Nephrol Dial Transplant (2005) 20: 1676–1685
doi:10.1093/ndt/gfh891
Advance Access publication 17 May 2005

Original Article

Nephrology Dialysis Transplantation

Associations of serum fetuin-A with malnutrition, inflammation, atherosclerosis and valvular calcification syndrome and outcome in peritoneal dialysis patients

Angela Yee-Moon Wang[1], Jean Woo[2], Christopher Wai-Kei Lam[3], Mei Wang[1], Iris Hiu-Shuen Chan[3], Ping Gao[4], Siu-Fai Lui[1], Philip Kam-Tao Li[1] and John E. Sanderson[1]

[1]Department of Medicine and Therapeutics, [2]Center for Nutritional Studies and [3]Department of Chemical Pathology, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, New Territories, Hong Kong and [4]Epitope Diagnostics, Inc., San Diego, CA, USA

Abstract

Background. Fetuin-A (α_2-Heremans Schmid glycoprotein) has recently been identified as a circulating inhibitor of calcification and is regulated as a negative acute phase protein. However, its relationships with cardiac valvular calcification and atherosclerosis and outcome have not been evaluated in peritoneal dialysis (PD) patients.

Method. We performed a prospective follow-up study in 238 PD patients with echocardiography done at baseline to detect cardiac valvular calcification and biochemical analysis performed for serum fetuin-A, albumin and C-reactive protein (CRP).

Results. Baseline serum fetuin-A concentration was (mean ± SD) 0.309 ± 0.068 g/l (normal range 0.4–0.95). Across the three tertiles of increasing serum fetuin-A, a significant trend effect was observed for age ($P = 0.023$), diabetes ($P = 0.008$), background atherosclerotic vascular disease ($P = 0.010$), cardiac valvular calcification ($P = 0.002$), serum albumin ($P < 0.001$), subjective global assessment ($P = 0.005$) and CRP ($P < 0.001$). Adjusting for CRP and calcium × phosphorus product, every 0.01 g/l increase in serum fetuin-A remained independently associated with a 6% decrease in the risk of valvular calcification (95% confidence intervals, 0.90–0.99; $P = 0.028$). Furthermore, serum fetuin-A showed a significant decrease across the four groups of patients with increasing components of the malnutrition, inflammation, atherosclerosis/calcification (MIAC) syndrome ($P < 0.001$) and was the lowest among patients with all components of the MIAC syndrome (0.263 ± 0.055 g/l) and highest among those who do not have the MIAC syndrome at all (0.338 ± 0.063 g/l). Lower serum fetuin-A was associated with greater all-cause mortality ($P = 0.0011$) and fatal and non-fatal cardiovascular events ($P = 0.0017$), but its significance was lost when atherosclerotic vascular disease, valvular calcification, inflammation and malnutrition were included in the model.

Conclusions. Serum fetuin-A showed important associations with valvular calcification, atherosclerosis, malnutrition and inflammation, and was linked to mortality and cardiovascular events in PD patients via its close relationships with the MIAC syndrome.

Keywords: atherosclerosis; calcification; fetuin-A; inflammation; malnutrition; peritoneal dialysis

Introduction

According to data from the USRDS, the mortality rate of end-stage renal disease (ESRD) patients is at least 10–20 times higher than age- and gender-matched controls and nearly half of the deaths are accounted for by cardiovascular causes [1]. Vascular and valvular calcification were identified recently as important predictors of mortality in ESRD patients on maintenance dialysis [2,3]. A study by Goodman and colleagues showed that >80% of young patients on dialysis had extensive coronary artery calcification on electron beam computed tomography, and the coronary calcification showed rapid progression with increasing time on dialysis [4]. Even though hyperphosphataemia and increased calcium intake with resulting high calcium × phosphorus product are important causes of calcification in ESRD patients [4], there are recent

Correspondence and offprint requests to: Dr Angela Y. M. Wang, Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, New Territories, Hong Kong. Email: awang@cuhk.edu.hk

data to suggest that inflammation also contributes to the calcification process [5]. A previous study by our group demonstrated a direct link between valvular calcification and inflammation in patients on continuous peritoneal dialysis (PD) [6]. Furthermore, pro-inflammatory cytokines have been shown to enhance *in vitro* calcification of vascular cells [7], suggesting a close relationship between inflammation and calcification.

Human fetuin-A (α_2-Heremans Schmid glycoprotein; AHSG), a 59 kDa glycoprotein synthesized in the liver and ubiquitously present in the extracellular space, has been identified as a potent circulating inhibitor of the calcification process and is downregulated in the presence of inflammation [8]. A recent study by Ketteler and co-workers reported an inverse relationship between serum fetuin-A and C-reactive protein (CRP) [9]. Haemodialysis patients with low serum fetuin-A were noted to have greater all-cause and cardiovascular mortality [9]. Furthermore, serum from patients with a low serum fetuin-A concentration showed reduced capacity to inhibit calcification [9]. However, it remains unknown whether serum fetuin-A is associated with cardiac valvular calcification or atherosclerotic vascular disease (AVD) in dialysis patients.

With this background, we examined serum fetuin-A in relation to cardiac valvular calcification, atherosclerosis, inflammation and malnutrition or the so-called MIAC syndrome in chronic PD patients and evaluated serum fetuin-A and the MIAC syndrome in relation to the clinical outcomes of chronic PD patients.

Subjects and methods

This is a single centre study performed in 238 Chinese ESRD patients undergoing continuous ambulatory peritoneal dialysis (CAPD) for at least 3 months at the dialysis unit in the Prince of Wales Hospital in Hong Kong. They were prospectively enrolled into the study starting in April 1999 after obtaining approval from the Human Research Ethics Committee of the Chinese University of Hong Kong. Informed consent was obtained from all patients before study entry. These patients represented 88% of the total PD population at our unit. The remaining 12% of patients were excluded based on the exclusion criteria, namely patients with underlying malignancy, chronic liver disease, chronic obstructive airways disease, systemic lupus erythematosus, chronic rheumatic heart disease, congenital heart disease or patients with incomplete data. Peritoneal dialysis accounts for nearly 80% of the dialysis population in Hong Kong.

All patients underwent echocardiography, measurement of dialysis adequacy and biochemical analysis at study baseline. In patients who developed peritonitis, exit site infections, other infective complications or volume overload, all the above assessments were deferred for at least 1 month after complete resolution of the complication.

