Mini-Review

DOI: 10.5582/bst.2018.01078

Transcriptomic responses of peripheral blood cells to coronary artery disease

Mingshan Lin, Yuan Zhang, Zhaozhuo Niu, Yifan Chi, Qiang Huang*

Department of Cardiovascular Surgery, Qingdao Municipal Hospital, Qingdao, Shandong, China.

Summary **Transcriptomic response of peripheral blood cells to coronary artery diseases (CAD) is a long recognized phenomenon. Currently, accumulating evidence indicates that such response having significant clinical utility in CAD-associated events determination. In this review, we summarized the existing data of transcriptomic biomarkers at mRNA, microRNA, long non-coding RNA, and circular RNA for the diagnosis, progression and outcome prediction and treatment response of CAD. Furthermore, we also discussed the functional significance on the gene expression patterns caused by CAD, and emphasized the importance of inflammatory pathways in CAD tissues-blood cells interaction. Based on the current knowledge, we proposed a perspective on the future strategies to further improve the robustness and reproducibility of transcriptomic biomarkers in the personalized medicine of CAD patients.**

Keywords: Coronary artery diseases, transcriptome, biomarkers, peripheral blood cells, non-coding RNA

1. Introduction

Coronary artery disease (CAD) represents a major public health problem and remains the main cause of morbidity and mortality globally. Despite remarkable advances in the management, the success of prognostic and therapeutic options remains modest (*1*). Therefore, more personalized strategies are still needed for this condition, in particular risk-assessment strategies to detect or predict a substantial number of clinical outcome events such as recurrence and heart failure.

In the last decade, many efforts have been devoted to identify novel diagnostic or prognostic biomarkers among the entire transcriptome of peripheral blood cells of the CAD patients (*2*). These discovery studies are based on the assumption that gene expression profiling of circulating blood using microarrays and deep RNA sequencing technologies might reflect physiological and pathological events occurring in different tissues of the body including CAD-related tissues. Currently, many CAD-associated RNAs confined in blood cell have been identified at mRNA, microRNA (miRNA), long non-coding RNA (lncRNA), and circular RNA (circRNA) levels. Noteworthy, based on these biomarkers, several diagnostic and predictive gene models with good performance have been developed. For example, a prospective multi-center study validated that a peripheral blood-based 23-gene expression model can provide modest but statistically significant improvement in determining the likelihood of obstructive CAD in non-diabetic patients as compared to clinical factors and other non-invasive imaging methods (*3*). Furthermore, another independent prospective multi-center trial demonstrated that integrating this gene signature with age and sex to calculate a diagnostic score can significantly predict near-term revascularization procedures (*4*). Therefore, as a new, quick, reproducible and robust method, the gene expression-based classifiers show promising clinical utility potentials in the diagnosis and prognosis prediction in CAD.

Although advances in screening, before these blood RNA biomarkers can revolutionize the clinical laboratory medicine and patient management of

Released online in J-STAGE as advance publication August 26, 2018.

**Address correspondence to:*
Dr. Qiang Huang, Department of Cardiovascular Surgery, Qingdao Municipal Hospital, #1 Jiaozhou Road, Qingdao 266011, China.
E-mail: qdsslyyhuangqiang@163.com

CAD, some basic concept must be addressed first, such as whether and what extent of whole blood gene expression patterns can reflect the presence, severity and prognosis of infarcted tissues? Whether transcriptomic responses of peripheral blood cells to CAD have important functional significance?

Here, we reviewed the current state of knowledge regarding CAD transcriptomic characteristics from the growing literature, and discussed the promising results in their clinical utility in CAD events prediction. In addition, we also discussed the functional significance of "bloodomics" response to CAD, and challenges and future perspective in this field.

