Established in 1871
Swiss Medical Weekly
Formerly: Schweizerische Medizinische Wochenschrift
The European Journal of Medical Sciences

Original article | Published 11 January 2012, doi:10.4414/smw.2012.13323
Cite this as: Swiss Med Wkly. 2012;141:w13323

Hepatitis C virus co-infection and sexual risk behaviour are associated with a high homocysteine serum level in HIV-infected patients

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Summary

BACKGROUND AND AIMS: A better understanding of the relationship of homocysteine with cardiovascular risk factors is needed. The objectives of this study were to assess the serum level of homocysteine in HIV-infected patients and to analyse the possible association of increased levels of the amino acid with cardiovascular risk factors, demographic and clinical characteristics of participants.

METHODS: Cross-sectional study carried out as a supplementary task to the usual controls necessary in HIV-infected patients in the outpatient clinic of the Hospital General of Castellon, Spain. For two consecutive visits the demographic, clinical and HIV-related characteristics and blood analyses results were obtained for each participant. Homocysteine serum level was documented and the possible association of the amino acid with all the other study variables was assessed with a multiple linear regression analysis.

RESULTS: A total of 145 patients were included. The mean homocysteine serum level of all participants was 11.9 ± 5.9 µmol/L. A total of 54 patients (37%) presented homocysteine serum levels higher than the upper limit of normal. An association was found between higher homocysteine serum level and the following variables: family history of early coronary disease (P = 0.027), sexual HIV risk behaviour (P = 0.016), hepatitis C virus co-infection (P = 0.002), higher height (P = 0.002), higher diastolic blood pressure (P = 0.049), lower serum level of folic acid (P <0.001), and lower serum level of vitamin B12 (P = <0.001).

CONCLUSION: In the HIV population, increased homocysteine serum level is associated with sexual risk behaviour and hepatitis C virus coinfection.

Key words: *hepatitis C virus; homocysteine; HIV-infected patients*

Introduction

With the advent of antiretroviral therapy there has been a spectacular decrease in morbidity and mortality in HIV-infected individuals [1]. However, parallel to this an increased risk of cardiovascular disease has become evident in these subjects. Many HIV-infected patients have dyslipidaemia, hyperglycaemia and central obesity that may be a side effect of the antiretroviral medication, an effect of ongoing replication of the virus, or the influence of both [2]. In addition, a high prevalence of smoking and other cardiovascular risk factors has been found in these patients [3, 4]. As a result, cardiovascular disease is being increasingly reported in HIV-infected subjects, and the magnitude of the problem is expected to increase dramatically in the years to come [5]. For this reason, the HIV-infected population is particularly suitable to carry out studies on the influence of risk factors in the development of cardiovascular disease.

Homocysteine is an amino acid produced by the catabolism of methionine. Serum levels of homocysteine are determined by genetic and nutritional factors. They are increased by dietary deficiencies of folic acid, vitamin B6, vitamin B12 and other vitamins and by excessive methionine intake [6]. Hyperhomocysteinaemia has been considered a potential risk factor for cardiovascular disease for many years [7, 8], and epidemiological studies have suggested a beneficial role for homocysteine lowering strategies [9]. However, recent large randomised clinical trials have failed to demonstrate a clear effect of such strategies on clinical outcome in patients with atherosclerosis [10–13]. As a consequence the role of the substance as a cardiovascular risk factor has been seriously questioned [14]. Therefore, a better understanding of the true relationship of homocysteine with cardiovascular risk factors is needed.

In this study, carried out in HIV-infected subjects, the objectives were to assess serum levels of homocysteine, and to analyse the possible association of increased levels of the amino acid with cardiovascular risk factors and the demographic and clinical characteristics of patients.

Method

Study design and patients

We conducted this cross-sectional study from September 2007 until July 2008 in the HIV outpatient clinic of the Hospital General of Castellon, University of Valencia in Spain. The institution provides free of charge comprehensive medical care to HIV-infected people. We enrolled pa-

tients older than 18 years of age. We excluded pregnant women, patients with creatinine serum levels >2 mg/dl, and subjects who were taking vitamin supplements since all of these circumstances have been demonstrated to influence homocysteine serum levels [6, 14, 15]. Informed consent was obtained from every participant.

