into a chronic manageable disease[1], and the increasing availability of antiretroviral therapy (ART) resulted in a drastic reduction in the AIDS-related deaths[1,2]. The success of ART in suppressing the HIV in the body to almost undetectable levels encouraged scientists to explore the feasibility and application of test and treat policy. This was a major deviation from the then prevalent practice of prescribing ART based on immunological status defined by CD4 counts among the HIV-infected individuals. Initial encouraging results have indicated the need to focus on early detection and immediate initiation of ART among HIV-infected individuals[3].

It was estimated that approximately 37.9 million people were living with HIV in 2018[4]. It has also been estimated that each year, nearly 770,000 people die from HIV-related causes and 1.7 million people become newly infected with HIV[4]. India ranks third in terms of global HIV burden and over half of all new infections occur among key populations and their partners[5]. The 2017 HIV Estimation Report of National AIDS Control Organisation (NACO) of India identified higher HIV prevalence in the States which had documented low prevalence till recently[6].

The Joint United Nations Programme on HIV/AIDS has set a very ambitious goal of 90-90-90 for 2020 (90% HIV-infected diagnosed, 90% diagnosed brought under ART cover and 90% under ART cover suppressed virologically)[7]. It is, therefore, essential that coverage of HIV testing is widened by employing additional strategies such as community testing and HIV self-testing in addition to the ongoing initiatives.

Evidence[8] shows that in low- and middle-income countries, 30-40 per cent of people initiating ART have CD4 count of <200 cells/µl, indicating either late diagnosis in the course of the disease or lack of implementation of the test and treat policy. In addition, because many HIV-infected individuals are likely to be unaware of their HIV status, high viral load during the phase of early HIV infection (EHI) might result in a high possibility of transmission events[9]. Identification of HIV infection early by implementing the test and treat strategy would bring the infected individuals under ART early, thereby reducing secondary transmissions resulting in a reduction in the overall size of latent reservoir as well as reduction in immune activation and associated comorbidities. All these might contribute in changing the trajectory of the HIV epidemic.

For the routine diagnosis of HIV infection, the most commonly used tests are rapid spot tests or ELISA that detect HIV antibodies. The Western blot test is used as a confirmatory test which detects antibodies against all important HIV proteins separately. Molecular assays based on HIV RNA are primarily useful in the diagnosis of acute HIV infections as well as for the estimation of viral load. Both p24 antigen-based ELISA and RNA-based testing can identify EHI even before the anti-HIV antibodies are detectable in the circulation; these cannot necessarily discriminate between early and chronic infections. These platforms require sophisticated laboratory infrastructure and trained workforce. Western blot test has limited utility and specificity in diagnosing EHIs. Absence of p31 band in Western blot testing is suggestive of the patient being within 90 days of HIV infection if p24 antigen detection ELISA test is positive. The quest for simpler bedside assays for the diagnosis of EHI continues.

The assays such as limiting antigen (LAg)-avidity assay, BED capture immunoassay, LS-Vitros avidity and BioRad avidity assays are now available, which can identify recent HIV infection in the HIV seropositive individuals[10]. These assays measure the strength of

the bond between HIV proteins and HIV-specific antibodies (avidity) and are based on the principle that in EHI, the anti-HIV antibodies have low avidity. However, it is anticipated that such avidity assays would always demonstrate false recency either in case of individuals with controlled long-term HIV infection such as in elite controllers or in case of individuals on antiretroviral treatment, which could show the antibody pattern similar to that of recent infection[11]. It is, therefore, important that the false recency rate (FRR) of these assays is computed before using them to determine the rates of recent HIV infections among HIV-infected individuals. Different recent infection testing algorithms (RITA) have been proposed, which employ combinations of other biomarkers with the recency estimating tests or assays.

