Mean Corpuscular Volume (MCV) Values Reflect Therapeutic Effectiveness in Zidovudine-Receiving HIV Patients

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Background: An increase of the mean corpuscular volume (MCV) of erythrocytes and alterations in the lipid profiles have been described in HIV-infected patients under combination of anti-retroviral treatment (cART), particularly zidovudine (AZT). **Methods:** In 687 sera from 179 HIV-positive patients, MCV levels were correlated with the clinical outcome or therapeutic effectiveness. The sera were classified into three groups according to AZT treatment; cART with AZT ($n = 317$), cART without AZT ($n = 262$), and no anti-retroviral therapy ($n = 108$). **Results:** The MCV and lipid profile values were increased after cART. The AZT-based cART group had higher MCV levels (111.6 ± 7.0 vs. 97.8 ± 7.0 fl, $P < 0.001$) and a higher incidence of macrocytosis (>99 fl; 95.3%

vs. 38.2%, $P < 0.001$) than the non-AZT-based cART group. The receiver operating characteristic curve analysis showed that the area under the curve was 0.835 and the cut-off of MCV (>102 fl) had a sensitivity of 96.1% and specificity of 66.7% for detecting HIV-RNA (-) sera in AZT-based cART group. In the multivariate regression analysis, HIV-viral load and HDL-cholesterol were the only significant correlates to the MCV values in the AZT-base cART group ($P < 0.05$). **Conclusion:** The MCV values could be used as a surrogate marker to monitor the clinical outcome of HIV-infected patients receiving AZT-based cART. J. Clin. Lab. Anal. 27:373–378, 2013. © 2013 Wiley Periodicals, Inc.

Key words: zidovudine; HIV; MCV; HIV-RNA; macrocytosis

INTRODUCTION

The aim of anti-retroviral therapy in HIV-infected patients is the suppression of viral load and enhancement of immune function. The measurement of HIV-RNA levels and CD4 counts are essential for monitoring the treatment response. However, because of its high laboratory cost, the surrogate markers for early detection of reduced adherence or treatment resistance with cost effectiveness would be helpful where HIV viral load and immune monitoring tests are unavailable.

Mean corpuscular volume (MCV) is the standard initial test currently used to assess anemic patients. MCV measurements are performed by almost all fully automated hematology instruments. An increase of MCV has been reported in HIV-infected patients who received combination anti-retroviral treatment (cART), including nucleoside reverse-transcriptase inhibitors treatment, particularly zidovudine (AZT) (1–3).

AZT was an anti-retroviral agent used for HIV infection, and maintaining adherence to treatment is the most

important predictor of therapeutic efficacy. Several previous studies (1–3) have reported an association between MCV levels and self-reported adherence. Recent studies have found that MCV levels are useful for identifying nonresponders with a high adherence rate and may be associated with HIV-RNA levels (3,4). However, the clinical values of MCV as a surrogate marker of adherence or therapeutic effectiveness are not yet conclusive, and the

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treatment adherences reported in previous studies were mostly assessed by self-reporting, which is subjective to reporting bias.

Macrocytosis is known to be associated with liver diseases as well as vitamin B12 or folic acid deficiency (5). Nucleoside reverse transcriptase inhibitor (NRTI) treatment with AZT has been reported to be associated with mitochondrial toxicity through inhibition of cellular DNA synthesis (2, 6) and abnormal serum lipid profiles (7, 8). Although a significant association between increased MCV values and decreased hepatic mitochondrial function was reported (9), there has been no report on the correlation between MCV values and lipid profiles. Since cART-inducing abnormal lipid metabolism can lead to cardiovascular disease (10), the relation between the lipid profile and MCV may be interesting.

In this study, we analyzed the association between the MCV levels and lipid profile, HIV-RNA viral load, and CD4+ lymphocyte count after AZT-based cART. In addition, the diagnostic value of MCV measurement for therapeutic effectiveness in HIV-infected patients was investigated.

MATERIALS AND METHODS

Study Subjects

This study included consecutive 687 sera from 179 HIV-positive Asian patients (167 men; 12 women). All samples were collected for lymphocyte subset tests to assess the immunologic status or monitor the therapeutic response at Seoul St. Mary's Hospital from August 2008 to December 2010. The mean age of the patients was 42 years (range 20–80 years) and average of 3.8 (1–8) sera per patient was taken at three- to six-month intervals. A retrospective chart review was performed on concurrent medications and available laboratory parameters at each visit. Patients who had anemia, macrocytosis, and viral or alcoholic liver diseases before initiation of treatment were excluded.