Echocardiography

Two-dimensional echocardiography was performed with a GE-VingMed System 5 echocardiographic machine (GE-VingMed Sound AB, Horten, Norway) with a 3.3 mHz multiphase array probe in patients lying in the left decubitus position. All echocardiographs were performed according to the recommendations of the American Society of Echocardiography [10] and were analysed by a single experienced cardiologist who was blinded to all clinical details. Cardiac valvular calcification was defined as bright echoes of >1 mm on one or more cusps of the aortic valve, mitral valve or mitral annulus. Sensitivity and specificity for echocardiographic detection of calcium in the mitral valve, mitral annulus and aortic valve were reported to be 76 and 89–94%, respectively [11]. The intra-observer agreement for echocardiographic detection of valvular calcification was 90% (kappa = 0.76) in our study.

Clinical and demographic data collection

Patients' smoking habit, underlying cause of ESRD, presence of diabetes, AVD and duration of dialysis were recorded at study baseline. AVD was defined as the presence of ischaemic heart disease and history of angina, previous myocardial infarction, coronary artery bypass surgery or stenting, cerebrovascular event, transient ischaemic attack or peripheral vascular disease with or without amputation.

Biochemical analysis

At the time of echocardiography, fasting venous blood was collected for the measurement of serum fetuin-A (AHSG), high sensitivity CRP (hs-CRP), serum albumin, calcium, phosphorus and parathyroid hormone (PTH). Serum fetuin-A was determined using a human fetuin-A enzyme-linked immunosorbent assay (ELISA) kit (Epitope Diagnostics, San Diego, CA). The assay utilized the two-site 'sandwich' technique with two selected polyclonal antibodies that bind to different epitopes of human fetuin-A. Intra-assay precision is 4.8–5.5% and inter-assay precision is 5.7–6.8%. hs-CRP was measured using the Tina-quant CRP (Latex) ultra-sensitive assay (D & P modular analyser, Roche Diagnostics GmbH, Mannheim, Germany). Serum albumin was measured using the bromcresol purple method. Calcium and phosphorus concentrations were measured using dye-binding methods on the Dimension AR automatic analyser (D & P modular analyser). PTH was determined by chemiluminescence immunoassay on the Immulite analyser (Diagnostic Products Corp., Los Angeles, CA).

Indices of dialysis adequacy

Total weekly urea clearance (Kt/V) and creatinine clearance (CCr) were measured using standard methods as described previously [12].

Nutritional assessment

Nutrition status was assessed by serum albumin and subjective global assessment (SGA) [13]. SGA includes six subjective assessments, three based on the patient's history

of weight loss, presence of anorexia and vomiting, and three based on the physician's grading of muscle wasting, presence of oedema and loss of subcutaneous fat. Oedema is not a useful index of malnutrition [13] but its presence or absence has to be taken into account when assessing changes in body weight. Based on these assessments, each patient was graded a score that reflected the nutrition status, namely 1 = normal nutrition; 2 = mild malnutrition; 3 = moderate malnutrition; and 4 = severe malnutrition [13].

Study outcome

All patients were followed-up prospectively after the baseline assessments. No patient was lost to follow-up. Patients who underwent kidney transplant were censored at the time of transplantation. The clinical outcomes evaluated were all-cause mortality and fatal and non-fatal cardiovascular events. During follow-up, all deaths and cardiovascular events (including electrocardiographically documented angina, myocardial infarction, heart failure, arrhythmia, transient ischaemic attacks, strokes, other thrombotic events or peripheral vascular disease) were recorded accurately. The attending physician who had no knowledge of the serum fetuin-A result provided the exact cause of death and the nature of the cardiovascular event. The definition of sudden death is described elsewhere [12]. For patients who had multiple cardiovascular events, survival analysis was limited to the first event.

Statistical analysis

Continuous variables were tested for normality with the Kolmogorov–Smirnov test before further statistical analysis. Continuous variables were expressed as the mean ± SD or median (interquartile range) depending on the distribution of data. Patients were stratified into tertiles according to the single time point serum fetuin-A concentration. Comparisons across the three tertiles were performed using one-way analysis of variance (ANOVA) for continuous variables and χ^2 statistics for differences in proportion. Continuous variables that were not normally distributed were log-transformed before comparisons using ANOVA. Trend effect across the three tertiles was evaluated using the trend test. Comparisons among the four groups of patients stratified by the components of the MIAC syndrome were done by one-way ANOVA with Bonferroni's test for *post hoc* analysis. Multiple logistic regression analysis was done to evaluate the relationships between CRP, serum fetuin-A and calcium × phosphorus product with valvular calcification. Survival curves of the three tertiles were generated by the Kaplan–Meier method and compared by the log-rank test. The Cox proportional hazards model was used to estimate the relative risks of all-cause mortality and fatal and non-fatal cardiovascular events for different variables. Factors with $P < 0.05$ on univariate analysis for all-cause and cardiovascular mortality which met the assumptions of proportional hazards were considered further in the multivariate Cox regression analysis for all-cause mortality and fatal and non-fatal cardiovascular events. A *P*-value of <0.05 was considered to be statistically significant. Statistical analysis was performed using SPSS software, version 11.0 (SPSS, Inc., Chicago, IL).

Results

The baseline characteristics of the study population are summarized in Table 1. The causes of ESRD were chronic glomerulonephritis in 75 patients (31.5%), diabetic nephropathy in 58 patients (24.4%), hypertensive nephrosclerosis in 32 patients (13.4%), obstructive uropathy in 13 patients (5.5%), polycystic kidney disease in 12 patients (5.0%), tubulointerstitial nephritis in seven patients (2.9%) and not identified in 41 patients (17.2%). The mean ± SD serum fetuin-A concentration was 0.309 ± 0.068 g/l (reference range 0.4–0.95) and did not differ between men (0.308 ± 0.068 g/l) and women (0.310 ± 0.068 g/l; $P = 0.862$). CRP was 2.31 (0.90, 6.65) mg/l for men and 3.02 (0.92, 9.88) mg/l for women ($P = 0.714$).

