THEORY AND METHODS

Bimodal virological response to antiretroviral therapy for HIV infection: an application using a mixture model with left censoring

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Study objective: To assess whether HIV RNA levels (log_{10} scale) in highly active antiretroviral therapy (HAART) treated population have a bimodal distribution, suggesting optimal or suboptimal response to HAART.

Methods: The study population from two ongoing cohort studies comprised 564 men (4785 person visits) and 1173 women (8675 person visits) with known dates of HAART initiation and with HIV RNA measurements before and after initiation. Values below detection limit of assays were treated in the analysis as left censored. Maximum likelihood methods were used to estimate parameters and to determine possible bimodality of HIV RNA distributions.

Results: A two component mixture model fitted HIV RNA levels significantly better than did a single component distribution at different years from HAART initiation in both therapy experienced and therapy naive patients. In the fifth year after HAART initiation, 32% of men and 44% of women had HIV RNA in the higher component with medians of 5247 and 9253 copies/ml, respectively, suggesting suboptimal virological response to HAART, which was associated with poor adherence and lower frequency of CCR5 heterozygous genotype.

Conclusion: The bimodal distribution of HIV RNA persisted during the years after HAART initiation. The high occurrence of suboptimal virological response at the fifth year after HAART initiation underscore the needs for careful monitoring and patient education about the importance of treatment adherence. This data analysis overcomes limitations of measurement techniques of observations having values below detection limits and serves to characterise the dynamics of the virological response to therapies.

The goal of highly active antiretroviral therapy (HAART) is to suppress HIV RNA to undetectable levels, providing conditions for CD4 cell counts to increase, thereby reducing HIV related morbidity and mortality.[1–4] However, it is not always achievable as responses to HAART are heterogeneous.[5 6] In some people, HIV RNA levels are not suppressed below 50 copies/ml (the limit of detection of current widely used assays) after HAART, and in others, the levels rebound after an initial suppression[7 8]; both scenarios constitute suboptimal virological response, which predicts poor HIV disease prognosis.[9 10] Thus, it is important to investigate the extent of suboptimal virological response to HAART over time and to assess the extent of poor HIV disease prognosis.

In this study, the authors investigated whether the distribution of HIV RNA (log_{10} scale) is bimodal—that is, whether HIV RNA levels are composed of a mixture of two subpopulations reflecting different responses to HAART. If bimodality is identified in a HAART treated population, the subgroup with higher HIV RNA values would presumably represent suboptimal virological response. Bimodality of disease markers has been shown in other contexts. For example, Lim and coworkers[11] showed the bimodality of blood glucose reporting that ''diabetes is a distinct entity rather than an arbitrarily defined extreme end of a continuously distributed measurement''.

This report uses data from two ongoing cohort studies, the multicentre AIDS cohort study (MACS) and the women's interagency HIV study (WIHS), to examine (a) whether the distribution of HIV RNA (log_{10} scale) comprises two normal distributions; (b) assuming bimodality is identified, whether and how it would change over time from pre-HAART to post-HAART at different years, and how the distribution would differ between the MACS and WIHS; and (c) identification of exposures associated with suboptimal response to therapies. Here, we use statistical methods for appropriate handling of values below the limit of detection of current assays.

METHODS

Population and study design

The MACS was started in 1984 to study the natural history of HIV-1 infection among homosexual and bisexual men in the USA. The study design has been previously described.[12 13] A total of 2785 infected participants were either HIV positive at enrolment (80%) or infected with HIV during follow up (20%). Of the 1437 men who were alive after 1995, a total of 750 men started HAART before 31 March 2003. The WIHS was started in 1993 and it is a multicentre prospective cohort study of the natural history of HIV-1 infection in women. Methods and cohort characteristics of WIHS have been previously described.[14] A total of 2072 infected women were either HIV positive at enrolment (99%) or infected with HIV during follow up (1%). Of which 1277 women started HAART before 31 March 2003. The MACS and WIHS study protocols were approved by institutional review boards of each of the

Abbreviations: HAART, highly active antiretroviral therapy; ART, antiretroviral therapy; LD, limit of detection; MACS, multicentre AIDS cohort study; WIHS, women's interagency HIV study

participating centres, and informed consent was obtained from every participant.

