Osteoprotegerin Assessment Improves Prediction of Mortality in Stroke Patients

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Background: Elevated circulating osteoprotegerin (OPG) level is associated with an increased risk of hospitalization for ischemic stroke and coronary artery disease. The aim of the present study was to analyze whether OPG assessment may improve the prediction of mortality in patients with stroke. *Patients and Methods:* Serum OPG, fetuin A, 25-OH-D_3, intact parathyroid hormone levels were assessed in serum samples which were left over after routine tests in a hospital laboratory. This assessment was conducted in 240 consecutive patients with acute ischemic stroke, admitted within 24 hours after the onset of symptoms to the Stroke Unit. Mortality data were obtained from the local registry office. *Results:* The mean OPG serum level was 14.6 ± 6.0 pmol/L (range: 3.7-43.4). There were no significant differences in the OPG values between men and women (13.9 ± 5.0 versus 15.1 ± 6.7 pmol/L; $P = .12$). Therefore, tertiles were calculated for the whole group. During the follow-up, 85 (35.4%) patients died and 92 (38.3%) died or had recurrent stroke. OPG level appeared a significant predictors of death and composite end-point (death/recurrent stroke), in addition to the well-established once (age, atrial fibrillation, diabetes RANKIN at admission and discharge, severity of stroke). In multivariable stepwise backward analyses, the OPG level persisted as a significant and independent predictor of death (hazard ratio [HR] = 1.084 (95% confidence intervals: 1.036-1.134)] and composite and point (HR = 1.082 [1.037-1.129]). *Conclusions:* OPG level may be considered as a predictor of mortality in stroke patients.

Key Words: Osteoprotegerin—stroke—biomarker—mortality

Abbreviations: OPN, Osteopontin; MPG, matrix Gla protein; BMP-7, Bone morphogenetic protein-7; OPG, Osteoprotegerin; RANKL, Receptor Activator for Nuclear Factor κ B Ligand; RANK, Receptor Activator for Nuclear Factor κ B; TRAIL, TNF-related apoptosis-inducing ligand; NF-kB, Nuclear factor kappa-b; IkB, Inhibitor kappa -b; hsCRP, High-sensitivity C-Reactive Protein; Lp-PLA, lipoprotein associated phospholipase A2; TIMP, tissue inhibitor of metalloproteinases, IL-6, interleukin-6.

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Introduction

Atherosclerosis is a major risk factor for ischemic stroke. Particularly, as a result of chronic inflammatory response of white blood cells in the arterial wall, which is aggravated by hyperlipidemia, oxidative stress, and fibrin proliferation.[1,2]

Destabilization of an atherosclerotic plaque and its rupture results in thrombus formation, which is responsible for approximately 20%-25% of ischemic strokes.[3]

Unstable atherosclerotic plaque in the carotid artery is typically not calcified but highly laden with lipids and its calcification improves stabilization and reduces the risk of ischemic stroke.[4,5] The process of calcification involves interactions between mediators and inhibitors. Among inhibitors, there are numerous proteins including osteopontin, fetuin A, Klotho, matrix Gla protein, bone morphogenetic protein-7 (BMP-7) and osteoprotegerin (OPG).[6] The process of artery calcification is quite similar to that which is responsible for bone metabolism. This involves the mechanisms based on the relationship between receptor activator for nuclear factor (B Ligand (RANKL), receptor activator for nuclear factor (B (RANK), and OPG. In addition, RANKL probably modulates the atherosclerotic process as well.[7] The atherosclerotic plaque has been proved to contain both bone matrix proteins and bone metabolic mediators. These include osteocalcin, osteopontin, RANKL, OPG, several types of cytokines, and bone BMP.[6] RANKL and RANK complex is postulated to activate the intracellular mechanism leading to increased production of BMP-4 in smooth muscles that induces apoptosis and calcification.[8]

