Correlation Between Vascular Endothelial Growth Factor Level and The Severity of The Acute Thrombotic Events

Thesis Submitted by

Mohamed Hamdy Mohamed, MSc.

In Partial Fulfillment of
MD in Critical Care Medicine

Supervisors

Prof. Dr. Hossam Ahmad Mowafi, MD
Professor of Critical Care Medicine
Critical Care Medicine Department
Cairo University

Prof. Dr. Tarek El Gohary
Asst. Prof. of Critical Care Medicine
Critical Care Department
Cairo University

Dr. Emad Omar
Lecturer of Critical Care Medicine
Critical Care Department
Cairo University

Dr. Ahmed Battah, MD
Lecturer of Critical Care Medicine
Critical Care Medicine Department
Cairo University

Cairo University

2009

ACKNOWLEDGMENT

First of all I would like to thank god for his mercy and gifts, and for helping me to achieve this study.

I had the honour to achieve this study under supervision of ***Professor Dr. Hossam Ahmad Mowafi,*** Professor of critical care medicine, faculty of medicine, Cairo university. He offered me the idea and facilities to complete this work as well as the spirit of being eager to gain more experience and skills. No words can express my deepest gratitude and greatest appreciation for his continuous encouragement and guidance throughout the accomplishment of this study.

Special thanks to ***Professor Dr. Sanaa Abdel Shafi***, Professor of clinical pathology, faculty of medicine, Beni Sweif university. She exerted a big effort in actively participating in the laboratory part of this work. Also I would like to thank the whole molecular lab. team for their great help. Their kind support, effort and help made this work possible.

Many thanks to ***Professor Dr. Tarek Samir Elgohary***, Assistant professor of critical care medicine, faculty of medicine, Cairo university, for his great encouragement, valuable directions and outstanding help and for his sincere supervision throughout the work of this thesis.

Thanks to ***Dr Emad Omar Abdel Aziz***, lecturer of critical care medicine, faculty of medicine, Cairo university, who helped me achieving progress in this thesis through clarifying all details and supplying me with all the updates and meticulous review of all study details.

Also I would like to express my thanks and appreciation to ***Dr Ahmad Abdel Rahman Battah,*** lecturer of critical care medicine, faculty of medicine, Cairo university, for his meticulous revision of every part this thesis, his guidance and support.

Also, I would like to thank ***Mrs. Manal Youssef***, for her great efforts in performing the computer work of this thesis.

Mohamed Hamdy

To
My Parents

Contents

List of Abbreviation

2D: 2 dimensions

ACC: American college of cardiology

ACS: Acute coronary syndrome

AHA: American heart association

ALT: Alanine transaminase

Ang.: Angiopoietin

APO B100: Apolipoprotein B100

APO E: Apolipoprotein E

AST: Aspartate transaminase

BBB: Bundle branch block

CABG: Coronary artery bypass graft

CAD: Coronary artery disease

CD: Clusters of differentiation

CD40L: Clusters of differentiation 40 ligand

Ck-MB: Creatine kinase MB isoform

CRUSADE: Can Rapid risk stratification of Unstable angina patients Suppress ADverse outcomes with Early implementation of the ACC/AHA Guidelines

CT: Computed tomography

CVD: Cardiovascular disease

DM: Diabetes mellitus

DNA: Deoxyribonucliec acid

ECG: Electrocardiogram

EF: Ejection fraction

ELISA: Enzyme linked immunosorbant assay

EPCs: Endothelial progenitor cells

FGF: Fibroblast growth factor

Flt. 1: fms-like tyrosine kinase

FS: Fractional shortening

G-CSF: Granulocyte colony stimulating factor

GP IIb/IIIa: Glycoprotein IIb/IIIa

Hb: Haemoglobin

HDL: High density lipoprotein

HIP: Hypoxia inducible protein complex

HIF: Hypoxia inducible factor

HS: Heparan sulphate

IHD: Ischemic hear disease

IL: Interleukin

iNOS: Inducible nitric oxide synthase

KDR: Kinase damain receptor

LDL: Low density lipoprotein

LV: Left ventricle

MI: Myocardial infarction.

MMPs: Matrix metalloporteinases

MRI: Magnetic resonant image

mRNA: Messenger ribonucliec acid

NAD(P)H: Nicotinamide adenine dinucleotide phosphate

Neu: Neuropilin

NFkB: Nuclear factor kappa B

NO: Nitric oxide

NOS: Nitric oxide synthase

NSTEMI: Non ST elevation myocardial infarction

PC: Prothrombin concentration

PCI: Percutaneous coronary intervention

PDGF: Platelet derived growth factor

PG: Proteoglycan

PIT: Pathologic intimal thickening

PLGF: Placental growth factor

Plt: Platelet

PURSUIT: Platelet glycoprotein IIb/IIIa in Unstable angina: Receptor Suppression Using Integrilin Therapy

RBCs: Red blood cells

RTK: Receptor tyrosine kinase

SMC: Smooth muscle cell

STARS: STent Anti thrombotic Regimen Study

STEMI: ST elevation myocardial infarction

TGFβ: Transforming growth factor β

TGs: Triglyerides

TIMI: Thrombolysis in myocardial infarction

TLC: Total leucocytic count

TLRs: Toll like receptors

TNFα: Tumor necrosis factor-α

Tn: Troponin

UA: Unstable angina

VEGF: Vascular endothelial growth factor

VEGFR: Vascular endothelial growth factor receptor

VPF: Vascular permeability factor

VSMCs: Vascular smooth muscle cells

WHO: World health organization

WMSI: Wall motion score index

List of Tables

List of Figures

Introduction

In coronary atherosclerosis, angiogenesis within the adventitia of arterial walls is seen in the development of plaques, and extends into the media and intima as the lesions progress. Furthermore, the expression of vascular endothelial growth factor (VEGF), an essential component in angiogenesis, has been positively correlated with the number of intimal blood vessels found within coronary atherosclerotic plaques. ***[Moulton KS 2001]***

Myocardial necrosis or ischemia can trigger a response to improve myocardial perfusion by the formation of new capillaries (angiogenesis) and by the enlargement of preexisting collateral vessels (arteriogenesis). Both angiogenesis and arteriogenesis are highly regulated processes that require the orchestrated interaction of endothelial cells, extracellular matrix, and surrounding cells mediated by a cascade of growth factors, their receptors, and intracellular signals. ***[Helisch A, et al, 2003 & Yancopoulos GD, et al, 2000]***

Vascular endothelial growth factor (VEGF), a highly specific mitogen for endothelial cells, is a key regulator of angiogenesis. An ever growing interest has been focused to this growth factor because it is thought to be implicated in the pathogenesis of atherosclerotic plaque progression as it was suggested that VEGF mediates atherosclerotic plaque neovascularisation and thus promotes its infilteration by inflammatory cells. These events, through a complex mechanism trigger plaque destabilization. ***[Mofidi R, et al, 2001]***

VEGF acts via two tyrosine kinase receptors, VEGF receptor -1 and VEGF receptor -2, Biological response mediated by the activation of these two receptors are somewhat different, the activation of VEGFR-2 induce cell proliferation, while activation of VEGFR-1 does not. ***[Neufeld G, et al, 1999]***

Thus VEGF plays a key role in the cascade of angiogenesis, which is considered to promote plaque progression and destabilization. ***[Gille H, et al, 2001 & Carmeliet P, et al, 2000]*** Nonetheless VEGF mRNA, protein, and its receptors' expression can be rapidly upregulated in the myocardium within minutes of ischemia (or hypoxia) ***[Hashimoto E, et al, 1994]***

Plasma concentrations of VEGF and a soluble form of its receptor are quantifiable by an enzyme linked immunosorbent assay (ELISA). Plasma concentrations of both VEGF and a soluble form of its receptor may be abnormal in patients with coronary artery disease and peripheral vascular disease. Raised concentrations of VEGF have also been found in patients with risk factors for coronary artery disease, such as hypertension and hyperlipidaemia, but with no clinically overt disease. ***[Chung NA, et al, 2003, Belgore FM, et al, 2001, Roller RE, et al, 2001 & Ogawa H, et al, 2000]***

On the basis of the reported data it may be expected that it maybe positive correlation between the level of VEGF level and manifestations of complicated destabilized coronary atherosclerotic plaque.

Aim of work

- To investigate the level of the vascular endothelial growth factor in patients presented with non ST elevation acute coronary syndrome and to determine whether this level is higher than in the control group.

- To determine whether this level is positively correlated to the severity of the disease and the short in-hospital prognosis or not.

Chapter I

Acute Coronary Thrombotic Events

Introduction:

Heart disease is the major cause of death in the United States and many of other countries. Many patients with heart disease present at the hospital with an acute coronary syndrome (ACS) and many of them face a significant risk of morbidity and death. Although timely and appropriate treatment reduces the risk of an immediate or subsequent poor outcome, the high prevalence of risk factors for coronary artery disease (CAD) ensures that the prevalence of future ACS will also remain high. ***[Rogers WJ, et al, 2000]***

Theories of atherosclerotic plaque formation have changed, such that cholesterol is no longer the lone culprit in CAD. Cellular signaling associated with inflammation is increasingly implicated in the initiation and progression of atherosclerotic plaques. Atherosclerotic plaques have been shown to develop early in life and remain subclinical for years or decades, depending on the accelerating effects that risk factors and genetic predisposition to CAD will have on disease progression. ***[Rogers WJ, et al, 2000]***

This chapter reviews the epidemiology, pathophysiology, and different clinical presentation and classification of ACS and identifies areas of need that, if addressed, may help to reduce the high rate of morbidity and mortality in patients presenting acute coronary syndrome.

Epidemiology

According to the American Heart Association (AHA), 71.3 million Americans had some form of cardiovascular disease (CVD) in 2003. CVD was responsible for nearly one million deaths in 2003. Among Americans with CVD, 13.2 million are estimated to have CAD, which is responsible for the majority of deaths attributed to CVD. ***[Thom T, et al, 2006]***

ACS is a manifestation of CAD that encompasses acute myocardial infarction (AMI) whether ST elevation myocardial infarction (STEMI) or non ST elevation myocardial infarction (NSTEMI) and unstable angina (UA). The AHA estimates that 700 000 Americans had their first coronary event in 2006, and 500 000 had a recurrent event. ***[Thom T, et al, 2006]***

For those who experience a recurrent coronary event, the risk of death is 4 to 6 times that of the general population. Mortality in patients with AMI has been observed to increase for each 30 minutes that passes before appropriate intervention. Timely treatment upon presentation contributed to the reduced in-hospital mortality (11.2% to 9.4%) observed in the 1990s as "median door-to-drug time" was reduced by nearly 50% for patients requiring thrombolytic therapy or primary intervention. ***[Rogers WJ, et al, 2000]***

Despite efforts to improve patient management, there is no evidence suggesting that the risk of developing CAD is declining. The large study, for example, found that 9 modifiable risk factors accounted for 90% of first AMIs. ***[Yusuf S, et al, 2004]***

Winkleby et al reported health profiles among young adults that were particularly alarming. Between 1990 and 2000, this group showed large increases in the prevalence of smoking and obesity with corresponding low rates of physical activity. The high prevalence of major modifiable CVD risk factors among young adults is expected to exacerbate the health care crisis. ***[Winkleby MA, et al, 2004]***

On the other hand it is estimated that 40 million Americans will be age 65 or older by 2010. Coupled with the increased prevalence of obesity and diabetes across different age groups, the aging population and poor health profiles among young adults will lead to an increase in CVD and the most potent risk factors for poor cardiovascular outcomes, such as hypertension and dyslipidemia. ***[Thom T, et al, 2006]***

Overview of the Pathogenesis of acute coronary thrombotic events: Development of atherosclerotic plaque

As it was mentioned before the accumulation of atherosclerotic plaques is no longer considered to be the simple result of cholesterol storage. As inflammation is increasingly implicated in plaque formation. At the cellular level, plaque accumulates in response to many signals that cause blood cells, such as monocytes, to adhere to the endothelium of the arterial lumen. Inflammatory responses to insults such as bacterial toxins, in addition to traditional risk factors, such as dyslipidemia, hypertension, hyperglycemia, and obesity, can initiate monocyte adherence. ***[Libby P, et al, 2005]***

Once adhered to the endothelium, monocytes migrate into the vascular wall to the arterial intima, the muscular layer closest to the vessel lumen. At this point, they transform into macrophages and begin to ingest the modified lipoprotein particles, which accumulate in the intima naturally and at an accelerated rate in people with hyperlipidemia. These lipid filled macrophages are also known as foam cells, which are the hallmarks of atherosclerotic plaques. ***[Libby P, 2002]***

Foam cells typically come together to form a plaque within the intima. Many foam cells die by apoptosis, disintegrate with debris becoming membrane-bound, and then are eliminated by phagocytosis or by shedding. The original modified lipoproteins, macrophages, foam cells, and apoptotic debris, in addition to other important factors, such as collagen and von Willebrand factor, form the core of the plaque. ***[Libby P, 2002]***

Plaque Rupture as a dominant cause of acute coronary thrombosis

Plaque rupture is the principal cause of luminal thrombosis in acute coronary syndromes occurring in 75% of patients dying from an acute myocardial infarction. The plaques that are vulnerable to rupture are characterized by the same histopathologic signatures, except that they still have an intact but thin fibrous cap. ***[Kolodgie FD, et al, 2004 & Kolodgie FD, et al, 2001]***

In ruptured plaques, the fibrous cap is focally interrupted, allowing circulating blood to come in direct contact with the thrombogenic contents of the lipid-rich core, leading to thrombosis and subsequent

acute coronary syndromes. Certain populations such as diabetic individuals appear more susceptible to superficial erosion as a mechanism of plaque disruption and thrombosis. In spite of the presence of other mechanisms that account for a minority of fatal coronary thromboses such as superficial erosion, intraplaque hemorrhage, and the erosion of a calcified nodule. The physical disruption of the atherosclerotic plaque still accounts the main cause of all acute coronary thromboses. Figure (1) ***[Lippy P, et al, 2005 & Burke AP, et al, 2003]***

Ruptured plaques possess a large necrotic core with an overlying thin-ruptured fibrous cap heavily infiltrated by foamy macrophages. In ruptured lesions, the necrotic core occupies approximately one third to one half of the total plaque area, whereas in the majority of unruptured vulnerable plaques, it occupies less than one fourth of the lesion. This observation suggests that the progressive necrotic core expansion precedes plaque rupture. ***[Burke AP, et al, 2003]***

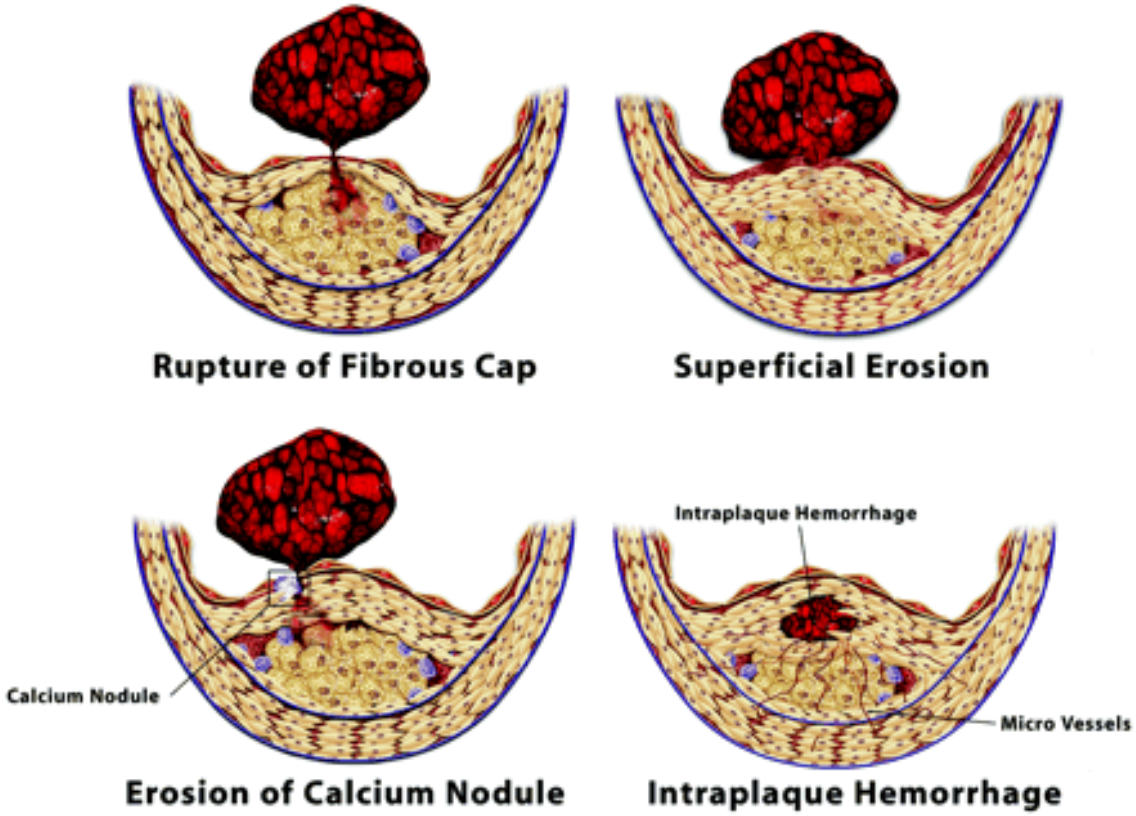

Figure (1): Illustrates the microanatomy of coronary arterial thrombosis and acute occlusion. Rupture of fibrous cap (upper left) causes some two thirds to three quarters of fatal coronary thromboses. Superficial erosion (upper right) occurs in one fifth to one quarter of all cases of fatal coronary thromboses. Erosion of a calcium nodule may also cause plaque disruption and thrombosis (lower left). In addition, friable microvessels in base of atherosclerotic plaque may rupture and cause intraplaque hemorrhage. Severe intraplaque hemorrhage can cause sudden lesion expansion by mass effect acutely as well.

