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Nervous System Autoantibodies and Vitamin D Receptor Gene Polymorphisms in Hemodialysis Patients

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Abstract

BACKGROUND—Cognitive deficits are prevalent in hemodialysis (HD) patients. Vitamin D deficiency and vitamin D receptor gene (*VDR*) SNPs have been linked to both neurodegeneration (ND) and neuroprotection, respectively. Autoantibodies (Ab) to myelin basic protein (MBP), glial fibrillary acidic protein (GFAP), and neurofilament (NF) triplet proteins arise secondary to nervous system (NS) damage providing a means to assess neurological injury. Characterization of Ab biomarkers of NS damage in HD patients, their association with *VDR* SNPs, and nutritional vitamin D (NVD) therapy was performed.

METHODS—*VDR* genotypes, cytokines, and Ab biomarkers to NS proteins in HD subjects receiving ergocalciferol (n=40) were compared to non-users (n=71). IL-6, TNF- α , and IgG titers against NFs, GFAP and MBP were measured by immunoassay. Subjects were genotyped for *VDR* SNPs *BsmI* (rs1544410) and *FokI* (rs2228570).

RESULTS—Subjects (age 63.3 $\pm$ 16.1 years, 66% male) who were C allele carriers of *BsmI* had higher values of NF-68 antibody titers (p = 0.027). Ergocalciferol users (n=40) compared to non-users (n=71) had lower Ab titers to NS proteins, however, only anti-NF160 and anti-MBP titers were significantly (p < 0.05) higher.

CONCLUSIONS—IgG against NS proteins in HD patients suggests neuronal and glial insult and a relationship with *VDR* alleles. NVD may provide some neuroprotection, indicated by anti-NF160 and anti-MBP, which was markedly lowered in ergocalciferol patients. This preliminary study suggests Ab detection may be useful in monitoring ND and the potential of NVD for neuroprotection in HD patients.

Keywords

Autoantibodies; Ergocalciferol; Nervous system; Polymorphism; Vitamin D; *VDR*

Introduction

Neurological deficits such as cognitive impairment and structural changes in the brain are highly prevalent in dialysis patients.^{1,2} Similar to the kidney disease, these neurological abnormalities progress as kidney function declines, thus, several studies have recognized the need for increased awareness and early screening.^{3,4} Cognitive impairment affects up to 70% of dialysis patients and is associated with poor medication adherence, impaired executive decision making, and increased hospitalizations and mortality.^{2,3} Structural changes include cerebral brain atrophy and silent infarcts.⁵ Despite our continued awareness of the neurological deficits in dialysis patients, few studies have identified biological markers specific to nervous system (NS) damage.

Although the exact cause of cognitive impairment in CKD is unknown, the prevalence of several factors predisposes CKD patients to neurological complications and injury. Older age, uremic neurotoxins, anemia, vascular disease, a significant stroke prevalence, and inflammation provide reasonable explanations to the high prevalence of neurological dysfunction in CKD. In a seven-year study, Griva et al recently demonstrated that not only elderly but also younger aged dialysis patients (mean age ~ 50 years old) display a significant association of mortality and cognitive impairment.³ With the improvements in dialysis technology, a ceiling-effect may have been reached in terms of uremic toxin removal, thus, recent studies have failed to demonstrate improvement in cognitive impairment and increased dialysis adequacy.^{6,7} Despite historical reports demonstrating improved cognitive function with anemia correction^{8,9}, contemporary studies where the majority of patients reach target hemoglobin goals, cognitive impairment association with hemoglobin concentration is absent.³ Lastly, the significant burden of cerebral- or cardiovascular disease in CKD (with risk factors including inflammation, oxidative stress, and vascular calcification) provides additional insults that could contribute in the development and progression of cognitive impairment.

