Ongoing changes in HIV RNA levels during untreated HIV infection: implications for CD4 cell count depletion

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Background: Understanding of the interplay between plasma HIV RNA level and CD4 cell count depletion in untreated infection remains incomplete.

Methods: We studied 1169 people with HIV seen for care at a major London clinic while naive to antiretroviral therapy. We considered pairs ($n = 5940$) of consecutive simultaneously measured CD4 cell count and plasma HIV RNA values from patients who had never started therapy. Baseline was the first date when both measures were known.

Results: HIV RNA levels increased variably and often substantially from baseline (60% experience an increase of over 50 000 copies/ml by 5 years of follow-up). The current HIV RNA level (i.e. first value of the pair) was strongly associated with the time-standardized change in CD4 cell count, with a mean 106 cells/μl per year greater rate of CD4 cell count decline per log-copy/ml higher current HIV RNA level ($P < 0.0001$). After adjustment for the current level, higher baseline HIV RNA was not associated with CD4 cell count decline. There was no average CD4 cell count decline with current HIV RNA level below 3.0 log-copies/ml, compared with a 159 cells/μl per year decline for those with HIV RNA at least 5.5 log-copies/ml ($P < 0.0001$). Further, the current CD4 cell count predicted subsequent changes in HIV RNA level (0.04 log-copies/year greater increases per 100 cells/μl lower CD4 cell count; $P < 0.0001$).

Conclusion: The often substantial increases in HIV RNA level observed in untreated HIV infection appear fundamentally linked to CD4 cell count depletion. Research into mechanisms by which HIV RNA levels rise over time should yield insights into the causes of CD4 cell count depletion, as the two processes are intimately linked.

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Introduction

Differences in rates of peripheral blood CD4 cell count depletion explain variability between untreated HIV-positive individuals in the time for infection to lead to AIDS [1,2], but understanding of the interplay between ongoing HIV replication, measured by plasma HIV RNA level, and CD4 cell count depletion remains incomplete [3,4]. Although the level of plasma HIV RNA at a fixed point during chronic infection has been shown to predict the rate of subsequent CD4 cell count decline in HIV-infected people [5–8], it has been suggested that only a small proportion of variability in CD4 cell count slope can be explained [6]. Several studies have considered trends over time in HIV RNA level and CD4 cell count [9–16]. HIV RNA level increases during the natural course of

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HIV infection, at varying rates between individuals [12,14,15]. The relevance of such increases for potentially driving ongoing CD4 cell count depletion remains to be determined [11,16], as do the factors associated with HIV RNA increases. We provide some further insights into these issues by studying in detail the ability of current HIV RNA levels to predict future CD4 cell count changes, and vice versa, in a large observational cohort of people with HIV followed while antiretroviral naive.

Patients and methods

We studied 1169 patients who attended for care at the Royal Free Hospital NHS Trust (in north London, UK) while naive to antiretroviral therapy, between 1996 and 2007. The cohort has been described in detail [17–19] and generally contains patients representing the broad range of demographic characteristics of people with HIV in the UK, mainly comprising men who have sex with men and heterosexual men and women who have acquired HIV in sub-Saharan Africa. Patients usually attend clinic approximately every 3 months, and HIV RNA levels and CD4 cell counts were measured at the time of each visit. CD4 cell counts have been measured using standard flow cytometry, whereas plasma HIV RNA levels have been measured using reverse transcription polymerase chain reaction-based approaches throughout, although the exact assay used has changed over time (from mainly Roche PCR assays to a recent change to the Abbott PCR assay from 2006 – other assays have not been used with appreciable frequency).

