

Vitamin B-12 metabolism in HIV-infected patients in the age of highly active antiretroviral therapy: role of homocysteine in assessing vitamin B-12 status¹⁻³

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ABSTRACT

Background: Before the advent of highly active antiretroviral therapy (HAART), 20% and 10% of HIV-infected patients had low vitamin B-12 and red blood cell folate (RBCF) concentrations, respectively. However, few patients had real vitamin B-12 deficiency.

Objective: We evaluated the prevalence of low vitamin B-12 and RBCF concentrations in HIV-infected patients receiving HAART and the usefulness of serum homocysteine (sHcy) for differentiating patients with deficiency from those with harmlessly low vitamin B-12.

Design: The prevalence of low vitamin B-12 and RBCF was evaluated in 126 HIV-infected patients receiving HAART. Moreover, sHcy concentrations were evaluated in 40 HIV-infected patients with low vitamin B-12 and in 37 HIV-infected patients with low RBCF and were compared with those in 128 HIV-infected patients with normal vitamin B-12 and RBCF. sHcy was used to monitor treatment with vitamin B-12 and folic acid in 28 patients (24 with low vitamin B-12 and RBCF and 4 with hyperhomocysteinemia but normal vitamin B-12 and RBCF).

Results: The prevalence of low vitamin B-12 was significantly lower in patients receiving HAART than in previously studied patients who did not receive HAART (8.7% compared with 27%). Nine of the 40 patients (22.5%) with low vitamin B-12 (≤ 200 pmol/L) had hyperhomocysteinemia (> 17.5 μ mol homocysteine/L). Nineteen (51.4%) of the 37 patients with low RBCF (≤ 580 nmol/L, percentile 10) had hyperhomocysteinemia. Among the 9 patients with an RBCF concentration ≤ 450 nmol/L (percentile 2.5), all had hyperhomocysteinemia. The treatment with vitamin B-12 and folic acid normalized sHcy concentrations.

Conclusions: The prevalence of low vitamin B-12 decreased after the introduction of HAART. The study of sHcy is useful for detecting HIV-infected patients with low vitamin B-12 and real deficiency. *Am J Clin Nutr* 2003;77:420-4.

KEY WORDS HIV, vitamin B-12, folate, homocysteine, highly active antiretroviral therapy, HAART

INTRODUCTION

Low concentrations of vitamin B-12 and red blood cell folate were found in 20% and 10% of HIV-infected patients, respectively (1). However, during the clinical evaluation of HIV-infected patients who have a low vitamin B-12 concentration, it is neces-

sary to ascertain whether these patients have a real deficiency or merely a harmlessly low vitamin B-12 concentration. This situation resembles that of iron deficiency during pregnancy and in persons with multiple myeloma (2), in which a low vitamin B-12 concentration can be found without a real vitamin B-12 deficiency. Some tests can help to distinguish patients with a real deficiency from those without a deficiency.

The deoxyuridine suppression test (dUST) is used to determine megaloblastic alterations by measuring changes in the incorporation of labeled thymidine (3). Our group showed that among HIV-infected patients with low vitamin B-12, most did not have a deficiency, except when a low red blood cell folate concentration was observed. However, the dUST demands a bone marrow sample, which is time consuming to obtain; as a result, few laboratories currently use this test routinely (4).

Some authors have suggested that the study of abnormalities in vitamin B-12 metabolites and in folate metabolism is an alternative to the dUST (5). In this regard, methylmalonic acid and homocysteine (Hcy) were found to have excellent sensitivity for diagnosing vitamin B-12 or folate deficiency (5, 6).

Because Hcy concentrations increase in vitamin B-12 and folate deficiencies (6), Hcy measurement may be a good tool for detecting and managing low vitamin B-12 concentrations in HIV-infected patients. Moreover, Hcy evaluation is routinely used in many laboratories and could easily be used in HIV-infected patients with low vitamin B-12 (7-9) and in monitoring the response to treatment.

