Neural Networks morbidity and mortality modeling during loss of HIV T-Cell Homeostasis

G.E. Hatzakis, Ph.D., C.M. Tsoukas, C.M., M.D., M.Sc.

Immune Deficiency Treatment Center, McGill University Health Center, Montreal, Canada

Despite the proven clinical benefits of HAART, mortality may still occur; particularly in those with less than 50 CD4+ cells/mL and, in some cases, with a viral burden below detectable plasma levels of HIV-1 RNA. Multiple factors may predict mortality including initial response to therapy, viral factors and host immune parameters. Due to the complexity of this problem, we developed Artificial Intelligence based tools/Neural Network (NN) to optimally evaluate outcomes of therapy and predict morbidity and mortality. To further validate the accuracy of these tools, we challenged their performance with that of Cox regression modeling (RM). Our study population involved 116 HIV+ individuals who consistently maintained CD4+ count < 50cells/mL for over 6 months. All patients were treated with antiretrovirals. To assess clinical outcomes, we developed a feedforward back-propagation Neural Network. We then compared the performance of this network to a Cox regression model. The Neural Network outscored the Cox regression model in the ROC curve areas: 0.888 vs 0.760 (HIV+ first Seropositivity to AIDS), 0.901 vs 0.758 (HIV+ first Seropositivity to Last Assessment incl. death) and 0.832 vs 0.799 (AIDS to Last Assessment incl. death), for the NN & Cox, respectively. In patients with a history of AIDS defining events and with severe TCell depletion, mortality occurs despite therapy. Although Neural Networks and Cox modeling were successful in predicting mortality, the Neural Network was superior in assessing risk in this population.

INTRODUCTION

Medical Decision Making on HIV therapeutics requires the consideration of various combinations of medical and non-medical factors. During the course of HIV infection, CD4 T-Cells are progressively lost and their function is diminished while CD8 T-Cells proliferate, become activated and ultimately become depleted late in disease. Loss of HIV-specific CD4 T helper response is presumed to be at the core of events leading to disease progression. The sum of both CD4 and CD8 T-Cells accounts for almost all the circulating T-Lymphocytes. Depletion of this subset defined as CD3 T-Cells, is related to loss of T-Cell Homeostasis and is an important mechanism associated to the loss of immune competence that is seen in advanced stages of this infection.

Four phases of CD4 cell loss have been described in large natural history studies of HIV disease. The first phase occurs immediately following Seroconversion. The mean CD4 cell count declines from about 1,000 to about 600 cells/mL. The CD8 cell count increases in a compensatory fashion, absolute T-Cell (CD3) numbers and percent remain either at normal or higher than normal levels. During the second phase, the CD4 cell count slowly declines and this occurs over many years. In the third phase, a rapid decline in CD4 is accompanied, in the majority of individuals, with a loss of CD8 cells. This decline of both T-Cell populations is generally accepted as the onset of failure of T-Cell Homeostasis and usually heralds symptomatic disease within 12-18 months. In the last phase, T-Cell loss continues with a further reduction of CD4 and CD8 T-Cell numbers. This phase encompasses the period between symptomatic disease and death. Although severe immunodeficiency, and end stage AIDS, is characterized by CD4 T-Cells < 50cells/mL, patients in this low CD4 stratum can be further divided into: a) those who are also CD8 T-Cell depleted and thus have loss of T-Cell Homeostasis and b) those with normal proportions of CD8 and CD3 cells. The use of potent antiretroviral combinations in this late stage of the disease has been usually associated with a modest increase in CD4 counts yet with also a very impressive reduction of morbidity and mortality. Despite the clinical improvement, several clinical trials have shown that the majority of these late stage patients experience treatment failure and some ultimately progress and die. Since not all individuals with < 50 CD4 cells/mL experience loss of T-Cell Homeostasis, it is important to evaluate the relative risk of disease progression and death in these two groups of individuals.

We have previously developed a feedforward backpropagation Neural Network which, based on biochemical, virologic and immunologic surrogate markers, could assess - over time - the variation of immunologic phenotypic indexes - as continuous variables - used in clinical practice today[1]. We wished to evaluate the role of T-Cell Homeostasis as expressed by the decline of CD3 T-Cell percentage below normal values and the utility

of this marker as a classification factor in predicting mortality in AIDS patients. We then decided to assess this model in predicting binary outcomes (morbidity, mortality). To challenge the accuracy of this model, we compared its performance versus a Cox Regression model (RM).

MATERIALS AND METHODS

Study Population: We systematically followed 116 HIV Seropositive individuals between 1983 and 2001 in the Immune Deficiency Treatment Center, McGill University Health Center. All patients eventually developed AIDS and 94 of them ultimately died.

