

A STUDY OF SERUM LEVELS OF RANKL AND OPG INPATIENTS WITH ACUTE CORONARY SYNDROME WITH AND WITHOUT PSORIATIC ARTHRITIS

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Abstract: Receptor Activator of Nuclear Factor Kappa B Ligand (RANKL) is a member of the tumor necrosis factor alpha (TNFα) family. It signals by its RANK receptor on monocyte/macrophage progenitors and stimulates the formation of bone-resorbing osteoclasts [1]. In psoriatic arthritis (PsA) RANKL is produced by activated Th17 cells, synovial fibroblasts, macrophages, dendritic cells and activated lymphocytes [2,3]. These are cells involved in systemic and local inflammation and atherogenesis.

Osteoprotegerin (OPG) is a glycoprotein first described in 1997 by Simonet et al. [1] who refer to it as a bone-protecting protein. OPG is a new member of the TNFα superfamily, a soluble receptor which acts as a decoy for RANKL and other ligands. It is also synthesized in many other tissues such as kidney, placenta, bones, vessels, lungs, and vascular structures. It circulates in serum and in plasma, although the concentration is much lower than locally in the bone and arterial tissue [4,5,6]. It has been found that vascular smooth muscle cells synthesize it as a result of various stimuli. OPG also protects large blood vessels from medial calcification [7]. The biological effect of OPG is to oppose RANKL and thus prevent subsequent stimulation of the receptor, therefore the level of OPG increases and acts as a mechanism of self-defense.

The role of RANKL and OPG in cardiovascular disease is still insufficiently defined and remains contradictory [8]. Also, the relationship between high serum levels of OPG in acute coronary syndrome (ACS) and their relation to inflammatory joint disease (IJD) is still discussed [9]. Evidence has been collected demonstrating the active involvement of RANKL and OPG in vascular pathology, including atherogenesis, arterial calcification and plaque instability.

Epidemiological studies show that elevated OPG levels in the circulation have a prognostic function in cardiovascular mortality and morbidity and may be a serum prognostic marker for future vascular events. On the other hand, the involvement of RANKL and OPG in the pathogenesis of local and systemic inflammation in ACS and IJD could link them to the amplification of cardiovascular risk and the severity of acute coronary syndromes, especially in patients with PsA. It is possible that the serum OPG level is increased in response to vascular wall damage and ongoing inflammation process within the atherosclerotic plaque lesion as a component of a complex compensatory mechanism in which RANKL plays a central role [11]. Serum OPG levels may be indicative of persistent damage of endothelial cells as well as the activation of VSMCs in advanced atherosclerotic plaque lesion. Together with other inflammatory mediators, RANKL and OPG can play a crucial role in the formation of the atherosclerotic plaque, its maturation and calcification [12, 13].

The present study aimed to examine and compare levels of sRANKL and sOPG in patients with ACS, divided into two groups, with and without PsA. Associations with cardiovascular mortality risk measured by the Global Registry of Acute Coronary Events (GRACE) were sought. ACS patients with and without PsA were compared in relation to sRANKL (24 and 48hrs) and sOPG (24 and 48hrs) to explore the effect of ACS.

Keywords: Receptor Activator of Nuclear Factor Kappa B Ligand, psoriatic arthritis, osteoprotegerin, acute coronary syndrome, inflammatory joint disease

ИЗСЛЕДВАНЕ НА СЕРУМНИТЕ НИВА НА RANKL И OPG ПРИ ПАЦИЕНТИ С ОСТЪР КОРОНАРЕН СИНДРОМ И ПСОРИАТИЧЕН АРТРИТ

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Резюме: Лигандът на рецепторния активатор на ядрения фактор капа бета (RANKL) е член на фамилията тумор некрозис фактор алфа (TNFα). Той се свързва с RANK рецептора си, разположен на повърхността на моноцит-макрофаг прогениторните клетки и стимулира образуването на остеокласти [1]. При псориатичния

артрит (PsA) RANKL се продуцира от активирани Th17 клетки, синовиални фибробласти, макрофаги, дендритни клетки и лимфоцити [2,3]. Това са клетки, участващи в системното и локално възпаление, и в атерогенезата.

