

**Total lymphocyte count for informing when to initiate antiretroviral therapy in HIV-
infected children: a meta-analysis of longitudinal data**

HIV Paediatric Prognostic Markers Collaborative Study

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Abstract

Background: Total lymphocyte count (TLC) has been proposed as an alternative to CD4 percent for deciding when to initiate antiretroviral therapy (ART) in HIV-infected children in resource-limited settings. However, few studies have evaluated TLC thresholds at which ART should be considered or compared TLC with CD4 percent monitoring.

Methods: Longitudinal data on 3917 HIV-infected children were pooled from observational and randomised studies conducted in Europe and the USA. The 12-month risks of death and AIDS by most recent TLC and age were estimated by parametric survival models, based on measurements before ART initiation or during zidovudine monotherapy. Risks were derived and compared at TLC and CD4 percent thresholds for starting ART recommended in WHO (2003) guidelines.

Findings: TLC was a powerful predictor of the risk of disease progression despite a weak correlation with CD4 percent ($r=0.08-0.19$ dependent on age). For children >2 years, the 12-month risk of death and AIDS increased sharply at TLC values $<1,500-2,000$ cells/mm³, with little trend at higher values. Younger children had higher risks and TLC was less prognostic. Mortality risk at TLC thresholds recommended by WHO was substantially higher than at corresponding CD4 percent thresholds. Comparing the markers at threshold values at which mortality risks are approximately equal, TLC was as effective as CD4 percent in identifying children prior to death but resulted in starting ART slightly earlier.

Interpretation: In this population, TLC was a strong predictor of short-term disease progression, being only marginally less predictive than CD4 percent. Confirmatory studies in resource-limited settings are required to identify the most cost-effective markers to guide ART initiation.

Introduction

In developed countries the decision when to initiate antiretroviral therapy (ART) in HIV-infected adults and children is based on clinical symptoms and assessment of CD4 T-cell count or percent and HIV RNA viral load [1-4]. However, in many resource-limited settings these tests are not routinely available, and there has been considerable interest in the use of alternative prognostic markers, particularly total lymphocyte count (TLC) [5-13]. World Health Organisation (WHO) 2003 guidelines have recommended TLC thresholds for adults and children at which it might be appropriate to initiate ART in the absence of CD4 measurements, while recognising these require further evaluation [14].

Most studies assessing the value of TLC as a marker for disease progression have been cross-sectional and undertaken in adults [11]. Here we describe an analysis of almost 4000 HIV-infected children, followed longitudinally in studies in Europe and the USA, before they first received effective ART. A model was developed to estimate the 12-month risks of death and progression to AIDS based on age and most recent TLC measurement. We also assessed the consistency of TLC and CD4 percent thresholds for initiating ART recommended in WHO guidelines using this model and a previous model for CD4 percent [15]. Finally, we compared the clinical outcomes that would have been observed if ART initiation rules had been based on TLC monitoring alone or on CD4 percent monitoring alone.

Methods

All major European and USA cohort studies and randomised trials of HIV-infected children with data prior to the introduction of effective ART were invited to participate in the HIV Paediatric Prognostic Markers Collaborative Study (HPPMCS) [15]. 17 studies provided individual longitudinal data on children infected perinatally with HIV-1 regardless of the age when infection was diagnosed or the method of diagnosis. These broad inclusion criteria increase the generalisability of our findings. Variables provided by the studies which are used in the present analysis are: date of AIDS diagnosis (excluding lymphoid interstitial pneumonia) [16], date of death, date last known to be alive, date of last clinical assessment, dates started zidovudine and any other antiretroviral drug, TLCs, and CD4 percentages. Details of the laboratory methods used to obtain TLC and CD4 measures were not collected by the individual studies.

To reduce the effect of presentation bias, children in non-birth cohort studies were excluded if the clinical endpoint (death or AIDS diagnosis) occurred within one month of their first laboratory measurement. Also, since the study aims to reflect the natural history of infection, analysis was restricted to measurements taken before the start of ART or during receipt of zidovudine monotherapy, which has at most a marginal clinical effect [17]. Parametric survival models as described previously were used to estimate the 12-month risks of death or progression to AIDS (or death in the absence of AIDS) according to age and most recent TLC measurement [15].