	CD3%<50% Group-A	*50% ≤CD3% CD3%<60% Group-B*	*60% ≤CD3% CD3%<70% Group-C*	*CD3% ≥70% Group-D*
N	43	23	29	21
Deaths	35	18	19	12
HIV+ → AIDS	2.1±2.4	5.47±3.84	2.3±2.3	2.82±2.93
HIV+ → Last Assessment	4.1±4.0	7.82±4.13	4.0±3.5	5.88±4.43
AIDS → Last Assessment	2.3±2.1	2.35±2.18	1.9±2.2	3.19±3.55
Viral Load	5.40±5.59	5.16±5.09	5.15±5.32	4.88±4.89
CD3%	41±10	57±3	65±3	75±4
CD4%	3±2	2±1	4±5	4±2
CD8%	36±10	54±3	60±5	70±5
CD3 Absolute	271±150	449±270	610±355	754±341
CD4 Absolute	17±12	20±10	21±12	30±14
CD8 Absolute	241±137	452±241	558±332	698±332

Table 1: Population description, Time to Morbidity and Mortality (yrs) and Baseline Characteristics (Average±SD) for all 4 Groups. *Units:* Viral Load (Log_{10} Copies/mL), Absolute T-Cell counts (Cells/mL).

According to the selection criteria these individuals had to maintain CD4 cell absolute counts under 50 cells/mL (CD4 ABS < 50) for at least 6 consecutive months. The levels of CD3% were used for stratification of this study population into 4 different groups (A, B, C, D). Groups A, B and C had loss of T-Cell Homeostasis with Group A severe CD3+ T-Cell depletion, Groups B and C mild to moderate CD3+ T-Cell depletion and Group D normal CD3+ T-Cell percent (no loss of Homeostasis). Descriptive Statistics for these groups as well as Baseline characteristics are listed in Table 1. In spite of the shortened average length of time from: HIV+ to AIDS, HIV+ to Last Assessment and AIDS to Last Assessment, the maximum range of the relevant time periods was up to 14.4, 17.2 and 13.2 yrs, respectively.

Experimental - Immunology: The HIV-1 RNA was enumerated with an Amplicor assay (Version 2.0, Roche, Nutley, NJ) with a lower limit of quantitation of 400 copies/mL. To ensure improved accuracy, the Roche Ultrasensitive assay of 50 copies/mL was performed on samples below 400 copies/mL. Three color flowcytometry using a FACS Caliber instrument (B.D. Mountain View California) and the appropriate fluorochrome conjugated monoclonal antibodies were used to enumerate: CD3+, CD4+, CD8+. Frozen - stored at specific time points - peripheral blood mononuclear cells (PBMC) were used retrospectively for cytometric analyses.

Variables: The utilization of CD4+ count as a surrogate to the clinical state of late stage disease - by itself - is problematic since partial rebound of CD4+ counts cannot be equated with immune recovery. Post-HAART CD4+ counts may mask other aspects of immune system damage. Thus, to assess recovery or disease progression, a clinician ideally must assess the entire span of CD4+ counts. The same holds true for CD8+ cell counts and thus total lymphocytes CD3+. The absolute as well as the percentage cell counts of these T-Cell subsets were utilized along with measurements of HIV RNA levels. Namely, Viral Load, CD3%, CD4%, CD8%, CD3 Absolute, CD4 Absolute and CD8 Absolute levels were used to predict the Time of: a) HIV Seropositivity to AIDS, b) HIV Seropositivity to Last Assessment and c) AIDS to Last Assessment, respectively. These variables were used in both modeling Neural Networks and Cox Regression, to assess the response and status of the individual patient's immune system in relation to HIV T-Cell Homeostasis, leading to morbidity and eventually mortality.

Experimental - Informatics: We developed a feedforward backpropagation Neural Network featuring 1 input, 1 hidden and 1 output layer over a sigmoid transfer function. As per the aforementioned T-Cell subtypes, an equal number of nodes for the input and hidden layers were selected predicting a dichotomous event (0/1) for morbidity and mortality, respectively. Multiple values for learning factor (η) and momentum (α) were tested. Both were eventually set to 0.001. Adaptive learning was used but it was finally not adopted since constant learning factor and momentum produced more accurate predictions albeit at an increased central processing unit (cpu) cost due to the overall increased number of epochs. The maximum number of epochs was set at 100,000. After the completion of each epoch, the R^2-Norm of the Euclidean space was computed and subsequently stored together with the weight factors calculated for this specific epoch. The model stopped its training process at the epoch that the difference of two consecutive R^2 measurements satisfied the convergence criterion of 0.0001. Should this not occur prior to reaching the maximum number of epochs, as a target epoch would then be selected the point where the model approximated a general minima within these 100,000 epochs. The weight factors were selected accordingly. These were initialized - through a random number generator[2] - both asymmetrically in the space [0,1] and symmetrically in the ranges [-0.5,0.5] as well as

