Original Article

Clinical Study Of Neopterin With Cardiovascular Diseases In Iraq Patients

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Abstract

Objectives: The aim of our study was to determine neopterin concentration in patients with Cardiovascular Diseases and their relationship with clinical parameters.

Background: The release of various cytokines activates cellular defense in cardiovascular diseases. As well as in the vascular endothelium and cardiomyopathy, neutrophils and monocytes/macrophages have been found to be infiltrating the artery and the heart, An indicator for those activated macrophages is neopterin, a byproduct of the guanosine triphosphate-biopterin pathway.

Methods: blood samples of people with cardiovascular diseases, aged between (35-65) years, of both sexes, from the cardiac care unit at Ibn 75 Sina Teaching Hospital / Mosul / Iraq, and compared with 75 blood samples of apparently healthy people. Neopterin levels were determined with a commercially available enzyme-linked immunosorbent assay
method.

Conclusions: Our data support the hypothesis of activation of monocytes and macrophages in patients with cardiovascular diseases. neopterin as a marker for macrophage activation is significantly increased in patients with CAD .there was a correlation between neopterin and clinical parameters as markers for the extent of the Atherosclerosis

Keywords: Neopterin, Cardiovascular, BMI, antioxidants, troponin I aminotransferase.

Introduction

The induction of plaque rupture appears to be the mechanism through which atherosclerosis develops into an acute clinical episode, the number and activation state of macrophages and inflammatory cells have a greater impact on the risk of plaque rupture atherosclerotic plaques' primary rather than plaque size (1-2) Inflammatory cells may be responsible for the weakening of the fibrous cap by lytic enzymes and subsequently the atherosclerotic plaque (3).

Neopterin is produced by macrophages and is also expressed in Atheromatous plaques within the carotid artery Monocyte activation is believed to play an important role in the pathogenesis coronary artery, as well as the aorta. (4) of acute coronary syndromes (ACS), and neopterin is a marker of its activation, and elevated levels have predictive value (5-6).

Neopterin is involved in immune activation and is a marker of disease activity in many inflammatory conditions (7). Information about the activation of cellular immunity in humans under the control of T helper cells type 1. Production of neopterin is associated with Elevated by increasing production of reactive oxygen species (4, 8) .Neopterin is a more sensitive biomarker for coronary heart disease than C-reactive protein, fibrinogen, and homocysteine and is equivalent to malonaldehyde (an end product of lipid peroxidation) as a stroke biomarker (4,9).

No data are available on neopterin in patients with cardiovascular diseases in Iraq. We, therefore, compared neopterin levels in patients with healthy control subjects. Additionally, we examined whether neopterin correlated with noninvasive indexes for the extent of myocardial necrosis

Materials and methods:

This study included 75 blood samples of people with cardiovascular diseases, aged between (35-65) years, of both sexes, from the cardiac care unit at Ibn Sina Teaching Hospital / / Mosul / Iraq and compared them with 75 blood samples of apparently healthy people. The study samples were collected for the period from (1/12/2021) to (4/12/2022) from the city of Mosul.

Fasting and non-fasting blood were taken, and the serum was separated (10) and used to estimate the following clinical parameters:

Neopterin: Was determined by Sandwich enzyme-linked immunosorbent assay (ELISA), using My BioSource kit (USA).

Aspartate aminotransferase and Alanine aminotransferase: Were determined by enzymatic colorimetric method using a BIOLABS kit (France) .

Creatinine kinase: Was determined by enzymatic colorimetric method using RANDOX kit (U.K).

C-reactive protein: Was determined by using a Plasmatec kit (France)

Troponin I : Was determined by enzyme-linked fluorescent immunoassay (ELFA) using a device mini VIDAS, Biomerieux kit (France)

Malondialdehyde (MDA): was measured manually according to the modified method of (11).

