An abuse of surrogate markers for AIDS.

It should come as a shock, no doubt, to learn that if three laboratory tests somehow disappeared or were outlawed (HIV antibody test, CD4 cell count, PCR viral load test), then AIDS, as commonly understood, would formally vanish from the USA and Europe. The three laboratory tests in question are called surrogate markers because they stand in for either AIDS itself or for its supposed cause, HIV. According to the current definition of AIDS, no matter how sick an American is with AIDS-defining diseases, he or she cannot be classified as an AIDS case if antibodies to HIV are not present. In other words, for an American doctor to diagnose pneumonia, TB, dementia, cervical cancer, etc. as AIDS it is necessary to obtain laboratory test results that satisfy the definition of AIDS. I will limit my remarks to CD4 cell counts and viral load. The damnable HIV antibody tests will be discussed in detail elsewhere.

At the beginning of the AIDS epidemic, it was already recognized as probably a mistake to use CD4 as a marker of AIDS or even a measure of therapeutic effectiveness. In 1981, James Goodwin, MD, wrote what he called “a diatribe against the measurement of T-cell subsets in human diseases” [1]. His “diatribe” began:

“It’s starting again. …The T- and B-cell measures--having run through the sick, the elderly, the young, the pregnant, the bereaved--had finally run out of diseases. Each condition was the subject of many reports; so that now, to give but one example, we can conclude with some assurance that T-cell numbers are up, down, or unchanged in old folks. And it’s starting all over again, this time with T-cell subsets.”

“What will they find?” he asked. “Sometimes the suppressor cell markers will be up and helper cells down; sometimes the suppressor cells will be down and the helper cells up; sometimes they’ll be unchanged--and various combinations of the aforementioned. …My strongest argument is this: Measurement of T and B cells and their subsets in diseases has no clinical meaning.”

“Nonimmunologists have naturally assumed that any subject occupying so much journal space must be relevant in some way--a logical but incorrect assumption. …And while the identification of T-cell subsets in mouse and man represents a major breakthrough in the understanding of immunoregulation, the enumeration of these subsets in myriad diseases largely represents a waste of time”.

As recently as 1998, Mario Roederer of Stanford University confirmed Goodwin’s assessment that an obsession with T-cell subsets in AIDS patients has been a mistake: “[T]he facts (1) that HIV uses CD4 as its primary receptor, and (2) that CD4+ T cell numbers decline during AIDS, are an unfortunate coincidence that have led us astray from understanding the immunopathogenesis of this disease” [2].

Prior to Roederer’s remarks, the use of the CD4 (T-cell counts) as a surrogate marker of disease progression was also criticized by the authors of the Concorde Study, the largest clinical trial evaluating the use of AZT: The authors concluded that:
“The small but highly significant and persistent difference in CD4 count between the groups was not translated into a significant clinical benefit. Thus, analyses of the time until certain concentrations of CD4 were reached (eg, 200/mL, 350/mL, or 50% of baseline) revealed significantly shorter times in the Def[erred] group. Had such analyses been regarded as fundamental, the trial might have been stopped early with a false-positive result. This discrepancy in the differences between Imm[ediate] and Def groups in terms of changes of CD4 count and of long-term clinical response casts doubt on the uncritical use of CD4 counts as ‘surrogate endpoints’ in trials…” [3].

Thomas Fleming and David DeMets have stated that, “The use of surrogate end points has probably been more intensely discussed in the design and analysis of clinical trials of HIV infection and AIDS than in any other area” [4]. However, “Predictions having an accuracy of approximately 50%, such as the accuracy seen with the CD4 count in the HIV setting, are as uninformative as a toss of a coin.” With regards to clinical trials and FDA approval of anti-HIV drugs, Fleming and DeMets have warned that, “Surrogate end points are rarely, if ever, adequate substitutes for the definitive clinical outcome in phase 3 trials” [4].

Indeed, a summary result from a 1993 state-of-the-art conference had previously concluded that the effect of treatment on the most popular surrogate, CD4 cell count, did not accurately predict the effect of treatment on the clinical outcomes, that is, progression to AIDS or time to death [5]. Nevertheless, with the exception of the early AZT clinical trials, all subsequent anti-HIV drug trials and FDA approvals have relied exclusively on the measurements of these surrogate markers and not on the real clinical outcomes, such as morbidity and mortality, that matter to most people.

