Nephrol Dial Transplant (2005) 20: 1647–1652
doi:10.1093/ndt/gfh879
Advance Access publication 26 May 2005

Nephrology Dialysis Transplantation

Original Article

Predictive value of serum macrophage colony-stimulating factor for development of aortic calcification in haemodialysis patients: a 6 year longitudinal study

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Abstract

Background. Accelerated atherosclerosis is a major complication in patients on haemodialysis (HD). Macrophage colony-stimulating factor (MCSF) is a representative regulator of activation of monocytes and macrophages, and plays important roles in the development of atherosclerosis in HD patients. However, the long-term predictive value of the serum MCSF level for the development of aortic calcification under HD conditions has not been reported.

Methods. Serum MCSF level was measured in 40 HD patients. The aortic calcification index (ACI) was also calculated on computed tomography once each year for 6 years. Predictive value was examined by logistic regression analysis.

Results. At baseline, there was a significant correlation between serum MCSF and ACI ($r = 0.43$, $P < 0.01$). A significant increase in ACI was first noted at 4 years post-baseline and the increase was maintained thereafter in the high MCSF group. No such changes were noted in the low MCSF group. Univariate analysis identified high levels of calcium × phosphorus product, triglyceride, C-reactive protein (CRP), MCSF and presence of diabetes mellitus as significant predictors for increased ACI at 6 years. However, among these five factors, high levels of CRP and MCSF were the only independent and significant predictors (odds ratio = 24.0, $P = 0.03$ and odds ratio = 22.8, $P = 0.02$, respectively).

Conclusions. Our results demonstrated that MCSF is associated with the process of atherosclerosis in HD patients. Furthermore, the serum MCSF level is an independent long-term predictor of increased ACI. These results provide useful information for preventive strategies against atherosclerotic disease under HD conditions.

Keywords: aortic calcification index; atherosclerosis; haemodialysis; logistic regression analysis; longitudinal study; macrophage colony-stimulating factor

Introduction

In patients with chronic renal failure (CRF) who are on maintenance haemodialysis (HD), atherosclerotic disease is a major cause of morbidity and mortality [1,2]. In the general population, several pathological processes, such as hypertension, hyperlipidaemia and diabetes mellitus (DM), are associated with increased incidence of atherosclerosis. On the other hand, some risk factors for the development of atherosclerosis have been identified in patients with end-stage renal disease (ESRD) [1,3]. In addition, atherosclerosis is currently recognized as a complex pathological process, involving inflammation and a variety of cytokines, affecting the arterial wall [4].

Monocytes and macrophages are the first cells to accumulate in atherosclerotic lesions, and they play important roles in the development of atherosclerosis [5]. The macrophage colony-stimulating factor (MCSF) is known to regulate the survival, differentiation and proliferation of monocytes and macrophages [6,7]. Furthermore, MCSF is produced by arterial wall cells (endothelial cells and fibroblasts) and macrophages in atherosclerotic lesions [8,9], and is known to induce a variety of vasoactive factors [5]. The above background suggests that MCSF plays important roles in atherogenesis and promotion of atherosclerosis.

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Indeed, increasing evidence has been reported in support of this hypothesis in studies of human atherosclerotic lesions and experimental animal models [10].

Several reports have demonstrated that serum MCSF levels in patients with CRF and HD were higher than those in normal controls [11,12]. In addition, these levels were reported to be associated with the process of aortosclerosis in HD patients [12]. However, to our knowledge, there are no reports on the long-term predictive value of serum MCSF levels in the development of atherosclerosis in HD patients. In the present study, we investigated the relationship between serum MCSF levels and changes in aortic calcification index (ACI) in patients on HD for $\geq$6 years. In addition, the predictive value of MCSF was also examined by a multivariate analysis model, which included several other risk factors.

Subjects and methods

Patients

Between 1997 and 1998, we measured serum MCSF levels in 63 patients who were receiving maintenance HD three times or twice per week (4 h/day). Patients with short duration of HD (<6 months, $n=6$), presence of inflammatory disease or cancer ($n=3$) were excluded from the study. In addition, 14 patients were excluded because the data obtained in the 6 year observation period were not complete (patients who died during the course of the study, $n=4$, transferred to another hospital, $n=3$; declined to participate in the study, $n=3$; or patients with incomplete data, $n=4$). Thus, the present study consisted of 40 HD patients (21 men and 19 women). Their age at baseline was 52.4 ± 1.9 years (mean $\pm$ SEM), and duration of HD was 86.8 ± 12.6 months. In this population, the background kidney diseases were chronic glomerulonephritis ($n=30$), diabetic nephropathy ($n=8$), chronic pyelonephritis ($n=1$) and idiopathic ($n=1$). Systolic and diastolic blood pressure (BP) were measured immediately before starting the HD session. As a control group, 30 healthy age-matched volunteers (16 men and 14 women) also had their serum MCSF level assayed. All patients and volunteers were informed of the purpose of the study and the influence of radiation and consented to participate in our study, and a signed consent form was obtained from each subject. In addition, the study was approved by the Human Ethics Review Committee of Nagasaki University School of Medicine.

