# Increasing plasma homocysteine during follow-up in heart transplant recipients: effects of folate and renal function

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Key words:
**Homocysteine; Heart transplantation; Allograft vascular disease; Atherosclerosis.**

*Background.* **Hyperhomocysteinemia is a common finding in heart transplant recipients and may represent a risk factor for graft failure. However, the time-course, determinants and effects of medical therapy on total homocysteine plasma levels after heart transplantation remain undetermined. The aim of this study was to prospectively analyze 1) the time-course of total homocysteine in heart transplant recipients; 2) the effects of folate supplements and cyclosporine A on total homocysteine; 3) the relation among renal function, serum vitamin levels, and total homocysteine.**

*Methods.* **Fifty-two heart transplant recipients consecutively evaluated for routine follow-up during 1998 were included in the study (mean age 54 ± 12 years; 28% female). Among the 52 patients, 10 patients were treated with folate for the entire period of the study (Group F), while 26 patients never received folate (Group NF). The remaining 16 patients who did not take folate on a regular basis were excluded from subgroup analysis. Total homocysteine and creatinine plasma levels were assayed at entry into the study (time 0) and at the end of the study, 12 months later (time 12).**

*Results.* **Homocysteinemia increased significantly from time 0 to time 12 ($p < 0.001$), regardless of creatinine plasma levels ($p = 0.03$) and folate intake ($p < 0.01$). However, total homocysteine levels were lower in Group F compared to Group NF at time 0 and time 12 ($p < 0.02$). On multivariate analysis, time of follow-up, serum creatinine and lack of folate intake were positive independent predictors of total homocysteine.**

*Conclusions.* **Homocysteinemia increased over time in heart transplant recipients, regardless of renal function and folate administration. Lower total homocysteine levels were associated with folate intake, suggesting that folate supplements may play a role in the prevention of vascular allograft disease.**

(Ital Heart J 2000; 1 (5): 344-348)

Received December 20, 1999; revision received April 11, 2000; accepted April 20, 2000.

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## Introduction

Homocysteine is a sulphur-containing metabolite of methionine and is an emerging independent risk factor for atherosclerosis. Previous studies have demonstrated that age, gender, renal function, and folic acid intake are major determinants of total homocysteine plasma levels in the general population[1-4].

Allograft vascular disease is a rapidly and progressive occlusive process, affecting the coronary arteries of heart transplant recipients. Its pathogenesis is not entirely clear, but it appears to be related to immune and non-immune factors. Notably, it is one of the leading causes of long-term graft failure in heart transplant recipients[5,6].

Several authors have reported that total homocysteine is often increased in patients with heart transplantation, and that it may represent a risk factor for allograft vascular disease. However, its time-course, determinants and effects of treatment have not been fully clarified[7-12].

We prospectively evaluated 52 patients who underwent heart transplantation to determine 1) the time-course of total homocysteine following transplantation; 2) the effects of medical treatment and particularly of folate administration on total homocysteine; 3) the relation among renal function, serum vitamin levels, and total homocysteine in this group of patients.

## Methods

**Patients.** Patients with heart transplantation who were consecutively referred for routine follow-up within a standardized follow-up program from June through September 1998 were considered eligible for the study. Immunosuppressive therapy and folate administration were managed by physicians unaware of the ongoing study. Folate supplements (15 mg of calcium N-5-methyltetrahydrofolate) were given orally in order to prevent anemia.

Chronic immunosuppressive therapy was based on the association between cyclosporine, azathioprine, and prednisone. All patients received cyclosporine orally twice a day to achieve blood trough levels of 150 to 250 ng/ml. The dose was tailored according to indices of renal function. Azathioprine was given orally, at a dose of 2 mg/kg/day, in order to maintain the white blood cell count between 6000 and 4000/mm$^3$. Prednisone was administered at a maintenance dose of approximately 0.2 mg/kg.

**Laboratory measurements.** Total homocysteine plasma levels and serum creatinine concentrations were measured at the beginning of the study (time 0) and 12 months later (time 12).