OPG, discovered by Simoneta et al. in 1997, is a member of the tumor necrosis factor (TNF) receptor superfamily. This receptor superfamily acts as a decoy receptor for TNF-related apoptosis-inducing ligand and RANKL. It counteracts the processes activated by RANKL/RANK complex. Thus, it decreases the osteoclast's differentiation and activity of mature osteoclasts.[9-11] OPG is produced by osteoblasts, stroma cells, lymphocytes B and monocytes, fibroblasts, endothelial cells, and vascular smooth muscle cells.[12,13] According to data published by Jensen et al., circulating OPG may act as a marker of both bone homeostasis as well as vascular calcification and inflammation.[14] Additionally, the dual mechanism of OPG action, both protective (antiplaque formation) and pro-atherosclerotic, has been suggested.[15] The protective effect of endogenous OPG probably results from the restriction of atherosclerotic plaque formation and artery calcification. That mechanism is supported by the development of excessive artery calcification in OPG knock-out mice.[16] The increased level of OPG may also be protective. This is due to the improvement of plaque stability, hampering apoptosis of endothelium cells, and smooth muscle cells as well as anti-inflammatory action.[15] Regardless of the ambiguous role of OPG, an increased level of serum OPG, characterize individuals with cardiovascular diseases.[7,17-19]

An elevated circulating OPG level is associated with an increased risk of hospitalization for ischemic stroke and coronary artery disease. Additionally, it is also associated with increased overall mortality, independent of traditional risk factors, and C-reactive protein levels.[20] Higher levels of OPG have already been reported in patients who died of stroke[21] and in those with more severe neurological deficits.[22] Furthermore, a higher level of OPG was associated with the presence and severity of cerebral atherosclerosis (an increased number of cerebral arteries involved).[23] OPG is also considered to be a potential biomarker of atherosclerosis in patients with asymptomatic carotid stenosis, in addition to lipoprotein associated phospholipase A2 (Lp-PLA), tissue inhibitor of metalloproteinases, interleukin-6, and high-sensitivity C-reactive protein (hsCRP).[24] The aim of the present study was to analyze whether OPG assessment may improve the prediction of mortality in patients with stroke.

Patients and Methods

The prospective study was conducted between January 2013 and August 2015. It included 240 patients with first episode ischemic stroke diagnosed according to the WHO criteria and based on radiological images (computed tomography and/or magnetic resonance of the head). All patients were hospitalized during the acute phase of stroke in the Department of Neurology of the Provincial Hospital in Rybnik, where diagnostics and therapy were conducted in accordance with the current guidelines.[13,15] The most probable mechanism of stroke was established, according to the Trial of Org 10172 in Acute Stroke Treatment classification.[18] Patients with hemorrhagic stroke, a history of cancer, apparent inflammation, and impairment in activities of daily living before the stroke were excluded from the analysis.

The study was approved by the Bioethics Committee of the Medical University of Silesia.

The frozen serum samples used in this study were those that had been left over after routine laboratory tests (in the hospital laboratory), obtained at admission to Stroke Unit of the Department of Neurology, Provincial Hospital, in Rybnik. These frozen serum samples were obtained from 240 consecutive patients with acute ischemic stroke within the 24 hours after the onset of symptoms. Patients with hemorrhagic stroke, history of cancer, apparent inflammation, and impairment in activities of daily living before the stroke were excluded from the analysis. The study was performed from January 2013 to August 2015. The study was initiated after obtaining approval from the Bioethics Committee of the Medical University of Silesia for the utilization of serum samples and retrieval of data from medical reports without an informed consent from stroke

patients. This also made it possible to include unconscious patients and prevented a selection bias.

The diagnosis of acute ischemic stroke was based on neurological examination and was confirmed by computed tomography. The diagnosis was made after exclusion of other causes of acute neurological deficit and in accordance with the current guidelines.[17] The most probable mechanism of stroke was established according to the Trial of Org 10172 in Acute Stroke Treatment classification.[18]

The clinical course of stroke was assessed on the basis of two main criteria. The basis of the severity of neurological deficit according to the National Institutes of Health Stroke scale and functional status at admission and discharge using the modified Rankin scale.

The retrieved data from medical records included clinical status, comorbidities, medication, previous ischemic cerebrovascular episodes, cardiovascular risk factors, and routinely performed laboratory parameters (total blood count, serum glucose, creatinine, total cholesterol, low-density lipoprotein, high-density lipoproteins, triglycerides, and urine analysis).

Complete follow-up data for mortality were obtained from the Registry Office in Rybnik (for May 30, 2017).