Patients were stratified into tertiles according to the single time point serum fetuin-A concentration, namely those with serum fetuin-A ≤0.276 g/l (lower tertile), those with serum fetuin-A between 0.276 and 0.330 g/l (middle tertile) and those with serum fetuin-A ≥0.330 g/l (upper tertile). All patients in the lower and middle tertiles and 62 patients in the upper tertile had serum fetuin-A <0.4 g/l (lower limit of reference range). The clinical and demographic characteristics of patients across the three tertiles of serum fetuin-A are detailed in Table 2. A significant trend effect was observed across the three tertiles of increasing serum fetuin-A concentration for age, presence of diabetes, AVD, cardiac valvular calcification, serum albumin, SGA and CRP. No significant difference was observed in the residual glomerular filtration rate (GFR) across the three tertiles of increasing serum fetuin-A concentration. Vitamin D therapy was used in 36.7, 40.8 and 37.5% of patients in the lower, middle and upper tertile, respectively ($P = 0.859$). Althogether nine patients

Table 1. Baseline characteristics of the study population[a]

Age, years	56 ± 12
Male/female	122/116
Time on CAPD[b], months	26.5 (15–51)
Body mass index, kg/m^2	23.1 ± 3.4
Positive history of smoking, *n* (%)	88 (37)
Diabetes, *n* (%)	73 (31)
Background atherosclerotic vascular complications, *n* (%)	55 (23)
Cardiac valve calcification, *n* (%)	61 (26)
Serum albumin, g/l	28.6 ± 5.1
C-reactive protein[b], mg/l	2.72 (0.92–9.00)
Serum fetuin-A, g/l	0.309 ± 0.068
Calcium × phosphorus product, mg^2/dl^2	53 ± 17
Parathyroid hormone[b], pmol/l	41 (17–74)
Total weekly Kt/V	1.82 ± 0.45
Total weekly CCr, l/week/1.73 m^2	56 ± 22
Residual glomerular filtration rate[b], ml/min/1.73 m^2	0.57 (0–1.87)

[a]Continuous values are mean ± SD unless specified otherwise.
[b]Median (interquartile range).
CAPD = continuous ambulatory peritoneal dialysis; Kt/V = urea clearance; CCr = creatinine clearance.

Table 2. Clinical characteristics of patients across tertiles of serum fetuin-A[a]

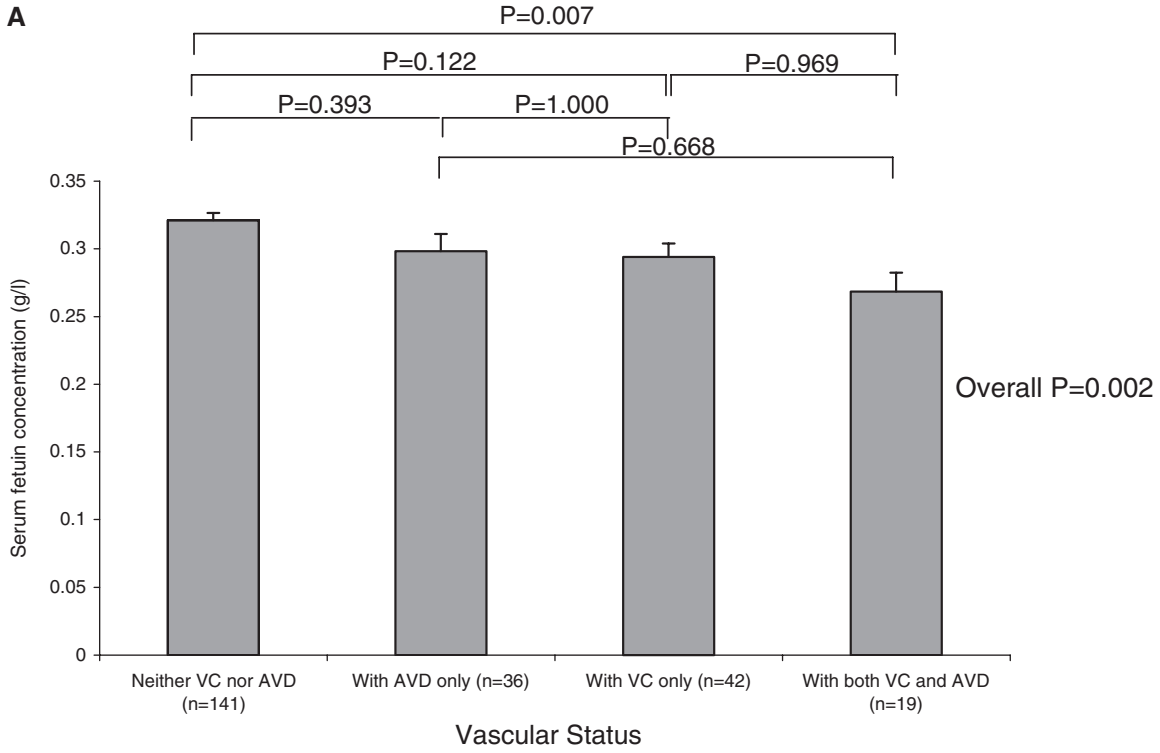
	Serum fetuin-A in tertiles			*P*-value for trend
	Lower ($n=79$)	Middle ($n=71$)	Upper ($n=88$)	
Age, years	58 ± 11	56 ± 12	54 ± 12	0.023
Male gender, %	50.6	56.3	47.7	0.686
Positive smoking history, %	36.7	39.4	35.2	0.832
Diabetes, %	43.0	25.4	23.9	0.008
Renal diagnosis, %				
Chronic glomerulonephritis	25.3	32.4	36.4	0.805
Diabetic nephropathy	31.6	25.4	17.0	
Hypertensive nephrosclerosis	10.1	19.7	11.4	
Others	32.9	22.5	35.2	
Dialysis duration[b], months	29 (13–51)	26 (13–49)	27 (19–54)	0.716
Body mass index, kg/m^2	23.4 ± 3.2	23.1 ± 3.2	22.8 ± 3.8	0.247
Atherosclerotic vascular disease, %	34.2	18.3	17.0	0.010
Valvular calcification, %	36.7	25.4	15.9	0.002
Serum albumin, g/l	26.8 ± 6.2	28.9 ± 4.2	30.0 ± 4.2	<0.001
Subjective global assessment, %				
Well nourished	44.3	57.7	64.8	0.005
Mildly malnourished	32.9	26.8	25.0	
Moderately/severely malnourished	22.8	15.5	10.2	
C-reactive protein[b], mg/l	5.2 (1.7–17.4)	2.8 (1.0–7.8)	1.2 (0.6–4.3)	<0.001
Calcium × phosphorus product, mg^2/dl^2	53 ± 17	52 ± 15	54 ± 18	0.685
Parathyroid hormone[b], pmol/l	40 (18–89)	41 (17–75)	40 (15–63)	0.409
Total weekly Kt/V	1.80 ± 0.48	1.77 ± 0.37	1.87 ± 0.49	0.331
Weekly PD Kt/V	1.52 ± 0.38	1.52 ± 0.34	1.53 ± 0.37	0.772
Total weekly CCr, l/week/1.73 m^2	56.8 ± 23.2	54.6 ± 19.9	57.8 ± 22.5	0.761
Residual GFR[b], ml/min/1.73 m^2	0.56 (0–1.86)	0.50 (0–1.70)	0.66 (0–2.07)	0.987

[a]Continuous values are mean ± SD unless specified otherwise.
[b]Median (interquartile range).
Kt/V = urea clearance; PD = peritoneal dialysis; CCr = creatinine clearance; GFR = glomerular filtration rate.

had previous total parathyroidectomy, with one, five and three patients in the lower, middle and upper tertile, respectively ($P=0.175$).

