Halaj-Zadeh J., ***J Anal Res Clin Med,*** 2014, 2(1), 17-24.
doi: 10.5681/jarcm.2014.003

*Original Article*

# Role of phosphor and GAS-6 in inflammation in hemodialysis patients in Tabriz, Iran

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**Article info**
***Article History:***
*Received: 8 Sep. 2013*
*Accepted: 17 Nov. 2013*

***Keywords:***
Hemodialysis,
Growth Arrest-Specific 6 (GAS-6),
Interleukin 6 (IL-6),
High Sensitivity C-Reactive Protein

**Abstract**

***Introduction:*** Inflammation is recognized in up to 50% of chronic kidney disease (CKD) patients, being a common feature of advanced renal disease and crucial mediator of vascular calcification which may be relevant in CKD. This study was aimed at evaluating the role of Growth arrest-specific 6 (Plasma GAS-6) and mineral metabolism abnormalities in hemodialysis (HD) patients.

***Methods:*** We enrolled a total of 92 adults including 46 (28 males and 18 females) clinically stable HD patients and 46 (23 males and 23 females) patients with normal kidney as control group. Plasma GAS-6, Interleukin 6 (IL-6), and high sensitivity C-reactive protein (hsCRP) concentration and biochemical alteration were quantified; as biochemical factors, GAS-6, IL-6, and hsCRP levels were determined by standard methods.

***Results:*** Levels of GAS-6 were significantly increased in HD patients compared with normal controls ($P < 0.001$). In HD patients, IL-6, and hsCRP levels were increased compared with controls ($P < 0.001$). The levels of GAS-6 were directly associated with IL-6 ($r = 0.560$, $P < 0.001$) in HD patients. No significant correlation was found between hsCRP and GAS-6 levels in HD patients ($r = 0.05$, $P = 0.742$). Multiple regression analysis demonstrated that serum P was independently associated with hsCRP and GAS-6 independently associated with IL-6.

***Conclusion:*** Elevated serum P and GAS-6 might play a role in the development of inflammation in CKD patients. Although our study shows that GAS-6 is directly associated with IL-6 and phosphor with hsCRP, their direct role in vascular calcification and type of their relationships need further studies in the future.

**Citation:** Halaj-Zadeh J, Ghorbanihaghjo A, Argani H, Dastmalchi S, Valizadeh Sh, Halaj N, et al. **Role of phosphor and GAS-6 in inflammation in hemodialysis patients in Tabriz, Iran.** J Anal Res Clin Med 2014; 2(1): 17-24.

## Introduction

In recent decades, intensive investigations have led to a paradigm shift in the interpretation of atherosclerosis from a purely metabolic process (i.e., mainly driven by hypercholesterolemia) to a disease where inflammation is the dominant pathophysiological and biochemical alteration.[1] Cardiovascular disease is up to 20-fold more frequent in End Stage Renal Disease (ESRD) patients and accounts for up to 50% of all

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deaths, with accelerated atherosclerosis being consistently implicated in this process.[2] Traditional cardiovascular risk factors cannot completely explain the prevalence of atherosclerosis, the elevated cardiovascular risk, and the disproportional predisposition for adverse cardiovascular outcomes in this population. Therefore, novel cardiovascular risk factors are suggested to contribute to atherogenesis and have been associated with the risk of cardiovascular disease.

Growth arrest-specific 6 (GAS-6) protein was the last addition to the family of plasma vitamin K–dependent proteins. GAS-6 was cloned and characterized in 1993 and found to be similar to plasma anticoagulant protein S.[3] Various cell types express GAS-6, including endothelial cells, vascular smooth muscles, leukocytes, and platelets. Soon after, it was recognized as a growth factor–like molecule, as it interacted with the subfamily of tyrosine kinases-AXL, TYRO3, and Mer as receptors for GAS-6.[4] GAS-6/AXL system imparts signals via the PI3K/Akt pathway, resulting in cell survival, proliferation, adhesion, and protection from cellular death.[5]

The GAS-6/TAM system regulates an intriguing mix of processes, including cell survival and proliferation, cell adhesion and migration, blood clot stabilization, and inflammatory cytokine release. Altered activity/expression of GAS-6/TAM components has been detected in a variety of pathologies such as inflammation, coagulopathy, cancer, autoimmune disease, diabetic vascular and renal disease, and chronic renal failure.[6-9] GAS-6 is elevated in sepsis and increased plasma concentration of GAS-6 in patients with septic shock correlated with disease severity and increased mortality.[10] The TAM ligands and receptors modulate inflammation, regulating toll-like receptor signaling and pro-inflammatory cytokine signaling in macrophages and dendritic cells.[5,11] Without the TAM receptors, animals develop unregulated immunity, autoimmunity, and inflammation.[12-14]

