HIV 1 VIRAL RNA QUANTITATION IN PLASMA WITH NON-B SUBTYPES INFECTION

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This paper discusses HIV 1 RNA quantitation in plasma with non-B subtypes infection. There have been a number of publications on comparison of RNA quantitation by different methods, most on subtype B. Only a few of these studies included non-B subtypes. Many of the studies have used one or two assays for comparative purposes and have involved limited numbers of specimens largely due to the high cost of these assays. In addition, the large specimen volume (almost 2.5 mL) needed to complete the three assays is a limitation to performing three different tests.

CDC has two international field stations, one in Bangkok, Thailand and another in Abidjan, Ivory Coast where HIV research is being conducted in collaboration with the local health ministries. Since some of our current projects there involve viral load measurements, we initiated this comparative study of different subtypes and different assays to understand which test would be the most appropriate for subtypes prevalent in those areas.

HIV exhibits great variability including intra- and inter-subtype variation as well as recombination in HIV 1. The implications of this variation are many. It can result in false negative immunologic diagnosis especially when assays involving peptide antigens are used. Some of the new assays do in fact use synthetic peptide antigens. The second implication is obvious difficulty in developing a universal vaccine and achieving cross protection against heterologous viruses. The variation can also affect viral load measurements, and is the topic of this presentation. There are also additional subtype issues such as differences in transmission and/or pathogenesis of different HIV 1 subtypes. The measurement of viral load is a very useful parameter that could assist in resolving these issues.

In addition to the HIV 1 diversity, methods used for RNA measurements are quite diverse themselves. Primers and probes used for amplification/hybridizations are based on specific sequences and any sequence variations in those sites may result in inefficient amplification and detection. Moreover, the different methods are based on very different principles and there are no standards available for different subtypes that could be used to calibrate and optimize the different assays. Although the VQA panel has helped significantly in standardizing quantitation of B subtype, we do not have standards for non-B subtypes. I believe development of such standards in the future will be very helpful.

To highlight the problem of viral load in non-B subtypes, I will discuss a case study involving a patient at Grady Hospital in Atlanta, GA. The patient was HIV 1 seropositive, had CD4+ counts of only 10/mm3, and had clinical AIDS. But the viral load was undetectable, which was surprising. The plasma sample was sent to us for confirmation and further evaluation. When viral load was repeated using the currently available Roche Amplicor Monitor Assay (Monitor-1.0), our results indicated only 500 copies of RNA/mL, close to the lower limit of quantitation, 400 copies/mL. Knowing the limitation of the current Monitor-1.0 on non-B subtypes, Roche Molecular Systems recently developed a primer set optimized for non-B

subtypes. Addition of this primer set to the master mix prior to RT-PCR (Monitor-1.0+) and repeat testing of the patient sample yielded 200,000 copies of RNA/mL of plasma (Figure 1). These results allowed initiation of anti-retroviral therapy in the patient. Since initiating this therapy in October 1997, we have been following this individual, and the viral load has become undetectable by the modified Roche method. The virus from this patient has been molecularly characterized as subtype A, a finding consistent with this patient being from Nigeria where this subtype is prevalent.

This demonstrates how important it is to use the right method. Without the appropriate method, the laboratory findings can be inconsistent with clinical findings. The right viral load method allows for the initiation of therapy and follow up monitoring of its effectiveness and development of resistance. Non-B subtype viruses are prevalent in Africa and Asia where large numbers of people are infected. In Ivory Coast, 95% of HIV infections are subtype A. In Uganda, the major subtypes are A (40%) and D (60%). In South Africa and India, subtype C is dominant among infected population. In Thailand, subtype E is the dominant virus with some injecting drug users infected with subtype B. Thai subtype B is somewhat different from US/European B and is referred to here as B.. Non-B subtypes are found with increasing frequency in the U.S. and in Europe. There have been several studies showing increased prevalence and incidence of non-B subtypes. One study examined blood donors in France. In 1985 there was only a 4% prevalence of non-B subtypes in French blood donors. In 1995, the prevalence of non-B subtypes increased to greater than 20%. In Germany, among those with recently acquired HIV 1 infection, 33% had non-B viruses, most of them subtype E.

