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Macrocytosis may be associated with mortality in chronic hemodialysis patients: a prospective study

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Abstract

Background: Macrocytosis occurs in chronic hemodialysis (CHD) patients; however, its significance is unknown. The purpose of this study was to establish the prevalence and distribution of macrocytosis, to identify its clinical associations and to determine if macrocytosis is associated with mortality in stable, chronic hemodialysis patients.

Methods: We conducted a single-centre prospective cohort study of 150 stable, adult CHD patients followed for nine months. Macrocytosis was defined as a mean corpuscular volume (MCV) > 97 fl. We analyzed MCV as a continuous variable, in tertiles and using a cutoff point of 102 fl.

Results: The mean MCV was 99.1 ± 6.4 fl, (range 66-120 fl). MCV was normally distributed. 92 (61%) of patients had an MCV > 97 fl and 45 (30%) > 102 fl. Patients were not B12 or folate deficient in those with available data and three patients with an MCV > 102 fl had hypothyroidism. In a logistic regression analysis, an MCV > 102 fl was associated with a higher Charlson-Age Comorbidity Index (CACI) and higher ratios of darbepoetin alfa to hemoglobin (Hb), [(weekly darbepoetin alfa dose in micrograms per kg body weight / Hb in g/L)*1000]. There were 23 deaths at nine months in this study. Unadjusted MCV > 102 fl was associated with mortality (HR 3.24, 95% CI 1.42-7.39, P = 0.005). Adjusting for the CACI, an MCV > 102 fl was still associated with mortality (HR 2.47, 95% CI 1.07-5.71, P = 0.035).

Conclusions: Macrocytosis may be associated with mortality in stable, chronic hemodialysis patients. Future studies will need to be conducted to confirm this finding.

Background

Anemia is a common consequence of end-stage renal disease (ESRD). Several causes have been identified including iron deficiency [1], reduced production of erythropoietin [2], shortened red cell survival [3] and folate deficiency [4]. Among these, endogenous erythropoietin deficiency is the predominant cause [2].

Erythropoiesis stimulating agents (ESAs) have been effective in treating the anemia of ESRD [5,6]. However, despite their use, anemia may still occur and is associated with significant morbidity and mortality in dialysis patients [7,8]. Furthermore, higher ESA doses have also been associated with increased mortality in hemodialysis patients [9-11]. Whether the increase in mortality observed with ESAs is due to achieved hemoglobin, ESA resistance due to inflammation, a direct effect of erythropoietin, concurrent administration of intravenous iron or some other mechanism, is unclear [12].

Morphologically, the anemia of ESRD is typically normocytic and normochromic [13], but up to 30% [14-16] may have macrocytosis. Proposed causes of macrocytosis in dialysis patients include intravenous iron [15,17], megaloblastic anemia due to B12 and folate deficiency [4] or dialysis-induced changes in red cell volume [18,19]. Reticulocytosis is associated with macrocytosis in the general population [20] and initial studies of ESAs suggested stable and predictable increases in reticulocyte levels with maintenance erythropoietin therapy [21]. However, reticulocytes can also be smaller than mature red cells [22], and no studies have specifically addressed the relationship between ESAs and macrocytosis.

While several studies have suggested causes, no studies have examined for clinical associations with macrocytosis in dialysis patients. Furthermore, an association between red cell size and mortality has not been identified in previous study [23].

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In our hemodialysis population, we have observed that many patients have an unexplained macrocytosis. The purpose of this study was to establish the prevalence and distribution of macrocytosis, to identify its clinical associations and to determine if macrocytosis is associated with mortality in stable, chronic hemodialysis patients.

Methods

We conducted a single-centre prospective cohort study of 150 adult patients on chronic hemodialysis (defined as greater than three months). The cohort was derived from all 195 patients undergoing hemodialysis in our local centre on October 1st, 2009. Exclusions are noted in figure 1. Three measurements of baseline laboratory data including red blood cell mean corpuscular volume (MCV) were collected monthly from October through December 2009. Values were subsequently averaged to avoid potential laboratory error associated with single measurements. Any individuals that died, were transfused or received a kidney transplant before completing three sets of blood tests were censored prior to study analysis (figure 1). Follow up began from the date of last blood work and patients were prospectively followed for nine months. This study was approved by the Queen Elizabeth II Health Sciences Centre research ethics board, our institutional research ethics committee.