Statistical analyses

We considered various analytical approaches. Our main approach was to consider pairs of consecutive CD4 cell count values from patients who had never started antiretroviral therapy. Included pairs of values were between 60 days and 2 years apart. Further, HIV RNA measures had to be performed from samples on the same day as the two CD4 cell counts, or at most 1 week apart. Thus, if a person had five such HIV RNA and CD4 cell count measures while antiretroviral naive, such a person would contribute four pairs of CD4 cell count/HIV RNA values. Baseline was defined to be the first date on which both the CD4 cell count and HIV RNA level were measured (within at most 1 week apart). Time-standardized changes in HIV RNA and CD4 cell count (expressed per year) were calculated by simply subtracting the first value from the second and dividing by the time span between the measures. Pairs were excluded if there was a decline in HIV RNA level of more than one log between the maximum value previous to the pair and either value in the pair, or a difference between the first and second value of the pair of more than 0.8 log-copies/ml, as this was adjudged to be a degree of change inconsistent with natural changes (although super-infection could in theory explain such a change) and would therefore suggest possible unrecorded antiretroviral treatment usage around the time of one or both of the measurements, although our antiretroviral treatment data are checked against case notes in a 100% audit carried out annually, so this is likely to be rare. Also excluded were pairs in which the first CD4 cell count value was less than 100/μl, to ensure there was sufficient scope for observing CD4 cell count decline in the interval. Using these pairs as the unit of analysis, we assessed the mean time standardized change in CD4 cell count according to the HIV RNA level at baseline (the first time point that HIV RNA level and CD4 cell count had both been measured) and the current HIV RNA level. These groups were log HIV RNA level less than 3, 3–3.49, 3.5–3.99, 4.0–4.49, 4.50–4.99, 5.00–5.49 and more than 5.5 log-copies/ml. Generalized linear models were also fitted to assess factors associated with the time-standardized CD4 cell count decline (using PROC GENMOD in SAS 9.1). Covariates considered were the first CD4 cell count value of the pair (although interpretation of this effect is difficult due to regression to the mean), the baseline and current HIV RNA level, age and sex. Generalized estimating equations were used to account for clustering between pairs within individuals, using an autoregressive correlation structure. We also fitted a model to assess factors associated with the time-standardized change in log HIV RNA level across the pair, with particular focus on the ability of the first CD4 cell count of the pair to predict the change in HIV RNA.

The relationship between current HIV RNA level and CD4 cell count was also assessed using a random effects model with CD4 cell count as the dependent variable. This model had a random intercept and fixed covariates of time from baseline (the first time point that HIV RNA level and CD4 cell count had both been measured), CD4 cell count at baseline and current log HIV RNA level (using PROC MIXED in SAS 9.1), similar to the approach of Lima *et al.* [16].

A further approach to assessing the association between the HIV RNA level and CD4 cell count decline was a time-to-event analysis in which we considered as time zero the first date when both the CD4 cell count and HIV RNA level had been measured, and considered the time taken for a drop of 100/μl in CD4 cell count The HIV RNA level was fitted as both a fixed baseline covariate and as a time-updated covariate in a Cox proportional hazards model. Other covariates included were age and sex.

We also fitted a random effects model to individual HIV RNA values to assess trends over time from baseline.

Results

Table 1(a) shows the characteristics of the 1169 patients included at baseline. These patients contributed a total of

Table 1. Characteristics of patients.

(a)	
Female sex	219 (19%)
Risk	
MSM	790 (68%)
Heterosexual	316 (27%)
Other	63 (5%)
Age at first visit (years)	33 (29–39)
Calendar date	Jan 2001 (Jul 1997 – Jan 2004)
Median time of baseline from date of first positive HIV test (year; IQR)	0.3 (0.0–2.8)
Mean baseline CD4 cell count (μl; median; IQR)	535 (501; 370–663)
Mean baseline HIV RNA (median; IQR) (log-copies/ml)	4.29 (4.42; 3.80–4.92)
Subsequently started ART	637 (54%)
Number of CD4 cell count/HIV RNA pairs contributed (median; IQR)	4 (2–7)
Median time span from baseline to 2nd value of last pair (year; IQR)	2.3 (1.1–4.4)
(b)	
Time span between values (day; median; IQR)	112 (90–159)
CD4 cell count (μl)	
Mean (median; IQR) value 1[a]	520 (476; 370–626)
Mean (median; IQR) value 2[a]	496 (458; 344–608)
Mean (median; IQR) time-standardized change in CD4 cell count	−66 (−63; −261 to +128)
HIV RNA level (log-copies/ml)	
Mean (median; IQR) value 1[a]	4.32 (4.44; 3.86–4.91)
Mean (median; IQR) value 2[a]	4.35 (4.46; 3.89–4.95)
Mean (median; IQR) time-standardized change	+0.09 (0.00; −0.46 to +0.68)

(a) Characteristics at baseline (first date HIV RNA level and CD4 count both known) and follow-up information for the 1169 patients included. Number (percent) or median [interquartile range (IQR)]. (b) Characteristics of the 5940 CD4 cell count/HIV RNA pairs. ART, antiretroviral therapy; IQR, interquartile range; MSM, men who have sex with men.
[a]Value 1 is first value of the pair; value 2 is the second.