2. Peripheral blood cells biomarkers for CAD at mRNA level

Dysregulation of a subset of genes including arachidonate 15-lipoxygenase (*ALOX15*), amphiregulin (*AREG*), BCL2 related protein A1 (*BCL2A1*), BCL2 like 1 (*BCL2L1*), carbonic anhydrase 1 (*CA1*), cytochrome c oxidase subunit 7B (*COX7B*), enoyl-CoA hydratase domain containing 3 (*ECHDC3*), interleukin 18 receptor 1 (*IL18R1*), immune response 2 (*IR2*), potassium voltage-gated channel subfamily E regulatory subunit 1 (*KCNE1*), matrix metallopeptidase 9 (*MMP9*), myosin light chain 4 (*MYL4*) and triggering receptor expressed on myeloid cells like 4 (TREML4) in peripheral blood cells showed a close correlation with the presence of acute coronary syndrome (ACS) at the very early stages (*5*). Gene annotation analysis reveals that these genes are significantly enriched in interleukins signaling, one of the key regulatory mechanisms accounting for the development of atherosclerosis. For discriminating progressive and stable CAD, an 8-gene risk prediction signature (X inactive specific transcript (*XIST*), glutathione S-transferase theta 1 (*GSTT1*), natriuretic peptide A (*NPPA*), kinesin family member 20B (*KIF20B*), *CR625615*, ankyrin 2 (*ANK2*), FLYWCH-type zinc finger 1 (*FLYWCH1*), A_24_P473972) combined with classic protein biomarkers and clinical indicators were suggested (*6*). Among this signature, two genes *ANK2* and *GSTT1* were independently validated using quantitative real-time PCR (*6*). It has been proposed that GSTT, as a detoxification enzyme, is responsible for the production of oxidative stress, and may contribute to the development of CAD (*7*).

Kiliszek *et al*. (*8*) revealed that dozens of genes involved in lipid/glucose metabolism, platelet function and atherosclerotic plaque stability significantly altered among gene expression pattern in peripheral blood mononuclear cells (PBMC) in the acute phase of ST-segment elevation myocardial infarction (STEMI). In particular, suppressor of cytokine signaling 3 (*SOCS3*) and golgi associated secretory pathway pseudokinase (*FAM20*) expression in PBMC were up-regulated in the first days of myocardial infarction in the vast majority of patients (*8*). Another study also linked gene expression patterns in peripheral blood of peripheral blood cells with the severity of CAD (*9*). This study identified 160 genes significantly correlated with CAD index, a validated angiographical measure of the extent of CAD that correlates with outcome. Most interestingly, this expression pattern could also accurately separate the aorta samples according to the severity of atherosclerosis in another independent gene expression dataset (*9*). This finding further support the notion that gene expression changed derived from peripheral blood can reflect similar pathophysiological changes in remote disease sites such as atherosclerotic arteries.

Classic protein biomarkers are less useful in predicting the long term events of CAD. A study by Suresh *et al*. (*10*) identified two subsets of genes belonging to epithelial mesenchymal transition (EMT) pathway, and cholesterol transport modulation associated with long-term (18 months) recurrent events following first-time myocardial infarction. Heart failure is another occurred consequence of CAD. Maciejak *et al*. (*11*) revealed that the up-regulation of ribonuclease A family member 1 (*RNASE1*), formin 1 (*FMN1*), and Jun dimerization protein 2 (*JDP2*) genes on the first day of STEMI can serve as potential prognostic biomarkers for the progression of heart failure after AMI. These findings support that the transcriptomic data of the circulating blood cells may provide new clues in the prognosis prediction of CAD.

3. Peripheral blood cells biomarkers for CAD at miRNA level

As mRNA levels, miRNA profiles of whole blood cells have shown potential utility in CAD diagnosis, prediction, and monitoring. From an unbiased screen of blood cells of CAD, miR-135a expression was significantly increased compared with unaffected controls, while miR-147 decreased (*12*). Dong *et al*. (*13*) identified miR-24, miR-33, miR-103a, and miR-122 correlated with blood lipids and their combination can provide a high diagnostic accuracy of CAD. Hoekstra *et al*. (*14*) indicated a cluster of 3 miRNAs, miR-134, miR-198 and miR-370, which can discriminate unstable CAD cases.

Downregulation of miRNA-8059, a miRNA with unknown function, can serve as a peripheral blood biomarker for the presence and extent of coronary artery calcification (*15*). Except for reflecting the severity of CAD, peripheral blood cells also have prognostic significance. A miRNA regulating development of myeloid *vs* lymphoid, miR-23a acts as a strong predictor for clinical outcomes in CAD patients after adjustment for baseline characteristics (*16*).

