The association between Oxidized low - density lipoprotein and high sensitivity C reactive protein in acute coronary syndromes patients

Abbas Mosad Ajeed MS.c, Clinical Biochemistry Al- Yarmouk College University/ Parmacy

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الخلاصة

الخلفية: المتلازمات التاجية الحادة للقلب (ACS) تمثل سلسلة خطر مرضي تشخيصي متصل من الذبحة الصدرية غير المستقرة من خلال احتشاء عضلة القلب (MI) مع أو بدون ارتفاعT شريحة-ST. وتشترك تلك الحالات الثلاثة بنفش الصورة المرضية برغم اختلاف العلاج. المؤشرلت الالتهابية المرتفعة ، ولا سيما بروتين سي التفاعلي، ترتبط مع زيادة خطر الحوادث القلبية الوعائية في المستقبل في الاصحاء. زيادة الاكسدة وتوليد الجذور الحرة للأكسجين يمكن أن يؤدي إلى تحول LDL إلى LDL المؤكسد التي يمكن أن تؤدي إلى تصلب الشرايين. زيادة مستويات الدم من البروتين الدهني منخفض الكثافة المؤكسد(oxLDL) يمكن أن تلعب دورا في هذه الظروف

الهدف: الهدف من هذه الدراسة قياس مستويات الدم للبروتينات الدهنية المتاكسدة منخفض الكثافة (LDL-ox) في المرضى الذين يعانون من احتشاء القلب (Q-MI) AMI وبدون(Q-MI)، والذبحة الصدرية غير المستقرة والضوابط، والتحقيق في العلاقة مع hs- CRP في متلازمة الشريان التاجي الحادة.

المرضى والطرق: احبل تسعون (٩٠) من مرضى القلب إلى وحدة العناية التاجية في ابن النفيس ومستشفى الكندي خلال الفترة من يوليو عام ٢٠١٣ ومارس ٢٠١٤ بعد التشخيص السريري لمتلازمة الشريان التاجي الحادة كانت اعمارهم تتراوح بين (٢٥-٨٤) سنة، و ضوابط صحية متطابقين (العمر، الجنس) التحق في هذه الدراسة. جميع مرضى القلب لديهم تخطيط القلب، المؤشرات الحيوية للقياسات القلبية خصوصا Troponine -I ، (CK-MB)، وقياسات مصليةHS-C-RP) و LDL-ox . ١٠مل من الدم تطلب لتقييم القياسات أعلاه.

النتائج: كل من LDL-ox و HS-CRP في المرضى الذين يعانون من متلازمات التاجية الحادة (ACS) تم العثور على ٩٠ ارتفاع كبير في المرضى الذين يعانون من ACS بالمقارنة مع الاشخاص الاصحاء، وكذلك هناك علاقة ايجابية ذات قيمة احصائية لوحظت بين البروتينات الدهنية المتاكسدة منخفض الكثافة (LDL-ox) و البروتين سي التفاعلي عالي الحساسية في مرضى احتشاء القلب (Q- MI) لكن علاقة ايجابية فقط في الذبحة الغير مستقرة و احتشاء القلب بدون Q- Segment

الاستنتاج: مستويات LDL-ox and HS-CRP أعلى في المرضى ACS، ولوحظ وجود علاقة إيجابية ذات دلالة إحصائية بين LDL-ox و HS-CRP في Q-MI، LDL-ox و HS-CRP قد تكون علامات لزعزعة استقرار ACS.

كلمات البحث: متلازمات الشريان التاجي الحادة، المؤكسد-LDL، HS-برو تين سي التفاعلي

Abstract

Background: Acute Coronary Syndromes (ACS) represents a pathological, diagnostic, and risk continuum from unstable angina through myocardial infarction (MI) with or without ST-segment elevation. These three conditions share a very similar pathology, although treatment differs. Elevated markers of inflammation, in particular CRP, are associated with an increased risk of future cardiovascular events in healthy subjects, Increased oxidative stress and the generation of the free oxygen radicals can result in modification of LDL to oxidized LDL that could lead to atherosclerotic. Increased blood levels of oxidized low density lipoprotein (ox-LDL) could play a role in these circumstances.

