Original Article

Evaluation of Non-Viral Surrogate Markers as Predictive Indicators for Monitoring Progression of Human Immunodeficiency Virus Infection: An Eight-Year Analysis in a Regional Center

Houshang Rafatpanah¹, Leila Essmailian¹, Mohammad Reza Hedayati-Moghaddam², Rosita Vakili¹, Mehdi Norouzi³, Mohammad Reza Sarvghad⁴, Ali Mohammad Hosseinpour⁴, Hiva Sharebiani¹, and S.A Rahim Rezaee^{1*}

¹Inflammation and Inflammatory Diseases Research Center, ⁴Health Department, HIV Division, Mashhad University of Medical Sciences, Mashhad; ²Research center for HIV/AIDS, HTLV and Viral Hepatitis, Iranian Academic Center for Education, Culture & Research (ACECR), Mashhad Branch, Mashhad; and ³Virology Division, Tehran University of Medical Sciences, Tehran, Iran

SUMMARY: Suitable methods for clinical monitoring of HIV-infected patients are crucial in resource-poor settings. Demographic data, clinical staging, and laboratory findings for 112 asymptomatic subjects positive for HIV were assessed at the first admission and the last visit from 2002 to 2010. Cox regression analysis showed hemoglobin (Hb) (HR = 0.643, $P = 0.021$) to be a predictive indicator for disease progression, while CD4, CD8, and platelet counts showed low HRs, despite having significant probability values. Hb and total lymphocyte count (TLC) rapidly declined from stage II to III (10.9 and 29.6%, respectively). Reduced CD4 and platelet counts and Hb during stage I were associated with disease progression, and TLC was correlated with CD4 counts at the last follow-up ($P < 0.001$). However, WHO TLC cutoff of 1,200 cell/mm³ had 26.1% sensitivity and 98.6% specificity. ROC curve analysis suggested that a TLC cutoff of 1,800 cell/mm³ was more reliable in this region. Statistical analysis and data mining findings showed that Hb and TLC, and their rapid decline from stage II to III, in addition to reduced platelet count, could be valuable markers for a surrogate algorithm for monitoring of HIV-infected subjects and starting anti-viral therapy in the absence of sophisticated detection assays.

INTRODUCTION

The Joint United Nations Programme on HIV/AIDS (UNAIDS) reported that an estimated 35 million people were infected with HIV worldwide by the end of 2013, with 230,000 infected people in the Middle East and North Africa combined. AIDS-related deaths have nearly tripled, from 8,300 in 2001 to 23,000 at the end of 2009, but gradually decreased by the end of 2013.

The number of people living with HIV in Iran has increased to 92,000 cases in 2009 (1,2). Finding reliable methods for efficient clinical monitoring of infected subjects is of great importance in order to improve the quality of life and survival in this expanding population. The gold-standard markers for monitoring HIV-infected individuals are CD4 T-cell count and HIV viral load, both of which require sophisticated and expensive equipment and materials. The World Health Organization (WHO) HIV/AIDS clinical staging system (3) is the preferred method for monitoring of patients in developing countries, and development of economical tests

could improve the prognostic significance of this system (4). In order to identify and monitor patients at risk of disease progression in resource-poor settings, the prognostic significance of various surrogate markers has been investigated (5–15). Researchers have proposed many alternative markers, including hemoglobin (Hb) (6–11), total lymphocyte count (TLC) (7,14,15), delayed-type hypersensitivity responses (5), serum albumin levels (13), neopterin (16), and body mass index (17). Due to the general availability of complete blood cell count (CBC) tests, particularly in resource-poor setting areas, this study investigated the prognostic value of CBC markers in HIV progression. The use of these markers for monitoring of HIV infection based on the WHO staging system was assessed in Iranian patients positive for HIV who were living in the Khorasan provinces (northeastern Iran). The prognostic value of these CBC test results was evaluated based on gold-standard CD4 and CD8 counts using flow-cytometry.

