FALSE POSITIVE VIRAL LOADS

What Are We Measuring?

By Matt Irwin

George Washington University School of Medicine, 2001

Abstract

Polymerase chain reaction (PCR) and other RNA assays are being used with increasing frequency in a variety of fields of science and medicine, especially in the study of the human immunodeficiency virus (HIV) and the acquired immunodeficiency syndrome (AIDS). In spite of the widespread use of these tests, however, there are several inconsistencies that raise serious doubts about their accuracy. RNA assays are perhaps most heavily relied upon in the medical management of people diagnosed with AIDS and in people who test positive on the HIV antibody tests, where they are used to measure a person's "viral load". Because many important clinical decisions are made based on these tests, the highest standards of sensitivity and specificity should be required.

The most significant inconsistency in RNA assays for people diagnosed HIV-positive is the presence of false positive viral loads, which occur commonly in 3% to 10% of people who have no risk factors for HIV and who test negative on the HIV antibody tests (people considered HIV-negative). In the United States, where the prevalence of HIV infection is about 0.4%, this false positive rate means that random screening using the viral load test would produce 30 to 100 false positives for every 4 true positives.

Other inconsistencies include the finding that between 99.99% and 99.9999% of the HIV virions estimated by this method are not infectious, which raises questions about their ability to cause disease. This paper will review a number of studies that focus on false positive results on HIV RNA assays, and will also briefly review some of the other inconsistencies that raise questions about their accuracy. This review is not meant to be a comprehensive review, but rather to highlight the most serious problems and discuss their implications for management of HIV infection as well as their implications for further research. The most likely explanation for the findings to be reviewed in this paper is that much of the RNA measured by viral load assays does not come from HIV, but rather comes from other microbes and from normal human cells.

Introduction

Monitoring of a person's viral load is used in a variety of ways in people diagnosed HIV-positive. It has become one of the primary methods, along with measuring people's CD4+ T-lymphocyte counts, for making treatment decisions such as starting or changing antiretroviral medications, or for deciding how advanced a person's HIV-infection is. If someone has been diagnosed HIV-positive, it can be used to diagnose them with AIDS. It is rarely used to diagnose someone as being HIV-infected, however, because of the high rate of false positives.

Most people, including many clinicans, believe that the numbers generated by a viral load test represent the number of active viruses present in each milliter of a person's blood, but this is not what viral loads actually represent. The viral load test is used to measure the quantity of RNA fragments that are believed to be specific to HIV which are present in each milliliter of a person's blood. Even this is not completely accurate, however, because the quantitative measurement is done indirectly using mathematical equations, as opposed to any method of direct counting. What actually happens is that probes are used to identify short RNA sequences thought to come from HIV. Then whatever is found by the probes is amplified exponentially by a string of replication steps. Only after all of these amplifications are completed can the RNA fragments be detected and counted. Then a complex mathematical estimation is used to try to acertain how many RNA fragments were present in the original sample of blood, which finally generates the number which represents a person's "viral load". Each one of these steps introduces the potential for inaccurate results, from the assumption that only RNA from HIV will be identified and amplified, to the assumption that the mathematical formula will accurately reveal how many of such RNA copies were originally present.

False positives occur with all of the available RNA assays, including the newer generation of tests (Mendoza et al 1998). When they are done on the serum of people considered HIV-negative, 3% to 10% of them commonly have positive viral loads, and the highest reported rate of false positive results is a remarkable 60% (HIV surrogate marker coll. group 2000). Although most cases reported have false viral loads of 10,000 or less, there have been reports of false positive viral loads as high as 100,000 copies per milliliter. In the United States, where the prevalence of HIV is about 1 in 250 people (0.4%), a false positive rate of only 2% would still mean that random screening of the population would result in 5 false positives for every true positive, and a false positive rate of 10% would result in 25 false positives for every true positive. The most likely explanation for this high false positive rate is that HIV-RNA assays commonly react with non-HIV RNA, such as that produced by normal human cells and other microbes.