Total homocysteine was measured in venous blood samples obtained after an overnight fast. The blood specimens were immediately placed in ice and centrifuged within 2 hours of collection. The resulting plasma was stored at -70°C. The assay was based on high performance liquid chromatography, as previously described[13]. The intra-day (inter-day) variability of the analytical method (calculated as the standard deviation of 6 assays from the same blood sample) was of 1.5% (4.0%), 1.4% (2.9%) and 1.3% (3.9%) for values of total homocysteine of 6.5, 15.5 and 32.5 μmol/l, respectively. The lowest measured value was 1 μmol/l and the sensitivity of the assay 0.5 μmol/l.

At time 12, serum folate and vitamin B12 concentrations were also determined using a chemio-luminescence based commercial kit (ACS-100 from Chiron Diagnostic, East Walpole, MA, USA). Whole blood cyclosporine and its metabolites were assayed at time 0, time 12 and every 1-3 months, as clinically appropriate. Mean cyclosporine blood levels assayed during the study period were related to total homocysteine at time 12.

**Statistical analysis.** Continuous variables are expressed as mean ± 1 SD and categorical data as percentages. According to the variable's distribution, group comparisons were performed using Wilcoxon's ranking sum, Mann-Whitney U test, or $\chi^2$ test, as appropriate. To correct for the influence of creatinine on total homocysteine values, the total homocysteine/creatinine ratio was analyzed over time. Linear regression analysis was used to test the relationship between continuous variables and total homocysteine. Multivariate linear regression analysis was used to identify variables independently related to total homocysteine. A p value $< 0.05$ was considered statistically significant.

## Results

**Patients.** Fifty-two heart transplant recipients were enrolled (mean age 54 ± 12 years, 28% female, 44 ± 30 months from transplant). The reason for transplantation was terminal heart failure due to idiopathic dilated cardiomyopathy in 28 cases (53%), coronary artery disease in 16 (30%), arrythmogenic right ventricular dysplasia in 4 (7%), valvular heart disease in 2 (4%), and congenital heart disease and acute graft failure in the remaining 2 cases. The study was completed in 100% of patients. No patient died during the study period.

**Time-course and determinants of total homocysteine.** In the entire group of 52 patients, total plasma homocysteine increased from time 0 to time 12 (16.1 ± 6.8 vs 18.6 ± 7.4 μmol/l, respectively; $p < 0.001$). Serum creatinine also increased over time (1.52 ± 0.39 mg/dl at time 0 and 1.61 ± 0.42 mg/dl at time 12, $p = 0.063$). The total homocysteine/creatinine ratio at time 12 was greater than at time 0 (11.8 ± 4.2 vs 10.6 ± 3.6, respectively, $p = 0.031$). On univariate analysis, the only predictors of total homocysteine were age ($r = 0.24$, $p = 0.007$), serum creatinine ($r = 0.48$, $p < 0.001$) and time from heart transplant ($r = 0.29$, $p = 0.001$). No other clinical or laboratory variable, including cyclosporine plasma levels ($r = - 0.122$, $p = 0.123$) and its metabolites ($r = - 0.198$, $p = 0.098$), were associated with total homocysteine. Similarly, mean cyclosporine was not correlated with total homocysteine measured at time 12 (214 ± 28 ng/ml, $r = -0.102$, $p = 0.231$).

On multivariate analysis, the time from heart transplant remained associated with increased total homocysteine plasma levels after the adjustment for creatinine plasma levels ($p = 0.045$).

**Effects of folate supplements and serum vitamin levels on total homocysteine.** Among the 52 patients, 26 never received folate supplements (Group NF), whereas 10 were treated with folate for at least 1 month before time 0 and continued until time 12 (Group F). In the remaining 16 patients, folate supplements were taken discontinuously and these patients were excluded from subgroup analysis. Baseline differences between the two groups are shown in table I.