MATERIALS AND METHODS

Study design: A total of 112 HIV-positive subjects were recruited from the triangular clinic at Mashhad University of Medical Sciences (MUMS) in Mashhad, Iran between January 2002 and January 2010. This clinic is the only center that provides a wide-range of counseling and therapy services for HIV-positive subjects in the Khorasan provinces. Only patients who were asymptomatic at the time of admission (WHO stage I) were included in this study. Their clinical and hematological

Received June 13, 2014. Accepted March 9, 2015. J-STAGE Advance Publication May 12, 2015.

DOI: 10.7883/yoken.JJID.2014.261

*Corresponding author: Mailing address: Inflammation and Inflammatory Diseases Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.

Tel: +98 511 8012768, Fax: +98 511 843 2666, E-mail: rezaee@mums.ac.ir

parameters were assessed at the first admission and the last visit prior to January 2010. In the subjects who underwent treatment or died, the last visit before starting antiretroviral therapy or death was included in the analysis. An infectious disease specialist assigned, clinical staging for each subject at both time-points based on the revised WHO staging system criteria (3). Disease progression was defined as shifting from stage I to stage III. Ethical approval for this study was granted by the MUMS ethics committee (Approval number: 86148).

Laboratory tests: Laboratory assessments included a CBC and CD4 and CD8 counts at the first visit (baseline) and at the end of the follow-up period. Anemia was defined as Hb values below 11.5 and 13.5 g/dL in women and men, respectively. CBC markers were measured using an automated Sysmex SE analyzer (Sysmex Corp, Kobe, Japan), and the CD4 and CD8 lymphocyte counts were measured using a FACS Calibur (BD Biosciences, Bergen Country, NJ, USA) flow cytometer.

Statistical analysis: The laboratory parameters were compared at 2 time-points by paired-sample t- and McNemar tests using SPSS Statistics for Windows, version 13.0. One-way analysis of variance (ANOVA, Chicago, IL, USA) was used to test for differences in laboratory marker levels across the 4 stages of HIV infection. Cox proportional hazards models were applied to assess the prognostic value of baseline hematological

and immunological markers. Correlations between TLC and CD4 counts were evaluated using the Pearson correlation coefficient. Differences were considered statistically significant for P values < 0.05 .

In addition, the study data were imported into a data-mining program, Rapidminer® (Rapi-INC, Cambridge, MA, USA). Two different algorithms were produced based on the results of TLC, Hb, and platelet (Plt) analysis (Fig. 1).

RESULTS

At baseline, the HIV-positive subjects had a mean age of 36.9 ± 8.6 years (range: 14–60 years). According to WHO clinical HIV/AIDS staging, 43 patients (38.4%) remained in stage I, 41 (36.6%) progressed to stage II, and 20 (25%) progressed to stage III or IV after a mean period of 21.7 ± 17.0 months. As shown in Table 1, Hb, hematocrit, white blood cell (WBC), TLC, and CD4 counts were significantly diminished; however, monocyte counts increased between the first and last visits. At first admission, 33.0% of subjects had anemia, which increased to 45.7% by the end of the follow-up period ($P = 0.038$).

One-way ANOVA revealed statistically significant differences for variables in the 4 stages of the disease at the last follow-up. The mean TLC for stages I, II, III, and IV were $2,254.3 \pm 765.2$, $2,235.3 \pm 850$, $1,573.4$

Fig. 1. (Color online) Algorithms for total lymphocyte counts (TLC), hemoglobin (Hb), and platelet (Plt). A) Decision tree algorithm for Hb, Plt, and TLC. B) Random forest algorithm for Hb and TLC.

Table 1. Comparison of laboratory values at first admission versus the last visit

Laboratory parameter	No.	First admission Mean $\pm$ SD	Last visit Mean $\pm$ SD	P -value ¹⁾
CD4 count (cell/mm ³)	95	492.7 $\pm$ 235.1	426.9 $\pm$ 272.5	0.013
CD8 count (cell/mm ³)	81	883.7 $\pm$ 377.7	892.75 $\pm$ 463.9	0.84
WBC count (cell/mm ³)	103	6,259.0 $\pm$ 2,652.0	5,646.6 $\pm$ 1,846.6	0.021
TLC (cell/mm ³)	90	2,394.2 $\pm$ 734.3	2,076.0 $\pm$ 794.5	< 0.001
Neutrophil count (cell/mm ³)	85	3,445.6 $\pm$ 2,117.2	3,094.5 $\pm$ 1,259.5	0.16
Monocyte count (cell/mm ³)	73	351.3 $\pm$ 289.1	467.1 $\pm$ 286.3	0.01
Hemoglobin (gr/dL)	103	13.9 $\pm$ 1.6	13.4 $\pm$ 1.9	0.015
Hematocrit (%)	103	42.8 $\pm$ 4.1	40.8 $\pm$ 4.8	< 0.001
Platelet (cell $\times 10^3$ /mm ³)	96	245.2 $\pm$ 391.2	204.7 $\pm$ 175.1	0.344

