whereas criteria based on a high cutoff were met later (Fig. 1, E and F). Marker kinetics with fast and medium rate constants were less influenced by background fluctuations.

The false-positive signals denoted TPA progression among events where TPA fluctuated in a steady state without any rate of increase (0.0000). The frequency and time point of the false-positive TPA signals were determined by the magnitude of the normal background fluctuations because larger fluctuations promoted the required critical difference. The criteria in Fig. 1, E and F, did not give any false-positive progression signals when the concentrations fluctuated in the middle of the normal range. Their robustness against false-positive signals of progression was therefore further investigated in situations where TPA concentrations fluctuated closely below the applied cutoff value. Indeed, both criteria provided false progression signals if steady-state concentrations fluctuated high within the normal range. However, the criterion in Fig. 1F gave false-positive signals at unusually large clinical fluctuations, whereas the criterion in Fig. 1E also gave false signals at small fluctuations. The criterion in Fig. 1F was robust against false-positive progression signals, owing to a higher cutoff value. In reviewing the data provided in Table 1, we believe it is clear that the criterion by Soléstornos et al. (3) (Fig. 1F), which adjusted the cutoff value and the critical difference to the higher background variation of TPA, performed better than criteria by other authors where this issue was left unconsidered (4–6) (Table 1). However, the importance of adjusting the algorithm to the individual marker also applied to the criteria elaborated by Soléstornos et al. (8) because the criteria that sufficed for CA 15.3-CEA (Fig. 1, B and C) gave false positives when applied to TPA data (Table 1). Conversely, the criteria that gave delayed CA 15.3-CEA information was more suitable for TPA (Fig. 1F; Table 1) (8).

In conclusion, the elaborated computer-simulation models have considerable potential because criteria can be compared in a variety of simulated conditions of steady-state and progressive disease. Varying the background variation and the tumor growth according to realistic patient data, as well as the cutoff value, enabled identification of criteria that were robust against false-positive signals of TPA progression. When data that define steady-state variability and rates of increase become available, the model systems should be used to generate assessment criteria for other markers and malignancies and compare and optimize diagnostic performance of the criteria. However, it is important to emphasize that computer-simulation studies cannot replace clinical tumor-marker trials. Computer-based comparison of assessment criteria is a supplement to clinical studies and relevant only if these studies have provided reliable estimates of basic performance characteristics. However, by simulating a large variety of new trial conditions, the robustness of a criterion can quickly be investigated at low costs, and a determination can be made as to whether the criterion is of limited use or should be implemented in routine clinical practice for patient monitoring.

References


Determinants of Increased Plasma Homocysteine in 221 Stable Liver Transplant Patients, Consuelo Fernández-Miranda, Marta Sanz, Angel de la Calle, Carmelo Loinaz, Pilar Gómez, Pilar Díaz-Rubio, Agustín Gómez de la Cámara, and Enrique Moreno [Departments of 1 Internal Medicine (Atherosclerosis Unit), 2 Surgery, and 3 Biochemistry, and the 4 Epidemiology Unit, Hospital Universitario 12 de Octubre, Madrid 28041, Spain; * address correspondence to this author at: Servicio de Medicina Interna, Hospital Universitario 12 de Octubre, Ctra. Andalucía km 5.4, Madrid 28041, Spain; fax 34-1-3908358, e-mail cfmiranda@inicia.es]

The plasma homocysteine concentration (tHcy) is affected by genetic and physiologic determinants, by life-style (including nutritional deficiencies of vitamins B6, B12, and folate), diseases, and by some drugs (1–4). Moderately increased tHcy is considered an independent risk factor for atherosclerotic disease in the coronary, cerebral, and peripheral arteries (1, 2) and a significant predictor of mortality in patients with coronary disease (5, 6). In renal and cardiac transplant recipients, tHcy is markedly increased and is associated with impaired renal function (7–12) and with low serum folate in some series (8–11). Some studies (7, 10), but not others (8, 11, 12), have reported that cyclosporine treatment and whole blood cyclosporine concentration were determinants of tHcy in renal and cardiac transplant recipients. Data on tHcy in liver transplant recipients are limited, and increased tHcy has been associated with renal dysfunction but not with folate concentrations or cyclosporine therapy (13).

Our aims were to evaluate the prevalence of increased
Lipoproteins were isolated by ultracentrifugation and assessed by RIA (Incstar) and whole blood tacrolimus by fluorescently. Whole blood cyclosporine was determined by RIA (Abbott). 

We studied 221 consecutive patients with clinically stable liver transplants seen between November 1999 and May 2000 at routine clinic visits. There were 141 men and 80 women. The immunosuppressive therapy for all patients was cyclosporine or tacrolimus. Twenty-five patients were also treated with mycophenolate, nine with azathioprine, and one with prednisone. Hypocaloric diets had been recommended for patients with diabetes and obesity, and alcohol abstention had been recommended for all patients. The patients who had received transplants <12 months before the study were excluded. Healthy volunteers (n = 50) with prudent lifestyles made up the control group, which included staff members of the University Hospital (Madrid, Spain) or their relatives. Controls were age- and sex-matched with the patients. Patients and controls who were being treated at the time of this study with drugs that could alter tHcy were excluded.