Participants returned every six months for a detailed interview, a physical examination, and collection of biological specimens. In MACS, HIV RNA was determined using the Roche Amplicor RNA kit (Hoffman-LaRoche, Nutley, NJ, USA) with a limit of detection (LD) of 400 copies/ml. If HIV RNA was not detected by this kit, the Roche Ultrasensitive RNA PCR assay (Hoffman-LaRoche, Nutley, NJ, USA) was performed (LD of 50 copies/ml). In WIHS, HIV RNA was measured using the NASBA assay (Organon Teknika, Cambridge, UK) with an LD of 4000 copies/ml[15] up to September 1998, and by the NucliSens assay (Organon Teknika) with an LD of 400 through March 1999 and 80 copies/ml beginning in April 1999.[16] Self reported use of antiretroviral drugs at each visit was summarised to define whether participants were using HAART. HAART was defined according to the DHHS/Kaiser Panel guidelines.[17] The date of HAART initiation was defined as the midpoint between the last visit without reporting HAART use (last no-HAART) and the first visit at which HAART use was reported (first HAART).

MACS and WIHS data collected up to 31 March 2003 (end of semi-annual visits 38th and 17th of MACS and WIHS, respectively) were included for the analysis, with further restriction that the interval between last no-HAART date and first HAART date was $\leqslant$ 1 year. We included all HIV RNA measurements if it was within a year before and the first five years after HAART initiation, and if HAART use was reported in the preceding six months.

Statistical method: a mixture model with left censoring

Analyses were conducted for MACS and WIHS separately to assess the presence and possible differences in the two cohorts. In addition, to determine the changes before and after starting HAART, analyses were performed for each of the following six intervals: (1) $\geqslant -1$ and <0 year (that is, within one year before HAART initiation), (2) $\geqslant 0$ and <1 year, (3) $\geqslant 1$ and <2 years, (4) $\geqslant 2$ and <3 years, (5) $\geqslant 3$ and <4 years, and (6) $\geqslant 4$ and <5 years after HAART initiation. Furthermore, to assess whether the putative bimodality was present in participants who were naive to antiretroviral therapy (ART) at HAART initiation, we repeated the analysis on that subset of people.

We conducted the analysis of HIV RNA in the $\log_{10}$ scale. Parameters for each cohort and each time interval were estimated using maximum likelihood methods. HIV RNA measurements below LD were treated as left censored. We allow different LD in analysis to accommodate the types of assays used over time. The primary purpose was to find out if the HIV RNA measurements in log scale were better described by a two component mixture distribution compared with a single component distribution. Let π be the probability HIV RNA measurement coming from the lower component of the mixture distribution, with its complement $(1-\pi)$ the probability of the measurement coming from the higher component. As the proportion of post-HAART HIV RNA measurements below LD was high, there might not be enough information for estimating both mean and variance in the lower component. Therefore, equal variances were assumed in the two components. The null hypothesis (that is, lack of heterogeneous response) corresponds to $\pi = 0$. Figure 1 provides an illustration of the expected shape of the HIV RNA distribution in $\log_{10}$ scale under the alternative hypothesis.

As the null value of probability π falls on the boundary of parameter space under the null hypothesis, the standard regularity conditions of hypothesis testing by the generalised likelihood ratio test do not hold.[18] Therefore, we selected the better fitting models by simulating the likelihood ratio statistic to obtain the p values.[19 20] Technical details are described in the appendix. S-plus 6.1 (Insightful Corporation, Seattle, WA) was used to perform all statistical analyses.

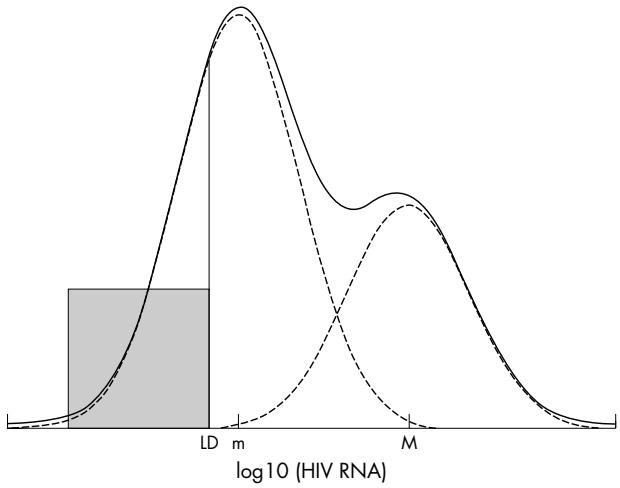

Figure 1 An illustration of the expected distribution of $\log_{10}$(HIV RNA)—a mixture of two normal distributions with equal variance. Solid and dashed lines denote the overall and component specific distributions, respectively. Shaded area represents the proportion below the limit of detection (LD).

