ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 4, Issue, 3, pp. 450-454, March, 2014

International Journal of
DEVELOPMENT RESEARCH

Full Length Research Article

RISK ASSESSMENT OF INFLAMMATION IN HEALTHY ASYMPTOMATIC FIRST DEGREE RELATIVES OF MYOCARDIAL INFARCTION PATIENTS

Rajesh G. Kumar, Shyamala Nivas, Mrudula K. Spurthi, Kishore G. Kumar, Saraswati, M., Srilatha, G., Chiranjeevi, P., Mohini T. Aiyengar and *Surekha H. Rani

Department of Genetics, Osmania University, Hyderabad, Andhra Pradesh, India-500 007

ARTICLE INFO

Article History:
Received 27th December, 2013
Received in revised form
22nd January, 2014
Accepted 13th February, 2014
Published online 05th March, 2014

Key words:
Inflammation,
Myocardial Infarction (MI),
First Degree Relative (FDR's),
C - reactive protein (CRP),
Tumor Necrosis factor (TNF).

ABSTRACT

Myocardial Infarction (MI) is both polygenic and multifactorial disorder and it is known that immunological processes play key role in initial phases of leukocyte recruitment, to eventual rupture of vulnerable atherosclerotic plaque. It is now universally recognized that inflammation within the lesions contributes importantly to the initiation and progression of atherosclerotic plaque. The aim of our study is to characterize the underlying mechanism in the development of MI by Estimating expression levels of CRP & TNF-alpha in MI patients and to identify the first-degree relatives (FDR's) at risk of the disease in comparison with controls. The results of the present study found significant mean levels of CRP (ng/ml) (4158.82±493.82 in MI patients, 3603.79±154.54 in FDR's and 2626.39±151.94 in controls). The mean ±SD of TNFα levels (pg/ml) in serum was found to be 16.32± 3.82 in MI patients, 13.10±1.5 in FDR's and 8.88± 2.7 of controls. The high levels of CRP and TNF-alpha may acts as potent independent inflammatory predictors of MI in FDR's and the study also shows the disease predisposing factors in first degree relatives. Hence, the study designed to screen FDR's of MI patients to evaluate the risk of future coronary events and to ignite various measures for delaying progression of disease by life style modification or by the drug therapies. Our study may help in new possibilities of anti-inflammatory treatment that might help in prevention of MI.

INTRODUCTION

Myocardial Infarction (MI) is both polygenic and multifactorial disorder and it is known that immunological processes play key role in beginning stages of leukocyte recruitment, to ultimate rupture of liable atherosclerotic plaque. Early on advancement of atherosclerotic lesion requires tethering and attachment of monocytes and followed by movement through the vascular endothelium. Progressive diversification of monocytes to macrophages and successive build-up of lipid results in foam cell production and fatty streak development (Blake & Ridker 2001). Immunological processes are also involved in inducing vulnerable plaque activation and thrombus formation, which in turn promotes acute coronary syndromes, such as myocardial infarction (Ross *et al*., 1999).

***Corresponding author: Surekha H. Rani,** *Department of Genetics, Osmania University, Hyderabad, Andhra Pradesh, India-500 007*

Plaque rupture is the most common type of atherosclerotic plaque complication, accounting for approximately 70% of all acute coronary syndromes (Naghavi *et al*., 2003). Cellular events such as adhesion, migration, and invasion of leukocytes into vascular cells occur concomitantly with the production of proinflammatory cytokines such as interleukin-6 (IL-6) and Tumor necrosis factor –alpha (TNF-α) which are thought to play a major role in the pathogenesis of atherosclerosis. Apart from their importance in vascular disease advancement, these cytokine circulating levels serve also as markers for unfavorable prognosis (Wassmann *et al*., 2004). CRP has been initially thought as a reliable marker of vascular inflammation and stimulate release of inflammatory cytokines as IL-1b, IL-6 and TNF-α by monocytes that may straightaway act as proinflammatory stimulus to phagocytic cells by binding to the FcγRII receptor (Blake & Ridker 2001). The positive family history relative risk of developing MI in a first degree relative (FDR's) is 3.8 to 12.1, with higher risk correlating with earlier

age-of-onset (Shah *et al*., 2009). The aim of our study is to characterize the underlying mechanism in the development of MI by Estimating expression levels of CRP & TNF-alpha in MI patients and to identify the first-degree relatives at risk of the disease in comparison with healthy controls.