Objective: The aim of this study was To measure circulating levels of oxidized-low-density lipoproteins (ox-LDL) in patients with AMI (Q-MI &Non-Q-MI), unstable angina and controls, and to investigate their correlation with hs-CRP in acute coronary syndromes.

Patients and Methods: Ninty (90) cardiac patients were admitted to the coronary care unit, Ibn alnafees Hospital and Al kindy Hospital over the period July 2013 and March 2014 with the clinical diagnosis of acute coronary syndrome their ages range was (25-84) years, healthy control (age,sex,matched) were enrolled in this study. All cardiac patients have routine ECG,cardiac biomarkers measurements especially Troponine I, (CK-MB),serelogical markers (hs-C-RP) and Ox-LDL.10 ml of blood needed for assessment of the above makers.

Results: : Ox-LDL and hs-CRP in Patients with Acute Coronary Syndromes (ACS) 90 were found significantly high in patients with ACS as compared to healthy subjects, and also a significant positive correlation was observed between Ox-LDL & hs-CRP in Q-MI but positive related in UA & Non-Q-MI .

Conclusion: OxLDLand hs-CRP levels are higher in ACS patients, and a significant positive correlation was observed between Ox-LDL & hs-CRP in Q-MI,Ox-LDL and hs-CRP might be markers of destabilization of ACS .

Keywords: acute coronary syndromes**,** Oxidized-LDL, hs-C-reactive protein.

1. Introduction

Acute coronary syndromes (ACS) occur because of the destabilization of atherosclerotic plaques, which may undergo rupture or erosion, with subsequent thrombosis and vessel occlusion [1]. Many findings indicate that plaque destabilization correlates with the location and activation of inflammatory cells both within the plaque [2,3] and in the systemic circulation [4]. Although the identification of vulnerable patients remains a challenge [5,6], a correlation between markers of systemic inflammation, such as C-reactive protein and pregnancy-associated plasma protein-A, with the presence of complex, vulnerable coronary plaques has recently been found in patients with stable and unstable angina. [7,8,9]. Among factors involved in the initiation, progression and destabilization of plaques, oxidized-low-density lipoproteins (ox-LDL) are thought to play a key role [10]. Increased circulating levels of ox-LDL have been shown to be associated with angiographically proven coronary artery

disease (CAD) [11, 12], thus suggesting that circulating ox-LDL is a significant marker of CAD. However, studies that measured ox-LDL (or auto-antibodies against ox-LDL) in the plasma of patients with different clinical manifestations of CAD, reported conflicting results [13, 14]. Recently, it has been shown that macrophage-rich, vulnerable, rupture prone carotid plaques have higher content of ox-LDL than macrophage-poor, stable plaques [15] and are associated with elevated levels of ox-LDL in plasma [11]. However, the correlation between circulating ox-LDL levels and presence of vulnerable coronary plaques has never been investigated. We therefore measured the plasma concentration of circulating ox-LDL in patients with ACS to evaluate the correlation between ox-LDL levels and hs-C-RP, Ox-LDL and hs-CRP were also determined in a group of control subjects without CAD.

2. Subjects and Methods

The population consisted of 120 subjects divided into three groups, 30 with UA their age range (25-75), 60 with AMI their age range (37-75). The other 30 subjects age and sex matched healthy subjects were studied as controls (This group includes 39 subjects who had no history or clinical evidence of cardiac diseases or any chronic disease).All patients had been admitted to the Coronary Care Units (CCU) of Ibn alnafees Hospital, between July 2012 and March 2013.The clinical examination and diagnosis were performed by physician specialized in in Ibn Al-Nafees Cardiac Specialty Teaching hospital and AL-KindyTeaching hospital. hs-C-RP was determined in serum using commercially available ELISA and performed as recommended in leaflet with kit. (Wiesbaden.Germany), Plasma circulating ox-LDL, collected from whole blood into Vacutainer tubes containing ethylenediamine tetraacetic acid (1 mg/mL) was measured with the enzyme-linked immunosorbent assay Mercodia Oxidized-lDL ELISA kit (Mercodia AB, Uppsala, Sweden), in which the wells of the microtiter plates are coated with the capture antibody mAb-4E6 [Holvoet et al.,2001]. No antioxidant were added to the plasma samples before blood collection