Arylesterase activity: Was determined by enzymatic hydrolysis of phenyl acetate to phenol and acetic acid (12)

Body Mass Index (BMI): Was calculated as weight in kilogram divided by square height in meters (13)

DATA ANALYSIS:

The data obtained in this study were analyzed using Statistical Package for Social Sciences (SPSS)

- Standard statistical methods were used to determine the mean and standard error.
- T-test was used to compare two parameters.
- Liner regression analysis [Pearson correlation coefficient (r)] was used to find the
- Relationship between different chemical parameters.
- P-Value ≤ 0.05 was considered to be statistically significant (14)

RESULT AND DISCUSION:

The concentration of Neopterin in serum of control group and its normal reference values

Neopterin is an indicator of cellular immune activity that can be measured in different body fluids where its concentration is stable for a long time and normal reference values vary serum neopterin according to researchers and sources (15-16). It was found that the normal range of neopterin concentration is (8.4 ± 1.3 nmol/L) in the blood serum of the control group, for both sexes and for all studied age groups, as shown in Figure (1), which agrees with (7.9 ± 1.9 nmol/L) found by (17-18).

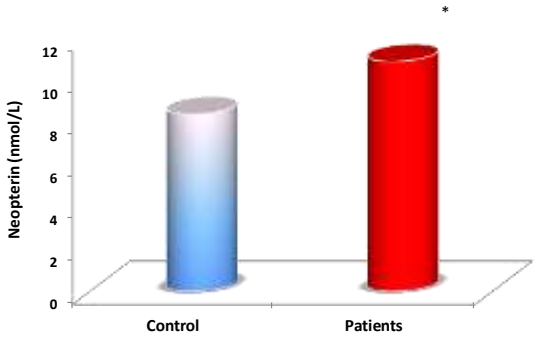
The neopterin concentration ranges from 5–8 (nmol/L), and most data indicate that the normal reference value neopterin concentration in serum is less than (10 nmol/L), because it represents its concentration in about 98% of healthy people (16, 19).

The concentration of Neopterin in serum of Patients group

It was found that the average neopterin concentration was (10.9 ± 1.5 nmol/L) in the blood serum of a group of patients with cardiovascular disease, for both sexes and for all studied age groups, as shown in Figure (1).

The concentration of Neopterin in serum of patients with cardiovascular diseases and its comparison with a control group

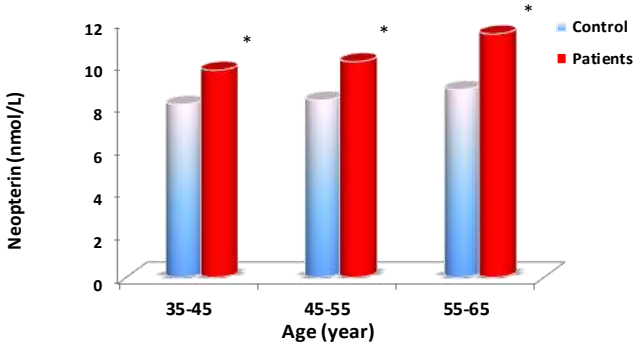
The results showed that there was a significant increase at the level of ($p \leq 0.01$) in the concentration of neopterin in the blood serum of the patient's group compared with the control group, according to the total average, as it was found that its concentration in the patient's group was ((10.9 ± 1.5 nmol / L), while in the control group, it was (8.4 ± 1.3 nmol/L) as shown in Figure (1).



* Significant difference at P≤**0.01**

Effect of age

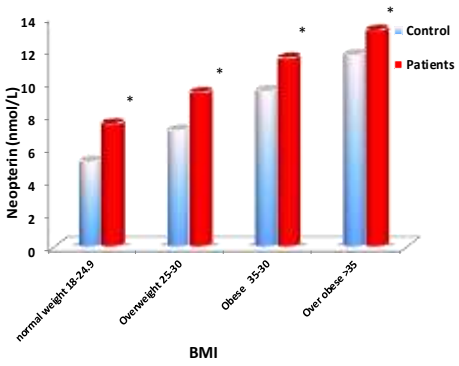
Figure (2) shows that there is a significant increase at the level of ($p \leq 0.01$) in the concentration of neopterin in the blood serum of the patients group compared with the control group, depending on age and for all studied age groups.