MATERIALS AND METHODS

The study includes 200 patients with Myocardial Infarction admitted at the cardiology unit of Durgabai Deshmukh Hospital and Research Center, Hyderabad. 200 asymptomatic first-degree relatives of the patients and 200 healthy individuals with no known history of any disease as controls were included in the study. The study has the approval of the institutional ethical committee, for biomedical research. All the patients were examined clinically and detailed history was recorded with particular reference to the known risk factors for MI, including family history, hypertension, diabetes mellitus, smoking, food habits, life style etc. in special case proforma. Clinical examination was followed by a series of laboratory investigations to carryout biochemical studies.

Estimation of Lipid profiles

Total cholesterol, high density lipoprotein cholesterol (HDL-C) and triglycerides (TG) were estimated using commercially available kits and low density lipoprotein cholesterol (LDL-C) was calculated according to friedewalds's equation.

Estimation of High sensitive C - reactive protein (Hs-CRP) levels

Estimation of CRP levels was carried out using commercially available ELISA (Enzyme Linked Immunosorbent Assay) with a typical two-step capture assay kit from Diagnostic Biochem Canada Inc. A monoclonal antibody specific for CRP is immobilized on to the micro well plate and another monoclonal antibody specific for a different region of CRP is conjugated to Horse Radish Peroxidase (HRP). CRP from the sample and standards were allowed to bind to the plate, washed, and subsequently incubated with the HRP conjugate. After a second washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured on a microtiter plate reader at 520nm. The intensity of the color formed by the enzymatic reaction is directly proportional to the concentration of CRP in the sample.

Estimation of TNF-α level

Estimation of TNF-α was carried out using commercially available OptEIA Human TNF-α ("sandwich" enzyme immuno assay) kit from Becton and Dickinson Company, Singapore. Samples and standards were incubated in micro titer plate wells, coated with the first monoclonal anti-TNF-α antibody and second-anti TNF-α monoclonal antibody linked to alkaline phosphatase. After incubation the

