CHAPTER V

DISCUSSION

The events of interest in this study were virological suppression after commencing a HAART regimen and virological failure following initial virological suppression in a cohort of HIV-positive, mainly Thai subjects who had never previously taken any antiretroviral medication. Virological suppression is a measure of the lack of viral replication. This has important clinical ramifications for patients. Active viral replication depresses the activity of and destroys T-cells, causing the depletion of CD4 T-cell lymphocyte numbers, as measured by CD4+ cell counts. Lower CD4+ cell counts are indicators of immunological depression and failure. Such immunological depression eventually leads to the emergence of opportunistic infections, infections that normally do not cause problems in people with healthy immune systems. A pVL greater than 55 000 copies is associated with a more rapid decline in CD4+ counts, disease progression and death. (Mellors et al., 1997; Mellors et al., 1996) In this study, virological failure was defined as a pVL >50 copies/mL measured on two consecutive occasions at least four weeks apart.

For individuals with HIV infection, being able to remain on therapy for a very long time is vital for survival. With ever-increasing access to potent ART in the developing world, more and more individuals are taking these medications and are living with HIV/AIDS for longer. However, despite the advances in recent years, the life span of HIV-infected individuals is less than average, even if they are on long-

term HAART. (Lohse, Hansen, Pedersen et al., 2007) In addition, there is still an excess mortality among HIV patients, which appears to be only partially attributable to immunodeficiency. (Lohse, Hansen, Gerstoft et al., 2007) Therefore, it is important to scrutinize all possible aspects of long-term therapy and to examine what elements of time may have a detrimental effect.

In the current study of time to virological failure in ARV-naïve subjects receiving HAART, 404 patients from the HIVNAT 006 cohort were included, and 69 (17.2%) experienced virological failure following initial virological suppression.

Time Courses to Studied Events

In survival analysis, as was employed in this study, shorter time to an event indicates increased likelihood (increased hazard), and longer time indicates decreased likelihood (decreased hazard) of that event's occurrence. This study used person-time logistic regression to assess the effect of time on virological outcomes. This is a technique not commonly used but it has been shown to be very effective for explicit modelling of the time variable itself. (Abbott, 1985; Halpern, Gillespie, & Warner, 1993).

A useful first step in the analysis of survival data is the estimation of the distribution of the survival times. The Kaplan-Meier technique is useful as a method of preliminary visual evaluation of the time course to the event of interest. The technique also is useful as it accommodates censoring of observations, that is failure to experience the event during follow-up, due either to withdrawal of subjects or to completion of the study. The analysis methodology should appropriately use the censored observations as well as the uncensored observations in order to maximise information in the analytical dataset.

The next step was to model the time variable. The Kaplan-Meier observations suggested the approach of cubic polynomial transformation of the time on study. In both time intervals in this study, polynomial transformation to the second and third degree achieved a better fit to the model, and this has been described above. Although further polynomial transformation is possible, it is not customary. (Allison, 1999).

These three time variables (first-, second- and third-order) were included in the person-time logistic regression analysis. A demonstration of the validity of this approach was conducted with the variable for gender. When gender was modelled for the suppression event with the Cox proportional hazards model, the estimation of gender effect was statistically significant, with females more likely to suppress more quickly than males (HR 1.28, P=0.014). When gender was modelled with logistic (and later by Poisson) regression for suppression, without the time variables, the outcome was not statistically significant (OR 1.11 and χ^2 1.04 respectively, P=0.306 and 0.307 respectively). When the time variables were included in the models for logistic and Poisson regression, gender regained its significance (OR 1.27 and χ^2 5.53 respectively, P=0.018 and 0.019 respectively).