Evidence demonstrating physiological and neuroprotective functions of vitamin D has grown substantially over the last ten years.¹⁰⁻¹² Vitamin D biosynthesis, degradative pathways and vitamin D receptor (VDR) discovery in the brain suggest a beneficial role for vitamin D in neurological development and function.¹³⁻¹⁵ An example of vitamin D function in the brain includes promoting upregulation of neurotrophins, proteins required for neurons survival in aging, development, and function; involvement in neurotransmitter metabolism and the synthesis of certain growth factors such as glial cell line derived neurotrophic factor (GDNF).¹⁶ Furthermore, there is mounting evidence of neuroprotective functions, immunomodulating properties and neurodegeneration (ND) protection. *In vitro* studies have shown 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) neuronal regulation protects neurons from excess calcium entry protecting neurons during ischemic events, excessive stimulative insults, and inducible nitric oxide synthase (iNOS) up-regulated during ischemic events.¹³ iNOS produces nitric oxide which at high levels damages neurons. Ischemic insults have been well characterized in CKD patients where multiple brain magnetic resonance imaging (MRI) studies have revealed a large burden of silent cerebral infarcts, lacunes strokes, and white matter lesions.¹⁷ For several years, vitamin D has shown immune stimulating properties in oncology and neurology. Masoumi et al demonstrated strong immunostimulation with 1,25(OH)₂D₃ in Alzheimer's patient's macrophages to phagocytosis and clear amyloid- β protecting neurons from apoptosis.¹⁸ Additionally, hippocampal cells in Alzheimer's disease display downregulation of VDR suggesting a potential role for these receptors in the pathophysiology of Alzheimer's. These previous results highlight the possibility that vitamin D deficiency may predispose to enhanced neuronal vulnerability and accelerated neurological degeneration.

Neurodegenerative diseases involve the loss of neurons involved in cognitive, emotional, motor and sensory functions. Several investigators have demonstrated a relationship between vitamin D [25 OH] deficiency and cognitive performance, primarily in elderly patients. Decreased performance in neurocognitive exams, increased white matter hyperintensity volume (marker of underlying cerebral microangiopathy), and prevalence of large vessel infarcts in elderly patients with vitamin D deficiency are illustrated by several authors.^{5,19-23} Additionally, polymorphisms in the *VDR* gene have been shown to be protective against certain neurological disease. Inferior performance in cognitive function (memory and attention impairments) was noted in *BsmI* (rs1544410) and *TaqI* (rs731236) polymorphism carriers in the Leiden 85-plus Study. Participants positive for the *Apal* (rs7975232) variant-allele and the haplotype 1 (baT) haplotype displayed better cognitive performance and less depressive symptoms.²⁴ In a study of late-onset Alzheimer's disease cases (n=104) and healthy controls (n=109), the polymorphism *Apal* genotype 'Aa' was associated with a 2.3 fold higher risk of developing Alzheimer's compared to the genotype 'AA'.²⁵ Parkinson's disease has shown a correlation with the 'b' allele and 'bb' homozygosity of the *BsmI* polymorphism in an 85-to-231 Korean case-control study.²⁶

While data concerning cognitive impairment being under-diagnosed and the effect on quality-of-life plentiful, fewer studies have provided biological evidence for NS damage in HD patients, apart from imaging studies. In spite of MRI studies illustrating associations with cognitive impairment and vitamin D deficiency, minimal invasive and inexpensive indicators for assessing HD patients risk for neurological damage are understudied. However, similarities exist between ND diseases such as Alzheimer's and dementia seen in HD patients, where antibodies indicating NS damage have been detected, as well as in other neurodegenerative disorders. These may be of utility in assessing the HD patient's development of neurological damage. Autoantibodies to myelin basic protein (MBP), glial fibrillary acidic protein (GFAP), and neurofilaments (NFs) triplet proteins have been detected in patients suffering from numerous neurological diseases.²⁷ In some reports, such Ab are pathogenic and can interfere with nerve conduction. Considering that there is a paucity of data on the association between biomarkers of vitamin D and neurological injury in the dialysis population. The objective of this exploratory study was to characterize biomarkers of neuropathy in HD patients and their association with vitamin D utilization and *VDR* SNPs.