5940 CD4 cell count per HIV RNA pairs. The characteristics of the pairs are shown in Table 1(b). The overall time-standardized mean change in CD4 cell count was −66 cells/μl per year ($P < 0.0001$). Although the median change in log HIV RNA was zero, the mean change was +0.091 log-copies/ml per year (a 23% rise in HIV RNA level, a doubling every 3.3 years; $P < 0.0001$); the mean change in log HIV RNA level from a random effects model was +0.087 log-copies/ml per year (95% CI +0.073–0.101; $P < 0.0001$; SD 0.15 log-copies/ml). HIV RNA rises are also illustrated in Fig. 1, showing the median change from baseline in log HIV RNA, considering all HIV RNA values before start of therapy and Kaplan–Meier plots of time to HIV RNA level rising to one log-copy/ml above baseline and to 50 000 copies/ml above the baseline level. In both cases, this probably does not fully reflect the average rate of change in individuals as those with greater rises are more likely to be started on therapy and are hence censored thereafter. Table 2 shows the probabilities of transition between viral load category from one measure to the next. Consistent with the regression to the mean phenomenon, those with HIV RNA in a lower category are more likely to increase to a higher category than decrease to a lower one, whereas those already in a higher category (log HIV RNA 5–5.49 copies/ml) are more likely to experience a decrease than increase in category.

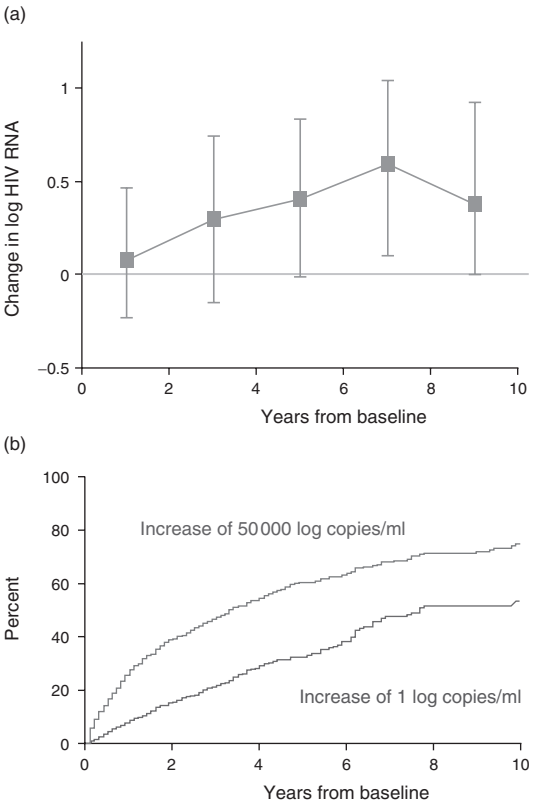

Fig. 1. Changes in HIV RNA. (a) Median [interquartile range (IQR)] change from baseline in log HIV RNA, considering all HIV RNA values before start of therapy. (b) Kaplan–Meier plots of time to HIV RNA level rising to 1 log-copy/ml above baseline and to 50 000 copies/ml above the baseline level.

The mean time-standardized change in CD4 cell count, according to the baseline HIV RNA level is shown in Fig. 2(a). There is a strong, graded relationship such that the rate of CD4 cell count decline is greater for people

Table 2. Probabilities of transition between viral load categories from current (first of pair) value to next value (second of pair), expressed as percentages (in bold), with numbers of observations.