The inflammatory reaction could be considered a vascular response to harmful stimuli, where the regulation of cell traffic through the vessel wall is a crucial regulatory step. GAS-6 has been shown to play an important role in this part of the inflammatory response. GAS-6 promotes inflammation by enhancing interactions between endothelial cells, platelets, and leukocytes. The role of Gas-6 in the vascular system is complex. The process of vascular calcification has also been related to Gas-6. Gas-6 signaling through AXL inhibits mineral deposition by cultured VSMCs (Vascular Smooth Muscle Cells).[15,16]

However, little is known about the clinical significance of the Gas-6/TAM system in patients with chronic kidney disease (CKD), especially end-stage renal disease, and its association with various inflammation that are common in hemodialysis (HD) patients. On the other hand, alteration of mineral metabolism is a prevalent condition in CKD. Large epidemiologic studies have shown a strong relationship between elevated levels of calcium (Ca), phosphorus (P), Ca-P product (Ca x P), and parathyroid hormone (PTH), and cardiovascular morbidity and mortality.[17-19] Although it has been shown that elevated serum P is related to cardiovascular morbidity and mortality in both HD and predialysis patients, the mechanisms by which serum P contributes to cardiovascular disease are not completely known.

We have addressed this issue by conducting a cross-sectional study to examine the interrelationships among the main parameters of mineral metabolism, serum GAS-6 level, and inflammatory factors [high sensitivity C-reactive protein (hsCRP)] and interleukin 6 (IL-6) in humans.

## Methods

This study was approved by the Department of Biochemistry of Tabriz University of Medical Sciences (TUMS), Iran. The ethics committee of Tabriz University of Medical Sciences approved the project protocol, and informed consents were obtained from all

patients or their close relatives. Then, blood samples were obtained from 46 patients with end-stage renal disease on chronic HD, and 46 healthy volunteers with no known medical history. The HD group participants were recruited from within the Tabriz University Nephrology Practice. Inclusion criterion for HD patients was being on HD for over 6 months. HD patients with vitamin D therapy, hormone therapy with PTH, and history of CVD were excluded. All patients from the HD group were on HD 3 times per week for 4-5-hour sessions. Bicarbonate buffered dialysate fluid containing 2-3 mmol/l potassium, 1.25 mmol/l calcium, 0.75 mmol/l magnesium, and low-flux polysulfone or cuprofane dialysis membranes were used in all patients.

Results are presented as mean ± standard deviation for parametric data and as median for nonparametric data. Numbers and their percentage were shown when appropriate. The Mann-Whitney test was used for evaluating the difference between different groups, and Pearson's correlation coefficient for evaluating correlations.

For all tests $P < 0.05$ was considered significant. Demographic data were analyzed and differences among groups were assessed by Mann-Whitney test for nonparametric data or Student's independent t-test for parametric data, and we further determined correlations between all variables with Pearson's correlation test. Differences between groups were evaluated, where appropriate, using ANOVA. SPSS for Windows (version 18; SPSS Inc., Chicago, IL, USA) was used for statistics.

Blood samples were drawn from each patient before breakfast in the morning (between 8 and 11 a.m.) prior to HD session, after an 8-hour to 12-hour overnight fast. Samples were collected in sterile tubes, centrifuged at 3000 g for 10 min at 4°C, and then stored at -80°C until assayed.