Almost all studies on vitamin B-12 and folate metabolism in HIV-infected patients were carried out before the advent of highly active antiretroviral therapy (HAART), a treatment with ≥ 3 antiretroviral drugs including a protease inhibitor (1, 10). Today, in the age of HAART, the prevalence of low serum vitamin B-12 concentrations and low red blood cell folate concentrations should be reappraised in HIV-infected patients undergoing this therapy because many HIV-infected patients develop

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macrocytosis (11). The present study was undertaken to ascertain whether the prevalence of low concentrations of these vitamins is lower now than it was before the advent of HAART and whether serum Hcy concentrations can be used to identify patients with a real vitamin B-12 deficiency and to monitor responses to treatment.

SUBJECTS AND METHODS

Evaluation of serum vitamin B-12 and red blood cell folate concentrations in HIV-infected patients receiving HAART

To evaluate the prevalence of abnormalities in these variables, a group of HIV-infected patients receiving HAART (taking ≥ 3 antiretroviral drugs) was compared with a group of previously studied HIV-infected patients who did not receive HAART. The HAART group consisted of all the HIV-infected patients who received HAART in the SANTPAU-CITRAN (Centre d'Investigació, Tractament i Rehabilitació Adictes a Narcòtics) program for 11 mo (126 patients). The non-HAART group was made up of 109 patients who either received treatment with one antiretroviral drug or received no treatment with antiretroviral drugs. The subjects in the non-HAART group were recruited from 1989 to 1992. The characteristics of these patients were published elsewhere (12, 13). The study was performed in accord with the Helsinki Declaration of 1975 as revised in 1983, and all subjects provided written informed consent.

Assessment of serum Hcy concentrations in the diagnosis of HIV-infected patients with low serum vitamin B-12 or red blood cell folate concentrations

Serum Hcy concentrations in HIV-infected patients with a low serum vitamin B-12 concentration (≤ 200 pmol/L; $n = 40$) or a low red blood cell folate concentration (≤ 580 nmol/L; $n = 37$) were compared with those in a group consisting of all the HIV-infected patients with a serum vitamin B-12 concentration > 200 pmol/L and a red blood cell folate concentration > 580 nmol/L who were recruited in the 11-mo period (128 HIV-infected patients who did or did not receive HAART). The patients with low concentrations of serum vitamin B-12 or red blood cell folate were recruited from patients who agreed to participate in the SANTPAU-CITRAN program from 1996 until 2001.

In this study, the patients were considered to have a low concentration of serum vitamin B-12 and red blood cell folate if serum vitamin B-12 and red blood cell folate concentrations were below percentile 10 (≤ 200 pmol/L and ≤ 580 nmol/L, respectively). The rationale for using percentile 10 was based on the fact that a considerable percentage of patients below this cutoff had hyperhomocysteinemia (14). We investigated serum Hcy concentrations in a population of 230 patients with different disorders. Ninety-two percent of patients with a red blood cell folate concentration below percentile 2.5 (60 patients) had hyperhomocysteinemia, as did 47% of patients ($n = 54$) with a red blood cell folate concentration between percentiles 10 and 2.5 (AF Remacha, unpublished observation, 2000). Moreover, the patients with concentrations below percentile 2.5 of serum vitamin B-12 (≤ 150 pmol/L) and red blood cell folate (≤ 450 nmol/L) concentrations were also evaluated. The reference values for red blood cell folate were calculated from 159 healthy persons ($\bar{x} \pm SD$: 837 ± 235 nmol/L; percentile 2.5: 450 nmol/L; percentile 97.5: 1750 nmol/L).