WHO 2003 guidelines for resource-limited settings recommend initiating ART in HIV-seropositive children if CD4 <20% (age <18 months) or CD4 <15% (age >18 months) [14]. Note that HIV-infected children are traditionally monitored by CD4 percentage rather than absolute CD4 count as the former is less age-dependent [3,4]. If CD4 testing

is not available, TLC values of $<2,500$ cells/mm³ (age <18 months) or $<1,500$ cells/mm³ (age >18 months) are recommended instead. We used the results of our models to assess the consistency of the predicted 12-month risks of death at these CD4 percent and TLC levels.

To compare the effect of different marker rules for starting ART we used the longitudinal data for each child to determine the hypothetical time point at which ART would have been triggered according to each rule and related this to the actual time of death. Since neither event was observed for some children, we used non-parametric competing risk analysis which simultaneously estimates the cumulative proportion of children who (a) would have started treatment before death, and (b) would have died before starting treatment [18]. To ensure the valid comparison of TLC and CD4 percent, visits at which only one marker was measured were excluded ($<2\%$ of visits). Finally, as some large gaps between measurements may have been due to incomplete reporting, data were censored at the first gap exceeding 12 months.

Results

Characteristics of the individual studies and the number of children per study have been described previously [15]. A total of 3917 children were included, of whom 2007 (51%) were female. 23% of children had their first reported TLC measurement before age 3 months, 22% between 3 months and 1 year, 39% between 1 and 5 years, and 16% after age 5 years. 28,300 TLC measurements were recorded, a median of six (IQR 3-10) values per child. The distribution of the interval between successive tests was <3 months for 58%, 3-6 months for 26%, 6-12 months for 10%, and >12 months for 6%.

Figure 1 shows the distribution of TLC values by age, based on each child's closest measurement to the target age. This reveals a marked decline in TLC with increasing age, a phenomenon also seen in uninfected children [19,20]. The proportion of measurements <1,500 cells/mm³ increased from 5% at 6 months, to 6% at 1 year, 8% at 2 years, 15% at 5 years, and 34% at 10 years. The corresponding proportions <2,500 cells/mm³ were 13%, 14%, 18%, 45%, and 77%. Results were essentially unchanged when the same analysis was limited to children followed prospectively from birth (not shown). In an analysis of all pairs of measurements within yearly age bands, TLC and CD4 percent were found to be weakly positively associated, with correlation coefficients ranging from 0.08 to 0.19.

In the analysis of progression to death, 559 events were observed among 3883 children over 9008 person-years of follow-up, and in the analysis of progression to AIDS 981 events were observed among 3347 children over 7216 person-years of follow-up. Data were sparse and estimates of disease progression risk therefore unreliable after about age 10 years. Figure 2A/B and Table 1 show the estimated risks of death and AIDS within 12 months according to the most recent TLC value. The general shape of the

relationship is strikingly similar to that previously found for CD4 percent [15]. Above the age of 2 years, mortality risk increases sharply at TLC values less than 1,500-2,000 cells/mm³, with little trend at higher values. For example, the estimated 12-month risk of death for a 5-year old child increases from approximately 1% for TLC >3,000 cells/mm³ to 2.4% at 2,000 cells/mm³, 5.3% at 1,500 cells/mm³, and 14% at 1,000 cells/mm³ (Figure 2A). In younger children, TLC is a less powerful predictor of death and, at an equivalent value of TLC, they experience higher mortality rates than older counterparts. These patterns are mirrored in the risk of progression to AIDS (Figure 2B), although incidence is typically three- to five-fold higher for the same values of age and TLC.