Остеопротегеринът (OPG) е гликопротеин, описан за първи път през 1997 г. от Simonet et al. , които го наричат протеин, предпазващ костите. OPG е нов член на TNF α суперфамилията, разтворим рецептор, който действа като примамка за RANKL и други лиганди [1] . Също така се синтезира от много други тъкани, като бъбрек, плацента, кости, съдове, бели дробове и съдови структури. Той циркулира в серума и в плазмата, въпреки че концентрацията му е много по-ниска от локалната в костната и артериалната тъкан [4,5,6]. Установено е, че съдовите гладкомускулни клетки го синтезират в резултат на различни стимули. OPG защитава и големите кръвоносни съдове от медиална калцификация [7]. Биологичният ефект на OPG е да се противопостави на RANKL и по този начин да предотврати последващо стимулиране на рецептора, поради което нивото на OPG се увеличава и действа като механизъм на самозащита.

Ролята на RANKL и OPG при сърдечно-съдовите заболявания все още е недостатъчно дефинирана и остава противоречива [8]. Също така, все още се обсъжда връзката между високите серумни нива на OPG при остър коронарен синдром (ОКС) и връзката с възпалителните ставни заболявания (ВСЗ) [9]. Събрани са доказателства за активното участие на RANKL и OPG в съдовата патология, включително атерогенеза, артериална калцификация и нестабилност на плаките.

Епидемиологичните проучвания показват, че повишените нива на OPG в кръвообращението имат прогностично значение за сърдечно-съдовата смъртност и заболяемост и могат да бъдат серумен прогностичен маркер за бъдещи съдови събития. От друга страна, участието на RANKL и OPG в патогенезата на локалното и системно възпаление в ОКС и ВСЗ може да ги свърже с усилването на сърдечно-съдовия риск и тежестта на ОКС, особено при пациенти с PsA. Възможно е серумното ниво на OPG да се увеличи в отговор на увреждането на съдовата стена и протичащия процес на възпаление в лезията на атеросклеротичната плака като компонент на сложния компенсаторен механизъм, в който RANKL играе централна роля [11]. Нивата на OPG в серума могат да бъдат показателни за персистиращо увреждане на ендотелните клетки, както и за активирането на VSMCs при напредналата лезия на атеросклеротична плака. Заедно с други възпалителни медиатори, RANKL и OPG могат да играят решаваща роля при образуването на атеросклеротичната плака, нейното узряване и калцификация [12, 13].

Настоящото проявяване е насочено към изследване и сравняване на нивата на sRANKL и sOPG при пациенти с ОКС, разделени на две групи, с и без PsA. Бяха потърсени асоциации със сърдечно-съдовия риск от смърт, измерен чрез глобалния регистър на остри коронарни събития (GRACE). Пациентите с PsA бяха сравнени по отношение на sRANKL (24 и 48 час) и sOPG (24 и 48 час), за да се проучат при ОКС.

Ключови думи: рецептор активатор на лиганда на ядрения фактор капа бета, псориазичен артрит, остеопротегерин, остър коронарен синдром, възпалително ставно заболяване

MATERIAL AND METHODS

Data was collected from cardiac surgical patients admitted and operated in the Clinic of Cardiac Surgery at the Medical University of Plovdiv, Bulgaria, in the period of 2014-2016. The procedures were conducted in accordance with the WMA Declaration of Helsinki. All patients provided written informed consent prior to the investigation. A total of 72 surgical patients with acute coronary syndrome (ACS) (mean age 69.59 ± 7.22), of whom 23 (mean age 70.09 ± 6.43) with psoriatic arthritis and 49 (mean age 70.39 ± 7.40) without psoriatic arthritis were included in the study. There was no significant difference between the groups in average age, $p = .868$. In both groups, the male patients were a majority as males constituted 77.5% of the ACS patients without PsA and 78.3% of the group with PsA, with no significant difference in the sex distribution between the groups, $p = .939$.

