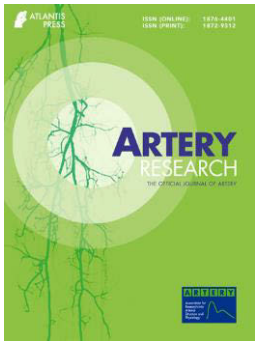

Artery Research

ISSN (Online): 1876-4401 **ISSN (Print): 1872-9312**
Journal Home Page: https://www.atlantis-press.com/journals/artres

Could BMP-2 and BMP-7 be biomarkers of coronary artery disease? A pilot clinical study

Saeed Nazemi, Atefeh Rezapour, Seyed Mohammad Hasan Moallem, Mohammad Afshar, Sepideh Elyasi, Elham Hashemi, Sheyda Golmohammadzadeh, Azadeh Zaerzadeh, Vahid Jomezadeh, Amir Hooshang Mohammadpour

To cite this article: Saeed Nazemi, Atefeh Rezapour, Seyed Mohammad Hasan Moallem, Mohammad Afshar, Sepideh Elyasi, Elham Hashemi, Sheyda Golmohammadzadeh, Azadeh Zaerzadeh, Vahid Jomezadeh, Amir Hooshang Mohammadpour (2018) Could BMP-2 and BMP-7 be biomarkers of coronary artery disease? A pilot clinical study, Artery Research 23:C, 14–19, DOI: https://doi.org/10.1016/j.artres.2018.05.006

To link to this article: https://doi.org/10.1016/j.artres.2018.05.006

Published online: 3 December 2019

Artery Research (2018) 23, 14–19

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/artres

Could BMP-2 and BMP-7 be biomarkers of coronary artery disease? A pilot clinical study

Saeed Nazemi [a], Atefeh Rezapour [b], Seyed Mohammad Hasan Moallem [c], Mohammad Afshar [d,e], Sepideh Elyasi [b], Elham Hashemi [f], Sheyda Golmohammadzadeh [a], Azadeh Zaerzadeh [b], Vahid Jomezadeh [g], Amir Hooshang Mohammadpour [b,h,*]

[a] Department of Cardiovascular Diseases, Razavi Hospital, Mashhad, Iran
[b] Department of Clinical Pharmacy, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
[c] Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[d] Department of Anatomy, Birjand University of Medical Sciences, Birjand, Iran
[e] Medical Toxicology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[f] Birjand Cardiovascular Disease Research Center, Department of Cardiology, Birjand University of Medical Sciences, Birjand, Iran
[g] Department of Surgery, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[h] Pharmaceutical Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

Received 31 December 2017; received in revised form 8 May 2018; accepted 14 May 2018
Available online 29 May 2018

KEYWORDS
Coronary artery calcification;
BMP-2 — BMP-7;
SMAD signaling pathway;
TGF-β

Abstract *Background:* Coronary artery calcification (CAC) is utilized as an important tool for the global risk assessment of cardiovascular events in individuals with intermediate risk. BMP-2 is a powerful inducer of bone formation and exposure to BMP-2 in the arteries leads to the loss of vascular smooth muscle cells (VSMC) markers and increase gene expression in favor of osteoblasts. BMP-7 is key factor in the bone and kidney and is suggested as inhibitor of vascular calcification. The main purpose of this clinical study was to find out the correlation between BMP-2 and BMP-7serum concentration and CAC in human for the first time.
Methods: In this study 84 patients with coronary artery disease who fulfilled inclusion and exclusion criteria, entered the study. For all patients a questionnaire consisting demographic data and traditional cardiovascular risk factors were completed. CT-Angiography was carried out to determine coronary artery calcium score and ELISA method was used for measuring BMP-2 and BMP-7serum concentrations.