Measurement of serum MCSF and estimation of ACI

Serum MCSF levels were measured by radioimmunoassay (RIA) in cooperation with the Otsuka Pharmacology Institute. This method was performed according to Itoh *et al.* [13]. Briefly, two 100 µl blood samples were each mixed with ^{125}I-labelled recombinant human (rh) MCSF (10 000 c.p.m./100 µl) and rabbit anti-serum against rhMCSF (200 µl) diluted 1:40 000. Then, the bound product was separated from the free ^{125}I-labelled rhMCSF by the addition of goat anti-rabbit IgG diluted 1:400 (100 µl). After centrifugation at 1000 *g* for 15 min, the precipitates were analysed for 1 min by an automated γ-spectrometer. The detection limit of this method is 0.1 ng/ml.

In addition, serum levels of calcium (Ca), phosphorus (P), total cholesterol (TC), triglyceride (TG) and C-reactive protein (CRP) were also measured in the same blood sample by an automatic analyser (JCA-RX 40, Nihon Densi Cooperation, Japan). The normal levels of the above factors according to our laboratory were as follows: Ca, 8.7–10.3 mg/dl; P, 2.5–4.7 mg/dl; TC, 128.0–220.0 mg/dl; TG, 38.0–150.0 mg/dl; and CRP, <0.17 mg/dl. Blood samples were obtained immediately after insertion of the dialysis needles into the vascular system, and these factors were measured on the same day.

It is the policy of our hospital that all HD patients undergo non-contrast computed tomography (CT) once a year. Since this CT is taken by scanning sequential consecutive 10 mm slices, it was considered more suitable for measurement of aortic calcification than the 1–3 mm thin slices of the coronary artery CT scan. In addition to this routine examination, CT of the chest (10 mm slices) was obtained in every patient at the same time. Details of the method of measurement of ACI were described previously [12]. Briefly, we measured the percentage area of the aortic circumference occupied by calcified material in each slice. Furthermore, we calculated the mean percentage of calcification of all slices for each patient and the group, and expressed these values as ACI. ACIs were determined by two of the authors (T.K. and Y.M.), and the average of the two values was used for analysis. For statistical evaluation, the ratios of ACI at 6 years and at baseline were calculated, and patients with ratios greater than the median were designated to be the high increase group.

Statistical analysis

All data are expressed as the mean $\pm$ SEM unless otherwise stated. Continuous variables were analysed by the Mann–Whitney U-test. Comparisons between different time points were made by using the repeated-measures analysis of variance and Scheffé test. The relationship between continuous variables was investigated by Pearson's correlation coefficient (r). Patients were divided into two separate groups according to the median values of age at baseline, duration of HD, Ca $\times$ P product and MCSF level for logistic regression analyses. Data of these parameters are presented as median and interquartile range (IQR), in addition to the mean $\pm$ SEM values. The crude and adjusted effects on risk factors including serum MCSF levels were estimated by logistic regression analysis, and were described as odds ratios (ORs) with 95% confidential intervals (CIs), together with the P-values. Variables that achieved statistical significance in the univariate analysis were entered in the multivariate analysis. All statistical tests were two-sided, and significance was defined as $P < 0.05$. Statistical analyses were performed on a personal computer with the statistical package StatView for Windows (version 5.0, Abacus Concept, Inc., Berkeley, CA).

Results

Patient characteristics

The baseline characteristics of the patients were as follows: age, 52.4 ± 1.9 (median 53.0, IQR 41–64); systolic BP, 144.3 ± 2.7 mmHg; diastolic BP, 76.8 ± 12.6 mmHg; Ca, 9.9 ± 0.2 mg/dl; P, 5.4 ± 0.2 mg/dl; TC, 163.8 ± 6.4 mg/dl; TG, 137.6 ± 7.2 mg/dl; CRP, 0.18 ± 0.03 mg/dl; and MCSF, 2.0 ± 0.1 ng/ml (median 1.9, IQR 1.6–2.2). Furthermore, the median and IQR of Ca × P products were 54.9 and 46.2–62.2 and of HD duration were 66 and 23–148 months, respectively. To investigate the relationships between MCSF and changes in ACI over 6 years, we divided the patients into two groups based on the MCSF level; patients with levels higher than the median value ($n = 20$) and those with levels lower than the median ($n = 20$). Table 1 summarizes the patient characteristics, medications and laboratory data for the low and high MCSF groups. These two groups did not differ with respect to the factors listed including medications.