The estimates from the previous analysis were utilised to assess the 12-month risk of death according to the thresholds for initiating antiretroviral therapy recommended by WHO guidelines (Figure 3). Mortality incidence is high in infancy using either the threshold for TLC (2,500 cells/mm³) or CD4 percent (20%), but to achieve substantially lower levels of risk much more conservative thresholds would be needed because of the intrinsically weak prognostic value of both TLC and CD4 percent in this age group (Figure 2A/B). In children aged 18 months or older, the estimated 12-month risk of death at a TLC of 1,500 cells/mm³ was more than double the risk at a CD4 of 15%. A similar discrepancy between the two markers was observed for the 12-month risk of AIDS (not shown). We ascertained that TLC values of 3,400 and 2,300 cells/mm³ for children aged below and above 18 months gave 12-month mortality estimates which were more consistent with CD4 values of 20% and 15% respectively. Alternatively, CD4 values of 15% and 10% gave estimates which were more consistent with TLC values of 2,500 and 1,500 cells/mm³.

Figures 4A/B (dark lines) depict the hypothetical outcomes that would have been observed if the childrens' paediatricians had strictly followed the WHO guidelines for initiating treatment based on CD4 percent or based on TLC. By six years after the first measurement, a cumulative 3.7% deaths would have occurred before treatment was triggered under CD4 percent monitoring compared with 6.3% under TLC monitoring, with a concentration of events in the first two to three years (Figure 4A). However, these figures need to be set against the average deferral of treatment, which an ideal marker rule aims to maximise. This was longer under TLC monitoring (12% of children would have started treatment following their first measurement, 50% by 6 years after their first measurement) than under CD4 percent monitoring (comparative values of 24% and 61%) (Figure 4B).

The relatively higher mortality and longer deferral of therapy under TLC monitoring is a consequence of the WHO treatment thresholds for CD4 percent being inherently more conservative than those for TLC. To enable a more direct comparison of the performance of the two markers, we evaluated (Figures 4A/B, light lines) the results of TLC monitoring using age-specific thresholds of 3,400 and 2,300 cells/mm³ (roughly comparable with CD4 thresholds of 20% and 15%) as well as the results of CD4 percent monitoring using age-specific thresholds of 15% and 10% (roughly comparable with TLC thresholds of 2,500 and 1,500 cells/mm³). Comparing the two markers at these levels, very similar numbers of deaths would have occurred before therapy was triggered (Figure 4A). The proportion of children who would have started treatment at their first measurement was also similar for the two markers (Figure 4B). However, the proportion starting treatment each year subsequently would have been lower under CD4 percent monitoring (10% per year at both threshold levels) than under TLC monitoring (13% per

year at lower threshold levels, 17% per year at higher threshold levels), opposite to the earlier finding using WHO thresholds.

Discussion

The availability of ART to treat HIV-infected individuals in resource-limited countries is increasing through various initiatives [21]. As demand will significantly exceed supply for the foreseeable future, deferring therapy while the patient is at low risk of clinical disease progression is the only practicable option. It may also benefit the individual patient in terms of reduced drug toxicity and resistance, preservation of future therapeutic options, and better adherence. In developed countries, recommendations on when to initiate ART in HIV-infected children are primarily based on CD4 percent measurements and, to a lesser extent, on HIV RNA viral load concentrations [3,4]. However, in many resource-limited settings issues of cost and lack of laboratory facilities/expertise often preclude the use of these tests. This has resulted in research into the use of alternative prognostic markers, particularly TLC [5-13], which is a much less expensive test and can be measured using an automated haematology analyser requiring low technical expertise.

Evidence supporting the use of TLC for clinical monitoring

Most studies assessing the value of TLC as a prognostic marker (in adults or children) have been cross-sectional, quantifying the sensitivity, specificity, and predictive value of TLC for “low” values of CD4 count (typically less than 200 or 350 cells/mm³) [6,7,9,11,13]. However, it is difficult to interpret these indices of diagnostic accuracy since the gold standard is itself imperfect, i.e., no value of CD4 count clearly demarcates individuals in whom clinical disease progression is imminent (and thus ART indicated) from those who will remain asymptomatic. Also, in children, there is the added complication of the effect of age on both CD4 values and disease progression risk. Much clearer knowledge is gained from the few longitudinal studies that have directly related TLC to the risk of disease progression [5,10,12]. A key finding from our study is

confirmation that TLC is indeed a strong predictor of death and AIDS in children despite its weak correlation with CD4 percent.