More meaningfully, miRNA profiling is also used

Table 1. Validated transcriptomic biomarkers for coronary artery diseases

Items	Acute coronary syndrome Biomarkers	Progression biomarkers	Clinical factors-associated biomarkers	Outcome predictor	Treatment response biomarkers
mRNA	ALOX15 AREG BCL2A1 BCL2L1 CA1 COX7B ECHDC3 IL18R1 IR2 KCNE1 MMP9 MYL4 TREML4 SOCS3 FAM20	XIST GSTT1 NPPA KIF20B CR625615 ANK2 FLYWCH1 A_24_P473972		Heart failure: RNASE1 FMN1 JDP2	Renin-angiotensin system blockade and statins treatment: IRAK1 TRAF6 TLR4
microRNA	miR-135 miR-147 miR-24 miR-33 miR-103a miR-122	miR-134 miR-198 miR-370	Extent of coronary artery calcification: miRNA-8059 lipid levels: miR-24 miR-33 miR-103a miR-122	Clinical outcome: miR-23a	Resveratrol supplementation: miR-21 miR-181b miR-663 miR-30c-2 miR-155 miR-34a Renin-angiotensin system blockade and statins treatment: miR-146a/b
LncRNA	LncPPARδ CoroMarker				
circRNA	circRNA11783-2				

to create biomarkers associated with treatments in CAD. Tomé-Carneiro *et al*. (*17*) found that after one-year supplementation with a grape extract containing resveratrol in CAD patients, the expression of pro-inflammatory cytokines C-C motif chemokine ligand 3 (CCL3), interleukin 1 beta (IL-1β) and tumor necrosis factor alpha (TNF-α) was significantly reduced in peripheral mononuclear blood cells (PMBCs), while a subset of inflammatory-related miRNAs including miR-21, miR-181b, miR-663, miR-30c2, miR-155 and miR-34a was also altered. In another clinical observation, after treated with renin-angiotensin system blockade and statins, the expression of miR-146a/b, interleukin 1 receptor associated kinase 1 (*IRAK1*), TNF receptor associated factor 6 (*TRAF6*) and toll like receptor 4 (*TLR4*) genes in PMBCs of CAD were markedly decreased (*18*). Therefore, these findings support a possibility of transcriptomic response to CAD may also be used as potential therapeutic biomarkers.

4. Transcriptomic biomarkers for CAD at lncRNAs and circular RNAs (circRNAs) levels

Compared with mRNA and miRNA, the studies investigating in the context of lncRNA expression pattern in CAD are limited. Cai *et al*. (*19,20*) examined the lncRNA profiles in PBMCs, and discovered two lncRNA (LncPPARδ, CoroMarker) as novel biomarkers for CAD, which can enhance the diagnostic specificity and sensitivity when combined with other risk factors. They further identified monocyte CoroMarker levels independent of known CAD risk factors and other cardiovascular diseases.

As a 'miRNA sponge', circRNAs have been validated to be functionally linked with CAD progression. However, the clinical significance of circulating circRNA for CAD has seldom been evaluated. Currently, only one study investigated the circRNA profiles of whole blood cells on CAD, and they found hsa-circRNA11783-2, a functionally unknown circRNA is closely related to CAD and diabetes (*21*).

5. The functional significance of transcriptomic response of peripheral blood cells to CAD

Although thousands of blood cell genes dysregulated in CAD, only a minority of them were validated as diagnostic and prognostic biomarkers (Table 1). The expression patterns of peripheral blood cells indirectly reflect the remote diseases status, and are induced by cytokines cascades from CAD. Several secretory factors are indicated to be independently associated