We collected baseline clinical data including age, gender, race, time on dialysis, hemodialysis access, urea reduction rate, cause of ESRD and comorbid medical conditions. Causes of ESRD and comorbid conditions were extracted from existing patient charts and definitions were at the treating physician's discretion. For medical comorbidities, we calculated a Charlson-Age Comorbidity Index (CACI) at baseline in all patients [24]. This index allocates different point scores to 19 individual medical conditions depending on the risk of mortality with each condition. In addition, one point is given for each decade > 40 years of age. Included in this score are common medical comorbidities such as diabetes, congestive heart failure, cancer, dementia and peripheral vascular disease. The CACI has been identified as a mortality predictor in dialysis patients. The lowest mortality is observed at scores ≤ 3 and the highest mortality at scores ≥ 8 [25]. All hemodialysis patients in our unit receive darbepoetin alfa, and either iron sucrose or iron dextran to maintain a target hemoglobin of 110 g/L (range 100-120 g/L). Doses of both monthly intravenous iron and darbepoetin alfa averaged over the first three months were recorded. Medication use including immunosuppressive agents, antiepileptic drugs, insulin, oral folate and multivitamins was also recorded. MCV was measured in femtolitres (fl). MCV was calculated using a Coulter LH 750 hematology analyzer, rounded to the nearest tenth. This analyzer has a within run coefficient of variation of ≤ 0.8 fl [26]. Our laboratory definition of macrocytosis is an MCV > 97 fl (normal range 80-97 fl). Additional baseline laboratory data available included the complete blood count (CBC), electrolytes, calcium, phosphate, parathyroid hormone, ferritin, percent saturation of transferrin (tsat), albumin, alkaline phosphatase, hepatocellular liver enzymes and serum glucose. We also collected reticulocyte counts, serum B12, red blood cell folate and serum thyroid stimulating hormone (TSH) levels available within one year of study onset (October 1st, 2009). Finally, we calculated a darbepoetin alfa to hemoglobin (Hb) ratio for each patient, [(weekly darbepoetin alfa dose in micrograms per kg body weight / Hb in g/L)*1000].

The primary outcomes of this study were all cause mortality and time to death in patients with and without macrocytosis at baseline. Causes of death were categorized at the treating physician's discretion according to the Canadian Organ Replacement Registry (CORR) diagnostic codes. CORR uses the ICD-10-CA [27], which is a modified version of the International Statistical Classification of Diseases and Related Health Problems, 10th Revision (ICD-10). In addition to mortality, we also examined for associations of macrocytosis with the clinical and laboratory parameters listed above. Patients were censored at the time of kidney transplantation.

All statistical analyses were performed using SPSS for Windows software (SPSS version 15.0, Chicago, IL). We initially examined MCV both as a continuous variable and using a cutoff point of > 97 fl. Recognizing the potential for a nonlinear relationship between macrocytosis and mortality, we also examined MCV in tertiles using a Kaplan Meier survival analysis. We subsequently used an ROC curve to determine the most statistically significant MCV cutoff point predictive of death. Differences in
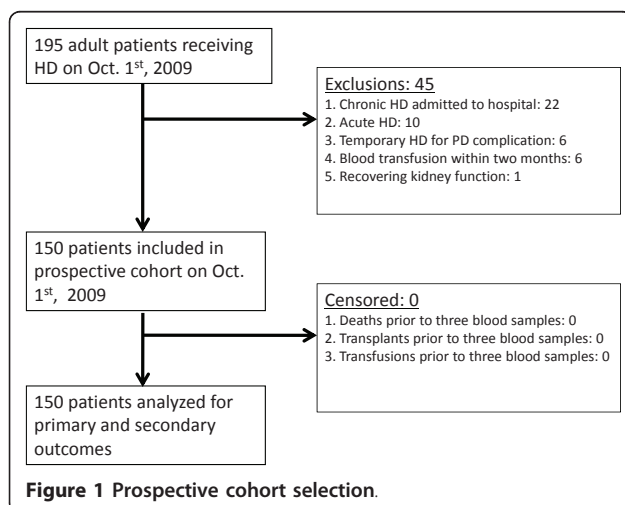
Figure 1 Prospective cohort selection.

baseline characteristics using this MCV cutoff point were calculated for continuous and categorical variables. Univariate clinical and laboratory associations were also determined using a binary logistic regression analysis. Spearman correlation coefficients were calculated to determine collinearity between association variables. For highly correlated variables, only those with the highest statistical significance were incorporated into a backward conditional multivariate model. A Cox survival analysis was used to evaluate time to death. Variables associated with macrocytosis and mortality were chosen for incorporation into the multivariate Cox survival analysis.