Person-time logistic regression employs a dataset in which a separate observation is constructed for every person-time unit during follow-up. For example, if the study includes 100 subjects and the mean time on study is 55.5 time-units, the analytical dataset contains 5 550 observations. Such a dataset is created using a "do-loop" in SAS. One or more independent variables then are created to model time on study. These variables can be modelled and interpreted in the same fashion as the other independent variables in the study. To examine consistency of the results from

person-time logistic regression, further analyses were performed using Poisson regression and the Cox proportional hazards model.

The Poisson distribution counts the number of events. The events are assumed to occur over time at a fixed rate on average and each event occurs independently and at random. The distribution's mean equals its variance. The distribution is asymmetric when the mean is small but is nearly symmetric when the mean is large and so is like the normal distribution. (Altman, 1991) Altman goes on to say that the Poisson distribution is appropriate for studying rare events. This is similar to the binomial distribution where the probability of the outcome of interest is small but there are a large number of events. The numbers of event occurrences in this study were small compared to the numbers of person-days.

The Cox proportional hazards model is a survival model. Survival models have two parts: the underlying hazard function, describing how hazard (risk) changes over time, and the effect parameters (β), describing how hazard relates to other factors. The proportional hazards assumption is that the hazard associated with each independent variable remains proportional throughout follow-up. For example, if taking drug X halves your hazard at time 1, it also halves your hazard at time 2, or time t for any value of t. The modelled effects typically are reported as hazard ratios. Sir David Cox observed that if the proportional hazards assumption holds (or, is assumed to hold) then it is possible to estimate the effect parameters without any consideration of the hazard function. (Altman, 1991; Cox, 1972) The Cox proportional hazards model generally cannot provide effect parameter estimates for time.

The tables below present the results of analysis by these three techniques. Results generally were very similar across techniques. Table 15 and Table 16 present the comparisons of the techniques for baseline to virological suppression. Table 17 and Table 18 present the comparisons of the techniques for virological suppression to virological failure.

Table 15: Comparison of logistic and Poisson regressions, baseline to virological suppression for time

Time variable alone (unadjusted)	**β Logistic regression**	**β Poisson regression**
Days on study	0.00959	0.0095
Days on study squared	-0.00002	-0.0000
Days on study cubed	8.124E-9	0.0000
Time variable adjusted for covariates	**β Logistic regression**	**β Poisson regression**
Days on study	0.0107	0.0106
Days on study squared	-0.00002	-0.0000
Days on study cubed	8.649E-9	0.0000

Table 16: Comparison of logistic, Poisson and Cox regressions, baseline to virological suppression for covariates other than time

Covariates	**β Logistic regression**	**β Poisson regression**	**β Cox model**
Gender	0.1370	0.1360	0.14878
Baseline CDC category (AIDS or not)	-0.1455	-0.1444	-0.07851
Year of starting HAART	0.00356	0.0036	-0.00578
Baseline CD4+ count (>200 cells)	-0.1190	-0.1178	-0.05557
Baseline pVL (>50,000 copies)	-0.3388	-0.3359	-0.41148
Baseline regimen	0.6268	0.6216	0.69379

Table 17: Comparison of logistic and Poisson regressions, suppression to virological failure for time

Time variable alone (unadjusted)	β Logistic regression	β Poisson regression
Days on study	0.0126	0.0126
Days on study squared	-9.95E-6	-0.0000
Days on study cubed	2.208E-9	0.0000
Time variable adjusted for covariates	**β Logistic regression**	**β Poisson regression**
Days on study	0.0125	0.0125
Days on study squared	-9.66E-6	-0.0000
Days on study cubed	2.132E-9	0.0000

Table 18: Comparison of logistic and Poisson regressions, baseline to virological suppression for covariates other than time, suppression to virological failure

Covariates	β Logistic regression	β Poisson regression	β Cox regression
Gender	-0.5599	-0.5597	-0.48828
Baseline CD4+ count (>200 cells)	1.1406	1.1403	1.11566
Baseline pVL (>50,000 copies)	0.6604	0.6602	0.68848
Baseline regimen	-0.6770	-0.6768	-0.91693
Suppressed by week 12	0.6570	0.6568	0.66787

Time to Suppression

This study investigated virological suppression in ARV-naive patients from the HIV-NAT 006 cohort initiating two HAART regimens (NNRTI- or PI-based) between January 1, 2000, and December 31, 2006, to investigate time to and predictors of virological suppression.