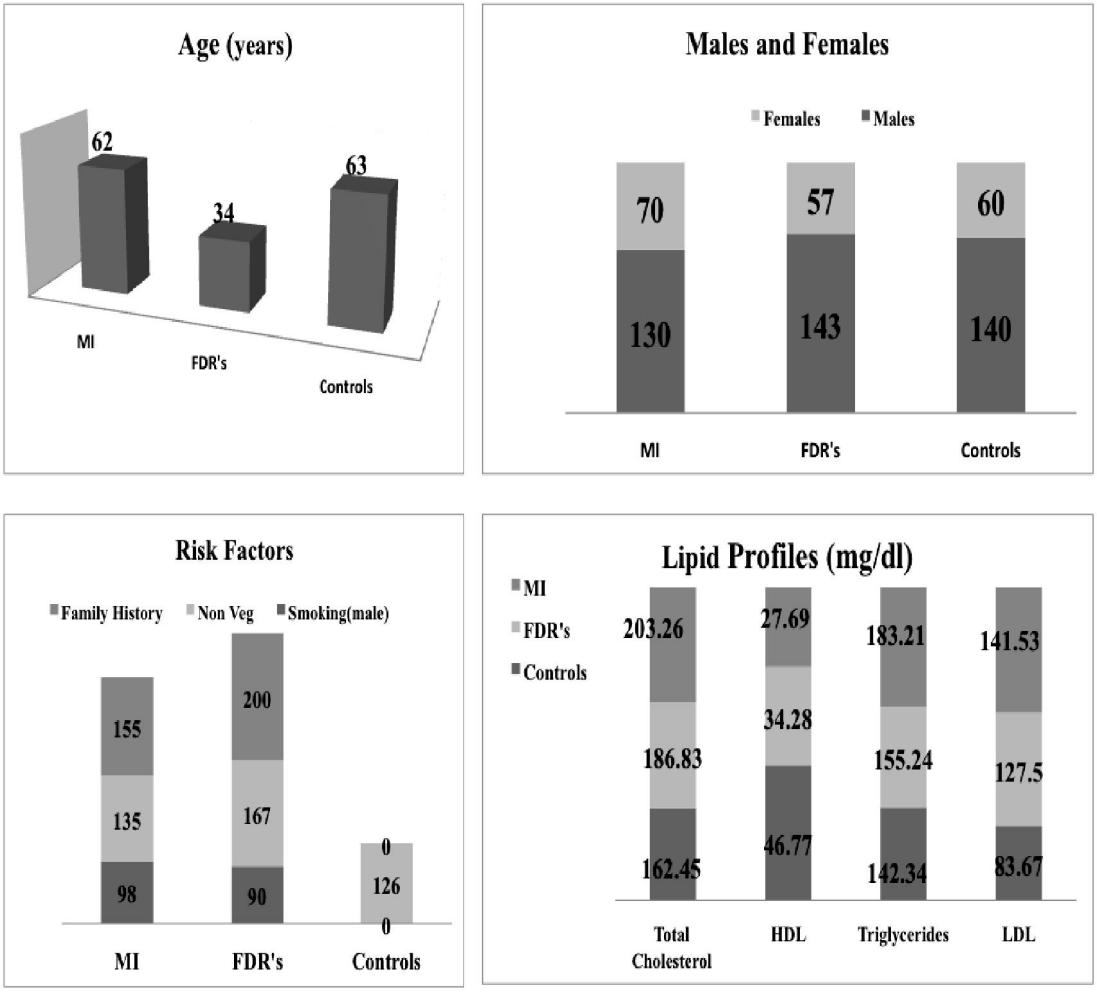


Figure 1. Demographic and Clinical Variables of Study Population

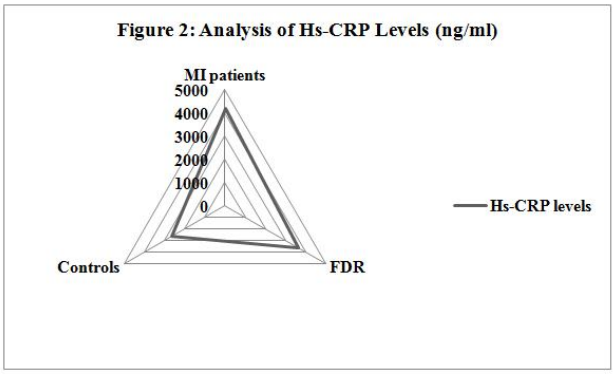
wells were washed and bound enzymatic activity was measured by adding a chromogenic substrate. The absorbance was read at 450nm within 30 minutes of stopping reaction in an ELISA reader. The intensity of the colour was proportional to the concentration of TNF-α in the sample or standard.

Statistical analysis: Statistical analysis was performed using SAS version 9, (SAS Institute Inc, Cary, NC, USA). The data was expressed as mean ± standard deviation.

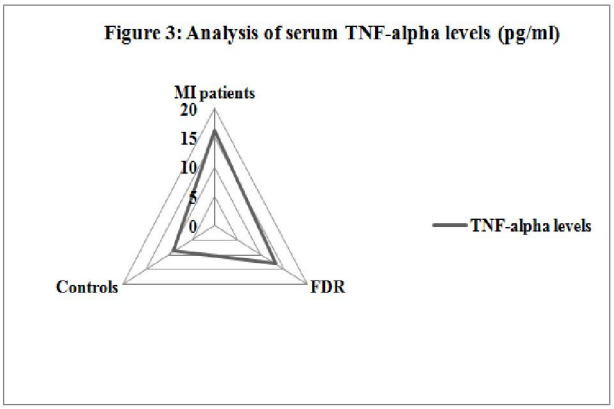
RESULTS

Demographic and Clinical Variables In Study Population: The demographic variables of subjects are presented in Figure 1.

Analysis of Hs-CRP: Analysis of serum Hs-CRP levels estimated in patients with MI, FDR's and controls are presented in Figure 2. The mean ±SD of Hs-CRP levels (ng/ml) was found to be 4158.82±493.82 in MI patients, 3603.79±154.54 in FDR's and the mean ±SD levels of controls was found to be 2626.39±151.94. The hs-CRP levels were found to be statistically significant at $p< 0.05$.



Serum TNF-α levels in MI patients, FDR's and Controls: TNF-α levels estimated in patients with MI, FDR's and controls are presented in Figure 3. The mean ±SD of TNF-α levels (pg/ml) in serum was found to be 16.32± 3.82 in MI patients, 13.10±1.5 in FDR's and 8.88± 2.7 of controls. The TNF-α levels were found to be statistically significant at $p< 0.05$.