Measurements

Additional assessments were performed in stored frozen samples at the Laboratory of the Department of Pathophysiology, Medical Faculty in Katowice, Medical University of Silesia. Serum OPG, fetuin A, intact fibroblast growth factor 23 (iFGF23), and hsCRP concentrations were measured by using commercially available ELISA kits from BioVendor (Brno, The Czech Republic)—OPG and fetuin A, Immutopics (San Clemente, CA), and immundiagnostik AG (Bensheim, Germany), respectively, with the mean intra- and interassay coefficients <4.9% and <9% (OPG), <8.6% and <11.2% (fetuin A), <4.4% and <6.1% (iFGF23), and <6% and <11.6% (HsCRP). Serum 25-OH-D_3 (limit of quantification 3 ng/mL) and intact parathyroid hormone level were assessed by electrochemiluminescence method using commercially available kits on the Cobas E411 analyzer (Roche Diagnostics GmbH, Mannheim, Germany) with interassay coefficients of variability <7.8% and 6.5%, respectively. Serum phosphorus and calcium were assessed by an automated system (Cobas 111, Roche Diagnostics GmbH, Mannheim, Germany) with interassay coefficients of variability <2.3% and 1.3%, respectively.

Data Analysis

Glomerular filtration rate (eGFR) was estimated according to the short Modification of Diet in Renal Disease formula.[20] Patients with eGFR <60 mL/min/1.73 m^2 or albuminuria in routine urine analysis were considered as having chronic kidney disease.

Vitamin D status was categorized by commonly used cut-offs and definitions of serum 25-OH-D_3: severe deficiency (values below 10 ng/mL), deficiency (between 10 and 19.9 ng/mL), insufficiency (between 20 and 29.9 ng/mL), and optimal values (above 30 and 100 ng/mL).[19] intact parathyroid hormone levels above 65 pg/mL were scored as increased based on the cut-off value suggested by the kit manufacturer. iFGF23 levels were scored in relation to median value (50 pg/mL).

Statistical Analysis

Statistical analysis was performed using STATISTICA 10.0 PL (StatSoft, Cracow, Poland), StataSE 13.0 (StataCorp LP, TX), and R software. Statistical significance was set at a P value below .05. All tests were 2-tailed. Imputations were not done for missing data. Nominal and ordinal data were expressed as percentages. Interval data were expressed as mean value ± standard deviation in the case of normal distribution. In the case of data with skewed or non-normal distribution, it was expressed as median, with lower and upper quartiles. Distribution of variables was evaluated by the Anderson-Darling test and the quantile-quantile (Q-Q) plot. Homogeneity of variances was assessed by the Levene test.

In order to show the survival rate and cumulative hazard estimates according to the follow-up time, Kaplan-Meier curves and Nelson-Aalen estimates were used. These were used with a log-rank test to compare survival distribution between 2 samples. Risk factors of death as well as composite end points (death or a recurrent stroke) were analyzed with univariable and multivariable stepwise backward Cox proportional hazard regression. Schoenfeld residuals were used to test proportional hazard assumption. The concordance probability, which is defined as the probability that predictions and outcomes are concordant, was calculated with Gönen and Heller's K concordance coefficient. The extended mean was obtained by extending the Kaplan-Meier product-limit survivor curve. This was extended to zero by using an exponentially fitted curve and then computing the area under the entire curve.

Results

Study Group Characteristics

The characteristics of the study group of 240 patients referred to the hospital with no or minor (the National Institutes of Health Stroke Scale ≤ 4 pts; N = 65), moderate (5-15 pts; N = 130), moderate to severe or severe stroke symptoms (≥16 pts; N = 45) is shown in Tables 1 and 2. There were 44.2% of patients with large vessel occlusion, 23.3% with lacunar, 22.9% with embolic stroke and 23 (8.6%) with other classified or unclassified causes.

A previous history of acute ischemic stroke or transient ischemic attack or intracerebral bleeding was observed in

20.9% of study patients. Hypertension and atrial fibrillation were diagnosed in 84.6% and 29.2% of subjects, respectively. One-third of patients with atrial fibrillation were treated with antithrombotic therapy. Over one-third of patients (35.8%) had chronic kidney disease and as much as 62.9% of patients had severe vitamin D deficiency.