Atherosclerotic plaque progression:

Pathologic intimal thickening (PIT) constitutes the earliest atherosclerotic change and is characterized by surface smooth muscle cells (SMCs) overlying relatively acellular lipid-rich pools. These pools of accumulated lipid likely occur from the loss of SMCs. ***[Kockx MM, et al, 1998]***

The proteoglycan matrix within the lipid pool remains intact with an excess of free cholesterol and the beginnings of focal calcification. When macrophages infiltrate the lipid pool, the entrapment and death of these cells is thought to be responsible for the conversion of PIT into early fibroatheroma. ***[Kockx MM, et al, 1998]***

The combination of greater macrophage infiltration and apoptotic death together with hypoxia-induced necrosis promotes development into the late fibroatheromatous lesion. As the plaque enlarges, the ensuing hypoxia or inflammatory cell infiltration is thought to promote neovascularization. These nascent immature blood vessels are inherently leaky and permit extravasation of erythrocytes into the plaque, further contributing to necrotic core enlargement. ***[Stary HC, et al, 1994]***

Necrotic core enlargement as a critical step for plaque rupture

It has been shown that macrophage infiltration is the first step toward the eventual formation of an atherosclerotic plaque. In vitro studies have shown that low-density lipoprotein (LDL) uptake by macrophages is facilitated by an oxidation process. ***[Rydberg EK, et al, 2004 & Steinberg D, 2002]***

Macrophages exposed to oxidized LDL are richer in free cholesterol than cholesterol esters. The threshold level of free cholesterol in macrophages is in part regulated by a re-esterification process involving acyl coenzyme A. Manipulating the activity or expression of Acyl coenzyme A in culture or animal models favors the accumulation of free cholesterol. The formation of necrotic core is attributed mostly to the

death of macrophages. As plaques progress from fatty streaks to those with necrotic cores, the free cholesterol content of the lesion increases, whereas cholesterol esters decrease. ***[Dove DE, et al, 2005]***

The increase in free cholesterol may be closely associated with lesion instability. In a study by Felton et al of human aortic atherosclerotic plaques, the progression from non disrupted to disrupted lesions is accompanied by increased free cholesterol, cholesterol esters, and free-to-esterified cholesterol ratio in the necrotic core while the triglyceride content is unchanged. ***[Felton CV, et al, 1997]***

It is generally accepted that apoptotic macrophages are a likely source of free cholesterol in plaques; however, it is entirely feasible that free cholesterol within the necrotic core could be derived from other sources, including erythrocyte membranes. ***[Tabas I, 2000]***

In one investigation of thromboembolic pulmonary hypertension, it was showed that necrotic cores in intimal plaques in large pulmonary arteries contain RBCs (as demonstrated by anti-glycophorin A staining) and macrophages. In addition, others have observed extravasated erythrocytes in disease processes are accompanied by deposits of free cholesterol and foamy macrophages. ***[Leon ME, et al, 2002]***

It is well appreciated that the cholesterol content of erythrocyte membranes exceeds that of all other cells in the body, with lipid constituting 40% of the weight. Moreover, erythrocyte membrane-derived cholesterol is elevated in patients with hypercholesterolemia and

is sensitive to short-term statin therapy. ***[Koter M, et al, 2002 & Fukumoto Y, et al, 2001]***

In the early to mid-20th century, several leading pathologists forwarded the hypothesis that intraplaque hemorrhage is a major contributor to the progression of coronary atherosclerosis; however, the precise nature of this relationship was not well understood. Later studies suggest that plaque hemorrhages are more frequent in the coronary vasculature in patients dying from ruptured compared with stable lesions with a >75% cross-section area of luminal narrowing. ***[Burke AP, et al, 2003]***

In an effort for understanding the influence of intraplaque hemorrhage on lesion progression, various types of human coronary plaques were examined for hemorrhagic events. In a relatively large series of human coronary plaques from sudden coronary death victim, there was a greater frequency of previous hemorrhages in coronary atherosclerotic lesions prone to rupture (as detected by glycophorin A) relative to lesions with early necrotic cores or plaques with PIT. ***[Kolodgie FD, et al, 2003]***

Importantly, the degree of reactive glycophorin A staining and the level of iron deposits in the plaque corresponded to the size of the necrotic core, and changes in these variables paralleled an increase in macrophage density, suggesting that hemorrhage itself serves as an inflammatory stimulus. ***[Kolodgie FD, et al, 2003]*** By contributing to the deposition of free cholesterol, macrophage infiltration, and enlargement of the necrotic core, the accumulation of erythrocyte membranes within

an atherosclerotic plaque may represent a potent atherogenic stimulus. And these factors together may increase the risk of plaque destabilization. ***[Burke AP, et al, 2003]***

Erythrocytes membrane derived free cholesterol and plaque progression

As proof of concept, an animal model of simulated intraplaque hemorrhage was used to assess the role of erythrocytes in lesion progression. The direct injection of packed erythrocytes (25 to 50 μL) into quiescent aortic atherosclerotic plaques produced excessive macrophage infiltration along with free cholesterol crystals. In contrast, control (non injected lesions) showed the characteristics of a regressed lesion with far fewer lesional macrophages and free cholesterol. Lipids identified by oil red O were also significantly greater in plaques with injected erythrocytes when compared with controls. Thus, the animal studies offer further evidence that episodic hemorrhages in plaques contribute to accumulated free cholesterol and macrophage infiltration. ***[Kolodgie FD, et al, 2003]***

The contribution of erythrocyte membrane cholesterol to necrotic core volume is predicted to be substantial because intraplaque hemorrhage is thought to occur repeatedly over years. Like internal bleeds, the bulk of the erythrocyte would be degraded over days, and because membrane cholesterol fraction cannot be metabolized internally, it would be available for absorption into the necrotic core. Moreover, the uptake of erythrocyte-derived cholesterol by macrophages, in turn, would

inevitably give up cholesterol to the core by apoptotic cell death. ***[Takaya N, et al, 2005]***

Consistent with this notion, recent MRI data of carotid plaques over an 18-month period showed evidence of intraplaque hemorrhage as contributing factor to necrotic core volume and lesion bulk. Further, patients with intraplaque hemorrhage at baseline showed a far greater susceptibility to repeat plaque hemorrhages. Therefore, accumulated RBC-derived cholesterol may represent a critical transition promoting the conversion of a stable plaque to an unstable phenotype. ***[Takaya N, et al, 2005]***

Association of plaque hemorrhage and vasa vasorum

Pathologic examination of unstable lesions has demonstrated that intraplaque hemorrhage and plaque rupture are associated with an increased density of microvessels. The concept of how RBCs precisely leak into the necrotic core is poorly understood. ***[Fleiner M, et al, 2004, Kockx MM, et al, 2003 & Kolodgie FD, et al, 2003]***

This finding suggests that microvascular disruption or leakiness may promote lesion progression by providing erythrocyte-derived cholesterol. In addition to leaky vasa vasorum, plaque fissuring can also account for the accumulation of erythrocytes, which has also been described to occur in the coronary vasculature of patients dying from sudden coronary death. ***[Fleiner M, et al, 2004]***

In one study more than 100 cases of sudden coronary death with serial sectioning of selected plaques were examined to better understand the relationship of intraplaque vasa vasorum in ruptured lesions. The number of vasa vasorum was increased 2-fold in vulnerable plaques and up to 4-fold in ruptured compared with stable plaques with severe luminal narrowing. ***[Fleiner M, et al, 2004]***

The entrance into the intimal space from the adventitia occurs specifically at breakpoints in the medial layer below sites of early necrotic core formation. The vessels divide as they approach the core with secondary and tertiary branches. Microvessels close to the medial wall appear to be well formed because they are typically accompanied by surrounding SMCs. This is in contrast to intimal vessels near the lumen, which appear immature. ***[Fleiner M, et al, 2004]***

Increased numbers of T cells are commonly found at breaks in the medial wall and base of the necrotic core compared with other regions of the plaque. It is thought that T helper cell–driven immune responses possibly through interferon-γ may inhibit SMC proliferation, contributing to medial disruption and absence of SMCs in perforating neovessels. ***[Fleiner M, et al, 2004]***

Vasa vasorum and coronary atherosclerosis

Studies more than a century ago described increased numbers of arterial microvessels in human atheroma, which have later been confirmed by several investigators. Different microscopic studies demonstrate that intimal microvessels arise more frequently from the

dense network of vessels in the adventitia adjacent to a plaque rather from the arterial lumen. ***[Kwon HM, et al, 1998 & Zhang Y, et al, 1993]***

Most of the intraplaque vasa vasorum are endothelialized, but only a few have mural pericytes and SMCs. Lack of mural cells and poorly formed endothelial cell junctions probably contribute to the leakiness of the intraplaque vasa vasorum. As well as porous microvessels may result from release of angiogenic factors from the closely associated macrophages. ***[Fleiner M, et al, 2004 ,Kockx MM, et al, 2003 & Kolodgie FD, et al, 2003]***

Emerging mechanisms of plaque angiogenesis

Angiogenesis depends on the combined action of various cytokines and growth factors secreted by infiltrating inflammatory cells which commonly accompanies chronic immune and inflammatory responses characterized by prominent T-cell and macrophage infiltration as described below.

- **T cell mediated signaling**

Varying degrees of T lymphocytes are consistently present in areas of neoangiogenesis specifically within the deep intima and below the necrotic core and shoulder regions. These morphological observations strongly suggest that T lymphocytes likely play an important role in the development and maturation of intraplaque vasa vasorum. Activated T cells are a known source of angiogenic factors, including vascular

endothelial growth factor, and can stimulate angiogenesis in association with early lymphocyte recruitment. ***[Hansson GK, 2001]***

The dependency of T cells in mediating angiogenic responses was demonstrated recently in CD4 knockout mice exposed to acute hind limb ischemia. As inflammation and collateral development in response to ischemia were significantly impaired in CD4 deficient mice. ***[Stabile E, et al, 2003]***

Moreover, experiments involving the infusion of spleen-derived purified CD4-positive T cells in CD4 deficient mice increased macrophage recruitment. These data highlight the importance to CD4+ cells as initiators of angiogenic responses in addition to their importance in the accumulation of macrophages, which then secrete a different types of cytokines and growth factors, including VEGFs, which facilitate angiogenic growth. ***[Stabile E, et al, 2003]***

- **Role of CD 40**

Several reports have established interactions between CD40 ligand (CD40L) and CD40 involving pluripotent functions on inflammation, including the production of cytokines as well as the angiogenesis factor, VEGF, by endothelial cells. Activated human T cells are reported to mediate contact-dependent expression of matrix metalloproteinases (MMPs) in endothelial cells through CD40L/CD40 signaling. ***[Mach F, et al, 1999]***

These interactions through CD40/CD40L were able to induce an angiogenic response in endothelial cells cultured in fibrin matrix gels. Moreover, stimulation of CD40 resulted in the expression of several angiogenic factors, including VEGF and fibroblast growth factor-2 and the receptors Flt-1 and Flt-4. These studies provide support for a proangiogenic function of CD40L–CD40 interactions. ***[Reinders ME, et al, 2003 & Melter M, et al, 2000]***

- **Toll like receptors**

The role of cytokine-driven inflammation and tissue destruction is becoming recognized as a major determinant of lesion instability. Production of these cytokines is initiated by signaling through Toll-like receptors (TLRs) that recognize host-derived molecules released from injured tissues and cells. ***[Hansson GK, et al, 2002]***

TLRs activate the pro-inflammatory transcription factor, nuclear factor κB (NF-κB), and the mitogen-activated protein kinase pathway, resulting in the production of cytokines that augment local inflammation. ***[Andreakos E, et al, 2004]*** In human atherosclerotic plaques, TLR1, TLR2, and TLR4 are shown to be upregulated in the endothelium and in areas infiltrated with inflammatory cells. ***[Edfeldt K, et al, 2002]***

The reduction in atherosclerosis was associated with lower levels of circulating pro-inflammatory cytokines IL-12 or monocyte chemo-attractant protein-1 accompanied by reduced numbers of plaque macrophages and expression of endothelial leukocyte adhesion molecules. Importantly, human studies suggest TLR4 expression is

upregulated in lipid-rich human plaques when compared with fibrous plaques. Moreover, the capacity of innate immune system to elicit inflammation reactions in response to endotoxins is impaired in patients with TLR4 polymorphisms. These studies and others suggest that TLRs may be essential for promoting the inflammatory component of atherosclerotic disease. ***[Xu XH, et al, 2001]***

However the role of CD40/CD40L and TLR in the promotion of plaque angiogenesis is still highly speculative. Experimental studies in animal models of coronary atherosclerosis (in mice) have clearly shown a role for CD40/CD40L or TLR pathway in the development or progression of atherosclerosis, but no study has shown an alteration of plaque or adventitial angiogenesis after inhibition of CD40/CD40L or inhibition of TLR signaling. ***[Moulton KS, et al, 2003 & Rosenfeld ME, et al, 2000]***

From the previous discussion it was found that neoangiogenesis is an important step in the pathogenesis of coronary atherosclerosis and is closely associated with plaque progression and is likely the primary source of intraplaque hemorrhage at sites of microvessel incompetence. Focal collections of T-cell– and macrophage-derived angiogenic factors contribute to:

(1) The arborization of vasa vasorum around the necrotic core.

(2) The formation of immature vessels; and

(3) The loss of basement membrane around functional capillaries.