* Corresponding author. P.O. Box 91775-1365, Mashhad, Iran. Fax: +985138823251.
E-mail address: MohamadpoorAH@mums.ac.ir (A.H. Mohammadpour).

https://doi.org/10.1016/j.artres.2018.05.006

Results: There was a significant positive correlation between BMP-2 serum concentration and total CAC score and also CAC of right coronary artery (RCA), left anterior descending (LAD), circumflex (CX), left main coronary artery (LMCA) ($P < 0.05$). Similar result was found for BMP-7 serum concentration except in LMCA ($P > 0.05$).
Conclusion: Based on our results, we can suggest BMP-2 and BMP-7 serum concentration as a probable biomarker for coronary artery disease. However, more studies with higher sample size are necessary for its confirmation.

Introduction

Vascular calcification is a life threatening complication of cardiovascular disease and the statistics of patients with vascular calcification, especially in the elderly people and people with metabolic disorders, are on the rise. This process not only leads to increased mortality, but also leads to physical disabilities and a decrease in the quality of life.[1,2] It is an inevitable process particularly in the advanced stages of atherosclerosis which can create break in the vessels and cause the plaque rupture. Coronary artery calcification (CAC) is a surrogate marker for subclinical atherosclerosis and is known to reflect atherosclerotic burden. Increased coronary artery calcium score (CACS) correlates with the risk of cardiovascular disease.[3] CAC determined by electron beam-computed tomography (EBCT). EBCT was recently determined as strong predictor that comforts the prediction of future cardiovascular events particularly in intermediate risk subjects.[4]

Recent studies have provided impetus to shift from cellular interaction based calcification models to models emphasizing on the important role of extracellular matrix in calcification.

The bone mineralization controlling proteins are also involved in vascular calcification.[5] Bone morphogenic proteins (BMPs) are proteins associated with growth factors and subset of TGF-β superfamily. These proteins, along with the angiogenesis-inducing factors and through the paracrine-endothelial-mesenchymal pathway, maintain bone structure.[6] BMPs signaling path is so powerful in bone formation that its induction in the muscle tissue leads to the formation of false bone tissue in the place.[7] BMPs are expressed by endothelial cells, smooth muscle cells and foamy cells in atherosclerotic vascular areas.[8] Among the proteins in this family, BMP-2 and BMP4 And at a later stage BMP 5, 6, 7 have the highest association with vascular disease due to calcification.[8]

BMP-2 is a powerful inducer of bone formation and exposure to BMP-2 in the arteries leading to the loss of vascular smooth muscle cells (VSMC) markers and increase gene expression in favor of osteoblasts.[8]

Different molecular pathways have been reported for BMP-2 that SMAD pathway is the most important one. Actually, two SMAD dependent and non-SMAD dependent mechanism pathways are available that both of them trigger calcification process by induction of Osx, Runx2, and Dlx5 transcription.[9]

The second molecular pathway that is listed for BMP-2 stimulates Msx2 gene expression through induction of ALP and Runx2/Cbfa1 track progress to increases in favor of bone formation.[2,8,10]

BMP-7 is key factor in the bone and kidney, and genetically modified mice with a defect in BMP-7, have bone disorders and hypo-mineralization patterns, renal dysplasia and kidney growth inhibition, and visual defects.[8]

BMP-7 and its derivatives by stimulating phosphate storage by increasing smad6, smad7 and p_{21} Using the SMAD signaling pathway in the skeletal system lead to reduce plasma phosphate levels, which play a major role in vascular calcification and inhibits it and also induce the VSMC phenotype.[8,11]

According to this, we evaluated the BMP-2 and BMP-7 as diagnostic biomarkers in human to determine the extent of coronary artery calcification.

