Intravenous Iron Therapy as a Possible Risk Factor for Atherosclerosis in End-stage Renal Disease

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SUMMARY

Atherosclerosis is a disease of the arterial wall, with increasing wall thickness representing an early event in the progression of the disease. It has been suggested that iron overload, as assessed by increased serum ferritin concentration, may be a risk factor for atherosclerosis.

The aim of this study was to investigate the relationship between the influence of intravenous (IV) iron therapy and ferritin levels and carotid intima media thickness (C-IMT) in dialysis patients. Sixty patients (51 ± 14) years were divided into two groups according to their IMT obtained by ultrasound; group I (high risk) and group II (low risk). The parameters assessed were serum creatinine, urea, calcium, phosphorus, hemoglobin, albumin, uric acid, iron, ferritin, and lipid levels. Thirty-eight patients (88%) in group I and 5 patients (12%) in group II received IV iron therapy while 5 patients (29%) in group I and 12 patients (71%) in group II ($P < 0.001$) did not receive IV iron therapy. Ferritin levels were higher in group I than in group II (581 ± 303 and 306 ± 224) ($P < 0.001$). C-IMT measurements correlated with serum ferritin and with the intravenous iron dose received during the 24 months preceding the study (r = 0.315, $P = 0.015$; r = 0.471, $P = 0.001$).

The findings indicate that IV iron therapy and elevated serum ferritin levels may cause an increase in the incidence of atherosclerosis. (Int Heart J 2005; 46: 255-264)

Key words: Atherosclerosis, Carotid intima media thickness, Ferritin, Iron

CARDIOVASCULAR disease is the major cause of death in patients with end-stage renal disease (ESRD) and the incidence of atherosclerosis-related complications is significantly higher in dialysis patients than in nonuremic controls.[1)] Atherosclerosis is a disease of the arterial wall, with increasing wall thickness representing an early event in the progression of the disease. B-mode ultrasound is a well-established method with which to evaluate atherosclerosis in peripheral

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arteries, and thickened common carotid IMT is also an indicator of early-phase coronary atherosclerosis.[2)]

There are several risk factors whose association with atherosclerosis is well established, including age, gender, lipid disorders, smoking, hypertension, diabetes mellitus, obesity, and lifestyle.[3)] Yet, about 25 percent of cardiovascular disease (CVD) deaths in men and 15 percent in women occur in persons with lower multivariate Framingham Study risk scores in the lowest two quintiles according to standard risk factors.[4)] It has been suggested that about half of atherosclerosis cases cannot be attributed to standard risk factors.[5)] This fact has recently stimulated a call for investigation of new risk factors.[6)]

Anemia is a major cause of morbidity and mortality in hemodialysis (HD) patients. Optimal anemia management, usually requiring erythropoietin (EPO) therapy, improves the life of end-stage renal disease patients.[7)] Functional iron deficiency clearly limits the ability of EPO to support erythropoiesis, therefore, avoiding functional iron deficiency has cost benefits associated with reduced EPO dose requirements.[8)] The National Kidney Foundation - Dialysis Outcomes Quality Initiative (NKF-DOQI) Guidelines for Anemia Management recommend that intravenous iron be used to prevent iron deficiency and support erythropoiesis.[9)] However, use of parenteral iron can produce iron overload, has been linked with tissue deposition of iron, and potentially can increase oxidative stress, thereby inducing cardiac disease.[7)] The purpose of this study was to investigate the relationship between the influence of intravenous (IV) iron therapy and ferritin levels and carotid intima media thickness (IMT) as assessed by ultrasound in a group of dialysis patients.

METHODS

We studied 60 ESRD patients (age, 51 ± 14) from the hospital dialysis unit of Gazi University Medical Center (Ankara, Turkey). The study protocol was approved by Gazi University, and the patients were informed of the purpose of the study before giving their voluntary consent. Patients who had been on dialysis three times weekly for at least 24 months (44.9 ± 26.7) were included. Seventy percent were male, 30% female, 20% had insulin dependent diabetes mellitus, and 68% were being treated with antihypertensive drugs. The patients were divided into two groups according to carotid intima-media thickness (C-IMT) obtained by ultrasound. Group I patients had an IMT ≥ 0.75 mm or abnormality of the arterial wall (eg, atherosclerotic plaque), group II patients had an C-IMT and no plaque of the arterial wall had an C-IMT < 0.75 mm. Intima-medial thickening is an abnormality of the arterial wall and a value of 0.75 mm was considered to be a critical threshold beyond which plaque formation is accelerated.[10)]