All 404 participants in this study achieved virological suppression. Median time to suppression was 119 days (17 weeks) (IQR 85 to 190 days, 12 to 27 weeks). As has been demonstrated, the modelled effect of time to suppression following the

initiation of HAART was highly statistically significant in this Thai cohort (P <0.0001). Additionally, it is clear that time does not have a linear relationship with suppression. In this cohort, there was a brief initial period during which few subjects suppressed, followed by an intermediate period of rapid suppression, followed in turn by a long period of relatively slow suppression. Specifically, hazards of suppression, in events per 1 000 person-days, were 3.1 in the first 12 weeks, 10.5 in weeks 12 to 24, 11.6 in weeks 24 to 36, 7.2 in weeks 36 to 48, and 2.3 after week 48. These results are reasonably consistent with the findings of others who have studied viral decay at the initiation of ART. (Perelson et al., 1997; Wei et al., 1995).

Baseline pVL and CD4+ cell count, initial HAART, HIV disease stage, adherence, gender, age and calendar year of commencing HAART have been reported to predict the likelihood of pVL suppression. (Dragsted et al., 2004; Fournier et al., 2005; Manegold et al., 2004; Oette et al., 2006; Paredes et al., 2000) The current study showed that women achieved suppression more quickly than men (OR 1.27, P=0.018), and this difference has been reported before. (Moore et al., 2001) However, this difference became only marginally significant in the multivariable model (OR 1.20, P=0.089). Removing the clearly non-significant covariates from the model (year of entry, CD4+ count and pVL) led to a reduction of the P value for this variable, increasing confidence that females achieved suppression faster than males (OR 1.22, P=0.059).

Two studies from Thailand by Kiertiburanakul et al. have examined time to virological suppression in treatment-naïve patients commencing a NNRTI-based (either efavirenz or nevirapine) ART. The 2006 prospective cohort study compared virological and immunological responses of an efavirenz-based regimen in patients

with baseline CD4+ <100 (n=21) and CD4+ ≥100 cells/mm^3 (n=25). (Kiertiburanakul et al., 2006) The primary outcome was time to a pVL <50 copies/mL. The Kaplan-Meier cumulative percent estimates of undetectable pVL at 12, 24, 36, and 48 weeks were 57.1, 76.2, 80.9, and 90.5 for the former group and 64.0, 92.0, 96.0, and 96.0 for the latter group. Median time to undetectable pVL was 12 weeks for both groups. This study found that subjects with a baseline pVL <100,000 copies/mL achieved suppression more quickly (12 weeks compared with 24 weeks, $P<0.01$) but none of the examined covariates were predictive of suppression.

The Kiertiburanakul et al. (2007) retrospective cohort study examined the efficacy and tolerability of GPO-VIR, the fixed-dose combination of stavudine 30/40 mg, lamivudine 150 mg and nevirapine 200 mg manufactured by the Thai Government Pharmaceutical Organization. (Kiertiburanakul et al., 2007) The primary study outcome was the time from initiation of this NNRTI-based regimen to achieve the goal of therapy, either a pVL <50 copies/mL or a 50% increase from baseline CD4+ cell count. Ninety individuals were identified from medical records, mean age was 35 years and 51% were male. In a median follow-up period of 15 weeks, 54% of patients achieved the goal of therapy. At 12, 24, 36 and 48 weeks, the Kaplan-Meier cumulative percent estimates of achieving the endpoint were 8.5, 62.7, 80.0, and 93.3, respectively. The median time to achieving the endpoint was 21 weeks. However, the authors found no significant association between any of the baseline characteristics and time to achieve the goal.