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3. Result

Serum serological markers(hs-C-RP), levels were estimated in 90 patients with ACS, (60 AMI & 30 UA) compared with 30 healthy control group, age and sex matched. The results reflect that patients with Q-MI have a higher CRP value than Non-Q-MI and unstable angina (see figure 1) but statistically there was no significant differences between the groups($p=0.39074$),but significant differences when compared ACS patients with control groups($p<0.001$).as shown in table(1).

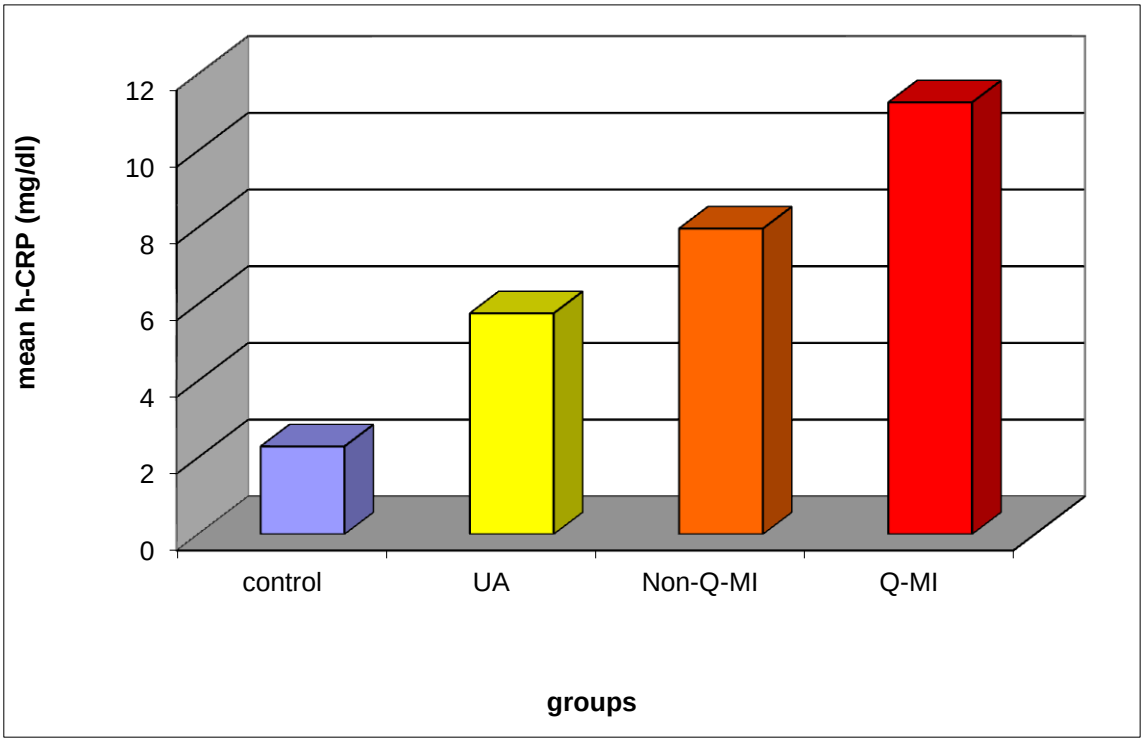

Fig.(1) :average of CRP in ACS patients and healthy control**.**

Table: (1) Serum hs- C-RP in ACS and Control Groups.