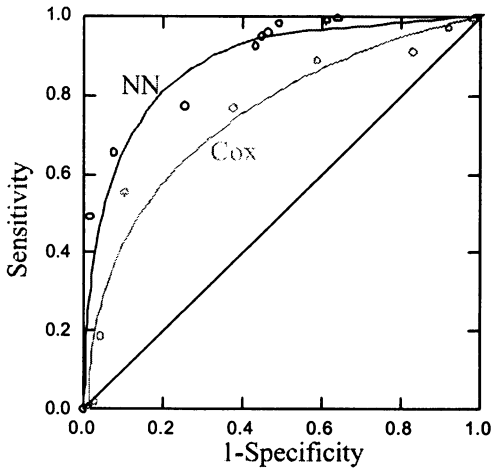

Figure 1: HIV+ to AIDS. ROC area, NN: 0.888, Cox RM:0.760

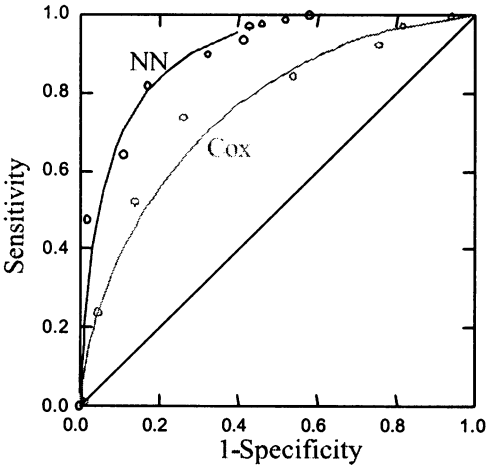

Figure 2: HIV+ to Last Assessment. ROC area, NN: 0.901, Cox RM:0.758

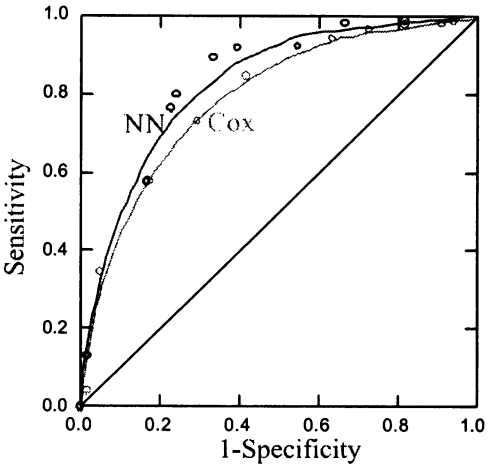

Figure 3: AIDS to Last Assessment. ROC area, NN: 0.832, Cox RM:0.799

$\left[-1/\sqrt{k_i}, 1/\sqrt{k_i}\right]$ (k_i : # of input links of processing element i)[3]. The latter was finally adopted since it seemed to improve the overall accuracy of the model. Through the aforesaid random number generator[2] we classified the entire population 10 times according to the proportion 3/4 vs 1/4 into training and validating datasets respectively, maintaining the same proportions - as per the sample size - for the selection of training and validating sets within all study groups A to D. For every single one of these 10 randomizations a Receiver Operating Characteristics Curve was computed. The aforementioned process was entirely repeated three times (30 randomizations

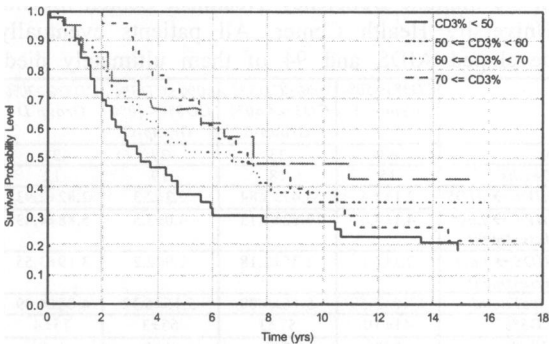

Figure 4:Kaplan-Meier Survival Analysis of HIV+ to Last Assessme

in total) to address: a) morbidity (0/1) from HIV Seroconversion to AIDS as well as b) mortality (0/1) from HIV Seroconversion to Last Assessment and c) mortality from AIDS to Last Assessment, respectively. Data was tested for high influential points. Despite of their existence, these could not be removed due to their clinical significance. NNs were programmed in GNU FORTRAN/Linux. Cox Regression was conducted in STATISTICA/ Windows[4]. Following the aforementioned randomizations, the generated datasets with the subsequent survival time, CD3% based stratification factor and the dichotomous morbidity and mortality were also used in the Cox RM.

