Cardiovascular Topics

Oxidative metabolism of neutrophils in acute coronary syndrome

Elena Proskurnina, Tatiana Danilova, Madina Sozarukova, Artem Snitsar, Anatoly Baranov

Abstract

Background: Inflammation in acute coronary syndrome (ACS) involves neutrophil activation and oxidative stress. Here, we studied the production of reactive oxygen species (ROS) by neutrophils in ACS.

Methods: The study included 42 patients, men and women aged 46–91 years with ischaemic heart disease (IHD), non-ST-segment elevation ACS and ST-segment elevation ACS. Neutrophil-derived ROS were quantified with double-step stimulated luminol-enhanced chemiluminometry.

Results: The specific indices of spontaneous and double-step stimulated chemiluminescence did not differ in the subgroups of IHD, non-ST-segment elevation ACS and ST-segment elevation ACS. The total double-step stimulated ROS production by neutrophils was significantly higher in ST-segment elevation ACS than in non-ST-segment elevation ACS and IHD.

Conclusions: In ACS, special activation mechanisms of peripherical neutrophils were not triggered in our study. The significant increase in free radical production by neutrophils in acute myocardial infarction was presumably a consequence of an increase in their number.

Research Centre for Medical Genetics, ul. Moskvorechye 1, Moscow, Russia
Elena Proskurnina, MD, PhD, proskurnina@gmail.com

Faculty of Fundamental Medicine, Lomonosov Moscow State University, Moscow, Russia
Tatiana Danilova, MD
Anatoly Baranov, MD, PhD

Kurnakov Institute of General and Inorganic Chemistry, Russian Academy of Sciences, Moscow, Russia
Madina Sozarukova, PhD

Demikhov City Clinical Hospital, Moscow, Russia
Artem Snitsar, MD
Anatoly Baranov, MD, PhD

Different types of the acute coronary syndrome (ACS) such as unstable angina and myocardial infarction must be differentiated diagnostically for selection of the best personalised treatment strategy. Despite the progress achieved, diagnosing ACS is often a complex process, in which inflammatory and oxidative markers might be useful.[1] However, to date, relevant biomarkers of cardiovascular inflammation useful for stratifying risk and prognosis or guiding therapy for patients with ACS have not been found.[2]

Inflammation substantially contributes to the pathogenesis of atherosclerosis and ACS; both innate and adaptive immunity regulate the progression of atherosclerosis, plaque stability and thrombus formation.[3,4] Clinical studies have demonstrated the activation of neutrophils, lymphocytes and monocytes, increased concentrations of pro-inflammatory cytokines and acute-phase reactants in patients with unstable angina and myocardial infarction.[5]

Following plaque rupture, activated platelets will induce a subsequent inflammatory process, including neutrophil recruitment via a mechanism involving the CD40-CD40 ligand.[6] In response to acute myocardial ischaemia, neutrophils infiltrate the infarcted myocardium, reaching a maximum number one day after the onset of the disease. Inflammatory Ly6Chi monocytes migrate to the myocardium later than the neutrophils and reach their maximum by the third day of the disease.[7]

Inflammatory leukocytes are considered the cellular protagonists of vascular inflammation, in triggering disease progression, and the destabilisation that causes ACS.[8] Neutrophil accumulation at the coronary culprit lesion site is a strong and independent predictor of mortality in patients with ACS.[9] In patients with repeat coronary events within a year, more pronounced activation of oxygen-dependent metabolism of neutrophils and lipid peroxidation was revealed by Kratnov.[10] The extensive inflammatory response that occurs during acute cardiac events is considered the process responsible, in part, for the development of depressive symptoms.[11] Therefore, treatment of inflammation might be useful for postinfarction myocardial repair.[12,13]

The neutrophil is an extremely active cell with pleiotropic functions involved in inflammation [phagocytosis, synthesis of reactive oxygen species (ROS) and chemotaxis]. Activated neutrophils and monocytes/macrophages produce ROS and, consequently, result in oxidative stress in the myocardium and blood vessels, which aggravates the inflammation, forming a vicious circle.[14] Undoubtedly, oxidative stress is an important pathogenetic event in ACS, along with inflammation.[15]