Methods

Patients

Eighty-three patients with diagnosis of coronary artery disease by angiography which was performed by the cardiologist, who aged higher than 40 years old, were enrolled in this study between November 2015 and March 2016. This test is the best way to detect CAD in the arteries, over 51% of which are blocked by atherosclerotic plaques and useful in detecting the vessels responsible for advanced CHD. However, it does not provide information about the artery wall and atherosclerosis may not be diagnosed that has not yet captured the duct. 12 patients with >50% coronary stenosis of at least one artery were considered as CAD+ and included in study. Patients with calcium and phosphor metabolic disorder or receiving medications which are effective on calcium and/or phosphate and immunosuppressant or antioxidant medications, intake of folic acid and methotrexate, malignancies, heart failure, hypo or hyper parathyroidisim, renal insufficiency, history of osteoarticular disorders and chronic inflammatory diseases, and acute infection during the study were excluded from the study. A questionnaire containing demographic data, laboratory data, drug and medical and familial history of cardiovascular risk factors was completed for all patients.

Patients were recruited from Cardiology ward of Razavi Hospital, Mashhad, Iran. This study was accepted by ethics

committee of Mashhad University of Medical Sciences (code of BMP-2: 931413).

A questionnaire containing demographic data, laboratory data, drug, medical, and familial history of cardiovascular risk factors was completed for all patients. All patients signed the consent form prior to entry in the study.

Determination of BMP-2 and BMP-7 serum concentration and CAC

Whole blood was collected from patients and centrifuged at 2500 rpm for 10 min. The plasma fraction was isolated and stored at −70 °C until required for analysis. Routine biochemical measurements such as plasma glucose, total cholesterol (TC), triglycerides, low density lipoprotein Cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c), and serum calcium and phosphorus level were carried out by routine laboratory methods. Serum level of soluble BMP-2 and BMP-7 were measured with an enzyme-linked Immunosorbant assay (ELISA) -kit (R&D, American); each assay was calibrated using BMP-2 standard curve following the manufacturer's instructions. Coronary Artery Calcification was determined by CT-Angiography. The development of a computerized tomography scanner of Electron beam computerized tomography (EBCT) in the early 1980s made it possible to track the calcification of coronary arteries with a quality and non-invasive approach. These imaging techniques often use the Agatston scoring system based on the area and density of calcification, to evaluate the quality of coronary artery calcification. The diagnostic accuracy is high in these methods, with a sensitivity of 90—94%, a 95—97% attribute and a negative predictive value of 93—99% to detect coronary artery occlusion that is 50% or more in angiography.[12]

Statistical analysis

Statistical analysis was carried out by SPSS 19, All measured values are presented as mean ± SD. Kolmogorov—Smirnov test was used to assess the normality of the variables distributions. Correlation between serum concentrations of BMP-2 and BMP-7 with CAC was analyzed using spearman correlation test (as their distribution was non-normal). To compare serum concentration of BMP-2 and BMP-7 between different groups, Independent-sample T Test was used. Results were considered significant at $p < 0.05$.

Results

Characteristics of the study population

The study population consists of 84 patients, 55 male (65.5%) and 29 female (34.5%). The mean age of population was 57.19 ± 10.18 years. Patients' characteristics, laboratory tests including biochemical parameters, and traditional cardiovascular risk factors are summarized in Table 1.

Serum concentration of BMP-2 and BMP-7 and calcium score Total, RCA, LAD, LMCA and CX are summarized in Table 2.

Correlation between BMP-2 and BMP-7 serum level and coronary artery calcification agatston score

There was a significant positive correlation between BMP-2 serum level and total coronary artery calcification score and CAC score of RCA, LMCA, CX, LAD ($P < 0.05$).

There was a significant positive correlation between BMP-7 serum concentration and total CAC score and CAC of RCA, LAD, CX ($P < 0.05$), but there was no significant correlation between BMP-7 serum concentration and CAC of LMCA ($P > 0.05$) (Table 3).

We evaluated the correlation of BMP-2 and BMP-7 serum level with patients' age. There was a small significant negative correlation between BMP-2 serum level and age ($P = 0.049$, $r = -0.215$) but not with BMP-7 ($P > 0.05$). No significant difference also was found between serum level of BMP-2 and BMP-7 in two genders (P value = 0.78 & 0.809, respectively).