The human genome has about 3 billion base pairs, while that of HIV has only about 10,000. Because of this difference, human cells produce a great deal more RNA than HIV does. RNA from human cells could be released in large quantities during times of rapid cell death, which is what occurs during the infectious and inflammatory processes commonly present in people diagnosed HIV-positive. This could greatly increase the potential for false positive viral loads in the very population being studied. The high rate of false positive results from HIV RNA assays suggests that some of the 3 billion base pairs in the human genome could be producing RNA that is mistakenly attributed to HIV. This argument is strengthened by the fact that typical RNA assays look for only about 3% of HIV's genetic material, or about 300 base pairs.

Another fact that increases the risk of false positive viral loads is that these tests use RNA sequences that are based on the antibody proteins detected by the ELISA and Western Blot antibody tests. This means that if a person has a false positive or indeterminate result on either of the antibody tests they are also very likely to have a false positive result on the viral load test. False positive and indeterminate results on these tests are well described. For instance, 20 to 40% of healthy blood donors with no risk factors for HIV infection and who test negative on the ELISA test will test indeterminate on the Western Blot test (Proffitt et al. 1993).

Another inconsistency is that studies have found that the number of viral copies estimated by these tests represent between 99.99% and 99.9999% non-infectious viruses (Piatak et al 1993). Non-infectious viruses are not considered to be able to cause disease, since by definition they cannot infect cells. It is also possible that these "non-infectious viruses", which may make up 99.99% to 99.9999% of a person's viral load count, are not really viruses at all, but rather represent the detection of RNA from non-HIV sources.

This paper will first present some inconsistencies in viral load measurements, as well as some alternative explanations for these inconsistencies which suggest the possibility that they are not accurately measuring HIV activity. Then it will review a number of studies documenting the relatively common occurrence of false positive viral loads in people who are considered HIV-negative. Some discussion of antiretroviral (anti-HIV) medications will be included. These medications work by interfering with RNA and DNA synthesis, and they have these effects in nearly all human cells as well as in other microbes, not only in HIV (Schmitz et al. 1994, Dalakas et al. 1994, Bacellar et al 1994, Physician's Desk Reference/PDR 1999, Cassone 1999, Atzori 2000, PDR 1999). This means that anti-HIV medications could reduce viral loads dramatically even if the RNA is coming from normal human cells or other microbes that are present in the person being tested.

I. Viral loads represent 99.99% to 99.9999% non-infectious virus

Viruses can only cause damage if they are infectious, because they need to infect cells in order to cause cell death. Researchers attempting to see what proportion of the huge numbers of HIV reported by quantitative PCR represent active, infectious viruses, have found that as few as 1 in 10 million (0.0001%) are actually infectious. A virus that cannot infect another cell is essentially sterile, since it cannot harm any cells if it cannot infect them. Following are some comments from a study published in Science in 1993 where researchers found that the vast majority of viral particles estimated by viral load assays were non-infectious and non-culturable (Piatak et al. 1993).

"Circulating levels of plasma virus determined by (quantitative) PCR correlated with, but exceeded by an average of 60,000-fold, numbers of infectious HIV-1 that were determined by quantitative culture of identical portions of plasma... Total virions have been reported (in other studies) to exceed culturable infectious units by factors of 10,000 to 10,000,000, ratios similar to those we observed in plasma." (Piatak et al. 1993, page 1752)

This means that these researchers estimated that only about 1 in 60,000 virions found using quantitative PCR were actually infectious, and that other studies have found as few as 1 in 10 million. The researchers were not able to culture any virus at all in more than half (35 of 66) patients, and people with no infectious virus at all had viral loads as high as 815,000 copies per milliliter. The study subjects had all tested positive on the ELISA and Western Blot antibody tests which are the two tests currently used to diagnose people as being HIV-positive, they all had high viral loads, and yet the majority of them had no culturable infectious units of HIV. This difficulty in finding active HIV particles has been encountered by many other researchers who have tried to confirm the presence of HIV in people's blood (Chiodi 1988, Gallo 1984, Learmont 1992, Popovic 1984, Sarngadharan 1984, Schupbach 1984).