Serum fetuin-A differed significantly among the four groups of patients stratified on the basis of the presence or absence of AVD and valvular calcification (overall; $P=0.002$). Those with neither AVD nor valvular calcification had the highest serum fetuin-A (0.321 ± 0.064 g/l) while those with both AVD and valvular calcification had the lowest serum fetuin-A (0.268 ± 0.061 g/l; $P=0.007$). Serum fetuin-A did not differ significantly between patients with either valvular calcification (0.294 ± 0.064 g/l) or AVD (0.298 ± 0.077 g/l) (Figure 1A). Adjusting for calcium × phosphorus product and CRP, every 0.01 g/l increase in serum fetuin-A was independently associated with a 6% decrease in the risk of valvular calcification (95% confidence intervals, 0.90–0.99; $P=0.028$; Table 3). Patients were also stratified into four groups on the basis of the presence or absence of inflammation and malnutrition. Inflammation was defined as those with hs-CRP ≥5 mg/l, while malnutrition was defined as those with serum albumin <30 g/l. Serum fetuin-A was significantly different across the four groups of patients stratified on the basis of inflammation and malnutrition (overall; $P<0.001$). Serum fetuin-A was the highest among patients without inflammation and malnutrition (0.336 ± 0.067 g/l) and lowest among those with both inflammation and malnutrition (0.271 ± 0.054 g/l; $P<0.001$). No significant difference was noted in serum fetuin-A between patients with either inflammation (0.315 ± 0.073 g/l) or malnutrition (0.307 ± 0.063 g/l). Patients were stratified into four groups on the basis of the presence of zero, one, two and all three components of the malnutrition, inflammation and atherosclerosis/calcification (MIAC) syndrome. Serum fetuin-A showed a significant decrease across the four groups of patients with increasing components of the MIAC syndrome, with serum fetuin-A being the lowest among patients having all three components (0.263 ± 0.055 g/l) and highest among those who do not have the MIAC syndrome (0.338 ± 0.063 g/l) (Figure 1B).

After follow-up for a mean ± SD of 31.7 ± 13.3 months, 89 patients (37.4%) had died and 28 patients (11.8%) had undergone kidney transplantation. Forty-seven men (38.5%) and 42 women (36.2%) died. During the follow-up period, 50.6% of patients in the lower tertile *vs* 39.4% of patients in the middle tertile *vs* 23.9% of patients in the upper tertile had died ($P=0.007$). The causes of death in our CAPD patients are shown in Table 4. Cardiovascular causes accounted for 60% of the total mortality, followed by infections in 16.9% and peritonitis in 13.5%. Twenty-six men and 27 women died from cardiovascular causes. There were 137 patients who developed



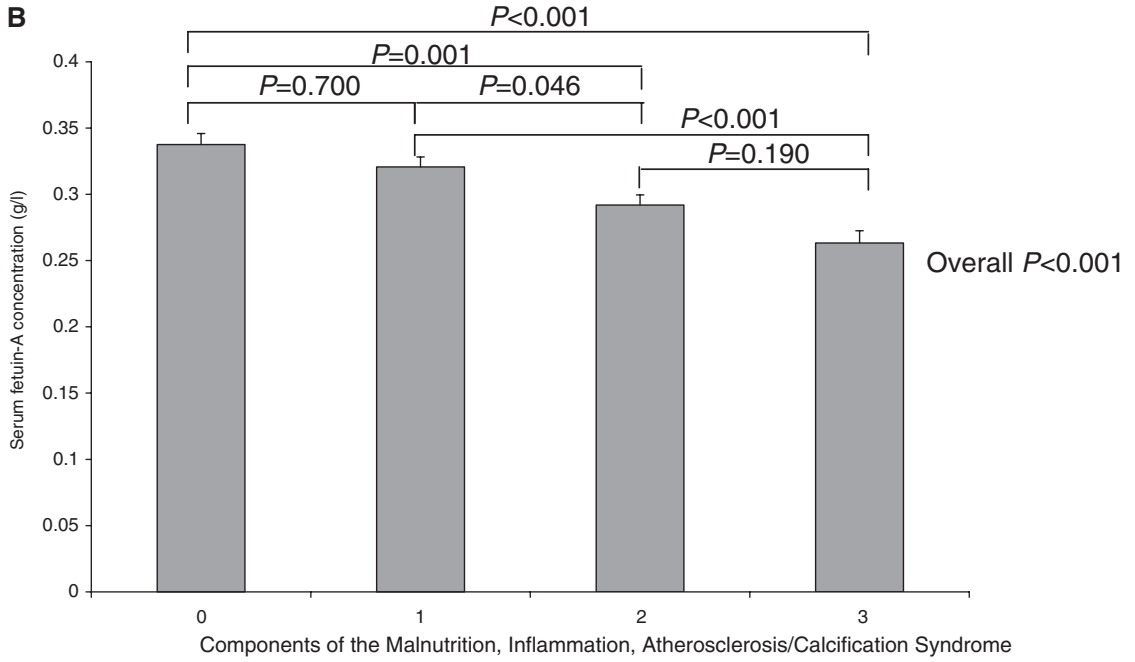


Fig. 1. (**A**) Serum fetuin-A concentration (mean ± SEM) in relation to presence or absence of valvular calcification (VC) and atherosclerotic vascular disease (AVD). (**B**) Serum fetuin-A concentration (mean ± SEM) in relation to the presence of zero ($n = 59$), one ($n = 81$), two ($n = 62$) and all three ($n = 36$) components of the malnutrition, inflammation, atherosclerosis/calcification syndrome. Malnutrition was defined as serum albumin <30 g/l, inflammation was defined as C-reactive protein ≥5 mg/l, atherosclerosis/calcification was defined by the presence of background AVD or cardiac valvular calcification.