Results
Patient Characteristics
All 150 eligible patients completed three sets of blood tests prior to study analysis (figure 1). The mean MCV (average of three values) for all patients was 99.1 ± 6.4 fl, (range 66-120 fl). MCVs for each of the three measurements were 99.0 ± 6.4 fl, 99.1 ± 6.3 fl and 99.2 ± 6.4 fl with an intra patient variation of 1 ± 0.7 fl. MCV was normally distributed (figure 2). Specifically, 92 (61%) of patients had an MCV > 97 fl, 59 (39%) > 100 fl, 45 (30%) > 102 fl and 24 (16%) > 105 fl. TSH, serum B12 and RBC folate levels were available at baseline in 22 (49%), 21 (47%) and 28 (62%) patients with an MCV > 102 fl. In those patients with available data, serum B12 and RBC folate levels were normal. Three patients had biochemical evidence of hypothyroidism (elevated
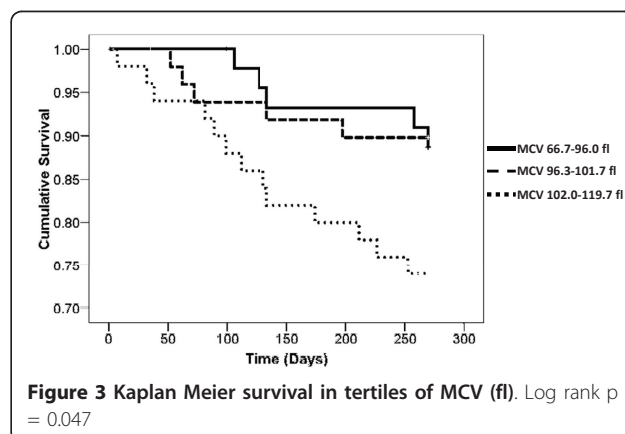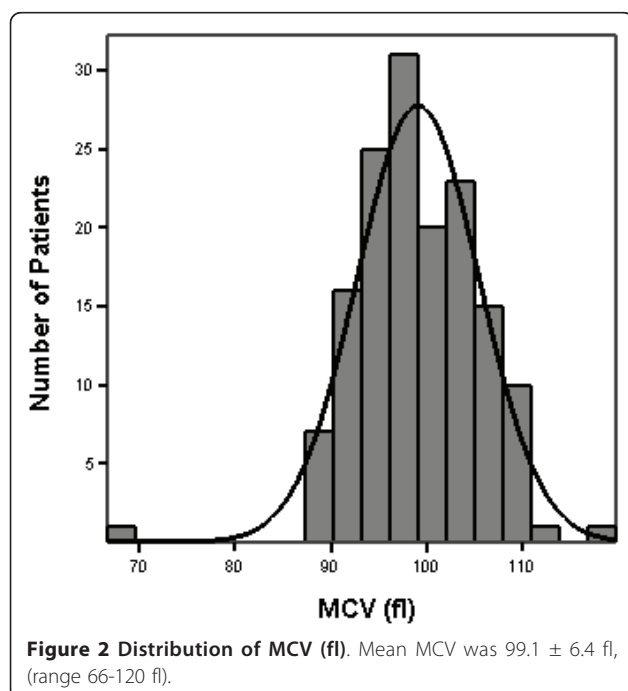
TSH and low free T4). Reticulocyte counts were only available in a few patients.