Acetylsalicylic acid was taken by 81% of patients with a previous history of acute ischemic stroke or ischemic heart disease. A total of 69% of patients diagnosed with dyslipidemia were on statin therapy.

Serum Osteoprotegerin (OPG) Levels

The mean OPG serum level was 14.6 ± 6.0 pmol/L (range: 3.7-43.4). There were no significant differences in the OPG values between men and women (13.9 ± 5.0 versus 15.1 ± 6.7 pmol/L; P = .12). Therefore, tertiles were calculated for the whole group. Some significant differences between the subgroups are shown in Table 1 and 2. Patients with the highest serum OPG concentration were significantly older, had a lower concentration of hemoglobin and triglycerides, as well as higher levels of CRP, phosphorus, and iFGF23, that cannot be explained by worse kidney function.

OPG as a Predictor of Death

OPG level appeared a significant predictors of death (for all reasons) and composite end point (death/recurrent stroke), in addition to the well-established once (age, atrial fibrillation—only death predictor, diabetes RANKIN at admission and discharge, severity of stroke).

Twenty-four patients (10%) died during hospitalization: 3 (3.7%) in the lowest OPG tertile subgroup, 5 (6.3%) in the middle OPG tertile subgroup, and 16 (20%) in the highest. In addition, there were 35 fatal outcomes during the first year of follow-up (N = 7%-8.6%; N = 12%-15.2%; N = 16%-20%; respectively). One year mortality was 12.3%, 21.5%, and 40% in OPG tertiles, respectively (P < .001).

Patients had died of stroke (N = 21%-24.7%), acute myocardial infarction (N = 25%-29.4%), heart failure (N = 18%-21.2%), infection (N = 16%-18.8%), and other or unknown cause (N = 5%-5.9%). Those with fatal outcome were older (77 ± 11 versus 69 ± 11 years; P < .001), more frequently underwent severe strokes (17.6% versus 1.9%; P < .001), had higher RANKIN scores at hospital discharge (3.9 ± 1.9 versus 2.1 ± 1.5; P < .001), and increased serum OPG levels (17.0 ± 6.8 versus 13.3 ± 5.1 pmol/L; P < .001) than stroke survivals.

Table 1. *Characteristics of study group and osteoprotegerin tertiles subgroups*

		Osteoprotegerin tertiles [pmol/L]			
	All	<11.7 [N = 81]	11.7-15.9 [N = 79]	≥15.9 [N = 80]	*P*
Osteoprotegerin [pmol/L]	14.6 ± 6.0	9.0 ± 1.9	13.7 ± 1.1	21.2 ± 5.2	–
Age [years]	72 ± 11	70 ± 11	70 ± 10	75 ± 12**	<**.01**
Sex [men/women]	109/131	37/44	38/41	34/46	.78
Previous stroke or transient ischemic attack [n/%]	45/18.7	15/18.5	16/20.2	14/17.5	.90
Previous intracerebral bleeding [n/%]	4/1.7	3/3.7	0	1/1.2	.12
Concomitant diseases					
Hypertension [n/%]	203/84.6	69/85.2	66/83.5	68/85.0	.95
Symptomatic atherosclerosis [n/%]	101/42.1	36/44.4	35/44.3	30/37.5	.60
Ischemic heart disease [n/%]	69/28.8	22/27.2	18/22.8	29/36.2	.16
Valvular diseases [n/%]	24 / 10.0	9/11.1	7/8.9	8/10.0	.89
Atrial fibrillation—AF [n/%]	70/29.2	28/34.6	15/19.0*	27/33.7	<**.05**
Obesity [n/%]	44/18.3	16/19.7	15/19.0	13/16.2	.83
Diabetes [n/%]	84/35.0	20/24.7	34/43.0*	30/37.5	<**.05**
Kidney diseases [n/%]	15/6.2	4/4.9	4/5.1	7/8.7	.53
COPD [n/%]	24/10.0	8/9.9	8/10.1	8/10.0	.99
Hypercholesterolemia [n/%]	107/44.6	41/50.6	30/38.0	36/45.0	.27
Other neurological diseases [n/%]	28/11.7	10/12.3	8/10.1	10/12.5	.87
Medication					
Aspirin[n/%]	114/47.9	36/45.0	35/44.9	43/53.7	.44
Statin therapy [n/%]	92/38.3	37/45.7	26/32.9	29/36.2	.22
Antithrombotic agents [n/%]	28/11.7	10/12.3	10/12.7	8/10.0	.85
Vitamin D supplementation [n/%]	1/0.4	0	1/1.3	0	–
Bisphosphonates [n/%]	2/0.8	0	2/2.5	0	–
Lifestyle					
Active smokers [n/%]	45/18.7	18/22.2	16/20.2	11/13.7	.35
Addicted to alcohol [n/%]	11/4.6	4/4.9	1/1.3	6/7.5	.17

*P < .05.
**P < .01.
#P < .001 in comparison to lower tercile group.