Table 1. Patient characteristics

	Low MCSF group (below median level)	High MCSF group (above median level)	*P*-value
Age at baseline (years)	53.7 ± 2.7	51.1 ± 2.7	0.53
Gender (*n*/% of males)	8/40	13/65	0.20
Duration of HD (months)	87.8 ± 18.2	85.8 ± 7.8	0.78
Systolic BP (mmHg)	144.0 ± 3.9	143.2 ± 4.3	0.90
Diastolic BP (mmHg)	77.9 ± 2.7	74.5 ± 3.0	0.41
Presence of HT (*n*/%)	6/30	6/30	0.99
Laboratory data			
Serum calcium (mg/dl)	9.7 ± 0.2	10.0 ± 0.2	0.21
Serum phosphorus (mg/dl)	5.0 ± 0.2	5.6 ± 0.2	0.11
Ca × P product	49.0 ± 3.0	55.5 ± 2.2	0.08
Serum total cholesterol (mg/dl)	150.1 ± 5.8	169.5 ± 10.0	0.10
Serum triglyceride (mg/dl)	132.8 ± 10.0	142.5 ± 9.0	0.48
C-reactive protein (mg/dl)	0.14 ± 0.02	0.22 ± 0.05	0.17
Presence of DM (*n*/%)	3/15.0	5/25.0	0.69
Medications			
Phosphorus binders	12/60.0	14/70.0	0.51
Vitamin D supplements	12/60.0	15/75.0	0.31

MCSF = macrophage colony-stimulating factor; BP = blood pressure; HD = haemodialysis; HT = hypertension; Ca = calcium; P = phosphorus; DM = diabetes mellitus.
Data are expresssed as mean ± SEM.

Changes in ACI

The ACI at baseline for the whole group of patients was 18.8 ± 2.7%. At baseline, the ACI of the high MCSF group (24.8 ± 4.1%) was significantly higher than that of the low MCSF group (12.8 ± 2.9%, $P < 0.01$). In addition, Pearson's correlation analysis showed a significant positive correlation between serum MCSF level and ACI at baseline ($r = 0.43$, $P < 0.01$). The changes in ACI over 6 years after baseline in the whole group of patients are shown in Figure 1a. A significant increase was first noted at 4 years, which was maintained thereafter. A similar trend was also found in the high MCSF group (Figure 1b). In the low MCSF group, the ACIs at all follow-up time points were not significantly different from that at baseline. Figure 2 shows the fold changes in ACI compared with the baseline level for both MCSF groups. Until 3 years after baseline, there was no significant difference in this change between the two MCSF groups. However, from 4 years, the rate of increase in ACI of the high MCSF group was significantly higher ($P < 0.01$) than that of the low MCSF group. In addition, the difference between the two groups increased with the duration of HD. The fold increase in ACI at 6 years (median level) for the whole group of patients was 1.4 ± 0.1 (1.3), and patients with a fold increase of >1.3 were considered as the high increase group.

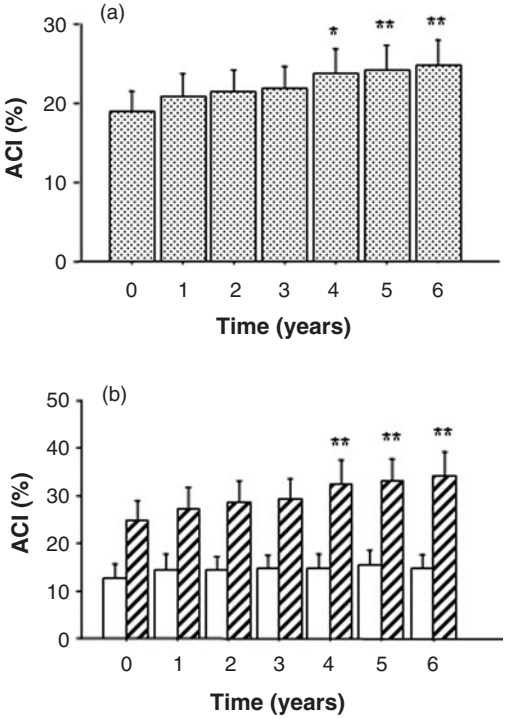

Fig. 1. Changes in ACI at baseline and 1–5 years after baseline. The ACI increased significantly from 4 to 6 years after baseline for the whole group of patients (**a**). A similar result was also found in the high MCSF group (hatched bars, **b**), but not in the low MCSF group (open bars, b). Data are expressed as the mean ± SEM. $^{*}P < 0.05$ and $^{**}P < 0.01$ *vs* baseline.