C-reactive proteins (CRP)				
Groups	**Control**	**UA**	**Non-Q-MI**	**Q-MI**
N	**30**	**30**	**28**	**32**
Mean	**2.29**	**5.76**	**7.97**	**11.26**
SEM	**0.15**	**0.16**	**0.21**	**0.82**
Range	**0.66-4.71**	**3.73-8.32**	**6.35-12.33**	**9.74-31.73**
Comparison with control		**P<0.001**	**P<0.001**	**P<0.001**
Comparison within the groups	**P=0.39074**			

Oxidized LDL was estimated by ELISA technique in the sera of patients at admission (n=90). The mean of Oxidized LDL of control, UA, non-Q-MI, and Q-MI are shown in table (4-9). There was a highly significant difference between controls and disease groups (P<0.001). Also there was a significant difference in the mean of Oxidized LDL among disease groups (P<0.001), with the highest level seen in the Q-MI group. See figure. (2).

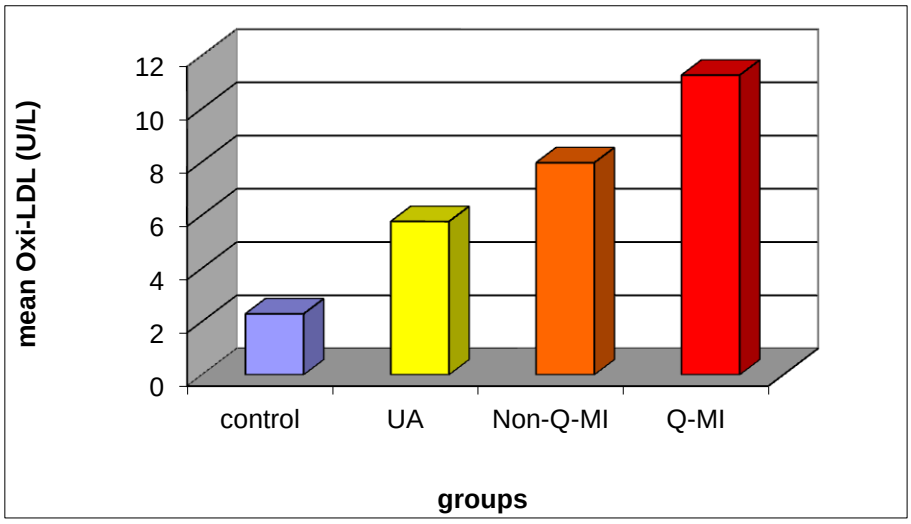

Figure. (2) Average of sera Oxid- LDL levels in patients at admission, and controls group.

Table: (2) Serum Oxid LDL in ACS and Control Groups.

Oxidized LDL(Oxi LDL)				
Groups	**Control**	**UA**	**Non-Q-MI**	**Q-MI**
N	**30**	**30**	**28**	**32**
Mean	**72.20**	**280.67**	**312.77**	**343.20**
SEM	**0.68**	**2.95**	**4.88**	**3.29**
Range	**62.90-78.60**	**257.60-326.40**	**265.00-362.92**	**299.00-380.08**
Comparison with control		**P<0.0001**	**P<0.0001**	**P<0.0001**
Comparison within the groups	**P<0.001**			

Table (2) shows the results of the laboratory tests in the three groups of patients. a significant positive correlation between Ox-LDL and C-RP in (Q-MI).but data showed a positive correlation in Non-Q-MI &UA patients. See.fig(3A),(3B),&(3C).