Mean age was 55 ± 13 in group I (n = 43) and 41 ± 13 in group II (n = 17) (P < 0.05). The parameters used to assess predialysis were serum creatinine, urea, calcium, phosphorus, hemoglobin, albumin, iron, ferritin, uric acid, parathyroid hormone (PTH), C-reactive protein, and lipids. C-reactive protein was measured using a high-sensitivity latex particle turbidimetric assay (COBAS INTEGRA 400, Roche Diagnostics, Turkey). Other standard hematological and biochemical laboratory tests were measured routinely. Patients who had high cholesterol levels and had received lipid-lowering agents or diet therapy were considered to have hypercholesterolemia. Ferritin, hs-CRP, and PTH levels were measured every six months and their medium values taken. These parameters were only measured every six months because the health insurance of the patients did not pay for more measurements. The other biochemical parameters were measured every month and their medium values were taken.

Patients with acute or chronic infections or chronic systemic disease, active liver disease, acute myocardial infarction, or cerebrovascular disease were excluded. Patients were dialyzed with hemophane membranes matched to body surface area (1.2 - 2.0 m^2) and bicarbonate-buffered dialysate. Intravenous iron sucrose treatment in conjuction with erythropoietin was performed according to the NKF/DOQI guidelines (Hgb levels 11 to 12 g/dL, Hct 33% to 36%). Intravenous iron sucrose was prescribed when the transferrin saturation was < 20% and/or serum ferritin was < 100 pg/mL. The iron therapy was interrupted when the ferritin levels were ≥ 800 pg/mL, and/or the transferrin saturation was ≥ 50%. Iron sucrose was diluted in 100 mL 0.9% sodium chloride for infusion and administered in the last 30 minutes of the hemodialysis session. After the loading dose, 50 to 100 mg of iron therapy was given weekly.

Sonographic examination of the bilateral carotid arteries was performed in the supine position by a single examiner who was blinded to the clinical and biochemical data. All patients were evaluated by high-resolution ultrasound using a Logic 9 (GE Corp. Waukesha, WI) system and a 7.5 MHz linear array transducer. Intima-media thickness was described as the distance from the leading edge of the lumen-intima interface to the media-adventitia interface of the carotid artery. Intima-media thickness of the right and left common carotid arteries was measured within the 1 cm segment proximal to the dilatation of the carotid bulb, at sagittal planes. The carotid plaques were excluded while measuring intima-media thickness. All measurements were made manually on still images obtained during sonographic scanning.

During the screening visit, weight and height were measured with the patients in light clothing without shoes. Body mass index was calculated by dividing weight by the square of height. The presence or absence of coronary artery disease (CAD) was recorded. Subjects were considered to have hyperten-

sion if a history of hypertension requiring treatment was recorded. A smoking habit was defined as current smoking and dialysis durations were recorded.

Statistical analysis: Results are expressed as the mean ± SD. Comparisons between groups were performed using the unpaired Student's *t* test. Correlations were evaluated using Pearson's correlation test. Significant risk factors were evaluated using logistic regression and multivariate regression analysis. Differences were considered significant at $P < 0.05$. Statistical analyses were performed using SPSS 9.0 software.

RESULTS

Demographic and clinical data, IMT measurements, and biochemical characteristics are presented in Table I. The incidence of diabetes mellitus was slightly greater in the high vascular risk group (group I), although the difference was not significant ($P = 0.081$). The duration of dialysis was longer in group I than in group II ($P = 0.001$). Ferritin levels were higher in group I than in group II (581 ± 303 and 306 ± 224) ($P < 0.001$).