one or more cardiovascular events during the follow-up period. Those who developed cardiovascular events subsequently had lower baseline serum fetuin-A (0.299 ± 0.069 g/l) than those who never developed a cardiovascular event during follow-up (0.322 ± 0.064 g/l; $P = 0.008$). The nature of the first cardiovascular event included 15 cases of electrographically documented angina or myocardial infarction, 19 cases of strokes, transient ischaemic attacks or other thrombotic events, six cases of

Table 3. Multiple logistic regression analysis showing relationships between C-reactive protein, fetuin-A, calcium × phosphorus product and cardiac valvular calcification in peritoneal dialysis patients

Variables	Unit increase	Odds ratio (95% CI)	*P*-value
C-reactive protein	1 mg/l	1.02 (1.00–1.04)	0.095
Serum fetuin-A	0.01 g/l	0.94 (0.90–0.99)	0.028
Calcium × phosphorus product	1 mg^2/dl^2	1.03 (1.01–1.05)	0.011

Table 4. Causes of death in continuous ambulatory peritoneal dialysis patients

Cardiovascular mortality	53 (22.1)
Ischaemic heart disease/myocardial infarction	7 (2.9)
Cerebrovascular disease	13 (5.5)
Heart failure	3 (1.3)
Sudden death	24 (10.1)
Arrhythmia	1 (0.4)
Peripheral vascular disease	5 (2.1)
Non-cardiovascular mortality	36 (15.1)
Peritonitis	12 (5.0)
Other infections	15 (6.3)
Malignancy	2 (0.8)
Other causes including dialysis termination	7 (2.9)
Total mortality	89 (37.1)

Expressed as *n* (% of the total study population of 238).

peripheral vascular disease, 83 cases of heart failure, seven cases of electrographically documented arrhythmia and seven cases of sudden cardiac death.

According to the Kaplan–Meier survival analysis, a significant decrease in all-cause mortality ($P = 0.0011$; Figure 2A) and fatal and non-fatal cardiovascular events ($P = 0.0017$; Figure 2B) was observed across the three tertiles of increasing serum fetuin-A. By univariate analysis, serum fetuin-A showed a highly significant association with all-cause mortality ($P < 0.001$) as well as fatal and non-fatal cardiovascular events ($P = 0.002$). Other factors relating to all-cause mortality and fatal and non-fatal cardiovascular events on univariate analysis are detailed in Table 5.

In the multivariate Cox regression analysis, serum fetuin-A was associated with all-cause mortality in the model adjusting for age, diabetes, residual GFR and AVD ($P = 0.002$). However, its significance was gradually lost when additional adjustment was made for cardiac valvular calcification, CRP and serum albumin in a stepwise fashion (Table 6). In the multivariate Cox regression models for fatal and non-fatal cardiovascular events, serum fetuin-A was significant when adjusting for age, positive smoking, diabetes and residual GFR, but did not retain its significance when AVD, valvular calcification, CRP and serum albumin were also controlled for in a stepwise fashion (Table 7).

Discussion

In this study, we observed an important relationship between serum fetuin-A and the MIAC syndrome in PD patients. Patients in the lowest tertile of serum fetuin-A not only had the highest CRP but also showed the greatest prevalence of valvular calcification and AVD and were the most malnourished. Of greater significance is the finding that patients having all components of the MIAC syndrome had the lowest serum fetuin-A. This suggests that fetuin-A not only is a potent inhibitor of extra-osseous calcium phosphate precipitation as shown previously [9], but may also be useful in identifying PD patients complicated with the MIAC syndrome. A previous study by Ketteler *et al.* has reported an inverse relationship between serum fetuin-A and CRP in chronic haemodialysis patients [9]. This agrees well with our current study also showing a negative correlation between serum fetuin-A and CRP ($r = -0.349$, $P < 0.001$) in PD patients. As shown by Lebreton *et al.*, serum fetuin-A is regulated as a negative acute phase protein and its serum concentration falls during the acute inflammatory response and normalizes when the infection is successfully treated [14]. The anti-inflammatory property of fetuin-A was evidenced further by the suppression of tumour necrosis factor release from lipopolysaccharide-stimulated macrophages in an *in vitro* study [15] as well as *in vivo* in a lipopolysaccharide-independent model of acute inflammation [16]. Even though low fetuin-A and high CRP may represent the same biological event, our data suggest that fetuin-A may be useful in further stratifying the severity of the MIAC syndrome in PD patients. Whether it has a causal relationship with the MIAC syndrome requires further investigation.

Fetuin-A is synthesized by hepatocytes and presents as a major soluble inhibitor of calcification in the extracellular space [17,18]. Targeted deletion of the fetuin gene in calcification-sensitive mice has been associated with severe calcification in various organs, suggesting that fetuin-A is an important inhibitor of ectopic calcification acting on the systemic level [17]. Price *et al.* showed that a specific complex of the calcium, phosphate, fetuin-A and matrix Gla protein in the serum prevents bone mineralization in etidronate-treated rats [19]. A recent study by Ketteler and co-workers demonstrated an impaired capacity of serum from AHSG-deficient patients on long-term dialysis with clinical evidence of extra-osseous calcifications to inhibit calcium × phosphorus precipitation *ex vivo*. The impaired capacity to inhibit calcification could be normalized by reconstituting the sera with purified AHSG/fetuin-A [9]. In our study, greater prevalence of valvular calcification was observed among patients with lower serum fetuin-A. Furthermore, the inverse relationship between serum fetuin-A and valvular calcification persisted even after adjusting for CRP and calcium × phosphorus product. This gave additional evidence that serum fetuin-A may