Procedure and variables included

This study was designed as a supplementary task to the usual controls required in HIV-infected patients. According to guidelines [16], these subjects must be monitored every three to six months in terms of clinical, immunological and virological status, and receive antiretroviral treatment and prophylaxis against *Pneumocystis jiroveci* pneumonia with trimethoprim/sulfamethoxazole when indicated. The study was completed at two consecutive visits in every patient. All patients were seen at both visits by one of the authors.

In the first visit demographic, clinical and HIV-related characteristics were obtained from each participant (table 1). At the second visit the results of blood analyses taken after overnight fasting two to four weeks before this visit were reviewed (table 2). Blood samples were collected from patients into gel-separator tubes and centrifuged 5 to 15 minutes later. Serum level of homocysteine was measured with a chemiluminescence immunoassay (Immulite 2500, normal value: 5 to 12 µmol/L, coefficient of variation of 8%). All the laboratory measurements were conducted by investigators who were blinded to the characteristics of the patients.

Statistics

Statistical analysis was performed using Minitab 15 (Minitab, Inc.). Continuous variables were summarised as mean and standard deviation and discrete variables were summarised as percentages. Data were normalised by log transformation for the following variables not showing a normal distribution: homocysteine, glucose, triglyceride, glycosylated haemoglobin and C-reactive protein serum levels.

To estimate the relationship between homocysteine serum levels of all patients and all the other study variables, we carried out a multiple linear regression analysis, using a best subsets procedure to find the best model. Previously we eliminated variables that showed no relationship at all with homocysteine and variables, other than homocysteine, that were highly correlated with other included variables to avoid multi-colinearity. For this purpose we used scatter plots and the Pearson correlation test or the Student t test, as appropriate. For bivariate analyses we used the following tests: chi square test (χ^2) for discrete variables, independent samples Student's t-tests for normally distributed continuous variables and Mann-Whitney U test, Wilcoxon signed ranks test or Kruskal-Wallis H test for non-normally distributed continuous variables.

A significance level of P = 0.05 was used in all analyses of the study.

Table 1: Demographical and clinical characteristics of the study patients.

Age, years, mean (± SD)	41.1 (± 8.1)
Gender, patients (%) – male – female	 103 (71) 42 (29)
Antiretroviral treatment, patients (%) – protease inhibitor based – non-protease inhibitor based therapy – none	 52 (36) 65 (45) 28 (19)
History, patients (%) – diabetes mellitus – hypertension – dyslipidaemia – cardiovascular disease	 24 (17) 17 (12) 23 (16) 3 (2)
Family history of early coronary disease *, patients (%)	26 (18)
Current smoker, patients (%)	107 (74)
Alcohol abuse **, patients (%)	32 (22)
HIV risk behaviour, patients (%) – parenteral – sexual	 82 (57) 63 (43)
Hepatitis C virus co-infection ***, patients (%)	89 (61)
Height (cm), mean (± SD)	169.8 (± 8.8)
Weight (kg), mean (± SD)	68.7 (± 13.5)
Body mass index (kg/m^2), mean (± SD)	23.8 (± 4.3)
Waist circumference (cm), mean (± SD)	87.3 (± 11.5)
Hip circumference (cm), mean (± SD)	95.4 (± 9.6)
Waist to hip ratio, mean (± SD)	0.92 (± 0.08)
Blood pressure (mm Hg), mean (± SD) systolic diastolic	 127.0 (± 19.2) 78.0 (± 13.2)
"HeartScore" **** ≥1%, patients (%)	56 (39)

SD indicates standard deviation; * coronary heart disease in first-degree relatives, male <55 years and female <65 years; ** ten or more alcoholic beverages per week; *** hepatitis C virus infection diagnosed on serology; **** 10-year risk of suffering a cardiovascular disease calculated at www.heartscore.org/es/Pages/welcome.aspx according to the 2007 European Guidelines on Cardiovascular Disease Prevention for Spanish patients [N].