Subjects and Methods

Study subjects

Maintenance hemodialysis patients from Rubin Dialysis Centers, Inc. (Clifton Park, Troy and Saratoga, NY) were recruited from July to August 2009 as part of a larger genomics study to evaluate the relationship between *VDR* and biomarkers. All patients from each dialysis shift were approached for study recruitment. Patients were eligible for enrollment if they were at least 18 years of age and had been on chronic hemodialysis for at least 3 months. This study was approved by the Institutional Review Boards at Albany College of Pharmacy and Health Sciences and the University of Illinois at Chicago (UIC). The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. Written informed consent was obtained from all participants prior to conducting any study procedures.

Laboratory and biomarker measurements

Pre-dialysis blood samples were collected from existing access sites of each study patient for measurement of inflammatory cytokines (interleukin-6 [IL-6] and tumor necrosis factor- α [TNF- α]), immunoglobulin G (IgG) titers against NFs (NF-68, NF-160, and NF-200), GFAP

and MBP. Plasma and sera were isolated, divided into aliquots and immediately stored at -80°C until biomarker analysis. Demographic data and current medication regimens were collected from each subject's medical records. All clinical laboratory work was performed at Spectra Laboratories (Rockleigh, NJ). Standard techniques were used to measure cholesterol, hemoglobin, albumin, calcium, phosphorus, intact parathyroid hormone (PTH) and 25-hydroxyvitamin D levels (25[OH]D). Plasma concentrations of inflammatory cytokines (IL-6 and TNF- α) were determined using commercially available multiplex bead array assay kits (Millipore Corporation; Billerica, MA). All multiplex assays were performed on a BioPlex 200 System (Bio-Rad Laboratories; Hercules, CA).

Serum IgG titers against NFs (NF-68, NF-160, and NF-200), GFAP and MBP were determined using as described previously.^{27,28} Details are available in supplemental file 1.

VDR Genotyping

BsmI [rs154410] and *FokI* [rs2228570] polymorphisms of the *VDR* gene were studied in our participants. Genomic DNA was extracted from peripheral blood leukocytes using the Gentra Puregene Blood Core Kit C (Qiagen Inc. catalog # 158389) according to the manufacturer's protocol. *VDR* polymerase chain reaction (PCR) assays were done as previously described.²⁹⁻³¹ *VDR* genotyping was performed and analyzed at the UIC Pharmacogenomics Laboratory in the College of Pharmacy with PyrosequencingTM Technology. Pyrosequencing primers were designed using Pyrosequencing single nucleotide polymorphism (SNP) Primer Design Version 1.06 software (<http://www.pyrosequencing.com>). Direct sequencing was performed to confirm and validate our Pyrosequencing assays. Additionally 15% of samples were genotyped in duplicate to assess genotyping precision. Genotyping calls were made blinded to clinical variables. Concordance between Pyrosequencing duplicates as well as direct sequencing was 100%. Call rates for individual assays ranged from 98-100% with an average of >99%.

Statistical analysis

A descriptive analysis of patient characteristics was conducted. Data were reported as mean $\pm$ standard deviation for all normally distributed continuous variables and as median (interquartile range) for all skewed variables. Comparisons of subjects age, dialysis vintage, albumin, corrected calcium, phosphorus, 25[OH]D, hemoglobin, total cholesterol, antiNF-68, antiNF-160, antiNF-200, antiGFAP and antiMBP between patients receiving ergocalciferol to those not taking ergocalciferol were made using Student's t-test. Comparisons of intact PTH and IL-6 between groups were made using the Wilcoxon rank-sum test, due to a significant departure from normality for these variables. Differences in race and intravenous doxercalciferol use were assessed using the χ^2 test. The relationship between Ab biomarkers was evaluated using Pearson's correlation analysis. Log transformations of skewed variables were used in correlation analyses.

The χ^2 test for goodness of fit was used to determine if genotype distributions deviated from Hardy-Weinberg equilibrium. Genotype allele frequencies were compared using χ^2 and Fisher's exact tests. Comparison of continuous variables between genotypes was examined assuming an additive model of inheritance using ANOVA or Kruskal-Wallis test where appropriate. Statistical analyses were conducted using SAS version 9.2 (SAS Institute Inc., Cary, NC). All statistical tests were performed at a two-sided 0.05 level of significance.