Discussion

In this study, the correlation of the BMP-2 and BMP-7 serum level with CAC was evaluated for the first time in human. As can be found from aforementioned results, there was a significant positive correlation between BMP-2 and BMP-7serum level and total CAC and CAC of RCA, LAD, LMCA, CX ($P < 0.05$), but, there was no significant correlation between BMP-7 serum level and CAC of LMCA ($P > 0.05$).

Different mechanisms have been proposed for the effects of BMP-2 on vascular calcification: The most important molecular pathway is the SMAD molecular pathway. In this path, two SMAD-dependent and non-SMAD-dependent mechanisms are expressed. In the SMAD path, the BMP-2 is connected to the type 2 receptor, and this connection causes phosphorylation of the receptor type 1 and the activation of its kinase. The receptor type 1, phosphorylates R-SMAD, and phosphorylated R-SMAD is transferred to the nucleus with SMAD, where it induces the transcription of Osx, Runx, Dlx, and initiates the calcification process. In this mechanism, I-SMAD inhibits signaling by inhibiting R-SMAD phosphorylation.[9] In a non-SMAD dependent pathway, MAPK triggers their activity by phosphorylation of mentioned genes, and these genes induce growth, differentiation and mineralization of the primary cells.[9] The second molecular pathway is Msx2 stimulation, which increases the expression of the gene in favor of osteosynthesis, as Msx2 induces ALP.[2,8] Also by progression of the Runx2/Cbfa1 pathway and inducing osterix in mesenchymal stem cells, ultimately leads to a favorable environment for vascular calcification.[9] In these pathways, Runx2 is required, and it has been observed that BMP-2 function is inhibited by removing Runx2 gene in mouse specimens.[13] The other pathways mentioned for BMP-2 are as follows: BMP-2 increases apoptosis and apoptosis is one of the key stages in the development of vascular calcification.[9] Moreover, BMP-2 by regulative increasing of Pit-1, which is sodium-dependent type 3 phosphate transporter and increases phosphate withdrawal in dose-dependent manner. As a result, BMP-2 increases the calcification induced by high dose of phosphate.[14] Besides, BMP-2 by stimulating

Table 1 Demographic data, laboratory tests, and traditional cardiovascular risk factors of patients.

Demographic data	Mean ± SD
Age (year) (mean ± SD)	57.2 ± 10.2[a] (37–89)[b]
BMI (kg/m[b]) (mean ± SD)	28.2 ± 4.7 (19.5–40.4)
Female/male ratio (%)	35%[c]
Laboratory tests	**Mean ± SD**
HDL-C (mg/dl) (mean ± SD)	43 ± 13 (25–121)
LDL-C (mg/dl) (mean ± SD)	92.3 ± 31.5 (42–191)
Total cholesterol (mg/dl) (mean ± SD)	164.9 ± 35.9 (109–292)
FBS (mg/dl) (mean ± SD)	105.3 ± 28 (75–257)
TG (mg/dl) (mean ± SD)	142.9 ± 53.8 (17–295)
Traditional risk factors	**Frequency (%)**
Hypertension (%)	48
Dyslipidemia (%)	62
Positive family history (%)	52
Diabetes (%)	19
Current Smoking (%)	34

BMI: Body Mass Index, HDL-C: High Density Lipoprotein-Cholesterol, LDL-C: Low Density Lipoprotein-Cholesterol, FBS: Fast Blood Sugar, TG: Triglyceride.
[a] Mean ± SD.
[b] Minimum and maximum of range.
[c] Frequency percent.

Table 2 Serum concentration of BMP-2 and BMP-7, calcium score Total, RCA, LAD, LMCA and CX.