Both of these studies by Kiertiburanakul et al. evaluated virological suppression at the same weekly time points (12, 24, 36 and 48 weeks) as in the current study. However, both studies involved fewer subjects than the current study and the

treatment regimens were both NNRTI-based and did not include any PIs. The current study includes subjects commencing both NNRTI- and PI-based regimens. Further, the 2007 study of GPO-VIR used a combined endpoint of either the achievement of a pVL <50 copies/ml or an increase in CD4+ cell count of 50%. The current study's endpoint was a pVL of <50 copies only. The 2006 study did not assess the effect of baseline pVL on time to suppression due to a lack of pVL results. The patients in the 2006 study had advanced HIV disease (baseline median CD4+ count of 52 and pVL 5.4 $\log_{10}$copies/mL) whereas the patients in the current study had less severe disease (median CD4+ count of 167 and pVL of 4.7 $\log_{10}$ copies/mL).

All participants in the current study achieved virological suppression, compared with 42 of 46 (91%) in the 2006 study and 49 of 90 (54%) in the 2007 study. Thus, those results were subject to censoring, which could have resulted in some downward bias in estimates of suppression rates at specific times on study. It is difficult to posit why the suppression rate in the other two studies from Thailand is lower, especially the study from 2007. However, both studies recruited subjects with relatively advanced HIV disease. The authors acknowledged small sample sizes and short periods of follow-up. Adverse events were reasons given for discontinuation of therapy, especially events associated with the use of nevirapine. There were financial restraints as to the availability of antiretroviral medications, especially medications needed in the case of failure of the initial regimen. The current study was conducted in a specialist research facility and not at a public hospital, and thus was not subject to many of the issues confronting these previous studies. On balance, there is only limited comparability between the current results and those previously reported from Thailand.

Porter et al. assessed virological response to HAART for antiretroviral-naive persons initiating therapy with low CD4+ counts (<50 cells/mm^3). At weeks 12, 24, 36, and 48 weeks, 80%, 83%, 85%, and 83% of participants, respectively, achieved pVL <400 copies/ml. The study also assessed the impact of the calendar year of starting HAART, gender, age, exposure category, ethnicity, baseline CD4+ count and pVL, and whether the regimen contained a PI, on achieving the endpoint by 48 weeks. The most important predictor of virological suppression in this study was calendar year of starting HAART (OR 2.49, 4.28, and 3.28 for 1999 to 2000, 2001 to 2002, and 2003 to 2005, respectively, compared with 1997 to 1998). (Porter et al., 2008).

Tolerance of some earlier HAART regimens was poor with frequent side effects that potentially had an adverse influence on adherence. By examining the year commencing ART, it is possible to determine if this factor had an influence on the virological outcomes, especially in the absence of data relating to specific regimens. The current study examined the year of commencing HAART as a possible indicator of the antiretroviral regimen commenced but did not find this a significant prognostic indicator. Most (207, 51%) of the subjects enrolled into the current study in 2000. This skewed distribution might potentially influence any estimates of the effect of calendar year.

Predictors of virological response were evaluated in a large multicenter cohort by the EuroSIDA Study Group (August 1996 to April 1999). (Paredes et al., 2000) The objective of the study was to assess the factors related to achieving and maintaining undetectable pVL levels in 1 469 treatment naïve patients commencing either a PI- or NNRTI-based regimen. The authors found that patients with higher baseline pVL levels (relative hazard [RH], 0.76 per $\log_{10}$ higher; 95% confidence

interval [95% CI], 0.69 to 0.84; $P<0.001$) were less likely to reach undetectable pVL levels. Older patients and those with higher CD4+ cell counts (RH per 50% higher, 1.09; 95% CI, 1.02 to 1.16; $P=0.008$) were more likely to achieve virological suppression. NNRTI- and PI-based regimens were equally associated with viral suppression, except those taking saquinavir mesylate hard gel capsules (SQHC) as a single PI (with no ritonavir) who were less likely to achieve undetectable HIV-1 RNA levels (RH, 0.62; 95% CI, 0.47 to 0.82; $P<0.001$).