¹⁾: Paired-Samples T-test.

WBC, White blood cell; TLC, Total lymphocyte count; SD, Standard deviation.

± 422.7 , and $1,567.3 \pm 711.5$ cell/mm³, respectively, a significant change between stages ($P = 0.002$; Fig. 2A) The mean CD4 T-cells counts were 515.6 ± 253.8 , 493 ± 282.6 , 225.8 ± 116.9 , and 134.3 ± 74.9 cell/mm³ for the 4 stages, respectively ($P < 0.0001$; Fig. 2B).

The mean Hb concentrations from stage I to IV were 13.9 ± 2.2 , 13.8 ± 1.4 , 12.3 ± 1.6 , and 12.0 ± 1.6 g/dL, respectively, a statistically significant decrease ($P = 0.001$; Fig. 2C), and for platelet were 220.2 ± 214.7 , 176.6 ± 60.6 , 216.1 ± 226.7 , and 200.0 ± 175.0 cell $\times 10^3$ /mm³, respectively ($P = 0.07$; Fig. 2D). As shown in Table 2, the greatest rates of decline in TLC, CD4, and Hb levels were observed from stage II to III; however, there were no significant changes in platelet counts between stages. Moreover, while there was a declining trend in total WBC and monocyte counts with increasing stage, a similar trend was not observed in the neutrophil counts (data not shown).

As shown in Fig. 3, there was a significant positive correlation between TLC and CD4 count at the last follow-up ($r = 0.54$, $P < 0.001$). ROC curve analysis revealed that the WHO TLC cutoff of 1,200 cell/mm³ as a surrogate marker for CD4 counts below 200 cell/mm³ had 26.1% sensitivity, 98.6% specificity, 85.7% positive predictive value (PPV), and 80.7% negative predictive value (NPV) (Fig. 4). However, statistical analysis

and data mining revealed 1,835 cell/mm³ to be the best cutoff concentration among the patients positive for HIV in northeast Iran assessed in the current study, offering 72.10% sensitivity, 72.08% specificity, 21.48% PPV, and 96.06% NPV, and corresponding to approximately 1,200 cell/mm³ (Fig. 1).

To assess the prognostic value of CBC markers for progression of HIV infection, stages III and IV were defined as the outcome variable in Cox regression analysis. As shown in Table 3, only Hb concentration had significant prognostic value ($P = 0.021$, HR = 0.643, 95% confidence interval [CI]: 0.442–0.935); while TLC, CD4, CD8, and platelet counts also showed significant

Table 2. Percent decrease of TLC, platelet, and hemoglobin across HIV/AIDS staging

Laboratory parameter	HIV/AIDS staging			
	I $\rightarrow$ II	II $\rightarrow$ III	III $\rightarrow$ IV	Total
TLC (cell/mm ³)	−0.8	−29.6	−0.4	−30.5
CD4 count (cell/mm ³)	−4.4	−54.2	−40.5	−73.9
Hemoglobin (gr/dL)	−0.7	−10.9	−2.4	−13.7
Platelet (cell $\times 10^3$ /mm ³)	−19.8	22.3	−7.5	−9.2

See footnote of Table 1.

Fig. 2. Changes in the mean of TLC (A), Hb (B), white blood cell (WBC) (C), and CD4 count (D) in HIV patients based on WHO staging system at the end of follow up. One way ANOVA revealed a statistically significant difference in these values between the 4 stages of disease.

Fig. 3. Correlation between TLC and CD4 lymphocyte counts based on WHO guidelines of TLC less than 1,200 cells/mm³ as a cutoff point to predict CD4 count less than 200 cells/mm³.