DISCUSSION

Coronary Heart Disease (CHD) is also called as Myocardial Infarction (MI) has an important inheritable element and considered as a multigenic complex disease (Allen *et al*., 2001). It is now universally recognized that inflammation within the lesions contributes importantly to the initiation and progression (Ross *et al*., 1993) of atherosclerotic plaque. Circulating factors related to inflammation may be predictors of cardiovascular disease in general populations (Woods *et al*., 2000). The inflammatory response may be promoted at several different sites. Although many markers of inflammation have been acquired from the liver, including CRP, serum amyloid-A and fibrinogen, low levels may also be predicted from other sources including the endothelium itself (Omoigui *et al*., 2007, Kishimoto *et al*., 2006). The fatty streak formed from the initial lesion of atherosclerosis, by the mechanism of inflammation plays a key role in the progression of fatty streak to complex plaque, and outcome of Acute Coronary Syndrome (ACS) (Correale *et al*., 2008).

C-reactive protein is the major human acute phase protein and is a sensitive indicator of inflammation occurring in the body. Its synthesis by the liver is regulated to a large extent by the pro-inflammatory cytokine IL-6 and also by TNF-alpha (Mendall *et al*., 1997). Coronary atherosclerosis has been influenced by various risk factors such as age, gender, family history, non-veg, smoking, alcohol playing a major role in future events of disease. Cardiovascular disease (CVD) family history appears to be a risk factor for successive disease development, and a powerful screening tool to recognize individuals with higher risk who may be candidates for effective prophylaxis (Yanez *et al*., 2009). A quantitative family risk score (≥1.0) is considered to be a strong predictor of disease in middle-aged men and women with positive family history. The relative risk of future MI is increased fivefold and patients with a positive family history will have multiple first- degree relatives who have or will develop clinically significant disease. The aim of our study is to outline the evidence that inflammation is a risk factor for coronary artery disease by focusing on CRP & TNF-alpha in MI patients and to identify the first-degree relatives at risk of the disease in comparison with healthy controls.

Several studies have shown that elevated serum levels of C-reactive protein (CRP), and Interleukin -6 (IL-6), TNF-alpha, interferon gamma, monocyte chemo attractant protein-1, cell adhesion molecules, nuclear factor kappa B, CD 40, lipoprotein associated phospholipase A2, myeloperoxidase, nitrotyrosine, and matrix metalloproteinase-9 are associated with the risk of MI and the severity of atherosclerosis (Anguera *et al*., 2002). It is widely known that CRP is present in plaques but not in the normal vessel wall and this deposition may precede the appearance of monocytes. Recently, it has been found that expression of chemokines in human endothelial cells and adhesion molecules are induced by CRP, it is also evident that CRP acts synergistically with lipopolysaccharide in the activation of endothelial cells (Yeh *et al*., 2001). Elevated levels of CRP might be a response to the inflammation, which could be primarily happening in the vessel wall at the area of the atherosclerotic lesion. Successively, this inflammation could be followed by a multiple fold increase in the traditional risk factors as secondary phenomena, irrespective of their role in disease consequence. The inflammatory mechanisms of which C-reactive protein is an indicator, may be acting at a distance to the blood vessel wall to produce elevations in conventional cardiovascular risk factors or in other inflammatory mediators,

such as TNF-α, which play a role in pathogenesis (Mendall *et al*., 2000). The results of the present study showed highly significant mean levels of CRP in MI patients followed by FDR's when compared to controls. The elevated levels of CRP in patients indicate the involvement of inflammation and its role in the pathogenesis of coronary artery disease (Figure 2). The presence of TNF-α in the majority of atherosclerotic lesions and absence from normal tissues suggests its involvement in atherogenesis (Barath *et al*., 1990, Barath *et al*., 1990). TNF-α may contribute to atherosclerosis by activation of growth factors, cytokines, and chemo attractants and by effecting the synthesis and stimulation of adhesion molecules (Shanley *et al*., 1995, Thurberg *et al*., 1998). TNF-α can trigger the excessive inflammatory response through the "cascade" that can be induced by a key pro-inflammatory cytokines, leading to inflammation development, and promoting plaque instability and thrombosis tendency.