Data from the current study did not support age or higher CD4+ counts as predictors of time to virological suppression. The median baseline CD4+ count was 167 cells/mm^3 (IQR 57 to 261). The current study included CD4+ cell count in the multivariable model but it proved to be not statistically significant in predicting virological suppression ($P=0.898$). By contrast, data from the current study did find that a baseline pVL $\geq$50,000 copies/mL was predictive of slower virological suppression ($P=0.005$), and a PI-based regimen was predictive of faster virological suppression ($P<0.001$) compared with a NNRTI-based regimen. In addition, the current study examined the effect of calendar year of starting HAART and this was not significant ($P=0.997$).

Time to Virological Failure

In the current study, 17.1% (69 of 404) of participants experienced virological failure after achieving suppression following the commencement of HAART. This cohort has allowed examination of virological failure rates up to more than four years after starting HAART. The median time to failure following suppression was 2.6 years, meaning that most (55%, 38 of 69 or 9.4% of the whole cohort of 404) failed in

the third year after starting therapy. As in the interval to suppression, time to subsequent virological failure in this Thai cohort is highly significant ($P<0.0001$).

The rate of virological failure in the current study was quite slow in the period immediately following commencement of HAART, increased in the middle period and then was slower at the end of the follow-up period. This outcome is not in accord with other studies that have shown virological failure to increase (linearly) with time (Le Moing et al., 2002) or to decrease with time (Mocroft et al., 2003). It is not apparent whether or not these studies used modelling techniques that incorporate the same opportunity for flexible analysis and comparison of event rates as this study. It is possible that in the current study, good adherence coupled with consistent monitoring of viral load facilitated a good initial response. When adherence faltered, indicated by virological rebound, monitoring allowed this to be observed quickly and remedial action instigated.

Many studies report shorter median follow-up times than this study (Le Moing et al., 2002; Nachega et al., 2007) meaning that the current study has increased power to make valid comparisons. Some studies have reported rates of virological failure much higher than the current study. (Le Moing et al., 2002; Mocroft et al., 2003; Paredes et al., 2000; Robbins et al., 2007) Why are the rates of failure in the current study so low? Possible reasons could be a more compliant patient population, motivated by the availability of effective therapy at no cost, as they are participants in clinical trials. Otherwise, ART would be very expensive for these patients, possibly unaffordable for most. Additionally, it is possible that the regimens used were more effective and/or better tolerated than in other studies. Further analysis of the results showed significant gender differences in virological outcomes (not shown, paper in

press), indicating that women were more likely to suppress faster than men and to subsequently fail more slowly than men. Nearly half of the participants in this study (45.3%) were women. As women have been shown virologically to be more successful, the numbers of women in this study must have strongly influenced the overall outcomes. Women were on study significantly longer than men (243 days, P=0.003) and this was mainly in the period suppression to failure, which was highly significantly longer for women when compared with men (261 days, P=0.001). The reasons for such better responses in females are not known.

In the current study, the factors that significantly predicted virological failure were gender, baseline CD4+ count, a baseline pVL >50,000 copies/mL and not being suppressed by week 12 after commencing therapy. A higher pVL as predictive of virological rebound is supported by other studies. (Le Moing et al., 2002; Mocroft et al., 2003; Robbins et al., 2007) Some studies have shown older age and an AIDS-defining event prior to baseline as predictors of failure but the results from this study do not support these findings (Bonnet et al., 2005; Gutierrez et al., 2006)