II. False positive viral loads

Studies examining false positive viral loads have found false positive rates varying widely from 0 to 60%, with the most common rates being about 3 to 10%. The numbers of viral copies per milliliter of blood found in people considered HIV-negative have ranged from 48 to over 100,000. These levels are much higher than the level used to make treatment changes in people diagnosed HIV-positive. Current recommendations are that if a person is on antiretroviral combination therapy and their viral load rises above undetectable, their medications should be changed. For instance, a recent article on the use of viral load in managing HIV-infection states:

"Failure to achieve the target level of 50 copies per milliliter after 16 to 24 weeks of treatment should prompt consideration of drug resistance, inadequate drug absorption, or poor compliance. ... For patients in whom a plasma viral load below detectable level has been achieved, a general guideline is to change antiretroviral drug therapy if the plasma HIV RNA concentration is found to be increasing. Ideally, any confirmed detectable plasma HIV RNA is an indication to change therapy. In some patients, it may be reasonable to wait until there is a documented increase in the plasma HIV RNA level to greater than 2000 to 5000 copies per milliliter." (Mylonakis et al. 2001, page 483).

This shows clearly that a level of only 50 copies per milliliter is considered significant, and yet it will be shown that false positive viral loads up to 100,000 copies per milliliter have been detected. Learning about what conditions predispose a person to have a false positive viral load would help a great deal in managing someone diagnosed HIV-positive, since it could help in determining how much of a person's viral load represents actual HIV activity. Although it is reasonable that false positive viral loads would appear any time there is a great deal of cell death because of the high quantities of RNA that are released when cells die in large numbers, no controlled studies were found that attempt to determine what factors influence the likelihood of false positive results.

IIa. False positive viral loads on three different viral load tests.

In 1998 Mendoza et al. published an article on false positive viral loads which is significant because they compare three different viral load assays (Mendoza et al. 1998). Several days after diagnosing a 5 month-old child with HIV-infection based on a positive viral load of 3044 copies per mL and starting him on antiretroviral medications, they discovered that he and his parents all tested negative on the antibody tests. After extensive follow-up testing of the child and his parents, they concluded that "a suspicious false-positive viral load result becomes the sole explanation for this controversy" (page 2076). This event prompted them to perform a simple experiment, which they describe as follows:

"Since viral load tests were approved for quantification of viraemia in already known HIV-positive individuals, we were interested to know their specificity. For this purpose, we selected 20 healthy volunteers, all of whom yielded negative results for HIV antibodies using different screening tests. Plasma from all of them were analyzed by three currently available HIV viral load tests." (Mendoza et al. 1998, page 2076)

The first assay, which used a branched DNA assay from Chiron laboratory, found that 2 of the 20 volunteers (10%) had a positive viral load, one with a viral load of 10,620 copies per mL and one with 2,020. The nucelic acid based amplification test, from

Organon Teknika, also yielded 2 of 20 false positives, although with smaller values of 150 and 480 copies per mL. The final assay, RT-PCR Monitor from Roche, was run in two different ways, once looking for only a particular HIV subtype and once looking for any type of HIV. When looking only for the subtype, only 1 of 20 (5%) was positive, but when looking for any type of HIV, 4 of 20 (20%) were positive. Although this rate of false positives (20%) was higher than that found for the other tests, the values measured were lower, ranging from 48 to 253 copies per mL. The authors do not reveal whether the same people who tested positive on one assay were more likely to test positive on another, but they do state that repeat testing reproduced the same results in more than half of the specimens that were able to be retested.

This study is significant not only because it found false positives in three different RNA assays, but also because it used healthy volunteers with no risk factors for HIV infection, in whom the possibility of acute HIV infection is exceedingly small. Most of the other studies to be reviewed only looked at the accuracy of the RT-PCR Monitor RNA assay in measuring viral loads, and they often studied people with known risk factors or known exposures to HIV.