Results

A total of 109 maintenance hemodialysis patients were studied. Characteristics of the study population are shown in Table 1. Demographic and clinical laboratory parameters were

evaluated to examine their relationship with biomarkers of ND. Markers of ND did not correlate with age, duration of dialysis, albumin, corrected calcium, phosphorus, log intact PTH, 25[OH]D, cholesterol, IL-6 or TNF- α . Titers of antiNF-68, antiNF-160, antiNF-200, anti-GFAP and anti-MBP were similar between those who were vitamin D replete (25[OH]D ≥ 30 ng/mL) to those who were vitamin D insufficient. However, in this dialysis population the respective biomarkers of ND correlated with one another (Table 2). There was no difference in circulating concentrations of ND biomarkers observed between the sexes or the races (Caucasian versus non-Caucasian), respectively.

The relationship between biomarkers of ND and vitamin D use was also evaluated. Patients on ergocalciferol were using $40,625 \pm 15,696$ IU/week for an average of 9.2 months. Subjects taking ergocalciferol ($n = 40$) had significantly lower concentrations antiNF-160 compared to patients not using ergocalciferol ($n = 69$), 1.48 ± 0.31 abs versus 1.62 ± 0.31 abs ($p = 0.0028$). Additionally subjects on ergocalciferol therapy had lower concentrations of anti-MBP compared to ergocalciferol nonusers, 1.63 ± 0.26 abs versus 1.75 ± 0.28 absorbance ($p = 0.0023$). There were no differences in ND biomarker levels between patients receiving intravenous activated vitamin D analog (VDA) therapy compared to subjects not receiving VDAs.

Since a potential relationship was observed between ergocalciferol use and both antiNF-160 and anti-MBP, we sought to further examine the characteristics of the ergocalciferol group (Table 3). There were no differences in clinical laboratory parameters between groups with the exception that 25[OH]D levels were higher in the ergocalciferol group ($p = 0.010$). A greater percentage of patients receiving ergocalciferol were vitamin D replete compared to those not receiving ergocalciferol (63% versus 39%, $p = 0.019$). Additionally, there were no differences in the proportions taking intravenous VDAs between groups.

BsmI (CC/CT/TT: 45/54/10) and *FokI* (CC/CT/TT: 45/44/20) genotype counts did not deviate from Hardy-Weinberg equilibrium ($p = 0.27$ and $p = 0.12$, respectively) for the entire cohort or when examined separately by race for *BsmI* or *FokI* ($p = 0.40$ and 0.67 , respectively). No differences across genotype groups were observed in clinical laboratory parameters or in titers of antiNF-68, antiNF-160, antiNF-200, antiGFAP or anti MBP were observed across the different *BsmI* or *FokI* genotypes. There were no differences in the proportions of patients taking ergocalciferol within each of the respective *BsmI* or *FokI* genotypes. However, carriers of the C allele in *BsmI* had higher values of the NF-68 antibody titers (Figure 1; $p = 0.027$).

Given that the majority of the study population was of Caucasian ancestry, a post hoc analysis evaluating clinical laboratory parameters and biomarkers in this population was conducted. Similar to the entire cohort, no differences in clinical laboratory parameters or IgG titers against NFs, GFAP or MBP across *BsmI* or *FokI* genotypes were observed. The association with higher NF-68 for *BsmI* C allele carriers held true for the Caucasian subpopulation (Fig. 1; $p = 0.036$).

Discussion

With increasing awareness of cognitive impairment in CKD, it is important to develop and validate noninvasive and sensitive markers for identifying patients at risk or with changes in markers that indicate neurological damage. Neuroautoantibodies arising secondary to nervous system damage may provide a means to assess neurological injury in HD patients. Furthermore, attempts should be made to determine therapies that may provide protection from neural insult. To our knowledge this is the first report to provide prospects for potentially identifying markers of NS damage in CKD and protective therapies. Although,

the optimal timing and frequency for instruments or markers to assess cognitive function in ESRD patients will depend on the clinical setting, our previous results demonstrate only injury.