This process initiates leakage of RBCs into the plaque and induces a cycle of inflammation and neovascularization. Understanding these

mechanisms of angiogenic growth within the neointima of atherosclerotic lesions may lead to the development of new therapies designed to stabilize plaques. ***[Moulton KS, et al, 2003]***

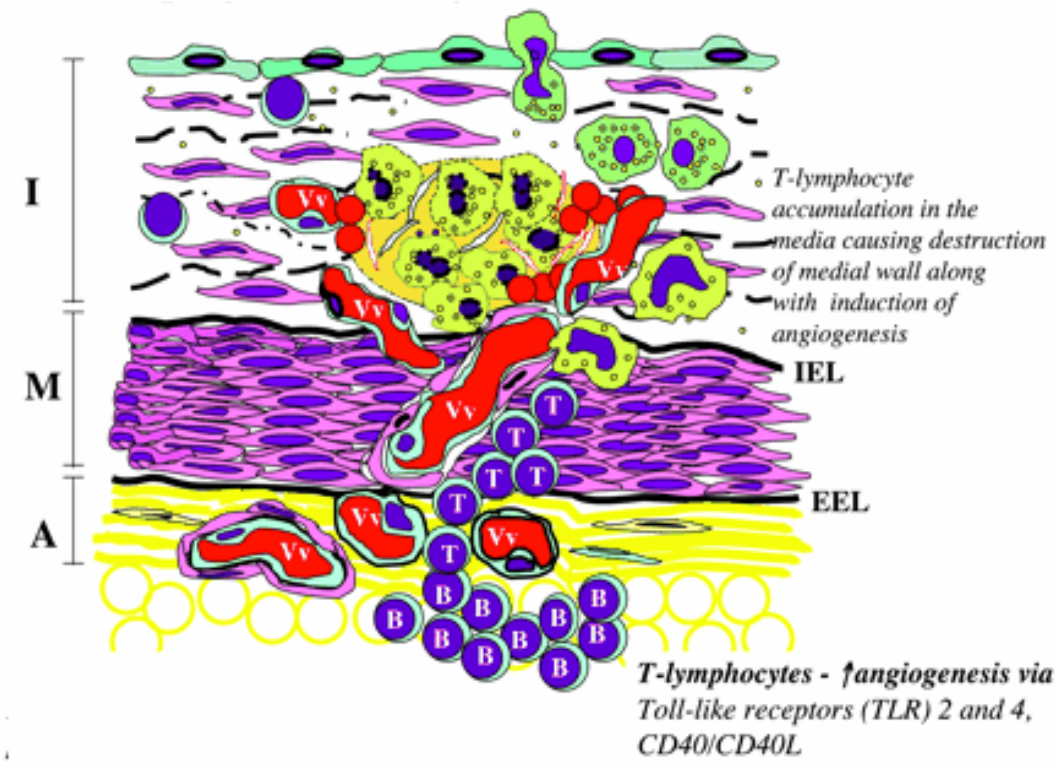

Figure (2): The diagram illustrates the origin of intraplaque vasa vasorum infiltrating from adventitial vessels through a disrupted medial wall. The inflammatory response in the adventitia is characterized mostly by B cells; however, as the vessel traverses the media, it becomes surrounded mainly by foci of T lymphocytes and perivascular macrophages as they approach the necrotic core. Leaky or ruptured vasa vasorum result in intraplaque hemorrhages and an accumulation of free cholesterol within the necrotic core derived from erythrocyte membranes. It is proposed that T lymphocytes via CD40/CD40L or TLRs induce angiogenesis by the release of angiogenic growth factors from macrophages.

I indicates intima; M, media; A, adventitia; EEL, external elastic lamina; IEL, internal elastic lamina; B, B lymphocytes; T, T lymphocytes; Vv, vasa vasorum.

Overview of different clinical presentation and classification of Non-ST elevation ACS

According to the World Health Organization (WHO) criteria that were revised in 1979, a patient with ischemic symptoms, no ST elevation or new Q waves, and a normal serum CK-MB was considered to have UA even if serum troponins were elevated. In recognition of the diagnostic and prognostic importance of elevations in serum troponins and that some patients have atypical symptoms and/or a nondiagnostic ECG, a joint American College of Cardiology/European Society of Cardiology committee proposed the following clinical definition of an acute, evolving, or recent MI: Typical rise and gradual fall (troponin) or more rapid rise and fall (CK-MB) of biochemical markers of myocardial necrosis with at least one of the following:

- Ischemic symptoms
- Development of pathologic Q waves on the ECG
- ECG changes indicative of ischemia (ST segment elevation or depression)
- Coronary artery intervention (eg, angioplasty) ***[Alpert JS, et al, 2000]***

According to this definition and the ACC/AHA guidelines, UA and NSTEMI differ primarily in whether the ischemia is severe enough to cause sufficient myocardial damage to release detectable quantities of a marker of myocardial injury. Unstable angina is considered to be present in patients with ischemic symptoms suggestive of an ACS and no elevation in troponins or CK-MB, with or without ECG changes indicative of ischemia (eg, ST segment depression or transient elevation

or new T wave inversion). Since an elevation in troponins and/or CK-MB may not be detectable for up to 12 hours after presentation, UA and NSTEMI are frequently indistinguishable at initial evaluation. ST segment and/or T wave changes are often persistent in NSTEMI while, if they occur in UA, they are usually transient. ***[Alpert JS, et al, 2000]***

Unstable angina can have a variety of different presentations which may correlate with prognosis in the absence of intervention. Regardless of type, the risk is greatest with angina that is refractory to or occurs despite maximal medical therapy and with an accelerating tempo of ischemic symptoms in the preceding 48 hours (crescendo angina). ***[Patel DJ, et al, 2001]***

The unstable angina and NSTEMI is classified into:

1. New onset angina.
2. Rest angina.
3. Early post infarciton angina.
4. Post revascularization angina.
5. Periprocedural angina.

The natural history of new onset angina depends in part upon the degree of exertion required to induce chest pain. Patients with new onset angina occurring only after heavy physical exertion have a prognosis similar to patients with chronic stable angina. In comparison, new angina occurring after minimal exercise or at rest, particularly if prolonged, carries a worse prognosis in the absence of intervention. ***[Patel DJ, et al, 2001]***

The rest angina, particularly if prolonged and/or associated with transient ST segment changes >0.05 mV, identifies patients at increased risk. Early postinfarction angina (defined as chest pain occurring within 48 hours after an acute MI) is typically associated with complex lesions and/or persistent intracoronary thrombus and with more severe coronary disease. The recurrent chest pain in this setting may signify either remaining viable myocardium in the infarct zone or a different area of myocardium at risk. ***[Patel DJ, et al, 2001]***

Angina occurring soon after an acute MI is associated with high risk in the absence of intervention. Patients with recurrent ischemia that was refractory to medical therapy had a higher rate of reinfarction at 30 days and 6 months. In addition, the occurrence of refractory ischemia was associated with a higher mortality compared to responsive ischemia or no ischemia at 30 days and one year. Similar findings were noted among patients with an STEMI. ***[Betriu A, et al, 1998 & Armstrong PW, et al, 1998]***

Postrevascularization angina that occurring after percutaneous coronary intervention (PCI) or coronary artery bypass graft surgery (CABG) can reflect a procedural event or, over the long-term, restenosis after PCI , stenosis in a graft (usually with saphenous vein grafts), or progression of native disease. Periprocedural angina refer to ischemic chest pain that occurring within 48 hours after stenting usually results from procedural events such as abrupt vessel closure (usually due to stent thrombosis or progression of an untreated dissection), transient coronary spasm, side branch occlusion, or distal embolization of atherosclerotic or thrombotic debris. ***[Robbins MA, et al, 1999]***

An important diagnostic consideration soon after PCI is the distinction between ischemic and nonischemic chest pain. Nonischemic chest pain is typically manifested at rest, without ECG changes or elevation of cardiac enzymes. Most patients describe pain characteristics different from their typical angina (more localized and frequently pleuritic). This discomfort lasts for less than 72 hours in about 80 percent of patients, and less than two weeks in the remainder. Overexpansion of the stent is thought to be responsible in most cases. ***[Kini, AS, et al, 2003]***

As after PCI, recurrent angina during the postoperative period after CABG is usually due to a technical problem with a graft or with early graft closure. It is therefore an indication for prompt catheterization with revascularization by PCI, if feasible. The diagnosis of recurrent ischemia may be difficult to make after CABG, since cardiac enzyme elevations occur as a result of the surgical procedure and since electrocardiographic changes may reflect postoperative pericardial inflammation. ***[Labinaz M, et al, 2002]***

The delayed onset of angina can reflect restenosis after PCI, graft stenosis after CABG, or progression of native disease. Affected patients typically present with the gradual and progressive return of effort angina. Prompt stress testing should be performed, since these patients are at increased risk. Stress myocardial perfusion imaging or echocardiography is preferred over exercise ECG testing, since these modalities can document both the site and extent of ischemia. While less common, some patients with recurrent ischemia present with UA. Such patients should be evaluated with cardiac catheterization after adequate medical

stabilization. Unstable angina can also occur in patients with prior CABG; such patients have an increased rate of significant coronary events. ***[Labinaz M, et al, 2002]***

This issue was best illustrated in the PURSUIT trial of almost 11,000 patients with a non-ST ACS, 12 percent (1134 patients) of whom had a prior CABG. Patients with a prior CABG had a significantly higher mortality at 30 days (5.2 versus 3.4 percent without a prior CABG) and six months (8 versus 6.6 percent). This difference may reflect a greater degree of cardiac disease. ***[Labinaz M, et al, 2002]***

Grafts are more likely than native vessels to show total occlusion, thrombus or complications that are more refractory to medical therapy. Among patients who undergo PCI for saphenous vein graft disease, the development of restenosis is manifested by an UA presentation in as many as 25 percent of patient. ***[Chandrasekar B, et al, 2000]***

On the other hand in different clinical trials and the CRUSADE registry, 9 to 14 percent of patients with a non-ST elevation ACS have, on coronary angiography, either normal vessels or no vessel with more than 50 to 60 percent stenosis. ***[Patel MR, et al, 2006]***

Possible mechanisms for the absence of marked coronary disease in these patients include coronary thrombosis with rapid clot lysis, vasospasm, microemboli, coagulopathy, vasculitis, small vessel disease, and coronary microvascular dysfunction. ***[Patel MR, et al, 2006 & Diver DJ, et al, 1994]***

When compared to patients with UA and a culprit coronary lesion on angiography, those with relatively normal coronary arteries were more likely to be women and to have no ST segment deviation. The characteristics of patients with UA who have mild or no coronary disease were further examined in a study of 5767 patients with a non-ST segment elevation ACS who were enrolled in the PURSUIT trial and who underwent angiography: 6 percent had mild coronary disease (>0 to 50 percent stenosis) and 6 percent had no disease. ***[Roe MT, et al, 2000]***

The strongest independent predictors of insignificant coronary disease were: Younger age, female sex, absence of enrollment MI, prior angina, diabetes, or ST segment depression. Similar predictors of insignificant coronary disease, as well as lack of current/recent smoking, were found in the CRUSADE registry. ***[Patel MR, et al, 2006 &Glaser R, et al, 2002]***

Patients with a non-ST elevation ACS who do not have significant coronary disease have a better outcome than those with a culprit coronary lesion. ***[Patel MR, et al, 2006***]

As mentioned above, UA and NSTEMI are frequently indistinguishable at initial evaluation of patients who present with suspected ischemic chest pain, since an elevation in serum troponins and/or CK-MB are usually not detectable for four to six hours after an MI and at least 12 hours are required to detect elevations in all patients. Thus, the above discussion of the classification of UA also applies to patients with NSTEMI. ***[Patel DJ, et al, 2001]***

Most patients with NSTEMI present with chest pain and ST segment depression. ST segment depression is an adverse predictor in patients with a non-ST elevation ACS. This is due in part to an increased incidence of left main or three vessel disease compared to those without ST segment depression. ***[Diderholm E, et al, 2002, Antman EM, et al, 2000 & Hamm, CW, et al, 2000]***

Another subset of patients present with chest pain and initial ST segment elevation but no Q waves. These patients are treated for an ST elevation MI, but some never develop Q waves. Such patients have a better prognosis than those who develop Q waves because of more frequent reperfusion and a less severe infarction. ***[Akkerhuis KM, et al, 2002]***

The absence of Q waves in patients with an NSTEMI reflects a high rate of spontaneous reperfusion, as 60 to 85 percent do not have occlusion of the culprit artery on arteriography performed soon after presentation. This presumably explains the lack of benefit from thrombolytic therapy in patients with an NSTEMI. ***[Akkerhuis KM, et al, 2002]***

NSTEMI can occur after percutaneous coronary intervention (PCI), a setting in which it is primarily manifested by an asymptomatic elevation in serum biomarkers. The reported incidence of periprocedural NSTEMI has ranged from 5 to 30 percent and is higher with stenting than balloon angioplasty alone. ***[Califf, RM, et al, 1998 & Shyu KG, et al, 1998]***

Among patients receiving a stent, the incidence is increased with suboptimal stenting (eg, 8.7 versus 4.2 percent with optimal stenting in the STARS trial), a shorter period of pretreatment with a thienopyridine, and a greater atherosclerotic burden. ***[Mehran, R, et al, 2000 , Cutlip, DE, et al, 1999 & Steinhubl SR, 1998]***

Cardiac troponins are a more sensitive marker than CK-MB for minor degrees of myocardial damage, and elevated values after PCI with or without stenting may be more common than increases in serum CK-MB. There is a graded relationship between the degree of enzyme elevation and increased six month mortality. ***[Akkerhuis KM, et al, 2002]***

It is thought that the most common cause of elevated serum enzymes after PCI is distal embolization of friable plaque constituents and occlusion of minor side branches, rather than reduced epicardial blood flow, and it has been suggested that patients with serum CK-MB more than three times the upper limit of normal or with ECG changes diagnostic of an MI and should be treated as if they had an acute MI. ***[Gibson, CM, et al, 2002 , Ricciardi MJ, et al, 2001 & Califf RM, et al, 1998]***

The diagnostic accuracy of serum biomarkers is reduced after CABG due to their release as a routine sequel of the procedure. As an example, an increase in serum CK-MB above the upper limit of normal has been noted in 60 to 90 percent of patients. As a result, the diagnosis of NSTEMI or STEMI after CABG usually requires characteristic ECG changes. However, patients with substantial elevations in serum markers

(eg, serum CK-MB more than 10 times the upper limit of normal) are generally considered to have an MI and a worse outcome. ***[Brener SJ, et al, 2002]***

Prognosis according to different types

The different types of non-ST elevation ACS are associated with different risks of adverse outcomes. This observation has led to attempts to risk stratify such patients. However, as will be described below, these efforts have limited applicability to current practice since almost all high or intermediate risk patients and many low risk patients now undergo coronary revascularization if available.