RESULTS

The analysis included 564 men from the MACS (4785 person visits) and 1173 women from the WIHS (8675 person visits) who started HAART and had HIV RNA measurements. Among the 564 men, 475 (84%) were white, 53 (9%) African American, and 32 (6%) Latino. Among the 1173 women, 216 (18%) were white, 630 (54%) African American, and 297 (25%) Latino. By 31 March 2003, 60 (11%) of the 564 men were deceased, 38 (63% of 60) from AIDS; 191 (16%) of the 1173 women died, 83 (44% of 191) from AIDS. In both cohorts, the annual mortality rate of AIDS was 1%−2% in HIV infected persons receiving HAART over the years after HAART initiation. Before HAART initiation, 455 (81%) of the 564 men and 965 (82%) of the 1173 women had received ART with 412 (73%) men and 763 (65%) women having ART experience for more than one year before HAART initiation.

Table 1 shows descriptive statistics of study population by years from HAART initiation. The calendar years corresponding to person visits stratified by different years from HAART initiation for the two cohorts were similar. Compared with women in the WIHS, men in the MACS were about four to five years older, had 30−100 more CD4 cells/mm^3 in each time period, and had higher HIV RNA levels before HAART initiation but lower HIV RNA levels after HAART.

The two component model fitted the $\log_{10}$(HIV RNA) significantly ($p<0.005$) better than the one component model for each time period in both cohorts. Table 2 summarises the parameter estimates of HIV RNA ($\log_{10}$ scale) as a mixture of two normal distributions. In the year before HAART initiation, there was an estimated 10% of men with median HIV RNA of 55 copies/ml and 90% with a median of 30 153 copies/ml; while an estimated 22% of women had median HIV RNA of 192 copies/ml and 78% had a median of 31 384 copies/ml.

From the year before to the first year after HAART initiation, both the percentage of HIV RNA in the higher component of the mixture distribution and the median HIV RNA in that component decreased dramatically. Specifically,

Table 1 Descriptive statistics (median and interquartile ranges (IQR) based on person visits) of study populations stratified by years from HAART initiation

Years from HAART initiation	⩾−1 and <0 year	⩾0 and < 1 year	⩾1 and <2 years	⩾2 and <3 years	⩾3 and <4 years	⩾4 and <5 years
Men (MACS cohort, n = 565)						
Number of persons	533	528	476	436	388	354
Number of person visits	970	922	854	759	667	613
Calendar year						
Median	1996.4	1997.4	1998.4	1999.4	2000.3	2001.3
IQR	1995.9–1997.3	1996.9–1998.3	1997.9–1999.2	1998.9–2000.1	1999.9–2001.0	2000.8–2001.9
Age (years)						
Median	42.9	43.8	44.6	45.6	46.8	47.5
IQR	38.4–47.4	39.2–48.3	40.2–49.0	41.4–49.9	42.4–50.9	43.4–51.7
CD4 (cells/mm^3)						
Median	303	376	440	479	484	518
IQR	165–459	234–549	282–629	315–662	328–673	330–724
HIV RNA (copies/ml)						
Median	26793	214	82	<50	<50	<50
IQR	5113–96219	<50–3115	<50–4996	<50–2504	<50–1437	<50–964
Women (WIHS cohort, n = 1173)						
Number of persons	1143	1127	852	769	692	588
Number of person visits	2049	1834	1406	1260	1181	945
Calendar year						
Median	1996.9	1997.9	1998.9	1999.8	2000.7	2001.6
IQR	1996.3–1998.1	1997.3–1999.2	1998.2–2000.0	1999.3–2000.6	2000.2–2001.4	2001.1–2002.2
Age (years)						
Median	38.6	39.6	40.4	40.9	42.2	43.0
IQR	33.4–43.6	34.4–44.4	35.2–45.2	35.9–46.0	37.1–47.2	37.9–47.6
CD4 (cells/mm^3)						
Median	270	319	369	397	408	417
IQR	141–430	182–484	211–549	228–586	239–608	248–632
HIV RNA (copies/ml)						
Median	16000	855	654	430	280	180
IQR	<4000–86000	<80–13000	<80–9800	<80–9900	<80–6100	<80–5700

the percentages in the higher component decreased from 90% to 48% in men and from 78% to 62% in women; and the corresponding medians reduced by a factor of 8 and 5 for men and women, respectively. Table 2 also shows that from the first (0–1) year to fifth (4–5) year after HAART initiation measurements in the higher component of HIV RNA further decreased from 48% to 32% for men and from 62% to 44% for women, while the median of the higher component fluctuated between 3868 and 9253 HIV RNA copies/ml. In turn, the median of the lower component was estimated to be comparatively stable at <20 copies/ml after HAART initiation in both cohorts. Specifically, in the fifth year after HAART initiation, 68% of the measurements from men were estimated to have a median HIV RNA of 6 copies/ml with an interquartile range (IQR) from 2 to 22 copies/ml and 56% of the measurements from women were estimated to have a median HIV RNA of 17 copies/ml with an IQR from 5 to 62 copies/ml. The remaining 32% of men had a median of 5247 copies/ml (IQR: 1252–22 000 copies/ml) and the remaining 44% of women had a median of 9253 copies/ml (IQR: 2436–35 159 copies/ml). The goodness of fit for modelling the $\log_{10}$(HIV RNA) as a mixture of two normal distributions with equal variance is displayed in figure 2, showing that the two component model fits the $\log_{10}$ (HIV RNA) well.