In Thailand, a couple of our large studies involve perinatal transmission and AZT intervention. Another prospective study involves injecting drug users monitored by the Bangkok Metropolitan Administration (BMA). Each study requires the use of a viral load method appropriate for that setting. In Abidjan, Ivory Coast there is high prevalence of both HIV 1 and HIV 2, and dually infected people. Our interests there have been 1) perinatal transmission, 2) viral RNA in breast milk, and 3) viral load in those infected with HIV 1 versus those that are dually infected.

Initial comparative work was done using normal human plasma spiked with culture supernatant representing five different HIV 1 subtypes (3 each of A, B., D, E, and F; Figure 2). These results showed that: 1) for subtype B. and D, RNA quantitation was similar and there were no significant differences when tested by different methods, 2) viral load measurements were variable for subtypes A, E and F. In particular, Roche Monitor-1.0 assay did poorly on subtype A and E. Inclusion of modified primers, as in the case of Roche Monitor-1.0+ assay, improved the quantitation of RNA of subtypes A, E and also F. Since our major concerns were subtypes prevalent in Thailand and Ivory Coast, our subsequent focus was on clinical specimens representing those subtypes. A total of 60 plasma specimens, representing 20 each of subtypes A, Thai B and E were tested by Roche Monitor v.1.0, NASBA and Chiron bDNA v.2.0 assays (Figure 3). The Chiron bDNA was used as the standard for comparison. Due to redundancy of >35 probes used in the assay, the bDNA assay was expected to be least affected by inter- and intra-subtype variation. Specimens were arranged in increasing copy number of RNA, as determined by the bDNA assay. Results showed the Chiron bDNA assay quantified RNA in 55 of 60 specimens. The five missed (1A, 3B., 1E) were all lower concentration (œ1000 copies/mL), close to the cut-off of the assay. Monitor-1.0 failed to quantify RNA in 8 specimens (5A, 3E) while NASBA failed to quantify RNA in 15 (7A, 2B., 6E) of 60 specimens. Most of those missed had viral RNA <10,000 copies/mL, with few exceptions. All 8 missed by Monitor-1.0 and 13 of 15 missed by

NASBA were non-B subtypes, suggesting that the current Monitor-1.0, and NASBA assays may fail to quantify RNA of non-B subtypes especially when the viral load is <10,000 copies/mL.

Pair-wise correlation between assays for different subtypes are shown in Figure 4 which includes correlation coefficients (R value) and the mean log differences (_) for each subtype. What is clear from this set of nine plots is that correlation coefficients are in the high range (0.675 to 0.774) and mean log differences are the lowest (0.04 to 0.2) when the comparisons are done for subtype B. (middle panels). Correlations were poorest for subtype A with R values of 0.0034 to 0.645 and _ values of 0.46 to 0.93 log (left panels). Correlations were fair to poor for subtype E (0.33 to 0.78), but _ values and lack of detection of 6 specimens by NASBA and Monitor-1.0 assays indicated a problem in efficiently quantifying subtype E (right panels). When these specimens were tested by the modified Roche Monitor assay (Monitor-1.0+, with added new primer set), all 60 specimens were quantified (Figure 5). In addition, overall comparison of the bar graphs suggest that quantitation between the Chiron bDNA and Monitor-1.0+ is quite similar.