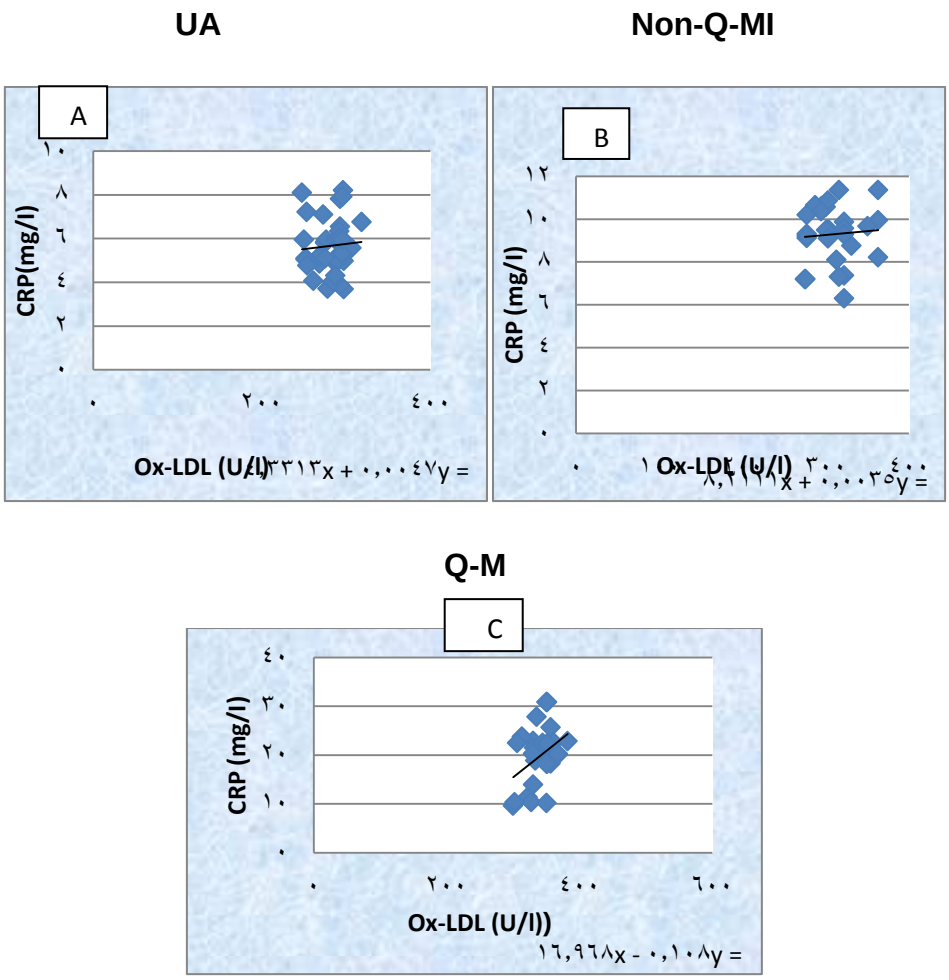

Figure (3): Correlation between Serum Ox-LDL Level & C-RP in UA (A), Ox-LDL Level & C-RP in Non-Q-MI patients (B), and Ox-LDL Level & C-RP in Q-MI patients (C).

Table (3): Correlation Coefficient (r) and significant value (p) of Serum C-RP vs. Ox-LDL in ACS.

Correlation C-RP & Ox-LDL	Type	r	P	Significance
UA	Positive	0.081	0.869	Non significant
Non-Q-MI	Positive	0.277	0.286	Non significant
Q-MI	Positive	0.384	0.021	Significant

Statistical Analysis

All data were expressed as mean ± SEM. The statistical significance was evaluated by Student's t- test using Statistical Package for the Social Sciences (SPSS Cary, NC, USA) version 16.0.

4. Discussion

Many inflammatory markers can be used as predictive markers for the risk of coronary heart diseases like:CRP: Patients with acute coronary syndrome have elevation in CRP in association with their presenting symptoms , in patients with AMI , CRP levels correlated with the presences of plaque rapture and an early study examine CRP in acute coronary syndrome found that CRP identified a subset of patients with severe unstable angina at increased risk for death and MI (16) which also showed that our lived patients have elevated level of CRP in circulation, and in patients with AMI is higher than unstable angina, (23) noted that primary cytokines (eg, IL-1ß or TNF-) stimulate production of "messenger" cytokine, IL-6, which induces expression of hepatic genes encoding acute-phase reactants found in blood, including CRP.our result also agreement with (24) who said that Greater early blood CRP moderately increases long-term risk of recurrent cardiovascular events or death, and may be a valuable prognostic predictor in patients after ACS. Our resuts also agreement with other results done by (31) who showed that C-reactive protein and a family history of myocardial infarction were positively associated with subsequent venous thrombosis.