TLC thresholds for initiating antiretroviral therapy

The large sample size of our study has allowed accurate estimation of the short-term risk of disease progression in the absence of effective therapy, which is a key but not the only consideration in the timing of ART initiation [22]. One approach is to select a TLC threshold at which the risk of disease progression rises sharply, although this is partly subjective because of the continuous relationship. Above two years of age the 12-month risks of death and AIDS increase sharply at 1,500-2,000 cells/mm³ (cf. 10-15% for CD4 [15]). However, as with CD4 percent, TLC is only weakly prognostic in younger children, and in the absence of any strongly predictive marker the optimal clinical management of this age group is uncertain [23]. As TLC and CD4 percent values are rarely below 2,500 cells/mm³ or 20% in the first 18 months of life (Figure 1, [15]), most children presenting early with HIV infection would not qualify for immediate treatment under WHO guidelines [14].

Our analysis revealed an inconsistency between the CD4 percent and TLC thresholds suggested by WHO in that the former trigger therapy at a much lower mortality risk than the latter. To achieve consistency either the TLC thresholds should be increased or the CD4 percent thresholds decreased. An alternative approach is to define a “tolerable” level of disease progression risk at which treatment is deferred and to select the marker thresholds accordingly. For instance, the 12-month risk of death exceeds 5% when TLC is below 3,900 cells/mm³ at 1 year, 2,600 cells/mm³ at 2 years, 1,500 cells/mm³ at 5 years, and 1,000 cells/mm³ at 10 years. The wide variation in values highlights the need for fine age categories under this approach to account for the close dependency between the risk of disease progression and age. It would also seem logical for the

threshold in the older paediatric age groups to be equivalent or close to the threshold in adults; WHO guidelines recommend 1,200 cells/mm³ for adults, although this is based on limited empirical evidence [14].

Comparison with CD4 monitoring

The use of TLC to monitor HIV infection is widely regarded as a compromise position until “.... the development and implementation of CD4 determination methods that are applicable to developing world settings” [14]. Several low-cost technologies are currently being developed or evaluated although most estimate absolute CD4 count and not CD4 percent [24] which, as noted earlier, is the marker traditionally used to monitor HIV-infected children. However, few studies (all in adults) have explicitly compared CD4 count and TLC as prognostic markers. In a South African cohort, a TLC cut-point of 1,200 cells/mm³ was as discriminating as a CD4 count of 200 cells/mm³ in predicting progression to AIDS and death [5]. Similarly, a study in Ethiopia found that the simple combination of the diagnosis of anaemia or a TLC less 1,200 cells/mm³ identified all 35 deaths that occurred during follow-up [10].

We used a similar approach to Mekonnen et al. [10] to compare the hypothetical effects of initiating treatment at thresholds for CD4 percent and TLC that predict a similar 12-month risk of progression to death. As expected, similar numbers of deaths would have occurred before each marker had crossed the relevant threshold. Although monitoring with CD4 percent would have resulted in a longer average deferral of therapy (Figure 4B), the difference was not dramatic and needs to be balanced against the greater cost of CD4 monitoring. It is important to emphasise that the threshold values used in this analysis are illustrative and not intended for clinical application. Also, the higher TLC

thresholds examined (3,400 and 2,300 cells/mm³) lie within the normal range (approximately the 10th centile) for uninfected children [19].

There are several other factors to consider in the interpretation of this analysis. First, the results depend on: (a) the distributions of age and marker values at presentation (b) the frequency of monitoring (c) the rule applied to the marker as well as the marker itself. However, broadly similar conclusions were reached in sensitivity analyses in which we allowed each of these factors to vary (not shown). Second, our analysis could not take clinical manifestations apart from AIDS into account, some of which could influence the decision when to initiate ART. Third, the cheaper CD4 counting methods being developed for use in resource-limited settings may be less accurate than the CD4 measurements available in our study which we presume were obtained by flow cytometry [24]. Conversely, in many laboratories in resource-limited settings TLC is derived from manual differential counts, which are less accurate than values obtained from automated haematology analysers [25].

Can our findings be generalised?