Correlation plots between the Chiron bDNA and Monitor-1.0+ (Figure 6) indicate that for different subtypes R value ranged from 0.85 (subtype A) to 0.99 (subtype B.) indicating very good performance of the Monitor-1.0+ assay (R=0.92 for all specimens). The comparison of modified Roche Monitor-1.0+ assay to Monitor-1.0 test indicated that for both subtypes A and E, the improvements in levels of quantitation of RNA was by a magnitude of a log or more, but the changes in quantitation of RNA in subtype B. was not significant (0.2 log). Recently, Roche introduced a new version of the assay called Monitor-1.5 with an optimized primer set for various subtypes and new thermocycling conditions. We evaluated this assay on 60 clinical specimens representing 3 subtypes. The results obtained were quite similar to Monitor-1.0+ (with added-in primers) with correlation coefficients of 0.815, 0.96 and 0.93 for subtypes A, B. and E, respectively (data not shown). The commercial availability of assays such as this would be important as an optimized tool in research projects as well as in clinical care of patients. We have applied modified Roche Monitor Assay (Monitor-1.0+) in the Thailand Perinatal Study. HIV 1 RNA was quantified in 278 of 280 delivery specimens from HIV-infected women (95% subtype E), exhibiting a 99.3% sensitivity. Results demonstrated a strong correlation between high viral load at delivery and HIV 1 perinatal transmission, with a p value of 0.0001. Examination of >450 sequential specimens collected from 68 infants who perinatally acquired HIV 1 infection showed, for the first time, a picture of viral load dynamics in infants at the population and individual levels (Figure 7). The data are indicative of very high viral load soon after birth, approaching >1,000,000 copies/mL by 2 to 4 months. During the follow-up, viral load did decline over time, however it remained at >100,000 copies/mL even at 3_ years of age in most children. This picture of very high viral load coupled with immature immune systems possibly results in persistence of virus and rapid progression of disease in infants. These data exemplify the application of appropriate viral load method to derive meaningful interpretations.

In conclusion, genetic variation in HIV 1 subtypes can significantly influence the viral RNA quantitation in clinical specimens. Extreme divergence of viruses within a subtype may result in undetectable viral load. Therefore, periodic evaluation of assays may be necessary to assess their performance and methods may have to be appropriately modified to accommodate divergent HIV 1 subtypes.

Acknowledgments

I would like to take this opportunity to acknowledge the people involved in this study. Susan Phillips performed most of the Roche Monitor tests, Tim Granade performed the NASBA assays, and Rich Respess provided logistic support. James Bagg performed the statistical analysis. We are also thankful to our collaborators in Thailand and Ivory Coast who provided valuable plasma specimens.