Evaluation of treatment with vitamin B-12 and folic acid in a group of HIV-infected patients with low vitamin B-12 or red blood cell folate concentrations

Twenty-eight patients were recruited from the same 11-mo period. This group consisted of 22 of 24 patients receiving HAART who had low vitamin concentrations, 2 patients who had low vitamin concentrations but were not receiving HAART, and 4 of the 8 control subjects (receiving HAART or not) who had hyperhomocysteinemia and vitamin B-12 and red blood cell folate concentrations ≥ 200 pmol/L and 580 nmol/L, respectively. Twenty-eight of the 34 possible candidates were treated (2 patients rejected the treatment, one patient died, and 3 candidates were lost before starting the treatment).

One milligram vitamin B-12 (cyanocobalamin; intramuscular) per week and 5 mg folic acid (oral)/d were administered to all patients for 6 wk. Vitamin B-12, red blood cell folate, and serum Hcy concentrations were measured before and after treatment.

Methods

Serum vitamin B-12 and red blood cell folate concentrations were measured by using CEDIA vitamin B-12 and folate in a Hitachi 911 automatic analyzer (Roche Diagnostics, Mannheim, Germany). In the historical group, a radioassay (Amersham, Buckinghamshire, United Kingdom) was used to measure concentrations of vitamin B-12 and folate (reference values: 450–1300 nmol/L) (12, 13).

Serum Hcy concentrations were measured by using 7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate (SBD-F) as the derivative and HPLC with fluorescence detection according to the method of Vester and Rasmussen (15). Homocysteic acid was used as the internal standard. This procedure was used for all serum samples, including those of a group of healthy persons who were used as a reference population.

For serum Hcy measurement, blood was collected in sterile, evacuated serum-separator tubes containing a gel and clot activator. The samples were kept at room temperature until centrifugation for 20 min at $2000 \times g$ and room temperature. The interval between the phlebotomy and the centrifugation ranged from 1 to 3 h, after which the sample was maintained at -20°C until use. The serum sample used in the Hcy measurement was also used for the vitamin B-12 measurement.

Several studies showed a difference in Hcy concentrations between samples maintained at room temperature and those maintained at 4°C (15, 16). Thus, with the use of the same HPLC procedure, the reference range for serum Hcy was calculated in 71 healthy persons (hemoglobin concentration > 120 g/L, mean corpuscular volume (MCV) < 100 fL, serum vitamin B-12 concentration > 200 pmol/L, red blood cell folate concentration > 600 nmol/L, and serum creatinine concentration < 100 $\mu\text{mol/L}$; age range: 20–84 y). The mean (\pm SD) reference value for serum Hcy was 7.5 ± 4.5 $\mu\text{mol/L}$. Because the Hcy distribution had a long tail on the right side, percentiles were used for calculating the reference range (percentiles 2.5–97.5: 2–17.5 $\mu\text{mol/L}$), and values for serum Hcy > 17.5 $\mu\text{mol/L}$ were considered to be elevated (hyperhomocysteinemia). This reference population was not related to the reference population used for red blood cell folate.

Statistical procedures

Calculations were made by using SPSS 10.0 (SPSS Inc, Chicago). Quantitative variables were expressed as the mean \pm SD, and qualitative variables were expressed as a percentage of



TABLE 1
Vitamin B-12 and folate metabolism in 2 groups of HIV-infected patients¹

	HAART group ² (n = 80 M, 46 F)	Non-HAART group ³ (n = 83 M, 26 F)
Age (y)	38 ± 9	30 ± 9 ⁴
Hemoglobin (g/L)		
Male	143 ± 16	117 ± 24 ⁴
Female	126 ± 19	105 ± 25
MCV (fL)	107 ± 11	92 ± 11 ⁴
Leukocytes (×10 ⁹ /L)	5683 ± 2085	4912 ± 2674 ⁴
CD4 (×10 ⁶ /L)	441 ± 250	212 ± 369 ⁴
CD8 (×10 ⁶ /L)	1005 ± 585	446 ± 460 ⁴
Vitamin B-12 (pmol/L)	402 ± 218	330 ± 219 ⁵
RBCF (nmol/L)	1473 ± 1087	1057 ± 665 ⁴

¹ $\bar{x} \pm$ SD. HAART, highly active antiretroviral therapy; MCV, mean corpuscular volume; RBCF, red blood cell folate.