MCV cutoff point most predictive of death
As a continuous variable in a Cox survival analysis, unadjusted mean MCV was associated with death (HR 1.08 for each 1 fl increase in MCV, 95% CI 1.01-1.15, P = 0.024). An MCV > 97 fl was not predictive of higher mortality, however, a Kaplan Meier survival analysis demonstrated a significant step up in mortality at the highest tertile of MCV (figure 3, log rank P = 0.047). This suggested a nonlinear relationship between MCV and mortality. Using an ROC curve, the optimal MCV cutoff point predictive of death was > 102 fl (area under curve 0.653 ± 0.066, P = 0.019, 95% CI 0.524-0.783). Baseline characteristics using this MCV cutoff point are listed in table 1. Univariate associations with an MCV > 102 fl are listed in table 2. After identifying those variables that were highly correlated, only a history of malignancy, the darbepoetin alfa to Hb ratio and the CACI were examined in a binary logistic regression analysis. A higher CACI and darbepoetin alfa to Hb ratio remained significantly associated with an MCV > 102 fl (table 2).

Mortality
There were seven kidney transplants and 23 deaths prior to last follow up (September 1st, 2010). The mean MCV of all transplanted and deceased patients was 94.1 ± 4.4 fl and 102.1 ± 5.9 fl respectively. Causes of death are noted in table 3. At last follow up, an MCV > 102 fl was associated with increased mortality (log rank P = 0.003). Kaplan-Meier survival curves above and below an MCV of 102 fl are noted in figure 4. In a Cox survival analysis, an MCV > 102 fl was strongly associated with death (unadjusted HR 3.24 95% CI 1.42-7.39, P = 0.005). Other variables significantly associated with mortality in this study included age, stroke, history of malignancy at baseline, a higher CACI, lower mean albumin, higher mean

Figure 2 Distribution of MCV (fl). Mean MCV was 99.1 ± 6.4 fl, (range 66-120 fl).

Figure 3 Kaplan Meier survival in tertiles of MCV (fl). Log rank p = 0.047

Table 1 Baseline characteristics

Variable	All (n = 150)	MCV ≤ 102 fl (n = 105)	MCV > 102 fl (n = 45)	P
Demographic				
Age (years)	65.0 ± 17.0	63.3 ± 17.6	68.9 ± 15.1	0.07
Time on dialysis (months)	34.5 ± 31.8	33.8 ± 34.4	36.4 ± 24.6	0.65
Male n (%)	76 (51)	58 (55)	18 (40)	0.09
Caucasian n (%)	135 (90)	91 (87)	44 (98)	0.04
Fistula as HD access n (%)*	45 (30)	33 (31)	12 (27)	0.56
Cause of ESRD n (%)				
Diabetes	51 (34)	40 (38)	11 (24)	0.11
Glomerulonephritis	29 (19)	18 (17)	11 (24)	0.30
Comorbidities n (%)				
Diabetes	63 (42)	45 (43)	18 (40)	0.75
Hypertension	114 (76)	79 (75)	35 (78)	0.74
Coronary artery disease	53 (35)	39 (37)	14 (31)	0.54
Peripheral vascular disease	33 (22)	23 (22)	10 (22)	0.97
Stroke	21 (14)	14 (13)	7 (16)	0.72
Cancer	25 (17)	11 (10)	14 (31)	< 0.01
Failed renal transplant	25 (17)	20 (19)	5 (11)	0.24
Charlson-Age Comorbidity Index	7 ± 3	6 ± 3	8 ± 3	0.01
Laboratory				
Mean MCV (fl)	99.1 ± 6.4	96.0 ± 4.6	106.3 ± 3.1	< 0.01
Albumin (g/L)	33.7 ± 3.5	34.0 ± 3.6	33.0 ± 3.3	0.14
Hemoglobin (Hb) (g/L)	108.6 ± 10.0	109.8 ± 10.0	105.7 ± 9.6	0.02
Hb btw. 100-120 g/L n (%)	103 (69)	73 (70)	30 (67)	0.65
Ferritin ug/L	729 ± 467	707 ± 454	782 ± 498	0.37
Tsat (%)	29.77 ± 11.80	28.05 ± 11.37	33.76 ± 11.93	< 0.01
Darbepoetin alfa to Hb ratio	5.37 ± 3.66	4.80 ± 3.49	6.69 ± 3.73	< 0.01
Medications				
Darbepoetin alfa dose mcg/week	43.0 ± 31.5	39.3 ± 31.1	51.6 ± 31.1	0.03
Receiving darbepoetin alfa n (%)[+]	141 (94)	97 (92)	44 (98)	0.27
Receiving intravenous iron n (%)[+]	137 (91)	96 (91)	41 (91)	0.95
Antiepileptic n (%)	5 (3)	1 (1)	4 (9)	0.01
On folate n (%)	148 (99)	103 (98)	45 (100)	0.81
On multivitamin	143 (95)	101 (96)	42 (93)	0.26