A classification of UA was proposed by Braunwald in 1989 to facilitate the assignment of patients to a particular risk group. This classification, which included patients with NSTEMI since troponins were not measured, takes into account the severity of symptoms, the clinical circumstances surrounding the anginal episode, and the intensity of treatment. ***[Braunwald E, et al, 1989]***

Severity Class I include patients with new onset, severe, or accelerated angina while Class II incude patients who have rest and subacute angina (no anginal episodes within the preceding 48 hours). Class III include patients who have rest and acute angina (angina within the preceding 48 hours) and according to Clinical circumstances, patients maybe furtherly classified into: Class A that include secondary UA (in the setting of anemia, infection, fever, etc), Class B that include Primary UA and Class C that include Post-MI angina. ***[Braunwald E, et al, 1989]***

Correlation with angiographic findings

The disease severity can be predicted by the original classification correlated with angiographic findings. As an example, angiographic correlations were evaluated in 238 consecutive patients admitted with the diagnosis of UA to a large tertiary care hospital: 2 percent had secondary angina (class A), 60 percent had primary angina (class B), and 38 percent had post-MI angina (class C). Multivariate regression analysis identified an angina score (assigned on the basis of one point for class I and class A, two points for class II and class B, and three points for class III and class C) as the most important predictor of angiographically demonstrable intracoronary thrombus and lesion complexity in the ischemia-related artery. ***[De Servi S, et al, 1996 & Ahmed WH, et al, 1993]***

In another report, patients with class III angina (rest angina within 48 hours) were more likely to have complex lesions (eccentric, ulcerated, irregular borders, or overhanging edges, associated with an intracoronary filling defect) and decreased Thrombolysis in Myocardial Infarction (TIMI) grade flow. Patients with class C angina (post-infarction angina) were more likely to have complex lesions, reduced TIMI flow, and intracoronary thrombus. Class C angina (refractory to medical therapy) was also correlated with intracoronary thrombus. ***[Dangas G, et al, 1997]***

Despite these general correlations between clinical presentation and angiography, approximately 12 to 14 percent of patients with a non-ST elevation ACS do not have significant coronary disease at angiography. ***[Cannon CP, et al, 2001 & Roe MT, et al, 2000]***

Prediction of outcome

Another prospective study of 417 patients attempted to identify the incidence and prognosis of different classification groups. After hospital admission, recurrence of chest pain increased with higher severity class (28, 45, and 64 percent for classes I, II, and III) but was the same in different clinical circumstances classes (49, 53, and 53 percent for classes A, B, and C). ***[Van Miltenburg-van Zijl, et al, 1995]***

Six month infarct-free and patient survival were significantly lower for class C than for classes A and B (89 versus 97 percent and 80 versus 89 percent, respectively). In addition to class C, other independent predictors of death were older age, male sex, hypertension, and maximal medical therapy. Similar findings were noted in a community-based series of 393 patients. Two other clinical factors, older age and diabetes, were also predictors of adverse outcomes. ***[Calvin, JE, et al, 1995]***

However the original Braunwald classification had a number of weaknesses including substantial overlap between the groups and difficulty in identifying very low risk patients. In addition, a number of important clinical considerations were not included, such as age, gender, the physical examination, the presence or absence of electrocardiographic (ECG) changes, the presence of comorbid illness known to increase the risk of coronary disease such as diabetes or peripheral vascular disease, and the prognostic importance of serum troponins which, according to the new definition of myocardial infarction, identifies patients with an NSTEMI. ***[Alpert, JS, et al, 2000]***

The prognostic importance of serum troponins and ST segment depression was demonstrated in a report in which patients with UA class IIIB were subdivided into troponin positive and troponin negative patients. The patients who were troponin-positive (who would now be considered to have an NSTEMI) had a much higher risk of cardiac death or MI at 30 days (15 to 20 versus less than 2 percent). ST segment depression also had independent adverse predictive value. The adverse outcome in troponin-positive patients could be related to a greater likelihood of complex lesion characteristics and visible thrombus at baseline. ***[Hamm CW, et al, 2000 & Heeschen, C, et al, 1999]***

Applicability to current practice

The original Braunwald classification remains valuable during the original assessment of patients with a non-ST elevation ACS. However, its predictive value for outcome is limited since almost all high or intermediate risk patients and many low risk patients now undergo coronary revascularization if available.

The TIMI (Thrombolysis In Myocardial Infarction) risk score is the most widely used and validated predictive instrument in patients with a non-ST elevation ACS. In TIMI risk Score seven independent risk factors were identified to predict the incidence of death, myocardial infarction and recurrent ischemia in patients with UA/NSTEMI. These Include:

1. Age > 65 years
2. Presence of 3 or more risk factors for coronary artery disease
3. Documented coronary stenosis > 50% at angiography
4. ST segment deviation > 0.5 mm

5. Two or more peisodes of angina in the last 24 hours
6. Aspirin use within the prior week
7. Elevated cardiac markers

The incidence of death, myocardial infarction or recurrent ischemia varies between 4.7% if none or only one of these factors is present to 40.9% if 6 or 7 factors are present. Patients with higher scores have significant reduction in adverse events when treated with fractionated heparin and GP IIb/IIIa inhibitors. The following table illustrates features of high, intermediate and low risk categories of UA/NSTEMI. The annual mortality rate for high risk patients exceeds 3%, for intermediate risk 1-3% and for low risk patients is < 1%.

Feature	High risk (At least 1 of the following features must be present)	Intermediate risk (Must have 1 of the following features)	Low risk (May have any of the following features)
History	Acclerating tmpo of ischemic symptoms in preceding 48 hrs.	Prior MI, peripheral or cerebrovascular disease, or CABG; prior aspirin use.	
Character of pain	Prolonged ongoing (>20 min)	-Prolonged (>20 min) rest angina, now resolved, with moderate or high likelihood of CAD -Rest angina (<20 min or relieved with rest or sublingual nitroglycerine)	New onset or progressive CCS class III or IV angina in the past 2 weeks with moderate or high likelihood of CAD
Clinical findings	-Pulmonary oedema, most likely related to ischemia -New or worsening MR murmur -Hypotension, bradycardia, or tachycardia -Age >75yrs	Age > 70 yrs	
ECG findings	-Angina at rest with transient ST-segment changes>0.05 mv -Bundle branch block, new or presumed new -Sustained ventricular tachycardia	-T wave inversion >0.2 mv -Pathological Q waves	Normal or unchanged ECG during an episode of chest discomfort
Cardiac markers	Elevated (eg Tn T or Tn I >0.1 ng/ml)	Slighly elevated (eg Tn T >0.01 but < 0.1 ng/ml)	Normal

Table (1): Illustrates the short term of death and non fatal MI inpatients with UA/NSTEMI

Chapter II

Role of Angiogensis in Atherosclerotic Coronary Artery Disease

Introduction

The role of blood vessel formation within diseased blood vessels has become one of the outstanding puzzles in the biology of cardiovascular disease. The generation of blood vessels is a prerequisite for embryonic development and is increasingly recognized to play essential roles in the pathogenesis of diverse chronic human diseases. ***[Carmeliet P, 2003, Kaiser M, et al, 1999 & Folkman J, 1995]***

For example, neovascularization is the integral part of the disease process of cancer, rheumatoid arthritis, ocular disorders, and many other diseases, and inhibition of angiogenesis is a major goal of therapeutic drug development. Efforts to develop an anti- angiogenic therapeutic approach to cancer have approved by the US Federal Drug Administration , Avastin, an antibody specific for VEGF (or VEGF-A), for the treatment of metastatic colorectal carcinoma. ***[Hurwitz H, et al, 2004]***

In atherosclerosis, however, the role of angiogenesis remains a highly debatable issue, and no consensus exists as to whether angiogenesis either is a key causative factor in the pathogenesis of atherosclerotic plaque formation or is a way to treat coronary heart disease. The controversy surrounding the role of angiogenesis in ischemic heart disease reflects, in part, the complexity of the underlying disease

process. However a growing body of evidence supports an association between intra-lesion angiogenesis with atherosclerotic plaques that cause acute coronary syndromes. ***[Tenaglia AN, et al, 1998]***

These vulnerable plaques are more likely to rupture and progress to cause intra-arterial occlusion. In the case of coronary arteries, this sudden and catastrophic restriction of the blood supply to the heart causes an acute coronary syndrome, often resulting in a fatal loss of cardiac function. The acute problem in the case of coronary artery disease is therefore vascular insufficiency, but this is the outcome of a complex pathophysiological process in which angiogenesis may itself play a vital, although as-yet undecided, role. ***[Boersma E, et al, 2003]***

Debate surrounding the pathogenic role of angiogenesis in atherosclerosis has been particularly energetic because a key therapeutic objective has been to use angiogenic cytokines such as VEGF or members of the fibroblast growth factor (FGF) family to stimulate collateral blood vessel formation in the ischemic heart and peripheral vascular disease, an approach called therapeutic angiogenesis. ***[Simons M, et al, 2003]***

Although this strategy is supported by an impressive body of preclinical research suggesting that VEGF, FGF-2, and other angiogenic cytokines can promote revascularization in diverse animal models of ischemic cardiovascular disease, the data from clinical trials have been inconclusive. ***[Celletti FL, et al, 2001]***

More debate still for proponents of therapeutic angiogenesis have been due to the presence of several studies suggesting that VEGF and other angiogenic factors can promote atherosclerosis in certain animal models and potentially destabilize coronary plaques by promoting intra-lesion angiogenesis. ***[Heeschen C, et al, 2001 & Moulton KS, et al, 1999]***

Angiogenesis and Ischemic heart disease

Many large human arteries possess a microvasculature in their adventitial layers called the vasa vasorum. Normal vasa vasorum originate from coronary artery branch points at regular intervals and run longitudinally along the vessel wall (first-order vasa vasorum). These vessels then separate to form circumferential arches around the main coronary lumen (second-order vasa vasorum). Because diffusion of blood nutrients from the lumen is limited to a distance of about 100 μm, a primary function of these vessels is thought to be the transport of nutrients to the vessel wall, although other roles are not precluded. ***[Carmeliet P, 2003]***

An association between intimal neovascularization and atherosclerosis was first noted by Koester in 1876; similar observations were made by Winternitz and coworkers in 1938. Then it was found that rupture of plaque capillaries could trigger intraplaque hemorrhage, leading to coronary thrombosis. It was later found that the intimas of adult human arteries are avascular until they exceed a certain thickness. ***[Carmeliet P, 2003]***

The study of Barger et al 1984 synthesized many of these earlier observations in the hypothesis that proliferation of the adventitial vasculature of coronary arteries allowed atherosclerotic plaques to develop beyond a critical thickness by supplying oxygen and nutrients to the core of the lesions. ***[Tenaglia AN, et al, 1998]***

Barger and Beeuwkes in 1990 subsequently proposed that the neovascular network in coronary atherosclerotic plaques may be more fragile and prone to rupture and therefore a potential cause of plaque destabilization and vascular spasm, leading to acute coronary syndromes. ***[Tenaglia AN, et al, 1998]***

An association between neovascularization and atherosclerosis has generally been confirmed by subsequent work showing a correlation between the extent of atherosclerosis and plaque neovascularization in human pathological samples, and in the coronary arteries of hypercholesterolemic animals. ***[Carmeliet P, 2003]***

For example, Williams et al 1988 found that progression of atherosclerotic plaques in hypercholesterolemic monkeys was associated with increases in blood flow through the vasa vasorum, whereas plaque regression induced by withdrawal of a high-cholesterol diet was associated with loss of vasa vasorum and a marked decrease in blood flow through the vasa vasorum to the coronary intima and media. ***[Sueishi K, et al, 1997]***

Later studies have revealed a more complex picture of the relationship between plaque neovascularization and atherosclerotic pathology where neovascularization is more common at sites of infiltration by chronic inflammatory cells such as macrophages and lymphocytes but less common in highly calcified or hyalinized plaques. ***[Kumamoto M, et al, 1995]***

Moreover, minor hemorrhage was frequent around newly formed vessels and intimal vasa vasorum was a frequent component of plaque pathology; in one study, intimal microvessels were detected in 42% of primary and 64% of restenotic human coronary atherectomy specimens. ***[O'Brien ER et al, 1994]***

Plaque vessels are found in the neointima, media, and adventitia, but most vessels appear to originate from the adventitial vasa vasorum rather than the luminal endothelium. However in-growth of new vessels from the luminal endothelium has also been observed. ***[Sueishi K, et al, 1997]*** However the relationship between neovascularization and the severity of disease is less certain, but one study that used micro-CT found a highly significant correlation between the number of vasa vasorum and wall thickness in hypercholesterolemic porcine coronary arteries. ***[Kwon HM, et al, 1998]***

Therefore the role for neovascularization in plaque instability has been widely hypothesized, but direct evidence for it is lacking, partly because the critical factors that precipitate plaque rupture remain largely unknown and partly because reliable animal models of plaque rupture

analogous to the human situation have not yet been developed. ***[Kwon HM, et al, 1998]***

Moreover, studies in human lesions suggest that there is a relationship exists between microvessels and the regions of plaques most vulnerable to rupture. As microvessels appear to have a predilection for the rupture in the shoulder regions of atherosclerotic plaques. ***[Sueishi K, et al, 1997 & O'Brien ER, et al, 1994]***

Whereas a recent study of neovascularization in 269 advanced human atherosclerotic plaques concluded that microvessel formation is strongly correlated with both plaque rupture and the signature features of vulnerable plaques. Not only, an increase in microvessel density occurred in ruptured compared with nonruptured plaques but was also found in the shoulder regions of plaques, where the fibrous cap joins the remainder of the arterial wall, and was strongly associated with a high degree of macrophage infiltration, intraplaque hemorrhage, and thin-cap lesions. In addition to an association between microvessels and vulnerable plaques, several proangiogenic cytokines are expressed in human lesions, making further weight to the argument that neovascularization is an active process in the atherosclerotic pathogenesis. ***[Moreno PR, et al, 2004]***

So it can be drawn from human and large animal studies that neovascularization is undoubtedly a common, although not invariable, feature of the pathology of human atherosclerotic lesions and often is found in experimental large animal (primate, pig, and dog) models of atherosclerosis and intimal thickening. ***[Brooks PC, et al, 1994]***

The strongest experimental evidence that angiogenesis plays a causative role in atherosclerosis has come from studies in the hypercholesterolemic mouse model. Moulton et al found that endothelium-specific inhibitors of angiogenesis, reduced plaque area in apolipoprotein E defecient mice. This study had a major impact because it provided the direct evidence that angiogenesis was involved in the process of plaque formation. The same laboratory extended its earlier findings by showing that another angiogenesis inhibitor, angiostatin, reduces atherosclerosis in the same mouse model. ***[Moulton KS, et al, 2003]***

The work of Moulton et al has raised as many questions to be answered. As subsequent commentators pointed out that their work did not test whether angiogenic factors promote atherosclerosis and antiangiogenic factors could have nonspecific effects that are not due solely to inhibition of vessel formation. Another important issue is the suitability of the mouse model for studying plaque neovascularization. In addition the incidence of lesions with intimal vessels reported by Moulton et al was relatively low. Because mouse aortas are relatively small and have a thin media. ***[Khurana R, et al, 2005]***

It therefore seems unlikely that all the effects of antiangiogenic agents on atherosclerosis in the cholesterol-fed Apo E deficient mouse are due to inhibition of plaque neovascularization. Therefore Moulton et al concluded somewhat cautiously that their study did not provide conclusive evidence of a causal link between angiogenesis and plaque development. ***[Khurana R, et al, 2005]***

Another widely discussed study seemed to provide the crucial evidence that angiogenesis is important for atherosclerosis by showing that intra-peritoneal administration of recombinant human VEGF protein promoted atherosclerosis doubly in ApoE and ApoB100 deficient mice. ***[Celletti FL, et al, 2001]***

The major effects of VEGF in this study were increases in bone marrow–derived endothelial progenitor cells (EPCs), plaque endothelial cell density, and macrophage infiltration. ***[Moulton KS, et al, 2003]***

In the ensuing vigorous debate, some scientists highlighted apparent discrepancies that call into question the mechanism underlying the proatherogenic effect of VEGF. For example, an increase in circulating bone-marrow derived EPCs was not evident until 2 to 3 weeks after VEGF administration, and plaque macrophage content did not increase until week 3, whereas significant increases in plaque area and endothelial density occurred 1 week after VEGF administration. So the causal relationships between the observed effects of VEGF in this study are therefore unclear. ***[Eppler SM, et al, 2002]***

While arguing that VEGF might cause "potential destabilization" of plaques, the study also failed to provide direct evidence that VEGF caused plaque destabilization or rupture. Another concern with the work of Celletti FL et al., 2001 is that long-term effects of VEGF protein administration to produce meaningful long-term biological effects was not examined. ***[Eppler SM, et al, 2002]***

Because animal and human pharmacokinetic studies of VEGF protein indicate that it is cleared from the circulation within a few hours after a bolus intravenous administration. ***[Eppler SM, et al, 2002]*** The biological efficacy of a single dose must be open to question. Interestingly, another study found that an antibody directed against the mouse homologue of KDR, had no effect on atherosclerotic plaque development in ApoE deficient mice. Because KDR is the major receptor mediating angiogenic effects of VEGF, this suggests that angiogenesis, at least driven by the VEGF/KDR pathway, is not a major contributor to atherosclerosis in the ApoE deficient mouse. ***[Luttun A, et al, 2002]***

The VEGF-related factor, placental growth factor (PlGF), is implicated in the promotion of early atherosclerosis in ApoE-deficient mice acting via Flt-1, but its proatherogenic effects are not mediated by increased angiogenesis. ***[Khurana R, et al, 2005]***