²Patients receiving HAART (≥ 3 new antiretroviral drugs).

³HIV-infected patients studied previously (from 1989 to 1992) who received either treatment with one antiretroviral drug or no antiretroviral treatment (12,13).

^{4,5}Significantly different from the HAART group: ⁴ $P < 0.0001$, ⁵ $P = 0.013$.

positive cases. For comparisons involving quantitative variables, the paired or unpaired Student's *t* test was used; CIs were calculated when the differences were significant. For comparisons involving qualitative variables, the chi-square test was used. A simple or multiple regression analysis was performed to study relations between variables.

RESULTS

Evaluation of serum vitamin B-12 and red blood cell folate concentrations in HIV-infected patients receiving HAART

Among the 126 patients in the HAART group, a serum vitamin B-12 concentration ≤ 150 and 200 pmol/L was detected in 2 (1.6%) and 11 (8.7%) patients, respectively. In contrast, among the 109 patients in the non-HAART group who were receiving treatment with only one or no antiretroviral drugs, 20 (18%) and 30 (27%) patients had a serum vitamin B-12 concentration ≤ 150

and 200 pmol/L, respectively. The 2 groups differed significantly in these percentages (chi-square test, $P < 0.0001$ for both concentrations of serum vitamin B-12).

A red blood cell folate concentration ≤ 450 or 580 nmol/L was observed in 1 (0.8%) and 13 (10.3%) patients in the HAART group, respectively, and in 19 (17.4%) and 24 (22%) patients in the non-HAART group, respectively. There was a significant difference between the 2 groups in the percentage of subjects who had a red blood cell folate concentration ≤ 450 nmol/L (chi-square test, $P < 0.0001$).

The patients in the non-HAART group had significantly lower values for hemoglobin, MCV, leukocytes, CD4, and CD8 (Table 1) than did the patients in the HAART group. As shown previously in the non-HAART group (12, 13), there was a relation in the HAART group between serum vitamin B-12 concentrations and granulocytes ($r = 0.33$, $P < 0.0001$); this relation was independent of other variables (hemoglobin, MCV, age, sex, red blood cell folate, and serum homocysteine).

Role of serum Hcy in the diagnosis of HIV-infected patients with low serum vitamin B-12 or red blood cell folate concentrations

Evaluation of patients with a low serum vitamin B-12 concentration

Only 9 of 40 patients (22.5%) with a low vitamin B-12 concentration (ranging from 60 to 198 pmol/L) had hyperhomocysteinemia (Table 2). The red blood cell folate concentration was also low in 6 patients with a low serum vitamin B-12 concentration. Five of 6 patients with both a low red blood cell folate concentration (≤ 580 nmol/L) and a low serum vitamin B-12 concentration had hyperhomocysteinemia (mean serum Hcy: 32.8 ± 16.8 $\mu\text{mol/L}$; range: 12.5–54 $\mu\text{mol/L}$). Hcy concentrations were significantly higher (Student's *t* test, $P = 0.017$) in these 6 patients than in the 34 HIV-infected patients with a low serum vitamin B-12 concentration and normal red blood cell folate concentration (mean serum Hcy: 8.7 ± 6.2 $\mu\text{mol/L}$; range: 1–24 $\mu\text{mol/L}$). Only 4 of 34 patients (11.8%) with a low vitamin B-12 concentration and a normal red blood cell folate concentration had hyperhomocysteinemia.