Figure 1

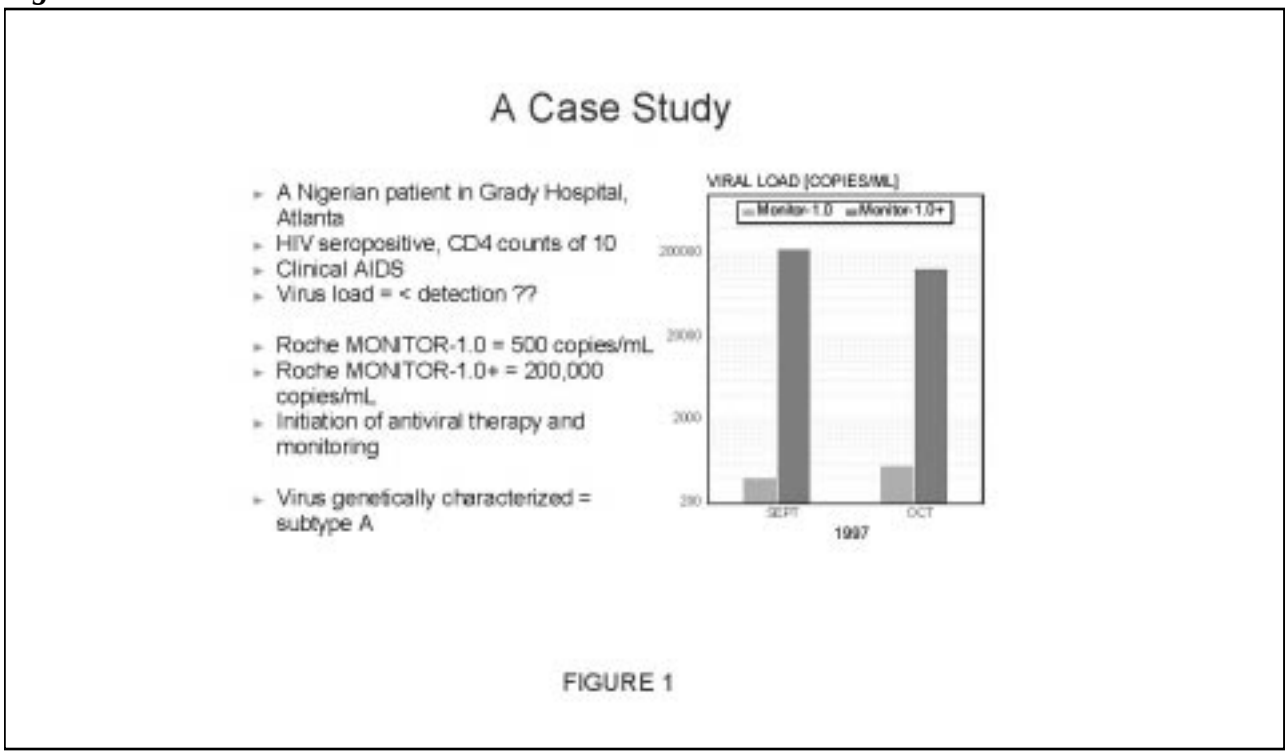

FIGURE 1

Figure 2

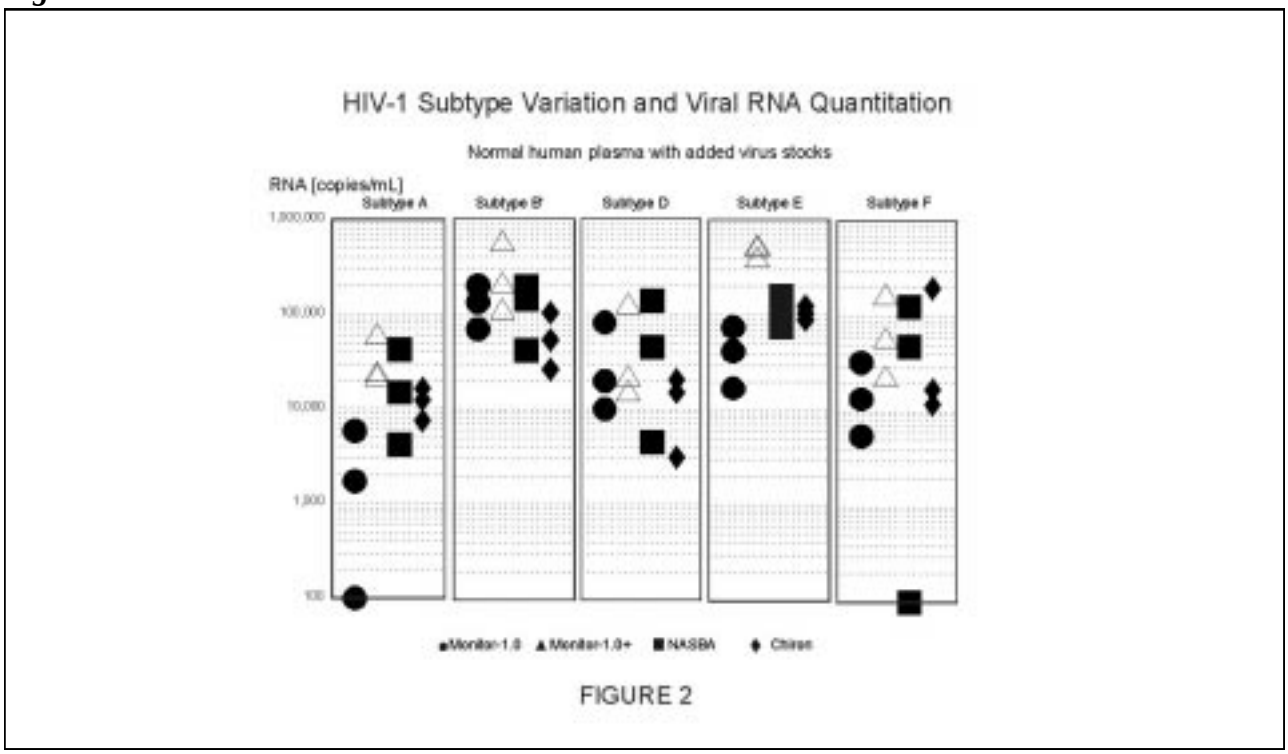

FIGURE 2

Figure 3