RANKL and OPG serum levels were tested at 24 and 48 hours after onset of ACS. RANKL serum was analyzed according to the following commercially available protocol: Tumor Necrosis Factor (Ligand) Superfamily, Member 11 (TNFSF11), Sandwich ELISA, Detection Range: 2.74-2.000 pg/ml, Supplier: Cloud-Clone.

OPG serum levels were determined by Tumor Necrosis Factor Receptor Superfamily, Member 11b (TNFRSF11B), Sandwich ELISA, Detection Range: 1-900 pg/mL - minimum detection 1 pg/mL, Supplier: RayBiotech. The preparation of all reagents and samples was done according to the manufacturer's instructions and at room temperature.

The risk and global registry of acute coronary events (GRACE) assesses the likelihood of death and myocardial infarction within 6 months after acute coronary syndrome. The calculation uses clinical data that has been found predictive of adverse events, including age, heart rate/pulse, systolic blood pressure, creatinine, cardiac arrest at admission, ST-segment deviation, abnormal cardiac enzymes, and Killip class [14].

DATA ANALYSIS

Data analysis was performed with the SPSS software package, version 24 (SPSS, Chicago, Illinois, USA). Normally distributed data is described in mean and standard deviation (SD); dichotomous data is expressed in number and percentage. The parameters of interest, including RANKL serum levels at 24 and 48 hours, OPG serum levels at 24 and 48 hours and RANKL/OPG ratio at 24 hours and 48 hours, were examined for skewness, kurtosis and normality. Due to high skewness in some cases and violation of normality in all distributions (Shapiro-Wilk, $p < .001$), non-parametric statistical tests were used: Mann-Whitney U test for between group comparisons and Spearman Rho correlation for exploring associations. Nominal variables were compared through chi-square test. Results are interpreted at level of significance $\alpha = .05$.

RESULTS

The two groups of ACS patients, with and without PsA, were compared on sRANKL at 24 and 48 hours, sOPG at 24 and 48 hours, and RANKL/OPG ratios at 24 and 48 hours. Table 1 summarizes the demographic and clinical data.

Table 1: Demographic and clinical information about the patients

Variables		ACS + Psoriatic arthritis N = 23	ACS - Psoriatic arthritis N = 49	Sig
Age: mean (SD)		70.09 (± 6.43)	70.39 (± 7.40)	.868
Sex : N (%)	Male	18 (78.3%)	38 (77.5%)	.939
	Fem ale	5 (21.7%)	11 (22.5%)	
sRANKL (24 hrs): pg/ml mean, (SD)		121.33 (± 124.52)	51.67 (± 41.05)	.030*
sRANKL (48 hrs): pg/ml mean, (SD)		89.21 (± 84.62)	36.94 (± 32.85)	.001**
sOPG (24 hrs): pg/ml mean, (SD)		207.71 (± 154.68)	99.30 (± 75.33)	.000**
sOPG (48 hrs): pg/ml mean, (SD)		143.36 (± 125.42)	69.37 (± 59.98)	.002**
RANKL/OPG 24 hours mean, (SD)		.50 ($\pm .22$)	.55 ($\pm .21$)	.275
RANKL/OPG 48 hours mean, (SD)		.62 ($\pm .20$)	.60 ($\pm .24$)	.422

* Significant at $p \leq 0.05$; **Significant at $p \leq 0.01$

The results showed statistically significant differences between the groups on four values. The group with PsA had a significantly higher level of sRANKL at 24 hours ($p = .030$) and at 48 hours ($p = .001$); and significantly higher levels of sOPG at 24 hours ($p < .001$) and at 48 hours ($p = .002$). (See Figure 1).

