ADVANCES IN THE DIAGNOSIS AND EVALUATION OF ACUTE HIV 1 INFECTION

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Diagnosis of HIV 1 Infection in Recently Infected Subjects.

Proper laboratory evaluation of subjects with primary viremia requires careful consideration of the sensitivity and specificity of the tests. Adding viral RNA testing will increase the sensitivity for diagnosing HIV infection by detecting people in the short window between viremia and seroconversion. In studies that recruit subjects with acute retroviral syndromes by referral, the number of additional diagnoses made in that way may be substantial, but in other settings, use of highly sensitive third generation antibody assays will detect nearly all of the cases of HIV 1 infection.

Early diagnosis is required if the benefits of early treatment are to be achieved. The relative benefit of initiating therapy in people during primary viremia is still controversial. In a series of six patients, it appears that treatment very early in the course of HIV infection does preserve certain anti-HIV immune responses.(1) CD4+ mediated proliferative responses against HIV 1 p24 antigens were preserved. These proliferative responses are generally lost extremely early in the course of HIV infection without treatment. Further, there is some possibility that very early treatment might lead to a lower set point of viremia, which is correlated with clinical prognosis. The possibility of early therapy decreasing the risk of clinical events is suggested by a study of AZT treatment initiated during primary viremia in which acute clinical events during the six months of AZT treatment were decreased in the treated group.(2) In addition, analysis of non-human primates treated with D4T immediately after SIV challenge led to a decrease in the set point of viremia.(3) In addition, the benefits of early diagnosis may include a decrease in the infectious period if counseling can be performed and is effective, and if treatment early in the course of HIV 1 infection decreases infectiousness. It is clear therapy that decreases plasma viremia also decreases viral load in genital secretions.(4) On this basis, it seems plausible that the infectiousness of the treated host may be somewhat less than an untreated host. Finally, early detection of HIV 1 infection is important for protection of the blood supply.

Unfortunately, opportunities for diagnosing early HIV infection are very often lost. For example, a group of twenty three subjects who were at risk for HIV infection were followedevery six months.(5, 6) Surprisingly, during that time the majority of them (87%) experienced a symptomatic acute infection, and 95% sought medical attention for the syndrome. This points out that even though the acute HIV viral syndrome is nonspecific, it is significant enough in the person's life to cause them to initiate contact with the medical care system. Only twenty five percent of those who sought medical attention were evaluated for acute HIV 1 infection at the time of their first visit. This lack of referrals is particularly surprising, given that these subjects were being followed in a

cohort because they were at risk for HIV 1 infection. In this setting, the pre-test suspicion that they could become infected with HIV 1 would have been high. The problem is that the acute syndrome of HIV 1 infection is relatively non-specific, with the majority of people having fevers, fatigue, and a rash. Less commonly present are headaches, lymphadenopathy, pharyngitis, myalgia, arthralgia, nausea, vomiting, and diarrhea. Laboratory abnormalities can include thrombocytopenia, leukopenia, and elevated liver function tests. Genital and oral ulcers are more common in this group than in people who are not primary HIV infected, but the ulcers are do not have specific characteristics. There is a rash, that is usually non-pruritic, painless, erythematous blotches mostly on the trunk and measuring about four to ten millimeters in diameter. Histologically, it is a dermal vasculitis with perivascular infiltrates of activated CD4+ cells.(7) The rash is not specific and can be observed in any acute viral illness. Taken together, these findings indicate that the diagnosis of acute HIV 1 infection should be considered in subjects presenting with acute viral syndromes especially if risk factors are present.