TABLE 2
Characteristics of HIV-infected patients with low serum vitamin B-12 or red blood cell folate (RBCF) concentrations¹

	Vitamin B-12 ≤ 200 pmol/L (n = 26 M, 14 F)	RBCF ≤ 580 pmol/L (n = 26 M, 11 F)	Control subjects ² (n = 76 M, 52 F)
Age (y)	41 ± 13 (24–78)	37 ± 9 (21–68)	38 ± 10 (25–76)
Hemoglobin (g/L)	129 ± 20 (90–170)	140 ± 16 (105–170)	128 ± 24 (43–169)
MCV (fL)	99 ± 13 (78–128)	103 ± 13 (79–135)	109 ± 14 (73–127)
Creatinine ($\mu\text{mol/L}$)	93 ± 19 (66–179)	97 ± 25 (66–179)	89 ± 16 (54–218)
Vitamin B-12 (pmol/L)	154 ± 37 (60–198)	413 ± 534 (100–3450)	520 ± 431 (201–3190)
RBCF (nmol/L)	1183 ± 1007 (324–5465)	505 ± 68 (313–580)	1529 ± 1130 (584–6760)
Serum homocysteine ($\mu\text{mol/L}$)	12.3 ± 12 (1–54)	22.3 ± 19 (4–81)	8.2 ± 7.8 (1–70)
Patients with hyperhomocysteinemia ³ (%)	22.5 ⁴	51.4 ⁵	6.25

¹ $\bar{x} \pm$ SD; range in parentheses. Six patients had low concentrations of both serum vitamin B-12 and red blood cell folate. MCV, mean corpuscular volume.

²HIV-infected patients who did or did not receive highly active antiretroviral therapy and who had a serum vitamin B-12 concentration > 200 pmol/L and a red blood cell folate concentration > 580 nmol/L.

³Serum homocysteine concentration > 17.5 $\mu\text{mol/L}$.

⁴Among patients with a serum vitamin B-12 concentration ≤ 150 pmol/L, 26.7% had hyperhomocysteinemia.

⁵Among patients with an RBCF concentration ≤ 450 nmol/L, 100% had hyperhomocysteinemia.

TABLE 3Effect of vitamin B-12 and folate on serum homocysteine (Hcy) concentrations in HIV-infected patients¹

	Serum Hcy	
	Before treatment	After treatment
	$\mu\text{mol/L}$	
Low vitamin B-12 or RBCF and high Hcy ($n = 14$)	24.7 \pm 10	6 \pm 2.5 ²
Low vitamin B-12 or RBCF and normal Hcy ($n = 10$)	11 \pm 3.6	6 \pm 2.8 ³
Normal vitamin B-12 and RBCF and high Hcy ($n = 4$)	34 \pm 24	17.2 \pm 17.4 ⁴

¹ $\bar{x} \pm \text{SD}$. RBCF, red blood cell folate.²⁻⁴Significantly different from before treatment: ² $P = 0.000022$, ³ $P = 0.001$, ⁴ $P = 0.022$.

Serum Hcy concentrations in the group with a low vitamin B-12 concentration without a low red blood cell folate concentration did not differ significantly from those of the control group (Student's t test, $P = 0.74$). Four of 15 patients (26.7%) with a serum vitamin B-12 concentration ≤ 150 pmol/L had a high Hcy concentration. However, 3 of 4 patients with an elevated serum Hcy concentration had a low red blood cell folate concentration. HIV-infected patients with a low vitamin B-12 concentration and hyperhomocysteinemia had neutrophil counts similar to those of patients with a low vitamin B-12 concentration and a normal Hcy concentration (Student's t test, $P = 0.152$).

Evaluation of patients with a low red blood cell folate concentration

Nineteen (51.4%) of 37 patients with a low red blood cell folate concentration (ranging from 313 to 580 nmol/L; the latter concentration corresponded to percentile 10 of the reference population) had hyperhomocysteinemia. Among the 9 patients who had a red blood cell folate concentration ≤ 450 nmol/L (percentile 2.5 of the reference population), all of them (100%) had an elevated serum Hcy concentration.