Total homocysteine increased from time 0 to time 12 both in Group F ($p = 0.011$, Fig. 1) and in Group NF ($p = 0.013$, Fig. 1).

However, absolute total homocysteine values in Group F were lower than in Group NF both at time 0 and time 12 ($p = 0.001$ and $p = 0.011$, respectively) (Fig. 1).

Serum vitamin B12 concentrations at time 12 were not significantly correlated with total homocysteine (461 ± 262 pg/ml, $r = -0.09$, $p = 0.912$).

**Table I.** Baseline (time 0) differences between Group NF and Group F.

| Variables | Group NF (n=26) | Group F (n=10) | p |
|---|---|---|---|
| Total homocysteinemia (μmol/l) | 19.1 ± 7.4 | 12.6 ± 3.2 | 0.001 |
| Creatininemia (mg/dl) | 1.55 ± 0.36 | 1.57 ± 0.41 | 0.45 |
| Age (years) | 52.5 ± 11.4 | 57.3 ± 5.7 | 0.35 |
| Time from transplant (months) | 55 ± 38 | 17 ± 21 | 0.012 |
| Cyclosporinemia (ng/ml) | 211.8 ± 24.7 | 219.1 ± 41.3 | 0.09 |
| Metabolites (ng/ml) | 555.0 ± 112.3 | 622.0 ± 100.3 | 0.07 |

Data are expressed as mean ± SD.

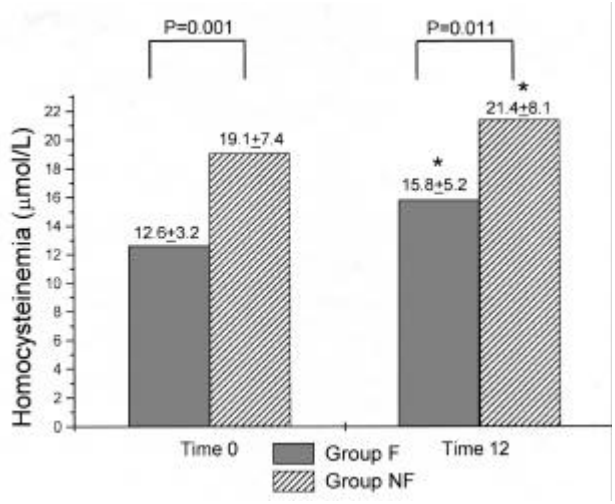


**Figure 1.** *Homocysteinemia in Group F and Group NF at time 0 and time 12. P values apply to the comparisons between groups. * statistically significant difference with respect to time 0.*