Significance of MCSF: relationship with CRP levels and predictive value

The mean serum MCSF level of the control group was 1.5 ± 0.3 ng/ml (median 1.4, IQR 1.2–1.6). The level in HD patients was significantly higher ($P < 0.01$) than in the control group. At baseline, the serum MCSF

levels were significantly associated with CRP levels ($r = 0.33$, $P = 0.03$). CRP levels also correlated with ACI ($r = 0.44$, $P < 0.01$).

To assess the risk factors for increased ACI, we used univariate logistic regression analysis (Table 2). High levels of Ca × P products, TG, CRP and MCSF, and the presence of DM were identified as significant predictors. Furthermore, among these five factors, high MCSF and high CRP levels were identified as independent and significant predictors for the increased ACI (MCSF, OR 22.8, 95%CI 1.5–345, $P = 0.02$; CRP, OR 24.0, 95%CI 1.4–409, $P = 0.03$). There was no significant association between baseline ACI and the increase in ACI after 6 years ($r = 0.28$ and $P = 0.08$).

Discussion

There is sufficient evidence to suggest that the serum MCSF level is increased and promotes aortic calcification under CRF or HD conditions [11,12]. With respect to the relationship between serum MCSF level and ACI in HD patients, Nitta *et al.* [12] reported a positive correlation between these variables ($r = 0.596$, $P < 0.01$). Our data at baseline also showed a similar trend, and support the role of MCSF in HD patients. In addition, the results of Nitta *et al.* [12] showed that the serum MCSF level did not correlate with various clinical parameters such as age, BP, serum levels of TC and TG, and Ca × P products. Our results are in agreement with the above findings. In the present study, we did not describe the type of HD membrane or frequency of HD per week as these factors varied according to serum parameters and general condition in several patients. Nitta *et al.* reported that the type of HD membrane did not influence serum MCSF levels. We speculated that these factors did not significantly influence our results.

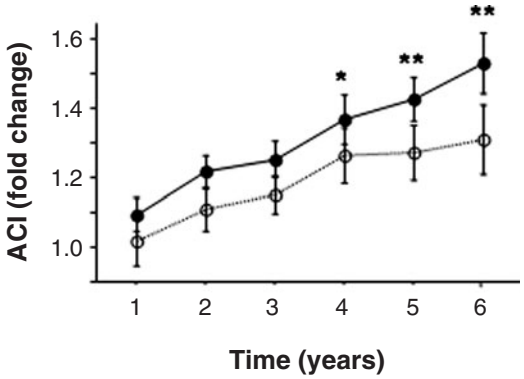

Fig. 2. Time course of fold changes in ACI compared with baseline. The rate of change in ACI gradually increased in the high MCSF group (thick line) and low MCSF group (dotted line). However, at 4 years and thereafter, the rate of changes of the high MCSF group significantly exceeded that of the low MCSF group. Data are expressed as the mean ± SEM. $*P < 0.01$ compared with the baseline levels. $*P < 0.05$ *vs* time-matched ACI of the low MCSF group. $**P < 0.01$ *vs* time-matched ACI of the low MCSF group.

To our knowledge, this study is the first report on the predictive value of elevated serum MCSF level over a long period in patients with HD. Furthermore, our main finding was that elevated serum MCSF level was a useful predictor for the development of aortic calcification, at least from 4 years after baseline. In addition, the fold change in ACI compared with baseline between the high MCSF group and low MCSF group increased with the duration of HD. Multivariate logistic regression analysis also identified a high serum MCSF level as a significant and independent predictor of aortic calcification. Our results showed that serum MCSF levels correlated significantly with aortic calcification from 4 years after baseline. While the exact mechanism of the post-4 year accelerated rate of calcification in the high MCSF group is not clear at present, our data indicate that the effect of MCSF on calcification of the aortic wall is slow and gradual, and that this process takes several years to become apparent on imaging studies.

In the present study, seven patients were excluded from analysis because of death or transfer to other hospitals. Among these seven patients, five developed severe atherosclerotic diseases, including cerebrovascular disease ($n = 3$) and coronary heart disease ($n = 2$).