Results

General data

A total of 145 patients were included in the study. 139 (96%) were natives of Spain, three (2%) were from other countries of Europe, two (1%) from Nigeria, and one (1%) from Brazil. All participants were white, except for the two Nigerians, who were black. No patient had missing data. Tables 1 and 2 summarise the study results. The overall mean homocysteine serum level was 11.9 µmol/L (median: 10.6 µmol/L, interquartile range: 8.4–13.5 µmol/L, and minimum to maximum range: 4.1–39.9 µmol/L). A total of 54 patients (37%) presented with homocysteine serum levels higher than the upper limit of normal. The mean homocysteine serum level in patients with parenteral HIV risk behaviour was 11.5 µmol/L, and in those with sexual HIV risk behaviour it was 12.3 µmol/L (P = 0.037). The mean homocysteine serum level in patients without hepatitis C virus co-infection was 11.5 µmol/L, and in those with hepatitis C virus co-infection it was 12.1 µmol/L (P = 0.042). A total of 31 patients (21%) were taking trimethoprim/sulfamethoxazole. The mean homocysteine serum level was 12.1 µmol/L in those patients, as compared to 11.8 in those who were not taking the medication (P = 0.793). We excluded two patients from the study because they were taking vitamin supplements.

Overall 117 patients (81%) were receiving antiretroviral medication. CD4 cell count was higher than 200 cells per mm^3 in 114 participants (79%), and HIV RNA was lower than 1000 copies/ml (3.0 log_{10}) in 115 participants (79%). A total of three of our patients (2%) had suffered a major cardiovascular event previously, two aged 70 and 71 respectively had a myocardial infarction. They were the two oldest patients in the study. The other patient with an antecedent of cardiovascular disease presented with an extensive ischaemic stroke at the age of 37. All 23 patients with a dyslipidaemia were on lipid-reducing medication. All 24 patients with diabetes mellitus were on treatment with insulin and/or oral antidiabetic drugs. And 15 of the 17 patients with hypertension were on blood pressure lowering medication. The remaining two patients with hypertension were on treatment with low salt diet.

Of the 89 patients with hepatitis C virus co-infection, 81 (91%) had parenteral HIV risk behaviour pattern and 8 (9%) a sexual risk behaviour pattern.

Multiple regression

A standard multiple linear regression was performed between homocysteine serum level as the dependent variable and a history of cardiovascular disease, family history of early coronary disease, HIV risk behaviour, hepatitis C virus co-infection, height, systolic blood pressure, diastolic blood pressure, total cholesterol, measured LDL-cholesterol, folic acid, and vitamin B_{12} serum levels as independent variables.

The other study variables were excluded from the model for the following reasons: A) age, gender, anti-retroviral treatment, history of diabetes mellitus, history of hypertension, history of dyslipidaemia, alcohol abuse, weight, body mass index, waist circumference, "HeartScore", CD4 lymphocyte count, glucose, HDL-cholesterol, triglyceride, glycosylated haemoglobin, and C-reactive protein because of lack of relationship with homocysteine serum level; B) hip circumference and waist to hip ratio, because they were highly correlated with other included variables; and C) current smoker, HIV RNA detectable and fibrinogen after application of the best subsets procedure.

The regression model was significantly different from zero: F = 5.98, P <0.001. Adjusted R^2 was = 27.6% and Mallows C-p was = 12.2. Table 3 displays detailed information regarding regression coefficients of independent variables.

The analysis of standardised residuals showed that they had a normal distribution with mean zero, they had approximately the same variance for each predicted value of homocysteine serum levels, and they were independent from each other.

A total of seven independent variables contributed significantly to prediction of homocysteine serum level as logarithmically transformed: family history of early coronary disease, sexual (versus parenteral) HIV risk behaviour, hepatitis C virus co-infection, height, folic acid and vitamin B_{12}.

Discussion

Serum level of homocysteine was higher than normal in 37% of our patients. Other studies carried out in HIV infected patients have found lower rates of this abnormality [17–20] and studies performed in other populations have shown great variability in the prevalence of the condition [14, 21–24].

Table 2: Blood analysis results of the study patients.