Figure 3 shows the superimposed estimated bimodal distributions of $\log_{10}$(HIV RNA) for the MACS and WIHS, suggesting that the mixture distributions of the two cohorts are significantly different (Likelihood ratio test: $p<0.001$) in each time interval. To find out if the bimodality was also present in the subset of people who were ART naive at HAART initiation, the analyses were repeated using only the data from this subgroup. The bimodality did persist at all periods from HAART initiation and in both men and women. Furthermore, after four years of HAART initiation, the median levels of the lower and higher components of HIV RNA were very similar to those reported in table 2. Specifically, for men ART naive at HAART initiation, the median HIV RNA levels of the lower and higher components were estimated to be 6 and 4514 copies/ml respectively; and,

Table 2 HIV RNA ($\log_{10}$ scale) as a mixture of two normal distributions*

	Men					Women				
		Lower component		Higher component			Lower component		Higher component	
Years from HAART initiation	Number of person visits	%	Median (copies/ml)	%	Median (copies/ml)	Number of person visits	%	Median (copies/ml)	%	Median (copies/ml)
⩾−1 and <0	970	10	55	90	30153	2049	22	192	78	31384
⩾0 and <1	922	52	19	48	3868	1834	38	9	62	6823
⩾1 and <2	854	58	10	42	7865	1406	43	12	57	6516
⩾2 and <3	759	64	10	36	8111	1260	47	13	53	7367
⩾3 and <4	667	65	4	35	5067	1181	49	7	51	6326
⩾4 and <5	613	68	6	32	5247	945	56	17	44	9253

*Observations below the limit of detection (LD) handled as censored. The two component model with equal variance fitted the $\log_{10}$ HIV RNA significantly ($p<0.005$) better than the one component model for each time period in both cohorts.