IIb. False positives of 100,000 copies per mL

In 1997 a study was published with a carefully documented false positive viral load of up to 100,000 copies per mL (Schwartz et al. 1997). The patient in question was a participant in an HIV vaccine clinical trial who was being carefully followed and whose blood had been tested for antibodies to HIV every few months for several years. A viral load test was first performed on his serum when the patient reported flu-like symptoms. Flu-like symptoms are thought to suggest the onset of acute infection with HIV, which is also called "acute retroviral syndrome". The viral load test was positive, and the authors decided to run viral load tests on all of the available samples of blood from that patient which had been stored over the course of the clinical trial. The antibody tests on these serum samples had all been negative, but they now found that four of the samples from several years prior had positive viral loads, with the largest viral load being "in the range of 10,000 to 100,000". This patient had repeated testing for the next year which continued to show negative results, so the likelihood that he actually was experiencing acute HIV infection is extremely low. While such a large false positive result is unusual, even one such result is significant enough to question the practice of choosing an arbitrary cutoff above which a viral load test is thought to accurately diagnose HIV infection. A cutoff that is commonly used is 10,000 copies per mL: for example, a very recent study that looked retrospectively at blood samples from people with risk factors for HIV infection and flu-like symptoms assumed that viral loads above 10,000 represented "true positives" while those below 10,000 did not (Daar et al. 2001). This was described by Daar et al. as follows:

"Follow-up was not available for these 127 patients (cohort 1); therefore, before testing any samples, we determined that an HIV RNA result above 10,000 copies/mL would be considered a true-positive result. ... Two of 127 patients in cohort 1 were negative for HIV antibody and negative for p24 antigen, but positive for HIV RNA with levels of greater than 100,000 copies/mL. For the purpose of this analysis, they were considered to be true positive for primary HIV infection." (Daar et al. 2001, page 26)

While it is possible that these patients would have eventually had positive HIV antibody tests, it appears inappropriate to assume that this is the case in the light of the studies above describing the high rate of false positive results. These two patients may represent more examples of false positive viral loads over 100,000, but it is impossible to be sure without furthur follow up data. This study by Daar et al. also looked at two other cohorts of people at risk for HIV infection. In the other two cohorts follow up antibody testing was available, and they found that 8 of 217 (3.7%) subjects had a false positive result, with viral loads ranging from 50 to 2000 copies/mL. Because the authors include cohort 1 in their data even though no follow-up data is available for this cohort, their conclusions and abstract report a lower false positive rate of 2.6%.

Although not the primary subject of this paper, the accuracy of p24 antigen testing was also called into question by Daar et al (2001). Some people in the study cohorts were found to be positive for HIV antibodies on initial screening, and were described as having "chronic HIV-infection". The large majority of these people (82%) were negative for p24 antigen, which is a protein thought to be a specific and integral part of the virus. People with viral loads at least as high as 631,000 copies/mL were still negative for p24 antigen, which again raises the question of how much virus was really present in these people.

Another recent study by Rosenberg et al. (1999) also found very high viral loads in people who were negative on the HIV antibody tests, with the highest being greater than 1.5 million copies per mL. This study was designed as an attempt to see if people diagnosed previously with acute mononucleosis were actually having symptoms of acute HIV-infection. They used a single stored blood sample, with no clinical outcome or follow-up to determine this. The authors found 4 of 563 (0.7%) subjects had positive viral loads with negative ELISA antibody tests. This rate of 0.7% is much smaller than the false positive rates mentioned above, which actually increases the probability that they were false positives, although no follow-up clinical data or testing was available. As with the authors just described, they assume that the people in their study are HIV-positive based solely on the viral load tests. While it is again possible that these people were newly infected with HIV, it is also possible that they

represent yet another example of false positives, this time with viral loads of over 1.5 million.