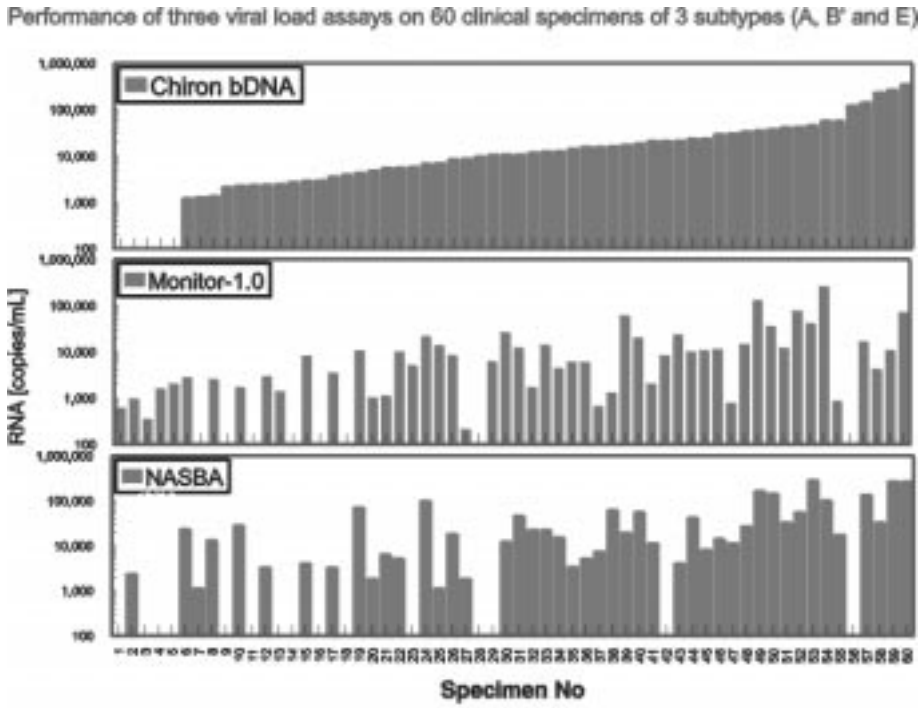

Figure 4

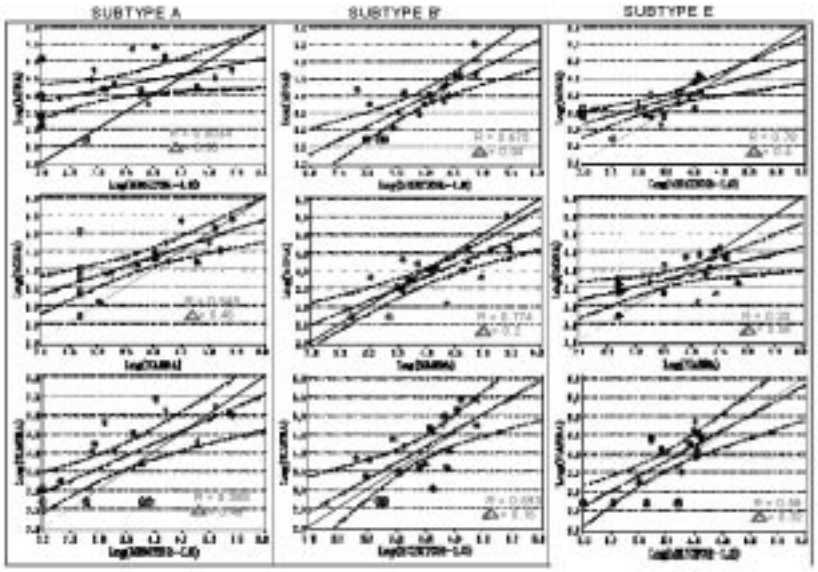

FIGURE 4

Figure 5

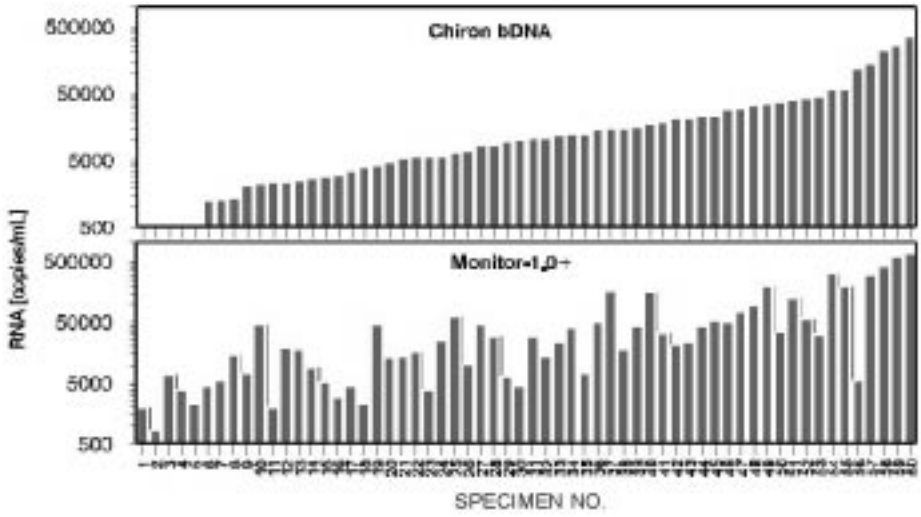