Table I. Clinical and Biochemical Characteristics

	Group I (high risk) $n = 43$	Group II (low risk) $n = 17$	*P* values
Age	55 ± 13	41 ± 13	0.001*
Sex, male/female	30/13	12/5	0.95
Hypertension ($n = 41$)	72%	59%	0.319
Diabetes mellitus ($n = 12$)	26%	6%	0.081
Smoking ($n = 26$)	55%	19%	0.014*
Duration of dialysis (months)	51 ± 29	31 ± 13	0.008*
Body mass index	23 ± 5	23 ± 4	0.77
Ferritin (ng/mL)	581 ± 303	306 ± 224	0.001*
C-reactive protein (mg/L)	7.56 ± 3.61	9.26 ± 4.96	0.205
Blood urea nitrogen (mg/dL)	71 ± 22	62 ± 22	0.15
Creatinine (mg/dL)	8.6 ± 2.4	8.9 ± 1.9	0.67
Uric acid (mg/dL)	6.2 ± 1.2	6.3 ± 1.6	0.77
Total cholesterol (mg/dL)	189 ± 54	169 ± 50	0.19
LDL cholesterol (mg/dL)	118 ± 40	104 ± 32	0.20
HDL cholesterol (mg/dL)	35 ± 8	32 ± 7	0.18
Hypercholesterolemia**($n = 24$)	47%	24%	0.101
Triglycerides (mg/dL)	169 ± 89	157 ± 75	0.64
Albumin (g/dL)	3.8 ± 0.2	3.7 ± 0.3	0.22
Parathyroid hormone (PTH) (1.5-6.4 pmol/L)	13.5 ± 6.5	14 ± 6.9	0.56
Calcium-phosphorus products	42.4 ± 7.6	41.9 ± 9	0.73

**Patients with high cholesterol levels who have received lipid lowering agents and/or diet therapy.
*Statistically significant.
Each group is defined depending on common carotid intima/media thickness: high vascular risk as thickness > 0.75 mm or arteriosclerotic plaque and low vascular risk as thickness < 0.75 mm.

Forty-three patients had received IV 100 mg ferric saccharate once a week or twice a month for the last two years while the other 17 patients had not received IV ferric saccharate. Of the patients who received IV iron therapy, 38 (88%) were in group I and 5 (12%) were in group II. Of the patients who did not receive IV iron therapy, 5 (29%) were in group I and 12 (71%) were in group II ($P < 0.001$). Age (OR 1.09, 95% CI: 1.02-1.2) ($P = 0.01$), smoking (OR 7.2, 95% CI: 1.3- 39.3) ($P = 0.02$) and ferritin (OR: 1.5 95% CI: 1.12-2.07) ($P = 0.006$) were found to be significant risk factors in logistic regression analysis of risk factors associated with group I and group II.

C-IMT was 0.98 ± 0.21 mm in the patients who received IV iron therapy and 0.7 ± 0.17 mm in the patients who did not receive IV iron therapy ($P < 0.001$). C-IMT was 0.99 ± 0.24 mm in the patients with hypercholesterolemia and 0.84 ± 0.21 mm in the patients without hypercholesterolemia ($P = 0.013$).

Serum ferritin was significantly correlated with the dose of elemental iron administered during the preceding 24 month period ($r = 0.430$, $P = 0.001$) (Figure 1), but it was not correlated with C-reactive protein ($r = 0.28$, $P = 0.831$). C-IMT was significantly correlated with age ($r = 0.389$, $P = 0.002$), ferritin levels ($r = 0.315$, $P = 0.014$), and the total dose of elemental iron ($r = 0.471$, $P = 0.001$) (Figures 2 and 3). In the multivariate regression analysis, risk factors such as age, ferritin, hypercholesterolemia, intravenous iron dose, and smoking that were

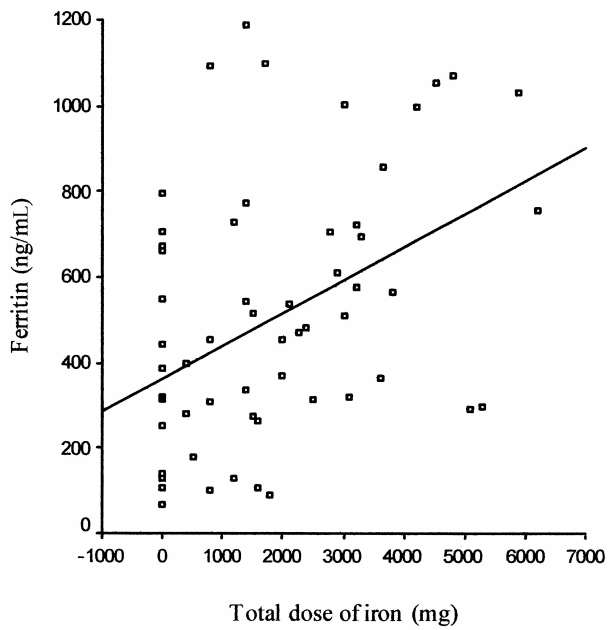


Figure 1. Correlation between annual intravenous iron dose and serum ferritin ($r = 0.430$, $P = 0.001$)