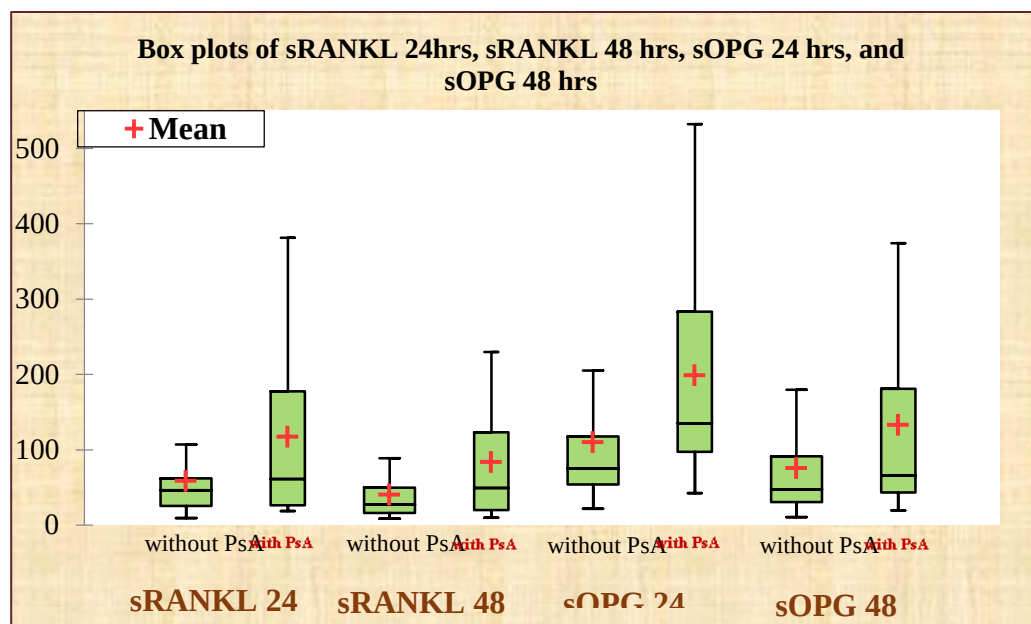


Figure 1 The serum levels of RANKL and OPG at 24 and 48 hours.

Spearman Rho correlation analysis was used to explore the relationship between cardiovascular mortality risk expressed as GRACE risk score, sRANKL at 24 & 48 hours, sOPG at 24 & 48 hours and RANKL/OPG ratio at 24 & 48 hours. GRACE risk score showed a significant association with two of the six parameters, including a positive correlation with OPG serum levels at 24 hrs ($r_s = 0.273$, $p = .024$) and a negative correlation with RANKL/OPG ratio at 24hrs ($r_s = -.365$, $p = .002$) - see Fig 2 and 3.