A year later, Fleming stated that, “It is very apparent one cannot simply consider establishment of statistically significant treatment effects on CD4 cell counts to be a valid surrogate for either of the two clinical endpoints. When the progression to AIDS/death endpoint was positive, the CD4 endpoint appropriately was significantly positive in 7 of 8 trials; unfortunately however, the CD4 endpoint was significantly positive in 6 of 8 trials in which the progression to AIDS/death endpoint was negative. The relationship of CD4 effects and survival is even more unsatisfactory. The CD4 endpoint was significantly positive in only 2 of 4 trials in which the survival endpoint was positive; yet it was significantly positive in 6 of 7 trials in which the survival endpoint was negative. In three other trials, survival trends were observed which were in the opposite direction of significant treatment effects on CD4” [6].

The well-recognized problems with CD4 counts eventually lead to its being replaced by the PCR viral-load test as the primary surrogate marker to be used in anti-HIV drug clinical trials. But, the “viral load” test has its share of problems. To start with, Roche’s “AMPLICOR HIV-1 MONITOR Test is not intended to be used as a screening test for HIV or as a diagnostic test to confirm the presence of HIV infection. …Quantitative culture has limited utility for monitoring virus levels in infected individuals since only a small fraction of virus particles is infectious in vitro. Infectious virus is often undetectable in asymptomatic individuals…The clinical specificity of the…test was determined by analysis of 495 anti-HIV-1 negative blood donors. None of these specimens was reactive…Assuming[/] a zero prevalence of HIV-1 infection in the seronegative
blood donors, the specificity of the test was 100%” (Roche Diagnostic Systems AMPLICOR HIV-1 MONITOR Test package insert, PMA No. BP950005/4).

References:


To save space, below is a list of some of the problems with the viral load test that were published in the scientific, medical literature:


“These findings represent a major departure from the notion that plasma HIV RNA level is a reliable predictor of rate of CD4 cell loss in HIV infection and challenge the concept that the magnitude of viral replication (at least as reflected by plasma levels) is the main determinant of the speed of CD4 cell loss at the individual level. The clinical implications are that in the majority of cases, an individual patient’s plasma HIV RNA level at the time of presentation for clinical care cannot predict, to a significant extent, the rate of CD4 cell decline that he or she will experience over the subsequent years and is therefore of limited clinical value in shaping the decision to initiate antiretroviral therapy. …The results of our study challenge the concept that CD4 cell depletion in chronic HIV infection is mostly attributable to the direct effects of HIV replication.”

“Quantitative PCR should not be used as a diagnostic test for HIV because false positives and false negatives can occur in these circumstances”.


“From April 1996 through December 2000, a total of 501 antiretroviral-naive [never taken AIDS drugs] HIV-seropositive patients who initiated HAART were recruited…at the Hospital Ramón y Cajal [Madrid, Spain]…After 24 months of follow-up, 42 (16.5%) of 255 patients were considered to have a discordant immune response [low CD4 cell counts with low viral load or high CD4 cell counts with high viral load]…Clinical progression of HIV disease was uncommon among the patients included in the analysis. Overall, 4 patients (1.6%) died of HIV infection-related complications, and 44 patients (17.3%) developed HIV infection-related clinical events…Most events (29 [65%] of 44 events) occurred within the first year after initiation of HAART. Overall, clinical events were not more frequent among patients with a discordant immune response than among patients with a good immunologic response.”


“We report in this work that HIV-1 and HIV-2 patients having a similar degree of CD4 depletion displayed similar levels of T cell hyperactivation and similar numbers of cycling cells in the peripheral blood despite great differences in the plasma viral load. These results and other recent reports call for re-evaluation of different hypotheses about causal relationships among virus concentration, CD4 depletion, and activation and turnover of T lymphocytes.”

Guidelines for Laboratory Test Result Reporting of Human Immunodeficiency Virus Type 1 Ribonucleic Acid Determination. MMWR. (2001) Nov 16;50(RR20):1-12

“[Table 2 shows that the results for identical material sent to multiple laboratories provided viral load results varying from 3,849 to 1,291,635 (Roche Amplicor HIV-1 Monitor), from 63,750 to 205,500 (Bayer HIV-1 3.0 RNA) and from 89,000 to 360,000 (Organon Teknika NucliSens)].”


“That a clinical benefit may not have been achieved with multi-drug rescue therapy calls into question the current wisdom of deeming an undetectable viral load the goal of therapy in the heavily pre-treated population. Even if it can be accepted that an undetectable viral load is an appropriate surrogate marker for clinically relevant outcomes in treatment-inexperienced patients
who are initiating combination therapy, it cannot necessarily be accepted without proof that it is a useful surrogate in heavily pre-treated patients...Therefore, until controlled trials are able to prove the utility of an undetectable viral load as a surrogate marker for clinically relevant outcomes in heavily pre-treated patients, we believe that clinicians should show caution before striving for complete viral suppression at any cost”

Saltus R. AIDS drug researchers say firm pressured them. Boston Globe. 2000 Nov 1

“When it came time to write up the data [on the AIDS 'therapeutic vaccine' called Remune] for publication, Kahn, Lagakos, and others on the team concurred that the analyses showed no benefit from the drug. But scientists from Immune Response performed their own analysis of blood tests on a sample of 250 patients in whom, the company argued, some benefit could be seen - not in longer survival, but in having lower levels of virus ['viral load'] in their blood…[Lead researcher] Lagakos said: "The company did not want our original analysis to go forward. We were put in a position where we had to agree to terms that were unacceptable to us. We decided to go forward with what we had.'’"