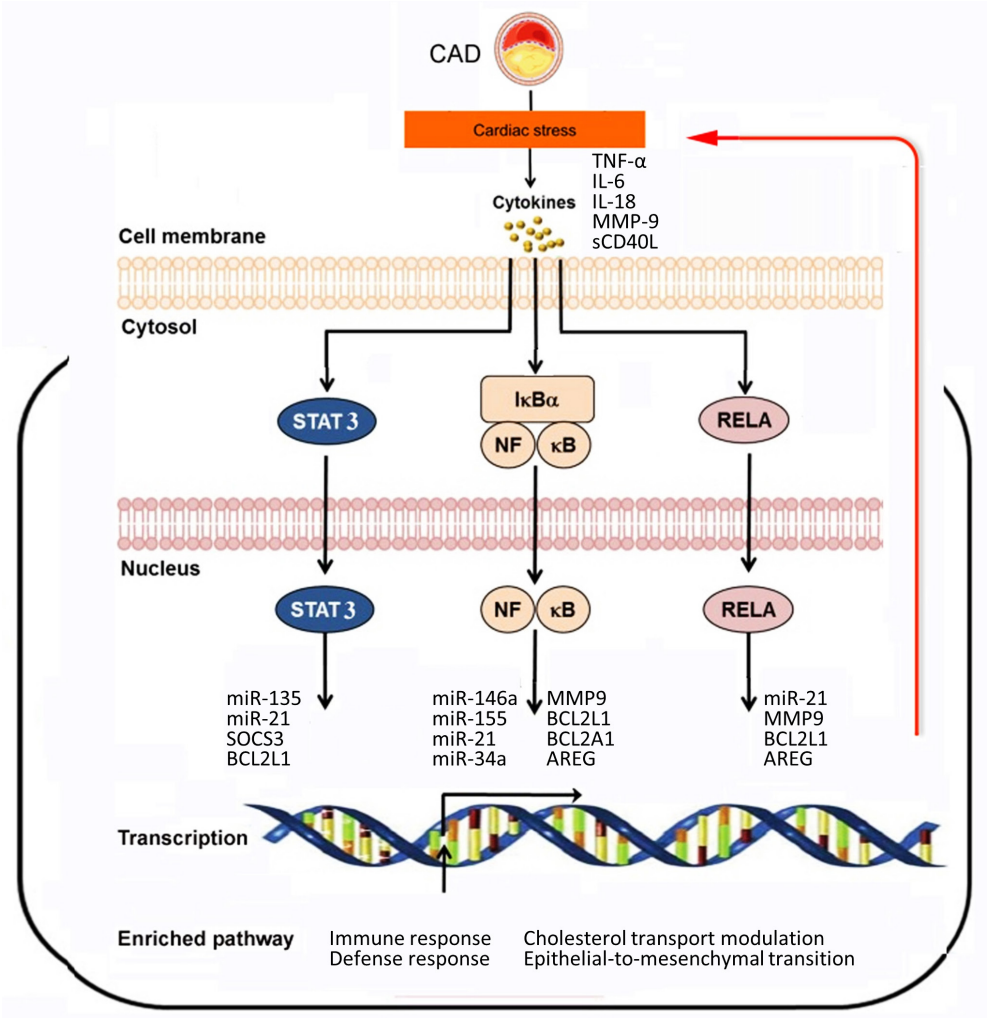

Figure 1. Functional analysis on the transcriptomic response of peripheral blood cells to coronary artery diseases.

with the severity of CAD. Among them, interleukin 6 (IL-6), interleukin 18 (IL-18), MMP-9, soluble CD40-ligand (sCD40L) and TNFα can theoretically induce the expression profiles of peripheral blood cells at all the transcriptomic levels (*22*). However, if these peripheral blood cells response can attribute or protect the damage of CAD has not been fully clarified.

GSTT1 is an enzyme regulating oxidative stress; its polymorphisms are associated with plasma malondialdehyde-conjugated low-density lipoprotein (MDA-LDL) levels and CAD risk (*23*). The overexpression of GSTT1 in PBMCs indicates that it may participate in the development of CAD through PBMCs. Significantly most of current validated peripheral blood cells mRNA and miRNA biomarkers are mediated by several important inflammatory transcription factors including signal transducer and activator of transcription-3 (STAT3), nuclear factor κB (NF-κB) (Figure 1). There is also strong coherence between mRNA and miRNA profiles. For example, EMT genes were enriched in CAD heart failure-related mRNA transcriptome; meanwhile some validated miRNA biomarkers also belong to EMT regulators such as miR-23a, miR-147, and miR-122. According to the current data, transcriptomic response of peripheral blood cells significantly focuses four biological process, immune response, defense response, cholesterol transport modulation, and EMT (Figure 1). Moreover, it can be also deduced that as a result of gene expression pattern variation, some peripheral blood cells-derived cytokines and secretory proteins such as MMP-9 would also participate in the development of CAD in a positive feedback manner.

6. Future perspective

Although widely used, the current classic protein biomarkers and invasive coronary angiography to diagnose and prognostic CAD still have limitations. Current evidence has demonstrated the clinical validity and utility of "bloodomics" data to reflect CAD status. In particular, a whole blood gene expression score has been developed and validated its robustness in discriminating CAD patients from other non-cardiac conditions in two prospective multi-center trials (*24*). Compared with the traditional assays, blood

transcriptome-based test might be very powerful in prognosis prediction, disease and treatment monitoring.

Although the current results of the evaluation are very promising, more cautions should be paid when explain the gene expression pattern of CAD peripheral blood cells, which can be affected by many complicated abnormal conditions. Currently transcriptomic biomarkers research differs tremendously among all reported studies. Therefore, biomarkers selection and algorithm developing from blood transcriptome of CAD must be based on strict validation test in multi-center, large cohorts of trails prospectively.