Serum creatinine levels, albumin, total protein, urea, and alkaline phosphatase were measured by enzymatic colorimetric method with an automated chemical analyzer (Abbott Analyzer, Abbott Laboratories, Abbott Park, North Chicago, IL). Serum total calcium and serum phosphorus were measured by using commercial kits (Pars Azmoon Co). Laboratory Analysis. To quantify total plasma GAS-6, we used the Human GAS-6 ELISA Kit (CAT No. SK00098-01, AVISCEERA BIOSCIENCE Inc., 2348 Walsh Ave, Suite C Santa Clara, CA 95051). The quantification was accomplished according to the manufacturer's protocol. In brief, the microtiter plates were coated with 100 µl of standards, specimens, and positive control and incubated for 2 hours on the plate shaker at room temperature. The wells were washed 4 times with washing buffer; 100 µl of detection antibody working solution was added to each well, gently mixed for 15 seconds, and incubated for 2 hours at 36°C. Then, the wells were again washed 4 times with washing buffer, and 100 µl of streptavidin-HRP conjugate working solution was added to each well, mixed for 15 seconds, and incubated for 60 minutes at 36°C and protected from light. Then, 100 µl of substrate solution was added to each well and they were incubated for 3-6 minutes on plate shaker. The reaction was then stopped by adding 100 µl of stop solution, followed by gentle mixing for 30 seconds until all the blue had changed to yellow. The absorbance was measured at 450 nm in a microplate reader within 15 minutes after the addition of stop solution. Intra-assay and inter-assay coefficients of variation of the test were 4-6% and 8-10%, respectively. Immunoenzymetric assay for the in vitro quantitative measurement of human IL-6 in serum (DIAsource IL-6-EASIA Kit, Cat No: KAP1261, DIAsource ImmunoAssays S.A, Belgium) uses monoclonal antibodies (MAbs) directed against distinct epitomes of IL-6.Calibrators (100 µl) and samples react with the capture MAb 1 coated on microtiter well and with a MAb 2 labeled with horseradish peroxidase (HRP). After an incubation period allowing the formation of a

sandwich, coated MAb 1–human IL-6–MAb 2–HRP, the microtiter plate is washed to remove unbound enzyme labeled antibody. Bound enzyme labelled antibody is measured through a chromogenic reaction.

Chromogenic solution (TMB) is added and incubated. The reaction is stopped with the addition of stop solution and the microtiter plate is then read at 450 nm wavelength. A calibration curve is plotted and IL-6 concentration in samples is determined by interpolation from the calibration curve. Detection limit was 2 pg/ml with inter-assay coefficient of variation (CV) 5.4% and intra-assay CV of 4.3%. Plasma analysis for a hsCRP was performed by the nephelometry method (Pars Azmoon Co).

For the quantitation of 25-OH vitamin D in serum, we used The IDS 25-Hydroxy Vitamin D EIA kit (Immunodiagnostic Systems Ltd., Cat No: AC 57F1, Germany). Calibrators, controls, and samples are diluted with biotin labelled 25-OH vitamin D. The diluted samples are incubated in microtitre wells which are coated with a highly specific sheep 25-OH vitamin D antibody for 2 hours at room temperature before aspiration and washing. Enzyme HRP labelled avidin, is added and binds selectively to complex avidin and to complex biotin; following a further wash step, color is biotin and is developed using a chromogenic substrate (TMB). The developed using a chromogenic substrate (TMB). The absorbance of the stopped reaction mixtures are read in a microtitre plate reader, color intensity developed being inversely proportional to the concentration of 25-OH vitamin D.

## Results

The clinical and laboratory characteristics of HD patients are shown in table 1. As shown in table 1, there was no significant difference in the mean age between the HD and control groups. The mean eGFR (Estimated Glomerular Filtration Rate) by MDRD (Modification of Diet in Renal Disease) was 5.5 ml/min/1.73 $m^2$. As a control group, we enrolled 46 age- and gender-matched healthy volunteers (mean age = 44.7 ± 13.5 years, 23 females and 23 males) with normal kidney function (mean eGFR-MDRD was 71.2 ± 21.7 ml/min/1.73 $m^2$) and without significant albuminuria. As shown in table 1, there was no significant difference in the mean age and gender between the two groups.

As shown in table 1, GAS-6 level were significantly increased in HD patients compared with controls (763 ± 187.91 pg/ml vs. 421 ± 189.91 pg/ml; $P < 0.001$). Table 2 shows that serum levels of GAS-6 did not differ between males and females in the HD patients (748.93 ± 175.04 pg/ml vs. 787.5 ± 209.29 pg/ml; $P = 0.490$) and also in the control group (473.3 ± 199.62 pg/ml vs. 369.9 ± 168.32 pg/ml; $P = 0.064$). As shown in table 3, our study confirmed an inverse linear relationship between GAS-6 levels and eGFR ($r = -0.424$; $P = 0.003$). Clinical markers associated with chronic inflammation and mortality in HD patients were linked to GAS-6. As has been shown in table 1, serum hsCRP level was lower in control group participants than the HD patients (1.38 ± 1.61 mg/l vs. 4.4 ± 1.26 mg/L; $P < 0.001$). Serum IL-6 level was higher in the HD group than the control group (5.77 ± 2.55 pg/ml vs. 1.59 ± 1.61 pg/ml; $P < 0.001$. Univariate correlation analysis in HD patients (Table 3) showed that the plasma IL-6 value was significantly, positively correlated with GAS-6 ($r = 0.56$; $P < 0.001$) and phosphor ($r = 0.292$; $P = 0.049$). The reverse association was found between IL-6 and eGFR ($r = -0.326$; $P = 0.027$) (Table 3). We found that hsCRP was positively correlated with calcium ($r = 0.432$, $P = 0.003$), phosphate ($r = 0.857$; $P < 0.001$), Ca × P ($r = 0.683$; $P < 0.001$), intact parathormone (iPTH) ($r = 0.468$; $P < 0.001$), and 25-OH vitamin D ($r = -0.585$; $P < 0.001$) (Table 3). There was no association between hsCRP and ALP (Alkaline Phosphatase) ($r = -0.145$; $P = 0.336$). The reverse association was found between hsCRP and 25-OH vitamin D ($r = -0.585$, $P < 0.001$). No significant correlation was