We evaluated age, sex, time since transplant, body mass index ([BMI]; kg/m²), smoking (any cigarette smoking in the past month) (14), presence of diabetes, hypertension, cardiovascular disease, immunosuppressive therapy, tHcy, serum creatinine, albumin and bilirubin, creatinine clearance, serum folate, vitamin B₁₂, and fasting (overnight) lipoprotein concentrations. Obesity was defined as BMI ≥30 kg/m². Hypertension was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg more than two times during the preceding year, or the need for antihypertensive treatment. Hypercholesterolemia was defined as a fasting LDL-cholesterol concentration ≥3.4 mmol/L or the need for antilipemic agents. Diabetes was defined as a fasting plasma glucose ≥7.0 mmol/L on 2 different days during the year before the study or the need for antidiabetic agents. Cardiovascular disease was defined as coronary disease (myocardial infarction), cerebrovascular disease (transient ischemic episode and/or thrombotic stroke), or peripheral vascular disease (occlusive or subocclusive artery disease and/or abdominal aorta aneurysm). tHcy was determined by fluorescence polarization immunoassay (15). Blood collected in Vacutainer Tubes (Terumo Europe N.V.) containing EDTA was immediately placed on ice and centrifuged within 1 h after collection at 1200g for 5 min. Plasma was stored frozen at −20°C until analysis. We defined increased tHcy as >2 SD above the mean of the controls; tHcy concentrations in controls showed a normal distribution.

Vitamin B₁₂ and serum folate were determined by RIA (SimulTRAC-SNB; ICN Pharmaceuticals). Serum creatinine, albumin, and bilirubin were measured on a Hitachi 747-200 analyzer (Roche). Creatinine clearance was estimated from the formula of Cockcroft and Gault (16). Lipoproteins were isolated by ultracentrifugation (17); cholesterol and triglycerides in the fractions were assayed enzymatically. Whole blood cyclosporine was determined by RIA (Incstar) and whole blood tacrolimus by fluorescence polarization immunoassay (Abbott).

The local ethical committee approved the protocol, and informed consent was obtained from all participants.

Means comparisons of continuous numeric variables were tested by the Student t-test. Categorical variables were tested by the χ² test. Associations between continuous variables were assessed by Pearson coefficients. P <0.05 was considered significant. Multiple linear regression analysis (backward) was used to evaluate the relationship between tHcy and the independent variables age, sex, serum creatinine, folate and vitamin B₁₂ concentrations, and treatment with cyclosporine or tacrolimus. A logistic regression analysis (outcome variable, increased tHcy: yes/no) was performed to determine the association between increased tHcy and the same independent variables.

Mean tHcy was increased in patients [mean ± SD, 15.2 ± 5.5 µmol/L; 95% confidence interval (CI), 14.5–15.8 µmol/L; geometric mean, 14.2 µmol/L] compared with controls (mean ± SD, 10.0 ± 2.2 µmol/L; 95% CI, 9.4–10.6 µmol/L; geometric mean, 9.8 µmol/L; P <0.0001). tHcy was higher in patients treated with cyclosporine (mean ± SD, 16.0 ± 5.1 µmol/L; 95% CI, 15.1–16.9 µmol/L; geometric mean, 15.2 µmol/L) than in those treated with tacrolimus (mean ± SD, 14.2 ± 5.7 µmol/L; 95% CI, 13.1–15.2 µmol/L; geometric mean, 13.2 µmol/L; P = 0.01). Creatinine concentration was higher and creatinine clearance lower in patients compared with controls (P <0.0001), as well as in patients on cyclosporine compared with those on tacrolimus therapy (P <0.001). Further details regarding the control and patient clinical characteristics and the patients on cyclosporine or tacrolimus therapy are available in an on-line supplement at Clinical Chemistry Online (http://www.clinchem.org/content/vol47/issue11).

tHcy was increased in 103 patients (47%). It was more prevalent in men, in older patients, in patients treated with cyclosporine, and in those with hypertension (Table 1), but in all these cases, creatinine concentration was more increased (P ≤0.001). Increased tHcy was not more prevalent in smokers or patients with obesity, diabetes, hypercholesterolemia, or a longer time since transplant (Table 1). Pearson correlation showed that tHcy was correlated with age (r = 0.20; P = 0.003), creatinine concentration (r = 0.62; P = 0.0001), creatinine clearance (r = −0.53; P = 0.0001), and folate concentration (r = −0.24; P = 0.0008). There was no correlation between tHcy and time since transplant, BMI, serum albumin, bilirubin, lipoproteins, vitamin B₁₂, or whole blood cyclosporine and tacrolimus concentrations.

A multiple linear regression showed that only creatinine (r² = 0.35; P = 0.0001) and folate (r² = 0.04; P = 0.0006) were independent predictors of tHcy, after simultaneous adjustment for age, sex, vitamin B₁₂ concentration, and cyclosporine or tacrolimus treatment. A logistic regression also showed that increased tHcy was associated with creatinine (odds ratio, 150; 95% CI, 29–785) and folate concentrations (odds ratio, 0.86; 95% CI, 0.76–0.98).