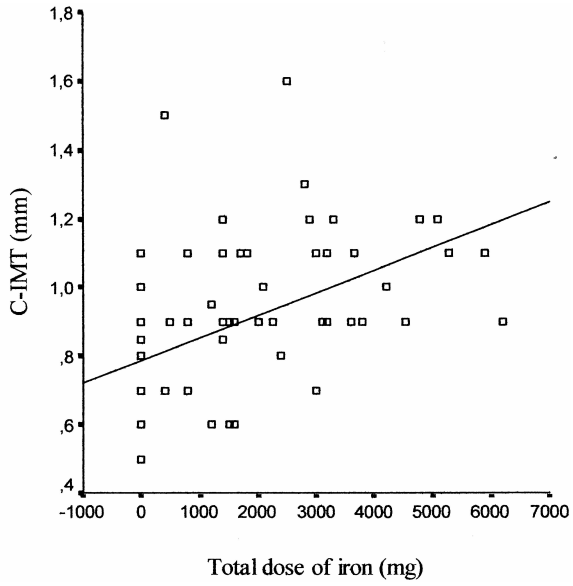


Figure 2. Correlation between iron dose and C-IMT (r = 0.471, *P* = 0.001)

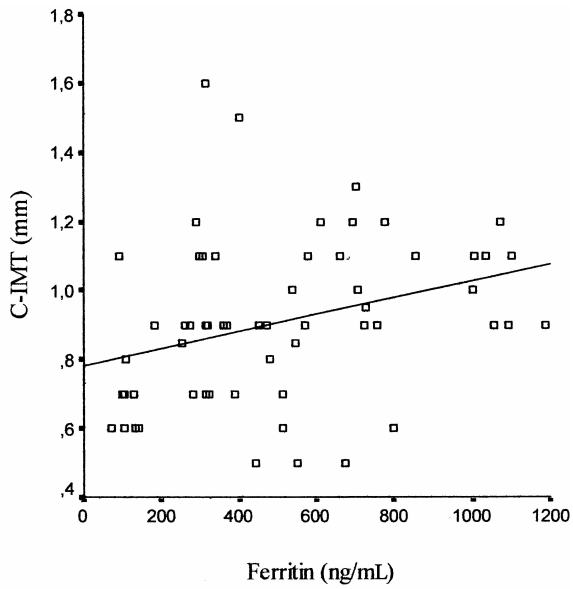


Figure 3. Correlation between serum ferritin and C-IMT (r = 0.315, *P* = 0.014)

significantly associated with C-IMT were included. The results of multivariate regression analysis of the relationship between the risk factors and C-IMT are shown in Table II. C-IMT was significantly associated with the total dose of elemental iron ($r^2 = 0.209$, $P = 0.001$), age ($r^2 = 0.293$, $P = 0.012$), and hypercholesterolemia ($r^2 = 0.337$, $P = 0.034$).

DISCUSSION

CCA-IMT is considered an early marker of the atherosclerotic process and is currently used to assess the presence and progression of atherosclerosis.[2,11] Epidemiological studies have showed that increased CCA-IMT was correlated with known cardiovascular risk factors and was associated with an increased prevalence and incidence of cardiovascular disease.[12,13] Confirming data in the literature, we found that the CCA-IMT increased with age and serum ferritin levels.[14-16] We also observed that the duration of dialysis was longer in group I patients than in group II patients. Group I patients were older than group II patients and C-IMT increased significantly with age. In our study, there were more diabetic and hypertensive patients in group I than in group II, although the differences were not significant. This statistical finding could provide a rational explanation for our observation that was caused by the relatively small number of patients (see Table II).