HIV RNA detectable *, patients (%)	63 (43)
CD4 lymphocyte count, per mm3, mean (± SD)	510 (± 380)
Glucose, mg/dL, mean (± SD)	105 (± 31)
Total cholesterol, mg/dL, mean (± SD)	174 (± 47)
HDL-cholesterol, mg/dL, mean (± SD)	41.2 (± 13.1)
Measured LDL-cholesterol, mg/dL, mean (± SD)	110 (± 36)
Triglyceride, mg/dL, mean (± SD)	154 (± 113)
Glycosylated haemoglobin, %, mean (± SD)	5.67 (± 0.98)
Fibrinogen, mg/dL, mean (± SD)	369 (± 113)
C-reactive protein, mg/dL, median (interquartile range)	3.33 (1.94 – 6.42)
Folic acid, µg/L, mean (± SD)	5.91 (± 5.37)
Vitamin B_{12}, ng/L, mean (± SD)	375 (± 200)
Homocysteine, µmol/L, mean (± SD)	11.9 (± 5.9)
* Limit of detection: 20 copies/ml	

The patients in our study were predominantly males in their fourth or fifth decade of life who had been parenterally infected with HIV. A majority of them were co-infected with hepatitis C virus. Similar epidemiological characteristics of HIV infected people have previously been found in our community and many other regions of Spain [25]. Most of our patients were on anti-retroviral medication, and HIV infection was acceptably controlled in a large number of them.

In our patients there was an increased prevalence of some cardiovascular risk factors. The high prevalence of the habit of smoking, present in 74% of participants, was especially relevant. This percentage was probably so high because most were intravenous drug users. Nonetheless, the figure is considerably greater than that observed in most other populations [26], and denotes the urgent necessity of specific programmes directed at diminishing the addiction in our community. Despite this, the calculated 10-year risk of suffering a cardiovascular disease, as estimated by the "HeartScore" equation, was, in general, low among our patients. This was presumably due to the acceptable serum levels of total cholesterol and the relatively young age of the majority of participants [27].

The association of increased serum homocysteine levels with increased serum folic acid and vitamin B12 levels is already well known [6, 7]. Therefore, the most relevant finding in our study was the association of high homocysteine serum levels with hepatitis C virus infection and with sexual (versus parenteral) HIV risk behaviour. Nevertheless, it must be noted that the differences in homocysteine serum levels were small between hepatitis C virus infected and non-infected patients, and between patients with sexual or parenteral HIV risk behaviour. Previous studies on HIV-infected patients did not find such associations, although only univariate analyses were carried out when the possible relationship of homocysteine serum level with those variables was evaluated [17, 19]. Our results emphasise the importance of performing multivariate analyses to correctly assess possible associations of this kind as they are much more reliable than univariate analyses.

In a review of the literature, we have found only two studies assessing the possible relationship between increased homocysteine serum level and hepatitis C virus infection in the general population. In one study, an association was found between the two variables in a univariate analysis, but not in a multivariate one [28]. In the second study, which included only a small group of subjects, no relationship was found in a univariate analysis [29].

We did not find a plausible explanation for the difference in homocysteine serum level between patients with sexual or parenteral HIV risk behaviour. Hopefully other studies in the future will help in understanding this result.

Interestingly high homocysteine serum level has been found to be associated with increased risk of liver fibrosis progression [30–32] and with a lower rate of sustained virological response to pegylated-interferon alpha2b plus ribavirin therapy, in patients with chronic hepatitis C [33].

Our study also showed an association of high homocysteine serum levels with the presence of a family history of early coronary disease, high diastolic blood pressure and low serum levels of folic acid and Vitamin B_{12}. All these associations had been well documented previously in HIV-infected patients [20, 34, 35] and in the general population [6]. Our results, however, emphasise once more the well established association of increased homocysteine level with cardiovascular risk factors.

Height was also found to be associated with high homocysteine serum level among our patients. However, the regression coefficient was very small, which probably implies a mild influence of the variable on the amino acid level. It is also possible that this is merely a chance result.

In contrast with other studies carried out in HIV-infected patients [19, 34, 36], we did not find an association between advanced age and higher homocysteine serum levels. Other cardiovascular risk factors, such as waist circumference or dyslipidaemia, previously found to be related to homocysteine serum level, did not show a significant association with homocysteine serum levels in our study. This was probably due to the relatively small number of patients in our study. Although it is also important to take into account that many of the associations found by other authors are based on univariate analysis. For this reason they must be interpreted with care [17].