Our data agreement with results done by (26) who showed that high sensitive C-reactive protein levels were higher in acute coronary syndrome patients compared to control also showed that ACS is associated with greater inflammation in the presence of myocardial necrosis than in cases of angina without necrosis.and agreement with (22) who showed that CRP level were found significantly high in patients with AMI as compared to healthy controls ($p<0.01$). Our findings suggest that early CRP is a valuable predictor in patients with ACS. However,. Moreover, admission electrocardiogram, a widely available measurement, was suggested that many simple clinical measurements were good predictors of prognosis of ACS. But, it was uncertain which clinical measurement was the most valuable or most cost-effective in clinical use since no study had compared their independent prognostic value under the same conditions. Further studies are needed to compare the prognostic values of common clinical markers or joint multiple markers in the same study.

There are several possible explanations for the prognostic value of CRP in patients with

ACS. It has been shown that CRP is related to the dysfunction of endothelial cells and the progression of atherosclerosis. Pasceri et al., 2000 found that CRP induced a significant increment of adhesion molecule expression in human endothelial cells, indicating the direct proinflammatory effect of CRP (29). Second, CRP has a role in the progression of atherosclerosis by decreasing the production of nitric oxide and prostacyclin produced by endothelial cells (34, 35) Moreover, CRP can amplify the immune response through complement activation,(28, 37). Pasceri et al., 2001 which has the effect of expanding the infarct size (29). Barrett et al., 2002 an animal study showed directly harmful effects on ischaemic myocardium (17). A significantly enlarged infarct size was found when human CRP was injected into rats after ligation of the coronary artery (19) In addition, elevated CRP might independently affect the coagulation system and increase mortality (18) When an ACS occurs, serum CRP concentration apparently increases and reaches a peak at 72 h (33, 20) CRP obtained within 72 h from the onset of symptoms is a reflection of the acute response of tissue injury. A higher level of CRP was related to more severe damage caused by the cardiovascular events and further damage caused by CRP itself (32) The early phase of the inflammatory response was also related to the ventricular function and remodeling (27). Ischaemia and reperfusion injury,(17) which can cause long-term. The present study demonstrates that plasma levels of oxidized LDL are significantly elevated in ACS patients compared with control groups, our results are agreement with other results done by (Paul et al.,2008 and Yamagishi et al .,2007) suggesting that their increases are independent of plaque instability (30, 36) also our results agreement with results done by (Maurizio et al.,2006) who said that Ox-LDL levels are higher in unstable patients and correlate with the presence of angiographically documented complex plaques (25). Ox-LDL might be markers of destabilization of CAD..also our data agreement with other results done by(Johannes Hulthe and Björn Fagerberg,2002) who showed Ox-LDL was associated with both subclinical atherosclerosis and inflammatory variables (21). Statistically our data also demonstrate a significant positive correlation between Ox-LDL and C-RP in (Q-MI).but data showed a positive correlation in Non-Q-MI &UA patients, see table (3).

References

1- Falk E, Shah PK, Fuster V. Coronary plaque disruption. Circulation 1995;92:657–71.

2- Moreno PR, Falk E, Palacios IF, et al. Macrophage infiltration in acute coronary syndromes: implications for plaque rupture. Circulation 1994;90:775–8.

3- Kovanen PT, Kaartinen M, Paavonen T. Infiltrates of activated mast cells at the site of coronary atheromatous erosion or rupture in myocardial infarction. Circulation 1995;92:1084–8.

4- Zalai CV, Kolodziejczyk D, Pilarski L, et al. Increased circulating monocyte activation in patients with unstable coronary syndromes. J Am Coll Cardiol 2001;38:1340–7.