All sera were classified into three groups according to anti-retroviral treatment: (a) group 1; sera with AZT-based cART for three months or more ($n = 317$), (b) group 2; sera with cART in the absence of AZT for three months or more ($n = 262$); and (c) group 3; sera before anti-retroviral therapy ($n = 108$). cART was defined as two or more NRTIs in combination with at least one protease inhibitor or one non-nucleoside reverse transcriptase inhibitor (NNRTI) or one integrase strand transfer inhibitor.

Laboratory Tests

In each group, MCV levels, lipid profiles (total cholesterol, triglyceride, HDL-cholesterol, and LDL-cholesterol), HIV viral loads, and lymphocyte subsets

(absolute cell counts and percent of CD3, CD4, and CD8 positive lymphocytes) were investigated. The MCV levels were measured using a Sysmex XE-2100 analyzer (Sysmex, Kobe, Japan). The normal range established in our laboratory for MCV is 80.0–99.0 fl, and macrocytosis was defined as a MCV greater than 99.0 fl. HIV-1 RNA viral loads were quantified with an Abbott real-time HIV-1 amplification reagent kit (Abbott Molecular, Des Plaines, IL) using an automated m2000sp and m2000rt real-time analyzer (Abbott Molecular). The lower limit of HIV-RNA detection was 40 HIV-1 RNA copies/ml. The lymphocyte subsets were measured with a BD Multitest 6-color TBNK reagent (BD Biosciences, San Jose, CA) using BD FACS Canto II flowcytometer (BD Biosciences). Lipid profiles were assessed with specific reagents (Sekisui Medical, Tokyo, Japan) using a Hitachi 7600-210 (Hitachi, Tokyo, Japan). Variables were determined every three to six months and the mean follow-up period was nine months (maximum 26 months) for the AZT-treated patients. This retrospective cohort study was approved by the Institutional Review Board of Seoul St. Mary's Hospital.

Statistical Analysis

Statistical analysis was performed with SPSS version 12.0 (SPSS, Chicago, IL). Between-group differences of each parameter were compared by Students' *t*-tests. Pearson's correlation test and multivariate regression analysis were used to investigate the correlation between MCV levels and laboratory parameters. The receiver operating characteristic (ROC) plot was used to estimate the ability of using the MCV measurements for detecting negative-plasma HIV-RNA. All *P* values were 2-tailed with $P < 0.05$ considered statistically significant.

RESULTS

Laboratory Results in Each Group

The laboratory results of all sera before and after cART comparison are shown in Table 1. MCV levels were significantly increased after cART (105.3 ± 9.8 vs. 88.9 ± 4.0 , $P < 0.001$) when compared to before cART. 69.4% of the 579 sera in the cART group had increased MCV values (>99 fl), while only one serum in the no treatment group had an increased MCV value (100.2 fl). There was a significant increase in the lipid profile levels after cART (Table 1). In addition, higher MCV levels (111.6 ± 7.0 vs. 97.8 ± 7.0 fl, $P < 0.001$) and higher incidence of macrocytosis (95.3% vs. 38.2%, $P < 0.001$) were observed in the AZT-based cART group than the non-AZT-based cART group. The levels of total cholesterol, HDL-cholesterol, and LDL-cholesterol were significantly higher in the non-AZT-based cART group.

TABLE 1. Laboratory Parameters of the 687 Sera From HIV-Infected Patients Who Had Received Different Types of Combination Anti-Retroviral Therapy (cART)

