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

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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
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 ± 765.2, 2,235.3 ± 850, 1,573.4 ± 404.5, and 1,566.4 ± 508.3, respectively ($P < 0.001$). The mean CD4 count for stages I, II, III, and IV were 582.8 ± 235.1, 572.3 ± 235.1, 291.4 ± 125.3, and 291.4 ± 125.3, respectively ($P < 0.001$). The mean Hb for stages I, II, III, and IV were 13.9 ± 1.6, 13.8 ± 1.6, 13.4 ± 1.9, and 13.4 ± 1.9, respectively ($P < 0.001$). The mean platelet count for stages I, II, III, and IV were 245.2 ± 391.2, 245.2 ± 391.2, 204.7 ± 175.1, and 204.7 ± 175.1, respectively ($P < 0.001$).

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

| Laboratory parameter | No. | First admission | Last visit | $P$-value$^{1}$ |
|----------------------|-----|----------------|------------|----------------|
| CD4 count (cell/mm$^3$) | 95 | 492.7 ± 235.1 | 426.9 ± 272.5 | 0.013 |
| CD8 count (cell/mm$^3$) | 81 | 883.7 ± 377.7 | 892.7 ± 463.9 | 0.84 |
| WBC count (cell/mm$^3$) | 103 | 6,259.0 ± 2,652.0 | 5,646.6 ± 1,846.6 | 0.021 |
| TLC (cell/mm$^3$) | 90 | 2,394.2 ± 734.3 | 2,076.0 ± 794.5 | <0.001 |
| Neutrophil count (cell/mm$^3$) | 85 | 3,445.6 ± 2,117.2 | 3,094.5 ± 1,259.5 | 0.16 |
| Monocyte count (cell/mm$^3$) | 73 | 351.3 ± 289.1 | 467.1 ± 286.3 | 0.01 |
| Hemoglobin (g/dL) | 103 | 13.9 ± 1.6 | 13.4 ± 1.9 | 0.015 |
| Hematocrit (%) | 103 | 42.8 ± 4.1 | 40.8 ± 4.8 | <0.001 |
| Platelet (cell × 10$^3$/mm$^3$) | 96 | 245.2 ± 391.2 | 204.7 ± 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 ± 711.5 cell/mm³, respectively, a significant change between stages \((P = 0.002; \text{Fig. 2A})\). 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 \((P<0.0001; \text{Fig. 2B})\).

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 \((P = 0.001; \text{Fig. 2C})\), and for platelet were 220.2 ± 214.7, 176.6 ± 60.6, 216.1 ± 226.7, and 200.0 ± 175.0 cell × 10⁹/mm³, respectively \((P = 0.07; \text{Fig. 2D})\). 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 \((P = 0.021, \text{HR} = 0.643, 95\% \text{ confidence interval [CI]}: 0.442–0.935)\); 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 → II | II → III | III → IV | Total |
|----------------------|-----------------|-------|---------|---------|-------|
| TLC (cell/mm³)       |                 | −0.8  | −29.6   | −0.4    | −30.5 |
| CD4 count (cell/mm³) |                 | −4.4  | −54.2   | −40.5   | −73.9 |
| Hemoglobin (gr/dL)   |                 | −0.7  | −10.9   | −2.4    | −13.7 |
| Platelet (cell × 10⁹/mm³) |           | −19.8 | 22.3    | −7.5    | −9.2  |

See footnote of Table 1.
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 progression.

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

| Variable             | HR   | 95% CI          | P-value<sup>1</sup> |
|----------------------|------|-----------------|---------------------|
| Age (y)              | 0.988| 0.910–1.073     | 0.77                |
| Gender               | 0.504| 0.074–3.442     | 0.485               |
| TLC (cell/mm³)       | 1.000| 0.999–1.001     | 0.776               |
| CD8 count (cell/mm³) | 1.002| 1.001–1.004     | 0.011               |
| CD4 count (cell/mm³) | 0.996| 0.993–1.000     | 0.033               |
| Neutrophil count (cell/mm³) | 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               |

<sup>1</sup>: P < 0.05.

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

### Surrogate Markers in HIV Infection

**Surrogate Markers in HIV Infection**

- Lym > 1,800
- Lym/mm$^3$
- 1,800 < Lym > 1,193
- Hb = 13.5
- Lym ≤ 1,193
- Hb < 11.5
- Lym > 1,193
- Hb < 11-12
- Lym < 1,193

- **No intervention**
- **Anti HIV Treatment**
- **Anemia Treatment**

**Surrogate Markers in HIV Infection**

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 1) 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 zidovudine 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.

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Conflict of interest None to declare.

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