In contrast with the patients with a low vitamin B-12 concentration, the 31 patients with a low red blood cell folate concentration without a low vitamin B-12 concentration had significantly higher (Student's t test, $P = 0.002$) serum Hcy concentrations than did the patients in the control group (20 \pm 19 compared with 8.3 \pm 7.8 $\mu\text{mol/L}$). A negative relation was found between serum Hcy concentrations and red blood cell folate concentrations. Multiple regression analysis that included the variables age, sex, hemoglobin, MCV, red blood cell folate, vitamin B-12, and creatinine showed that red blood cell folate concentrations ($r = -0.42$) and creatinine concentrations ($r = 0.3$) were independent variables related to serum Hcy concentrations.

Effect of treatment with vitamin B-12 and folic acid in a group of HIV-infected patients with low vitamin B-12 or red blood cell folate concentrations

Among the 24 patients who had a low serum vitamin B-12 concentration and a low red blood cell folate concentration, serum Hcy concentrations in the 14 patients who had a high Hcy concentration decreased to normal values after treatment with vitamin B-12 and folic acid (Table 3). The treatment decreased Hcy concentrations $> 50\%$ in 4 of 10 patients whose starting serum Hcy concentration was normal.

A decrease in Hcy concentrations was also observed in the 4 patients who had a high serum Hcy concentration and normal serum vitamin B-12 and red blood cell folate concentrations. The Hcy concentrations were normalized in 3 of the 4 patients. As expected, significant increases in serum vitamin B-12 (before and after treatment: 334 \pm 183 and 437 \pm 146 pmol/L, respectively; $P = 0.0002$) and red blood cell folate (before and after treatment: 880 \pm 778 and 1881 \pm 1115 nmol/L, respectively; $P < 0.0001$) occurred after treatment.

DISCUSSION

The present study, which is the continuation of our earlier work, addressed several issues concerning folate and vitamin B-12 metabolism in HIV-infected patients. In the previous studies, we showed that 20% of HIV-infected patients (before HAART) had a low vitamin B-12 concentration (12). Nevertheless, few of them had a deficiency as determined by the dUST (4). Abnormalities in vitamin B-12 binding proteins may have been the cause of these low vitamin B-12 concentrations (13).

Given the elevated Hcy concentrations in vitamin B-12 or folate deficiency (5–7), we sought to investigate this metabolite rather than to perform the time-consuming dUST in HIV-infected patients. Although some authors previously evaluated plasma Hcy concentrations in HIV-infected patients, few subjects with a low serum vitamin B-12 concentration were included and seldom did the subjects also have a low folate concentration (17, 18). In the present study, we measured serum Hcy concentrations in a large group of HIV-infected patients with a low vitamin B-12 or red blood cell folate concentration.

Not surprisingly, $< 30\%$ of the HIV-infected patients with a low serum vitamin B-12 concentration had hyperhomocysteinemia. Moreover, most of the patients with hyperhomocysteinemia and a low vitamin B-12 concentration had a low red blood cell folate concentration. In contrast, 100% and 51.5% of patients with a red blood cell folate concentration below percentiles 2.5 and 10, respectively, had hyperhomocysteinemia.

These results are similar to those obtained by using the dUST (4). Few HIV-infected patients with a low serum vitamin B-12 concentration alone have a real vitamin B-12 deficiency (1, 4). By contrast, serum Hcy concentrations confirmed the presence of a real deficiency in most of the HIV-infected patients who had a low red blood cell folate concentration and a normal vitamin B-12 concentration, as the dUST had shown in our earlier study. Moreover, the dUST showed that the deficiency was due to folate (4).

In patients with low concentrations of both red blood cell folate and vitamin B-12, Hcy concentrations cannot determine whether a patient has vitamin B-12 deficiency, folate deficiency, or both. The dUST or the serum methylmalonic acid concentration would probably be useful in these few patients (19). If these tests are not available, treatment with both vitamins is recommended. On the basis of our findings and on those of earlier studies (4), the measurement of red blood cell folate concentrations in HIV-infected patients who have a low serum vitamin B-12 concentration is probably as satisfactory as the measurement of serum Hcy concentrations, because among HIV-infected patients with a low vitamin B-12 concentration, those with a low red blood cell folate concentration are more likely to have a real deficiency.