A rise in 8-iso-PGF2a during ischaemia indicates the activation of lipid peroxidation.[16] In one study, advanced oxidation protein

products were significantly increased in patients with ACS with ST-segment elevation myocardial infarction and tended to increase in patients with non-ST-segment elevation myocardial infarction.[17] Oxidative stress markers (total thiol groups, catalase, superoxide dismutase and glutathione reductase) predict early left ventricular systolic dysfunction after acute myocardial infarction, treated with primary percutaneous coronary intervention.[18]

ACS patients had significantly higher levels of thiobarbituric acid reactive substances (TBARS), while levels of nitric oxide, hydrogen peroxide, superoxide dismutase and catalase activity were lowered.[19] Therefore, the development of various biomarkers of oxidative stress is important for the prevention and diagnosis of ACS, and patient stratification and treatment.[20]

In laboratory practice, the number of neutrophils and the ratio between neutrophils and lymphocytes or platelets are predominantly used. The ratio between neutrophil and lymphocyte counts (NLR) has been proposed as a new low-cost predictor for cardiovascular risk.[21] Higher neutrophil count, monocyte count and NLR, and lower lymphocyte count on admission were associated with the presence of coronary collateral vessels in patients with non-ST-segment elevation ACS.[22] In patients with a recent ACS, an increased pretreatment NLR value was effective in predicting the risk of mortality.[23] Awan *et al.* demonstrated a strong correlation between rising NLR and cardiac troponin-I increase in patients with non-ST-segment elevation ACS.[24]

The ratio and number of cells provide a simple and quick means of assessing neutrophil disturbances, but these indices are not sufficient since neutrophil activity varies widely. Therefore, the assessment of activity should be performed along with a clinical blood test. Respiratory metabolism of neutrophils can be assessed by the nitroblue tetrazolium test, but it has many drawbacks and, in general, is not informative. However, we have failed to find such studies in ACS. Myeloperoxidase, an enzyme of polymorphonuclear neutrophils and macrophages, was studied as a possible marker of plaque instability and a useful clinical tool in the evaluation of patients with coronary heart disease.[25]

A promising method for assessing oxidative metabolism of neutrophils is luminol-enhanced chemiluminescence, stimulated by stimuli such as zymosan, phorbol-12-myristate-13-acetate (PMA), *N-f*ormylmethionyl-leucyl-phenylalanine (fMLP) and others.[26] However, neutrophils are activated in two stages, and priming precedes the activation. Therefore, stimulation by one stimulus is incomplete and depends on the previous state of the neutrophils in the body. Therefore, it is impossible to obtain uniform indices of activity, even for healthy donors.

We previously proposed an original approach based on two-stage stimulation, which allows us to quantify the total oxidative resource of the neutrophils.[27] We have also determined some patterns of neutrophil response in pathology.[28] In this study, we aimed to apply this new protocol to quantitatively study the oxidative metabolism of neutrophils in ACS.

Methods

The study included 42 male and female patients, aged 46–91 years (mean age 61.5 ± 10.8 years), hospitalised in the Demikhov State Clinical Hospital, Moscow, Russia. The control group included 25 male and female volunteers, aged 42–55 years (mean age 49.2 ± 4.1 years) without acute or chronic disease. Blood samples for the study were taken on admission after the cardiologist's examination.

Inclusion criteria were patients over 18 years of age, verified diagnosis of non-ST-segment elevation ACS or ST-segment elevation ACS (the first 24 hours). Non-ST-segment elevation ACS symptoms included the clinical manifestations within a period of not more than 48 hours, plus electrocardiographic features of ischaemia. Exclusion criteria included patients under 18 years of age with acute infectious diseases, malignant neoplasms or manifested autoimmune disorders.

The patients were divided into three subgroups: ST-segment elevation ACS (*n* = 10; mean age 57.8 ± 7.9 years); non-ST-segment elevation ACS (*n* = 12; mean age 61.9 ± 13.7 years); and patients with verified ischaemic heart disease (IHD): stable angina of New York Heart Association functional class II–III (*n* = 20; mean age 62.7 ± 8.1 years). Information about the patients is presented in Table 1.

The study was performed in accordance with the Helsinki Declaration (adopted at the 64th General Assembly of the WMA in 2013), and approved by the Ethics Committee of Demikhov City Clinical Hospital. All subjects signed informed consent.

Chemiluminescence measurements were performed with a Lum-1200 chemiluminometer (DISoft, Russia) at 37°C. ROS production by neutrophils was assessed with the previously developed protocol based on the two-step sequential stimulation of neutrophils with PMA and fMLP.[27] Briefly, in all the experiments, 5 ml of blood was taken from the cubital vein into vacutainers with heparin (the final concentration of heparin was 5 U/ml). Blood samples were stored for up to two hours before analysis.