	Mean ± SD
Coronary artery calcium score	
Total calcification of coronary vessels (agatston score) (mean ± SD)	346.6 ± 557.8[a] (0–3756)[b]
Calcification in coronary LAD (agatston score) (mean ± SD)	174.7 ± 287.1 (0–1610.2)
Calcification in coronary RCA (agatston score) (mean ± SD)	65.2 ± 105.6 (0–476.8)
Calcification in coronary CX (agatston score) (mean ± SD)	45.9 ± 95.1 (0–615.2)
Calcification in coronary LMCA (agatston score) (mean ± SD)	28.2 ± 103.9 (0–749.8)
BMP-21 serum level	
Concentration of BMP-2 (pg/mL) (mean ± SD)	47.6 ± 30.6 (7–115.7)
Concentration of BMP-7 (pg/mL) (mean ± SD)	320.3 ± 307. (3.4–985.6)

LAD: Left Anterior Descending, RCA: Right Coronary Artery, CX: Circumflex, LMCA: Left Main Coronary Artery, BMP-2: Bone Morph genic Protein-2, BMP-7: Bone Morph genic Protein-7.
[a] Mean ± SD.
[b] Minimum and maximum of range.

endothelial reticuloma stress, results in increasing Nox in smooth muscle of the vessels and therefore cause regulative increasing of the Runx2 and induces calcification.[15] It has also been observed that BMP-2 has an unknown effect on the metabolism of cholesterol.[16]

Table 3 Correlation between BMP-2 and BMP-7 serum concentration with LAD, RCA, LMCA, and CX coronary artery calcification score.

	LMCA	RCA	CX	LAD	Total of CAC	Parameter
BMP-2	0.049	<0.001	<0.001	<0.001	<0.001	P value
	0.279	0.526	0.501	0.717	0.902	CC
BMP-7	0.077	<0.001	<0.001	<0.001	<0.001	P value
	0.253	0.587	0.485	0.753	0.911	CC

CAC: Coronary Artery Calcification, LAD: Left Anterior Descending, RCA: Right Coronary Artery LMCA: Left Main Coronary Artery, CX: Circumflex.

There have been a lot of *in vivo* and *in vitro* studies in this field. In one of these studies, Nakagawa et al. showed that in cultivating differentiated VSMCs, BMP-2 have increased and its endogenous inhibitors reduced.[17] Also in 2011, Balderman et al. stated that there is higher level of BMP-2 in coronary artery differentiated, calcified cells enhances its possible role in the process of vascular calcification.[18]

In two other studies, the effect of human uremic serum, which increases calcification in the arteries, has been studied on cultured cells. The effect of uremic serum on bovine VSMCs cultivation showed that its calcification effects increase BMP-2 and its signaling paths.[19] This effect has been also seen on mesenchymal stem cells, so that by interrupting the BMP-2 effects by its inhibitor, the effects of calcification induction of this serum on the cultured cells are inhibited.[20] It was also found that BMP-7 play a role in VSMCs differentiation and control of their differentiation associated with osteoblast phenotypes.[8]

Animal studies also indicate that increasing BMP-2 accelerates intima atherosclerotic calcification.[17] In experiments performed on mice, BMPs antagonists were useful for inhibition of vascular calcification and atherosclerosis.[16]

In another study which examined the relationship between diabetes and vascular diseases, the higher BMP-2 level was detected in patients with diabetes in comparison with general population, and BMP-2 has a significant positive correlation with coronary artery disease, type 2 diabetes, atherosclerotic plaque calcification and also has a significant negative correlation with the volume of blood flow within vessels.[21] This study is conducted based on a comparison between diabetic and non-diabetic patients and not providing any comprehensive results about the BMP-2 predictive capability.

Hruska et al., showed that BMP-7 and derivatives by stimulating the storage of phosphates in the skeletal system reduces the amount of phosphate in plasma that plays a major role in vascular calcification, and it inhibits the direct effects the VSMCs that significantly reduces vascular calcification. So that when uremic rats were treated with BMP-7, calcification was inhibited in these animals.[8,22] As well as in atherosclerosis rat models even when the mice were treated with BMP-7 for 15 weeks, vascular calcification reduced in the same or less than the control group.[23]

In a study conducted in 2008 on 18 children with chronic renal failure showed that serum concentrations of BMP-7 in patients had increased compared to control group,

probably due to a protective mechanism in kidney failure. This study has not mentioned to examine the concentrations of BMP-7 in coronary artery calcification which is the subject of this study.[24]

In general, very few clinical studies have been done on the relationship between BMPs and vascular calcification. To the best of knowledge, a comprehensive clinical study for investigation of relationship between the serum levels of two biomarkers BMP-2 and BMP-7 and coronary artery calcification was not performed in patients with chronic ischemic heart disease.