With this background, data on recent and established HIV-1 infections in the high risk behaviour populations of female sex workers (FSW), people who inject drugs (PWID) and men having sex with men (MSM) from northwestern part of India using LAg-avidity enzyme immuno assay based on RITA gain significance and should be critically examined. In the study by Chauhan *et al*[12] in this issue CD4 count has been used as an additional biomarker. It was previously used in another study[13] which had a small sample size and could not provide conclusive evidence on the utility of CD4 count as an additional immunological marker to correctly identify the recent HIV infections. Furthermore, wide variations in CD4 counts among patients with recent and long-term infections restrict the use of CD4 counts as the additional biomarker in avidity assays. Among the various other biomarkers tested, viral load has emerged as the most studied and reliable additional biomarker for accurate estimation of the incidence[14]. Use of viral load in this study would have increased the rationality of the results. The authors could have used even other additional biomarkers to strengthen their results, and it would have enabled them to make a good comparison between other biomarkers and CD4 counts. However, one limitation why viral load is difficult to be included in RITAs is that it cannot be done on the previously collected or stored samples in surveys such as sentinel surveys or samples stored following routine testing.

It was important to note that 52 per cent of the study population comprised PWID. The FSW and MSM populations were under represented in the study sample. Therefore, whether the EHI rates reported by the authors would also be applicable to all the above-mentioned typologies is questionable particularly if FRR are different in these three subpopulations. The issues such as how FRR can affect the EHI estimations and how confident are we about estimating FRR accurately are debatable. It is known that FRR can be greatly influenced by the type of sample and uniformity within the sample in terms of risk behaviour.

The present study[12] used the date of diagnosis which might be considerably different from the date of infection. For the correct estimation of incidence by an assay, both the FRR and the mean duration of recent infection (MDRI) that can be provided by these assays, are important. MDRI is the average time an individual spends in the phase of recent infection as defined by a biomarker or a set of biomarkers[15]. The choice of a recency discrimination threshold on a biomarker therefore relies on maximizing MDRI and minimizing FRR[15]. As this study[12] has not reported MDRI, the FRR might have been either overestimated or underestimated. In addition, recency was not compared with the predicted incidence in the population. These should be considered as limitations of the study. It is important and advisable to validate this assay on the samples collected from the participants who are shown to be recently infected in classical cohort studies with documented seroconversion.

As far as the applicability of this LAg RITA algorithm to the national-level sentinel surveillance is concerned, though conceptually it would have been prudent to use the resource of nation-wide sample collection for HIV incidence estimation, the currently available best protocols require plasma viral loads in addition to a given choice of recency assay for such estimations. It would be difficult in the setting of sentinel surveillance in India where serum samples are used for testing. The only option would be to perform the LAg assay and viral load on dried blood spots (DBS) collected during surveillance. For this to happen, the process of estimation of viral load from DBS will have to be standardized. The authors have also mentioned that this assay can be used to determine incidence in the cross-sectional surveys. This claim needs additional work and stronger evidence base. Another important point to be considered is that there is no assay for HIV-2 and hence, this assay may overestimate HIV incidence in populations having high proportions of HIV-2 infections like in Central Africa[16].

It would be interesting to explore if the results would differ by using higher or lower cut-off values for the optical density in the LAg assay. If serial samples from proven recent infections are available, it is possible to perform modelling using different cut-offs to determine the MDRI. In the study by Chauhan and colleagues[12], more people in the reproductive age group of 15-45 yr were infected with HIV, and there was a sharp decline thereafter. The presentation of age-stratified recent infection indicated that the proportion of recent infection increased with the increase in reproductive age until the age of 45 yr. This observation has policy and programmatic implications.

Although this assay has obvious limitations in correctly estimating incident or recent HIV infections estimations, but this might be useful in understanding the dynamics of HIV epidemic locally and regionally and might help in identifying HIV hot spots where suitable testing strategies could be implemented.

Conflicts of Interest: None.

**K.G. Murugavel[1], Madhuri Thakar[2] &
Sanjay Mehendale[3],***
[1]Division of Immunology, YRG CARE,
Chennai 600 113, Tamil Nadu, [2]Department of
Immunology & Serology, ICMR-National AIDS
Research Institute, Pune 411 026 & [3]Director
Research, P.D. Hinduja National Hospital &
Medical Research Center, Mumbai, 400 016,
Maharashtra, India
*For correspondence:
dr_sanjay.mehendale@hindujahospital.com

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