	AZT-based cART (group 1) <i>n</i> = 317	cART without AZT (group 2) <i>n</i> = 262	cART-naïve (group3) <i>n</i> = 108
MCV (mean $\pm$ SD, fL)	111.6 $\pm$ 7.0 ^a	97.8 $\pm$ 7.0	88.9 $\pm$ 4.0
Macrocytosis (number (%))	302 (95.3) ^a	100 (38.2)	1 (0.9)
Total cholesterol (mg/dl)	181.9 $\pm$ 33.7 ^a	198.1 $\pm$ 41.9	160.8 $\pm$ 31.0
Triglyceride (mg/dl)	236.4 $\pm$ 124.7	213.2 $\pm$ 117.3	188.2 $\pm$ 161.5
HDL-cholesterol (mg/dl)	44.0 $\pm$ 9.8 ^a	47.6 $\pm$ 14.8	37.1 $\pm$ 10.7
LDL-cholesterol (mg/dl)	96.5 $\pm$ 29.3 ^a	111.9 $\pm$ 31.4	94.4 $\pm$ 22.4
log HIV RT-PCR (log ₁₀ copies/ml)	1.7 $\pm$ 0.6	1.8 $\pm$ 0.7	3.7 $\pm$ 0.9
CD3 number (counts/ μ l (%))	1467.4 $\pm$ 495.4 ^a (73.3 $\pm$ 10.0)	1337.4 $\pm$ 542.6 (68.8 $\pm$ 10.4)	1466.1 $\pm$ 783.6 (75.8 $\pm$ 8.0)
CD4 number (counts/ μ l (%))	548.0 $\pm$ 263.9 ^a (27.2 $\pm$ 9.5)	468.3 $\pm$ 262.3 (23.6 $\pm$ 8.4)	398.7 $\pm$ 198.0 (22.1 $\pm$ 8.0)
CD8 number (counts/ μ l (%))	798.0 $\pm$ 331.0 (40.1 $\pm$ 10.5)	763.6 $\pm$ 358.0 (39.6 $\pm$ 11.7)	965.2 $\pm$ 669.2 (48.6 $\pm$ 11.2)

^aSignificant difference between group 1 and group 2 ($P < 0.05$).

AZT, zidovudine; MCV, mean corpuscular volume; RT-PCR, real-time polymerase chain reaction.

MCV and Lipid Profile Values in Each Time Interval After AZT Initiation

To analyze the changes in the MCV value following initiation of AZT-based cART, we averaged the MCV levels of sera within the same interval. Mean MCV levels initially increased above the reference range after AZT treatment, especially in the first one to three months and remained at higher levels than the pretreatment baseline levels throughout the follow-up period (Fig. 1). After AZT treatment for one and three months, the MCV levels in 52.9% and 94.4% of all sera had increased, respectively. In

terms of averaged lipid profile, no significant changes were observed at each interval after AZT treatment during the two-year follow-up period.

CD4+ Lymphocytes and HIV-RNA Results in Association of MCV Levels

Patients with macrocytosis had a significantly higher number of CD4+ lymphocytes than patients with normal MCV levels (mean $\pm$ SD; 562.2 $\pm$ 259.5 vs. 262.8 $\pm$ 181.7, $P < 0.001$). After cART, positive correlations between

Fig. 1. Change in the mean corpuscular volume (MCV) levels after the start of zidovudine (AZT) treatment for HIV patients.

Fig. 2. Correlation between CD4% and MCV levels in HIV patients taking anti-retroviral drugs with zidovudine (AZT) (A) and without AZT (B).

CD4+ lymphocyte % and MCV values were observed for both the AZT-based and non-AZT-based cART groups ($r = 0.338$, $P < 0.001$ and $r = 0.155$, $P = 0.012$, respectively) (Fig. 2).

In terms of the HIV-RNA assay, the average plasma HIV-viral loads and HIV-RNA detection rate were not different between the AZT-based cART group and non-AZT-based cART groups (Table 1). In the correlation analysis, MCV values were found to be negatively correlated with quantitative HIV-RNA viral loads in only the AZT-based cART group ($r = -0.390$, $P < 0.001$). The ROC analysis was performed to determine the cut-off level of MCV for detecting HIV-RNA (–) sera in the AZT-based cART group. The area under the curve was 0.835 (95% confidence interval (CI), 0.779–0.881) and the cut-off of MCV (>102 fL) had a sensitivity of 96.1% (CI,

Fig. 3. Receiver operating characteristic curves of mean corpuscular volume (MCV) for the detection of negative HIV-RNA for patients who received zidovudine (AZT)-based combination anti-retroviral therapy (cART). The area under the curve was 0.835 (95% confidence interval (CI), 0.779–0.881) and a cut-off MCV > 102 fL corresponded to a sensitivity of 96.1% (CI, 92.5–98.3) and specificity of 66.7% (CI, 38.4–88.2).

92.5–98.3) and specificity of 66.7% (CI, 38.4–88.2) for detecting HIV-RNA (–) (Fig. 3).