The lack of association between LMCA and serum levels of BMP-7 may be due to the small study population. The coronary artery calcium score of the studied patients in the sub-groups was not distributed uniformly. Perhaps if the calcium score distribution was balanced, a significant relationship could be found.

The results of this study as a small pilot one, can propose the relationship between serum concentrations of BMP-2 and BMP-7 and coronary artery calcification and thus these biomarkers may have the ability to predict the CAC. Actually, this study could be the basis for studies with larger sample size and more accurate assessment of these biomarkers.

Conclusion

In this study, the correlation of the BMP-2 and BMP-7 serum level with CAC was clinically evaluated for the first time in patients with coronary artery disease that there was a significant positive correlation between BMP-2 and BMP-7 serum level and total CAC and CAC of RCA, LAD, LMCA, CX ($P < 0.05$), but, there was no significant correlation between BMP-7 serum level CAC of LMCA ($P > 0.05$).

Funding

The authors are thankful for the funding of this study by the Research Council of Mashhad University of Medical Sciences.

Conflict of interest

None.

Acknowledgments

This study is part of a research thesis for a Pharm.D. degree at Mashhad University of Medical Sciences.

References

1. Santos RD, Nasir K, Carvalho JA, Raggi P, Blumenthal RS. Coronary calcification and coronary heart disease death rates in different countries, not only the influence of classical risk factors. *Atherosclerosis* 2009;**202**(1):32—3.
2. Towler DA, Shao JS, Cheng SL, Pingsterhaus JM, Loewy AP. Osteogenic regulation of vascular calcification. *Ann N Y Acad Sci* 2006;**1068**:327—33.
3. Abedin M, Tintut Y, Demer LL. Vascular calcification mechanisms and clinical ramifications. *Arterioscler Thromb Vasc Biol* 2004;**24**(7):1161—70.
4. Budoff MJ, Achenbach S, Blumenthal RS, Carr JJ, Goldin JG, Greenland P, et al. Assessment of coronary artery disease by cardiac computed tomography a scientific statement from the American heart association committee on cardiovascular imaging and intervention, Council on cardiovascular radiology and intervention, and committee on cardiac imaging, Council on clinical Cardiology. *Circulation* 2006;**114**(16):1761—91.
5. Abedin M, Tintut Y, Demer LL. Vascular calcification: mechanisms and clinical ramifications. *Arterioscler Thromb Vasc Biol* 2004;**24**:1161—70.
6. Shao JS, Aly ZA, Lai CF, Cheng SL, Cai J, Huang E, et al. Vascular Bmp Msx2 Wnt signaling and oxidative stress in arterial calcification. *Ann N Y Acad Sci* 2007;**1117**:40—50.
7. Katagiri T, Yamaguchi A, Komak M, Abe E, Takahashi N, Ikeda T, et al. Bone morphogenetic protein-2 converts the differentiation pathway of C2C12 myoblasts into the osteoblast lineage. *JCB Home* 1994;**127**(6):1755.
8. Hruska KA, Mathew S, Davies MR, Lund RJ. Connections between vascular calcification and progression of chronic kidney disease: therapeutic alternatives. *Kidney Int Suppl* 2005;**99**:142—51.
9. Rawadi G, Vayssiere B, Dunn F, Baron R, Roman-Roman S. BMP-2 controls alkaline phosphatase expression and osteoblast mineralization by a Wnt autocrine loop. *J Bone Miner Res* 2003;**18**:1842—53.