**Table 1. Main demographic and clinical characteristics of HD subjects**

| Variable | HD group (n = 46) | HC group | P |
|---|---|---|---|
| Age (years) (mean ± SD) | 61.08 ± 13.92 | 61.84 ± 1152 | 0.776* |
| Sex (male/female) | 28/18 | 23/23 | 0.14** |
| Underlying diagnoses [n (%)] | - | - | |
| Diabetic nephropathy | 18 (40.0%) | - | - |
| Chronic glomerulonephritis | 3 (6.7%) | - | - |
| Polycystic kidney disease | 5 (11.1%) | - | - |
| Hypertensive ischemic nephropathy | 10 (22.2% ) | - | - |
| Obstructive nephropathy | 7 (15.6%) | - | - |
| Unknown etiology | 2 (4.4%) | - | - |
| eGFR (ml/min per 1.73 $m^2$) [median (min–max)] | 5 (3-9) | 68 (34-136) | < 0.001*** |
| Time on dialysis (month) (mean ± SD) | 44 ± 34.4 | - | - |
| Alkaline phosphatase, (Iu/l) | 411.15 ± 310.05 | 185.76 ± 59.93 | < 0.001* |
| Calcium (mg/dl) (mean ± SD) | 8.81 ± 0.90 | 9.50 ± 0.56 | < 0.001* |
| Ca × P product (mg/dl) | 53.43 ± 9.74 | 38.73 ± 7.82 | < 0.001* |
| Albumin (g/dl) (mean ± SD) | 3.48 ± 0.77 | 3.98 ± 0.49 | < 0.001* |
| Total protein (g/dl) (mean ± SD) | 8.25 ± 1.01 | 7.88 ± 1.30 | 0.130* |
| Phosphorus (mg/dl) (mean ± SD) | 6.05 ± 0.91 | 4.08 ± 0.87 | < 0.001* |
| iPTH (pg/dl) (mean ± SD) | 367.29 ± 133.38 | 26.04 ± 15.34 | < 0.001* |
| Creatinine (mg/dl) (mean ± SD) | 9.20 ± 2.44 | 1.10 ± 0.28 | < 0.001* |
| Urea (mg/dl) (mean ± SD) | 104 ± 17.47 | 39.56 ± 16.05 | < 0.001* |
| Triglyceride (mmol/l) (mean ± SD) | 169.1 ± 53.90 | 97.40 ± 35.60 | < 0.001* |
| hsCRP (mg/l) (mean ± SD) | 4.40 ± 1.26 | 1.38 ± 1.61 | < 0.001* |
| IL-6 (pg/ml) (mean ± SD) | 5.77 ± 2.55 | 1.59 ± 1.61 | < 0.001* |
| glucose (mg/dl) | 126 ± 7.21 | 92.43 ± 28.83 | 0.753* |
| ET-l (pg/ml) (mean ± SD) | 2.31 ± 0.87 | 0.75 ± 0.48 | < 0.001* |
| 25(OH) vit D (nmol/l) (mean ± SD) | 22.72 ± 6.95 | 35.77 ± 14.95 | < 0.001* |
| GAS-6 (pg/ml) (mean ± SD) | 763.52 ± 187.91 | 421.63 ± 189.91 | < 0.001* |

iPTH: Intact parathormone; hsCRP: High sensitivity C-reactive protein; GAS-6: Growth arrest specific-6; IL-6: Interleukin 6
ET-1: Endothelin-1; HC: Health control; HD: Hemodialysis patients; eGFR: Estimated glomerular filtration rate
*Performed by independent-sample t-test; **Performed by Chi-square test; ***Assessed by Mann-Whitney test