Now, the present "bloodomics" studies of CAD only focus on individual levels. Considering the inherent correlation between mRNA, miRNA, lncRNA and circRNA expression pattern, discovery and evaluation of more generalized biomarkers at multiple levels of transcriptomic data would further increase the robustness of current strategies.

In the emerging field of peripheral blood cells transcriptome of CAD, increased biomarkers and models show clinical potential utility in diagnosis, outcome prediction and treatment monitoring, and with functional significance in the disease development. Before the further clinical use of transcriptomic biomarkers in personalized healthcare of CAD patients, more elaborate studies are required to improve their powers through well-designed large multicenter trial cohort studies.

Acknowledgement

This study was supported by Qingdao Medicine Research Project (2016-WJZD022).

References

1. van den Hoogen PC, Feskens EJ, Nagelkerke NJ, Menotti A, Nissinen A, Kromhout D. The relation between blood pressure and mortality due to coronary heart disease among men in different parts of the world. Seven Countries Study Research Group. N Engl J Med. 2000; 342:1-8.
2. Chen HH, Stewart AF. Transcriptomic signature of atherosclerosis in the peripheral blood: Fact or fiction? Curr Atheroscler Rep. 2016; 18:77.
3. Rosenberg S, Elashoff MP, Daniels SE, *et al*. Multicenter validation of the diagnostic accuracy of a blood-based gene expression test for assessing obstructive coronary artery disease in nondiabetic patients. Ann Intern Med. 2010; 153:425-434.
4. Voora D, Coles A, Lee KL, Hoffmann U, Wingrove JA, Rhees B, Huang L, Daniels SE, Monane M, Rosenberg S, Shah SH, Kraus WE, Ginsburg GS, Douglas PS. An age- and sex-specific gene expression score is associated with revascularization and coronary artery disease: Insights from the Prospective Multicenter Imaging Study for Evaluation of Chest Pain (PROMISE) trial. Am Heart J. 2017; 184:133-140.
5. Silbiger VN, Luchessi AD, Hirata RD, Lima-Neto LG, Cavichioli D, Carracedo A, Brión M, Dopazo J, García-García F, dos Santos ES, Ramos RF, Sampaio MF, Armaganijan D, Sousa AG, Hirata MH. Novel genes detected by transcriptional profiling from whole-blood cells in patients with early onset of acute coronary syndrome. Clin Chim Acta. 2013; 421:184-190.
6. Nührenberg TG, Langwieser N, Binder H, Kurz T, Stratz C, Kienzle RP, Trenk D, Zohlnhöfer-Momm D, Neumann FJ. Transcriptome analysis in patients with progressive coronary artery disease: Identification of differential gene expression in peripheral blood. J Cardiovasc Transl Res. 2013; 6:81-93.
7. Kadıoğlu E, Taçoy G, Özçağlı E, Okyay K, Akboğa MK, Çengel A, Şardaş S. The role of oxidative DNA damage and GSTM1, GSTT1, and hOGG1 gene polymorphisms in coronary artery disease risk. Anatol J Cardiol. 2016; 16:931-938.
8. Kiliszek M, Burzynska B, Michalak M, Gora M, Winkler A, Maciejak A, Leszczynska A, Gajda E, Kochanowski J, Opolski G. Altered gene expression pattern in peripheral blood mononuclear cells in patients with acute myocardial infarction. PLoS One. 2012; 7:e50054.
9. Sinnaeve PR, Donahue MP, Grass P, Seo D, Vonderscher J, Chibout SD, Kraus WE, Sketch M Jr, Nelson C, Ginsburg GS, Goldschmidt-Clermont PJ, Granger CB. Gene expression patterns in peripheral blood correlate with the extent of coronary artery disease. PLoS One. 2009; 4:e7037.
10. Suresh R, Li X, Chiriac A, Goel K, Terzic A, Perez-Terzic C, Nelson TJ. Transcriptome from circulating cells suggests dysregulated pathways associated with long-term recurrent events following first-time myocardial infarction. J Mol Cell Cardiol. 2014; 74:13-21.
11. Maciejak A, Kiliszek M, Michalak M, Tulacz D, Opolski G, Matlak K, Dobrzycki S, Segiet A, Gora M, Burzynska B. Gene expression profiling reveals potential prognostic biomarkers associated with the progression of heart failure. Genome Med. 2015; 7:26.
12. Hoekstra M, van der Lans CA, Halvorsen B, Gullestad L, Kuiper J, Aukrust P, van Berkel TJ, Biessen EA. The peripheral blood mononuclear cell microRNA signature of coronary artery disease. Biochem Biophys Res Commun. 2010; 394:792-797.
13. Dong J, Liang YZ, Zhang J, Wu LJ, Wang S, Hua Q, Yan YX. Potential role of lipometabolism-related microRNAs in peripheral blood mononuclear cells as biomarkers for coronary artery disease. J Atheroscler Thromb. 2017; 24:430-441.
14. Hoekstra M, van der Lans CA, Halvorsen B, Gullestad L, Kuiper J, Aukrust P, van Berkel TJ, Biessen EA. The peripheral blood mononuclear cell microRNA signature of coronary artery disease. Biochem Biophys Res Commun. 2010; 394:792-797.
15. Howlett P, Cleal JK, Wu H, Shah N, Horton A, Curzen N, Mahmoudi M. MicroRNA 8059 as a marker for the presence and extent of coronary artery calcification. Open Heart. 2018; 5:e000678.
16. Satoh M, Nasu T, Takahashi Y, Osaki T, Hitomi S, Morino Y, Nakamura M. Expression of miR-23a induces telomere shortening and is associated with poor clinical outcomes in patients with coronary artery disease. Clin Sci (Lond). 2017; 131:2007-2017.
17. Tomé-Carneiro J, Gonzálvez M, Larrosa M, Yáñez-Gascón MJ, García-Almagro FJ, Ruiz-Ros JA, Tomás-Barberán FA, García-Conesa MT, Espín JC.