Thus, anti–Flt-1 antibodies inhibited early lesion growth in Apo E defecient mice without affecting plaque neovascularization, whereas mice doubly deficient in Apo E and PlGF exhibited a reduction in the size, number, and macrophage content of specifically early atherosclerotic lesions but with no effect on either the number of plaque microvessels or the growth of advanced lesions. Therefore the pro-atherogenic effect of VEGF and its related factor PlGF could be mediated by other mechanisms other than angiogenesis. ***[Khurana R, et al, 2005]***

Role of Angiogenesis in the development of Neointima

Increased neovascularization has been observed at sites of intimal hyperplasia in models after arterial stenting, angioplasty, and venous bypass graft failure. In pig coronary arteries, adventitial neovascularization correlated with vessel stenosis after balloon injury and stent implantation, and VEGF expression was reported to be increased after stenting. ***[Shigematsu K 2001, Westerband A, et al, 2000 & Pels K, et al, 1999]*** Neointimal angiogenesis was also observed in autologous vein grafts and their anastomoses in the dog femoral artery when the neointima exceeded certain limit in thickness. ***[Ohta O, et al, 1997]***

The oxygen deprivation hypothesis proposes that adventitial neovascularization is an adaptive response to hypoxia resulting from vessel wall thickening. Hypoxia induces multiple biological responses, including the upregulation of several growth factors and cytokines that are implicated in endothelial and VSMC proliferation and migration. ***[Pugh CW, et al, 2003]***

However, the existence of a more complex relationship between function of the adventitial vasa vasorum and intimal thickness is indicated by studies showing that adventitial damage may itself trigger neointimal formation. ***[Shigematsu K 2001]***

As occlusion of the vasa vasorum in the pig femoral artery stimulated intimal hyperplasia, and the intimal thickening increased on removal of the adventitia from the rabbit carotid artery and regressed concomitantly with adventitial regeneration. These findings suggest that

the adventitia may exert an inhibitory effect on intimal hyperplasia, whereas a decreased blood supply through the adventitial vasa vasorum could trigger atherogenic intimal thickening. ***[Barker SG, et al, 1994]***

The role of angiogenesis in the development of neointima has been studied in several animal models by the intravascular or periadventitial delivery of either angiogenic growth factors, particularly VEGF and FGF, or antiangiogenic agents. These studies have not produced a consensual picture of the role of angiogenesis in neointimal formation, largely because the outcomes of such studies vary depending on the nature of the model, species, and type of stimulus. ***[Hutter R, et al, 2004 & Pugh CW, et al, 2003]***

In balloon-injury or stent-implantation models in which neointimal lesions are induced by endothelial denudation and arterial injury, the rate of re-endothelialization is a critical determinant of neointima formation. ***[Hutter R, et al, 2004, Van Belle E 1997 & Asahara T, et al, 1995]***

In a porcine coronary balloon injury model, local periadventitial liposome-mediated VEGF-A_{165} gene transfer had no significant effect on angiogenesis or intimal hyperplasia. ***[Pels K, et al, 2003]*** On the contrary, other studies of the influence of VEGF on injury-induced intimal hyperplasia have reached many conclusions, that Intramuscular administration of VEGF protein increased intima-to-media ratios in the balloon-injured rabbit femoral artery, whereas VEGF blockade with sFlt1 attenuated neointima formation in rabbits, mice, and rats. ***[Ohtani K, et al, 2004 & Celletti FL, et al, 2002]***

The study of Khurana et al, 2004 together with previous work Grosskreutz CL et al., 1999, indicates that VEGF can also promote VSMC migration, suggesting that where VSMC injury is extensive, the balance may shift in favor of a neointima formation. ***[Khurana, et al, 2004]***

There are many evidences about the effect of angiogenic cytokines on graft-induced intimal thickening at an anastomosis, as adenoviral VEGF-A transfer was shown to increase intimal thickening in a rat cardiac allograft model, possibly secondary to increased intra-graft influx of macrophages and neovascularization within the intimal lesions. ***[Lemstrom KB, et al, 2002]***

Booth RF and coworkers in 1989 demonestrated a different approach to this problem as they examined the effects of angiogenic stimuli in a model in which intima formation is induced by placement of a perivascular Silastic collar around the adventitia of the rabbit carotid artery without damaging the endothelium. Advantages of this model are that it allows the collar to serve as a localized delivery reservoir for the candidate agent and that the luminal endothelium remains structurally and macroscopically intact. ***[Khurana, et al, 2004]***

Liposome-mediated periadventitial VEGF-A gene transfer in this model was associated with an adventitial angiogenic response in the collared arteries of hypercholesterolemic rabbits. However, angiogenesis inhibitors were ineffective in preventing neointimal thickening when administered in the absence of angiogenic growth factor, suggesting that

although a strong adventitial angiogenic stimulus can enhance intimal thickening, angiogenesis is not required to initiate it. On the basis of these results, it was proposed that intimal thickening has an initial angiogenesis-independent phase that is followed by an angiogenesis-dependent phase, regardless of endothelial integrity (See figure 3). ***[Khurana, et al, 2004]***

Apparently contradictory findings obtained with VEGF in different animal models may be related to distinct biological effects of VEGF in the cardiovascular system with different concentration. ***[Yla-Herttuala S, et al, 2003]***

At low concentrations resulting from low-efficiency gene transduction methods, VEGF elicits a mainly arterioprotective effect and a weak angiogenic response; at higher concentrations produced by high-efficiency adenoviral delivery, the arterioprotective effect of VEGF may be impaired, and the angiogenic effects may predominate; at still higher concentrations, an excessive and pathophysiological neovascular response may prevail and possibly mediate proatherogenic effects such as intimal thickening and accelerated atherosclerosis. ***[Ozawa CR, et al, 2004]***

Although such a model cannot explain all findings with VEGF in models of cardiovascular disease, it is consistent with other studies showing a critical role of VEGF concentration in determining its biological effects in vivo. ***[Ozawa CR, et al, 2004]***

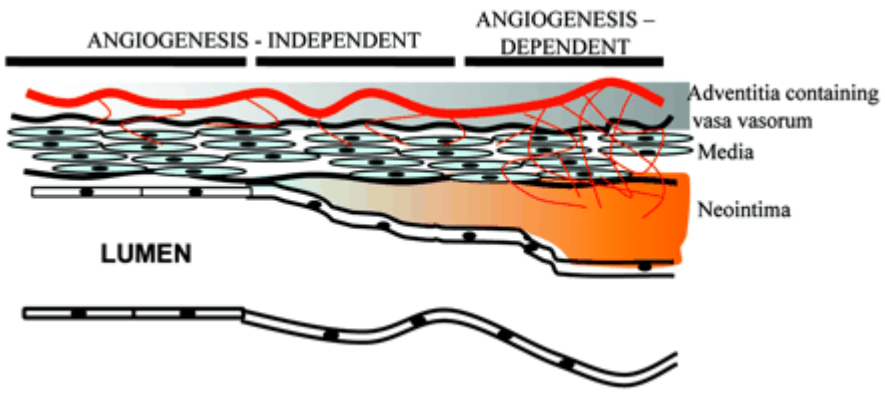

Figure (3): Illustrates the role of adventitial angiogenesis in intimal thickening. The formation of intimal thickening has distinct phases, regardless of endothelial integrity. After an initial injury-induced and angiogenesis-independent early phase, adventitial angiogenesis contributes to a later phase of intimal hyperplasia.

Inducible factors for neointimal angiogenesis

- Endothelial progenitor cells
- Hypoxia
- Inflammation

Role of Endothelial Progenitor Cells

Endothelial progenitor cells (EPCs) have been widely thought as having immense promise for the treatment of coronary artery disease, largely because of their ability to regenerate endothelial cells after angioplasty and their potential for the revascularization of ischemic tissue. ***[Melo LG, et al, 2004& Aicher, et al, 2005]***

However, recent findings indicate that both circulating and bone marrow–derived EPCs and stem cells are a major source of lesion-associated VSMCs, endothelial cells, and plaque microvessels in mouse models of atherosclerosis. In some respects, the contrasting findings with

EPCs parallel the controversy surrounding the role of angiogenesis in atherosclerosis. ***[Melo LG, et al, 2004, Hillebrands JL, et al, 2001 & Zachary I, et al, 2000]***

Furthermore, most of the powerful mobilizers of EPCs such as G-CSF are also proinflammatory and there is currently too little evidence to say whether EPCs either are a major cause of plaque neovascularization in general or play a role in atherogenesis; equally, the lack of clinical trial data precludes statements about the safety or efficacy of EPC therapy for cardiovascular disease. ***[Epstein SE, et al, 2004]***

Role of Hypoxia

The notion that angiogenesis may contributes to the progression of lesion formation remains attractive, because it helps the developments in the understanding the role of hypoxia in regulating angiogenesis. As theoretical arguments suggest that once vessel wall thickness exceeds a critical depth as a result, for example, of intimal thickening induced by hypercholesterolemia or injury, the supply of oxygen and other nutrients to the media and neointima will be restricted by the increased distance either from the lumen or from adventitial vasa vasorum. ***[Sueishi K, et al, 1997 & Torres Filho IP, et al, 1994]***

A study of oxygen profiles in balloon-injured rabbit iliofemoral arteries found that the arterial wall oxygen supply is impaired after injury but is later compensated for by the formation of new adventitial vasa vasorum. When the critical threshold distance of 100 μm between the

adventitia and vessel lumen is exceeded, a hypoxic environment will form in the interior of the artery. ***[Torres Filho IP, et al, 1994]***

Which in turn will provide a stimulus for the accumulation of hypoxia-inducible transcription factors (HIFs) such as HIF-1α that induce expression of VEGF and other angiogenic regulators. ***[Pugh CW, et al, 2003]***

Secretion of VEGF stimulates angiogenesis, thereby promoting plaque growth by increasing the oxygen supply to the media and neointima. Consistent with this hypothesis, expression of VEGF, FGF, and HIF-1α has been demonstrated in atherosclerotic lesions. It is important to emphasize that, according to this model, angiogenesis does not initiate plaque formation but serves as a permissive factor allowing later plaque growth once a critical arterial thickness has been reached. ***[Belgore F, et al, 2004 & Brogi E, et al, 1996]***

Oxidant stress has been reported as an alternative hypoxia independent pathway to trigger an angiogenic switch and to enhance arterial lesion formation in a transgenic mouse model overexpressing NAD (P) H oxidase. Extensive neointimal vascularization was observed in the transgenic mice model that over expressing NAD (P) H oxidase compared with controls, but the correlation between the extent of neovascularization and lesion size was not discussed. ***[Khatri JJ, et al, 2004]***

Role of inflammation

Plaques vulnerable to rupture are commonly composed of a lipid core separated from the vessel lumen by a thin fibrotic cap and contain higher macrophage counts. Monocytes and macrophages release mitogenic, proinflammatory, prothrombotic, and tissue lytic factors that enhance progression of atherothrombotic lesions. Macrophages and vascular smooth muscle cells (VSMCs) also secrete angiogenic factors, several of which are expressed in human atherosclerotic lesions. ***[Libby P, 2002 & Varnava AM, et al, 2002]***

The shoulder regions of these plaques, where the cap joins the remainder of the arterial wall, constitute rupture-prone "hot spots" where fissures and tears are most likely to occur. These shoulder regions usually possess the greatest density of inflammatory cells, which are capable of secreting extracellular matrix-degrading metalloproteinases (MMPs) that weaken the fibrous cap, thereby facilitating rupture of the atherosclerotic plaque. ***[Rajagopalan S, et al, 1996]***

MMPs and their cosecreted tissue inhibitors have been reported to be critical in atherogenesis and in VSMC migration across the basement membrane. Interleukin (IL)-8, also produced by macrophages infiltrating atherosclerotic plaques, has been shown to have angiogenic activity equipotent to that of VEGF and FGF-2. ***[Galis ZS, et al, 2002]***

IL-8 was detected almost exclusively in atheromatous human coronary atherectomy specimens compared with normal coronary arteries

and was associated with an angiogenic response that was completely inhibited with neutralizing anti–IL-8 antibodies. ***[Simonini A, et al, 2000]***

An alternative explanation for the occurrence of plaque vessels is the local production of proangiogenic factors in the plaque by infiltrated inflammatory cells such as activated monocytes/macrophages, creating a "neovascular" milieu. In a similar fashion, inflammatory cell release of MMP in tumor progression models mobilizes VEGF and thereby initiates the angiogenic switch. ***[Bergers G, et al, 2000]***

Nonetheless the view that inflammatory cells drive angiogenesis in atherosclerotic plaques is supported by the finding that vasa vasorum density in the atherosclerotic lesions of hypercholesterolemic mice is highly correlated with the occurrence of foci of inflammatory cells rather than atheroma size. ***[Moulton KS, et al, 2003]***

In addition that plaque vessels could promote atherosclerosis by providing a conduit for leukocyte entry, thereby facilitating the recruitment of inflammatory cells to the plaque. In support of this hypothesis, microvessels within lipid-rich plaques strongly express adhesion molecules that would facilitate transendothelial migration of inflammatory cells into the plaque microenvironment. ***[De Boer OJ, et al, 1999]***

According to such a model, angiogenesis is both the result of and an amplifier of inflammation. However, effects of angiogenic cytokines in atherosclerosis-associated inflammation may not be mediated solely by

angiogenesis. As mentioned, administration of an inhibitory antibody directed against the VEGF Flt-1 receptor limited growth of atheromatous plaques in hypercholesteolemic mice through a reduction in the mobilization and infiltration of hematopoietic cells, including monocytes, granulocytes, and progenitor cells, but without any concomitant inhibition of plaque neovascularization. ***[Luttun A, et al, 2002]***

Nonetheless a more recent research proved that inflammatory cells and mediators are key players in the mechanisms mediating both atherogenesis and collateral vessel formation, something that forms a key component in the "Janus phenomenon" in which interventions that enhance collaterogenesis also increase atherosclerosis. ***[Epstein SE, et al, 2004]***

Therefore the role of angiogenesis in destabilization and rupture of atherosclerotic lesions remains a debatable and refractory problem, also the underlying causes of plaque instability may bring a fresh perspective. It has been argued that VSMC-rich lesions are stable because of their high cellular content, whereas relatively acellular lesions, with a higher degree of calcification, fibrosis, and lipids, are more prone to fracture and rupture. ***[Shanahan CM, et al, 1999]***

However, it could be argued that, by enriching the supply of nutrients to the plaque core, plaque neovascularization may increase plaque cellularity and thereby act as an underlying cause of plaque stabilization. On the contrary atheromas develop microvascular channels as a result of neovascularization; these new vessels are both fragile and

prone to hemorrhage. Intraplaque deposition of fibrin, fibrin-split products, and hemosiderin provides evidence of intraplaque hemorrhage. ***[Jeziorska M, et al, 1999]***

Thrombosis in situ leads to generation of thrombin, which potently triggers the release of PDGF, further stimulating the migration and proliferation of VSMCs. Activated platelets also elaborate transforming growth factor-ß, the most potent stimulus known for interstitial collagen synthesis by VSMCs. Hence, plaque neovascularization and subsequent silent microvascular hemorrhage could possibly participate in a vicious circle of growth spurts that might contribute plaque instability rather than plaque stability. ***[Blakytny R, et al, 2004]***

It remains to ask whether inhibition of angiogenesis could be a therapeutic target in atherosclerotic disease. The available evidence suggests that, although antiangiogenic therapies may potentially have some effect on the growth of atherosclerotic and neointimal lesions, particularly in vein graft stenosis and restenosis after angioplasty, however any benefit is likely to be nullified by the harmful effects of inhibiting endothelial function and regeneration. This is also supported by work from a number of laboratories indicating that VEGF exerts protective effects on the arterial endothelium. ***[Khurana R, et al, 2004 & Zachary I, et al, 2000]***

Recent evidence from clinical trials of the VEGF inhibitory antibody Avastin for cancer indicates that up to 5% of all patients treated with Avastin may have an increased risk of thromboembolism, including

cerebrovascular events, myocardial infarction, and deep vein thrombosis. ***[Ratner M, 2004 & Simons M, 2005]***

These findings suggest that endogenous VEGF may play an arterioprotective role in the adult human vasculature. The multiplicity of the biological roles of VEGF and the importance of endothelial integrity for vascular function are both strong arguments against an antiangiogenic approach to the treatment of cardiovascular disease. ***[Ratner M, 2004 & Simons M, 2005]***