* Significant difference at P≤**0.01**

Effect of body mass index (BMI)

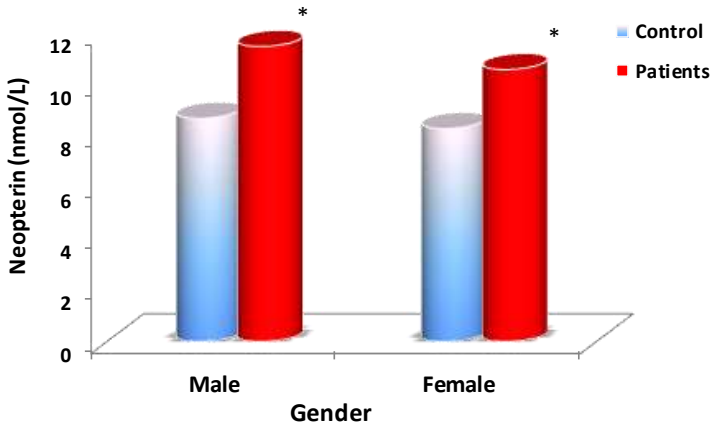
There was a significant difference at the level of ($p \leq 0.01$) in the concentration of neoptrin in the blood serum of the patients group compared with the control group, depending on the body mass index and for all levels, as shown in Figure (3).



* Significant difference at P≤**0.01**

Effect of gender

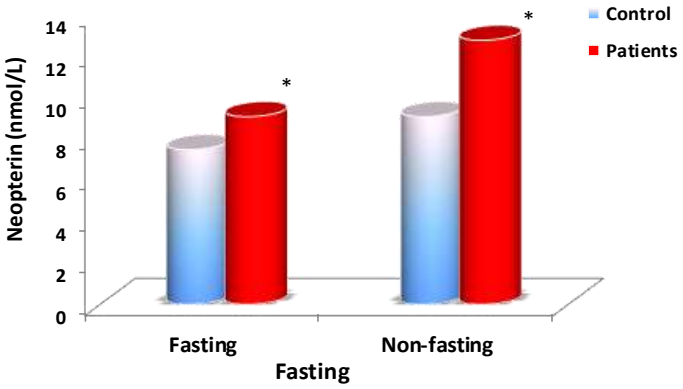
Figure (4) shows that there is a significant increase at the level of ($p \leq 0.01$) in the concentration of neopterin in the blood serum of the patients group compared with the control group based on the division by gender. Its concentration is high in male and female patients when compared with its concentration in healthy males and females.



* Significant difference at P≤**0.01**

Effect of fasting

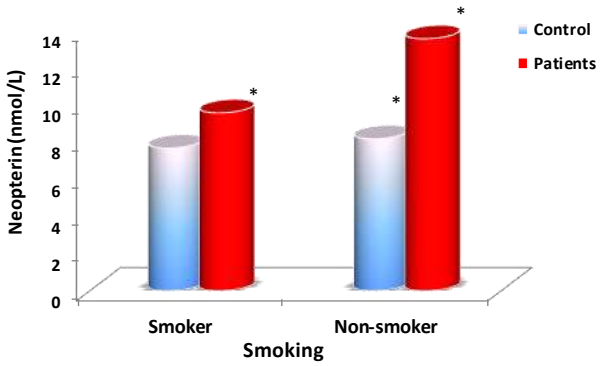
The results of the effect of fasting showed a significant increase at the probability level ($p \leq 0.01$) in the concentration of neoptrin in the blood serum of the patient's group, compared with the control group, as shown in Figure (5) .



* Significant difference at P≤**0.01**

Effect of smoking

There was a significant difference at the probability level ($p \leq 0.01$) in the concentration of neopterin in the blood serum of the patient's group compared with the control group, depending on smoking, as shown in Figure (6).



* Significant difference at P≤**0.01**

The concentration of neopterin is elevated in patients with cardiovascular disease, and this is consistent with its findings (5,20).

Inflammation plays a major role in the pathogenesis of atherosclerosis and cardiovascular disease, and the inflammatory state has a negative impact on patient outcome, and neopterin is produced by monocyte/macrophages upon activation of the cellular immune response to pro-inflammatory stimuli such as the cytokine interferon-gamma, (6,21).