IIc. A Meta-Analysis of RNA assay false positive results

In 1996 Owens et al. published a meta-analysis of 96 different studies that looked at the specificity and sensitivity of the polymerase chain reaction (PCR) in diagnosing HIV infection (HIV surrogate marker coll. group 2000). They found that the specificity of PCR varied widely in these studies from a low of 40% to a high of 100%, which means that false positive rates varyed from 60% to 0%. They would have had even higher false positive rates if they had included "indeterminate" PCR results as being positive. In the studies of highest quality, according to the authors, the false positive rate ranged from 5% to 0%. The authors also found that studies using more recent PCR technology were no more accurate than older studies, and that publication bias may have prevented studies with worse results from being published. Here are their descriptions of these findings:

"Our subgroup analysis show that studies published only as abstracts provided lower estimates of the sensitivity and specificity of PCR. This may indicate publication bias - the preference for publishing favorable rather than unfavorable studies. ... We did not find evidence that performance of PCR improved over time." (HIV surrogate marker coll. group 2000, page 810)

They also discuss a common factor that can lead to claims of falsely high specificities. This comes about because the PCR test result is called positive or negative based on a threshhold value, and is not a "yes or no" result. If the threshhold is chosen so that even a very mild reactivity is interpreted as positive, then many people who are not actually positive will be mistakenly identified. If a high threshhold is required and only very strongly reactive samples are counted, then specificity will increase, but more people who are actually positive will be missed resulting in poor sensitivity. As described by the authors:

"Because both sensitivity and specificity are determined by the choice of the threshhold for an abnormal test result, there is an inherent tradeoff between them. The threshhold can be chosen so that PCR is 100% sensitive or so that it is 100% specific, but not normally both (unless the test is perfect...). Thus, a study that only evaluates the sensitivity of PCR or only evaluates the specificity of PCR provides insufficient information for evaluation of the test's performance." (HIV surrogate marker coll. group 2000, page 812)

IId. False positive viral loads - a case series

Rich et al. (1999) published a case series describing three patients with false positive viral loads. While the authors do not give information that would allow an estimation of the rate of false positives, their series is significant because it demonstrates that false positives on viral load may be likely to occur in conjunction with false positives on both the ELISA and Western Blot HIV antibody tests. Since the RNA assays look for RNA that is based in the amino-acid sequence of the same proteins used in the ELISA and Western Blot, this would not be surprising. The ELISA is used as a screening test and the Western Blot, which separates the same proteins that are used in the ELISA into 10 separate bands, is used as a confirmatory test. The Western Blot test is only done if the ELISA is positive. While the first two cases had negative antibody tests, but the third case had a positive ELISA and an indeterminate Western Blot test. This case was a 20 year old healthy woman whose test results were described as follows:

"During a four month period after her indeterminate result on the Western Blot test, she had positive results on ELISA and indeterminate results on Western Blot on separate occasions. Five months later, both the ELISA and Western Blot tests yielded negative results, but the patient had a plasma viral load of 1300 copies/mL." (Rich et al. 1999, page 38).

The possibility that false positive viral load tests are more likely when false positive ELISA or indeterminate Western Blot tests occur is reasonable, and furthur studies would not be difficult. Western Blot tests are indeterminate in 20 to 40% of healthy blood donors who are negative on the ELISA test (Proffitt et al 1993). While this extremely high false indeterminate rate raises questions about this most heavily relied upon test, it would make future research easy to perform because of the plentiful supply of people with indeterminate tests in whom viral loads can be measured.

IIe. False positive viral loads after needle sticks with HIV positive blood

Gerberding et al. (1994) conducted a study of HIV contaminated needle sticks, and in the process also uncovered data that call into question the value of viral load/PCR testing. They did PCR tests on 133 of the 327 healthy workers who had experienced needle sticks in their clinic. All of these 133 subjects remained HIV negative on the ELISA antibody test, but seven of them had "indeterminate" PCR results, and four others had one or more actual positive results, for a false positive rate of 3%. If the indeterminate results are counted as well, the false positive rate is 8%. Gerberding et al. comment on their findings with PCR as follows:

"The failure to demonstrate seroconversion... among those with positive PCR tests suggests that false positives occur even under stringent test conditions. The low

predicitive value of a positive or indeterminate PCR test... contraindicates the routine use of gene amplification in this clinical setting." (Gerberding et al. 1994, page 1415)