Grape resveratrol increases serum adiponectin and downregulates inflammatory genes in peripheral blood mononuclear cells: A triple-blind, placebo-controlled, one-year clinical trial in patients with stable coronary artery disease. Cardiovasc Drugs Ther. 2013; 27:37-48.
18. Takahashi Y, Satoh M, Minami Y, Tabuchi T, Itoh T, Nakamura M. Expression of miR-146a/b is associated with the Toll-like receptor 4 signal in coronary artery disease: Effect of renin-angiotensin system blockade and statins on miRNA-146a/b and Toll-like receptor 4 levels. Clin Sci (Lond). 2010; 119:395-405.
19. Cai Y, Yang Y, Chen X, He D, Zhang X, Wen X, Hu J, Fu C, Qiu D, Jose PA, Zeng C, Zhou L. Circulating "LncPPARδ" from monocytes as a novel biomarker for coronary artery diseases. Medicine (Baltimore). 2016; 95:e2360.
20. Cai Y, Yang Y, Chen X, Wu G, Zhang X, Liu Y, Yu J, Wang X, Fu J, Li C, Jose PA, Zeng C, Zhou L. Circulating 'lncRNA OTTHUMT00000387022' from monocytes as a novel biomarker for coronary artery disease. Cardiovasc Res. 2016; 112:714-724.
21. Li X, Zhao Z, Jian D, Li W, Tang H, Li M. Hsa-circRNA11783-2 in peripheral blood is correlated with coronary artery disease and type 2 diabetes mellitus. Diab Vasc Dis Res. 2017; 14:510-515.
22. Rodgers GP, Kopecky S. The future of cardiovascular biomarkers. Am Heart Hosp J. 2006; 4:228-231.
23. Ueno T, Watanabe H, Fukuda N, Tsunemi A, Tahira K, Matsumoto T, Takayama T, Chiku M, Saito S, Sato Y, Hirayama A, Matsumoto K, Soma M. Influence of genetic polymorphisms in oxidative stress related genes and smoking on plasma MDA-LDL, soluble CD40 ligand, E-selectin and soluble ICAM1 levels in patients with coronary artery disease. Med Sci Monit. 2009; 15:CR341-348.
24. Vargas J, Lima JA, Kraus WE, Douglas PS, Rosenberg S. Use of the Corus® CAD Gene Expression Test for Assessment of Obstructive Coronary Artery Disease Likelihood in Symptomatic Non-Diabetic Patients. PLoS Curr. 2013; 5. pii: ecurrents.eogt.0f04f6081905998fa92b99593478aea.

(Received April 24, 2018; Revised July 21, 2018; Accepted August 8, 2018)