We found no association between antiretroviral treatment with high homocysteine serum levels. Some other authors have found such an association, although again with univariate analysis [18], whilst others have not [17, 34, 36]. Moreover we found no association of advanced HIV infection with homocysteine serum level. This also implied

Table 3: Information regarding the coefficients of the independent variables of the multiple regression model*.

Independent variable	Coefficient	SE	T	P
Constant	0.0471	0.2662	0.18	0.860
History of cardiovascular disease	0.1712	0.0881	1.94	0.054
Family history of early coronary disease	0.0751	0.0337	2.23	0.027
Sexual HIV risk behaviour	0.1004	0.0410	2.45	0.016
Hepatitis C virus co-infection	0.1272	0.0410	3.10	0.002
Height, cm	0.0045	0.0014	3.10	0.002
Blood pressure systolic, mm Hg	–0.0013	0.0009	–1.45	0.149
Blood pressure diastolic, mm Hg	0.0026	0.0013	1.99	0.049
Total cholesterol, mg/dL	0.0014	0.0008	1.67	0.097
Measured LDL-cholesterol, mg/dL	–0.0014	0.0010	–1.35	0.180
Folic acid, µg/L	–0.0094	0.0027	–3.79	<0.001
Vitamin B_{12}, ng/L	–0.0002	0.0001	–2.98	0.003

* Dependent variable: homocysteine serum level, µmol/L; SE denotes standard error; T denotes t test; P denotes significance level.

an unlikely association between trimethoprim/sulfamethoxazole and homocysteine serum level among our patients. Other authors have found a similar result [18, 37].

Despite including several well known determinants of homocysteine serum level, our multivariate model only explained 27.6% of the variability of the amino acid. Other similarly designed studies have shown comparable findings [17]. This means that knowledge of the determinants of serum levels of homocysteine is still very limited and more studies are needed to clarify the real relationship of the amino acid to cardiovascular risk factors.

The main limitations of the present study are its cross-sectional design and the relatively small population analysed. Failure to include other variables that may influence homocysteine serum level [14, 35] is another potential problem. Despite those reservations, our study provides some new information regarding the association of homocysteine with cardiovascular risk factors and other conditions in the HIV-infected population. Our results cannot be generalised to the rest of the population, as clinical presentation of diseases and response to treatments are not always the same in the HIV and the general population [38].

In brief, our results show that homocysteine serum level was higher than normal in 37 % of patients and we found an association between higher homocysteine serum level and HIV sexual risk behaviour and hepatitis C virus co-infection.

Funding / potential competing interests: Support for the preparation of this work has been provided by the Spanish VACH Cohort and the ISCIII-RETIC (RD06/006). No other potential conflict of interest relevant to this article was reported.

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References

1 Buchacz K, Rangel M, Blacher R, Brooks JT. Changes in the Clinical Epidemiology of HIV Infection in the United States: Implications for the Clinician. Curr Infect Dis Rep. 2009;11:75–83.

2 Fichtenbaum CJ. Metabolic Abnormalities Associated With HIV Infection and Antiretroviral Therapy. Curr Infect Dis Rep. 2009;11:84–92.

3 The Data Collection on Adverse Events of Anti-HIV Drugs (DAD) Study Group. Combination antiretroviral therapy and the risk of myocardial infarction. N Engl J Med. 2003;349:1993–2003.

4 Domingo P, Suarez-Lozano I, Teira R, Lozano F, Terrón A, Viciana P, et al. Dyslipidemia and Cardiovascular Disease Risk Factor Management in HIV-1-Infected Subjects Treated with HAART in the Spanish VACH Cohort. Open AIDS J. 2008;2:26–38.

5 Boccara F. Cardiovascular complications and atherosclerotic manifestations in the HIV-infected population: type, incidence and associated risk factors. AIDS. 2008;22(Suppl 3):S19–S26.

6 McNulty H, Pentieva K, Hoey L, Ward M. Homocysteine, B-vitamins and CVD. Proc Nutr Soc. 2008;67:232–7.

7 Wald DS, Wald NJ, Morris JK, Law M. Folic acid, homocysteine, and cardiovascular disease: judging causality in the face of inconclusive trial evidence. BMJ. 2006;333:1114–7.