IIf. False positive tests for HIV-DNA

Another assay which was once heavily promoted is an HIV-DNA assay, which is similar to an HIV-RNA assay and uses the same polymerase chain reaction (PCR) technology. A study looking at this assay was published in 1992 by Busch et al.. They did PCR-DNA tests on 151 ELISA-negative people and found that 18.5% (28 people) had positive PCRs. Furthurmore, they found that only 25.5% of people diagnosed HIV-positive had positive PCR's. In their conclusion section they draw attention to how close the two numbers, 18.5% and 25.5%, are:

"This study of PCR detection of HIV-DNA in serum identified a disturbingly high rate of nonspecific positivity with a widely employed gag primer pair system [gag is a protein considered to be specific to HIV]. In fact, the overall positivity was not significantly different for serum specimens from seropositive patients and seronegative control donors (25.5% vs 18.5%). ... In contrast to the high rate of false positive results observed with gag primers, env DNA [env is another protein thought to be specific to HIV] was not detected by laboratory B in any of the specimens from either seronegative or seropositive individuals. Absence of reactions with both primer pairs from all 59 specimens from seropositive persons meant that no serum sample could be confirmed positive for HIV-DNA, i.e. 0% sensitivity. This finding is in marked contrast to the high sensitivity reported previously by Laboratory B for both gag and env primers." (Busch et al. 1992, pages 874-875).

Although HIV-DNA testing is not used for viral load measurements, it is of interest to note the significant problems that developed with this test even though the laboratories that produced it claimed that it was highly accuracte, sensitive, and specific. The fact that they found 0% sensitivity for one of the key proteins thought to be specific to HIV again suggests that these assays are mostly reacting with non-HIV DNA and RNA, and mistakenly attributing it to HIV.

III. Alternative explanations for variations in viral loads and improved clinical outcomes

IIIa. The placebo effect

Although people whose viral loads are reduced successfully by antiretroviral drugs do have better clinical outcomes (Gilbert et al 2001), there are several other possible explanations for this besides the widely accepted one. The conventional explanation is

that these reduced viral loads represent reduced HIV activity and reduced numbers of HIV particles, and this results in improved clinical health.

One factor that is ignored by this model is the placebo affect. Although many HIV drug trials are double blind placebo controlled trials, viral load scores are not blinded. Because viral loads are commonly thought to represent the number of viruses per milliliter of blood, it can be terrifying to hear that one's viral load is in the thousands, hundreds of thousands, or even in the millions. Receiving good news, such as being informed of a dramatically lowered viral load, can have a direct effect on a person's physiology, even if the RNA being measured is not coming from HIV. Hearing that one's viral load has been dramatically lowered can reduce the emotional and psychological problems such as anxiety and depression which can be severe in people diagnosed HIV-positive. Enhancing psychological and emotional well-being may promote various health behaviors such as exercise, good nutrition, improved medical care, and self-care.

There is also good reason to believe that the patients in the clinical trials in question can see through the double blind. It has been shown that most participants in drug studies can correctly guess whether they are getting active or placebo medications (Greenberg and Fisher 1997). There are several reasonable explanations for this finding. In the case of HIV, one is that the viral load is often reduced more by active medications. Another is that the groups receiving the active medications also have significantly more side effects.

IIIb. RNA reductions in normal human cells and other microbes

The number of viruses estimated by viral load tests is based on measurements of RNA fragments, so that any change in overall RNA levels in the blood could potentially alter a person's viral load, even if this RNA does not come from HIV. Many antiretroviral drugs have a short-term antimicrobial effect, which can result in a temporary improvement in health, and they do this by directly inhibiting RNA and DNA synthesis. These drugs also cause reduced RNA and DNA synthesis in a wide variety of human cells including red blood cells, white blood cells, nerve cells, bone building cells, and muscle cells, which result in some of their most common adverse effects as reported in clinical trials (Schmitz et al. 1994, Dalakas et al. 1994, Bacellar et al 1994, Physician's Desk Reference/PDR 1999). Microbes that have been found to be suppressed by these drugs include Pneumocystis carinii, Candida albicans, Enterobacter, Shigella, Salmonella, Klebsiella, Citrobacter, and E-coli, and many other microbes that have not yet been studied may also be affected (Cassone 1999, Atzori 2000, PDR 1999). The reduced RNA and DNA synthesis in the microbes will result in reduced infection, while in human cells it will result in reduced activity, reduced cell division, and reduced inflammatory response to infection. This reduced