Aliquots of 450 µl of Hank's buffer with Hepes (HHBS), 25 µl of 1 mM luminol solution (5-amino-1,2,3,4-tetrahydro-1,4-phthalazinedione; Fluka, Germany) (final concentration = 45 µM), and 25 µl of whole blood were placed into the chemiluminometer cuvette and spontaneous chemiluminescence was recorded for 10 minutes. Next, 50 µl of 0.5 mg/ml PMA (final concentration = 0.081 nM) was added. After 20 minutes of incubation, 55 µl of 100 mg/ml fMLP (final concentration = 10 nM) was added and the chemiluminescence response was recorded for at least 60 minutes.

Table 1. Clinical information about the patients		
Parameters	*Subgroup 1 + 2 (ST-segment elevation ACS and non-ST-segment elevation ACS)* (n = 22)	*Subgroup 3 (IHD: stable angina class II–III)* (n = 20)
Age, years (mean ± SD)	60.8 ± 12.3	62.7 ± 8.1
Smoking, *n* (%)	7 (31.8)	2 (10.0)
Males/females, *n* (%)	14/8 (63.6/36.4)	13/7 (63.6/36.4)
PCI during hospitalisation, *n* (%)	1 (4.5)	8 (40.0)
PCI before hospitalisation, *n* (%)	3 (13.6)	10 (50.0)
Post-infarction cardiosclerosis, *n* (%)	3 (15.1)	14 (70.0)
Hypertension, *n* (%)	16 (72.7)	20 (100)
Acute heart failure (Killip 1), *n* (%)	14 (63.6)	2 (10.0)
Type 2 diabetes mellitus, *n* (%)	2 (9.1)	8 (40.0)
Congestive heart failure, *n* (%)	9 (40.1)	18 (90.0)

ACS, acute coronary syndrome; IHD, ischaemic heart disease; PCI, percutaneous coronary intervention.

A kinetic plot of neutrophil response to double-step stimulation involves three parts (Fig. 1A): a spontaneous (or basal) chemiluminescence (A_{bas}); a PMA-stimulated response; and a double-step stimulated response ($A_{PMA+fMLP}$). A_{bas} values were determined from blank chemiluminograms (blood samples with luminol but without stimuli); $A_{PMA+fMLP}$ values were determined at peak maximum after double-step sequential stimulation. Specific values (divided by the number of neutrophils) of A'_{bas} and $A'_{PMA+fMLP}$ were calculated and compared with clinical data and laboratory results.

Statistical analysis

The size of the groups was not previously determined. For statistical analysis, Statistica for Windows v.10.0 (StatSoft Inc, USA) was used. The normality was checked according to the Shapiro–Wilk test. Normally distributed variables are presented as the arithmetic mean ± standard deviation (SD). Otherwise, medians and first and third quartiles were calculated. Comparative analysis of two independent groups was carried out using the parametric t-test. The relationship was evaluated by the Spearman correlation coefficient (r_s). The differences were considered statistically significant at $p \leq 0.05$.

Results

In patients with ischaemic heart disease and non-ST-segment elevation ACS, the neutrophil response was similar to chemiluminograms of blood of healthy donors (Fig. 1A), whereas the neutrophil response for patients with ST-segment elevation ACS had qualitative and quantitative differences (Fig. 1B). In ST-segment elevation ACS, the amplitude of two-step stimulated chemiluminescence was several times higher. In several cases of ST-segment elevation ACS, there was a slight 'slow flash' on the chemiluminogram, which indicates the activation of phagocytosis. Total and specific (divided by neutrophil count) parameters of neutrophil activity are presented in Table 2.