“Because viral RNA levels ['viral load'] are quantified as copies of RNA per milliliter, it is possible that nonviable, but persisting, HIV-1 residues are being detected by such testing. This raises an even more fundamental question as to the validity of viral RNA monitoring in general.”


“In contrast to previous reports...the viral load in the majority of the [long-term survivors] tested was detectable and, in some [long-term survivors], quite high...and variable over time.”


“Participants with baseline RNA levels below the level defining virological response were excluded from the analysis [which creates an artificial downward trend in the viral load, by eliminating people whose viral load might well go up on therapy]...In the ZDV-naive population [had not taken AZT before this trial] the maximum median fall in HIV RNA occurred by 4 weeks for all three [treatment] arms [AZT, AZT+ddl, AZT+ddC]. Thereafter, the median HIV RNA increased towards baseline levels [so you take these drugs for a lifetime for a theoretical benefit that lasts a month!]... The proportions responding (i.e. achieving HIV RNA ['viral load'] less than 800 copies/ml) in the first [year] in Delta 1 were 61% on ZDV-ddI and 45% on ZDV-
ddC [and 10% on ZDV alone]. In Delta 2 [had previously taken AZT], the proportion of responders [whose viral load dropped] was lower; 23% on ZDV-ddI, and 30% on ZDV-ddC [and 2% on ZDV alone].


“The results obtained for patients with a broad range of plasma viral loads before and after antiretroviral therapy reveal a constant mean viral (v)RNA copy number (3.6 log10 copies) per infected cell, regardless of plasma virus load or treatment status.”


“18 subjects had 1 or 2 positive results with v.2.0 and an undetectable confirmatory test for a false positive rate of 4.4%. The rate is similar at baseline (9/183 subjects = 4.9%), wk. 4 (7/162= 4.3%) and wk. 26 (2/44 = 4.5%). Of the 18 pos. specimens, 9 tested pos. once and 9 twice. With version 3.0, 11 of 67 samples tested were pos. (16.4%). 6 were pos. once and 5 twice. The range of false pos. rates was 9.1% at wk. 4 (total of 22 specimens) to 26.7% at wk. 26 (total of 15 specimens). A week 4 sample with two values of 8,000 copies/ml on v.2.0 was neg. by DNA PCR, p24 antigen and Western Blot. Follow-up testing of this subject at wk. 26 was negative for HIV antibody and RNA. The emotional impact of a false positive screening RNA test in a recently exposed person is significant. With the high false positive rate, we do not advocate the routine use of HIV RNA tests to screen asymptomatic people. The high rate of repeat false positive tests in a given sample (50%) suggests a possible biologic mechanism.”


“We selected 20 healthy volunteers, all of whom yield negative results for HIV antibodies using different screening tests. Plasma from all of them were analysed by three different currently available HIV viral load tests: branched DNA (bDNA) signal amplification assay (Chiron), nucleic acid sequence-based amplification (NASBA) Nuclisens (Organon Teknika), and Ultradirect reverse transcriptase (RT)-PCR Monitor (Roche)…2 samples [10%] yielded positive results by the bDNA assay…Another 2 specimens [10%] yielded false-positive results by the NASBA Nuclisens…[and] one of the 20 samples [5%] was interpreted as positive by the Ultradirect RT-PCR Monitor assay…using the Monitor test with non-B primers, up to 4 of the 20 samples [20%] yielded positive values…Results were reproduced in more than half of tested specimens for which plasma volumes were enough for repeat testing.”

“This study was a] Prospective follow-up of a cohort of 162 unselected, protease inhibitor-naive, antiretroviral-experienced patients with advanced HIV disease, treated with indinavir combined with two nucleoside analogues...21% of patients exhibited discrepant virological and immunological responses to treatment, of whom one-half failed to exhibit significant increases in CD4 cells despite a virological response to therapy and one-half exhibited increased CD4 cell counts in the absence of significant decrease in plasma viral load. The incidence of AIDS-defining events in the latter group of patients was similar to that of responder patients, whereas their incidence was higher in patients who failed to exhibit a virological and immunological response and those who failed to increase CD4 cells despite a significant decrease in viral load. [calling into question the common use of viral load to estimate the rate of progression to AIDS]”

False positive or false negative? It depends on the answer you want. Apparently, absence of antibodies to HIV trumps a high viral load result.