**Table 2. Comparison of the serum level of GAS-6 between males and females in the HD and HC study groups**

| Parameters | Male [mean ± SD] (n%) | Female [mean ± SD] (n%) | P* |
|---|---|---|---|
| GAS-6 (pg/ml) (HD) | [748.93 ± 175.04] (60.86) | [787.5 ± 209.25)] (39.13) | 0.493 |
| GAS-6 (pg/ml) (HC) | [473.30 ± 199.62] (50) | [369.9 ± 168.32] (50) | 0.064 |
| P** | < 0.001 | < 0.001 | - |

Differences among groups were assessed by Mann-Whitney test
GAS-6: Growth arrest specific-6; HD: Hemodialysis patients; HC: Health control; *Serum GAS-6 (males vs. females); **Serum GAS-6 levels (HD vs. HC)

**Table 3. Correlation coefficients (r) between inflammatory parameters and clinical characteristics in HD**

| Variable | C-reactive r | Protein P | IL-6 r | P |
|---|---|---|---|---|
| Age (year) | -0.009 | 0.951 | -0.039 | 0.795 |
| Total protein (g/dl) | 0 .093 | 0.539 | 0.163 | 0.278 |
| Albumin (g/dl) | -0.105 | 0.487 | -0.152 | 0.315 |
| GAS-6 (pg/ml) | 0.050 | 0.742 | 0.560** | < 0.001 |
| eGFR (ml/min per 1.73 $m^2$) | -0.136 | 0.366 | -0.326* | 0.027 |
| Phosphorus (mg/dl) | 0.857** | < 0.001 | 0.292* | 0.049 |
| Calcium (mg/dl) | 0.432** | 0.003 | 0.077 | 0.610 |
| Ca × P | 0.683** | < 0.001 | 0.100 | 0.508 |
| iPTH (pg/dl) | 0.468** | 0.001 | 0.177 | 0.240 |
| 25(OH) vit D (ng/ml) | -0.585** | < 0.001 | -0.172 | 0.252 |
| ALP (Iu/l) | -0.145 | 0.336 | 0.021 | 0.891 |

*Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed)
GAS-6: Growth arrest specific-6; iPTH: Intact parathormone; 25(OH) vit D: 25-OH vitamin D; ALP: Alkaline phosphatase
eGFR: Estimated glomerular filtration rate; IL-6: Interleukin 6

**Table 4. Multiple regression analysis for C-reactive protein and IL-6 as dependent variable**

| Independent variable | Beta-regression coefficient | SE of regression coefficient | T | P |
|---|---|---|---|---|
| IL-6 | | | | |
| Age | 0.018 | 0.028 | 0.115 | 0.909 |
| eGFR | -0.112 | 0.241 | -0.722 | 0.475 |
| iPTH | 0.091 | 0.003 | 0.539 | 0.594 |
| Calcium | 0.382 | 0.976 | 1.901 | 0.066 |
| Phosporous | 0.400 | 1.205 | 0.928 | 0.360 |
| Ca × P | -0.267 | 0.057 | -1.233 | 0.226 |
| 25(OH) vit D | 0.048 | 0.062 | 0.284 | 0.778 |
| ALP | 0.092 | 0.001 | 0.639 | 0.529 |
| GAS-6 | 0.509 | 0.002 | 3.395 | 0.002 |
| C-reactive protein | | | | |
| Age | -0.117 | 0.00 | -1.168 | 0.251 |
| iPTH | 0.080 | 0.001 | 0.72 | 0.474 |
| Calcium | 0.087 | 0.31 | 0.669 | 0.508 |
| Phosporous | 0.898 | 0.387 | 3.215 | 0.003 |
| Ca × P | -0.053 | 0.018 | -0.379 | 0.707 |
| 25(OH) vit D | -0.200 | 0.020 | -1.838 | 0.075 |
| ALP | -0.073 | 0.000 | -0.774 | 0.444 |
| GAS-6 | 0.107 | 0.001 | 1.102 | 0.279 |
| Albumin | -0.068 | 0.168 | -0.664 | 0.511 |
| Total protein | -0.010 | 0.125 | -0.101 | 0.920 |

iPTH: Intact parathormone; 25(OH) vit D: 25-OH vitamin D; GAS-6: Growth arrest specific-6
ALP: Alkaline phosphatase; eGFR: Estimated glomerular filtration rate; IL-6: Interleukin 6