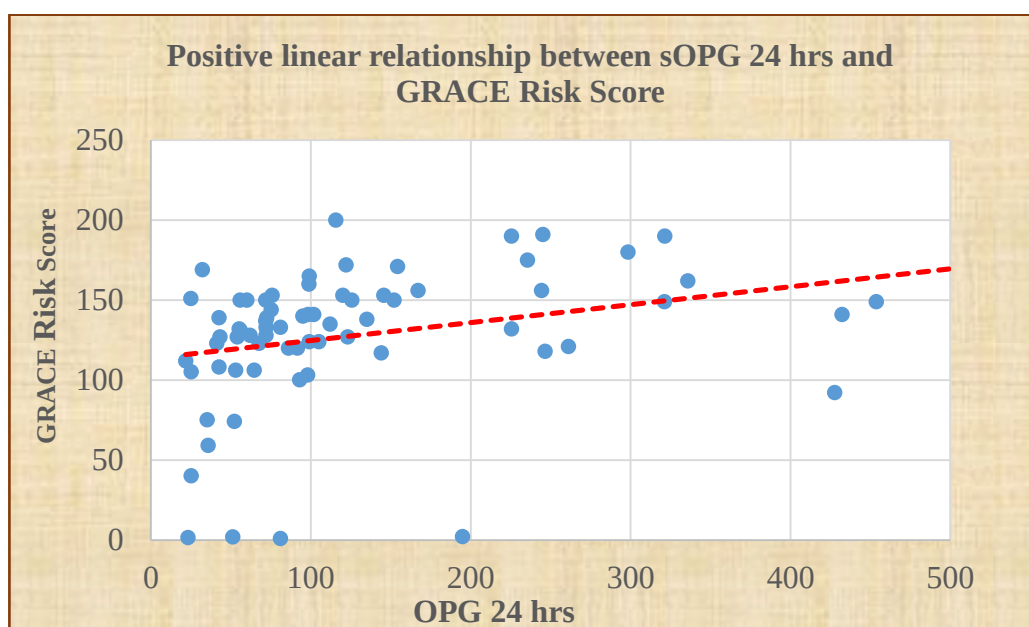


Figure 2- The linear relationship between sOPG and GRACE Score Scale

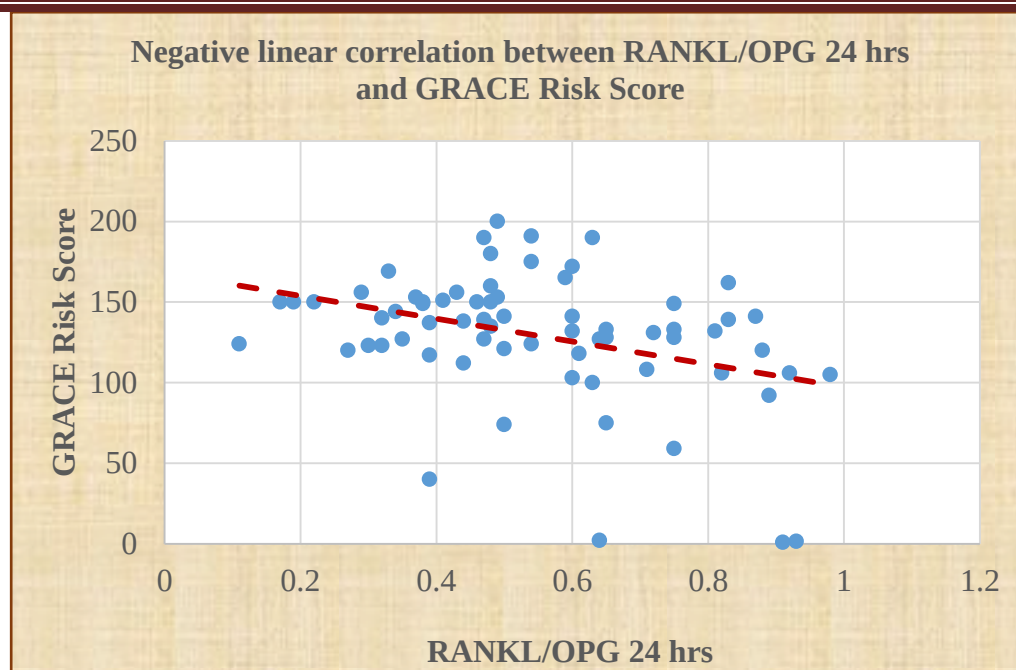


Figure 3- The negative linear relationship between RANKL/ OPG- 24h and GRACE Score Scale

DISCUSSION

This study provided a comparative analysis between two groups of ACS patients, with and without psoriatic arthritis in relation to sRANKL, sOPG, and RANKL/OPG ratio. The results revealed significantly higher levels of sRANKL, and sOPG in ACS patients with psoriatic arthritis. This finding provides evidence for the involvement of RANKL and OPG serum levels in systemic and local inflammation and atherogenesis [5, 7]. No significant difference existed between the two groups of patients regarding the RANKL/OPG ratio as the mean values were very similar.

A significant positive correlation was observed between cardiovascular mortality risk (GRACE risk score) and OPG serum levels at 24 hours. Higher OPG levels were associated with an increased mortality risk. On the other hand, higher mortality risk was negatively associated with the RANKL/OPG ratio at both 24 hours. Higher GRACE risk score was associated with lower RANKL/OPG ratio and vice versa. On this basis, it can be extrapolated that the RANKL - OPG balance plays an important role in cardiovascular disease, and that OPG levels in the circulation have a prognostic function in cardiovascular mortality and morbidity and may be a serum prognostic marker for future vascular events[11]. Related studies have indicated that the serum OPG level is increased in response to vascular wall damage and ongoing inflammation process within the atherosclerotic plaque lesion [13]. Our results corroborate these previous findings and also suggest that alterations in the RANKL/OPG ratio can be linked to an amplified cardiovascular risk, especially in patients with PsA. However, this extrapolation also requires further research involving a larger cohort of patients.

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