Neopterin also has pro-oxidant properties, which have been demonstrated in physicochemical and cell culture studies.

An increase in neopterin secretion is associated with an increase in the production of active oxygen species (8, 22) , and decreased levels of serum antioxidants. Therefore, neopterin production may also indirectly reflect the severity of oxidative stress as Reactive oxygen species (ROS) are key signaling molecules that have an important role in the development of inflammatory disorders.

ROS by polymorphonuclear neutrophils (PMNs) at the site of inflammation leads to endothelial dysfunction and tissue injury as a result of which neopterin levels are elevated in body fluids in a range of diseases where T cells or

macrophages are activated, and has been identified as a marker of disease and inflammation e.g. viral infections Accordingly, Neopterin has been widely used as a clinical marker for immune activation during inflammation in a wide range of conditions and stress (6,23-24).

Oxidizing and antioxidants agents

The results showed a significant increase in the concentration of malonaldehyde at the level of (P≤0.01) in the patient group compared with the control group as shown in Table (1). This is consistent with (25-27), and is attributed to the presence of an increase in the levels of lipid peroxidation in patients with cardiovascular disease, which causes a high concentration of malonhyde as a by-product of lipid peroxidation (28).

There was also a significant decrease in the activity of the aryl esterase enzyme in patients with cardiovascular diseases at the level of (p≤0.01)), compared with the activity of the enzyme in the control group. The enzyme binds to high-density lipoprotein HDL and acts as an antioxidant through the group of free sulfhydryls in its reduced form, and when the fat is associated with it, it turns it into the inactive oxidized form, and thus the enzyme's effectiveness decreases (29-30).

Table (1): The concentration of oxidizing agents and antioxidants in the control and patients groups

Oxidizing and antioxidants agents	**Controls Group** **Mean± SE**	**Patient Group** **Mean± SE**
Arylesterase(U/ml)	95.5 ± 7.5	62.4±8.8*
MDA (µmol/L)	13.8 ± 2.7	17. 6 ±3.5*

* Significant at the level P≤0.01

Cardiac indications

The results shown in Table (2) showed a significant increase at the level of (P≤0.01) in the activity of the two enzymes that transport the amino group AST and ALT in patients with cardiovascular diseases compared with the control group, and the reason for this is that the two enzymes are secreted from liver and muscle cells and their effectiveness increases As a result of damage and dissolution of mitochondria and necrosis in cardiac muscle cells or in liver cells, causing these enzymes to be released into the blood, this is consistent with (31-32)

A significant increase was found at the level (P≤0.01) in the activity of creatine kinase enzyme in the patient group compared to the control group as shown in Table (2), and this is consistent with what was found (33-34) that the activity of the enzyme increases in heart disease, and the reason may be due to necrosis of the heart muscle, then its death, and the enzyme is released in additional quantities to the plasma.

The level of C-reactive protein can be an indicator of the risk of developing cardiovascular problems because the development of atherosclerosis (the placement of cholesterol within the walls of blood vessels) is associated with inflammation within the walls of blood vessels, and C-reactive protein is an indicator of the state of Inflammation,(3, 35-36) a significant increase was found at the level (P≤0.01) in the patient group, as shown in Table (2). CRP, which indicates the level of inflammation and its role in the formation of sclerosis and its complications.

The results shown in Table (2) also indicate that there is a significant increase in the level of troponin I at the level (P ≤ 0.01) in patients, and this is consistent with what was shown by (37-38) that when the heart muscles are damaged, troponin is sent into the bloodstream and with increased heart damage more amounts of troponin are released into the blood which is indicative of a heart attack that occurs when blood flow to the heart is blocked, which is a criterion for detecting heart muscle damage and for predicting Acute myocardial infarction or death in patients with unstable coronary heart disease (CHD) and heart failure (HF) correlates with the amount of damaged myocardium, which is a predictor of the risk of death.