Other cardiovascular risk factors (smoking, obesity, diabetes, hypertension, and hypercholesterolemia) were
Impaired renal function. Ignatescu et al. showed that a potential immunosuppressive therapy, an observation consistent with other trials. Multivariate analysis, which included creatinine, showed a lower prevalence of vitamin B12 deficiency, a fact that may be attributable to the fact that the great majority of the patients were not vitamin B12 deficient, a fact that may be related to the relatively young age of the study population. Folate supplementation with or without the addition of vitamin B12 and/or B, decreases tHcy in renal and liver transplant patients, even in the absence of folate deficiency (12, 13, 22, 23). Although some researchers recommend treatment of increased tHcy with vitamin supplements in patients with coronary disease or multiple cardiovascular risk factors (24), the preventive effect of such therapy still remains to be determined. Randomized trials are currently under way (25).

In renal transplant recipients, increased tHcy may be associated with increased risk of cardiovascular disease (8, 26), a major complication in these patients (27). In liver transplant recipients, atherosclerotic disease has not been considered as an important problem (28–30). A recent large study of these patients (31), however, found that cardiovascular and cerebrovascular diseases were the second most important cause of late graft loss. In our study, atherosclerotic disease was present in only seven patients (5%); all were ≥50 years of age. This complication increases with the age of the liver transplant recipients (30). Our series included rather young participants, 38% of whom were <50 years, which could explain the low incidence of cardiovascular events. The high prevalence of high tHcy and other cardiovascular risk factors suggests the need for careful follow-up for atherosclerotic disease.

In summary, increased tHcy is frequently found in liver transplant patients. The prevalence of increased tHcy is even higher in patients treated with cyclosporine compared with those undergoing tacrolimus treatment. It

### Table 1. Determinants of increased plasma Hcy concentrations in liver transplant patients. a

<table>
<thead>
<tr>
<th>Determinants</th>
<th>Increased tHcy (%)</th>
<th>Odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men (n = 141)/Women (n = 80)</td>
<td>75 (53)/28 (35)</td>
<td>2.11 (1.16–3.88)</td>
</tr>
<tr>
<td>Age ≥55 years (n = 136)/Age &lt;55 years (n = 85)</td>
<td>72 (85)/31 (23)</td>
<td>1.96 (1.09–3.56)</td>
</tr>
<tr>
<td>Months since transplant ≥60 (n = 98)/&lt;60 (n = 123)</td>
<td>49 (50)/54 (31)</td>
<td>1.28 (0.72–2.25)</td>
</tr>
<tr>
<td>Cyclosporine (n = 119)/Tacrolimus therapy (n = 102)</td>
<td>69 (58)/34 (33)</td>
<td>2.76 (1.54–4.97)</td>
</tr>
<tr>
<td>Obesity (n = 48)/Nonobesity (n = 173)</td>
<td>21 (46)/82 (47)</td>
<td>0.95 (0.47–1.93)</td>
</tr>
<tr>
<td>Smokers (n = 66)/Nonsmokers (n = 155)</td>
<td>35 (53)/68 (44)</td>
<td>1.59 (0.85–2.99)</td>
</tr>
<tr>
<td>Diabetes (n = 55)/No diabetes (n = 166)</td>
<td>28 (51)/79 (48)</td>
<td>1.14 (0.59–2.20)</td>
</tr>
<tr>
<td>Hypertensive (n = 97)/Normotensive (n = 124)</td>
<td>54 (56)/49 (39)</td>
<td>1.92 (1.08–3.42)</td>
</tr>
<tr>
<td>Hypercholesterolemic (n = 80)/Nonhypercholesterolemic (n = 141)</td>
<td>37 (46)/66 (47)</td>
<td>0.98 (0.54–1.77)</td>
</tr>
</tbody>
</table>

a 2 test was used for statistical analysis. Smoker was defined as any cigarette smoking in the past month. Obesity was defined as BMI ≥30 kg/m2. Hypertension was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg more than two times during the preceding year or the need for antihypertensive treatment. Hypercholesterolemia was defined as a fasting LDL-cholesterol concentration ≥3.4 mmol/L or the need for antilipemic agents. Diabetes was defined as a fasting plasma glucose ≥7.0 mmol/L on two different days during the year preceding the study or the need for antidiabetic agents.

b Homocysteine values greater than means ±2 SD of control values (≥14.5 μmol/L).

c p < 0.05.

d p < 0.001.
remains unclear whether cyclosporine directly affects homocysteine metabolism or whether increased tHcy may be attributable to impaired renal function or higher prevalence of other confounders in these individuals. Serum creatinine is the most important predictor of tHcy, whereas serum folate had only minor influence. Avoidable, unhealthy lifestyle factors (e.g., smoking and obesity) and associated diseases (e.g., diabetes, hypertension, and hypercholesterolemia) did not influence tHcy significantly in our study.

The authors are grateful to Alicia Muñoz, Marisol Sillió, Blanca Naválon, Manuela Gómez and Trinidad Rodríguez for skillful technical assistance.

References