Table 2. Logistic regression analysis for increased aortic calcification index in patients on haemodialysis

	Univariate analysis			Multivariate analysis		
	OR	95% CI	*P*-value	OR	95% CI	*P*-value
Age at baseline (>3 years)	0.4	0.1–1.6	0.21	–	–	–
Gender (female)	0.8	0.2–2.8	0.75	–	–	–
Duration of HD (>66 months)	1.5	0.4–5.2	0.53	–	–	–
Presence of HT	0.6	0.2–2.4	0.49	–	–	–
Ca × P product (>549)	4.9	1.2–19.9	0.03	1.8	0.2–12.8	0.54
TC (>220 mg/dl)	2.3	0.1–14.0	0.38	–	–	–
TG (>150 mg/dl)	5.7	1.3–25.6	0.02	10.9	0.7–158.9	0.08
CRP (<1.7 mg/dl)	4.5	1.2–17.4	0.03	240	1.4–408.8	0.03
Presence of DM	10.2	1.1–93.4	0.04	14.9	0.4–573.2	0.15
MCSF (>1.9 ng/ml)	5.4	1.4–21.1	0.01	22.8	1.5–344.8	0.02

OR = odds ratio; CI = confidence interval; HD = haemodialysis; HT = hypertension; Ca = calcium; P = phosphorus; TC = total cholesterol; TG = total triglyceride; CRP = C-reactive protein; DM = diabetes mellitus; MCSF = macrophage colony-stimulating factor.

The serum MCSF levels of these five patients were 2.3, 2.4, 2.9, 3.2 and 3.8 ng/ml, all of which were higher than the 75th percentile of the study group.

The initial studies on the primary roles of MCSF suggested that this factor stimulates monocyte activation and proliferation [6,7]. However, MCSF subsequently was reported to be associated with several other important factors that regulate the development of atherosclerosis, such as interleukins and lipid metabolism [14,15]. Based on these reports, we speculated that serum MCSF levels in HD patients might reflect such complex functions and inter-relationships, and that it might be a significant predictor of aortic calcification. However, because we measured aortic wall calcification on CT scans, which provides only a measure of calcium contents of the vessel wall, one limitation of this study is that we could not correlate serum MCSF with increased plaque formation and aortic occlusion. Another limitation of our study is the small sample size. Despite these limitations, we speculate that the functional and clinical significance of the serum MCSF level in the development of atherosclerosis in HD patients is a real effect.

In the present study, the pathogenic role of MCSF in atherosclerosis was emphasized. However, monocytes and monocyte-derived macrophages also have anti-atherogenic functions. Briefly, these cells can remove the surplus lipid and clear macrophages that are filled with lipid in early atherosclerotic lesions [16]. The balance between anti- and pro-atherogenic effects depends on the biological milieu, for example the presence of a variety of cytokines, nitric oxide (NO) and lipid accumulation [15,17]. However, these factors are presumed to have adverse effects in HD patients, i.e. increased serum levels of inflammatory cytokines, including interleukin-1 (IL-1) and tumour necrosis factor, and decreased NO production have been reported in patients with CRF and HD [18,19]. Under these conditions, MCSF and macrophages activated by MCSF might act mainly as stimulators of atherosclerosis. Indeed, Ando *et al*. [20] reported that uraemic serum enhanced and activated the scavenger receptor, which plays a leading role in atherogenesis, in the human monocyte cell line. To our knowledge, there are no reports indicating that MCSF has anti-atherogenic functions or inhibits the development of atherosclerosis in HD patients. In addition to these factors, CRP also plays important roles in the development of atherosclerosis and activation of macrophages. Our results also support these early findings. Thus, the process of atherosclerogenesis is very complex, and our results add the involvement of MCSF in the inflammatory and immune processes.

In conclusion, we showed that the serum MCSF level correlated positively with aortic calcification at baseline. Furthermore, in HD patients with elevated MCSF levels, a significant increase in calcification was first noted at 4 years and the increased levels were maintained thereafter. Using logistic regression analysis, an elevated serum MCSF level was identified as a useful predictor for the development of atherosclerosis. MCSF was also identified as an independent factor in the multivariate analysis model that included several risk factors. Our results may help in planning preventive strategies for atherosclerotic disease in HD patients.

Acknowledgements. The authors thank Dr F. G. Issa (www.word-medex.com.au) for the careful reading and editing of the manuscript.

Conflict of interest statement. None declared.

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Received for publication: 23.10.04
Accepted in revised form: 8.4.05