infection and inflammation, as well as the direct suppression of RNA production, is likely to result in dramatic reductions of RNA levels in the blood stream. If viral load assays commonly measure RNA from normal human cells and other microbes and mistakenly attribute it to HIV, as is suggested by the articles to be reviewed in this paper, then the reduced RNA and DNA synthesis that they cause could obviously result in a lowered viral load, even if there is no HIV present.

Unfortunately, the antimicrobial effect of these drugs is short lived as microbial resistance develops quickly (PDR 1999). This may be another explanation for people whose viral loads increase while taking anti-HIV drugs, since microbes and human cells could adapt and increase their RNA production in spite of the presence of anti-HIV medications. Other drugs that interfere with RNA synthesis, such as many cancer chemotherapeutic agents, would also cause viral loads to fall dramatically, even in a person who is HIV-negative.

Another possibility raised by these arguments is that the rebound in viral loads that is often seen soon after a person stops taking antiretroviral drugs may not represent renewed HIV activity as is commonly thought. When human or microbial RNA and DNA production is suppressed by artificial means, the cells will naturally try to compensate by increasing their production of RNA and DNA. When the inhibiting effect of the drug is removed, this accelerated production may become dominant and cause a rapid increase in viral load even if HIV is not present.

IIIc. Large reductions in viral load are no better than small reductions

Comparisons of studies showing positive effects from lowered viral loads show another inconsistency: dramatic reductions in viral load do not offer any better clinical benefit than small reductions. An analysis of all 16 randomized trials that compared outcomes based on drug-induced lowering of viral load found that drugs that cause marked lowering of viral loads do not show better clinical results than studies with only mild reductions and drugs that cause similar reductions in viral loads have widely varying clinical outcomes (HIV Surrogate Marker Collaborative Group 2000). If a surrogate marker such as viral load is a reliable indicator of drug efficacy, then more dramatic reductions in the viral load should result in better clinical outcomes, but this is not the case. Here are some quotes from the authors of a study looking at this question, published in AIDS Research and Human Retroviruses in 2000.

"If a prognostic marker is reliable as a surrogate endpoint, then comparisons of randomized treatments that show large differences in marker levels should also show large differences in the hazard of AIDS/death. ... (In our analysis), trials that show similar differences in marker effects may have quite varied differences in clinical outcome." (HIV Surrogate Marker Collaborative Group 2000, pages 1129-1130)

In their abstract they state simply:

"Short-term changes in these markers (HIV-1 RNA and CD4 count) are imperfect as surrogate end points for long-term clinical outcome because two randomized treatment comparisons may show similar differences between treatments in marker changes but not similar differences in progression to AIDS/death." (HIV Surrogate Marker Collaborative Group 2000, page 1123)

IIId. Alternative explanations for reduced AIDS death rates

Although antiretroviral combination medical regimens are credited with the dramatic reduction in AIDS death rates in the United States, there are some alternative explanations for the reduced rates that are often overlooked. The first problem is that the reduction began before the new drugs were introduced. In 1995, the AIDS death rates began to drop (CDC 1997), but the first protease inhibitor was not approved by the FDA until December of 1995. In 1996, only 20% of people diagnosed HIV-positive were taking the new medications, which is not enough of a proportion to account for the large drops that occurred (McNaughten et al. 2001).