The data show that the specific indices of spontaneous and stimulated chemiluminescence did not differ for the subgroups of healthy donors, and ischaemic heart disease, unstable angina

Table 2. Descriptive statistics for the parameters of ROS production neutrophil activity, where A_{bas} and A'_{bas} are the total basal and specific basal activity of neutrophils, respectively, and $A_{PMA+fMLP}$ and $A'_{PMA+fMLP}$ are the total and specific stimulated activity of neutrophils, respectively			
	A_{bas}	A'_{bas}	$A_{PMA+fMLP}$	$A'_{PMA+fMLP}$
Groups	mean (± SD)	mean (± SD)	mean (± SD)	mean (± SD)
Ischaemic heart disease ($n = 20$)	0.79 (0.58)	0.18 (0.15)	9.11 (4.40)	2.01 (1.06)
Non-ST-segment elevation ACS ($n = 12$)	0.87 (0.55)	0.16 (0.13)	9.79 (5.77)	1.86 (0.98)
ST-segment elevation ACS ($n = 10$)	1.29 (1.08)	0.14 (0.09)	17.8 (10.07)* ($p = 0.04$)	2.00 (0.98)
Control group ($n = 25$)	0.75 (0.45)	0.15 (0.10)	6.85 (3.02)	1.78 (0.88)
*Significant differences in relation to the non-ST-segment elevation ACS subgroup according to the t-test.			

pectoris and acute myocardial infarction patients. Total indices did not differ for healthy donors, and ischaemic heart disease and unstable angina patients, but were significantly higher for acute myocardial infarction patients. This increase was caused, not by increased activation of the neutrophils, but by an increase in their numbers.

The correlation coefficient between $A_{PMA+fMLP}$ and the neutrophil number was 0.63 for the total ACS subgroup. However, the correlation between $A_{PMA+fMLP}$ and NLR was weak ($r = 0.35$). A significant correlation was found between $A_{PMA+fMLP}$ and lactate dehydrogenase (LDH-1) in ACS patients but not in IHD patients. The correlation coefficients were 0.72 (ACS) and 0.09 (IHD).

Discussion

The complex and ambiguous role of neutrophils makes them therapeutic targets, but clearly requires the distinction of beneficial effects from harmful side effects. Many strategies are being developed, including enhancing, inhibiting or restoring neutrophil function.[29] For this purpose, adequate methods for assessing the activity of neutrophils are needed.

In addition to the haemogram indices, neutrophils are assessed by the activity of their enzymes, for example, elastase,[30] or by the amount of ROS (NBT test,[31] stimulated chemiluminescence.[32]). A distinctive feature of our method is the two-step stimulation,
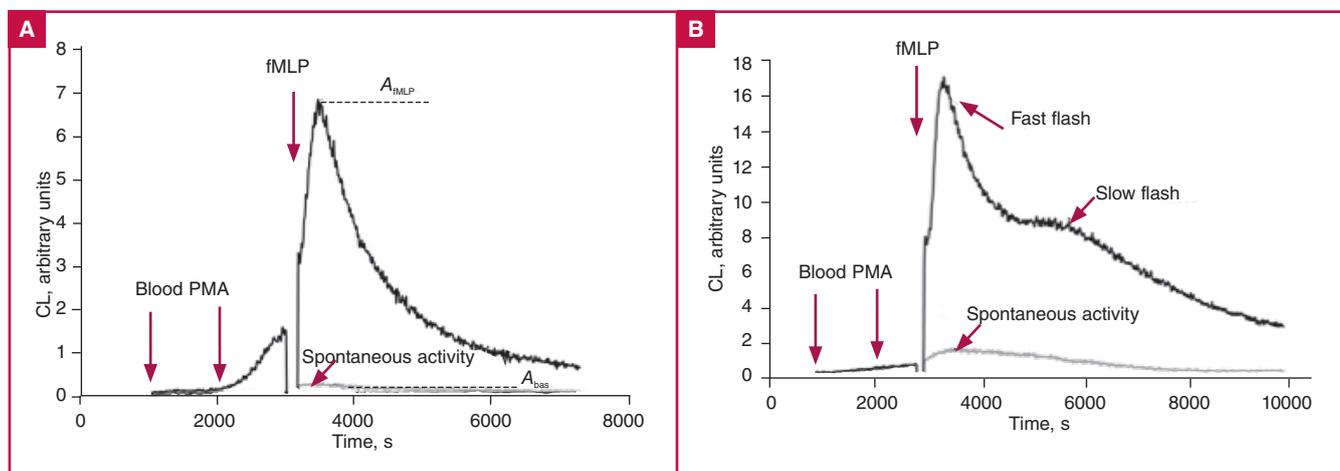

Fig. 1. Spontaneous activity (grey line) and response of blood neutrophils to two-step stimulation (black line) of patients with IHD (A) and ST-segment elevation ACS (B). The arrows indicate the moments of adding a blood sample, PMA and fMLP to the cuvette. CL, chemiluminescence.

which allows, in contrast to the one-step one, to assess the full resource of the neutrophil after priming with the first stimulus, to unify the response, and to quantify reference intervals.[27] Unlike the NBT test, two-step chemiluminometry makes it possible to also assess the kinetics of the response.