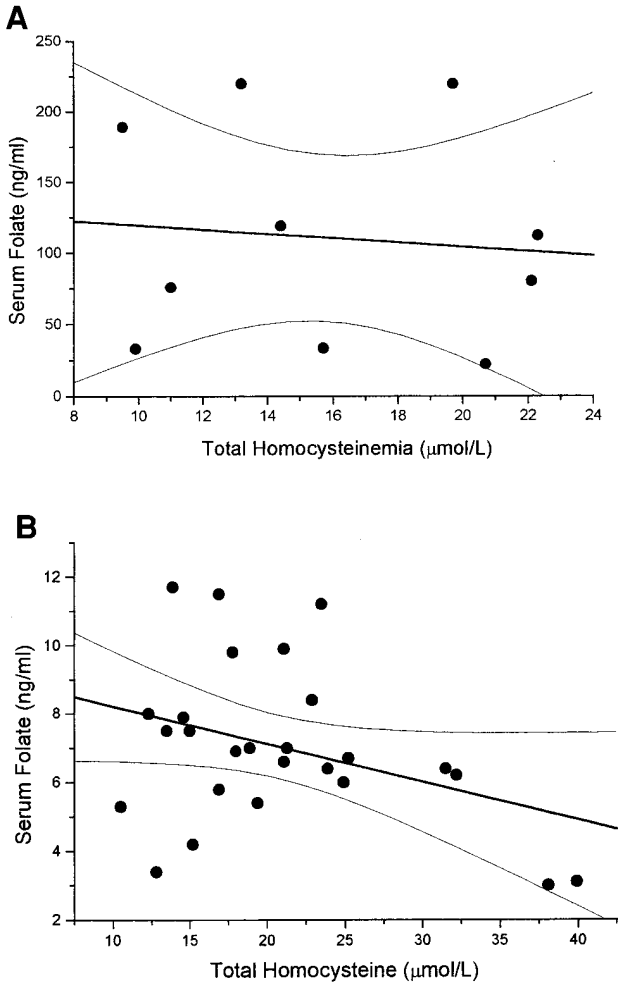


**Figure 2.** *Relation between total homocysteine and folate plasma levels. Lines of best fit and 95% confidence intervals are shown in the figure. A: patients who took folate during the study (r = -0.10, p = 0.923). B: patients who did not take folate during the study (r = -0.37, p = 0.069).*

Mean serum folate at time 12 was 110.4 ± 76.1 ng/ml in Group F and 7.1 ± 2.4 in Group NF ($p < 0.001$). No significant correlation was found between total homocysteine and serum folate ($p = 0.20$) in the entire group of patients. However, when the analysis was restricted to Group NF, a trend towards an inverse linear relationship was observed between serum folate and total homocysteine ($r = -0.37$, $p = 0.069$) (Fig. 2).

Notably, on multivariate analysis, folate intake remained associated with lower total homocysteine even after adjustment for creatinine plasma levels and time from heart transplant ($p < 0.001$).

## Discussion

The present study prospectively analyzed the time-course of total homocysteine plasma levels after heart transplantation. It demonstrates that total homocysteine increases during follow-up in heart transplant recipients, independently of renal function, cyclosporine plasma levels and folate intake. However, folic acid supplements appeared to favorably influence total homocysteine in this group of patients.

**Rationale of the study.** Heart transplantation is an increasingly common therapeutic option for patients younger than 65 years of age affected by terminal heart failure. Due to improvements in the treatment of acute rejection and nosocomial infections, the survival of heart transplant recipients has constantly improved over the

years. However, the long-term outcome of these patients is still negatively influenced by a rapidly progressive form of atherosclerosis affecting their coronary arteries[5]. The etiology of allograft vascular disease is still debated, but appears to be related not only to "traditional" risk factors (i.e. dyslipidemia or diabetes), but also to the immune response towards allograft antigens[6].

Homocysteine is a metabolite of the methionine transsulphuration pathway, and represents an independent risk factor for occlusive artery disease[1-4]. Its plasma levels are influenced by several factors, including genetic traits, vitamin intake, renal function, and the use of several drugs[1-4].

Recently it has been reported that total homocysteine is increased in heart transplant recipients[7-12]. Furthermore, it has been hypothesized that total homocysteine could represent a risk factor for allograft vascular disease[9,11]. However, available data are limited by retrospective design[9,12], non-uniform methodology[9], short follow-up[8] and small patient populations[7]. Moreover, no study has investigated the effect of folate supplements on total homocysteine in this group of patients[8-12]. Therefore, a comprehensive analysis of the time-course, determinants, and effects of medical therapy on total homocysteine in heart transplant recipients, to the best of our knowledge, is not yet available.

**Homocysteine time-course.** The time-course of total homocysteine after heart transplantation is not well defined. Berger et al.[8] showed that total homocysteine increases within 3 months of heart transplantation, but then remains constant. Conversely, an inverse relationship between total homocysteine and time of follow-up was found in another series[10].

The present data demonstrate that total homocysteine increases over time after transplant. Although a positive correlation between serum creatinine and total homocysteine has previously been described[1,8,10], renal function did not appear to be the main determinant of total homocysteine in our series. When the total homocysteine/creatinine ratio was considered in order to reduce the effect of renal function on homocysteine plasma levels, the increase in total homocysteine was confirmed. Moreover, time from transplant remained a positive predictor of total homocysteine on multivariate analysis, even after adjustment for serum creatinine levels, suggesting that increasing total homocysteine was not simply a marker of renal function status.