It is important to emphasise that the children in this analysis were followed in clinics in Europe or the USA, whereas we aim to draw inferences relevant to resource-limited settings, especially sub-Saharan Africa which has borne the brunt of the HIV epidemic. There is a broad ethnicity distribution in our study (40% white, 36% black, 17% Hispanic, 7% other/missing) and this factor was found not to be associated with disease progression after adjustment for CD4 percent [15]. However, the limited paediatric natural history data available from Africa indicate much higher mortality rates than those observed in developed countries prior to the use of effective ART [26,27]. This would suggest that our estimates for the risk of progression to death are too low in an African

context, although this depends on the extent to which the increased mortality is mediated via lower TLC and CD4 percent values. Also, several studies have noted higher and more variable lymphocyte levels in African than in Caucasian populations, possibly due to the endemicity of other infections [28,29].

While we caution against direct extrapolation of our quantitative results, there is no obvious reason why our major finding that TLV predicts clinical progression almost as well as CD4 percent should not extend to resource-limited settings. If so, this would challenge the received wisdom that it is necessary to introduce expensive CD4 technologies to such settings. It is important to pool natural history data from Sub-Saharan Africa to confirm or refute this finding. Such a study would help to identify other cost-effective markers and to define monitoring strategies that optimise clinical outcomes. For example, simple measurements such as haemoglobin or weight have been shown to be highly prognostic and could be jointly considered with TLC in simple algorithms [10,13,30]. Finally, policy makers deciding whether to invest in CD4 technology need to consider the most appropriate tools both to monitor response to ART and to guide the timing of its initiation [8,11].

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Appendix

Steering Committee

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Full details of study organisation at www.hppmcs.org.

Contributors

All members of the Steering Committee were involved in the iterative process of devising statistical analyses to illuminate the study question and the interpretation of these analyses. T Duong did the statistical analyses under the supervision of D Dunn and DM Gibb. D Dunn, DM Gibb, and T Duong drafted the paper which was extensively revised following comments by Steering Committee members.

Conflict of interest statement

None declared.

Funding source

The study received no specific funding. T Duong was partly funded by St Stephen's AIDS Trust.

Table 1. Estimated risk (percent) of AIDS and death within 12 months at selected values of age and TLC.

Age years	Endpoint	Total lymphocyte count (cells/mm ³)							
		500	1000	1500	2000	2500	3000	4000	6000
0.5	AIDS	69 (61-77)	61 (54-68)	53 (48-59)	46 (41-50)	39 (35-43)	34 (30-38)	26 (23-29)	17 (15-20)
	Death	56 (9.5-69)	44 (9.4-53)	34 (9.4-39)	26 (9.5-30)	20 (9.4-23)	15 (9.5-19)	9.4 (7.1-11)	4.6 (3.6-9.3)
1	AIDS	67 (58-73)	55 (49-61)	44 (39-48)	35 (32-39)	28 (25-32)	23 (20-26)	16 (15-19)	11 (10-13)
	Death	52 (7.3-60)	38 (7.3-42)	26 (7.3-29)	18 (7.3-21)	12 (7.3-15)	8.7 (7.0-10)	4.8 (3.8-7.5)	2.6 (2.0-7.5)
1.5	AIDS	64 (56-71)	50 (44-55)	37 (33-41)	28 (25-51)	21 (19-24)	17 (15-19)	12 (11-13)	8.6 (7.7-14)
	Death	49 (6.1-55)	32 (6.1-36)	20 (6.2-23)	13 (6.2-15)	8.2 (6.2-9.7)	5.5 (4.3-6.5)	3.0 (2.4-6.4)	1.9 (1.5-6.5)
2	AIDS	62 (53-68)	45 (39-49)	32 (28-35)	22 (20-25)	16 (15-19)	13 (11-14)	9.0 (8.2-12)	7.3 (6.5-12)
	Death	46 (5.4-52)	28 (5.4-31)	16 (5.4-18)	9.4 (5.4-11)	5.7 (4.7-6.6)	3.7 (3.0-5.6)	2.1 (1.7-5.7)	1.6 (1.2-5.8)
5	AIDS	50 (42-57)	26 (22-29)	13 (12-15)	8.2 (7.1-9.3)	6.0 (5.2-7.0)	5.2 (4.5-6.2)	4.8 (4.0-5.7)	4.7 (3.9-5.7)
	Death	34 (3.5-39)	14 (3.5-16)	5.3 (3.6-6.6)	2.4 (1.8-3.7)	1.4 (1.0-3.6)	1.1 (0.8-3.6)	0.9 (0.6-3.7)	0.9 (0.6-3.7)
10	AIDS	36 (29-43)	12 (8.5-15)	5.2 (4.2-6.3)	3.8 (3.1-4.6)	3.4 (2.7-4.3)	3.4 (2.6-4.3)	3.3 (2.5-4.3)	3.3 (2.5-4.3)
	Death	22 (2.5-27)	5.2 (2.5-7.3)	1.5 (0.9-2.6)	0.8 (0.4-2.2)	0.6 (0.4-2.6)	0.6 (0.4-2.6)	0.6 (0.4-2.6)	0.6 (0.3-2.6)