found between hsCRP and GAS-6 levels in the HD group (r = 0.05, P = 0.742). To test the hypothesis of an independent association between variables of mineral metabolism, GAS-6, and inflammatory parameters, forward stepwise multiple regression analysis was performed with CRP and IL-6 as the dependent variables (Table 4). Age, gender, albumin, total protein, eGFR, and serum concentrations of Ca, P, iPTH, 25-OH vitamin D, and GAS-6 were considered as possible predictors of inflammatory parameters. The result showed that from the different independent variables, serum P was independently associated with hsCRP, and serum GAS-6 was independently associated with IL-6.

## Discussion

Few studies to date have analyzed the possible association of GAS-6 with different diseases. In a mouse model, GAS-6 was suggested to be involved in the progression of nephrotoxic nephritis, because lower mortality, proteinuria, and fibrin deposition were observed in GAS-6 mice than in normal mice.[20] It has been demonstrated that vascular calcification and inflammation are common complications in CKD patients.[21] Presence and the extent of vascular calcifications are strong predictors of cardiovascular disease and all-causes of mortality and morbidity in these patients. It has been shown that GAS-6 is highly expressed in atherosclerotic lesions and in activated endothelial cells exposed to pro-inflammatory cytokines. The presence of GAS-6 in precursor T cell lines and monocytes suggests a potential role of activating endothelial cells during the initial inflammatory response following vascular injury. Lee et al. showed that GAS-6 level is markedly elevated in the non-HD CKD and HD patients. Moreover, in their study, there was a trend toward positive correlation of GAS-6 levels with previous history of coronary artery disease in maintenance of HD patients.[22]

In our study, the GAS-6 level in HD patients, in comparison with normal subjects, was significantly higher. Increased GAS-6 levels did not seem to be a direct product of

the dialysis procedure itself.[22] Several studies have demonstrated that various inflammatory biomarkers, such as hsCRP, IL-6, and serum albumin, are robust and independent predictors of both all-cause and CVD mortality in ESRD patients. Inflammation is recognized in up to 50% of CKD patients, being a common feature of advanced renal disease.[23,24]

The most extensively studied biomarkers of inflammation in cardiovascular disease are hsCRP and IL-6. The Cardiovascular Health Study reported that levels of hsCRP and IL-6 were significantly higher in patients with renal insufficiency compared with patients with normal kidney function.[25] GAS-6/TAM signaling is known to be involved in triggering systemic inflammation in diverse human diseases (such as infection, acute stroke, and acute coronary syndrome). Our findings provide new and important clinical evidence that circulating GAS-6 protein may be involved in CKD-associated inflammation. In addition, our study found a significant correlation between circulating GAS-6 and IL-6 levels. The results of the present study indicate that elevated serum GAS-6 is an independent predictor of increased levels of IL-6 in these patients, suggesting that increased levels of GAS-6 may promote and/or facilitate the development of inflammation in CKD patients. Studies on endothelial cells, monocytes-macrophages, and smooth muscle cells support the direct role of hsCRP in atherogenesis. The study by Avanzi et al. demonstrated an association between GAS-6 levels and hsCRP in patients with critical limb ischemia.[26]

However, recently, the study by Lee et al. did not show such an association between hsCRP and GAS-6 in the uremic milieu, non-dialysis and dialysis patients.[22] Our study did not find an association between GAS-6 and hsCRP in HD patients.

The results of the present study indicate that elevated serum P is an independent predictor of increased levels of inflammatory parameters in these patients. Interesting data derive from studies report the potential anti-inflammatory properties of phosphate binders, specifically sevelamer. The study by Yamada et al. on HD subjects showed that there was a significant decrease in hsCRP during sevelamer therapy, and that the reduction rate of hsCRP was significantly correlated with the change of P.[27]

## Conclusion

The potential association between mineral metabolism and inflammation in advanced CKD patients, who were undergoing dialysis, showed that serum P is a risk factor for the presence of an inflammatory state in these subjects. Our observation on the association between serum P and pro-inflammatory cytokines requires further exploration and confirmation by longitudinal prospective studies.

Plasma GAS-6 concentration may be an independent risk factor of HD patients and a potential surrogate marker of inflammation. These results support the hypothesis that modulation of GAS-6 activity may provide an important point for intervention. GAS-6/TAM signaling represents a new class of therapeutic targets.

## Conflict of Interests

Authors have no conflict of interest.

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