Vascular endothelial is one of the important targets for TNF-α and endothelial injury induced by TNF-α has important significance in many cardiovascular diseases (Junping *et al.,* 2004). In the present study we have observed highly significant levels of TNF-α in MI patients followed by FDR's when compared to controls (Figure 3). It has been well derived that positive family history considered as a major risk factor for disease, and has integrated reflection of various environmental and genetic factors shared by members in a family. The risk of MI that arises from genetic cause should result on an average from the same risk factor within a single family (Williams *et al*., 2001). In the elderly, parental medical history may be difficult to obtain or is often inaccurate, and a positive sibling history of CVD is a stronger independent predictor of incident cardiovascular events than parental history. Sibling's health history has been proposed as a marker to stratify populations for genetic research (Yanez *et al*., 2009). Familial aggregation of CHD is thought to account for 50% to 60% of total documented CHD before the age of 60 years. First-degree relatives of people with premature CHD (proband) exhibit a risk that is 2 to 12 times greater than that of the general population (Ton *et al*., 2011).

It is the first kind of study to investigate the risk prediction of MI in FDR's by evaluating the levels of CRP and TNF-alpha on a large sample (600) size. The high levels of CRP and TNF-alpha may acts as potent independent inflammatory predictors of MI in FDR's and the study also shows the disease predisposing factors in first degree relatives. The study briefs the role of inflammation in atherosclerosis as well as in the precipitation of an acute events and to our knowledge, ours is the first kind of study to investigate the role of circulating levels of TNF-alpha and CRP in asymptomatic healthy FDR's of MI patients to identify individuals at risk of the disease. Our study supports that positive family history of CHD is associated with increased risk of heart disease as various parameters suggestive for higher future risk of developing coronary artery disease in FDR's and our study may help in to slow the momentum of MI in developing countries like India, particularly among the working-age population, major initiatives are needed to combat MI, by promotion of healthy diet and physical activity, generation of awareness or development of guidelines for risk factors, therapeutic and surgical strategies.

Conclusion

MI is a multifactorial disease associated with various environmental and genetic factors. Hence the study designed to screen FDR's of MI patients to evaluate the risk of future coronary events and to ignite various measures for delaying progression of disease by life style modification or by the drug therapies. This study also supports the new possibilities of anti-inflammatory treatment that might help in prevention of cardiovascular disease.

Abbreviations

Myocardial Infarction (MI)
First degree relative (FDR's)
Interleukin -6 (IL-6)
High sensitive C - reactive protein (Hs-CRP)
High density lipoprotein cholesterol (HDL-C)
Triglycerides (TG)
Low density lipoprotein cholesterol (LDL-C)
ELISA ("Enzyme Linked Immunosorbent Assay)
Horse Radish Peroxidase (HRP)
Coronary heart disease (CHD)
Acute Coronary Syndrome (ACS)

Competing Interest

There is no Competing Interest

Acknowledgements

Author acknowledges the ICMR-New Delhi for granting SRF, OU-DBT –ISLARE-Hyderabad, India, OU-DST-PURSE, Hyderabad, India, and CAS-Phase II.

REFERENCES

Allen, R.A., Lee, E.M., Roberts, D.H., Park, B.K., Pirmohamed, M. Polymorphisms in the TNF-αand TNF-receptor genes in patients with coronary artery disease. 2001. *European Journal of Clinical Investigation*., 31:843-51.

Anguera, I., Miranda-Guardiola, F., Bosch, X, et al. Elevation of serum levels of the anti inflammatory cytokine interleukin-10 and decreased risk of coronary events in patients with unstable angina. 2002. *Am Heart J*., 144,:811–17.

Barath, P., Fishbein, M.C., Cao, J., Berenson, J., Helfant, R.H., Forrester, J.S. Detection and localization of tumor necrosis factor inhuman atheroma. 1990. *Am J Cardiol*., 65:297–302.

Barath, P., Fishbein, M.C., Cao, J., Berenson, J., Helfant, R.H., Forrester, J.S. Tumor necrosis factor gene expression in human vascular intimal smooth muscle cells detected by in situ hyridization. 1990. *Am J Pathol*., 137:503–09.