8 Humphrey LL, Fu R, Rogers K, Freeman M, Helfand M. Homocysteine level and coronary heart disease incidence: a systematic review and meta-analysis. Mayo Clin Proc. 2008;83:1203–12.

9 Yang Q, Botto LD, Erickson JD, Berry RJ, Sambell C, Johansen H, et al. Improvement in stroke mortality in Canada and the United States, 1990 to 2002. Circulation. 2006;113:1335–43.

10 Ebbing M, Bleie Ø, Ueland PM, Nordrehaug JE, Nilsen DW, Vollset SE, et al. Mortality and cardiovascular events in patients treated with homocysteine-lowering B vitamins after coronary angiography: a randomized controlled trial. JAMA. 2008;300:795–804.

11 Albert CM, Cook NR, Gaziano JM, Zaharris E, MacFadyen J, Danielson E, et al. Effect of folic acid and B vitamins on risk of cardiovascular events and total mortality among women at high risk for cardiovascular disease: a randomized trial. JAMA. 2008;299:2027–36.

12 Bonaa KH, Njolstad I, Ueland PM, Schirmer H, Tverdal A, Steigen T, et al. Homocysteine lowering and cardiovascular events after acute myocardial infarction. N Engl J Med. 2006;354:1578–88.

13 Lonn E, Yusuf S, Arnold MJ, Sheridan P, Pogue J, Micks M, et al. Homocysteine lowering with folic acid and B vitamins in vascular disease. N Engl J Med. 2006;354:1567–77.

14 Antoniades C, Antonopoulos AS, Tousoulis D, Marinou K, Stefanadis C. Homocysteine and coronary atherosclerosis: from folate fortification to the recent clinical trials. Eur Heart J. 2008;30:6–15.

15 Selhub J. Public health significance of elevated homocysteine. Food Nutr Bull. 2008;29(2 Suppl):S116–S125.

16 Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. Department of Health and Human Services. November 3, 2008; 1-139. Available at www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf. Accessed January 22, 2009.

17 Guaraldi G, Ventura P, Garlassi E, Orlando G, Squillace N, Nardini G, et al. Hyperhomocysteinaemia in HIV-infected patients: determinants of variability and correlations with predictors of cardiovascular disease. HIV Med. 2009;10:28–34.

18 Bernasconi E, Uhr M, Magenta L, Ranno A, Telenti A. Homocysteinaemia in HIV-infected patients treated with highly active antiretroviral therapy. The Swiss HIV Cohort Study. AIDS. 2001;15:1081–2.

19 Bongiovanni M, Casana M, Pisacreta M, Tordato F, Cicconi P, Russo U, et al. Predictive factors of hyperhomocysteinemia in HIV-positive patients. J Acquir Immune Defic Syndr. 2007;44:117–9.

20 Uccelli MC, Torti C, Lapadula G, Labate L, Cologni G, Tirelli V, et al. Influence of folate serum concentration on plasma homocysteine levels in HIV-positive patients exposed to protease inhibitors undergoing HAART. Ann Nutr Metab. 2006;50:247–52.

21 Başkan BM, Sivas F, Aktekin LA, Doğan YP, Ozoran K, Bodur H. Serum homocysteine level in patients with ankylosing spondylitis. Rheumatol Int. 2009;29:1435–9.

22 Fimognari FL, Loffredo L, Di Simone S, Sampietro F, Pastorelli R, Monaldo M, et al. Hyperhomocysteinaemia and poor vitamin B status in chronic obstructive pulmonary disease. Nutr Metab Cardiovasc Dis. 2009;19:654–9.

23 Fujimaki C, Hayashi H, Tsuboi S, Matsuyama T, Kosuge K, Yamada H, et al. Plasma total homocysteine level and methylenetetrahydrofolate reductase 677C>T genetic polymorphism in Japanese patients with rheumatoid arthritis. Biomarkers. 2009;14:49–54.

24 de Bree A, van der Put NM, Mennen LI, Verschuren WM, Blom HJ, Galan P, et al. Prevalences of hyperhomocysteinemia, unfavourable cholesterol profile and hypertension in European populations. Eur J Clin Nutr. 2005;59:480–8.

25 Roca B, Suarez I, Gonzalez J, Garrido M, de la Fuente B, Teira R, et al. Hepatitis C virus and human immunodeficiency virus coinfection in Spain. J Infect. 2003;47:117–24.