Current (first) viral load category (log-copies/ml)	Next (second) viral load category (log-copies/ml)						
	<3	3–3.49	3.5–3.99	4.0–4.49	4.5–4.99	5.0–5.49	≥5.5
<3	**81.5**	**17.3**	**1.3**				
	392	83	6				
3–3.49	**12.3**	**50.2**	**31.5**	**6.0**			
	51	209	131	25			
3.5–3.99	**1.0**	**12.4**	**53.9**	**30.5**	**2.2**		
	9	109	472	267	19		
4–4.49		**0.5**	**16.1**	**54.1**	**27.1**	**2.1**	
		7	226	758	380	29	
4.5–4.99			**1.0**	**19.9**	**55.3**	**22.2**	**1.7**
			15	306	851	341	26
5–5.49				**2.8**	**28.1**	**51.0**	**18.2**
				25	249	452	161
≥5					**5.3**	**38.4**	**56.3**
					18	131	192

Probabilities of transition between viral load categories from current (first of pair) value to next value (second of pair), expressed as percent (in bold), with numbers of observations. Note that transitions for empty cells are not possible due to inclusion criteria for pairs.

with higher baseline HIV RNA level. There was a mean 46 cells/μl per year increased rate of decline in CD4 cell count per log-copy/ml higher baseline HIV RNA level, after adjustment for other factors (model 1, Table 3(a); $P < 0.0001$). We also evaluated the CD4 cell count change, according to the current (i.e. first value of the pair; Fig. 2(a)) HIV RNA level, and this shows a stronger association than with the baseline level. There was no evidence of any CD4 cell count decline, on average, when the current HIV RNA level was below 3.0 log-copies/ml, compared with a decline of 159 cells/μl per year for those with HIV RNA ≥at least 5.5 log-copies/ml (Fig. 2(a)). In a multivariable model (Table 3(a), model 2) which included both baseline and current HIV RNA level, there was a mean 106 cells/μl per year greater rate of CD4 cell count decline per log-copy/ml higher current HIV RNA level ($P < 0.0001$). After adjustment for the current level, there was a 30 cells/μl lesser CD4 cell count decline per 1 log-copy/ml higher baseline HIV RNA level; 95% CI (+13–47); $P = 0.0007$). To illustrate the reason for this, we fitted a reparameterized model in which we included the current HIV RNA level and the change from baseline in HIV RNA, instead of the baseline level. The difference in rate of CD4 cell count decline per log-copy/ml higher current HIV RNA level was −77, 95% CI (−93–60; $P < 0.0001$), whereas that for the change from baseline in log HIV RNA was −30 (−47–−13; $P = 0.0007$). Thus, the more the HIV RNA level has risen from baseline to the current time, the greater the subsequent rate of CD4 cell count decline, even after standardizing for current HIV RNA level, further emphasizing the likely significance of rises in HIV RNA level for determining the rate of CD4 cell count depletion. This finding of a much greater predictive importance of the current, compared with the baseline, HIV RNA level was consistent when using a time-to-event analytical approach and when using random effects models (Table 3).

In addition, findings were similar when fitting a random effects model of factors associated with the CD4 cell count; based on all available CD4 cell counts in ART-naive people for which the HIV RNA level was also known, there was an estimated 43/μl (95% CI 38–48) lower CD4 cell count per 1 log higher (time-updated) HIV RNA level ($P < 0.0001$).

Next, we considered the median (interquartile range) log HIV RNA level according to the CD4 cell count (Fig. 2). The tendency for HIV RNA levels to be higher at lower CD4 counts is strong, and continues across the entire CD4 cell count spectrum, even within the range of values found in uninfected people [20]. In addition, for 95.5% of CD4 cell counts less than 200/μl the person had previously had an HIV RNA level above 4 log-copies/ml, and for 86.2% of people with CD4 cell count below 50/μl the person had an HIV RNA level above 4.7 log-copies/ml (50 000 log copies/ml), suggesting that CD4 cell count depletion can generally only continue below a certain level if the HIV RNA level is sufficiently high.

Because changes in HIV RNA appear to be important in driving CD4 cell count depletion, we studied factors predicting the change in HIV RNA level in the 5940 pairs of measures. There was a 0.040 log-copies/ml per year lesser rise in HIV RNA per 100/μl higher current CD4 cell count (1st value of the pair; $P < 0.0001$), indicating that the current level of CD4 cell count predicts the change in HIV RNA level. In addition, women had a 0.14 log-copies/ml lesser increase in log HIV RNA level per year ($P < 0.0001$) but age did not predict the change in HIV RNA level.