Blake, G.J., Ridker, P.M. Novel Clinical Markers of Vascular Wall Inflammation. 2001. *Circ Res*., 89:763-71.

Correale, M., Brunetti, N.D., Gennaro, L.D., Biase M.D. Acute Phase Proteins In Atherosclerosis (Acute Coronary Syndrome). 2008. *Cardiovascular & Hematological Agents in Medicinal Chemistry*., 6:272-77.

Junping, L., Shuoren, W., Zeng-Chun, M.A., et al. Proteomic analysis of the effects of tumor necrosis factor-α on endothelial cells. 2004. *Chinese Journal of Pathophysiology*., 20: l121-l12.

Kishimoto, T. Interleukin-6: discovery of a pleiotropic cytokine. 2006. *Arthritis Research & Therapy*., 8 (Suppl 2):S2.

Mendall, M., Patel, P., Asante, M., et al. Relation of serum levels of cytokines to cardiovascular risk factors and coronary heart disease. 1997. *Heart*., 78: 273–77.

Mendall, M.A., Strachan, D.P., Butland, B.K., Ballam, L., Morris, J., Sweetnam, P.M., Elwood P.C. C-reactive protein: relation to total mortality, cardiovascular mortality and cardiovascular risk factors in men. 2000. *European Heart Journal*., 21: 1584–590.

Naghavi, M., Libby, P., Falk, E., Casscells, S.W., Litovsky, S., Rumberger, J. From vulnerable plaque to vulnerable patient: a call for new definitions and risk assessment strategies: part I. 2003. *Circulation*., 108:1664 –672.

Omoigui S. The Interleukin-6 inflammation pathway from cholesterol to aging Role of statins, bisphosphonates and plant polyphenols in aging and age-related diseases. 2007. *Immunity & Ageing*., 4:1.

Ross, R. The pathogenesis of atherosclerosis: a perspective for the 1990s. 1993. *Nature*., 362:802– 09.

Ross, R. Atherosclerosis: an inflammatory disease. 1999. *N Engl J Med*., 340:115–26.

Shah,S.H., Freedman, N.J., Zhang, L., Crosslin, D.R., Stone, D.H. Neuropeptide Y Gene Polymorphisms Confer Risk of Early-Onset Atherosclerosis. 2009. *PLoS Genet*., 5: e1000318.

Shanley, T.P., Warner, R.L., Ward, P.A. The role of cytokines and adhesion molecules in the development of inflammatory injury. 1995. *Mol Med Today*., 1:40–45.

Thurberg, B.L., Collins, T. The nuclear factor-B/inhibitor of B autoregulatory system and atherosclerosis. 1998. *CurrOpinLipidol*., 9:387–96.

Ton, T.G. N., Fogg, T.T., Fong, C., John, C., Shirley, X.L., Marshall, J.A., Peters, K., Neal, W., Pearson, T.A. Knowledge, Perception, and Behaviors of Relatives of People with Premature Heart Disease; A Systematic Literature Review. 2011. *Circulation*., 124:958-64.

Wassmann, S., Stumpf, M., Strehlow, K., Schmid, A., Schieffer B., Bohm, M. Nickenig, G., Interleukin-6 Induces Oxidative Stress and Endothelial Dysfunction by Overexpression of the Angiotensin II Type 1 Receptor. 2004. *Circ Res*., 94: 534-41.

Williams, R.R., Hunt, S.C., Heiss, G., Province, M.A., Bensen, J.T., Higgins, M., et al., Usefulness of Cardiovascular Family History Data for Population-Based Preventive Medicine and Medical Research. 2001. *Am J Cardiol*., 87:129–35.

Woods, A., Brull, D.J., Humphries, S.E., Montgomery, H.E. Genetics of inflammation and risk of coronary artery disease: the central role of interleukin-6. 2000. *European Heart Journal*., 21:1574–583.

Yanez, N. D., Burke, G.L., Manoio, T., Gardin J.M., Polak, J. Sibling History of Myocardial Infarction or Stroke and Risk of Cardiovascular Disease in the Elderly: The Cardiovascular Health Study. 2009. *Ann Epidemiol*. 19:858–66.

Yeh, E.T.H., Anderson, H.V., Pasceri, V., Willerson J.T. C-Reactive Protein; Linking Inflammation to Cardiovascular Complications. 2001. *Circulation*. 9: 974-75.

****