An alternative explanation for the reduction in death rates that started in 1995 is that the number of new cases of AIDS began dropping in 1993 (CDC 1997). The drop in AIDS deaths starting two years later in 1995 would be a logical extension of the drop in new AIDS cases. In addition, in 1993 a new definition of AIDS was introduced that allowed people with no clinical illness to be diagnosed with AIDS - people with CD4 counts below 200. This group of people has represented about half of all AIDS diagnoses since that time. This means that people diagnosed since 1993 are not as sick as people diagnosed before 1993 and that more people would be able to be diagnosed with AIDS. In spite of this loosening of the diagnosis, the incidence of new AIDS cases began to fall which suggests that the number of AIDS cases would have been dropping even more steeply if this new definition had not been introduced.

Conclusions

While this paper does not explain the cause of false positive viral loads, it does demonstrate that there is a surprisingly high rate of false positives. This finding raises enough questions to advise caution regarding the current heavy reliance placed on them when making treatment decisions for people diagnosed HIV-positive.

False positive viral loads occur commonly in 3 to 10% of people who are HIV negative, with the highest reported rate being 60%. The highest false positive viral load reported was in the range of 10,000 to 100,000 copies per milliliter, and it is possible that some values over 1.5 million also indicated false positives although no

follow up data is available for these cases. This fact must be contrasted with the current practice of changing antiretroviral regimens if a person's viral load does not fall below 50, as described in Mylonakis et al.'s (2001) description of current practice guidelines.

One hypothesis that could explain these findings is that HIV viral load assays commonly misidentify RNA from normal human cells and from other microbes as being from HIV. This hypothesis could be tested by measuring viral loads in acutely ill people with high RNA levels in their blood. Because anti-HIV medications reduce RNA synthesis in a wide variety of cells, the reductions in viral load that accompany the use of these medications may indicate a non-specific reduction in total RNA burden, as opposed to a specific reduction in HIV RNA. This argument is supported by the finding of Piatak et al (1993) and others that most people with high viral loads do not have culturable/infectious virus, and that even in people who do have culturable virus, between 99.99% and 99.9999% of the viruses are non-culturable and non-infectious. These "non-infectious" viruses may represent falsely elevated viral loads due to misidentification of RNA from human cells and other microbes.

Another implication of the findings is that the diagnosis of HIV-infection continues to rely heavily on the ELISA and Western Blot antibody tests. The accuracy of these antibody tests and the experimental methodology used to determine their sensitivity and specificity should thus be carefully examined, especially since some authors consider false positives to be a problem with these tests as well (Proffitt et al. 1993, Challakeree et al. 1997, de Harven 1998a&b, Giraldo 1998, MacKenzie 1992, Papadopulos-Eleopulos et al. 1993, Sayre et al. 1996). A strong correlation between positive viral loads and positive HIV antibody tests is expected because the viral load tests are designed to look for RNA sequences that come from the proteins used in the antibody tests. If a person tests positive for the antibodies, they are likely to have RNA with the same code sequences in their blood because this RNA is used by the cells to code for these proteins. This means that a false positive HIV-antibody test is very likely to increase the risk of a false positive viral load.

Furthur examination of what factors increase the risk of false positive or falsely elevated viral loads would be extremely valuable since many treatment decisions are currently based in viral load measurements. Until such research is undertaken, however, it is advisable to make treatment decisions based on a person's symptoms and on the presence of clinical illness, and not to rely heavily on viral load test results. If a person appears to be clinically worse even though their viral load has gone down, it may be advisable to reduce or stop the medications being administered. Much of the reduced viral load observed in this situation may be due to toxic effects on human cells. Likewise, if a person is clinically healthy even though their viral load is high and they are not on any anti-HIV medications, it may be advisable to withhold

medication and instead encourage conservative health promoting measures that focus on nutritional, social, psychological, and spiritual health, rather than focusing on treatments whose primary goal is to reduce the person's "viral load".

Matt Irwin MD is a family practice resident who wrote several literature reviews on HIV and AIDS while attending medical school at George Washington University. He also holds a Master's degree in social work from the Catholic University of America. In addition to his interest in alternative views of HIV and AIDS, he specializes in health promotion with nutritional, psychological, social, and spiritual interventions, as well as classical homeopathy. He has a practice near Washington, D.C.

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