In HIV-infected patients, as in patients with other pathologies (9, 20), a low vitamin B-12 concentration may be related to changes in the vitamin B-12 binding proteins and not to a real

deficiency. This decrease in serum vitamin B-12 concentration is correlated with neutrophil counts, the main source of cobalophilin, which is the major vitamin B-12 binding protein in plasma (13).

Furthermore, a low vitamin B-12 concentration is an adverse prognostic factor in HIV-infected patients (21). Low vitamin B-12 concentrations may be attributed to low white blood cell counts, a factor associated with more advanced HIV disease (12, 22).

The present study showed that patients receiving HAART have a lower prevalence of reduced vitamin B-12 (8.7% compared with 27%) and higher concentrations of hemoglobin, leukocytes, CD4, and CD8. Moreover, as in the non-HAART group, vitamin B-12 concentrations in the HAART group were positively related to neutrophil counts, and this relation was independent of Hcy concentrations. The new and old data correlate well with the hypothesis that vitamin B-12 is a prognostic factor (12, 21, 22) and that leukocytes (12, 22), as the main source of cobalophilin, are related to the vitamin B-12 concentrations found in these patients.

Finally, the response to vitamin B-12 and folate treatment was assessed in a group of HIV-infected patients. As expected, all patients with a low red blood cell folate or vitamin B-12 concentration and hyperhomocysteinemia responded to treatment. However, because the decrease in Hcy concentration occurred in response to treatment with both vitamins, it was not possible to ascertain whether the response was specifically due to one vitamin or to the other or to both. In patients with hyperhomocysteinemia and normal concentrations of these vitamins, a decrease in or a normalization of Hcy concentration was observed. A similar response was observed in patients with hyperhomocysteinemia and vitamin B-12 and folate concentrations within their respective reference ranges when vitamin treatment was administered (23–25).

The potential implications of hyperhomocysteinemia for HIV-related cardiovascular disorders warrant further investigation. In this regard, hyperhomocysteinemia may contribute to the cardiovascular morbidity (8) of HIV-infected patients receiving HAART. In summary, our data lend support to the assessment of Hcy and red blood cell folate concentrations in HIV-infected patients with a low vitamin B-12 concentration in order to detect patients with a real vitamin B-12 deficiency. 