Fig. 4. ROC curve for TLC. WHO cutoff point of 1,200 cell/mm³ for TLC, as a surrogate marker for determining of a CD4 count below 200 cell/mm³ had 26.1% sensitivity, 98.6% specificity, 85.7% positive predictive value (PPV), and 80.7% negative predictive value (NPV).

Table 3. Results from Cox regression analysis for variables associated with the risk of disease progression

Variable	HR	95% CI	P-value ¹⁾
Age (y)	0.988	0.910–1.073	0.77
Gender	0.504	0.074–3.442	0.485
TLC (cell/mm ³)	1.000	0.999–1.001	0.776
CD8 count (cell/mm ³)	1.002	1.001–1.004	0.011
CD4 count (cell/mm ³)	0.996	0.993–1.000	0.033
Neutrophil count (cell/mm ³)	1.000	1.000–1.001	0.518
Hemoglobin (gr/dL)	0.643	0.442–0.935	0.021
Platelet (cell/μL)	1.000	1.000–1.000	0.007

¹⁾ P < 0.05.

CI, Confidence interval; HR, Hazard ratio; TLC, Total lymphocyte count.

probability values, they had low hazard ratios.

However, data mining results using decision tree and random forest algorithms revealed that TLC, HB, and platelet counts had significant prognostic value for monitoring clinical progression (Fig. 1A and B). Finally, an algorithm for monitoring of HIV infected subjects was developed based on these statistical and data mining findings (Fig. 5).

DISCUSSION

CD4+ T-cells, the main targets of HIV infection, play a key role in orchestrating cellular immune responses. These cells activate anti-HIV specific CD8+ T-cells (cytotoxic T-lymphocytes) involved in viremia control (17,18).

HIV-1 infection can interfere with hematopoiesis, which leads to hematological complications including anemia, neutropenia, lymphopenia, and thrombocytopenia (19). In the current study, 33% of asymptomatic patients were anemic at the time of admission to the triangular clinic; this percentage increased to 45.7% during the average 21.7-month follow-up. The incidence of anemia in asymptomatic individuals in our study is similar to previous reports from Europe and North America (20). Different factors may contribute to anemia in HIV patients, including decreased serum erythropoietin levels, autoantibodies to erythropoietin, use of myelosuppressive medications such as zidovudine, nutritional deficiencies, bone marrow suppression by opportunistic infections, neoplasms, and the impact of HIV itself (21–23). Furthermore, the current study showed that lower baseline Hb concentrations were associated with increased risk of disease progression. This finding is consistent with those of several studies that reported anemia to be an independent predictor of disease

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Fig. 5. According to statistical analysis (Table 3) and data mining using Rapidminer® and decision tree algorithm (Fig. 4A) and Random forest algorithm (Fig. 4B), it is noted that platelet less than 127,800 mm^3 in data mining and less than 230,000 mm^3 in statistical analysis are prognostic factor for more monitoring of HIV patients.

progression and death in individuals with HIV (4,11,20–24). It is not clear why anemia is associated with risk of HIV progression, although it is unlikely that it has a direct effect. However, similar to advanced stages of other diseases such as cancer, anemia could be a useful index for disease progression in HIV (22–24).

In the present study, unlike the strong association between baseline (stage I) Hb levels and disease progression, statistical analysis revealed that TLC and platelet counts had weaker prognostic value. However, the results of data mining indicated that a combination of TLC, Hb and platelet counts were more reliable measures for monitoring of HIV infected subjects. Thrombocytopenia can occur in HIV-related diseases as a result of platelet destruction by antibodies (25,26). Tong et al. assessed hepatitis B treatment guidelines to show that monitoring of platelet counts at baseline and during the course of disease reduced death rates by 20%, suggesting the potential of this marker as a useful tool for prognosis of liver failure and hepatocellular carcinoma. Therefore, it has been recommended to add this marker to the current guideline criteria for monitoring of chronic hepatitis B patients (27). Furthermore, accumulating evidence suggests that monitoring of platelet levels in HIV and SIV infections may serve as a novel hematological marker for predicting central nervous system diseases associated with HIV (28,29). These data would highlight the probable role of platelet count in monitoring of HIV positive individuals. As the data mining results of the current study shows, platelet counts between 127,800 and 230,000/ mm^3 are prognostic factors for additional monitoring of HIV patients.