Estimates in grey are unreliable as these TLC values are rarely observed at these ages
95% CIs (based on 1000 non-parametric bootstrap samples) shown in parentheses

Legends to Figures

Figure 1

Distribution of TLC by age.

Footnote: Window of ± 3 months at 6, 12, 18, 24 months, and ± 6 months at all other ages. The number of measurements at each age point ranges from 385 to 1527.

Figure 2A/B

Estimated risk within 12 months of (A) death (B) progression to AIDS. The curves are truncated at the 2nd and 98th centiles for that age.

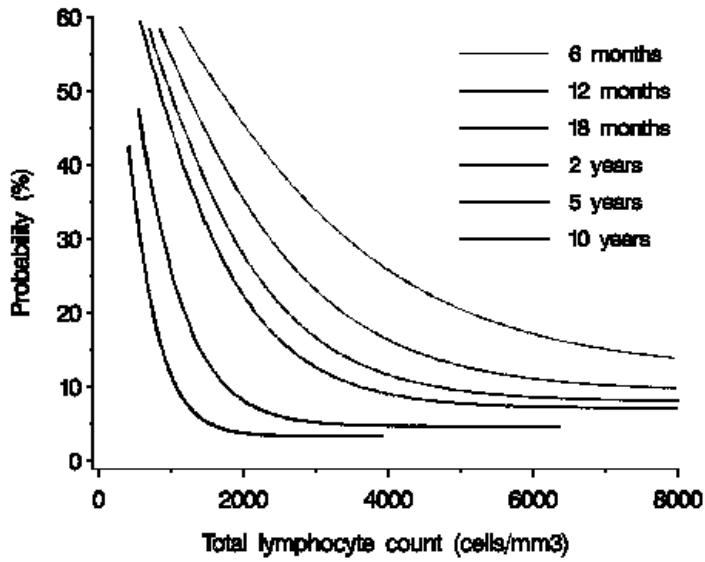
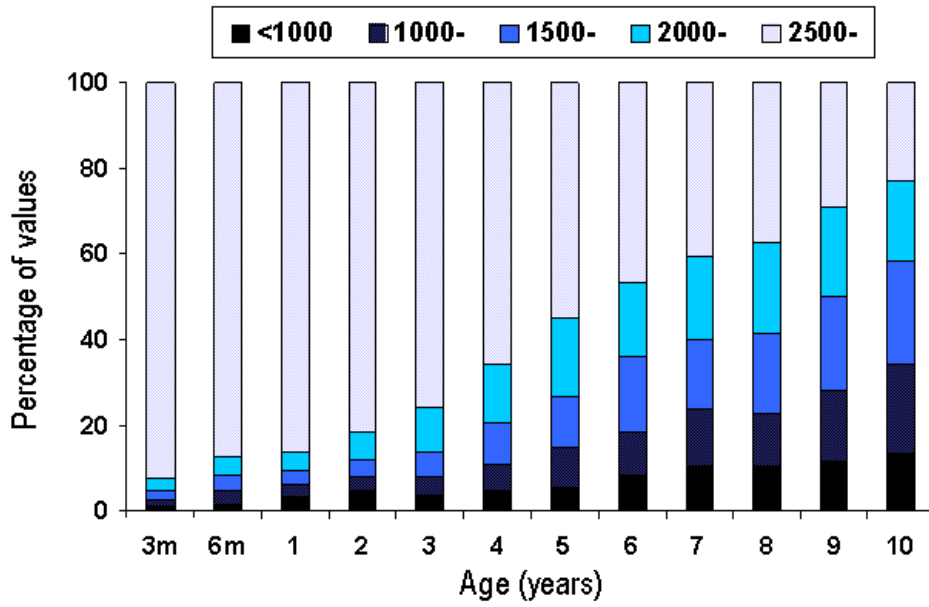
Figure 3