Chapter III

Vascular Endothelial Growth Factor; The key role of angiogenesis: Implications in IHD

Introduction:

The development of new blood vessels is essential to embryonic growth and throughout life for physiological repair processes such as wound healing, post-ischemic tissue restoration, and the endometrial changes of the menstrual cycle. However, abnormal development of new blood vessels has been implicated in numerous pathophysiological processes. For example, inhibited growth of blood vessels is associated with bowel atresia and peptic ulcers. ***[Folkman J, 1995]***

Furthermore, although generally focusing on tumour growth, increased vascular growth has been demonstrated in many other non-malignant diseases such as rheumatoid arthritis, systemic lupus erythematosus, psoriasis, proliferative retinopathy as well as in ischemic heart disease in which the formation of new capillaries (angiogenesis) may be of clinical importance in facilitating reperfusion and regeneration of hibernating cardiac tissue after myocardial infarction and in microvascular ischemia. ***[Ton J Rabelink, et al, 2004& CouffinhalT, et al, 1997]***

VEGF is a key regulator of angiogenesis and the majority of the knowledge of VEGF originates from work done as part of studies in cancer research, as the ability of a tumor to metastasize seems be related to the quantity of VEGF produced as VEGF has been detected in

numerous tumor cells and in the plasma of patients with various cancers. Hypoxia appears to play an important part as the expression of VEGF mRNA and production of the growth factor is intensified in regions neighbouring the necrotic area. ***[Belgore FM, et al, 2001, Faller DV, 1999, Molica S, et al, 1999 & Millauer B, et al,1993]***

In addition, VEGF may also have a role in the regulation of inflammatory repair processes as VEGF increases vascular permeability and acts as chemotactic agent for phagocytic cells, both processes of eminent importance during inflammation. Again hypoxia, a common feature in damaged tissue, seems to be the underlying mechanism. ***[Ausprunk DH, et al, 1997 & Detmar M, et al, 1997]***

Angiogenesis related research in cardiovascular medicine has been initially linked to ischemic heart disease and atherosclerosis. The observed raised angiogenic markers resulted in a theory of impaired angiogenesis in cardiovascular disease. Therefore one therapeutic direction in ischemic vascular disease has been to use various angiogenic growth factors in an effort to improve vascularization and more recently the role of angiogenesis in the pathophsiology of hypertension and atherosclerosis has also been investigated. ***[Tabibiazar R, et al, 2001 & Belgore F, et al, 2001]***

Atherosclerosis eventually results in progressive arterial occlusion which leads to ischemia, hypoxia and subsequently to necrosis. These processes trigger the expression of a variety of vasoactive substances,

matrix proteins and growth factors, which mediate neovascularization, remodelling of the vasculature and surrounding tissue. ***[Faller DV, 1999]***

Animal studies of VEGF in various aspects of cardiovascular disease have provided pilot data for studies in man. For example, histological studies of coronary atherosclerotic plaques, saphenous vein bypass grafts, and areas of recent myocardial infarction that demonstrated increased VEGF expression gave a way to observational clinical studies. ***[Bobryshev YV, et al, 2001, Burton PBJ, et al, 2000]***

Pathophysiological possibilities include the suggestion that acute myocardial ischaemia rapidly induced up-regulation of VEGF and its receptors VEGFR-1 and VEGFR-2. These data would suggest that VEGF plays a role in neovascularization in connection with myocardial ischaemia and atherosclerotic arteries. ***[Lee SH, et al, 2000 & Chen YX, et al, 1999]***

Moreover, in patients with coronary artery disease there is a correlation between the directly measured index of collateral blood flow and intracoronary levels of VEGF, suggesting that VEGF is influenced by degree of coronary atherosclerosis. ***[Fleisch M, et al, 1999]*** However, generally, the precise role(s) of large amounts of circulating VEGF in the plasma of subjects with long-standing peripheral or coronary atherosclerosis, or in acute myocardial infarction compared to asymptomatic controls is unclear. ***[Roller RE, et al, 2001 & Hojo Y, et al, 2000]***

Overview of the formation of the vascular system

The formation of the vascular system is fashioned by three processes:

- Vasculogenesis.
- Angiogenesis.
- Arteriogenesis.

During embryogenesis, there is differentiation of embryonic mesenchymal cells (the endothelial precursor cells or angioblasts) into endothelial cells resulting in de novo development of blood vessels (vasculogenesis). ***[Risau W, et al, 1995]***

The second step is angiogenesis, which refers to the formation of new blood vessels by sprouting from pre-existing small vessels in adult and embryonic tissue (sprouting angiogenesis) or by intravascular subdivision. The existing vasculature can be transformed into a mature network by processes of pruning and remodelling. Table (2) Thirdly, arteriogenesisis defined as rapid proliferation of pre-existing collateral vessels. The Both processes: angiogenesis and arteriogenesis occur as a result of occlusion of a vessel, thus improving blood delivery and local perfusion of ischemic tissue. ***[Ton J Rabelink, et al, 2004 & Risau W, 1997]***

Angiogenesis related research in cardiovascular medicine has initially been linked to ischemic heart disease and atherosclerosis. The observed raised angiogenic markers resulted in a theory of impaired angiogenesis in cardiovascular disease. Therefore one therapeutic

direction in ischemic vascular disease has been to use various angiogenic growth factors in an effort to improve vascularization and then the role of angiogenesis in hypertension and atherosclerosishas also been investigated. ***[Tabibiazar R, et al, 2001 & Belgore F, et al, 2001]***

Phase	***Key events***
Endothelial cell activation	Morphological changes of endothelial cells priming them for proliferation and secretion, local vasodilatation, increased vascular permeability, accumulation of extravascular fibrin
Degradation of basement membrane	Angiogenic stimulus results in proteolytic vascular basement membrane degradation
Migration of endothelial cells	Chemotactic factors produced by fibroblasts, monocytes and platelets induce endothelial cell migration and sprouting
Proliferation of endothelial cells	Locally produced mitogens induce endothelial cells DNA synthesis and mitosis
Differentiation of endothelial cells	Endothelial cell proliferation decreases and cell–cell contact re-establish, which leads to lumen development
Reconstitution of basement membrane	Vessel maturation achieved by reconstitution of basement membrane synthesized by endothelial cells
Vasculature maturation and stabilization	Capillary remodelling by stabilization and regression

Table (2): This table illustrates the key events in the process of angiogenesis.

Angiogenic Growth factors

The existence of angiogenic factors was first observed with the isolation of a tumour factor that generated mitogenic activities in

endothelial cells and later found to be a member of the Fibroblast growth factor (FGF) family. Angiogenic growth factors are produced by a variety of different cells, and their functions include close involvement in tumor development as well as tumor angiogenesis. ***[Folkman J, et al, 1992]***

Indeed, angiogenic growth factors such as vascular endothelial growth factor (VEGF), FGF and angiopoietin are essential to angiogenesis. Further to the initiation of angiogenesis, these growth factor regulators establish the rate and extent of angiogenesis. However, little data are available about the resolution phase of angiogenesis and it is unclear if this process results from exhaustion of these growth factors or if negative regulators predominate in this phase. ***[Fong G, et al, 1995]***

Angiogenic growth factors are so-called because of their varying ability to induce the proliferation of various cells in vitro, which contribute to the process of angiogenesis in vivo, as demonstrated by studies of animal models. These growth factors are produced by various cell types and include a diverse range of proteins in addition to VEGF and FGF: platelet derived growth factor, tumour necrosis factor, insulin like growth factor-1, transforming growth factor, angiogenin, hepatocyte growth factor, placental growth factor and several others. ***[Friesel RE, et al, 1995]***

Of the previous number of angiogenic growth factors described, the FGF and VEGF families have been most extensively researched and will be described in more detail.

- **Fibroblastic growth factor**

The first angiogenic growth factor to be discovered, this family currently comprises at least 20 molecules with extensive mitogenic potentials representing some of the most potent angiogenic peptides.
They are produced by vascular endothelial and smooth muscle cells, hence their almost widespread distribution. With numerous biological activities, including induction of proliferation of a wide range of cells, the FGFs are closely involved in several developmental and pathophysiological processes. ***[Lim HS, et al, 2004 & Friesel RE, et al, 1995]***

They stimulate fibroblast as well as endothelial cell growth and are therefore of vital importance in the process of angiogenesis, and also play a significant part in the three phases of wound healing: inflammation, repair and regeneration. Further important functions of FGFs include tumour development and progression. ***[Ichimura T, et al, 1996]***

The biological responses of FGF are mediated through the activation of four specific receptors, membrane tyrosine kinases resulting in an increase of multiple isoforms of FGF due to alternative mRNA splicing. The two most widely researched isoforms are FGF-1 and FGF-2. ***[Xu X, et al, 1999]***

Fibroblastic growth factor - 1

Also known as the acidic FGF, in its mature form it is a 16kD peptide. FGF-1 stimulate DNA synthesis without signalling through a cell surface receptor, suggestive of an intracrine mechanism transmitting a

nuclear localization signal. ***[Lim HS, et al, 2004 & Wiedlocha A, et al, 1994]***

Like other members of the family, FGF-1 has mitogenic and chemotactic effects especially on fibroblasts, endothelial cells and smooth muscle cells. It also contributes to the control of capillary progression, wound healing and tumour progression. Not surprisingly, FGF-1 expression is increased during regeneration of endothelial cells, hypoxia and collateral formation. However, in vivo studies looking into its potential therapeutic use have been disappointing. ***[Kuwabara K, et al, 1995]***

Fibroblastic growth factor - 2

This single chain 18kDa polypeptide is also referred to as basic FGF and has a 55% sequence identity with FGF-1. Hypoxia, in addition to a number of other growth factors, increases its activity. FGF-2 is one of the most potent mitogens and chemotactic factors of the vascular endothelial cell. It has been demonstrated that basic FGF and VEGF have synergistic effects on angiogenesis in vivo. Numerous studies are investigating the potential role of FGF-2 and VEGF in the pathophysiology of coronary artery disease. ***[Asahara T, et al, 1998]***

- **Vascular endothelial growth factor**

Vascular endothelial growth factor is considered the major putative angiogenic growth factor involved in angiogenesis and neovascularization. It was initially known as vascular permeability factor (VPF) which purified from tumour cell ascites, its biological effects were

subsequently shown to extend to endothelial cell mitogenesis, prompting the name change to VEGF. ***[Nouran El Ghandour, et al, 2006, Blann AD, et al , 2000 & Ferrara N, et al, 1992]***

VEGF is now known to be a multifunctional peptide capable of inducing receptor-mediated endothelial cell proliferation and angiogenesis both in vivo and in vitro. In addition to its crucial role in embryonic vascular development, VEGF has been implicated in the process of neovascularization in adult pathophysiology. VEGF is a basic, 45kDa disulfide-linked dimeric glycoprotein, and is structurally related to platelet derived growth factors. ***[Carmeliet P, et al, 1997 & Neufeld G, et al, 1999]***

VEGF loses all biological activities following reduction and dissociates into monomeric units between 17 and 23kDa. The various VEGF iso-proteins have been described to have a circulating half-life of between 10 min and 6h, depending upon the isoform, and the exogenous stimulus. The whole VEGF family currently consists of at least five members whose effects are mediated mainly via two high affinity membrane spanning receptors: fms-like tyrosine kinase (Flt-1) and kinase domain receptor (KDR), these receptors communicate with the cell interior via transmembrane receptor tyrosine kinases (RTKs). ***[Nouran EL Ghandour, et al, 2006, Enholm B, et al, 1998 , Levy AP, et al, 1997 & Levy NS, et al, 1997]***

VEGF – A: Interestingly, the human chromosome 6, that encodes for the VEGF-A gene, the first VEGF protein identified, is also a location

giving origin to several human disorders with different unidentified genetic defects. ***[Vincenti V, et al, 1996 & Volz A, et al, 1994]***

The VEGF gene sequence extends over approximately 14kb, encoding eight exons that are separated by seven introns. Through alternate exon splicing of this gene, different mRNA are encoded producing five biologically active proteins ($VEGF_{121}$, $VEGF_{145}$, $VEGF_{165}$, $VEGF_{189}$ and $VEGF_{206}$). ***[Lim HS, et al, 2004 & Park JE, et al, 1993]***

All VEGF-A transcriptions have a common signal peptide terminal enabling its identification by VEGFR Flt-1 and KDR. Exons six and seven code for peptides determining the capability of binding to the extra-cellular matrix and/or heparan sulphate proteoglycan (Heparan sulphate (HS) is a linear polysaccharide that found in all animal tissues as a proteoglycan (PG) in which two or three HS chains are attached in close proximity to cell surface or extracellular matrix proteins. It binds to a variety of protein ligands and regulates a wide variety of biological activities, including developmental processes, angiogenesis, blood coagulation and tumour metastasis). All VEGF isoforms are secreted glycoproteins. They are able to homodimerize and bind to heparan sulphate (except $VEGF_{121}$). ***[Keyt BA, et al, 1996]***

$VEGF_{165}$, often referred to as VEGF-A or simply VEGF, is the predominant human isoform secreted by a variety of normal and transformed cells. Although all human VEGF-A isoforms are able to induce in vivo angiogenesis, there are, however, differences in their capability to bind VEGFR (Flt-1). The soluble glycoproteins $VEGF_{121}$,

$VEGF_{145}$ and $VEGF_{165}$ can be detected by biochemical assays (e.g. ELISA) of fluid samples such as human serum and plasma. ***[Blann AD, et al, 2002, Seko Y, et al, 1997, Lip PL, et al, 2000 & Webb NJ, et al, 1998]***

$VEGF_{121}$is a weakly acidic polypeptide failing to bind to heparan sulphate, whereas the VEGF isoforms $VEGF_{189}$ and $VEGF_{206}$ are more basic and exhibit higher affinity to heparin sulphate than $VEGF_{165}$. The differences in the affinity for heparan sulphate and in the isoelectric point have a profound effect on the bioavailability of VEGF, leaving larger VEGF isoforms almost completely cell associated and bound to extra-cellular matrix. ***[Park JE, et al, 1993]***

Only the isoform $VEGF_{165}$ is freely diffusible and able to bind to heparan sulphate, which is an indicator of its mitotic activity for vascular endothelial cells. There is also evidence to suggest that the stability of the VEGF–heparan sulphate receptor complex may contribute to effective signal transduction and therefore proliferation of the vascular endothelial cells. In contrast, $VEGF_{206}$ is the rarest isoform and has only been discovered in human foetal liver. ***[Keyt BA, et al, 1996 & Park JE, et al, 1993]***

VEGF – B: This member of the VEGF gene family is composed of 188 amino acids and can be expressed as homodimer or heterodimer with VEGF-A. Alternate splicing of the VEGF-B gene, situated on chromosome 11, results in two isoforms. $VEGF\text{-}B_{167}$ is a soluble peptide and $VEGF\text{-}B_{189}$ is bound to the cell and extra-cellular matrix and has been shown to stimulate vascular endothelial cell proliferation. VEGF-B may

contribute to the regulation of angiogenesis in muscle tissue. ***[Olofsson B, et al ,1996 , Olofsson B, et al, 1996 & Joukov V, et al,1997]***

VEGF – C: VEGF-C is a protein composed of 419 amino acids, with a predicted molecular mass of 47kDa. Its gene is located on chromosome 4. VEGF-C shares 30% of the VEGF homology domain and can be found in small quantities in myocardium, placental tissue, skeletal muscle, ovaries, in certain tumour cell lines and is present in platelets. ***[Wartiovaara U, et al, 1998, Chilov D, et al, 1997 & Joukov V, et al ,1997]***

It is involved in the formation and maintenance of the venous and lymphatic systems and promotes lymphatic endothelial cell proliferation and vessel enlargement. Nonetheless, there is also data to suggest that VEGF-C may possess angiogenic properties relating to capillaries. The actions of both VEGF-C and VEGF-B are mediated via their receptors Flt-1 and Flt-4. ***[Aase K, et al ,1999, Witzenbichler B, et al,1998 & Kukk E , et al, 1996]***