Interestingly, the Hb and TLC in this study were nearly stable in subjects with stage I and II HIV, and rapidly declined in subjects with stage III infections. The decreasing trend between stages II and III is shown in Fig. 2, which may be a suitable marker for initiation of HIV anti-viral treatment.

The results of statistical analysis and data mining suggest that TLC of 1,800 cell/mm^3 and approximately 1,200 cell/mm^3 , respectively, provided the best sensitivity and specificity as an indicator of TCD4+ cells lower

than 200 cell/mm^3 in Iranian patients with HIV (Figs. 2 and 4). Lau et al. have previously described a similar pattern of changes in TLC and Hb using segmented regression models (7). Further studies have revealed that the accelerated decline in these biomarkers was highly associated with the risk of AIDS (8,10). TLC and Hb may therefore be suitable markers for monitoring and evaluating HIV-infected subjects, as they are economical to measure and require less sophisticated laboratory techniques. Increasing Hb has been proposed as a predictive marker for treatment success when accompanied by increasing TLC. However, normal variations in Hb and differences in endemic diseases, genetics, nutrition, infections, viral characteristics, and side effects of antiretroviral treatment such as ziduvodine must be taken to account (6,7,17). In contrast to TLC and Hb, WBC count decreases steadily during the course of infection and cannot serve as a laboratory marker for monitoring patients. This study used a combination of data mining and statistical results to identify TLC, Hb, and platelet biomarkers and introduce a surrogate algorithm for monitoring of HIV infected subjects in low-income counties (Fig. 5).

In conclusion, this study demonstrated that reduced CD4 and platelet counts and Hb during stage I HIV infection are associated with disease progression and may have prognostic value for monitoring of HIV-positive subjects. Furthermore, rapid declines in Hb levels and TLC from stage I to III and platelet count fluctuation may be informative markers for starting anti-viral therapy in the absence of flow cytometry and viral load detection techniques. This finding is particularly valuable for clinicians in resource-poor settings, where equipment and materials to measure CD4 counts are unavailable and WHO staging is the preferred method of monitoring patients.

Acknowledgments The authors would like to have a great thank to Dr. Zahra Reyazi for her great help during sampling. This study was granted by Mashhad University of Medical Sciences (Ap. number: 86148).

Conflict of interest None to declare.