If signs and symptoms of primary HIV infection are present, what tests should be ordered? A laboratory algorithm has not been established. The most sensitive and specific test for the diagnosis of HIV 1 infection continues to be anti-HIV 1 antibody assays, discussed elsewhere in this report. Special considerations may be required for the evaluation of patients with acute HIV 1 infection who may have negative antibody tests. The time course of assay results during acute HIV 1 infection is reviewed elsewhere.(8) Briefly, plasma viral RNA becomes detectable above four hundred copies per ml probably typically between four and fourteen days after exposure and infection. HIV 1, p24 antigenemia may be three days to a week later. Positive third generation EIA antibody assays typically become reactive three days to a week after that. These third generation EIAs are more sensitive than the second generation EIAs because they detect IgM class antibodies as well as IgG. Finally, the less sensitive EIA typically becomes reactive 129 days after seroconversion by the more sensitive EIAs.

The specificity of viral RNA assays becomes an issue when they are used for the diagnosis of primary HIV infection. Indeed, none of the RNA viral load assays are FDA approved for use in the diagnosis of HIV 1 infection. There appear to be differences in the specificity of different viral load assays, although this remains to be fully evaluated in all laboratory settings. RT-PCR based assays (Roche, Pleasanton, CA) appear to have 100% specificity when performed under ideal circumstances, but considerably less specificity in clinical laboratories participating in proficiency testing programs. The difference may be that clinical laboratories run HIV 1 infected specimens in the same batch asHIV 1 uninfected samples which creates opportunities for contamination. Importantly, negative control specimens are not routinely included in RT-PCR based assays, apparently to save space on the assay plate. These negative controls which lack RNA templates but contain all other components of the PCR mix, are important for the detection of contamination in PCR assays in general.

The branched chain DNA (bDNA) assays (Chiron, Emeryville, CA) differ in principle from the RT-PCR assays. The bDNA assays involve hybridization of probes to the viral RNA followed by amplification of the probe signal. Because the original nucleic acid template is not amplified, the risk of contamination of future assay runs with the products of previous assay runs is eliminated. Nevertheless, the false positive bDNA assay results do occur and likely represent non-specific binding of the probes to components of the specimen being analyzed.

Fortunately, the majority of symptomatic patients that are identified during primary HIV infection have truly massive viral loads. In a

small series in San Francisco, nine out of nine patients with symptoms of primary HIV infection had viral loads over 300,000 RNA copies/mL.(9) Seven out of nine had viral loads more than one million. These very high levels of viremia are unlikely to be due to false positive viral RNA tests. Nevertheless, asymptomatic primary HIV infection can occasionally be associated with low viral loads that are in a range that may be due to falsely positive assay values. The latest generation of viral RNA assays provides more sensitive detection and quantification of low viral loads down to 50 to 100 RNA copies/mL of plasma. Although the more sensitiveassays have a role in predicting the risk of virologic failure of therapy, it is unclear if the more sensitive assays provide any substantial additional diagnostic value in the setting of primary HIV 1 infection. The majority of subjects who are symptomatic with primary HIV 1 viremia will have viral loads that are much higher than the detection limit of available assays. In addition, there is a rapid doubling time of plasma viremia early after infection, estimated to be less than one day from animal and human studies. In this situation, the time between a positive ultra sensitive viral load test and a positive standard viral load test would be only two or three days. This is the extent to which the window period may be decreased using the ultrasensitive assays rather than the standard viral load assays. There are rare cases of recently infected subjects who present with very low viral loads, below 500 RNA copies/mL of plasma. For these subjects, the ultrasensitive viral load assays would be important for detecting HIV 1 infection prior to seroconversion.

In conclusion, there is no HIV 1 RNA assay that is currently FDA approved for the diagnosis of HIV 1 infection. Serologic assays for detection of anti-HIV 1 antibodies are clearly the gold standard for HIV 1 diagnosis because of their high sensitivity and specificity. The HIV 1 p24 antigen assay is also very specific (~99.9%), although less sensitive than antibody assays and viral RNA assays. The p24 antigen assay typically becomes positive when the viral RNA increases above 10,000 copeis/mL. Antigenemia is detectable for only three to five months, so the antigen assays have to be used with serologic assays to confirm HIV infection.

Drug Resistance Testing in Recently Infected Subjects.