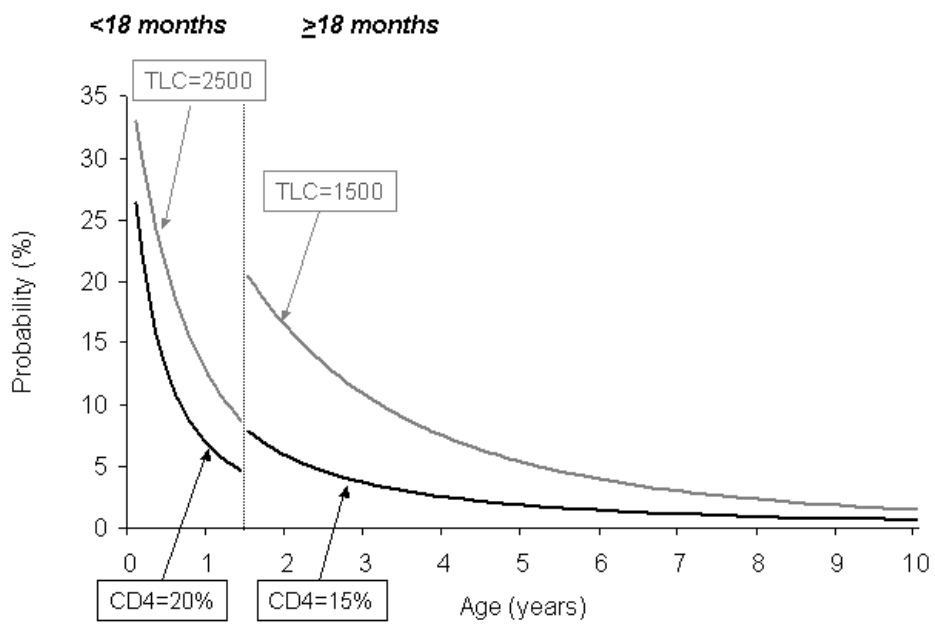
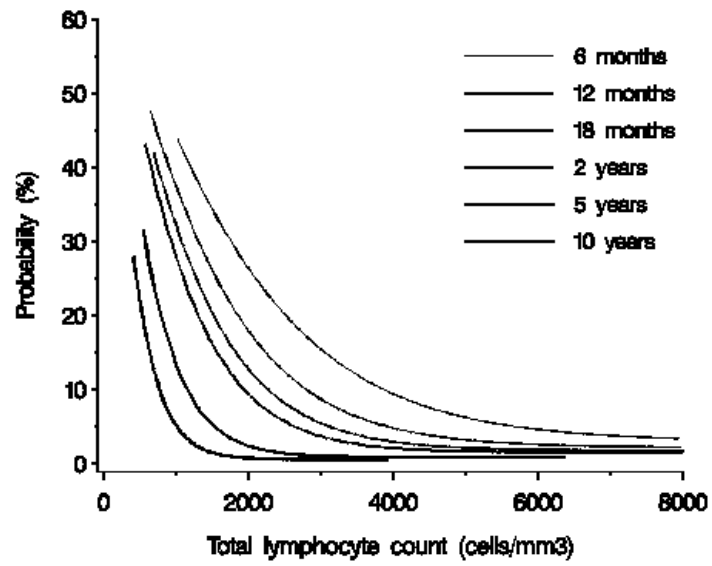
Estimated risk of death within 12 months at WHO recommended CD4 percent and TLC thresholds [14].