According to the response kinetics, we did not see any principal differences between neutrophils from patients with IHD or ACS and healthy donors. In some patients with ACS, a slow flash (a delayed ROS release) was expressed. According to our assumptions, the slow flash is due to intracellular ROS.[28] However, the intensity of this slow flash was too low to be clinically significant. In sepsis and trauma, the intensity of a slow flash can be an order of magnitude greater than a fast flash.[28]

Quantitatively, the specific double-step activity of neutrophils did not differ significantly in patients with ACS and IHD, which indicates the absence of mechanistical changes in the function of ROS production of neutrophils. Therefore, an increase in their number leads to an increase in the total production of ROS. This is in line with other studies where the number of neutrophils or the NLR was used as a marker of ACS.[23-25] In our study, the amplitude of the stimulated signal was positively correlated ($r = 0.63$) with the number of neutrophils. However, the correlation with the NLR was weak.

The activity of neutrophils is related positively with LDH-1 ($r = 0.72$). An increase in LDH-1 in clinical practice is most often used to diagnose ACS.[33] Recently, studies have been published that propose the combined use of NLR and LDH activity in assessing sensitivity in chemotherapeutic treatment of lung cancer,[34] lymphomas,[35] as components of a prognostic marker in colon cancer,[36] and as prognostic radiation treatment for oesophageal cancer.[37] We believe that additional studies of combined parameters of neutrophil activity and LDH-1 will also be useful for ACS.

Here, significant differences were obtained between the double-step stimulated activity of neutrophils in ST-segment elevation ACS relative to non-ST-segment elevation ACS, but not between spontaneous activity. Takeshita *et al.* also found that spontaneous chemiluminescence in ST-segment elevation ACS relative to non-ST-segment elevation ACS did not differ.[38] Therefore, double-step stimulated chemiluminescence can be proposed as an additional parameter for the differential diagnosis between non-ST-segment elevation and ST-segment elevation ACS.

Increased activity of peripheral neutrophils in the bloodstream may lead to oxidative stress and endothelial damage. When total free radical production by neutrophils is high, antioxidant therapy aimed at protecting the endothelium may be useful. Therefore, assessment of neutrophil free radical production by our method may be useful when considering antioxidant support in patients with ACS.

Conclusions

Since the specific indices of spontaneous and double-step stimulated chemiluminescence did not differ in the subgroups of patients with IHD, non-ST-segment elevation ACS and ST-segment elevation ACS, we hypothesise that in ACS, special activation mechanisms of peripherical neutrophils are presumably not triggered. The total double-step stimulated activity of neutrophils was significantly higher in ST-segment elevation ACS related to non-ST-segment elevation ACS and

IHD patients, which makes it possible to use this parameter for a differential diagnosis. Since an increase in ROS production is caused precisely by an increase in the number of neutrophils, numerical indices remain important in the diagnosis of ACS. We plan to study the oxidative metabolism of neutrophils in larger groups in the dynamics of ACS.