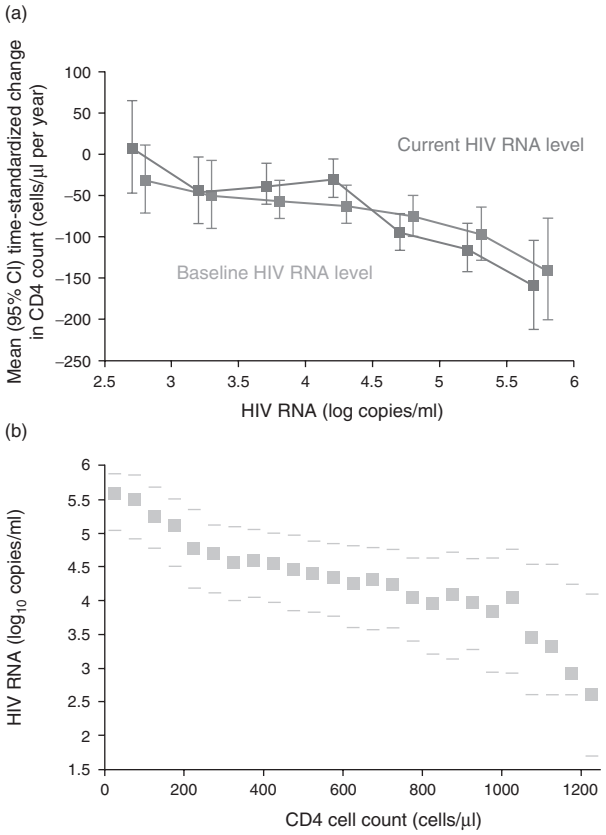

Fig. 2. Association between HIV RNA level and CD4 count. (a) Mean time-standardized change in CD4 cell count within pairs of consecutive values, according to level of HIV RNA at baseline and the 1st value of the pair and (b) median [interquartile range (IQR)] HIV RNA level according to concurrent CD4 cell count in natural HIV infection, based on 11 595 concurrent measures. Numbers of pairs contributing to the analysis, according to baseline and current $\log_{10}$ HIV RNA level, respectively, are: less than 3 (803, 481), 3–3.49 (469, 416), 3.5–3.99 (1142, 876), 4.0–4.49 (1320, 1400), 4.50–4.99 (1348, 1539), 5.00–5.49 (599, 887) and more than 5.5 (259, 341). CI, confidence interval.

Discussion

The analyses we present here provide documentation of the extent and significance of ongoing changes in HIV RNA level in untreated infection, demonstrating an intimate link between HIV RNA increases and CD4 cell count depletion. The HIV RNA level was considerably more strongly discriminatory for subsequent CD4 cell count decline in our study than found in studies based on a single HIV RNA level at a fixed time [5–8]. Mellors *et al.* found the mean rate of decline ranged from 36 cells/μl per year in the lowest HIV RNA group, compared with 76 cells/μl per year in the highest of the five groups, a 40 cells/μl per year difference [5] compared with a difference of 167 cells/μl per year across the range of our seven groups. Investigators for one study that related HIV RNA level to subsequent CD4 cell count decline over a mean of 2.2–5.1 years argued that HIV RNA level only minimally predicts CD4 cell count decline, because of the high variability in observed CD4 cell count decline for a given HIV RNA level [6]. Our analysis suggests that part of the reason for this variability, and the much stronger predictive power of the current compared with the baseline HIV RNA level, is likely to be the fact that the HIV RNA level changes during the follow-up, by different amounts for people with the same baseline HIV RNA level. Nevertheless, there is a very high level of variability in our time-standardized CD4 cell count changes (IQR -258 ± 128 cells/μl per year), which is due to measurement variability and intraindividual variation caused by factors including intercurrent illness, premeasurement exercise level and diurnal variation [21–23]. The fact that our CD4 cell count changes are based on two consecutive values only makes the variability observed particularly high. The advantage of this approach, however, is that short-term changes in CD4 cell count can be related to the current HIV RNA level. The variability means that, for example, for a person with HIV RNA level below 3 log-copies/ml, the change in CD4 cell count between consecutive measures is likely to be highly variable, but so long as the HIV RNA level remains below 3 log-copies/ml it will on average remain at around zero. Likewise, a person with HIV RNA maintained more than 5.5 log-copies/ml will experience change averaging CD4 cell count decline of 159 cells/μl per year.