10. Bostrom KI, Rajamannan NM, Towler DA. The regulation of valvular and vascular sclerosis by osteogenic morphogens. *Circ Res* 2011;**109**(5):564—77.
11. López-Cabrera M. Mesenchymal conversion of mesothelial cells is a key event in the pathophysiology of the peritoneum during peritoneal dialysis. *Adv Met Med* 2014:473134.
12. Kasper DL, Fauci AS, Hauser SL, Longo DL, Jameson JL, Loscalzo J. *Harrison's principles of internal medicine*. 19th ed. New York: McGraw Hill Education; 2015.
13. Bae JS, Gutierrez S, Narla R, Pratap J, Devados R, Van Wijnen AJ, et al. Reconstitution of Runx2/Cbfa1-null cells identifies a requirement for BMP-2 signaling through a Runx2 functional domain during osteoblast differentiation. *J Cell Biochem* 2007;**100**(2):434—49.
14. Li X, Yang HY, Giachelli CM. BMP-2 promotes phosphate uptake, phenotypic modulation, and calcification of human vascular smooth muscle cells. *Atheroscler* 2008;**199**(2):271—7.
15. Liberman M, Johnson RC, Handy DE, Loscalzo J, Leopold JA. Bone morphogenetic protein-2 activates NADPH oxidase to increase endoplasmic reticulum stress and human coronary artery smooth muscle cell calcification. *Biochem Biophys Res Commun* 2011;**413**(3):436—41.
16. Derwall M, Malhotra R, Lai CS, Beppu Y. Inhibition of bone morphogenetic protein signaling reduces vascular calcification and atherosclerosis. *Arterioscler Thromb Vasc Biol* 2012;**32**:613—22.
17. Nakagawa Y, Ikeda K, Akakabe Y, Koide M, Uraoka M, Yutaka KT, et al. Paracrine osteogenic signals via bone morphogenetic protein-2 accelerate the atherosclerotic intimal calcification in vivo. *Arterioscler Thromb Vasc Biol* 2010;**30**(10):1908—15.
18. Balderman J, Lee HY, Mahoney CE. Bone morphogenetic protein-2 decreases microRNA-30b and microRNA-30c to promote vascular smooth muscle cell calcification. *J Am Heart Assoc* 2012;**1**(6):3905.
19. Chen NX, Duan D, O'Neill KD, Wolisi GO, Koczman JJ, Laclair R, et al. The mechanisms of uremic serum-induced expression of bone matrix proteins in bovine vascular smooth muscle cells. *Kidney Int* 2006;**70**(6):1046—53.
20. Kramann R, Couson SK, Neuss S, Kunter U, Bovi M, Bornemann J, et al. Exposure to uremic serum induces a

procalcific phenotype in human mesenchymal stem cells. *Arterioscler Thromb Vasc Biol* 2011;**31**(9):45—54.
21. Zhang M, Sara JD, Wang FL, Liu LP. Increased plasma BMP-2 levels are associated with atherosclerosis burden and coronary calcification in type 2 diabetic patients. *Cardiovasc Diabetol* 2015;**14**:64.
22. Kang YH, Jin JS, Yi DW, Son SM. Bone morphogenetic protein-7 inhibits vascular calcification induced by high vitamin D in mice. *Tohoku J Exp Med* 2010;**221**(4):299—307.
23. Davies MR, Lund RJ, Hruska KA. BMP-7 is an efficacious treatment of vascular calcification in a murine model of atherosclerosis and chronic renal failure. *J Am Soc Nephrol* 2003; **14**(6):1559—67.
24. Musiał K, Fornalczyk K, Zwolińska D. Osteopontin (OPN), PDGF-BB (platelet-derived growth factor) and BMP-7 (bone morphogenetic protein) as markers of atherogenesis in children with chronic kidney disease (CKD) treated conservatively—preliminary results. *Pol Merkur Lek* 2008;**4**:25—7.