References

1. Centurion OA. Serum biomarkers and source of inflammation in acute coronary syndromes and percutaneous coronary interventions. *Cardiovasc Revasc Med* 2016; **17**(2): 119–128.
2. Dominguez-Rodriguez A, Abreu-Gonzalez P. [Inflammatory biomarkers in ischemic acute coronary syndrome]. *Emergencias* 2015; **27**(5): 319–324.
3. Meuwissen M, van der Wal AC, Siebes M, Koch KT, Chamuleau SA, van der Loos CM, *et al.* Role of plaque inflammation in acute and recurrent coronary syndromes. *Neth Heart J* 2004; **12**(3): 106–109.
4. Carter AM. Inflammation, thrombosis and acute coronary syndromes. *Diab Vasc Dis Res* 2005; **2**(3): 113–121.
5. Biasucci LM, Liuzzo G, Angiolillo DJ, Sperti G, Maseri A. Inflammation and acute coronary syndromes. *Herz* 2000; **25**(2): 108–112.
6. Setianto BY, Hartopo AB, Gharini PP, Anggrahini DW, Irawan B. Circulating soluble CD40 ligand mediates the interaction between neutrophils and platelets in acute coronary syndrome. *Heart Vessels* 2010; **25**(4): 282–287.
7. Nahrendorf M, Swirski FK, Aikawa E, Stangenberg L, Wurdinger T, Figueiredo JL, *et al.* The healing myocardium sequentially mobilizes two monocyte subsets with divergent and complementary functions. *J Exp Med* 2007; **204**(12): 3037–3047.
8. Sager HB, Nahrendorf M. Inflammation: a trigger for acute coronary syndrome. *Q J Nucl Med Mol Imaging* 2016; **60**(3): 185–193.
9. Distelmaier K, Winter MP, Dragschitz F, Redwan B, Mangold A, Gleiss A, *et al.* Prognostic value of culprit site neutrophils in acute coronary syndrome. *Eur J Clin Invest* 2014; **44**(3): 257–265.
10. Kratnov AE. Markers of inflammation and intracellular metabolism of neutrophils in patients with acute coronary syndrome and normal troponin T levels. *Bull Exp Biol Med* 2011; **151**(4): 467–469.
11. Poole L, Dickens C, Steptoe A. The puzzle of depression and acute coronary syndrome: reviewing the role of acute inflammation. *J Psychosom Res* 2011; **71**(2): 61–68.
12. Rymer JA, Newby LK. Failure to launch: targeting inflammation in acute coronary syndromes. *J Am Coll Cardiol Basic Transl Sci* 2017; **2**(4): 484–497.
13. Wang H, Liu Z, Shao J, Lin L, Jiang M, Wang L, *et al.* Immune and inflammation in acute coronary syndrome: molecular mechanisms and therapeutic implications. *J Immunol Res* 2020; **2020**: 4904217.
14. Aukrust P, Berge RK, Ueland T, Aaser E, Damas JK, Wikeby L, *et al.* Interaction between chemokines and oxidative stress: possible pathogenic role in acute coronary syndromes. *J Am Coll Cardiol* 2001; **37**(2): 485–491.
15. Armstrong EJ, Morrow DA, Sabatine MS. Inflammatory biomarkers in acute coronary syndromes: part III: biomarkers of oxidative stress and angiogenic growth factors. *Circulation* 2006; (8):e289–292.
16. Berg K, Jynge P, Bjerve K, Skarra S, Basu S, Wiseth R. Oxidative stress and inflammatory response during and following coronary interventions for acute myocardial infarction. *Free Radic Res* 2005; **39**(6): 629–636.
17. Skvarilova M, Bulava A, Stejskal D, Adamovska S, Bartek J. Increased level of advanced oxidation products (AOPP) as a marker of oxidative stress in patients with acute coronary syndrome. *Biomed Pap Med Fac*

Univ Palacky Olomouc Czech Repub 2005; **149**(1): 83–87.

18. Rajic D, Jeremic I, Stankovic S, Djuric O, Zivanovic-Radnic T, Mrdovic I, *et al*. Oxidative stress markers predict early left ventricular systolic dysfunction after acute myocardial infarction treated with primary percutaneous coronary intervention. *Adv Clin Exp Med* 2018; **27**(2): 185–191.

19. Koprivica Z, Djordjevic D, Vuletic M, Zivkovic V, Barudzic N, Andjelkovic N, *et al*. Von Willebrand factor and oxidative stress parameters in acute coronary syndromes. *Oxid Med Cell Longev* 2011; **2011**: 918312.

20. Lubrano V, Pingitore A, Traghella I, Storti S, Parri S, Berti S, *et al*. Emerging biomarkers of oxidative stress in acute and stable coronary artery disease: levels and determinants. *Antioxidants* (Basel) 2019; **8**(5).

21. Zazula AD, Precoma-Neto D, Gomes AM, Kruklis H, Barbieri GF, Forte RY, *et al.* An assessment of neutrophils/lymphocytes ratio in patients suspected of acute coronary syndrome. *Arq Bras Cardiol* 2008; **90**(1): 31–36.

22. Tenekecioglu E, Yilmaz M, Karaagac K, Bekler A, Aslan B, Demir S, *et al*. Predictors of coronary collaterals in patients with non ST-elevated acute coronary syndrome: the paradox of the leukocytes. *Cent Eur J Immunol* 2014; **39**(1): 83–90.

23. Dong CH, Wang ZM, Chen SY. Neutrophil to lymphocyte ratio predict mortality and major adverse cardiac events in acute coronary syndrome: A systematic review and meta-analysis. *Clin Biochem* 2018; **52**: 131–136.