Noninvasive biomarkers measurements which would offer valuable prognostic information can help identify patients at risk and could be used as secondary surrogate markers for treatment trials. Changes or expression of autoantibodies precede clinical signs and symptoms of neurological disease providing a much more sensitive marker of damage than late presenting clinical detection as suggested by some animal studies.³² Loss of these structural proteins, indicated by serum IgG, if pathogenic, may contribute to ND. The present neurological markers have been utilized in several studies illustrating either toxic exposure or disease progress. MBP is found in the myelin sheath of neurons (Figure 2). Serum IgG antibodies against MBP are cytotoxic and present in early multiple sclerosis.³³ GFAP is the intermediate filament of astrocytes cell structure and movement, cell communication, and the functioning of the blood brain barrier. Astrogliosis, the hypertrophy or astrocytes secondary to neuronal injury or degeneration, can be successfully measure by GFAP. GFAP has been proposed to detect and characterize the cellular response to toxicant-induced in neurotoxicity safety evaluations.³⁴ NFs are a unique triplet of proteins that are widely distributed in the neuron either separately, or as triplet bundles that polymerize and form cross-bridges. NFs proteins sustain mechanical stability resisting compression of the axon and are highly specific biomarkers for neuronal death and axonal degeneration. NFs are classified according to their molecular weight (in kD) as NF-68 (light weight approximately 68kD), NF-160 (medium weight of approximately 168 kD), and NF-200 (heavy weight approximately 200 kD). They vary quantitatively in different neuronal structures and in their antigenicity, which may explain the variable titers of anti-NF IgG in ergocalciferol users.²⁸

Subsequent neuronal death and axonal degeneration results in the release of intracellular proteins into the interstitial fluid. Neurodegenerative disorders target selective populations of neurons, with ensuing loss of function. However, the correlation of the neuroAb markers in our study is consistent with the process of neurodegeneration (Table 2) since loss of NF triplet proteins is expected to occur in the presence of a primary neuronal insult, followed by a secondary loss of myelin and glial involvement. Threshold values for the neuroAb markers have not been reported or determined, any presence of neuroAb, particularly the IgG isotype, would indicate injury³⁵. Furthermore, persistently elevated levels of IgG vs. IgM would signify continued neurological damage. The universal and significantly elevated levels of IgG raise concerns as to the causes of such persistent potential neurological damage. All subjects (100% frequency) in our cohort displayed positive and considerably elevated IgG levels of each neuroAb, exceeding frequencies seen in diseases such as Alzheimer's disease and vascular dementia.³⁶ Although such markers are rarely present or elevated in healthy controls studies, some investigators have noted elevated levels in only 0 to 17% of reference subjects.³⁷ However, control subjects levels remain lower than individuals with active or chronic disease and NS injury.³⁸⁻⁴⁰ The detection of autoantibodies in a small percentage of the reference population probably reflects natural autoantibodies, usually IgM, which are found in some individuals³⁵.

Serum levels of the neurological markers were significantly elevated for each group (ergocalciferol users vs. nonusers). However, the ergocalciferol users demonstrated lower absorbance values (i.e. lower concentrations of antibodies) for each of the neurological markers, albeit only two demonstrated significance (Table 3: AntiNF-160 and AntiMBP). Despite the inability to infer causal mechanisms from our report, it is important to identify neuroprotective therapies. Several reports have demonstrated associations with 25-hydroxyvitamin D levels and neurological abnormalities (e.g. dementia, MRI indicators,

etc.), yet none have illustrated the importance of correcting vitamin D levels or treatment with vitamin D supplements.^{5,23,41,42} Additionally, the benefits of NVD were seen in spite of patients currently receiving activated vitamin D analogs. The widespread distribution of 1 α -OHase (the enzyme responsible for converting 25-hydroxyvitamin D to 1,25-hydroxyvitamin D) in the human brain suggest that local conversion of vitamin D to the active form is of significance.¹¹ The variable efficacy of vitamin D precursors to the activate form in the nervous system is unknown, yet may explain the additional benefits of nutritional vitamin D supplementation in addition to activated VDA administration.