There is increasing epidemiological evidence concerning the role of iron in atherosclerosis and ischemic heart disease. Experimental models show that increased tissue iron amplifies free radical-mediated tissue damage. This tissue injury leads to the production of superoxide radicals which can reduce ferric ion to ferrous ion and generate free radicals.[7] In 1981, Sullivan first proposed the so-called “iron hypothesis” suggesting that the regular menstrual loss of iron, rather than other effects of estrogen, protects women against CVD.[17] Iron is an essential metal involved in a wide spectrum of physiological functions in the body, such as oxygen transport and enzymatic reactions. Nevertheless, excess iron can be dangerous because it promotes the generation of free radicals which subsequently lead to tissue damage.[18]

Table II. Multivariate Analysis of Risk Factors Associated With C-IMT

Dependent variable Independent variable	*P*	Incremental Sequential r^2	Cumulative Sequential r^2	Partial r^2
C-IMT				
Annual iron dose	0.001	0.222	0.222	0.209
Age, years	0.012	0.095	0.317	0.293
Hypercholesterolemia	0.034	0.053	0.370	0.337

$r^2 = 0.370$, F ratio = 10.97, $P < 0.001$

We found that ferritin levels were higher in group I than in group II. Our results show that the annual dose of intravenous iron was significantly correlated with serum ferritin and C-IMT. This association was independent of serum C-reactive protein, which was used as a marker of systemic inflammation.

Oxidative modification of low-density lipoprotein (LDL) cholesterol appears to be one of the pivotal steps in atherogenesis.[19] Native LDL cholesterol, whose serum levels correlate well with CHD risk, has a weak atherogenic effect.[19,20] Oxidized LDL cholesterol has several biological effects that may contribute to the progression of atherosclerotic lesions, such as cytotoxicity to endothelial and smooth muscle cells, inhibition of endothelial cell-dependent arterial relaxation, and reduced motility of macrophages.[21,22] In this study, the total cholesterol and LDL cholesterol levels in group II patients were higher than in group I patients, however, the difference was not significant. We expect that these results may be related to the patients who had high cholesterol levels and received lipid lowering agents and/or diet therapy.

The routine administration of intravenous iron preparations, as a means for anemia correction in most HD patients, has been shown to improve both survival and quality of life.[23,24] Iron saccarate is considered a safe and efficacious preparation.[25,26] However, parenteral iron overload has been linked with tissue deposition of iron, and potentially can increase oxidative stress, thereby inducing cardiac disease.[7] Drueke, *et al* found that C-IMT and the carotid wall-to-lumen ratio were associated with plasma advanced oxidation protein products, serum ferritin, and annual intravenous iron dose administered.[16] Prospective results from the Bruneck study in humans provided strong evidence for a role of iron status in coronary heart disease.[27] In contrast, an analysis of prospective studies in nonuremic populations did not provide good evidence of a strong association between iron status and coronary heart disease.[28] In the present study, we found that there is a significant relationship between C-IMT, ferritin, and the cumulative dose of intravenous iron given to treat anemia. In patients given IV iron therapy, C-IMT was higher than in patients who were not.

In conclusion, the findings indicate that IV iron therapy and elevated serum ferritin levels may cause an increase in the incidence of atherosclerosis. We acknowledge that to confirm these preliminary results and to firmly establish the relationship between supplemental iron administration, excess iron, and cardiac disease, large prospective studies are needed.