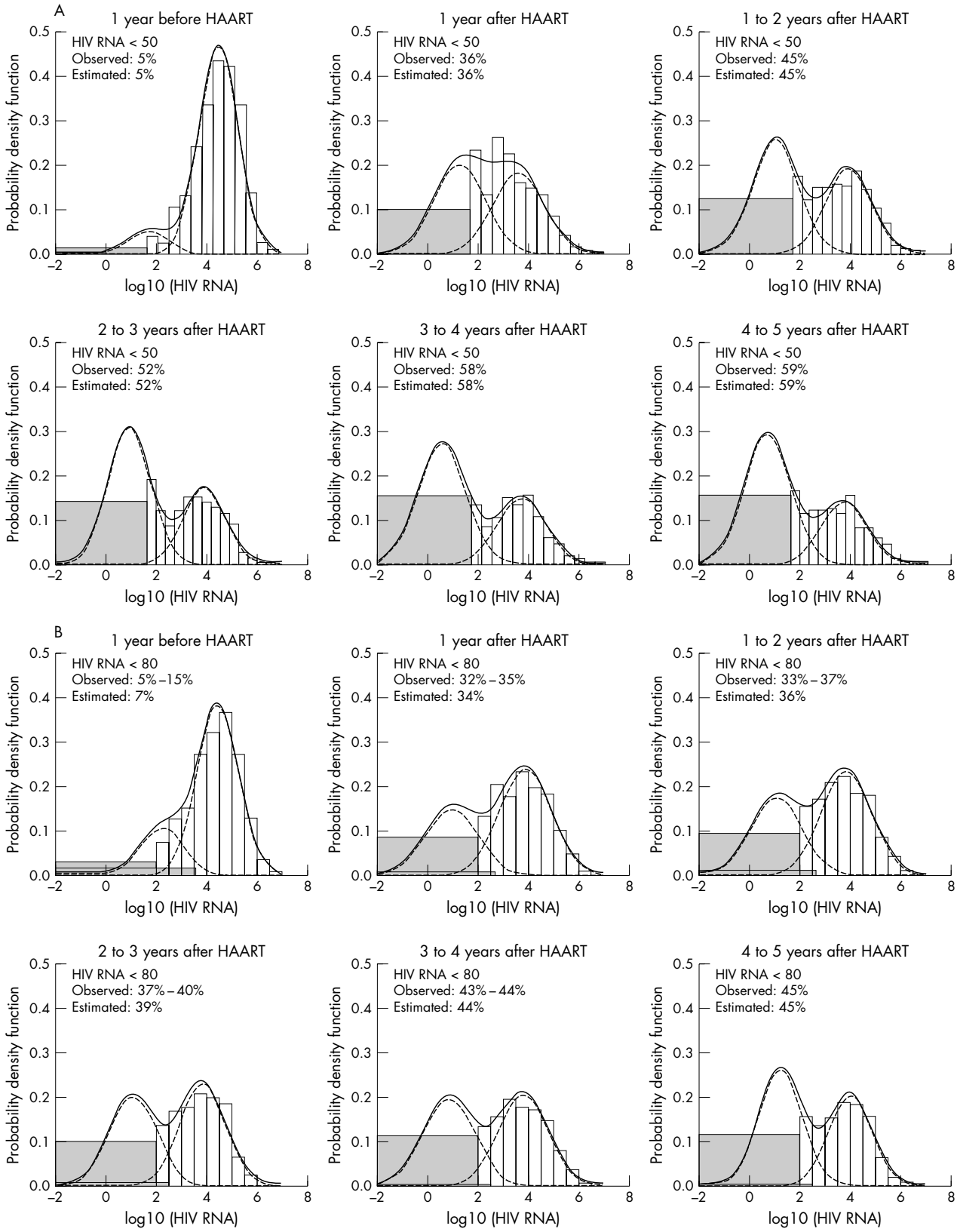

Figure 2 Goodness of fit for modelling $\log_{10}$(HIV RNA) as a mixture of two normal distributions with equal variance for the MACS (panel A) and the WIHS (panel B), respectively. The histograms are observed data and the lines are fitted values. Shaded areas depict the observations below the limits of detection (LD). LD is 50 HIV RNA copies/ml ($\log_{10}(50) = 1.7$) in the MACS, and is 80 ($\log_{10}(80) = 1.9$) or 400 or 4000 HIV RNA copies/ml in the WIHS. The observed % below 80 in the WIHS ranges from the percentage <80 copies/ml alone to the sum of the percentages of undetected HIV RNA <80, or <400, or <4000 copies/ml.