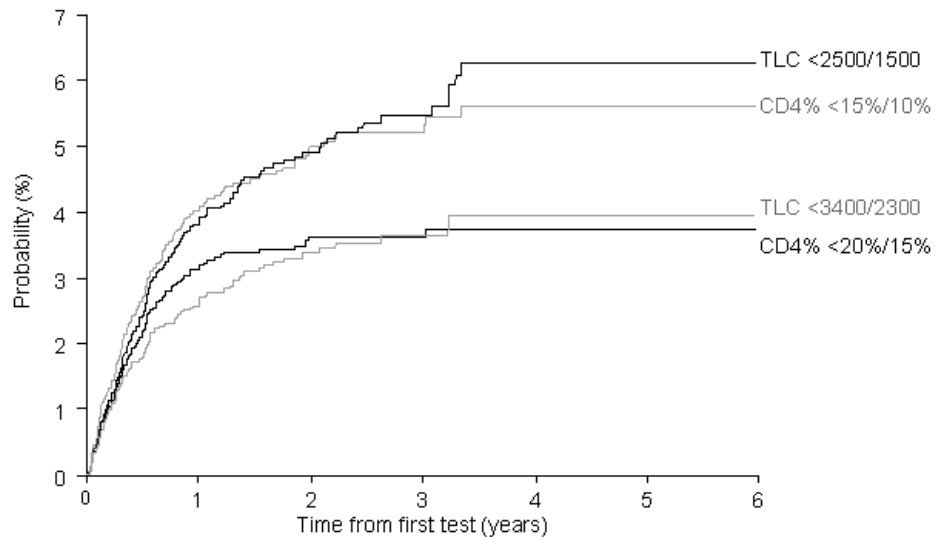
Figure 4A/B

Hypothetical outcomes based on CD4 percent and TLC monitoring at different threshold values: (A) Cumulative risk of death before observing marker value triggering therapy (B) Cumulative probability of observing marker value triggering therapy (before death).

Values below graph show number of children at risk.

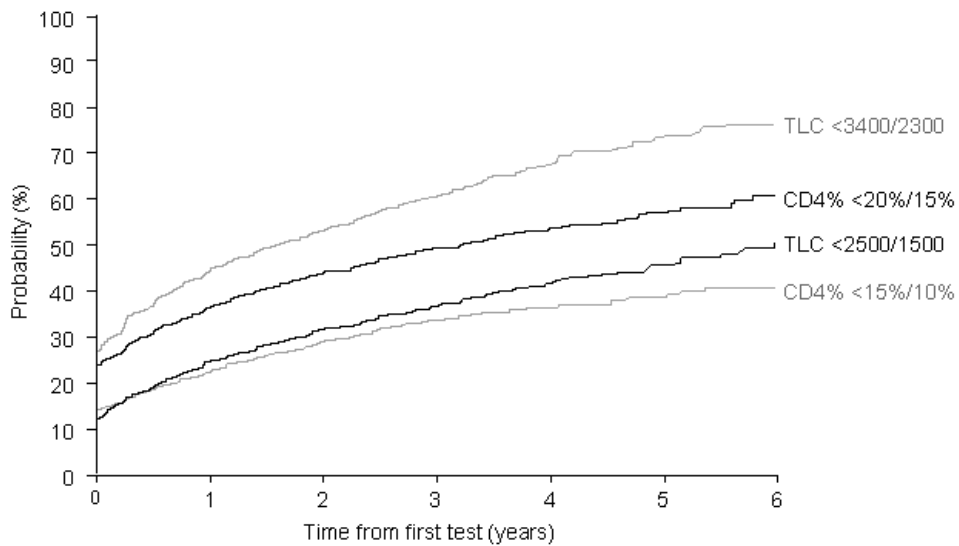






No. at risk

TLC<2500/1500	3883	2223	948	461	160	78	41
CD4%<15%/10%	3883	2306	1024	531	185	92	46
TLC<3400/2300	3883	1637	622	278	94	39	21
CD4%<20%/15%	3883	1897	817	424	146	74	35



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