An individual tested positive by PCR, but was antibody negative. Therefore, the patient’s viral load of 100,000 copies of RNA per ml was called false-positive. It took $5000 worth of PCR testing in several labs to get the "right" answer: negative.


“In this study of 78 HIV-positive people with high CD4 cell counts and no symptoms] there were no differences in viral load with regard to time of HIV-1 infection [i.e. amount of virus does not grow over time]...10 patients fulfilled the criteria for LTNP [Long Term Non-Progressors]. 7 of these 10 patients had viral loads above 10,000 RNA copies/ml and 2 above 30,000 RNA copies/ml. The level of viral load of LTNP was not statistically different compared with the other 68 patients”


“maintenance of plasma HIV RNA levels below 10,000/ml in early HIV disease appears to be associated with decreased risk of progression to AIDS. However, in patients with more advanced disease [low CD4 cell counts], disease progression occurred in up to 30% of patients with fewer than 10,000 HIV RNA copies/ml [In other words, viral load is not terribly well associated with progression to AIDS]...HIV RNA levels should not be measured within a month of acute illnesses or within a month after influenza and pneumococcus immunizations. Increases in HIV RNA levels in blood of as much as 300-fold have been observed within two weeks of routine
immunizations against influenza, tetanus, or pneumococcus.” [In other words, viral load results are accurate only in asymptomatic people.]


“Our investigation produced two main findings. First, the false-positive and false-negative rates of PCR that we determined are too high to warrant a broader role for PCR in either routine screening or in the confirmation of diagnosis of HIV infection. This conclusion is true even for the results reported from more recent, high-quality studies that used commercially available, standardized PCR assays...We did not find evidence that the performance of PCR improved over time”


“the majority (83%) of [flu] vaccinated [HIV-positive] individuals experienced a significant increase in plasma HIV-1 RNA levels within 1-2 [weeks after] immunization and returned to their prevaccination levels within 4 weeks after immunization...patients on antiretroviral therapy were not noticeably different from those not in therapy with regard to increases in plasma viremia.”

Christine Defer et al., "Multicentre quality control of polymerase chain reaction [viral load] for detection of HIV DNA" (1992) AIDS 6: 659-663

"False-positive and false-negative results were observed in all laboratories (concordance with serology ranged from 40 to 100%)."


"The results indicate that current techniques for detecting cell-free HIV-1 DNA in serum lack adequate sensitivity, specificity, and reproducibility for widespread clinical applications."

"In any event, the levels of viral (and cellular) DNA in serum appear to be so low that reproducible detection, even with use of PCR, is not currently possible."

"The availability of sensitive assays for plasma HIV viral load and the trend toward earlier and more aggressive treatment of HIV infection has led to the inappropriate use of these assays as primary tools for the diagnosis of acute HIV infection."

"Physicians should exercise caution when using the plasma viral load assays to detect primary HIV infection..."

"Plasma viral load tests for HIV-1 were neither developed nor evaluated for the diagnosis of HIV infection... Their performance in patients who are not infected with HIV is unknown."

M. Piatak et al., "High levels of HIV-1 in plasma during all stages of infection determined by competitive PCR" (1993) Science 259: 1749-1754.

"Plasma virus levels determined by QC-PCR correlated with, but exceeded by an average of 60,000-fold, virus titers measured by endpoint dilution culture."

In fact, 53% of the viral load positive patients had no culturable HIV.

"For HIV-1 propagated in vitro, total virions have been reported to exceed culturable infectious units by factors of 10,000 to 10,000,000, ratios similar to those we observed in plasma."


"...the high level of plasma virus observed by Piatak et al. [reference above] was about 99.9 per cent non-culturable, suggesting that it was either neutralized or defective. Therefore, rather than supporting a cytopathic model, this observation actually may help explain the relatively slow dissemination of the infected cell burden and thus the relative ineffectiveness of therapy with nucleoside analogues which target this process.

"...we question the longitudinal conclusions some of these investigators have drawn from cross-sectional data. The results presented are equally consistent with the conclusion that higher viraemia is a consequence of, rather than the proximate cause of, defective immune responses."
Simply put: the AIDS surrogate markers are being abused. These surrogate markers are causing a great deal of harm by labeling people with myriad diseases and conditions—even healthy people who only have antibodies to HIV—as having incurable AIDS, which is said to be invariably fatal. The surrogate markers are also being used to obtain FDA approval of clinically ineffective AIDS chemotherapies that are highly toxic and even lethal if taken long enough.

David Rasnick