*Patients without a fistula were dialyzed using a tunnelled, cuffed catheter, except for one using an AV graft
+Received at least one dose during initial three months of study
Continuous variables are reported as means ± standard deviation

Table 2 Univariate and Multivariate Associations with MCV > 102 fl

	Univariate (OR [95% CI])	P	Multivariate (OR [95% CI])	P
Malignancy*	3.86 [1.59, 9.38]	0.003	2.12 [0.73, 6.17]	0.168
Darbepoetin alfa to Hb ratio	1.15 [1.04, 1.27]	0.005	1.19 [1.07, 1.32]	0.001
Charlson-Age Comorbidity Index	1.18 [1.04, 1.33]	0.012	1.23 [1.07, 1.40]	0.003
Transferrin saturation	1.04 [1.01, 1.07]	0.013	—	
Age 60 and above	3.34 [1.42, 7.86]	0.006	—	
Antiepileptic medication	10.15 [1.10, 93.51]	0.041	—	
Mean hemoglobin (g/L)	0.96 [0.93, 1.00]	0.025	—	
Weekly darbepoetin alfa dose (mcg)	1.01 [1.00, 1.02]	0.033	—	
Weekly weight adjusted darbepoetin alfa dose (mcg/kg)	3.21 [1.29, 7.98]	0.012	—	

* Hematologic and non-hematologic
Variables included in the multivariate binary regression analysis: Malignancy, Darbepoetin alfa to Hb ratio and the Charlson-Age Comorbidity Index.

Table 3 Cause of Death at Last Follow Up

	All (n = 150)	MCV < 102 fl (n = 105)	MCV > 102 fl (n = 45)
All Cause	23	10	13
Cardiac	7	3	4
Withdrawal	8	5	3
Sepsis	5	1	4
Cancer	2	1	1
Stroke	1	0	1

ferritin, lower levels of uric acid and lower body weight. The darbepoetin alfa to Hb ratio was not associated with mortality in this study. After adjusting for the CACI, an MCV > 102 fl remained significantly associated with mortality (HR 2.47, 95% CI 1.07-5.71, p = 0.035).

Discussion

In this study of stable, chronic hemodialysis patients, we identified a high prevalence of unexplained macrocytosis associated with a higher CACI and higher ratios of weekly weight adjusted darbepoetin alfa to Hb. Macrocytosis both as a continuous variable and using a cutoff point of 102 fl was associated with higher all-cause mortality.

While the etiology of macrocytosis or its association with mortality in our population is unclear, several possibilities need to be considered. Macrocytosis was associated with a higher CACI at baseline that in turn, was associated with increased mortality. Perhaps macrocytosis is a response to underlying illness, and serves as a "window" into the bone marrow of an elderly dialysis patient with multiple comorbidities. The macrocytic response may not be a spontaneous development, rather, an effect of

**Figure 4 Kaplan Meier survival for MCV > 102 fl**. Log rank p = 0.003

exogenous ESA administration to a chronically unwell patient. This would explain the association between MCV > 102 fl and higher darbepoetin alfa to Hb ratios, the latter reflecting ESA resistance perhaps due to underlying medical illness.

Regarding other possible causes, we did identify a univariate association between tsat and macrocytosis. Intravenous iron has been linked to increased mortality in dialysis patients [12,28,29], and has been suggested as a cause of macrocytosis [15,17]. However, in this study, mean iron dose over three months at baseline was not associated with an MCV > 102 fl. As well, there did appear to be a univariate association between macrocytosis and a history of malignancy, which in turn, was associated with mortality in this study. Macrocytosis occurs in certain malignancies, either independently or as a consequence of chemotherapy [30]. However, 11/14 macrocytic patients in our study with a history of malignancy did not have active cancer at the time of study, and no patients were actively receiving chemotherapy. Furthermore, only one death was directly attributable to cancer amongst the patients with an MCV > 102 fl.