VEGF –D: The latest member of the human VEGF family to be described in detail, VEGF-D, shares 61% homology with VEGF-C and its gene is located on chromosome 22. Human VEGF-D seems to be generated by proteolytic processing of precursor polypeptides. VEGF-D is recognized by VEGFR-2 and VEGFR-3, which are present on endothelial cells, and appears to be capable of stimulating lymphangiogenesis. There is further evidence to suggest that VEGF-D

may promote the spread of tumour cells via the lymphatic system. ***[Staker SA, et al, 2001, Karkkainen MJ, et al, 2000 & Achen MG, et al, 1998]***

VEGF – E: Based on the sequence of VEGF-A_{121}, a further VEGF variant**,** VEGF-E, was discovered in the genome of Orf virus. The Orf virus is an epitheliotropic parapoxvirus which induces proliferative skin lesions in goats, sheep and humans (seen as 'milker's nodules'). VEGF-E binds with high affinity to VEGFR-2 resulting in stimulation of angiogenesis and vascular permeability, therefore enhancing viral infection. ***[Savory LJ, et al, 2000 & Meyer M, et al, 1999]***

VEGF receptors

In humans, the effects of VEGF on endothelial cells is mediated mainly via two high-affinity membrane receptors, VEGFR-1 and VEGFR-2. They are also referred to as RTK. Both receptors have a high affinity for VEGF and possess seven characteristic immunoglobulin-like domains that form the extra-cellular section. In addition to, a kinase-insert domain that links the transmembrane region. ***[Park M, et al, 1999]***

VEGFR-1 and VEGFR-2 are 33% identical in their extra-cellular domain and 80% in their kinase domains. Both receptors are predominantly expressed on endothelial cells, but have also been detected on human uterine, colonic and aortic smooth muscle cells, trophoblasts and in foetal kidney. VEGFR-3 is a further RTK with seven immunoglobulin-like domains. This receptor is mainly expressed in lymphatic vessels and binds only VEGF-C and –D. ***[Simon M, et al, 1995 & Charnock-Jones D, et al, 1994]***

VEGFR-1: Vascular endothelial growth factor receptor-1 (VEGFR-1), also known as fms-like tyrosine kinase-1 (Flt-1), is a 180kDa. The human gene is located on chromosome 13. VEGFR-1 and VEGFR-2 are predominantly expressed on the vascular endothelium, but traces of mRNA have been located in monocytes, renal mesangial cells and stroma of human placenta. VEGF-A_{121}, VEGF-A_{165}, and VEGF-B, associate with this receptor with varying affinity. VEGF-A_{165} binds to VEGFR-1 with high affinity than VEGF-A_{121}. The ability of the receptor to attach heparan-sulphate proteoglycan is eluded after the removal of the second immunoglobulin-like domain of VEGFR-1. ***[Barleon B, et al,1996, Keyt BA, et al,1996, Sawano A, et al,1996, Gitay-Goren H, et al, 1996 & Wilting J, et al, 1996]***

In addition to the full-length receptor, the VEGFR-1 gene encodes for a soluble form carrying only six immunoglobulin domains. This form results from differential splicing of the Flt-1 mRNA and was first discovered in human umbilical vein endothelial cells. This soluble receptor, referred to as soluble Flt-1 (sFlt-1), attaches itself to $VEGF_{121}$ with a high affinity, and is present in human plasma and amniotic fluids from pregnant women. ***[Hornig C,et al,2000 & Vuorela P, et al, 2000]***

The biological implications of sFlt-1 remain unknown although in vitro studies have demonstrated that it is capable of reducing VEGF-induced mitogenesis. Therefore, sFlt-1 may correspond to a physiological regulatory mechanism for reducing VEGF action. ***[Kendall RL, et al, 1996]***

VEGFR-2: The gene of the second VEGF tyrosine-kinase receptor, VEGFR-2, is located on chromosome 4. VEGFR-2 is also known as kinase- domain containing receptor (KDR), and is homologous to the foetal liver kinase-1 (flk-1) receptor in mice. KDR is predominantly expressed in endothelial cells and was cloned from a human endothelial cell. ***[Millauer B, et al, 1993]*** However, the mRNA for this receptor can also be detected in haematopoietic stem cells, megakaryocytes and retinal progenitor cells. ***[Khaliq A,et al,1996 , Vuckovic , et al,1996 & Yang XJ , et al ,1996]***

VEGFR-1 and VEGFR-2 transduce signals for endothelial cells in response to ligands of the VEGF family. Their individual reaction is distinctively different. Unlike Flt-1, the final glycosylated form of KDR undergoes VEGF-triggered auto-phosphorylation, which may explain the much weaker response of VEGFR-1 activation. KDR binds $VEGF_{121}$, $VEGF_{145}$, $VEGF_{165}$VEGF-C and VEGF-D. Despite numerous similarities between VEGFR-1 and VEGFR-2, a naturally occurring soluble form of KDR comparable to sFlt-1 has not been described. ***[Millauer B,et al,1996]***

VEGFR-3: The VEGFR-3 gene is encoded in the chromosomal 5. VEGFR-3 is also known as fms like tyrosine kinase 4 (Flt-4) and its extra-cellular domain is 80% homologue to the other VEGFR Only VEGF-C and VEGF-D of the VEGF family are associated with Flt-4. ***[Park M, et al, 1999 & Achen MG, et al, 1998]***

Unlike VEGFR-1 and VEGFR-2, Flt-4 is predominantly expressed in lymphatic endothelium in adult tissue. However, in most vascular endothelial cells low levels of VEGFR-3 are detectable. Its presence, particularly on lymphatic endothelial cells and on developing vessels of several organs suggests that Flt-4 together with its ligands may have a role in the regulation of growth and differentiation of the lymphatic system. ***[Staker SA, et al, 2001]***

Neuropilins

In addition to VEGFR-1 and VEGFR-2, endothelial cells express neuropilin-1 (Neu-1) and neuropilin-2 (Neu-2), which selectively bind (but with low affinity) VEGF-A_{165}. Due to a short intracellular domain of these receptors they are not likely to operate as an independent receptor. This is further supported by lack of cellular response when stimulating only the neuropilins. ***[Soker S, et al, 1998]***

However, during the embryonic stages of angiogenesis neuropilin-1 seems to regulate blood vessel development, suggesting a role as coreceptor for VEGFR-2The genetic encoding and exact biological purpose has yet to be discovered. ***[Kitsukawa T, et al, 1997]***

- **Placental growth factor**

The first VEGF-related protein, placenta growth factor (PlGF), discovered in 1991, owes its name to the predominance in placental tissue. It was later identified as a member of the VEGF family as the molecule shares 53% of a homologous domain with the platelet derived growth factor-like region of VEGF. ***[Lim HS, et al, 2004]***

Three isoforms arise by means of alternate splicing, PlGF-1, PlGF-2 and PlGF-3. These molecules are, like VEGF, dimeric glycoproteins. However, the PlGF expression pattern is limited to the placenta and some forms of tumours such as brain tumours and renal cell carcinoma. PlGF homodimers bind VEGFR-1 (Flt-1), but have little effect on angiogenesis in vitro. ***[Cao Y, et al, 1997, Takahashi A, et al, 1994 & Weindel K, et al, 1994]***

On the other hand, naturally occurring VEGF/PlGF heterodimers, identified in rat glioma cells, are mitogenic; their potency is approximately seven fold lower than that of the VEGF homodimer. Taking into consideration, the reports of hypoxia-induced up-regulation of VEGF/PlGF in vitro, it seems possible that PlGF and VEGF may be coexpressed in vivo. ***[Cao Y 1996]***

- **Angiopoietin**

A further family of growth factors involved in the early processes of angiogenesis and vasculogenesis are the angiopoietins. One isotype, angiopoietin 1 (Ang1) is present in tissues adjacent to blood vessels suggesting a paracrine mode of action, whilst another, angiopoietin 2 (Ang2) is only found at sites of tissue remodeling. ***[Maisonpierre PC, et al, 1997 & Davis S, 1996]***

Both angiopoietins, as well as the more recently discovered angiopoietin-3 (in mouse) and angiopoietin-4 (in humans), have been identified as ligands for 2 types of receptors. ***[Valenzuela DM 2000]***

In vitro neither Ang1 nor Ang2 have mitogenic effects. However, Ang1 facilitates endothelial cell sprouting and vascular network maturation. Ang2 antagonises Ang1 by blocking Ang1-induced phosphorylization. ***[Lim HS, et al, 2004, Koblizek TI, et al, 1998 & Maisonpierre PC, et al, 1997]***

On the other hand Ang2, in combination with VEGF, promotes neovascularization. ***[Asahara T et al., 1998]*** Knock-out mice for Ang1 genes demonstrate an embryonic lethal phenotype caused by defective embryonic development of the vasculature resulting in immature vessels and lack of branch network. ***[Sato TN et al., 1995 & Suri C 1996]***

The findings indicate a contribution of the angiopoietin system at later stages in the vascular development. This system appears to be particularly involved in the determination of the subdivision of the initially homogeneous capillary network into larger arterioles and venules. ***[Suri C, et al, 1996]***

A mutation of the angiopoietin receptor in mice leads to vascular dysmorphogenesis, possibly resulted by a lack of peri-endothelial support cell recruitment, resulting in underdevelopment of smooth muscle cell layers. ***[Vikkula M, et al, 1996]***

Regulation of VEGF production

As a key regulator, it is essential that the expression of VEGF is itself correctly controlled in order to prevent uncontrolled angiogenesis. There are a plethora of cytokines, growths factors and physiological

parameters modulating the production of VEGF, depending on the different states. In the mature organism, VEGF expression is limited and a balance between angiogenic and anti-angiogenic stimuli is maintained. ***[Blann AD et al., 2002]***

However, in response to tissue damage, a wide array of growth factors, cytokines and other molecules are released stimulating angiogenesis directly or indirectly via VEGF which is essential for the repair process. ***[Blann AD, et al, 2002]***

In pathophysiological situations such as cancer and diabetes mellitus, stimulated VEGF expression might result in increased pathological angiogenesis. This hypothesis is further supported by data demonstrating a suppression of neovascularization by inhibition of VEGF or its effects. ***[Adamis AP, et al, 1996 & Aiello LP, et al, 1995]***

However, in other circumstances, such as atherosclerosis and diabetes, the increased plasma VEGF concentration might be an attempt to compensate for tissue damage or hypoxia, or may simply reflect endothelial cell damage apparent in these conditions. ***[Blann AD, et al, 2002]***

- **The interaction of VEGF with cytokines and other growth factors**

Factors that can increase VEGF production include platelet derived growth factor, tumour necrosis factor-α (TNF-α), fibroblast growth factor 4 (FGF 4), bFGF, transforming growth factor-ß (TGF-ß), PDGF,

angiotensin-2, insulin-like growth factor I, keratinocyte growth factor, interleukin 1 (IL-1) and IL-6. ***[Felmeden D, et al, 2003 & Williams B 1997]*** A few substances, such as the cytokines IL-10 and IL-13, decrease VEGF production. ***[Matsumoto K 1997]***

The angiopoietins also influence VEGF release. Ang-1 stimulates vessel sprouting whereas Ang-2 inhibits this effect, also they mediates destabilization of vessel integrity, which in turn facilities vessel sprouting in response to VEGF. These effects are mediated via the angiopoietins receptors. The combination of VEGF, Ang-1 and Ang-2 is essential for successful angiogenesis as established in vivo experiments. ***[Asahara T et al., 1998, Maisonpierre PC, et al, 1997, Suri C, et al, 1996 & Davis S, et al, 1996]***

- **Effect of oxygen on VEGF expression**

Apart from growth factors there is a variety of chemical stimuli affecting the release of VEGF. Hypoxia, which occurs in pathophysiological processes such as atherosclerosis, solid tumours and proliferative retinopathy, is a major stimulator of VEGF expression resulting in neovascularization. Hypoxia induces a protein called hypoxia inducible protein complex (HIPC) or hypoxia-inducible factor (HIF). ***[Belgore F, et al, 2001]***

This transcriptional regulator is activated by reduced oxygen tension and up-regulates the transcription of VEGF mRNA. HIF increases production of VEGF mRNA with enhanced stability by directly attaching to a HIF binding-site located in the VEGF promoter region. Furthermore

VEGFR-1 seems to be up-regulated through hypoxia induced HIF. ***[Gerber HP, et al, 1997 &. Levy AP, 1997]***

Hypoxia not only increases VEGF production but it also seems to increase the stability of some VEGF isoforms. With regard to stability, VEGF-A isoforms are hypoxia sensitive whereas hypoxia has little or no effect on VEGF-B and VEGF-C mRNA. This variation in the behaviour of VEGF isoforms may be another regulatory mechanism, that ensures that the different VEGF species are tissue and/or functionally specific. ***[Enholm B, et al, 1998]***

The importance of oxygen as a regulator of VEGF production is further emphasized by demonstrating inhibitory properties of the normoxic or even hyperoxic environment. Hypoxia-induced VEGF increase returns to baseline levels within 24h of the return of the cells to normoxia. ***[Gu JW, Adair TH 1997]***

VEGF expression is decreased in vitro and in vivo studies following hyperoxia. Additionally, hyperoxia-induced retinopathy in prematurely born mice can be prevented by intraoccular VEGF injection. These data clearly demonstrate the importance of oxygen as a regulatory mechanism of VEGF expression. ***[Klekamp JG, et al, 1999 & Yue X 1999]***

The same rationale may also partly explain raised plasma VEGF in certain cancers as the demands of the growing tumour may create a local hypoxia. ***[Belgore F, et al, 2001]***

- **Regulation of VEGF by nitric oxide**

VEGF is known to induce the release of nitric oxide (NO) from endothelial cells, and vascular endothelium and inducible NO synthase (iNOS) production are amplified during VEGF-induced angiogenesis. Therefore the physiological effects of VEGF may, at least in part, be mediated by endothelium derived NO. ***[Belgore F, et al, 2001 & Blann AD, et al, 2002]***

The vital role of NO in VEGF-induced angiogenesis has also been demonstrated in NOS knock-out mice as well as after NOS inhibition, both resulting in reduction of angiogenesis. ***[Miyazaki H, et al, 1999 & Murohara T, et al, 1998]***

NO, on the other hand, also has regulatory effects on VEGF production. As it leads to reduced stimulation of the promoter region of the VEGF gene, hence lower VEGF expression. ***[Blann AD, et al, 2002]***

Pathological circumstances coupled with impaired NO availability, such as atherosclerosis, are associated with increased VEGF levels consistent with the presence of a negative feedback loop. Increased levels of plasma VEGF have been demonstrated in patients with various risk factors for atherosclerosis such as diabetes mellitus and hypertension. ***[Belgore F, et al, 2001 & Blann AD, et al, 2002]***

- **Effect of glucose on VEGF expression**

Hypoglycaemia increases VEGF expression, which was initially thought to be an indirect consequence mediated via associated hypoxia.

However, up-regulation and increased production of VEGF have been described in cells exposed to hypoglycaemia independently of HIF (hypoxia). ***[Satake S, et al, 2001]***

After equilibration of the glucose concentrations, VEGF production returned to pre-experimental levels suggesting that acute hypoglycaemia may trigger VEGF mediated angiogenesis. ***[Satake S, et al, 1998]***

Furthermore increased intracellular Ca^{2+}levels in a glucose-deprived environment leads to activation of protein kinase C. This process induces an increase of VEGF expression, thus not only confirming previous studies but exposing its underlying mechanism. ***[Park SH, et al , 2001]***

Remarkably, not only lack of glucose but also high glucose levels result in an upsurge of VEGF mRNA, as well as production of VEGF and VEGFR-2. Several studies have demonstrated that hyperglycaemia can directly increase VEGF expression via a protein kinase C dependent mechanism, and this effect can be abolished by a protein kinase C inhibitor. ***[Hoshi S, et al, 2002 , Cha DR, et al, 2000 & Kim NH, et al, 2000]***

Hyperglycaemia induced VEGF up-regulation is also reversible by normalizing the extra-cellular glucose concentration in SMC. Hence the apparent relationship between angiogenesis, VEGF and diabetes. ***[Blann AD, et al, 2002]***

Patients and methods

This study was conducted on 78 patients (53 males and 25 females). Their age ranged between 35 and 77 years with a mean age of 54.7±9 yrs. Of these patients 57 of them were admitted to critical care department of the cairo university with acute coronary syndrome and were divided into 2 groups:

Group 1: included 31 patients who presented by unstable angina, that defined as the presence of typical angina at rest or on minimal exertion associated with acute and transient ST-T segment ECG changes but with normal cardiac enzymes, including troponin levels.