24. Awan MS, Daud MY, Khan M, Jehangiri AU, Adnan, Jalil S. Usefulness of neutrophils to lymphocytes ratio for predicting troponin-I elevation in patients presenting with suspected nSTe-acute coronary syndrome. *J Ayub Med Coll Abbottabad* 2019; **31**(Suppl 1)(4): S674–S677.

25. Loria V, Dato I, Graziani F, Biasucci LM. Myeloperoxidase: a new biomarker of inflammation in ischemic heart disease and acute coronary syndromes. *Mediators Inflamm* 2008; 2008:135625.

26. Eagleson JS, Moriarty KM. Neutrophils, oxidative killing mechanisms and chemiluminescence. *N Z Vet J* 1984; **32**(18): 41–43.

27. Obraztsov IV, Godkov MA, Polimova AM, Dyomin EM, Proskurnina EV, Vladimirov YuA. Evaluation of functional activity of whole blood neutrophils by two-stage stimulation: a new approach to chemiluminescent analysis. *Russian J Immunol* 2015; **9**(18)(4): 418–425.

28. Obraztsov IV, Godkov MA, Kulabukhov VV, Vladimirova GA, Izmailov DYu, Proskurnina EV. Early diagnosis of burn sepsis: experience of using a new chemiluminescent method for analyzing the functional activity of neutrophils with two-stage stimulation. *Gen Reanimatol* 2017; **XIII**(1): 40–51.

29. Nemeth T, Sperandio M, Mocsai A. Neutrophils as emerging therapeutic targets. *Nat Rev Drug Discov* 2020; **19**(4): 253–275.

30. Taylor S, Dirir O, Zamanian RT, Rabinovitch M, Thompson AAR. The role of neutrophils and neutrophil elastase in pulmonary arterial hypertension. *Front Med* (Lausanne) 2018; **5**: 217.

31. Szczepanski Z, Sychlowy A. [Clinical use of nitroblue tetrazolium (NBT) reduction by neutrophils]. *Pol Tyg Lek* 1972; **27**(40): 1548–1550.

32. Allen RC. Neutrophil leukocyte: combustive microbicidal action and chemiluminescence. *J Immunol Res* 2015; **2015**: 794072.

33. Petersen JR, Smith E, Okorodudu AO, Valbuena G, Bissell MG. Utilization of LDH isoenzymes in the diagnosis of myocardial infarction. *Clin Lab Manage Rev* 1997; **11**(2): 103–106.

34. Banna GL, Signorelli D, Metro G, Galetta D, De Toma A, Cantale O, *et al*. Neutrophil-to-lymphocyte ratio in combination with PD-L1 or lactate dehydrogenase as biomarkers for high PD-L1 non-small cell lung cancer treated with first-line pembrolizumab. *Transl Lung Cancer Res* 2020; **9**(4): 1533–1542.

35. Kaito S, Kanemasa Y, Sasaki Y, Okuya T, Yamaguchi T, Funasaka C, *et al*. A new prognostic score comprising lactate dehydrogenase, albumin and neutrophil to lymphocyte ratio to predict sensitivity to first-line chemotherapy in patients with peripheral T-cell lymphomas. *Int J Hematol* 2018; **107**(4): 451–459.

36. Casadei-Gardini A, Scarpi E, Ulivi P, Palladino MA, Accettura C, Bernardini I, *et al*. Prognostic role of a new inflammatory index with neutrophil-to-lymphocyte ratio and lactate dehydrogenase (CII: colon inflammatory index) in patients with metastatic colorectal cancer: results from the randomized Italian Trial in Advanced Colorectal Cancer (ITACa) study. *Cancer Manag Res* 2019; **11**: 4357–4369.

37. Luo HS, Xu HY, Du ZS, Li XY, Wu SX, Huang HC, *et al*. prognostic significance of baseline neutrophil count and lactate dehydrogenase level in patients with esophageal squamous cell cancer treated with radiotherapy. *Front Oncol* 2020; **10**: 430.

38. Takeshita S, Isshiki T, Ochiai M, Ishikawa T, Nishiyama Y, Fusano T, *et al*. Systemic inflammatory responses in acute coronary syndrome: increased activity observed in polymorphonuclear leukocytes but not T lymphocytes. *Atherosclerosis* 1997; **135**(2): 187–192.