Significantly lower NF-68 protein expression seen with the T allele of the *BsmI* SNP highlights a potential protective allelic effect. No other reports in the literature have illustrated a relationship with *VDR* SNPs and neurofilaments. Although several studies have demonstrated allelic difference in other NS diseases with the *BsmI* polymorphism, a larger sample size is warrant to confirm our association.

The current investigation has its limitations. First, the cross-section design prevents the interpretation of cause and effect as our investigation is only a marker of insult. Furthermore, the sample size is modest for a genetic study, limiting our ability for further genomic stratifications and increasing the potential for a type 2 error. To check whether our non-significant results across genotype groups could be due to a lack of statistical power, we conducted post hoc power analyses with the *BsmI* C allele which was associated with higher NF-68 levels. A post hoc power analysis revealed that on the basis of the mean NF-68 antibody titers, *BsmI* and *FokI* genotypes sample sizes were not adequately powered, with an observed statistical power of 0.47 and 0.36, respectively. The lack of a neuropsychological, or brain imaging measures limits our ability to correlate these antibody biomarkers with clinical measures. Without cognitive data (e.g. MiniMental Status examination, neuropsychology testing, clinical psychiatric ratings of mood) the neuroautoantibodies cannot be correlated to clinically detectable cognitive impairment.

Conclusion

IgG against NS proteins (NFs, GFAP and MBP) in HD patients suggests neuronal and glial insult. These Abs may serve as markers of neural insult in hemodialysis patients. However, the markers would need to be tested in earlier stages of chronic kidney disease to determine their sensitive to neurological changes prior to the initiation of dialysis. Lower neuromarker titers in patients receiving ergocalciferol or T allele of the *BsmI* SNP suggest a neuroprotective effect. Prospective trials are needed to verify these neuroprotective effects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Anti-NF68 absorbance values according to the *BsmI* alleles for all subjects with T (n=10) or C (n=99) allele, respectively, and Caucasians with T (n=10) or C (n=84) allele, respectively. Mean $\pm$ SD; *p< 0.05.

Figure 2.

Mechanism for generation of autoantibodies against nervous system proteins (NSP). In the presence of neural insult (I) due to different etiological factors, structural proteins, such as the neuronal neurofilament (NF) triplet proteins (L=light or 68kD; M=medium or 160 kD; H=heavy or 200 kD), myelin sheath proteins (MBP=myelin basic protein), or astrocytic protein (GFAP=glial fibrillary acidic protein) may undergo (II) immunological processing *in situ* by microglia/macrophages, or outside the blood brain barrier (BBB) in the periphery by dendritic cells and/or B-lymphocytes. Antigen presentation to T helper cells (T_h) and T-help through cytokine production (e.g., IL-2 and IL-6) stimulates the development of humoral immunity, with the elaboration of antibodies (Y) detectable in the peripheral blood serum.

Table 1

Patient characteristics

Parameter	All Patients (n = 109)
Age (years)	63.1 ± 16.2
Male (n, [%])	64 (58.7)
White (n, [%])	94 (86.2)
Dialysis vintage (years)	3.4 ± 3.0
Albumin (g/dL)	4.3 ± 0.6
Corrected calcium (mg/dL)	9.0 ± 0.6
Phosphorus (mg/dL)	5.5 ± 1.8
Intact PTH (pg/mL)	329 (218 to 462)
25-hydroxyvitamin D (ng/mL)	31.9 ± 15.9
Hemoglobin (g/dL)	11.7 ± 1.3
Total cholesterol (mg/dL)	149 ± 35
Doxercalciferol use (n [%])	76 (69.7)
IL-6 (pg/mL)	3.2 (1.9 to 6.0)
TNF-α (pg/mL)	12.5 ± 6.6
AntiNF-68 (absorbance 405 nm)	1.68 ± 0.30
AntiNF-160 (absorbance 405 nm)	1.57 ± 0.31
AntiNF-200 (absorbance 405 nm)	1.70 ± 0.30
AntiGFAP (absorbance 405 nm)	1.74 ± 0.39
AntiMBP (absorbance 405 nm)	1.70 ± 0.28