Genotypic evidence of resistance to reverse transcriptase inhibitors has been detected in drug naive, recently infected subjects.(10-15) Polymorphisms in HIV 1 protease, some of which are selected by protease inhibitors, have also been detected in drug naive subjects.(16) However, in the summer of 1998, HIV with decreased susceptibility and/or multiple protease mutations had not been reported in recently infected, drug naive persons.

We initiated surveillance for transmission of multi drug resistant variants in a population in San Francisco where antiretroviral therapy is widely used and frequently associated with virologic failure.(17) Subjects who have virologically failed therapy maybe at higher risk of transmitting drug resistant viruses. Subjects were identified through the Options Project in San Francisco, which is a study of primary HIV 1 infection.(18) Primary HIV 1 infection is defined as seroconversion in the last six months, or a positive RNA test and negative serology, or a positive EIA that is non-reactive in a less sensitive EIA assay.(19) Recruitment into this study is by a referral from an anonymous testing sites, emergency rooms, private practices, and the telephone hotline. Drug resistance was initially assessed in screening assays using high density probe assays (Affymetrix, Cupertino, CA) which determine the entire sequence of protease and the 250 codons of reverse transcriptase. The genotypic analysis was confirmed using standard cycle sequencing and a novel phenotypic assay. Reverse transcriptase mutations associated with AZT resistances were present in 16% of this

population which is at the higher end of the range that has been reported in other settings. We also found that3/37 subjects had evidence of 3TC resistance. There was one patient who had seven protease mutations, two of which (L90M and 88D) have not been associated with naturally occurring polymorphisms in the protease gene of HIV 1.(12, 18)

Decreased drug susceptibility to protease inhibitors, AZT, and 3TC was confirmed using a novel drug resistance phenotyping assay (ViroLogic, South San Francisco, CA). The assay tests drug susceptibility using HIV 1 protease and reverse transcriptase genes that have PCR amplified from the patient's plasma and cloned as a population into a test vector that contains DNA from a standard laboratory strain (NL4-3) and a luciferase marker gene. The hybrid test vector is then transfected into 293T cells in the presence or absence of each antiretroviral drug. Infectivity of these virus stocks is determined using a luciferase readout in a 293T target cell. In this way, the inhibitory concentration to a large variety of drugs can be determined.

Drug resistance was also assessed in the partner of the subject who was infected with multi-drug resistant HIV 1. The drug resistance studies were performed 22 weeks after the sexual exposure and 13 weeks after all antiretroviral therapy had been discontinued. Genotypic analysis indicated that the majority of the partner's virus had reverted to drug-sensitive wild-type variants, although genotypic evidence of drug resistance was detectable with analysis of 20 independently sequenced clones. Standard drug resistance genotyping assays did not reliably detect the presence of drug resistant variants in the source case which reflects the limited capacity of these assays for the detection of viral sequence variants that comprise less than 20-50% of the viralpopulation. This limitation is expected to be a problem in subjects who have stopped therapy and in those who become infected with mixtures of wild type and resistant viral variants. Under these circumstances, the resistant viral variants are likely to be partially overgrown by wild-type viral variants.

In conclusion, a variant of HIV 1 having multiple genetic markers of resistance was detected after sexual exposure in a recently infected person.(12) Decreased susceptibility *in vitro* and slower virologic response *in vivo* confirmed drug resistance. Genotypic evidence of drug resistance was no longer evident using standard assays in the source subject 22 weeks after putative transmission, and 13 weeks after stopping all antiretroviral medications. This probably represents overgrowth of the wild-type virus in the absence of therapy.

Detection of sexual transmission of multi-drug resistant HIV 1 in a community where antiretroviral therapy is widely utilized indicates that more intensive surveillance for primary drug resistance is warranted. The clinical utility of drug resistance testing however, remains unclear. Prior to transfer from research laboratories to clinical laboratories, the drug resistance assays will have to demonstrate reproducibility, robustness in multiple laboratory settings, and will have to provide prognostic information that is not available from the antiretroviral history alone. Clinical trials to prospectively assess the value of drug resistance testing for guiding antiretroviral therapy are currently in progress.

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