Table (2): Cardiac indicators in the two control groups of patients

Cardiac indications	**Control group** **Mean ± S.E**	**Patients group** **Mean ± S.E**
AST (**U/L**)	25.3 ± 4.8	69.7±8.2*****
ALT (**U/L**)	29.9 ± 8.4	60.2±7.7*****
Creatine kinase (CK) (**U/L**)	55.8±6.9	108.8+10.5*****
C-reactive protein (CRP) (mg/L)	4.1 ± 2.2	36.4 ±5.8*****
Troponin I (ng/L)	3.3±0.2	95.2±11.5*****

* Significant at the level P≤0.01

Study the relationship between Neopterin and Clinical indications in control and patients group

Table (3) shows that there is a positive significant correlation at the level of ((P≤0.05) between the concentration of **neopterin** with the level of active C protein in the group of patients. The presence of infections, therefore, rises as a result of damage to the heart muscle, as well as n Neopterin rises as a result of inflammation and the pathological condition (39-40).
and no correlation was shown between neopterin and other indicators..

Table (3): the relationship between Neopterin and Clinical indications in control and patients group

Clinical indications	**Control group** **r-value**	**Patients group** **r-value**
AST (**U/L**)	0.826	0.842
ALT (**U/L**)	0.558	0.518
Creatine kinase (CK) (**U/L**)	0.636	0.681
C-reactive protein (CRP) (mg/L)	0.572	0.245*****
Troponin I (ng/L)	0.742	0.755

Arylesterase(U/ml)	0.615	0.667
MDA (µmol/L)	0.593	0.554

* Significant at the level P≤0.05

Conclusion

It was concluded that neopterin has a major role in cardiovascular diseases and can be represented as a marker of this disease. Also, the positive relationship between neopterin with C-reactive protein in patients group. This indicates the importance of neopterin as a predictive diagnostic marker in cardiovascular diseases and related diseases.

References

1- Jebari-Benslaiman, Shifa, Unai Galicia-García, Asier Larrea-Sebal, Javier Rekondo Olaetxea, Iraide Alloza, Koen Vandenbroeck, Asier Benito-Vicente, and César Martín. 2022. "Pathophysiology of Atherosclerosis" International Journal of Molecular Sciences 23, no. 6: 3346. https://doi.org/10.3390/ijms23063346

2- Bentzon, J. F., Otsuka, F., Virmani, R., & Falk, E. (2014). Mechanisms of plaque formation and rupture. Circulation research, 114(12), 1852–1866. https://doi.org/10.1161/CIRCRESAHA.114.302721

3- Rana F. Jasim, Thikra A. Allwsh. Orexin A hormone and its relation to Coronary heart diseases. Research J. Pharm. and Tech 2021; 14(3):1417-1422. doi: 10.5958/0974-360X.2021.00253.5

4- Takuya Watanabe. 2021 . Neopterin derivatives – a novel therapeutic target rather than biomarker for atherosclerosis and related diseases. Vasa 50:3, 165-173

5- Lanser, L., Pölzl, G., Fuchs, D., Weiss, G., & Kurz, K. (2019). Neopterin is Associated with Disease Severity and Outcome in Patients with Non-Ischaemic Heart Failure. Journal of Clinical Medicine 8(12): 2230

6- Gieseg, Steven P., Gregory Baxter-Parker, and Angus Lindsay. 2018. "Neopterin, Inflammation, and Oxidative Stress: What Could We Be Missing?" Antioxidants 7, no. 7: 80.

7 - Molero-Luis, M., Casas-Alba, D., Orellana, G. et al. (2020). Cerebrospinal fluid neopterin as a biomarker of neuroinflammatory diseases. Sci Rep 10, 18291

8- Martins Roberta de Paula, Karina Ghisoni, Chai K. Lim, Aderbal Silva Aguiar, Gilles J. Guillemin, Alexandra Latini,. 2018 ,Neopterin preconditioning prevents inflammasome activation in mammalian astrocytes,. Free Radical Biology and Medicine,Volume 115,Pages 371-382,

9- Hajsl M, Hlavackova A, Broulikova K, Sramek M, Maly M, Dyr JE, et al. 2020. Tryptophan metabolism, inflammation, and oxidative stress in patients with neurovascular disease. Metabolites.;10:208

10- Thikra A. Allwsh (2013). Clinical Study of Adiponectin Hormone and its Relation with some Variables in Cardiovascular Patients in Nineveh Province. Rafidain Journal of Science, 24(4), 50-64. doi: 10.33899/rjs.2013.71021

11 - Guidet B. shah SV. Am J. Physiol. 1989;257(26) : 440.