Our results do not tell us the means by which increased HIV replication leads to CD4 cell count depletion. HIV RNA increases could be secondary to increased generalized immune activation, for example as measured by $CD38^{++}$ expression on CD8 cells, which could itself be the most direct cause of CD4 cell count loss [24,25]. Importantly, we also found that a lower current CD4 cell count is predictive of a larger increase in HIV RNA level, providing evidence for feedback such that HIV RNA rises lead to lower CD4 cell counts which, in turn, lead to greater rises in HIV RNA level, probably due to reduced ability of CD4 cells to prompt the HIV-specific cellular immune response.

We show that HIV RNA levels tend to increase throughout untreated HIV infection. Thus, although the long-term course of HIV is to some extent already predictable soon after the time of infection, and some of this is due to genetic factors [26], there is much variability in HIV RNA course after the 'set point' used as a phenotype in genetic studies. It would be of interest to investigate host genetic predictors of these HIV RNA increases.

Changes in HIV RNA assays over time are unlikely to affect the association between HIV RNA level and CD4 cell count depletion. We also studied the change in HIV

Table 3. Association between HIV RNA level and CD4 count depletion.

(a)	Difference (95% CI) in mean time standardized CD4 change per 1 log copies/ml difference in HIV RNA level*	*P*-value
Model 1:		
Baseline HIV RNA level**	−46 (−59–33)	<0.0001
Model 2:		
Baseline HIV RNA level**	+30 (+13–47)	0.0007
Value 1 HIV RNA level**	−106 (−129–84)	<0.0001

* adjusted for the 1st CD4 cell count of the pair (−50 and −55 difference in mean time-standardized CD4 change per 100 cells/μl higher in models 1 and 2, respectively; $P < 0.0001$), sex and age (both not statistically significant), ** per 1 log higher.

(b)	Relative hazard* (95% CI; *P*-value)
Model 1:	
Baseline HIV RNA**	1.34 (1.20–1.49; $P < 0.0001$)
Model 2:	
Baseline HIV RNA**	0.88 (0.74–1.05; $P = 0.16$)
Time updated HIV RNA**	1.71 (1.43–2.03; $P < 0.0001$)

* adjusted for baseline CD4 cell count (relative hazard 1.30 and 1.33 per 100/μl higher in models 1 and 2, respectively), sex and age (both not statistically significant), ** per 1 log higher.

(a) Adjusted difference in mean time-standardized CD4 change within pairs per 1 log-copies/ml difference in HIV RNA level (5940 pairs included). (b) Relative hazard of a 100 cells/μl decline in CD4 cell count from baseline, according to baseline and time-updated HIV RNA level (534 individuals experienced a decline of at least 100 cells/μl, out of 1048 people with baseline CD4 cell count more than 150 cells/μl.

RNA level between pairs restricting to those pairs for which the same assay was used for each measure and the highly statistically significant increase in HIV RNA levels remained (data not shown).

Over half (54%) of the patients in this study eventually started antiretroviral therapy (ART). Selection of patients for timing of initiation of ART could influence our findings, in that people with rapid declines in CD4 cell counts will tend to contribute fewer pairs to our analysis. This would likely lead to underestimation of the overall average rate of CD4 cell count decline but should not bias the association between current HIV RNA level and CD4 cell count change between this and the next measure. Guidelines for the CD4 cell count at which to initiate ART have not changed greatly in the UK during the time period of the observations analysed, although there has been a tendency for earlier initiation within the CD4 200–350/ μl range [27,28]. It seems unlikely that our results will be affected by changes over time in indications for ART initiation.

The time taken for AIDS to develop is long. Risk of AIDS is closely linked to the CD4 cell count in peripheral blood and it is the time taken for this depletion to occur that explains both the length of, and the variability in, the time taken for AIDS to occur in untreated infection [1,2]. CD4 cell count depletion in peripheral blood does not increase in rate as the CD4 cell count declines [14,29]. We show here that variability in CD4 cell count decline is linked more closely to viral replication than has previously been documented. Further study of predictors of increases in HIV RNA levels may help us understand the causes of CD4 cell count depletion.

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Authors contributions: Concept of analysis (all), ongoing data cleaning and merging for cohort (F.L., C.S., A.P., R.L., V.C), clinical input (M.J., A.R., A.M.G), statistical input (A.P., F.L., C.S., R.L., V.C), drafting of manuscript (A.P.), critical input into draft manuscript (all), final approval of manuscript (all), concept and ongoing planning and implementation of cohort (all), specialist information technology input (R.T.) and virology expertise (A.M.G.).

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