Table 2. *Characteristics of stroke symptom severity and its type, as well as laboratory parameters in study group and osteoprotegerin tertiles subgroups*

		Osteoprotegerin tertiles			
	All	<11.74 [N = 81]	11.74-15.88 [N = 79]	≥15.88 [N = 80]	*P*
The National Institutes of Health Stroke Scale index (NIHSS-I)	9.2 ± 6.5	8.8 ± 6.1	9.1 ± 6.7	9.8 ± 6.7	.62
No stroke symptoms [n/%]	3/1.3	2/2.5	0	1/1.2	.75
Minor stroke [n/%]	62/25.8	19/23.5	23/29.1	20/25.0	
Moderate stroke [n/%]	130/54.2	47/58.0	41/51.9	42/52.5	
Moderate to severe stroke [n/%]	27/11.2	9/11.1	7/8.9	11/13.7	
Severe stroke [n/%]	18/7.5	4/4.9	8/10.1	6/7.5	
Type of strokes					
Large vessel occlusion [n/%]	106/44.2	33/40.7	40/50.6	33/41.2	.50
Lacunar [n/%]	56/23.3	18/22.2	19/24.0	19/23.7	
Embolic [n/%]	55/22.9	19/23.5	13/16.5	23/28.7	
Other classified [n/%]	8/2.3	5/6.2	2/2.5	1/1.2	
Unclassified [n/%]	15/6.3	6/7.4	5/6.3	4/5.0	
Laboratory data					
Hemoglobin [g/dL]	13.7 ± 1.6	14.1 ± 1.4	13.6 ± 1.6*	13.4 ± 1.6*	<**.05**
Neutrophils [*10^3/μL]	6.0 ± 2.6	6.0 ± 2.6	5.9 ± 2.2	6.0 ± 2.8	.95
Lymphocytes [*10^3/μL]	1.9 ± 1.3	2.1 ± 1.9	1.8 ± 0.7	1.7 ± 0.8	.15
Total cholesterol [mmol/L]	5.1 ± 1.5	5.1 ± 1.6	5.0 ± 1.6	5.0 ± 1.3	.89
LDL-cholesterol [mmol/L]	3.2 ± 1.3	3.3 ± 1.2	3.3 ± 1.4	2.9 ± 1.1	.14
HDL-cholesterol [mmol/L]	1.4 ± 0.4	1.4 ± 0.3	1.3 ± 0.3	1.4 ± 0.4	.35
Triglycerides [mmol/L]	1.38 (1.04-1.96)	1.36 (1.07-2.01)	1.73 (1.26-2.18)	1.16* (0.94-1.59)	<**.01**
Creatinine [mg/dL]	1.90 (1.84-1.97)	1.91 (1.85-1.95)	1.90 (1.84-1.97)	1.93 (1.84-1.98)	.75
Estimated glomerular filtration rate—Egfr [ml/min/1.73m^2]	70 ± 25	73 ± 24	71 ± 24	67 ± 26	.37
CKD (eGFR < 60 ml/min/1.73m^2 or albuminuria) [n/%]	86/35.8	30/37.0	25/31.6	31/38.7	.62
CRP [mg/L]	4.2 (1.8-9.3)	3.0 (1.4-7.8)	4.6 (1.5-9.4)	5.3** (2.5-13.7)	<**.01**
Calcium [mg/dL]	2.32 (2.19-2.45)	2.32 (2.23-2.47)	2.28 (2.20-2.43)	2.29 (2.17-2.44)	.38
Phosphorus [mg/dL]	1.11 (0.96-1.33)	1.08 (0.94-1.23)	1.07 (0.89 − 1.32)	1.23# (1.04 − 1.52)	<**.01**
Intact parathyroid hormone—iPTH [pg/mL]	40.7 (26.7-56.4)	40.0 (26.6-56.3)	38.2 (27.2-54.7)	42.6 (26.5-64.7)	.35
iPTH > 65 pg/mL [n/%]	48/20.0	16/19.7	12/15.2	20/25.0	.30
Intact fibroblast growth factor 23—iFGF23 [pg/mL]	50.1 (17.6-84.9)	26.5 (9.4-57.5)	60.7 (20.2-91.0)#	67.8 (35.4-95.2)#	<**.001**
iFGF23 > 50 pg/mL [n/%]	120 (50%)	24/29.6	46/58.2#	50/62.5#	<**.001**
Fetuin A [mg/mL]	292 ± 84	289 ± 79	296 ± 91	291 ± 82	.87
25-OH-D [ng/mL]	9.9 ± 7.1	9.3 ± 6.3	9.9 ± 7.6	10.6 ± 7.3	.51
25-OH-D < 10 ng/mL [n/%]	151/62.9	48/59.3	54/68.3	49/61.2	.39
25-OH-D: 10-19.9 ng/mL [n/%]	61/25.4	27/33.3	15/19.0	19/23.7	
25-OH-D: 20-29.9 ng/mL [n/%]	25/10.4	5/6.2	9/11.4	11/13.8	
25-OH-D ≥ 30 ng/mL [n/%]	3/1.3	1/1.2	1/1.3	1/1.2	