The current study was not designed to assess determinants of the clinical implications of virological outcomes. However, some implications are apparent. The main laboratory marker for clinical measurement in HIV infection is the CD4+ cell count. Median baseline CD4+ count was 167 cells/mm^3. Median CD4+ cell count at exit was 462 cells/mm^3, a highly significant increase of 295 cells/mm^3 (P=0.000). In the current study, a CD4+ count <200 cells/mm^3 at baseline was highly significantly predictive of faster virological failure (P<0.001). This has very positive implications for the sustained clinical success of these patients. Treatment with HAART before the CD4+ count falls below 200 cells/mm^3 is supported by the results from the current

study. These results are supported by other studies. Bonnet et al. reported that in the first 6 months after starting HAART, a CD4+ count <50 at baseline was highly predictive of virological failure (HR 13.0, 95% CI 3.8 to 44.3) and a CD4+ count between 50 and 199 at baseline also was predictive of failure (HR 5.1, 95% CI 1.6 to 16.3) when compared with those who commenced HAART with a CD4+ count >350 cells/mm^3. (Bonnet et al., 2005)

In addition, only 17.1% of the 404 patients failed in the six years of this study, 55% of these in the third year after commencing HAART. Of those 69 who failed, all subsequently re-suppressed (data not shown) following intensified adherence counselling, a change of regimen or both. With continued emphasis on high levels of adherence to therapy and the availability of clinical support and monitoring, the prognosis for these patients should be highly optimistic.

This prospective cohort study of antiretroviral-naïve HIV-infected Thai patients demonstrates that time has a highly significant effect, both time to suppression and time to failure. Faster suppression was found to be highly significantly predictive of subsequent slower virological failure and sustained virological success.

Limitations

The implications of the results on HIV disease progression in this cohort may not be representative of other populations in resource-limited settings. These subjects were participants in clinical trials at a well-established clinical trials centre in a major city, with access to most of the available antiretroviral medications. This is not the case in all resource-limited settings, even in Thailand. The study did not address which particular regimens were used apart from general class distinctions of NNRTI

and PI. Even though a PI-based regimen was more predictive of virological success in this group, it is not known what an ideal regimen might be.

Some possible determinants that were not considered include place of residence (Bangkok or outside), ethnicity, previous antiretroviral therapy (only naive subjects were included in the analysis), other laboratory markers such as haemoglobin levels, and the re-achievement of viral suppression.

Virological failure due to the stopping or modification of an ARV regimen owing to adverse events (adverse effects and laboratory abnormalities) can be considered a competing risk event. There are insufficient data available to examine these effects in this study. Adherence (good or not good) affects virological response but there are insufficient data regarding adherence in this cohort to address adequately this question. However, the low rate of virological failure and the long period of suppression before virological rebound indicate that this cohort probably were mostly highly compliant. The fact that 55% of the failures occurred in the third year possibly indicate that some amount of treatment fatigue may have had an impact on compliance levels.

Too few patients experiencing viral rebound may restrict power. In this study, only 69 of the 403 (17.1%) who achieved virological suppression subsequently failed. In comparison with the decades that patients potentially now remain on treatment, the period of observation in this study (six years) is short. Further follow-up of larger numbers of patients over longer periods may meaningfully address this question.

It is conceivable that patients who had virological failure could have a higher or lower chance of being lost to follow-up compared with those who did not fail. Such differential censoring could introduce bias into the analysis. In this study of 404

participants, approximately 10% were lost to follow-up and non died during the period of observation.

Higher pVLs at baseline can be predictive of slower and less sustained virological response. (May et al., 2007) This study included baseline pVL in the model. However, this study did not consider all the factors related to baseline pVL that can be prognostic, especially the time from infection to the time of diagnosis and commencement of therapy. This time interval may well influence subsequent outcomes.

Further study is warranted. There are few published data from Asia and further studies from this region would provide valuable information. The characteristics not considered in this study could provide further information that would add to the results presented here. This study was able to follow patients for up to six years. Studies with longer follow-up periods should provide more powerful evidence, especially if there are more failure events that could more accurately predict clinical outcomes.