12- Thikra A. Allwsh ; Rana F. Jasim. (2008). Study of Arylesterase and It's Relationship With Some Clinical Variables in Atherosclerotic Patients in Mosul (Part I). Rafidain Journal of Science, 9(4), 143-157. doi: 10.33899/rjs.2008.42011

13- Kolimechkov S. Body Mass Index. STK Sport UK. 2016.

14-Kirkwood B R. Essentials of Medical Statistics. 1st ed., Black well Scientific Publication, Oxford. 1988; pp. 43-56

15 - Fuchs, D. & Gisslen, M. (2021). Laboratory diagnostic value of neopterin measurements in patients with COVID-19 infection. Pteridines, 32(1), 1-4.

16 - Plata-Nazar, K., Luczak, G., Borkowska, A., Delinska-Galinska, A., Kozielska, E., Marek, K. & Korzon, M. (2007). Reference Standard of Serum Neopterin Concentration in Healthy Children. Pteridines, 18(1), 19-24.

17 - Liu, Z. Y., & Li, Y. D. (2013). Relationship between serum neopterin levels and coronary heart disease. Genetics and molecular research : GMR, 12(4), 4222–4229.

18 - Martins Roberta de Paula, Karina Ghisoni, Chai K. Lim, Aderbal Silva Aguiar, Gilles J. Guillemin, Alexandra Latini,. 2018 ,Neopterin preconditioning prevents inflammasome activation in mammalian astrocytes,. Free Radical Biology and Medicine,Volume 115,Pages 371-382,

19 - Blankson, S.O.; Rietmeyer, L.; Tettey, P.; Dikroh, L.; Tornyigah, B.; Adamou, R.; Moussiliou, A.; Padounou, C.; Amoussou, A.; Mensah, B.A.; et al. 2023,High Plasma Levels of Neopterin Are Associated with Increased Mortality among Children with Severe Malaria in Benin. Diagnostics 13, 528.

20 - Dogheim, G.M., Amralla, M.T. & Werida, R.H. (2022). Role of neopterin as an inflammatory biomarker in congestive heart failure with insights on effect of drug therapies on its level. Inflammopharmacol 30, 1617–1622

21 - Hailemichael W, Kiros M, Akelew Y, Getu S, Andualem H. 2021. Neopterin: A Promising Candidate Biomarker for Severe COVID-19. J Inflamm Res.;14:245-251.