*$P < .05$.
**$P < .01$.
#$P < .001$ in comparison to lower tercile group.

In multivariable stepwise backward analyses, the OPG level persisted as a significant and independent predictor of death (hazard ratio = 1.084 [95% confidence intervals: 1.036-1.134]) and composite and point (hazard ratio = 1.082 [1.037-1.129]; Table 3).

Discussion

Our data show that serum OPG levels are a significant and independent predictor of death in patients with stroke assessed on admission to the stroke unit. Thus far, only a few studies demonstrated that serum OPG level is a potential biomarker of the type and extent of stroke, as well as a further prognosis. Song et al. observed a correlation between serum OPG concentration and the type of stroke, when samples were taken immediately after admission to the stroke unit.[22]

Serum OPG levels are considered to be an independent risk factor of atherosclerosis and increased peripheral arterial pressure as a surrogate of arterial stiffness.[25,26] In addition, in patients treated for ischemic stroke with multiple risk factors, OPG levels were significantly higher than in patients with lacunar or embolic stroke.[22] However, our data did not support this finding. The structure of stroke types and their severity was similar in OPG tertile subgroups. Of interest, patients with the highest serum OPG levels were significantly older and had higher levels of hsCRP, phosphorus, iFGF23 regardless of similar kidney function (eGFR).

The association between OPG and hsCRP is not a new finding. It was previously described by Kadoglou et al.[4] According to Jensen et al., plasma concentration of OPG is not only a biomarker of bone homeostasis, but also of vascular calcification and inflammation.[14] Therefore, the circulating OPG level may reflect increased activation of inflammatory mechanisms.[27] In line with this hypothesis, Mogelvang et al. showed that patients with elevated plasma concentrations of both OPG and hsCRP had 5 times greater risk of cardiovascular disease and death.[20]

Of note, we have demonstrated very high prevalence of severe vitamin D deficiency (25-OH-D levels <10 ng/mL), that was much greater than in the general Polish population aged 65 year or above (63% versus 12.7% in women and 7.9% in men).[28] It can partially be explained by very high prevalence of diabetes and cardiovascular diseases in the study cohort. It can also be attributed to low seafood consumption, functional disability and impaired mobility,[28] which is frequent among seniors, as well as a lower number of sunshine hours per year (1350 hours) and greater air pollution in Upper Silesia than in the other parts of Poland.[29]

The co-occurrence of increased OPG and hsCRP levels corresponded with lower hemoglobin levels (possibly related to functional iron deficiency) and increased iFGF23 concentration in the highest OPG tertile subgroup. The association between low-grade inflammation and increased iFGF23, as well as cFGF23, was previously described by our group in a large cohort of community dwelling seniors.[30] It was also found in an in vitro study that IL-1 and TNF-α stimulate osteocyte FGF23 secretion, which is in line with our findings.[31] This mechanism explains the increased serum iFGF23 level in patients with the highest OPG levels, but not without phosphorus retention related to impaired eGFR. Perhaps inflammation may also induce kidney resistance for phosphaturic effect of FGF23. This interesting hypothesis requires further studies.