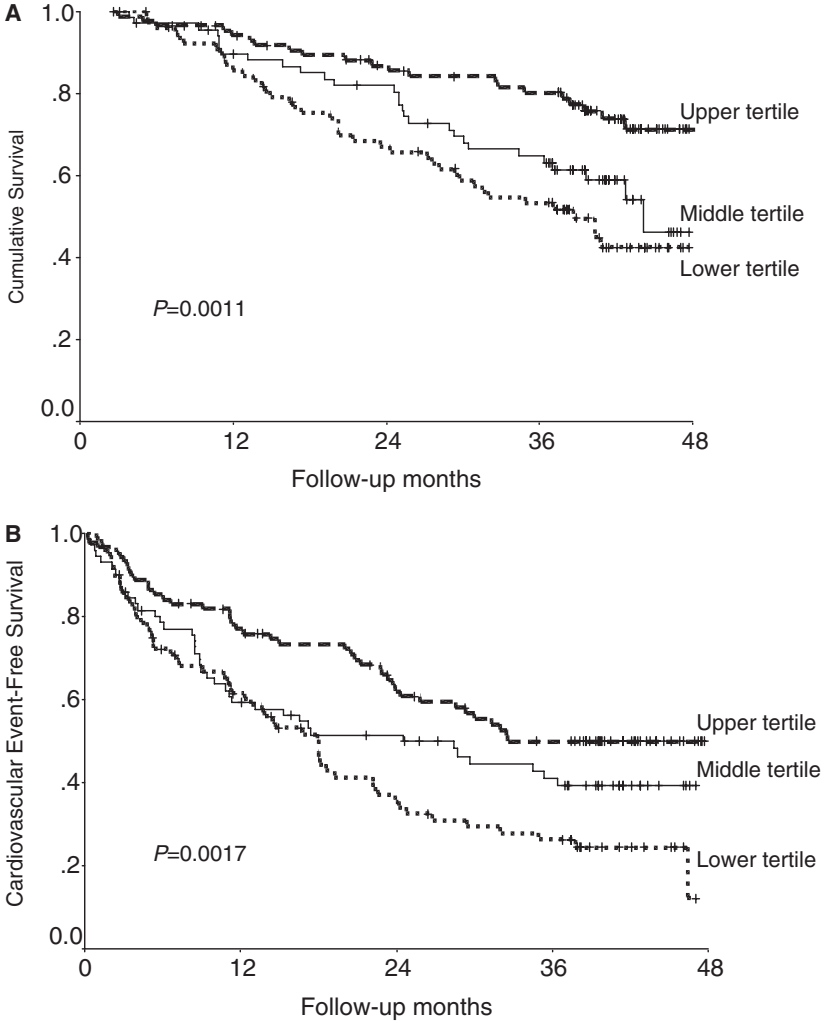


Fig. 2. Kaplan–Meier survival curves in relation to (**A**) all-cause mortality and (**B**) fatal and non-fatal cardiovascular events, according to tertiles of serum fetuin-A. Log-rank test showed significant difference in all-cause mortality between the lower and upper tertiles ($P = 0.0005$) and between the middle and upper tertiles ($P = 0.0292$) but not between the lower and middle tertiles ($P = 0.2151$). Log-rank test showed a significant difference in fatal and non-fatal cardiovascular event rate between the lower and upper tertiles ($P = 0.0002$) but not between the middle and upper tertiles ($P = 0.0911$) or between the lower and middle tertiles ($P = 0.1178$).

indeed be an important inhibitor of calcification in dialysis patients. On the other hand, our study also showed lower serum fetuin-A among PD patients with AVD. In view of the fact that valvular calcification represents a marker of atherosclerosis in dialysis patients other than reflecting an excess calcium × phosphorus load [20], this raises an interesting hypothesis of whether fetuin-A may have a regulatory role in atherogenesis via its anti-inflammatory property and inhibition of calcification. Fetuin-A has been shown to antagonize transforming growth factor and bone morphogenetic proteins that are potent osteogenic growth and differentiation factors possibly involved in atherosclerotic calcification [21]. Fetuin-A/AHSG also inhibits insulin receptor tyrosine kinase activity and is involved in insulin resistance [22], another important risk factor for atherosclerosis. All these are indirect evidence that serum fetuin-A may also be involved in the atherosclerotic type of calcification and warrant further investigation.

Elevated CRP is a well-known predictor of mortality and cardiovascular deaths in dialysis patients [12]. A recent report also linked cardiovascular mortality in haemodialysis patients to lower serum fetuin-A [9] although its significance was lost when CRP was also entered into the model, suggesting its close relationship with CRP as a biological marker of inflammation and extra-osseous calcification. In this study, lower serum fetuin-A was associated with an increased risk of all-cause mortality as well as fatal and non-fatal cardiovascular events in patients on PD, but its significance was lost when the MIAC syndrome was included in a stepwise fashion in the model. This gave additional evidence that serum fetuin-A, being a calcification inhibitor and a negative acute phase reactant, is indeed closely related to the MIAC syndrome that

Table 5. Univariate Cox regression analysis in relation to all-cause mortality as well as fatal and non-fatal cardiovascular events

Factors	Unit increase	All-cause mortality ($n = 89$)		Fatal and non-fatal cardiovascular events ($n = 137$)	
		Hazard ratio (95% CI)	*P*	Hazard ratio (95% CI)	*P*
Age	1 year	1.055 (1.033–1.078)	<0.001	1.022 (1.007–1.038)	0.004
Male gender	–	1.305 (0.860–1.979)	0.210	1.091 (0.780–1.526)	0.611
Positive smoking history	–	1.451 (0.953–2.209)	0.083	1.418 (1.007–1.997)	0.046
CAPD duration	1 month	1.002 (0.995–1.009)	0.537	0.999 (0.993–1.005)	0.690
Diabetes mellitus	–	1.924 (1.265–2.925)	0.002	2.219 (1.573–3.129)	<0.001
Atherosclerotic vascular disease	–	3.029 (1.969–4.658)	<0.001	2.696 (1.866–3.894)	<0.001
Cardiac valvular calcification	–	2.835 (1.856–4.330)	<0.001	2.044 (1.428–2.926)	<0.001
Calcium × phosphorus product	1 mg^2/dl^2	1.000 (0.988–1.012)	0.976	1.005 (0.996–1.015)	0.280
Parathyroid hormone	1 pmol/l	1.003 (0.999–1.007)	0.108	1.003 (1.000–1.006)	0.070
C-reactive protein	1 mg/l	1.029 (1.017–1.041)	<0.001	1.015 (1.004–1.026)	0.008
Serum albumin	1 g/l	0.909 (0.875–0.945)	<0.001	0.940 (0.910–0.971)	<0.001
Serum fetuin-A	0.01 g/l	0.923 (0.891–0.957)	<0.001	0.959 (0.933–0.984)	0.002
Total weekly Kt/V	1 unit	0.474 (0.281–0.799)	0.005	0.572 (0.384–0.853)	0.006
PD Kt/V	1 unit	0.922 (0.527–1.612)	0.775	0.859 (0.551–1.340)	0.504
Residual GFR	1 ml/min/1.73 m^2	0.766 (0.645–0.911)	0.003	0.855 (0.763–0.958)	0.007

CAPD = continuous ambulatory peritoneal dialysis; Kt/V = urea clearance; GFR = glomerular filtration rate.