Among the immunosuppressive drugs routinely administered to heart transplant recipients, cyclosporine has frequently been investigated as potentially involved in homocysteine metabolism due to its deleterious effects on the kidney[14-16]. However, a direct correlation between cyclosporine and total homocysteine has been confirmed by some authors[10,12] and denied by others[8]. In the present series, cyclosporine and cyclosporine metabolite blood levels were not related to total homocysteine. Moreover, renal function appeared to only marginally influence total homocysteine, therefore attenuating the eventual effect of cyclosporine on total homocysteine through renal impairment[14,15].

Our data support the hypothesis of a progressive impairment of homocysteine metabolism, linked to a "heart transplant status", independent of renal function or immunosuppressive therapy. This hypothesis is consistent with the previously described higher plasma levels of homocysteinemia detected in heart transplant recipients compared to kidney recipients with similar values of creatinine[7]. The exact mechanism of hyperhomocysteinemia in patients with heart transplant has not yet been clarified, however a potential contributory role could be played by folic acid and vitamin B12 concentrations detected in this group of patients[8,9].

**Effect of folate supplements.** Folic acid and vitamin B12 are the main dietary determinants of total homocysteine in the general population and in heart transplant recipients[3,9]. Moreover, previous studies have demonstrated that folate supplements are effective in reducing total homocysteine levels in subjects with and without cardiovascular disease[1-4]. However, the effects of folate supplements on total homocysteine in heart transplant recipients have never been investigated.

The current study demonstrates that, although total homocysteine increases over time regardless of folate supplements, folate intake was associated with lower total homocysteine. When Group F and Group NF patients were compared both at time 0 and time 12, total homocysteine concentration in Group F was significantly lower ($p < 0.02$). Moreover, on multivariate analysis, folate intake remained associated with lower total homocysteine after adjustment for the other independent predictors of total homocysteine ($p < 0.001$), suggesting that folate supplements may positively influence total homocysteine in heart transplant recipients.

When serum folate was analyzed, no correlation was found with total homocysteine. However, when the analysis was limited to Group NF, a trend towards a significant correlation between these two variables was observed.

The relation between serum folate and total homocysteine has extensively been reported to be linear only for concentrations < 6-7 ng/ml[9,10,17]. Serum folate levels exceeding this threshold may saturate the remethylation pathway and represent an adequate folate status. Thus it is not surprising that serum folate did not correlate with total homocysteine in our Group F patients, in whom folate levels were > 20 ng/ml.

**Clinical implications.** An important issue is how these results should apply to clinical practice. Although prospective, this study was observational, and therefore any conclusions must be cautious. Moreover, the time from transplant in Group F was significantly shorter than in Group NF. Although multivariate analysis provides

the unique opportunity to adjust for differences in baseline characteristics, selection bias cannot be confidently excluded. Nonetheless, some relevant considerations can be made. Heart transplant represents nowadays a valid therapeutic opportunity for patients with end-stage heart failure, who would otherwise have an extremely poor life expectancy. Unfortunately, allograft vascular disease continues to negatively influence the long-term prognosis of these patients and total homocysteine may represent a risk factor for graft failure. Whether the reduction of total homocysteine due to folate supplements may prevent graft failure in heart transplant recipients needs to be prospectively investigated in appropriately sized randomized trials.

**Limitations of the study.** The assignment to treatment with folate was not randomized but left to the clinical judgment of the physicians in charge of the patient's care. Moreover, total homocysteine levels measured before starting folate treatment in Group F were not available. However, total homocysteine plasma levels in Group F were lower than in Group NF both at time 0 and time 12. Furthermore, after adjustment for other total homocysteine determinants, folate intake remained strongly associated with lower total homocysteine, suggesting that folic acid supplements play a positive role in total homocysteine reduction after heart transplant.