RESULTS

The area under the ROC curve for NN and Cox RM was computed - based on the validation datasets - from HIV+ to AIDS (Figure 1), HIV+ to Last Assessment (Figure 2) and AIDS to Last Assessment (Figure 3). It is noted that these Figures represent the average ROC of those generated for each of the 10 randomizations (30 in total), as described. In all cases, NN outperformed the Cox RM. Parameter estimates through Cox RM - when assessing HIV+ to AIDS, HIV+ to Last Assessment and AIDS to Last Assessment - resulted in p-levels with corresponding Wald statistics that suggested: a) absolute TCell counts were better predictors than percentage levels, b) CD3 and CD8 were better predictors than CD4 absolute and percentage levels

and c) Viral Load was not a good predictor(Table 2).

	HIV+ → AIDS		*HIV+ → Last Assessment*		*AIDS → Last Assessment*	
	P	*Wald*	*P*	*Wald*	*P*	*Wald*
Viral Load	0.999	0.000	0.180	1.801	0.324	0.972
CD3%	0.025	5.034	0.004	8.135	<0.001	21.361
CD4%	0.254	1.299	0.042	4.142	0.001	11.492
CD8%	0.037	4.363	0.006	7.486	<0.001	20.451
CD3 ABS	<0.001	12.177	<0.001	18.700	<0.001	22.429
CD4 ABS	0.959	0.003	0.070	3.283	<0.001	12.445
CD8 ABS	0.001	11.891	<0.001	18.364	<0.001	22.103

Table 2: P-levels and Wald statistics from parameter estimates through Cox Regression. Statistical significance at P<0.05.

Kaplan-Meier risk analysis was also performed for the same periods from HIV+ to AIDS (not shown here), HIV+ to Last Assessment (Figure 4) and AIDS to Last Assessment (Figure 5).

CONCLUSIONS

This data demonstrates that strata of CD3+ over time are indicative of maintenance or relative loss of T-Cell Homeostasis, an important process in the pathogenesis of HIV. During the early stages seen with this infection, CD8+ compensate for the loss of CD4+ by maintaining stable levels of circulating CD3+ T-Cells (blind T-Cell Homeostasis) while at an advanced phase, concomitant and irreversible loss of CD4+ and CD8+ clearly indicate permanent and un-repairable damage to the immune system. It has been demonstrated that - at least - during blind T-Cell Homeostasis, regulation of CD3+, incurred from a rising CD8+, is not strongly associated to other surrogate phenotypic markers such as CD38+, CD69+, CD25+, CD26+, CD27+, CD28+, CD71+, CD122+ and HLA-DR+ despite the relation of CD8+ with these markers[5;6]. It has also been demonstrated in natural history studies of HIV infection that: 1) the levels of T-Cells (CD3+) remain constant years after Seroconversion[7] and 2) plummeting CD3+ counts herald loss of T-Cell Homeostasis occurring at a period estimated about 18 months prior to the onset of AIDS[8]. To evaluate the role of non-viral factors in the loss of Homeostasis, Margolick et al previously showed that smoking and use of AZT was not a significant predictor in the modeling of T-Cell trajectories[8].

Our findings are in accordance with previous studies demonstrating the significance of CD3+ T-Cell levels as an independent predictor to HIV disease progression[9]. In fact, CD3+ is a better predictor of HIV natural course, leading to morbidity and mortality, than CD4+ and Viral Load levels used extensively in HIV research and therapeutics today.

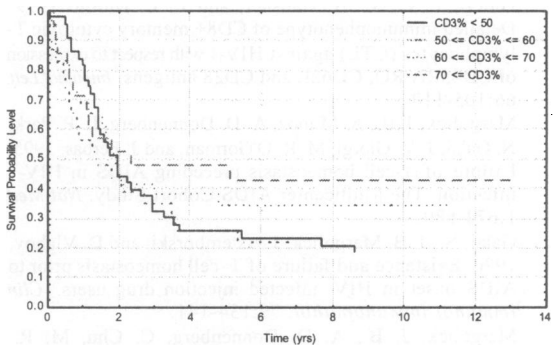

Figure 5: Kaplan-Meier Survival Analysis of AIDS to Final Assessment

Furthermore, our findings demonstrated superiority of the NN model versus that of the Cox RM in predicting morbidity and mortality. Comparison of the present results with our own previously published work[1] would suggest that Neural Networks can more effectively predict dichotomous rather than continuous outcomes, in concordance with the literature[10-12].

The NN outscored RM in Receiver Operating Characteristics curve when predicting morbidity from HIV Seropositivity to AIDS and mortality from HIV Seropositivity to Last Assessment as well as from AIDS to Last Assessment. We concluded that NN based modeling can be at least as accurate as regression modeling in predicting morbidity and mortality that occurs in late stages of HIV infection following loss of T-Cell Homeostasis. Also, due to the predictive value of CD3+ T-Cell count, the use of this marker should be considered in clinical practice and especially in late disease. It may prove useful in deciding when to initiate therapy, evaluating the response to antiretroviral treatment and eventually in the prediction of morbidity and mortality.

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