5- Pearson TA, Mensah GA, Alexander RW, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation 2003;107:499–511.

6- Idem. From vulnerable plaque to vulnerable patient: a call for new definitions and risk assessment strategies. Part II. Circulation 2003;108:1772–8.

7- Zairis MN, Papadaki OA, Manousakis SJ, et al. C-reactive protein and multiple complex coronary artery plaques in patients with primary unstable angina. Atherosclerosis 2002;164:355–9.

8- Arroyo-Espliguero R, Avanzas P, Cosin-Sales J, et al. C-reactive protein elevation and disease activity in patients with coronary artery disease. Eur Heart J 2004;25:401–8.

9- Cosin-Sales J, Christiansen M, Kaminski P, et al. Pregnancyassociated plasma protein A and its endogenous inhibitor, the proform of eosinophil major basic protein (proMBP), are related to complex stenosis morphology in patients with stable angina pectoris. Circulation 2004;109:1724–8.

10- Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. Circulation 2002;105:1135–43.

11- Weinbrenner T, Cladellas M, Covas MI, et al. The SOLOS Study Investigators. High oxidative stress in patients with stable coronary heart disease. Atherosclerosis 2003;168:99–106.

12- Holvoet P, Mertens A, Verhamme P, et al. Circulating oxidizedlDL is a useful marker for identifying patients with coronary artery disease. Arterioscl Thromb Vas Biol 2001;21:844–8.

13- Ehara S, Ueda M, Naruko T, et al. Elevated levels of low-density lipoprotein show a positive relationship with the severity of acute coronary syndromes. Circulation 2001;103:1955–60.

14- Rossi GP, Cesari M, De Toni R, et al. Antibodies to oxidized-lowdensity lipoproteins and angiographically assessed coronary artery disease in white patients. Circulation 2003;108:2467–72.

15- Nishi K, Itabe H, Uno M, et al. Oxidized-lDL in carotid plaques and plasma associates with plaque destabilization. Arterioscler Thromb Vas Biol 2002;22:1649–54.

16- Armstrong E. J., David A. Morrow and Marc S. Sabatine. (2006): Inflammatory Biomarkers in Acute Coronary SyndromesPart II: Acute-Phase Reactants and Biomarkers of