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REFERENCES

1. Pascazio L, Bianco F, Giorgini A, Galli G, Curri G, Panzetta G. Echo color doppler imaging of carotid vessels in hemodialysis patients: evidence of high levels of atherosclerotic lesions. Am J Kidney Dis 1996; 28: 713-20.
2. Sonoda M, Yonekura K, Yokoyama I, Takenaka K, Nagai R, Aoyagi T. Common carotid intima-media thickness is correlated with myocardial flow reserve in patients with coronary artery disease: a useful non-invasive indicator of coronary atherosclerosis. Int J Cardiol 2004; 93: 131-6.
3. Meyers DG. The iron hypothesis: does iron play a role in atherosclerosis? Transfusion 2000; 40: 1023-9. (Review)
4. Leaverton PE, Sorlie PD, Kleinman JC, *et al*. Representativeness of the Framingham risk model for coronary heart disease mortality: a comparison with a national cohort study. J Chronic Dis 1987; 40: 775-84.
5. Heller RF, Chinn S, Pedoe HD, Rose G. How well can we predict coronary heart disease? Findings in the United Kingdom Heart Disease Prevention Project. Br Med J 1984; 288: 1409-11.
6. Hennekens CH. Increasing burden of cardiovascular disease: current knowledge and future directions for research on risk factors. Circulation 1998; 97: 1095-102.
7. Besarab A. Iron and cardiac disease in the end-stage renal disease setting. Am J Kidney Dis 1999; 34(Suppl): s18-24. (Review)
8. Khan A, Besarab A, Amin NB, Vogel SE, Gupta A, Zazra J, Frinak S, Anandan JV, Zasuwa G. Are the optimal iron parameters for erythropoiesis during maintenance erythropoietin (epo) therapy? J Am Soc Nephrol 1998; 9: 152-A (Abstr).
9. National Kidney Foundation-Dialysis Outcomes Quality Initiative: NKF-DOQI clinical practice guidelines for the treatment of anemia of chronic renal failure. Am J Kidney Dis 1997; 30(Suppl): s192-240.
10. Perret F, Depairon M, Bovet P, Darioli R, Despland P, Abel M, Kappenberger I. Carotid and femoral intima-media thickness (IMT) in the Seychelles heart study 1994: reproducibility and mean values according to age and sex. *1st International Symposium on Arterial Wall Thickening*. Paris; France: 1995.
11. Salonen R, Salonen JT. Progression of carotid atherosclerosis and its determinants: a population-based ultrasonography study. Atherosclerosis 1990; 81: 33-40.
12. Burke GL, Evans GW, Riley WA, *et al*. Arterial wall thickness is associated with prevalent cardiovascular disease in middle-aged adults. The Atherosclerosis Risk in Communities (ARIC) Study. Stroke 1995; 26: 386-91.
13. Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE. Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. Circulation 1997; 96: 1432-7.
14. Schmidt-Trucksass A, Grathwohl D, Schmid A, *et al*. Structural, functional, and hemodynamic changes of the common carotid artery with age in male subjects. Arterioscler Thromb Vasc Biol 1999; 19: 1091-7.
15. Kiechl S, Willeit G, Egger G, Poewe W, Oberhollenzer F. Body iron stores and the risk of carotid atherosclerosis: prospective results from the Bruneck study. Circulation 1997; 96: 3300-7.
16. Drueke T, Witko-Sarsat V, Massy Z, *et al*. Iron therapy, advanced oxidation protein products, and carotid artery intima-media thickness in end-stage renal disease. Circulation 2002; 106: 2212-7.
17. Sullivan JL. Iron and the sex differences in heart disease risk. Lancet 1981; 1: 1293-4.
18. Lee TS, Shiao MS, Pan CC Chau LY. Iron -deficient diet reduces atherosclerotic lesions in apoE-deficient mice. Circulation 1999; 99: 1222-9.
19. Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. N Engl J Med 1989; 320: 915-24. (Review)
20. Holvoet P, Collen D. Oxidation of low density lipoproteins in the pathogenesis of atherosclerosis. Atherosclerosis 1998; 137(suppl): S33-8. (Review)
21. Esterbauer H, Ramos P. Chemistry and pathophysiology of oxidation of LDL. Rev Physiol Biochem Pharmacol 1996; 127: 31-64. (Review)
22. Watanabe T, Pakala R, Katagiri T, Benedict CR. Oxidized low-density lipoproteins potentiate the mitogenic effect of 5-hydroxytryptamine on vascular smooth muscle cells. Jpn Heart J 2002; 43: 35-42.
23. Fishbane S, Maesaka JK. Iron management in end-stage renal disease. Am J Kidney Dis 1997; 29: 319-33. (Review)
24. Macdougall IC, Chandler G, Elston O, Harchowal J. Beneficial effects of adopting an aggressive intravenous iron policy in a hemodialysis unit. Am J Kidney Dis 1999; 34(suppl 2): S40-6.

25. Charytan C, Levin N, Al-Saloum M, Hafeez T, Gagnon S, Van Wyck DB. Efficacy and safety of iron sucrose for iron deficiency in patients with dialysis-associated anemia: North American clinical trial. Am J Kidney Dis 2001; 37: 300-7.
26. Chandler G, Harchowal J, Macdougall IC. Intravenous iron sucrose: establishing a safe dose. Am J Kidney Dis 2001; 38: 988-91.
27. Kiechl S, Willeit G, Egger G, Poewe W, Oberhollenzer F. Body iron stores and the risk of carotid atherosclerosis: prospective results from the Bruneck study. Circulation 1997; 96: 3300-7.
28. Danesh J, Appleby P. Coronary heart disease and iron status: meta-analyses of prospective studies. Circulation 1999; 99: 852-4.