26 Fu M, Martínez-Sánchez JM, Pérez-Ríos M, López MJ, Fernández E. A comparison of the Fagerström test for nicotine dependence and smoking prevalence across countries: updated data from Spain. Addiction. 2009;104:326–7.

27 Graham I, Atar D, Borch-Johnsen K, Boysen G, Burell G, Cifkova R, et al.; European Society of Cardiology (ESC); European Association for Cardiovascular Prevention and Rehabilitation (EACPR); Council on Cardiovascular Nursing; European Association for Study of Diabetes (EASD); International Diabetes Federation Europe (IDF-Europe); European Stroke Initiative (EUSI); Society of Behavioural Medicine (ISBM); European Society of Hypertension (ESH); WONCA Europe

(European Society of General Practice/Family Medicine); European Heart Network (EHN); European Atherosclerosis Society (EAS). European guidelines on cardiovascular disease prevention in clinical practice: full text. Fourth Joint Task Force of the European Society of Cardiology and other societies on cardiovascular disease prevention in clinical practice. Eur J Cardiovasc Prev Rehabil. 2007;14(Suppl 2):S1–S113.

28 Herrero JI, Quiroga J, Sangro B, Beloqui O, Pardo F, Cienfuegos JA, et al. Hyperhomocysteinaemia in liver transplant recipients: prevalence and multivariate analysis of predisposing factors. Liver Transpl. 2000;6:614–8.

29 Bernhard MC, Junker E, Hettinger A, Lauterburg BH. Time course of total cysteine, glutathione and homocysteine in plasma of patients with chronic hepatitis C treated with interferon-alpha with and without supplementation with N-acetylcysteine. J Hepatol. 1998;28:751–5.

30 Adinolfi LE, Ingrosso D, Cesaro G, Cimmino A, D'Antò M, Capasso R, et al. Hyperhomocysteinaemia and the MTHFR C677T polymorphism promote steatosis and fibrosis in chronic hepatitis C patients. Hepatology. 2005;41:995–1003.

31 Roblin X, Pofelski J, Zarski JP. Role de l'homocysteine au cours de la steatose hepatique et de l'hepatite chronique C. Gastroenterol Clin Biol. 2007;31:415–20.

32 Toniutto P, Fabris C, Falleti E, Cussigh A, Fontanini E, Bitetto D, et al. Methylenetetrahydrofolate reductase C677T polymorphism and liver fibrosis progression in patients with recurrent hepatitis C. Liver Int. 2008;28:257–63.

33 Borgia G, Gentile I, Fortunato G, Borrelli F, Borelli S, de Caterina M, et al. Homocysteine levels and sustained virological response to pegylated-interferon alpha2b plus ribavirin therapy for chronic hepatitis C: a prospective study. Liver Int. 2009;29:248–52.

34 De Larrañaga G, Alonso B, Puga L, Benetucci J. Homocisteína plasmática en infectados con el virus de la inmunodeficiencia humana. Medicina (B Aires) 2003;63:393–8.

35 Ganji V, Kafai MR. Demographic, lifestyle, and health characteristics and serum B vitamin status are determinants of plasma total homocysteine concentration in the post-folic acid fortification period, 1999–2004. J Nutr. 2009;139:345–52.

36 Raiszadeh F, Hoover DR, Lee I, Shi Q, Anastos K, Gao W, et al. Plasma Homocysteine is not associated with HIV serostatus or antiretroviral therapy in women. J Acquir Immune Defic Syndr. 2009;51:175–8.

37 Smulders YM, Spoelstra-de Man AM, Slaats EH, Weigel HM, Stehouwer CD, Jos Frissen PH. Trimethoprim-sulphamethoxazole as primary *Pneumocystis carinii* prophylaxis does not increase serum homocysteine levels in HIV-positive subjects. Eur J Intern Med. 2001;12:363–5.

38 Gonvers JJ, Heim MH, Cavassini M, Müllhaupt B, Genné D, et al. Treatment of hepatitis C in HCV mono-infected and in HIV-HCV co-infected patients: an open-labelled comparison study. Swiss Med Wkly. 2010;140:w13055. doi: 10.4414/smw.2010.13055.