Table 6. Multivariate Cox regression models for all-cause mortality in CAPD patients [expressed as hazard ratios (95% confidence intervals), *P*-value]

	Unit increase	Model 1	Model 2 (adding atherosclerosis)	Model 3 (adding calcification)	Model 4 (adding inflammation)	Model 5 (adding malnutrition)
Age	1 year	1.06 (1.04–1.09), <0.001	1.06 (1.03–1.08), <0.001	1.05 (1.03–1.08), <0.001	1.05 (1.02–1.07), <0.001	1.05 (1.02–1.07), <0.001
Diabetes mellitus	–	1.57 (1.03–2.40), 0.038	1.30 (0.83–2.02), 0.248	1.21 (0.78–1.88), 0.390	1.35 (0.86–2.12), 0.188	1.41 (0.91–2.20), 0.126
Residual GFR	1 ml/min/1.73 m^2	0.71 (0.59–0.86), <0.001	0.70 (0.58–0.85), <0.001	0.73 (0.61–0.88), 0.001	0.72 (0.60–0.87), 0.001	0.74 (0.62–0.88), 0.001
Serum fetuin-A	0.01 g/l	0.94 (0.91–0.98), 0.001	0.95 (0.91–0.98), 0.002	0.95 (0.92–0.99), 0.010	0.96 (0.93–1.00), 0.055	0.98 (0.94–1.02), 0.229
Atherosclerotic vascular disease	–		2.31 (1.46–3.65) <0.001	2.53 (1.60–4.02), <0.001	2.40 (1.50–3.82), <0.001	2.44 (1.54–3.89), <0.001
Cardiac valvular calcification	–			1.93 (1.23–3.05), 0.004	1.82 (1.15–2.87), 0.011	1.70 (1.07–2.72), 0.026
C-reactive protein	1 mg/l				1.02 (1.00, 1.03), 0.015	1.02 (1.00–1.03), 0.018
Serum albumin	1 g/l					0.93 (0.88–0.98), 0.004

GFR = glomerular filtration rate.

strongly predicts mortality and cardiovascular death in PD patients.

After adjusting valvular calcification, fetuin-A remains a significant predictor of all-cause mortality. This may be interpreted as fetuin-A contributing to mortality by mechanisms independent of calcification or fetuin-A capturing residual confounding. In other words, it is possible that valvular calcification reflects imperfectly the calcification burden of the cardiovascular system in CAPD patients and that fetuin-A, being so involved in this process, reflects calcification risk beyond valvular calcification.

Compared with the study by Ketteler *et al.* in haemodialysis patients [9], our current study showed that the serum fetuin-A concentration was slightly lower among Chinese PD patients and may be partly related to a difference in the fetuin-A assay method used in the two studies. Whether this reflects a generally lower production of fetuin-A and possibly a greater risk of calcification in PD compared with the haemodialysis population or rather relates to an inter-ethnic difference in fetuin-A production requires further evaluation. As shown in previous studies, CRP is lower in Asian than Caucasian ESRD patients [12,23]. Whether a similar inter-ethnic difference is observed for serum fetuin-A needs further investigation.

Our study showed no association between the duration of dialysis and serum fetuin-A, suggesting that production of this negative acute phase protein did not increase with time on dialysis. The increase in age across the three tertiles of decreasing serum

Table 7. Multivariate Cox regression models for fatal and non-fatal cardiovascular events in CAPD patients [expressed as hazard ratios (95% confidence intervals), *P*-value]

	Unit increase	Model 1	Model 2 (adding atherosclerosis)	Model 3 (adding calcification)	Model 4 (adding inflammation)	Model 5 (adding malnutrition)
Age	1 year	1.01 (1.00–1.03), 0.085	1.01 (1.00–1.03), 0.176	1.01 (0.99–1.03), 0.231	1.01 (0.99–1.03), 0.289	1.01 (0.99–1.03), 0.350
Positive smoking	–	1.38 (0.97–1.97), 0.074	1.41 (0.99–2.01), 0.060	1.31 (0.92–1.89), 0.139	1.33 (0.92–1.90), 0.126	1.25 (0.87–1.80), 0.233
Diabetes mellitus	–	2.04 (1.43–2.91), <0.001	1.78 (1.24–2.56), 0.002	1.67 (1.16–2.41), 0.006	1.71 (1.19–2.47), 0.004	1.80 (1.25–2.60), 0.002
Residual GFR	1 ml/min/1.73 m^2	0.82 (0.73–0.92), 0.001	0.81 (0.72–0.91), <0.001	0.83 (0.73–0.93), 0.002	0.82 (0.73–0.93), 0.001	0.83 (0.74–0.93), 0.002
Serum fetuin-A	0.01 g/l	0.97 (0.94–0.99), 0.032	0.98 (0.95–1.00), 0.069	0.98 (0.95–1.01), 0.125	0.98 (0.96–1.01), 0.223	0.99 (0.97–1.02), 0.521
Atherosclerotic vascular disease	–		2.22 (1.50–3.29), <0.001	2.31 (1.56–3.42), <0.001	2.25 (1.52–3.35), <0.001	2.19 (1.47–3.27), <0.001
Cardiac valvular calcification	–			1.51 (1.03–2.22), 0.035	1.48 (1.01–2.18), 0.044	1.41 (0.96–2.08), 0.082
C-reactive protein	1 mg/l				1.01 (0.99–1.02), 0.327	1.01 (0.99–1.02), 0.410
Serum albumin	1 g/l					0.96 (0.92–0.99), 0.019

GFR = glomerular filtration rate.

fetuin-A is well in accord with the age-related changes in CRP demonstrated in our previous study [12] and may be partly explained by the greater prevalence of the MIAC syndrome with increasing age. Like albumin, fetuin-A is predominantly synthesized by the liver and is downregulated during inflammation [17,18]. It is therefore not surprising to find that serum fetuin-A and albumin were positively correlated. In contrast to other inflammatory markers such as CRP or interleukin-6 that show a close negative correlation with renal clearance [24,25], fetuin-A was not related to residual renal function, PD or total solute clearance in our PD patients. This suggests that neither residual renal function nor PD makes any significant contribution to the clearance or production of serum fetuin-A, although a prospective study is needed to confirm this observation.

Our study has several limitations that require consideration. First, a single time point measurement of serum fetuin-A was performed and may not reflect changes over time or time-averaged exposure. Secondly, the cross-sectional relationship observed between serum fetuin-A and the MIAC syndrome, however strong, did not allow causal inferences. Thirdly, the inclusion of prevalent but not incident PD patients may introduce survival bias.