FIGURE 5

Figure 6

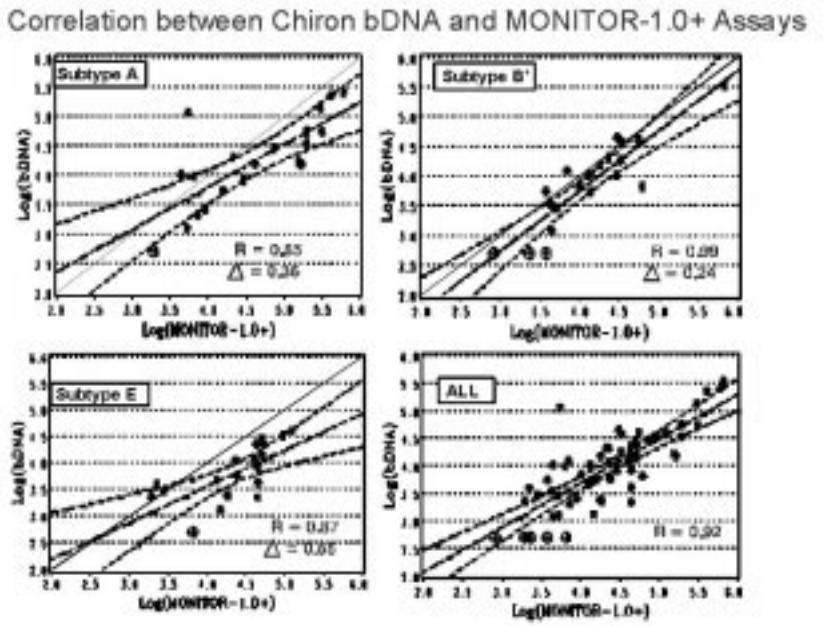

FIGURE 6

Figure 7

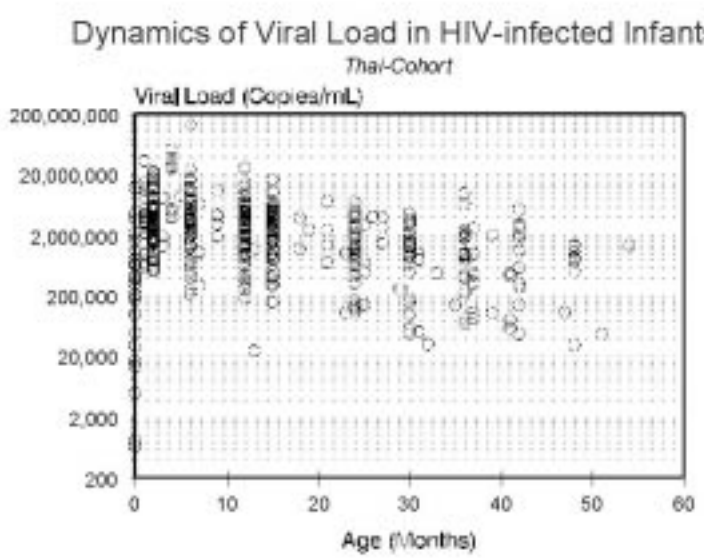

FIGURE 7