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REFERENCES

1. Remacha AF, Cadafalch J. Cobalamin deficiency in patients infected with the human immunodeficiency virus. *Semin Hematol* 1999;36:75–87.
2. Snow CF. Laboratory diagnosis of vitamin B12 and folate deficiency. *Arch Intern Med* 1999;159:1289–98.
3. Wickramasinghe SN, Saunders JE. Results of three years' experience with the deoxyuridine suppression test. *Acta Haematol* 1977;58:193–206.
4. García-Die F, Remacha A, Cadafalch J, Barceló MJ, Parellada MM, Arzá B. The deoxyuridine suppression test: the role of azydothymidine (AZT). *Eur J Haematol* 1996;56:208–12.
5. Savage DG, Lindenbaum J, Stabler SP, Allen RH. Sensitivity of serum methylmalonic acid and total homocysteine determinations for diagnosing cobalamin and folate deficiencies. *Am J Med* 1994;96:239–46.
6. Green R. Metabolite assays in cobalamin and folate deficiency. *Bailliere's Clin Haematol* 1995;8:533–66.
7. Moller J, Rasmussen K, Christensen L. External quality assessment of methylmalonic acid and total homocysteine. *Clin Chem* 1999;45:1536–42.
8. Christen WG, Ajani UA, Glynn RJ, Henekens CH. Blood levels of homocysteine and increased risks of cardiovascular disease. *Arch Intern Med* 2000;160:422–34.
9. Tripodi A, Chantarangkul V, Lombardi R, Lecchi A, Mannucci PM, Cattaneo M. Multicenter study of homocysteine measurement—performance characteristics of different methods, influence of standards on interlaboratory agreement of results. *Thromb Haemost* 2001;85:291–5.
10. Baldewicz TT, Goodkin K, Blaney NT, et al. Cobalamin level is related to self-reported and clinically rated mood and to syndromal depression in bereaved HIV-1(+) and HIV-1(–) homosexual men. *J Psychosom Res* 2000;48:177–85.
11. Geene D, Sudre P, Anwar D, Goehring C, Saaidia A, Hirschel B. Causes of macrocytosis in HIV-infected patients not treated with zidovudine. *Swiss HIV Cohort Study. J Infect* 2000;40:160–3.
12. Remacha AF, Riera A, Cadafalch J, Gimferrer E. Vitamin B12 abnormalities in HIV-infected patients. *Eur J Haematol* 1991;47:60–4.
13. Remacha AF, Montagud M, Cadafalch J, Riera A, Martino R, Gimferrer E. Vitamin B12 transport proteins in patients with HIV-1 infection and AIDS. *Haematologica* 1993;78:84–8.
14. Ueland PM, Refsum H, Stabler SP, et al. Total homocysteine in plasma or serum: methods and clinical applications. *Clin Chem* 1993;39:1764–79.
15. Vester B, Rasmussen K. High performance liquid chromatography method for rapid and accurate determination of homocysteine in plasma and serum. *Eur J Clin Chem Clin Biochem* 1991;29:549–54.
16. Fermo I, De Vecchi E, Arcelloni C, D'Angelo A, Paroni R. Methodological aspects of total plasma homocysteine. *Haematologica* 1997;82:246–50.
17. Müller F, Svardal AM, Aukrust P, Berge RK, Ueland PM, Froland SS. Elevated plasma concentration of reduced homocysteine in patients with immunodeficiency virus infection. *Am J Clin Nutr* 1996;63:242–6.
18. Paltiel O, Falutz J, Veilleux M, Rosenblatt DS, Gordon K. Clinical correlates of subnormal vitamin B12 levels in patients infected with the human immunodeficiency virus. *Am J Hematol* 1995;49:318–22.
19. Carmel R, Rasmussen K, Jacobsen DW, Green R. Comparison of the deoxyuridine suppression test with serum levels of methylmalonic acid and homocysteine in mild cobalamin deficiency. *Br J Haematol* 1996;93:311–8.
20. Hansen M, Gimsing P, Ingeberg S, Jans M, Nexø E. Cobalamin binding proteins in patients with HIV infection. *Eur J Haematol* 1992;48:228–31.
21. Tang AM, Graham NMH, Chandra RK, Saah AJ. Low serum vitamin B12 concentrations are associated with faster human immunodeficiency virus type 1 (HIV-1) disease progression. *J Nutr* 1997;127:345–51.
22. Baum MK, Shor-Posner G, Lu Y, et al. Micronutrients and HIV-1 disease progression. *AIDS* 1995;9:1051–6.
23. Ubbink JB, Vermaak WJH, Van der Merwe A, Becker PJ. Vitamin B-12, vitamin B-6, and folate nutritional status in men with hyperhomocysteinemia. *Am J Clin Nutr* 1993;57:47–53.
24. Woodside JV, Yarnell JW, McMaster D, et al. Effect of B-group vitamins and antioxidant vitamins on hyperhomocysteinemia: a double-blind, randomized, factorial-design, controlled trial. *Am J Clin Nutr* 1998;67:858–66.
25. Wald DS, Bishop L, Wald NJ, et al. Randomized trial of folic acid supplementation and serum homocysteine levels. *Arch Intern Med* 2001;161:695–700.