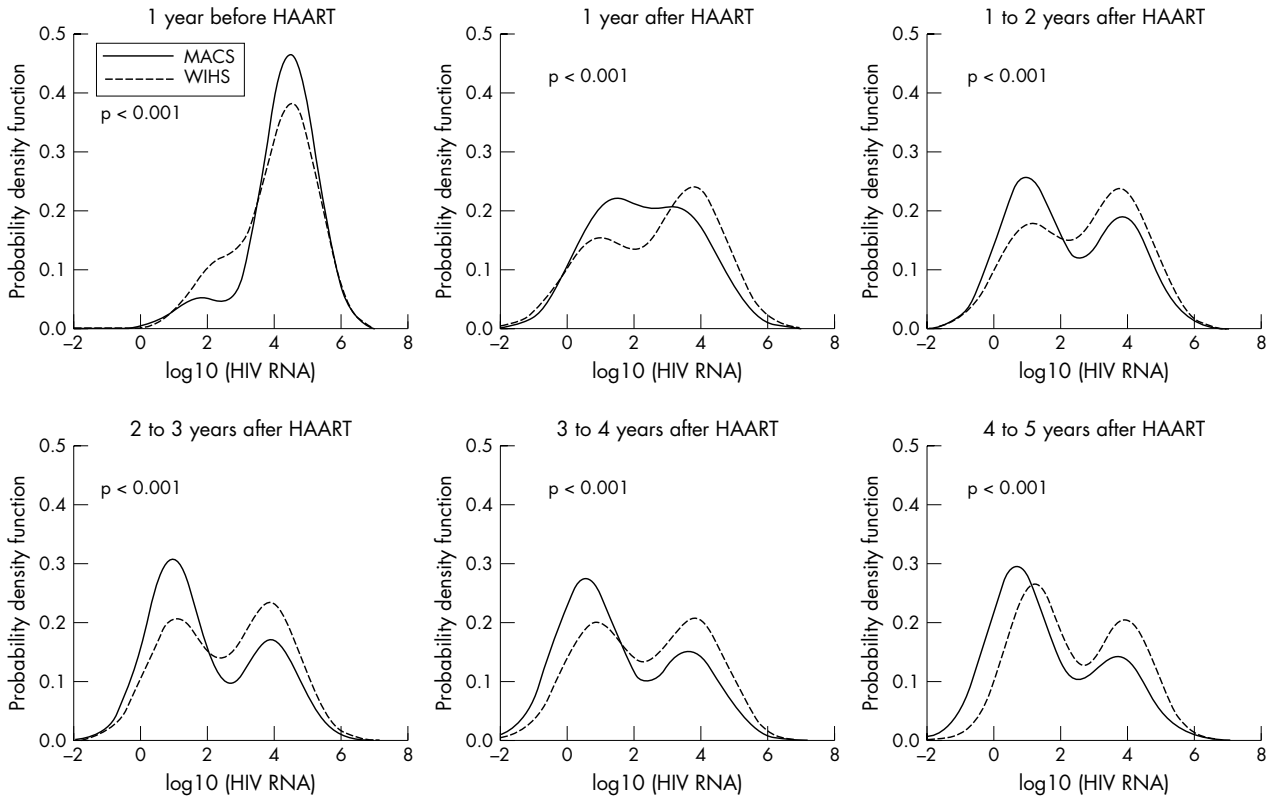

Figure 3 Superimposed estimated bimodal distributions of $\log_{10}$(HIV RNA in copies/ml) for the MACS and WIHS by different years from HAART initiation. Negative values on the x axis represent less than 1 copy/ml (for example, −2 corresponds to 1 copy/100 ml). The solid line is for the MACS and the dashed line is for the WIHS. The p value in each panel was obtained from likelihood ratio test to examine whether the mixture distributions from the two cohorts were different.

in women, they were 28 and 10034 copies/ml respectively. An important difference of the people who were ART naive at HAART initiation was that the percentage in the lower component after four years was higher. Specifically, 87% men (19% above the 68% in table 2) and 62% women (6% above the 56% on table 2) were estimated to be in the lower component of the bimodal distribution at the fifth year after HAART initiation.

As shown in figure 3, at the fifth (4–5) year after HAART in both cohorts, the two components of $\log_{10}$(HIV RNA) separated at about 2.5, which corresponded to 317 HIV RNA copies/ml. Therefore, we chose 300 HIV RNA copies/ml at the fifth year after HAART as the cut off point to partition our study population into two groups: (1) optimal virological responders—if their median HIV RNA measurements taken within the fifth year after HAART initiation <300 copies/ml, and (2) suboptimal virological responders—if their median HIV RNA ⩾300 copies/ml.

Using 300 copies/ml as the cut off value, we identified 517 optimal virological responders (54% of them women), and 423 suboptimal virological responders (72% of them women). Of the 517 subjects with optimal virological response, 51% were white, 25% African American, and 20% Hispanic; while among the 423 subjects with suboptimal virological response, 34% were white, 44% African American, and 21% Hispanic. The differences in the percentages of white people and African Americans between the two groups were because more women, most of whom were African Americans, were in the group with suboptimal virological response. Regarding AIDS restriction genes,[21] CCR5 heterozygous genotype was found in 11.5% of subjects with optimal virological response, but in only 6.6% of those with suboptimal virological response (χ^2 test p = 0.014).

Table 3 shows antiretroviral therapy use, adherence status, immunological and virological markers by time since HAART initiation for the two groups (HIV RNA <300 or ⩾300 copies/ml in the fifth year after HAART—that is, with optimal or suboptimal virological response). Before HAART initiation, the group with suboptimal virological response had lower CD4 cell count (p<0.001), higher HIV RNA level (p<0.001), and more exposure to ARTs before HAART (p = 0.029). After HAART the suboptimal virological response group had higher rate of poor adherence (<95%) to HAART (p<0.001 during both one to three and three to five years after HAART initiation). Furthermore, the rates of increase of CD4 cell count in the two groups (386−313 = 73 cells/mm^3 in the optimal virological response group and 318−249 = 69 cells/mm^3 in the suboptimal virological response group) were similar in the first year after HAART. However, the rate of increase of the group with suboptimal virological response was substantially lower (352−318 = 34 cells/mm^3 compared with 470−386 = 84 cells/mm^3; p<0.001) between one to three years after HAART; and the CD4 cell count of the group with suboptimal virological response actually declined afterwards (p<0.001).

DISCUSSION

Our data suggest that the overall distribution of $\log_{10}$ HIV RNA among HAART treated persons in the MACS and the WIHS might be better represented as a mixture of two normal distributions. Although it is well known that persons have heterogeneous responses to HAART, the significant finding of bimodality accentuates the classification of the population in two subgroups (that is—optimal and suboptimal responses). While most HAART users achieved optimal virological response (median HIV RNA <20 copies/

Table 3 Antiretroviral therapy use, adherence, immunological and virological markers by time since HAART initiation stratified by HIV RNA ⩾ 300 or <300 copies/ml at the fifth year after HAART initiation