The present study did not include a control population, documenting the possible changes in total homocysteine over time during an equivalent span of time.

In conclusion, homocysteinemia is a potential risk factor for allograft vascular disease. In the present study, it increased over time in heart transplant recipients, regardless of renal function, immunosuppressive therapy, and folate supplements.

However, folate intake was independently associated with lower total homocysteine plasma levels.

These data suggest that folate supplements could play a role in the prevention of allograft vascular disease after heart transplantation.

## Acknowledgments

The authors wish to thank the nurses Gianna Canu, Paola Marchesini and Sandra Sassi for their precious help in collecting the data.

## References

1. Malinow M. Hyperhomocyst(e)inaemia: a common and easily reversible risk factor for occlusive atherosclerosis. Circulation 1990; 81: 2004-6.
2. Mayer E, Jacobsen D, Robinsom K. Homocysteine and coronary atherosclerosis. J Am Coll Cardiol 1996; 27: 517-27.
3. Malinow MB, Bostom AG, Krauss RM. Homocysteine, diet, and cardiovascular disease. Circulation 1999; 99: 178-82.
4. Andreotti F, Burzotta F, Mazza A, Manzoli A, Robinson K, Maseri A. Homocysteine and arterial occlusive disease: a concise review. Cardiologia 1999; 44: 341-5.
5. Hosenpud J, Bennet L, Keck B, Fiol B, Novick R. The registry of the International Society for Heart and Lung Transplantation: fourteenth official report. J Heart Lung Transplant 1997; 16: 691-712.
6. Andersen H. Heart allograft vascular disease. An obliterative vascular disease in transplanted hearts. Atherosclerosis 1999; 142: 243-63.
7. Ambrosi P, Barlatier A, Habib G, Garçon D. Hyperhomocysteinemia in heart transplanted recipients. Eur Heart J 1994; 15: 1191-6.
8. Berger P, Jones J, Olson L, et al. Increase in total plasma homocysteine concentration after cardiac transplantation. Mayo Clin Proc 1995; 70: 125-30.
9. Gupta A, Moustapha A, Jacobsen D, Goosemastic M. High homocysteinemia, low folate and low vitamin B6 concentration: prevalent risk factors for vascular disease in heart transplant recipients. Transplant 1998; 65: 544-50.
10. Cole D, Ross H, Evrowsky LJ, et al. Correlation between total homocysteine and cyclosporine concentrations in cardiac transplant recipients. Clin Chem 1998; 44: 2307-12.
11. Ambrosi P, Garçon D, Riberi A, Habib G. Association of mild hyperhomocysteinemia with cardiac graft vascular disease. Atherosclerosis 1998; 138: 347-50.
12. Cook R, Tupper J, Parker S. Effect of immunosuppressive therapy, serum creatinine and time after transplant on plasma homocysteine in patients following heart transplantation. J Heart Lung Transplant 1999; 18: 420-4.
13. Araki A, Sako Y. Determination of free and total homocysteine in human plasma by high performance liquid chromatography with fluorescence detection. J Chromatogr 1987; 422: 43-52.
14. Mc Nally P, Feehally J. Pathophysiology of cyclosporin anephrotoxicity: experimental and clinical observations. Nephrol Dial Transplant 1992; 7: 791-804.
15. Arnadottir M, Hultberg B, Vladov V, Nisson-Ehle P, Thysell H. Hyperhomocysteinemia in cyclosporine treated renal transplant recipients. Transplantation 1996; 61: 509-12.
16. Chung G, Walker S, Vadher B, Murphy F, Leaver N, Banner N. Effect of sandimmune cyclosporine on renal blood flow and renal function in heart transplant recipients. Transplant Proc 1998; 30: 1147-8.
17. Selhub J, Jacques P, Rosenberg I, et al. Serum total homocysteine concentration in the third National Health and Nutrition Examination Survey (1991-1994): population reference ranges and contribution of vitamin status to high serum concentrations. Ann Intern Med 1999; 131: 331-9.