REFERENCES

- UNAIDS. Global report: UNAIDS report on the global AIDS epidemic 2010. UNAIDS Geneva 2010.
- UNAIDS. Epidemic publications, Epi slides from 2014 gap report. 2014. Available at <http://www.unaids.org/sites/default/files/media_asset/01_Epi_slides_2014July.pdf>
- DEFINITIONS WC. HIV/AIDS Programme. 2007.
- Lundgren JD, Mocroft A, Gatell JM, et al. A clinically prognostic scoring system for patients receiving highly active antiretroviral therapy: results from the EuroSIDA study. *J Infect Dis*. 2002;185:178-87.
- Anastos K, Shi Q, French AL, et al. Total lymphocyte count, hemoglobin, and delayed-type hypersensitivity as predictors of death and AIDS illness in HIV-1-infected women receiving highly active antiretroviral therapy. *J Acquir Immune Defic Syndr*. 2004;35:383-92.
- Florence E, Dreezen C, Schrooten W, et al. The role of non-viral load surrogate markers in HIV-positive patient monitoring during antiviral treatment. *Int J STD AIDS*. 2004;15:538-42.
- Lau B, Gange SJ, Phair JP, et al. Rapid declines in total lymphocyte counts and hemoglobin concentration prior to AIDS among HIV-1-infected men. *AIDS*. 2003;17:2035-44.
- Lau B, Gange SJ, Phair JP, et al. Use of total lymphocyte count and hemoglobin concentration for monitoring progression of HIV infection. *J Acquir Immune Defic Syndr*. 2005;39:620-5.
- Chen RY, Westfall AO, Hardin JM, et al. Complete blood cell count as a surrogate CD4 cell marker for HIV monitoring in resource-limited settings. *J Acquir Immune Defic Syndr*. 2007;44:525-30.
- Gange SJ, Lau B, Phair J, et al. Rapid declines in total lymphocyte count and hemoglobin in HIV infection begin at CD4 lymphocyte counts that justify antiretroviral therapy. *AIDS*. 2003;17:119-21.
- Obirikorang C, Yeboah FA. Blood haemoglobin measurement as a predictive indicator for the progression of HIV/AIDS in resource-limited setting. *J Biomed Sci*. 2009;16:102.
- Post FA, Wood R, Maartens G. CD4 and total lymphocyte counts as predictors of HIV disease progression. *QJM*. 1996;89:505-8.
- Shah S, Smith CJ, Lampe F, et al. Haemoglobin and albumin as markers of HIV disease progression in the highly active antiretroviral therapy era: relationships with gender. *HIV Med*. 2007;8:38-45.
- Daka D, Loha E. Relationship between total lymphocyte count (TLC) and CD4 count among peoples living with HIV, Southern Ethiopia: a retrospective evaluation. *AIDS Res Ther*. 2008;5:26.
- Susaengrat W, Mitchai M, Leerattanapetch N. Total lymphocyte count: a surrogate marker for predicting CD4+ count in enrolled process for antiretroviral therapy in resource-limited settings. *J Med Assoc Thai*. 2008;91:1514-7.
- Mildvan D, Spritzler J, Grossberg SE, et al. Serum neopterin, an immune activation marker, independently predicts disease progression in advanced HIV-1 infection. *Clin Infect Dis*. 2005;40:853-8.
- Langford SE, Ananworanich J, Cooper DA. Predictors of disease progression in HIV infection: a review. *AIDS Res Ther*. 2007;4:11.
- Lane HC. Pathogenesis of HIV infection: total CD4+ T-cell pool, immune activation, and inflammation. *Top HIV Med*. 2010;18:2-6.
- Moses A, Nelson J, Bagby GC Jr. The influence of human immunodeficiency virus-1 on hematopoiesis. *Blood*. 1998;91:1479-95.
- Berhane K, Karim R, Cohen MH, et al. Impact of highly active antiretroviral therapy on anemia and relationship between anemia and survival in a large cohort of HIV-infected women: women's interagency HIV study. *J Acquir Immune Defic Syndr*. 2004;37:1245-52.
- Mocroft A, Kirk O, Barton SE, et al. Anaemia is an independent predictive marker for clinical prognosis in HIV-infected patients from across Europe. EuroSIDA study group. *AIDS*. 1999;13:943-50.
- Moyle G. Anaemia in persons with HIV infection: prognostic marker and contributor to morbidity. *AIDS Rev*. 2002;4:13-20.
- Sullivan P. Associations of anemia, treatments for anemia, and survival in patients with human immunodeficiency virus infection. *J Infect Dis*. 2002;185 Suppl 2:S138-42.
- Semba RD, Shah N, Klein RS, et al. Prevalence and cumulative incidence of and risk factors for anemia in a multicenter cohort study of human immunodeficiency virus-infected and -uninfected women. *Clin Infect Dis*. 2002;34:260-6.
- Passos AM, Treitinger A, Spada C. An overview of the mechanisms of HIV-related thrombocytopenia. *Acta Haematol*. 2010;124:13-8.
- Costantini A, Giuliodoro S, Mancini S, et al. Impaired in-vitro growth of megakaryocytic colonies derived from CD34 cells of HIV-1-infected patients with active viral replication. *AIDS*. 2006;20:1713-20.
- Tong MJ, Hsien C, Hsu L, et al. Treatment recommendations for chronic hepatitis B: an evaluation of current guidelines based on a natural history study in the United States. *Hepatology*. 2008;48:1070-8.
- Wachtman LM, Skolasky RL, Tarwater PM, et al. Platelet decline: an avenue for investigation into the pathogenesis of human immunodeficiency virus -associated dementia. *Arch Neurol*. 2007;64:1264-72.
- Wachtman LM, Tarwater PM, Queen SE, et al. Platelet decline: an early predictive hematologic marker of simian immunodeficiency virus central nervous system disease. *J Neurovirol*. 2006;12:25-33.