A final consideration is that macrocytosis may be a manifestation of altered hematopoiesis. In one study of non-dialysis elderly patients, 25/49 with unexplained macrocytosis had dysplastic changes or findings consistent with MDS on bone marrow biopsy [31]. Alternatively, altered hematopoiesis may be due to high doses of ESAs. Macrocytosis due to suspected abnormal rapid cellular maturation in states of massive erythropoietic stimulation has been observed in rat models [22], and we did observe an association between darbepoetin alfa dose and MCV > 102 fl. However, altered hematopoiesis was not confirmed with bone marrow biopsy in this study. Furthermore, bone marrow dysplasia was not identified in an earlier study of dialysis patients treated with recombinant erythropoietin for 24 months who subsequently underwent bone marrow biopsy [32]. In addition, macrocytosis in dialysis patients has been identified prior to the introduction of ESAs [17].

As a predictor of increased mortality, macrocytosis does have advantages over other laboratory markers commonly used in dialysis patients. In our study, macrocytosis was a strong predictor of mortality evidenced by a high hazard ratio. In addition, as it is included in the routine CBC, measurement of the MCV requires no additional laboratory tests and is cheaper compared to conventional laboratory markers of mortality.

There are limitations to this study. With only 23 outcomes, we were unable to do extensive multivariate analyses without risk of over-fitting. Therefore, there is the possibility of residual confounding. However, this is primarily a pilot study and the main purpose is to stimulate hypothesis generation for future analyses. Another
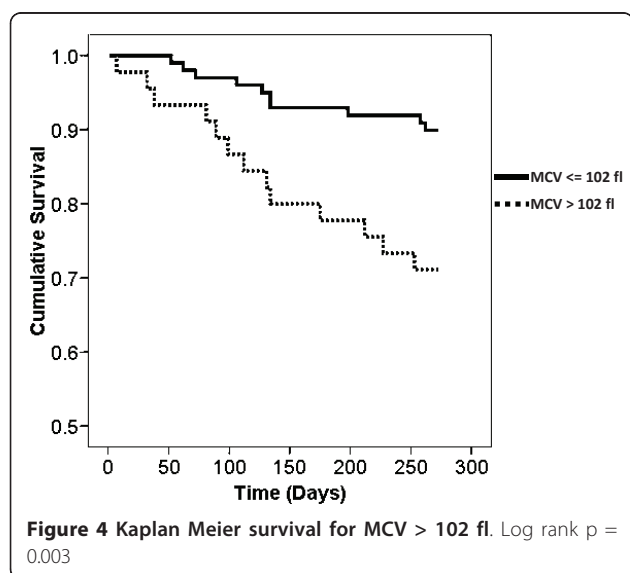
limitation is that the lack of serum B12 and red cell folate levels in all patients makes it difficult to completely exclude vitamin deficiency as a cause of macrocytosis. In addition, only a few patients had reticulocyte levels measured at baseline (a known cause of macrocytosis). However, 93 and 100% of patients with an MCV > 102 fl were on multivitamin and folate supplementation, and at six months, RBC folate and serum B12 levels were normal in over 90% of macrocytic patients (data not shown). In addition, a reanalysis at six months revealed comparable reticulocyte counts between macrocytic and normocytic patients, and an absolute reticulocytosis in only a few macrocytic patients (data not shown). Finally, as this is a single-centre study, we acknowledge that the findings of this study cannot necessarily be generalized to other dialysis populations. A recent study in a larger cohort of dialysis patients identified a lower mean MCV than our cohort, and did not find an association between MCV and mortality [23]. However, our study is unique as we analyzed MCV both as a continuous and categorical variable. Furthermore, our population is not unlike other dialysis cohorts with respect to its identified predictors of mortality. These predictors include age [33-36], stroke [33,34], cancer [33,34], albumin [35] and ferritin [37,38]. Nonetheless, our population may be unique with respect to a high prevalence of macrocytosis and validation with another dialysis population may increase the strength of our findings. In addition, if macrocytosis is associated with higher comorbidity it would fluctuate based on patient clinical status. Thus, a study using marginal structural models that adjust for time dependent confounding would be clinically relevant.

Conclusions

Unexplained macrocytosis may be associated with mortality in stable, chronic hemodialysis patients. Future studies will need to be conducted in larger numbers of incident hemodialysis patients to determine the significance of this finding.

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