Endothelial Cell Activation. Circulation 113;152-155.
17- Barrett TD, Hennan JK,Marks RM, et al. (2002):C-reactive-protein-associated increase in myocardial infarct size after ischemia/reperfusion. J Pharmacol Exp Ther;303:1007–13.
18- Di Napoli M, Papa F, Bocola V. (2001): Prognostic influence of increased C reactive protein and fibrinogen levels in ischemic stroke. Stroke;32:133–8.
19- Griselli M, Herbert J, Hutchinson WL, et al. (1999): C-reactive protein and complement are important mediators of tissue damage in acute myocardial infarction. J Exp Med;190:1733–40.
20- Jahn J, Hellmann I, Maass M, et al. (2004): Time-dependent changes of hs-CRP serum concentration in patients with non-ST elevation acute coronary syndrome. Herz;29:795–801.
21- Johannes Hulthe; Björn Fagerberg. (2002): Circulating Oxidized LDL Is Associated With Subclinical Atherosclerosis Development and Inflammatory Cytokines (AIR Study) Arteriosclerosis, Thrombosis, and Vascular Biology. 22:1162.).
22- Kasap,S.,Gonenc,A.,Sener,D.E., et al.(2007).Serum Cardiac Markers in patients with Acute Myocardial infarction:Oxidative Stress,C-Reactive protein and N-Terminal Probrain Natriuretic peptide;J Clin Biochem Nutr.;41(1)50-57.
23- Libby P., Paul M. Ridker (1999): Novel Inflammatory Markers of Coronary Risk. Circulation; 100:1148-1150.
24- Li-ping He1, Xin-yi Tang2, Wen-hua Ling3, Wei-qing Chen1,Yu-ming Chen; (2010): Early C-reactive protein in the prediction of long-term outcomes after acute coronary syndromes: a meta-analysis of longitudinal studies, Heart;96:339-346.
25- Maurizio Anselmi a,∗, Ulisse Garbin b, Pierfrancesco Agostoni a, Massimiliano Fusaro a, Anna Fratta Pasini b, Cristina Nava b,Dritan Keta a, Marco Turri a, Piero Zardini a, Corrado Vassanelli a, Vincenzo Lo Cascio b, Luciano Cominacini b. (2006): Plasma levels of oxidized-low-density lipoproteins are higher in patients with unstable angina and correlated with angiographic coronary complex plaques; Atherosclerosis 185 ;114–120.
26- Mehmet Yunus Emiroglu, MD[1], Özlem Batukan Esen, MD[2], Mustafa Bulut, MD[1], Hekim Karapinar, MD[3], Meier CA, Bobbioni E, Gabay C, Assimacopoulos-Jeannet F, Golay A, Dayer JM(2010):IL-1 receptor antagonist serum levels are increased in human obesity: a possible link to the resistance to leptin? J Clin Endocrinol Metab 87:1184–1188.
27- Nian M, Lee P, Khaper N, Liu P. (2004): Inflammatory cytokines and postmyocardial remodeling. Circ Res; 94: 1543–1553.
28- Nakagomi A, Freedman SB, Geczy CL. (2000): Interferon-gamma and lipopolysaccharide potentiate monocyte tissue factor induction by C-reactive protein: relationship with age, sex, and hormone replacement treatment. Circulation;101:1785–91.
29- Pasceri V, Chang J, Willerson JT, Yeh ET. (2001): Modulation of c-reactive protein-mediated monocyte chemoattractant protein-1 induction in human endothelial cells by anti-atherosclerosis drugs. Circulation; 103: 2531–2534.
30- Paul Holvoet, PhD,† Dieuwke De Keyzer, MECGB, and David R Jacobs, Jr, PhD. (2008): Oxidized LDL and the metabolic syndrome; Lipidol; 3(6): 637–649.
31- Petter Quist-Paulsen, Inger Anne Næss, Suzanne C. Cannegieter, Pål R. Romundstad, Sverre C.

Christiansen, Frits R. Rosendaal, Jens Hammerstrøm' Arterial mcardiovascular risk factors and venous thrombosis: results from a population-based, prospective study (the HUNT 2) Haematologica, Vol 95, Issue 1, 119-125 doi:10.3324/haematol.2009.011866 Copyright © 2010 by Ferrata Storti Foundation.

32- Simes RJ, Marschner IC, Hunt D, et al.(2002): Relationship Between Lipid Levels and Clinical Outcomes in the Long-Term Intervention With Pravastatin in Ischemic Disease (LIPID) Trial. To What Extent Is the Reduction in Coronary Events With Pravastatin Explained by On-Study Lipid Levels? Circulation;105:1162–1169.

33- Soeki T, Tamura Y, Shinohara H, et al. (2000): Serial changes in serum VEGF and HGF in patients with acute myocardial infarction. Cardiology;93:168–74.

34- Verma S, Wang CH, Li SH, et al (2002): A self-fulfilling prophecy: C-reactive protein attenuates nitric oxide production and inhibits angiogenesis. Circulation;106:913–9.

35- Venugopal SK, Devaraj S, Jialal I (2003): C-reactive protein decreases prostacyclin release from human aortic endothelial cells. Circulation;108:1676–8.

36- Yamagishi SI, Matsuoka H, Kitano S, et al. (2007): Elevated circulating oxidized LDL levels in Japanese subjects with the metabolic syndrome. Int J Cardiol. 118(2):270–272.

37- Yeh ET, Anderson HV, Pasceri V, et al. (2003): C-reactive protein: linking inflammation to cardiovascular complications. Circulation;104:974–5.