22 - Takuya Watanabe. 2021 . Neopterin derivatives – a novel therapeutic target rather than biomarker for atherosclerosis and related diseases. Vasa 50:3, 165-173
23 - Kuryltsiv, N. B., & Zborovska, O. V. (2021). Neopterin, a promising biomarker for the diagnosis of intraocular inflammation. blood, 78, 79.
24 - Lin-lie Meng, Li Cao, 2021, Serum neopterin levels and their role in the prognosis of patients with ischemic stroke: A systematic review and meta-analysis,Journal of Clinical Neuroscience,Volume 92,Pages 55-60,
25 - Fadel MA, Kafafy TA, Essam SM, Abdel-Aal AM. 2022. Serum superoxide dismutase and malondialdehyde as oxidative stress biomarkers in coronary artery disease. J Curr Med Res Pract;7:215-20
26 - Kong, Amanda Shen-Yee, Kok Song Lai, Cheng-Wan Hee, Jiun Yan Loh, Swee Hua Erin Lim, and Maran Sathiya. 2022. "Oxidative Stress Parameters as Biomarkers of Cardiovascular Disease towards the Development and Progression" Antioxidants 11, no. 6: 1175.
27- Jasim Rana F& Allwsh Thikra A. (2020) Relation between orexin A and obesity in coronary heart patients. Relation. 25,5:2377-2389
28- Thikra Ali Allwsh, & Noori Mohammed Aziz. (2023). Clinical study of copeptin in serum patiants of heart diseases. Tikrit Journal of Pure Science, 20(3), 99–107. https://doi.org/10.25130/tjps.v20i3.1192
29 - Dan-Dan Zhou, Min Luo, Ao Shang, Qian-Qian Mao, Bang-Yan Li, Ren-You Gan, Hua-Bin Li, 2021, "Antioxidant Food Components for the Prevention and Treatment of Cardiovascular Diseases: Effects, Mechanisms, and Clinical Studies", Oxidative Medicine and Cellular Longevity, vol. 17 pages, 2021.
30- Jasim, R. F., Mohammad, S. S., & Allwsh, T. A. (2021). The Relation Between Fibroblast Growth Factor 21 and Insulin Resistance in hyperlipidemia Patients. Egyptian Journal of Chemistry, 64(12), 7091-7097. doi: 10.21608/ejchem.2021.80062.3947
31- Bekkelund, S.I. (2021). Serum alanine aminotransferase activity and risk factors for cardiovascular disease in a Caucasian population: the Tromsø study. BMC Cardiovasc Disord 21, 29
32 - Ndrepepa G. 2021. Aspartate aminotransferase and cardiovascular disease—a narrative review. J Lab Precis Med;6:6.
33 - Brewster, L.M., Haan, Y.C., Zwinderman, A.H., van den Born, B.H., & van Montfrans, G.A. (2020). CK (Creatine Kinase) Is Associated With Cardiovascular Hemodynamics. HYPERTENSION. Volume 76, Number 2, 8, pp. 373-380(8)
34 - Cao, F., Maguire, M.L., McAndrew, D.J. et al. (2020). Overexpression of mitochondrial creatine kinase preserves cardiac energetics without ameliorating murine chronic heart failure. Basic Res Cardiol 115, 12
35 - Melnikov, Ivan, Sergey Kozlov, Olga Saburova, Yuliya Avtaeva, Konstantin Guria, and Zufar Gabbasov. 2023. "Monomeric C-Reactive Protein in Atherosclerotic Cardiovascular Disease: Advances and Perspectives" International Journal of Molecular Sciences 24, no. 3: 2079.
36 - Pierpaolo Pellicori, Jufen Zhang, Joe Cuthbert, Alessia Urbinati, Parin Shah, Syed Kazmi, Andrew L Clark, John G F Cleland, 2020. High-sensitivity C-reactive protein in chronic heart failure: patient characteristics, phenotypes, and mode of death, Cardiovascular Research, Volume 116, Issue 1, Pages 91–100
37 - Empana J P I Lerner, M C Perier, P Jabre, M Andrieu, R E Climie, T Van Sloten, B Vedie, D Geromin, E Marijon, N Danchin, F Thomas, P Boutouyrie, X Jouven, Ultra-sensitive troponin-I and incident coronary heart disease, stroke, heart failure, cardiac arrhythmias, arterial aneurysms and venous thromboembolism hospitalizations, European Heart Journal, Volume 43, 544.
38 - Chauin A. 2021.The Main Causes and Mechanisms of Increase in Cardiac Troponin Concentrations Other Than Acute Myocardial Infarction (Part 1): Physical Exertion, Inflammatory Heart Disease, Pulmonary Embolism, Renal Failure, Sepsis. Vasc Health Risk Manag.;17:601-617.
39 - Dogheim, G.M., Amralla, M.T. & Werida, R.H. (2022). Role of neopterin as an inflammatory biomarker in congestive heart failure with insights on effect of drug therapies on its level. Inflammopharmacol 30, 1617–1622
40 - Aasbrenn, M., Farup, P.G. & Videm, V. (2019). Changes in C-reactive protein, neopterin and lactoferrin differ after conservative and surgical weight loss in individuals with morbid obesity. Sci Rep 9, 17695