Table 2

Relationship between biomarkers of neurodegeneration

	AntiNF-68	AntiNF-160	AntiNF-200	AntiGFAP	AntiMBP
AntiNF-68	1.00	r = 0.1906 p = 0.0471	r = 0.0689 p = 0.4766	r = 0.3285 p = 0.005	r = 0.3285 p = 0.005
AntiNF-160	r = 0.1906 p = 0.0471	1.00	r = 0.3914 p < 0.0001	r = 0.4678 p < 0.0001	r = 0.5012 p < 0.0001
AntiNF-200	r = 0.0689 p = 0.4766	r = 0.3914 p < 0.0001	1.00	r = 0.5165 p < 0.0001	r = 0.5267 p < 0.0001
AntiGFAP	r = 0.3285 p = 0.005	r = 0.4678 p < 0.0001	r = 0.5165 p < 0.0001	1.00	r = 0.7818 p < 0.0001
AntiMBP	r = 0.3285 p = 0.005	r = 0.5012 p < 0.0001	r = 0.5267 p < 0.0001	r = 0.7818 p < 0.0001	1.00

Pearson's correlation analysis was used to assess the relationship of biomarkers of neurodegeneration with one another.

Table 3

Patient characteristics grouped by ergocalciferol use

Parameter	No Ergocalciferol (n = 69)	Ergocalciferol (n = 40)	pa
Age (years)	62.1 ± 15.9	64.7 ± 16.7	0.4208
Male (n, [%])	45 (65.2)	19 (47.5)	0.0702
Caucasian (n, [%])	58 (84.1)	36 (90.0)	0.3854
Dialysis vintage (years)	3.5 ± 3.3	3.1 ± 2.5	0.4883
Albumin (g/dL)	4.3 ± 0.6	4.4 ± 0.5	0.4579
Corrected calcium (mg/dL)	9.0 ± 0.6	8.9 ± 0.6	0.2616
Phosphorus (mg/dL)	5.5 ± 1.8	5.6 ± 1.9	0.8630
Intact PTH (pg/mL)	315 (218 to 430)	364 (247 to 547)	0.2286
25-hydroxyvitamin D (ng/mL)	29.0 ± 15.0	37.1 ± 16.4	0.0101
Hemoglobin (g/dL)	11.8 ± 1.3	11.6 ± 1.5	0.3618
Total cholesterol (mg/dL)	150 ± 40	148 ± 28	0.7903
Intravenous vitamin D analog use (n [%])	47 (68.1)	29 (72.5)	0.6311
IL-6 (pg/mL)	3.0 (1.7 to 5.6)	4.1 (2.0 to 6.7)	0.2086
TNF-α (pg/mL)	13.0 ± 7.1	11.6 ± 6.6	0.2761
AntiNF-68 (absorbance 405 nm)	1.68 ± 0.26	1.71 ± 0.35	0.6245
AntiNF-160 (absorbance 405 nm)	1.62 ± 0.31	1.48 ± 0.31	0.0218
AntiNF-200 (absorbance 405 nm)	1.71 ± 0.27	1.67 ± 0.33	0.3858
AntiGFAP (absorbance 405 nm)	1.78 ± 0.42	1.69 ± 0.34	0.2245
AntiMBP (absorbance 405 nm)	1.75 ± 0.28	1.63 ± 0.26	0.0232

The χ^2 test was used for comparisons of proportions among gender, race and intravenous doxercalciferol use, whereas Student's t-test was used for comparisons of age, dialysis vintage, albumin, corrected calcium, phosphorus, 25-hydroxyvitamin D, hemoglobin, total cholesterol, antiNF-68, antiNF-160, antiNF-200, antiGFAP and antiMBP between groups. These values are shown as mean ± standard deviation. The Wilcoxon rank-sum test was used for comparisons of intact PTH and IL-6. These values are shown as median (interquartile range) between groups.

^a Comparison of No Ergocalciferol versus Ergocalciferol groups.