Serum OPG level has to reflect the existence of more important mechanisms than inflammation itself. In the regression analysis OPG, but not hsCRP, predicted survival in the follow-up period. It has been shown that OPG reflects the severity of arteriosclerosis in various vascular regions. It is well known that atherosclerosis of the carotid artery, which often also contains calcified areas, is strongly predisposed to the occurrence of ischemic stroke.[32,33] In line with this supposition, Kwon et al. described increased serum OPG levels in human subjects with calcified carotid plaques.[34] The survival in stroke survivors is not only determined by infections in disabled, and stroke recurrence, but also other cardiovascular complications related to atherosclerosis in other vascular beds, particularly in coronary arteries.

Later mortality after stroke is mainly related to cardiovascular causes.[35] The mortality within the first year after stroke is mostly determined by direct neurological effect

Table 3. *Results of multivariable stepwise backward Cox proportional hazard regression for death and composite end point adjusted to 20(OH)D level*

	HR	95% CI	3*P*
Death predictors			
Age [per 10 years]	1.027	1.005-1.050	<.05
Osteoprotegerin [per 10 pmol/L]	2.505	1.745-3.595	<.001
RANKIN out > 3 pts [Y/N]	3.745	2.355-5.955	<.001
Composite end point predictors			
Age [per 10 years]	1.028	1.007-1.050	<.05
Osteoprotegerin [per 10 pmol/L]	2.465	1.744-3.493	<.001
RANKIN out > 3 pts [Y/N]	3.021	1.955-4.668	<.001

of the initial event with its secondary consequences. After the first year, the most frequent cause of death is still recurrent strokes followed by a cardiovascular event, mostly myocardial infarction. The long-term risk of death is highest for cardioembolic stroke and for undetermined ischemic stroke.[36] After an initial stroke the risk of subsequent event is higher than the risk of cardiac event over a 2-year follow-up period.[37]

The difficulty in proving the existence of an association between OPG plasma levels and ischemic stroke development may be that vascular calcification may not be involved in stroke development per se, and equally increases the risk of cardiovascular and cerebrovascular events.

In addition, there exists a factor limiting the utility of OPG as a prognostic biomarker in patients with stroke. This is exhibited in its inverse relationship with the glomerular filtration rate in chronic kidney disease,[20] due to reduced renal clearance of OPG.[38-40] Therefore, in a patient with chronic kidney disease an adjustment for eGFR would be mandatory. Of interest, even after adjustment for glomerular filtration, Mogelvanga et al., was still able to demonstrate the predictive value of OPG. That suggests that OPG truly may improve risk stratification after stroke.

Limitations

Our study has some limitations. We analyzed OPG and other biomarkers only at single time point. It would be interesting if OPG maintained its predictive value in stroke survivals at the discharge from the stroke unit. Additionally, the size of our Caucasian cohort is relatively small, precluding analyses in various stroke types. Therefore, the results of our research have to be confirmed in larger trials including other ethnic groups. Finally, we did not test polymorphisms of the OPG gene that were shown to affect OPG levels in the circulation.

In conclusion, OPG level may be considered as a predictor of mortality in stroke patients.

Declarations

Ethics approval and consent to participate

The study was approved by the Bioethics Committee of the Medical University of Silesia.

Consent to Publish

Consent provided upon request.

Availability of Data and Materials

The datasets analyzed during the current study are not publicly available due to the continuation of analyses but are available from the corresponding author on reasonable request.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

J.W.: data acquisition, drafting of the manuscript. M.S.: data interpretation, critical revision for important intellectual content. A.J.O.: data analysis and statistical analysis. A.B.: laboratory analyses. M.O.G.: critical revision for important intellectual content. I.C.: conception and design, obtaining funds, data interpretation, manuscript editing and final approval. All authors have read and approved the final version of the manuscript.

Supplementary Materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.jstrokecerebrovasdis.2019.01.006.

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