In summary, our study shows important associations between serum fetuin-A and cardiac valvular calcification, atherosclerosis, inflammation and malnutrition in PD patients, and serum fetuin-A is related to the clinical outcomes of chronic PD patients via its close relationships with the MIAC syndrome. Further study is needed to explore a possible mechanistic link between fetuin-A and calcification, atherosclerosis, malnutrition and inflammation in patients on PD.

Acknowledgements. This study was supported by the Hong Kong Health Services Research Fund and the Bristol Myers Squibb Foundation: Unrestricted Nutrition Grant Program.

Conflict of interest statement. P. Gao holds stock of Epitope Diagnostics. All other authors declare no conflict of interest.

References

1. US Renal Data System. *USRDS 1998 Annual Data Report*. National Institute of Diabetes and Kidney Diseases, Bethesda, MD; 1999: 63–70
2. London GM, Guerin AP, Marchais SJ, Metivier F, Pannier B, Adda H. Arterial media calcification in end-stage renal disease: impact on all-cause and cardiovascular mortality. *Nephrol Dial Transplant* 2003; 18: 1731–1740
3. Wang AY, Wang M, Woo J *et al*. Cardiac valve calcification as an important predictor for all-cause mortality and cardiovascular mortality in long-term peritoneal dialysis patients: a prospective study. *J Am Soc Nephrol* 2003; 14: 159–168
4. Goodman WG, Goldin J, Kuizon BD *et al*. Coronary artery calcification in young adults with end-stage renal disease who are undergoing dialysis. *N Engl J Med* 2000; 342: 1478–1483
5. Tintut Y, Patel J, Territo M, Saini T, Parhami F, Demer LL. Monocyte/macrophage regulation of vascular calcification *in vitro*. *Circulation* 2002; 105: 650–655
6. Wang AY, Woo J, Wang M *et al*. Association of inflammation and malnutrition with cardiac valve calcification in continuous ambulatory peritoneal dialysis patients. *J Am Soc Nephrol* 2001; 12: 1927–1936
7. Tintut Y, Patel J, Parhami F, Demer LL. Tumour necrosis factor-α promotes *in vitro* calcification of vascular cells via the cAMP pathway. *Circulation* 2000; 102: 2636–2642
8. Schafer C, Heiss A, Schwarz A *et al*. The serum protein alpha 2-Heremans–Schmid glycoprotein/fetuin-A is a systemically acting inhibitor of ectopic calcification. *J Clin Invest* 2003; 112: 357–366
9. Ketteler M, Bongartz P, Westenfeld R *et al*. Association of low fetuin-A (AHSG) concentrations in serum with cardiovascular mortality in patients on dialysis: a cross-sectional study. *Lancet* 2003; 361: 827–833

10. Schiller NB, Shah PM, Crawford M *et al*. Recommendations for quantification of the left ventricle by two-dimensional echocardiography. American Society of Echocardiography Committee on Standards, Subcommittee on Quantitation of Two-Dimensional Echocardiograms. *J Am Soc Echocardiogr* 1989; 2: 358–367
11. Wong M, Tei C, Shah PM. Sensitivity and specificity of two-dimensional echocardiography in the detection of valvular calcification. *Chest* 1983; 84: 423–427
12. Wang AY, Woo J, Lam CK *et al*. Is a single time-point C-reactive protein predictive of outcome in peritoneal dialysis patients? *J Am Soc Nephrol* 2003; 14: 1871–1879
13. Enia G, Sicuso C, Alati G, Zoccali C. Subjective global assessment of nutrition in dialysis patients. *Nephrol Dial Transplant* 1993; 8: 1094–1098
14. Lebreton JP, Joisel F, Raoult JP, Lannuzel B, Rogez JP, Humbert G. Serum concentration of human α2HS glycoprotein during inflammatory process. Evidence that α2HS glycoprotein is a negative acute-phase reactant. *J Clin Invest* 1979; 64: 1118–1129
15. Wang H, Zhang M, Soda K, Sama A, Tracey KJ. Fetuin protects the fetus from TNF. *Lancet* 1997; 350: 861–862
16. Ombrellino M, Wang H, Yang H *et al*. Fetuin, a negative acute phase protein, attenuates TNF synthesis and the innate inflammatory response to carrageenan. *Shock* 2001; 15: 181–185
17. Schinke T, Amendt C, Trindl A, Poschke O, Muller-Esterl W, Jahnen-Dechent W. The serum protein alpha2-HS glycoprotein/fetuin inhibits apatite formation *in vitro* and in mineralizing calvaria cells: a possible role in mineralization and in calcium homeostasis. *J Biol Chem* 1996; 271: 20789–20796
18. Jahnen-Dechent W, Schinke T, Trindl A *et al*. Cloning and targeted deletion of the mouse fetuin gene. *J Biol Chem* 1997; 272: 31496–31503
19. Price PA, Lim JE. The inhibition of calcium phosphate precipitation by fetuin is accompanied by the formation of a fetuin–mineral complex. *J Biol Chem* 2003; 278: 22144–22152
20. Wang AY, Ho SYY, Wang M *et al*. Is cardiac valvular calcification a marker of atherosclerosis and arterial calcification in end-stage renal disease patients? *Arch Intern Med* 2005; 165: 327–332
21. Binkert C, Demetriou M, Sukhu B, Szweras M, Tenenbaum HC, Dennis JW. Regulation of osteogenesis by fetuin. *J Biol Chem* 1999; 274: 28514–28520
22. Mathews ST, Singh GP, Ranalletta M *et al*. Improved insulin sensitivity and resistance to weight gain in mice null for the AHSG gene. *Diabetes* 2002; 51: 2450–2458
23. Stenvinkel P, Wanner C, Metzger T *et al*. Inflammation and outcome in end-stage renal failure: does female gender constitute a survival advantage? *Kidney Int* 2002; 62: 1791–1798
24. Herbelin A, Urena P, Nguyen AT, Zingraff J, Descamps-Latscha B. Elevated circulating levels of interleukin-6 in patients with chronic renal failure. *Kidney Int* 1991; 39: 954–960
25. Pecoits-Filho R, Heimburger O, Barany P *et al*. Associations between circulating inflammatory markers and residual renal function in CRF patients. *Am J Kidney Dis* 2003; 41: 1212–1218

Received for publication: 12.2.05
Accepted in revised form: 13.4.05