Lipid Profiles in Association of MCV Levels

The total cholesterol and HDL-cholesterol levels showed a weak positive correlation with the MCV values in the AZT-based cART group ($r = 0.131$, $P = 0.048$ and $r = 0.257$, $P < 0.001$, respectively). However, no correlation between MCV and lipid profile was observed for the non-AZT-based cART group. When we divided the sera according to the MCV results (macrocytosis (+) (>99 fL) and macrocytosis (–) (≤ 99 fL)), sera from patients with macrocytosis were found to have significantly higher concentrations of total cholesterol.

We utilized multivariate regression analysis to determine the independent laboratory factors in association MCV values. Among the variables, which included lipid profiles, CD4 count, HIV-viral loads, and duration of treatment, only HIV-viral load (slope, -3.180 ; $P < 0.001$) and HDL-cholesterol (slope, 0.177 ; $P = 0.003$) were significantly associated with MCV values in the AZT-based cART group.

DISCUSSION

By analyzing the association between the MCV levels and HIV-RNA viral loads, lymphocytes subset or lipid

profiles after cART, we found that the MCV levels may be used as a surrogate marker to evaluate the effectiveness of AZT-based cART.

AZT inhibits mammalian cellular DNA polymerases as well as viral reverse transcriptase and may induce macrocytosis by impairing cell division. In this study, MCV levels were increased after cART and there was a significant difference between the AZT-based and non-AZT-based cART group. Although stavudine as well as AZT have been previously reported as a cause of macrocytosis (1,2), the identification of macrocytosis with other cART than AZT was not focused in this study due to the small number of sera.

The therapeutic goal of anti-retroviral treatment includes achieving an undetectable HIV-viral load (11, 12). We found that MCV values had a positive-correlation with CD4+ lymphocyte counts and a negative-correlation with HIV-RNA viral loads after AZT-based cART. The patients with increased MCV values above the normal range (>99 fl) had a significantly higher number of CD4+ lymphocytes when compared to patients with normal MCV levels, suggesting that the MCV values may be used as an indicator of immune recovery or therapeutic effectiveness after cART. In the ROC analysis, the MCV was shown to have a good diagnostic value with an area under the curve of 0.835, sensitivity of 96.1%, and specificity of 66.7%. Since it was reported that good adherence might be useful for detecting virologic failure (13), inclusion of adherence data would improve the diagnostic value of MCV.

We also found that dyslipidemia occurred after cART, supporting the previous studies (7, 8). No significant change in the averaged lipid profile at each interval after AZT treatment was observed during the follow-up period, which is in agreement with a previous study that reported rapid increases in serum cholesterol and triglyceride following cART initiation (7). In contrast to a previous study that reported insignificant changes in HDL-cholesterol levels after cART (7), we observed an increase in the HDL-cholesterol levels after cART and MCV values were found to be correlated with HDL-cholesterol as well as total cholesterol in the AZT-based cART group. This may be due to other factors, such as different populations, other medical conditions, lipid-lowering medications, or diet. We found that a higher concentration of total cholesterol, HDL-cholesterol, and LDL-cholesterol results was observed in non-AZT-based cART group than the AZT-based cART group. This might be due to the effect of nucleoside analogues on mitochondrial function and adipocyte metabolism in previous studies where PI-based highly active anti-retroviral therapy was reported to be associated with moderate to severe changes in lipid profiles (10, 14). However, no correlations between lipid profile values and MCV levels were ob-

served in the non-AZT-based cART group in the present study.

In the multivariate analysis, HIV-viral load and HDL-cholesterol were found to be significantly correlated with MCV values, but serum cholesterol, triglyceride, duration of AZT treatment, or CD4+ lymphocyte count was not correlated with MCV values.

Potential limitations of this study include the relatively small number of newly HIV-infected patients, relative short-term follow-up, lack of self reporting adherence, absence of vitamin B12 and folic acid data, and potential influence from other factors. In addition, this study included only Korean and middle-aged patients and only focused AZT treatment. Similar studies that focus on other anti-retroviral agents would be useful in terms of better understanding the diagnostic value of MCV measurements after HIV treatment. Despite these limitations, our data do indicate the need for additional prospective studies, including measurements of longitudinal changes for laboratory parameters in a randomized evaluation.

In conclusion, our study demonstrated that for AZT-treated HIV-infected patients, increased MCV values may correspond to negative-HIV-RNA and improved immune function, and also may be related with other adverse effects of the anti-retroviral agents, such as hyperlipidemia. Therefore, the MCV values hold promise for use as a surrogate marker to monitor clinical outcomes in patients receiving AZT-based cART.

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