Years from HAART initiation	HIV RNA <300 copies/ml at fifth year after HAART (n = 517)				HIV RNA ⩾300 copies/ml at fifth year after HAART (n = 423)			
	⩾−1 and <0	⩾0 and <1	⩾1 and <3	⩾3 and <5	⩾−1 and <0	⩾0 and <1	⩾1 and <3	⩾3 and <5
Number of person visits	913	887	1660	1753	757	705	1307	1332
NRTI, %								
=0	31	0	0	0	26	0	0	0
=1	17	2	9	9	22	4	12	10
⩾2	52	98	91	91	52	96	88	90
PI, %								
=0	95	12	16	41	93	9	16	31
=1	4	76	68	43	7	75	61	45
⩾2	1	12	16	16	<1	16	23	24
NNRTI, %								
=0	>99	83	68	54	99	85	65	54
=1	<1	17	32	45	1	15	34	44
⩾2	0	0	<1	1	0	0	1	2
Adherence, %		n = 125	n = 1207	n = 1740		n = 77	n = 902	n = 1317
100	NA	39	42	40	NA	36	36	34
95–99	NA	40	45	47	NA	38	39	41
75–94	NA	15	9	12	NA	22	18	17
<75	NA	6	4	1	NA	4	7	8
CD4 (cells/mm^3)								
Median	313	386	470	528	249	318	352	339
IQR	178–461	246–550	306–656	351–728	150–400	199–461	213–532	196–541
HIV RNA (copies/ml)								
Median	13244	115	<80	<80	28000	2554	3500	4365
IQR	3744–64000	<80–1551	<50–505	<50–80	5500–110000	200–19000	320–23000	690–23195

ml), a substantial percentage of them (32% in men and 44% in women) had suboptimal response (median HIV RNA >5000 copies/ml) even in the fifth year after HAART initiation. These percentages with suboptimal response after prolonged use of HAART are the people on whom emergence of resistance may be seen in the future. In general, using the bimodal mixture model that we have proposed here for characterising failures (that is, progressing to disease under HAART) is likely to be a more efficient way in cohort studies following up persons under HAART for a long term.

This report presents the first characterisation of the distribution of HIV RNA using a mixture of parametric models with left censoring and relates it to the extent of suboptimal virological response to HAART at different years after HAART initiation, thereby providing a unique historical look at the distribution of HIV RNA levels in the HAART treated population.

In this analysis over 80% of the participants had received ART before HAART. Some of them might have had partial or no virological response to ART.[22 23] This may account for the presence of bimodality of the distributions of HIV RNA levels in the year before HAART initiation. However, bimodality is not fully explained by antiretroviral experience before HAART initiation because, when we restricted the analysis to the subgroups who were ART naive at HAART initiation, the bimodality persisted with similar median values after four years from HAART initiation but with higher percentages in the lower component of HIV RNA for the ART naive HAART initiators. This is consistent with ART naive persons having a better response to treatment.

In our analyses we included only the visits where people reported receiving HAART in the previous six month interval, thereby eliminating the suboptimal virological response because of recent withdrawal of HAART. We also found that those with suboptimal virological response had more advanced HIV disease and ART exposure before HAART; and they had lower adherence after HAART, which is consistent with prior reports.[24–29] In addition, we identified that there were fewer subjects with CCR5 heterozygous genotype among the suboptimal virological responders regardless of their level of adherence to HAART. This showed that poor adherence to HAART and lower frequency of CCR5 heterozygous genotype might be independently associated with suboptimal virological response.

In this analysis, the variances of two components were assumed to be equal because of a large proportion of censored data. As the left censored data (that is, below the LD) were unobservable, there is uncertainty about the actual number

What this paper adds

The bimodal distribution of HIV RNA persisted during the years after HAART initiation. The high occurrence of suboptimal virological response at the fifth year after HAART initiation underscore the needs for careful monitoring and patient education about the importance of treatment adherence. Our approach overcomes limitations of measurement techniques of observations having values below detection limits and serves to characterise the dynamics of the virological response to treatments.

Public policy implications

High percentages (32%–44%) of persons with moderate levels of HIV RNA after four years receiving HAART underscore the need for careful monitoring, as they have the potential for increasing resistance and consequently reducing treatment options over the long term. Given the relation of suboptimal response to lower adherence to HAART, patient education is needed to emphasise the importance of treatment adherence. Overall, the low levels of HIV RNA in people treated with HAART over four years, many of whom did not fully adhere to the recommended use, makes it imperative to offer HAART to people infected with HIV and who fulfilled the guidelines recommended for who should be receiving treatment.

of components of the overall distribution and the actual distribution of the censored data. Although unlikely, it is not unfeasible that the true distribution could be unimodal with median below 50 and a heavy right tail to incorporate what here we have identified as the second component of the suboptimal responders. The inferences reported here could be validated once methods to detect HIV RNA below 50 and 80 copies/ml become widely available and properly standardised.

Most men in the MACS are white, and women in the WIHS are mostly African Americans, who differ in many social and demographic aspects. Despite these differences, it is comforting to note that the general shape and changes of the distributions of $\log_{10}$(HIV RNA) (fig 3) are present in both cohorts of HIV infected persons receiving HAART. The significant findings in figure 3 showing more suppression of HIV RNA in the cohort of men cannot be ascribed to sex only but have to be interpreted in the context of the differences between the two cohorts. Specifically, women participating in the WIHS cohort relative to the men in the MACS cohort are of substantially lower socioeconomic status with more difficulties to adhere to the complexities of HAART therapy.

In summary, high percentages (32%–44%) of persons with moderate levels of HIV RNA after four years receiving HAART underscore the need for careful monitoring, as they have the potential for increasing resistance and consequently reducing treatment options over the long term. Give the relation of suboptimal response to lower adherence to HAART, patient education is needed to emphasise the importance of treatment adherence. Overall, the low levels of HIV RNA in people treated with HAART over four years, many of whom did not fully adhere to the recommended use, makes it imperative to offer HAART to people infected with HIV and who fulfilled the guidelines recommended for who should be on treatment.

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APPENDIX

SIMULATING THE LIKELIHOOD RATIO STATISTIC

For normal component densities, McLachlan[19] proposed a Monte Carlo approach for the calculation of a p value of the likelihood ratio test when testing whether the number of components G is 1 under the null hypothesis compared with 2 under the alternative hypothesis (that is, a mixture of two normal distributions with common variance). In this report, we use McLachlan's approach to assess the p value of the likelihood ratio test for $\pi = 0$ under the null hypothesis compared with $\pi > 0$ under the alternative hypothesis. Based on the data $\mathbf{Y} = (Y_1, \ldots, Y_N)$, let $\hat{\boldsymbol{\theta}}^{(0)} = (\hat{\mu}^{(0)}, \hat{\sigma}^{(0)})$ and $\hat{\boldsymbol{\theta}}^{(1)} = (\hat{\pi}, \hat{\mu}_1^{(1)}, \hat{\mu}_2^{(2)}, \hat{\sigma}^{(1)})$ are the maximum likelihood estimators of the parameters under H_0 (that is, $\pi = 0$) and H_1 (that is, $\pi > 0$), respectively. The distribution of the likelihood ratio statistic to test the null hypothesis $\pi = 0$ compared with the alternative of $\pi > 0$ can be simulated as follows. Under the null hypothesis, a simulated sample $\mathbf{Y}_s^{(0)} = (Y_{1s}^{(0)}, \ldots, Y_{Ns}^{(0)})$ for $1 \leq s \leq S$ replications, each with sample size N is generated from the density $f(\mathbf{y}, \hat{\boldsymbol{\theta}}^{(0)})$. Let $\widehat{\boldsymbol{\theta}}_s^{(0)}$ and $\widehat{\boldsymbol{\theta}}_s^{(1)}$ be the MLEs for θ under H_0 and H_1 based on the simulated sample $\mathbf{Y}_s^{(0)}$, respectively. It is expected that $\widehat{\boldsymbol{\theta}}_s^{(0)}$ be very close to $\widehat{\boldsymbol{\theta}}^{(0)}$, and $\widehat{\boldsymbol{\theta}}_s^{(1)}$ will encode the variability of $\widehat{\boldsymbol{\theta}}$ under H_1 when H_0 is true. In particular, the variance of $-2\log\lambda_s = -2\left\{\log f(\mathbf{Y}_s^{(0)}, \widehat{\boldsymbol{\theta}}_s^{(0)}) - \log f(\mathbf{Y}_s^{(0)}, \widehat{\boldsymbol{\theta}}_s^{(1)})\right\}$ will represent the variability of the likelihood ratio statistic (LRS) $-2\log\lambda = -2\left\{\log f(\mathbf{Y}, \widehat{\boldsymbol{\theta}}^{(0)}) - \log f(\mathbf{Y}, \widehat{\boldsymbol{\theta}}^{(1)})\right\}$ when H_0 is true. If the value of the LRS from the original data is between the $(j-1)^{\text{th}}$ and the j^{th} smallest values of its S replications, then the p value for the test that rejects H_0 is approximately $1-j/(S+1)$. A total of $S = 200$ simulations were run to provide the p values reported here.

APHORISM OF THE MONTH

The problem with school health

There is a problem with school health and with Healthy Schools, because they never talk about medical care and yet the school is the first line of medical care for ordinary people. The combination of school based health education and self health care can produce expert, active consumers and health citizens. Why don't we do it? Is it part of the conspiracy of health professionals against the public?

Lowell Levin and JRA