Group 2: included 26 patients who presented by non ST segment elevation myocardial infarction, The clinical diagnosis of NSTEMI was based on the occurrence of typical rise of troponin and/ or rapid rise and gradual fall of CK and CK-MB with at least one of the following:

- Ischemic symptoms
- ECG changes indicative of ischemia (ST segment elevation < 1 mm or depression)

As statin treatment decreases the level of VEGF ***[HF Alber, et al, 2002]***, patients in group 1 and 2 were furtherly divided according to the prior statin use into:

Group 1A: included statin treated patients in group 1, this group included 16 patients.

Group 2A: included statin treated patients in group 2, and this group included 13 patients.

Group 1B: included non statin treated patients in group 1, and this group included 15 patients.

Group 2B: included non statin treated patients in group 2, and this group included 13 patients.

Group 3 (control group): Twenty one patients without any known myocardial disease who referred to the cath. Lab for chest pain evaluation and their results revealed normal coronaries were served as a control group.

Exclusion criteria:

All patients had any of the following were excluded from the study:

1. patients who were subjected to urgent revascularization
2. patients who had history of cerebrovascular accidents
3. patients who had history of critical ischemia of lower extremities
4. patients who had history of recent infection
5. patients who had history of autoimmune disease or malignancy

All patients were subjected to all of the following:

1. Full history taking.
2. Routine general and systemic examination.

3. 12-lead ECG.
4. Routine labs including cardiac biomarkers and troponin.
5. Lipid profile: total cholesterol, LDL, HDL and TGs.
6. Measurement of VEGF plasma concentrations:

Venous blood sample were collected from the patients into syringes by needle aspiration. The blood were kept at 4c for 30 minutes to clot and then centrifuged. Concentration of VEGF were measured by quantitative enzyme linked immunosorbant assay.

7. Echocardiography:

Standard 2D, M mode, pulsed and color Doppler using parasternal and apical views to measure dimensions and evaluate global and regional left ventricular function.Regional left ventricular function were evaluated according to the guidelines of the American echocardiographic society using a 16-segment model. Each segment were visually graded using a semiquantitative scoring system where: 1 = normal, 2 = hypokinesia, 3 = akinesia, and 4 = dyskinesia. A global wall motion score index (WMSI) were calculated as the sum of the scores of each segment divided by the number of the visulsized segments.

8. Coronary angiogram:

Coronary angiogram was undertaken by the percutaneous transfemoral approach and all images were recorded digitally. Coronary angiograms were scored visually into a severity score (0–3) which defined the number of vessels with a luminal stenosis ⩾50% (for right, left anterior descending, and circumflex arteries or its main branch eg 1st diagonal branch of left anterior descending or obtuse marginal of left

circumflex). Then the severity and extent of CAD were graded using a modified Gensini score. The most severe stenosis in each of the three coronaries and in the left main was graded from 0 to 6 (0, no stenosis; 1, 1–29% stenosis; 2, 30–49% stenosis; 3, 50–69% stenosis; 4, 70–89% stenosis; 5, 90–99%; 6, 100% occlusion) and summed to yield a score of 0–24.

Hospital course: During hospitalization, the 5 major cardiovascular complications assessed were the following:

1) Recurring myocardial ischemia, translated by the reappearance of angina or acute myocardial infarction.
2) Heart failure defined by signs and symptoms of pulmonary congestion, requiring the use of specific therapy, such as diuretics, vasodilators, or inotropic support.
3) Cardiogenic shock defined as systolic blood pressure < 90 mm Hg for more than 30 minutes, requiring the use of vasopressors or the development of metabolic acidosis.
4) Arrhythmias requiring pharmacological treatment, electrical cardioversion or use of pacemaker.
5) death.

Statistical analysis:

Computer software package SPSS 15.0 was used in the analysis. For quantitative variables, mean/median (as a measure of central tendency), standard deviation/range, minimum, and maximum (as measures of variability) were presented. Frequency and percentages were presented for qualitative variables. Mann-Whitney, Kruskal Wallis and

Anova tests were used to estimate differences in quantitative variables. Chi-square and Fisher Exact tests were used to estimate differences in qualitative variables. Correlation to estimate association between quantitative variables was presented in the form of correlation coefficient and its significance. A probability value (p value) less than 0.05 was considered significant.

Results

This study was conducted on 78 patients (53 males & 25 females). Their age ranged between 35 & 77 yrs with a mean age of 54.7±9 yrs.

The patients were classified into:

Group 1: included 31 patients who presented by unstable angina

Group 2: included 26 patients who presented by non ST segment elevation infarction.

Patients in group 1 and 2 were furtherly divided according to the prior statin use into:

Group 1A: included statin treated patients in group 1, this group included 16 patients.

Group 2A: included statin treated patients in group 2, and this group included 13 patients.

Group 1B: included non statin treated patients in group 1, and this group included 15 patients.

Group 2B: included non statin treated patients in group 2, and this group included 13 patients.

Group 3: included 21 patients with no previous history of cardiac disease who presented to the cath. Lab for chest pain evaluation & their results revealed normal coronary angiogram.

The results will be presented as the following:

1. General characteristics of the different studied groups.
2. Clinical & laboratory data of the different studied groups as regards the following:

⇒ Risk factors of ischemic heart disease.

⇒ ECG findings.

⇒ Results of routine labs including cardiac enzymes & troponin.

⇒ Serum level of VEGF.

3. Echocardiographic findings.
4. Coronary angiogram.
5. In hospital course & prognosis.

General characteristics of the different groups:

Age: The patients age in group 1A ranged between 42 & 77 years with mean age 60.5±9.5 years, group 1B ranged between 35 & 65 years with mean age 55.33±7.68, group IIA ranged between 48 & 71 years with mean age 55.85±6.73, Group IIB ranged between 36 & 70 years with mean age 54.77±11.25, and in group III ranged between 40 & 61 years with mean age 49.19±6.43. Regarding the age there was no significant difference between the studied groups.

Age	***IA***	***IB***	***IIA***	***IIB***	***III***
Range	42-77	35-65	48-71	36-70	40-61
Mean±SD	60.5±9.5	55.33±7.68	55.85±6.73	54.77±11.25	49.19±6.43
P value	>0.05				

Table (3): Illustrates the mean age of the different studied groups

Sex: The sex distribution among the different studied groups is shown in table (4) and figure (4). There was an overall predominance of males in group 1 & 2 compared to group 3.

Sex	Group 1		Group 2		Group 3	
	N	%	N	%	N	%
Males	21	67.7	22	84.6	10	47.61
Females	10	32.3	4	15.4	11	52.38
Total	31	100.0	26	100.0	21	100.0

Table (4): Illustrates the sex distribution among the different studied groups

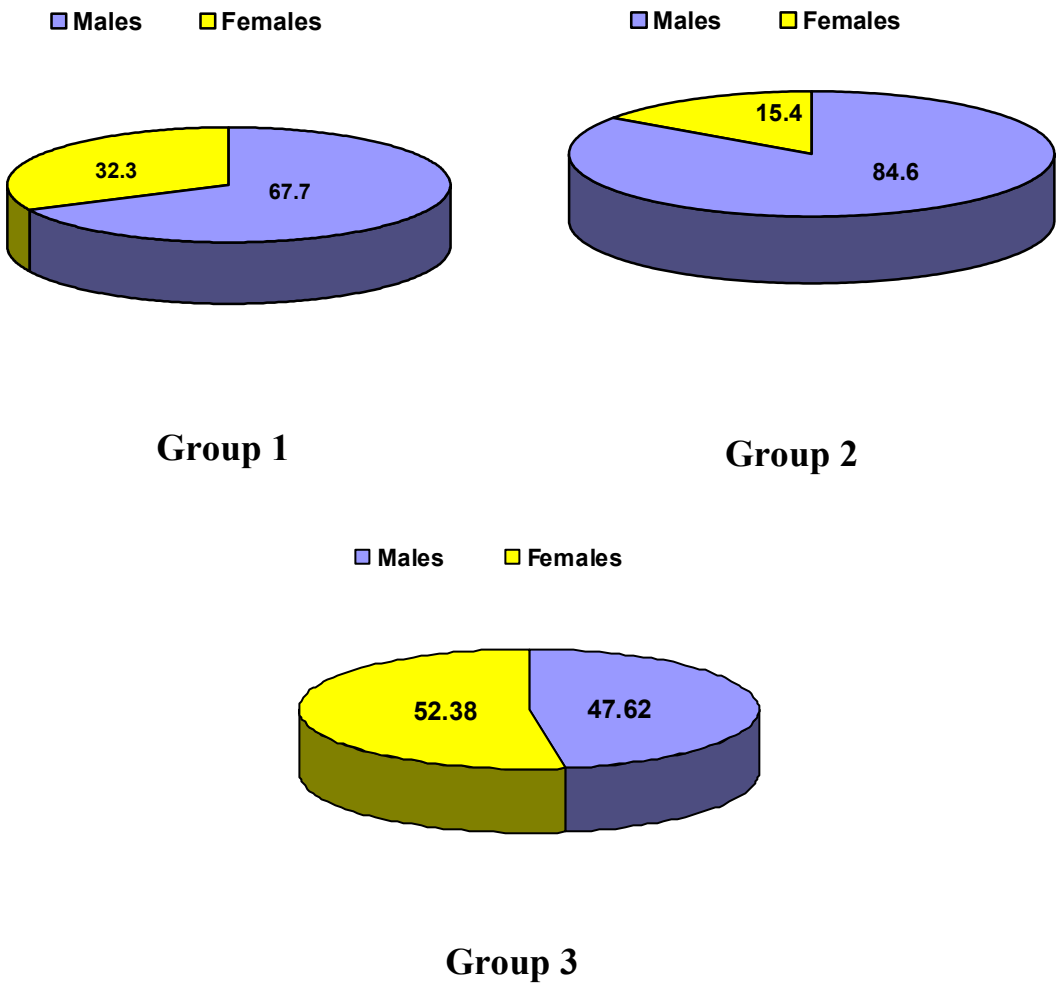

Figure (4): Illustrates the sex distribution among the different studied groups

Risk factors:

Hypertension was the main risk factor encountered among the studied patients. It was presented in 77.4%, 57.7% & 52.4% of patients in group 1, 2 & 3 respectively. Dyslipidemia was the second risk factor and it was presented in 77.2% of patients, on the other hand type I diabetes mellitus was the least risk factor encountered, that was presented only in 3.5% of the whole studied patients. These data showed that there was no stastistical significant difference between the studied groups as regards these variables. The P value for all of these variables is > 0.05. Table (5) and figure (5)

Risk factors	**Group 1**		**Group 2**		**Group 3**		**Total**	
	N	%	N	%	N	%	N	%
Type I DM	1	3.2	0	0.0	1	4.8	2	3.5
Type II DM	15	48.4	9	34.6	5	23.8	29	50.8
HTN	24	77.4	15	57.7	11	52.4	50	87.7
Smoking	12	38.7	15	57.7	6	28.6	33	57.9
Dyslipi-demia	21	67.7	16	61.5	7	33.3	44	77.2
Family History	7	22.6	5	19.2	8	38.1	20	35.1

P Value > 0.05

Table (5): Illustrates the different risk factors encountered among the studied groups.

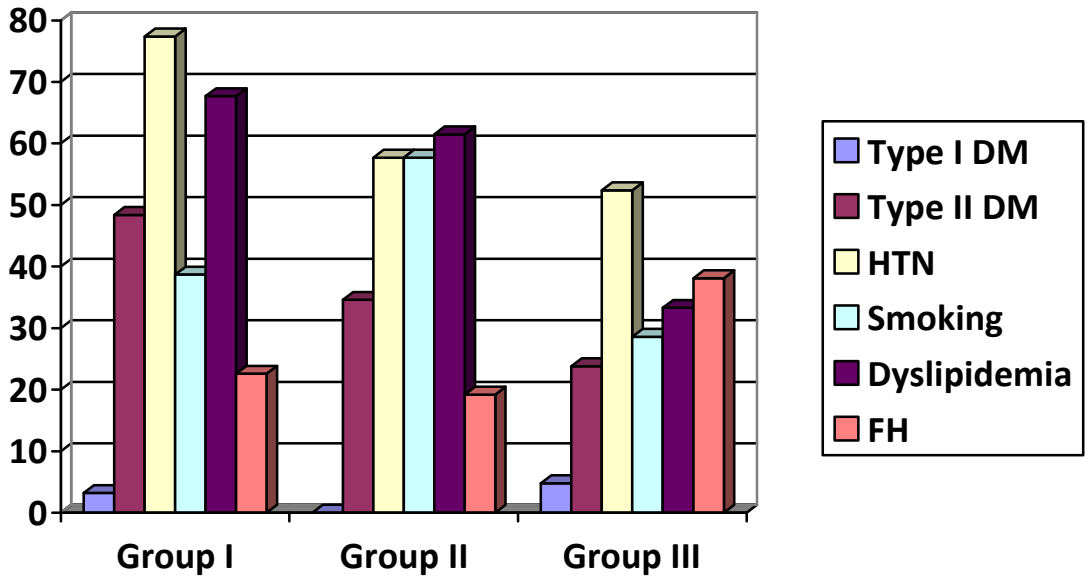

Figure (5): Illustrates different risk factors encountered among the studied groups.

Comparison between group 1& 2 regards their admission ECG findings:

Both group 1 and group 2 were comparable to each other regarding their admission ECG findings. Anterior followed by lateral ST, T wave changes was the most encountered admission ECG findings. Anterior ST, T wave changes were presented in 45.2% and 61.5% of patients in group 1 and 2 respectively while lateral ST, T wave changes were presented in 47.4% of patients in both groups. Previous Q wave infarction and Inferior ST, T wave changes were minimally presented in both groups, 38.6% and 36.8% of patients respectively. Only one patient of each group had bundle branch block. Table (6)

ECG findings	Group 1		Group 2		Total	
	N	%	N	%	N	%
Previous Q wave infarction	12	38.7	10	35.8	22	38.6
BBB	1	3.2	1	3.8	1	3.5
Anterior ST, T wave changes	14	45.2	16	61.5	30	52.6
Lateral ST, T wave changes	16	51.6	11	42.3	27	47.4
Inferior ST, T wave changes	11	35.5	10	35.5	21	36.8

P value> 0.05

Table (6): Illustrates comparison between group 1 & 2 as regards their admission ECG findings.

Results of routine labs in different studied groups

No statistical significant difference among the studied groups as regards the results of routine labs, except for TLC and AST. As expected they were found to be higher in group 2 patients compared with group 1 as the former patients had myocardial injury that always associated with elevation of cardiac biomarkers & leucocytosis. Table (7)

Lab. Results	Group 1	Group 2	Group 3	P value
Hb	12.9±2	13.5±1.5	13.9±9	0.07
TLC	7.0±2	8.7±2.2	6.7±1.3	0.001
PLT	241.3±68.9	218.4±61.7	205.3±46.9	0.106
PC	82.0±12.7	83.3±9.8	80.2±6.9	0.556
AST	31.2±12.2	102.6±94.9	29.7±7.51	0.001
ALT	37.7±17.5	55.9±34.9	28.7±7.5	0.055
Urea	43.6±51.1	30.9±10	24±5	0.797
Creat.	1.58±1.7	1.1±0.23	1±0.2	0.741
Na	137.2±3.5	138.9±3.4	137.9±3.3	0.16
K	4.3±0.5	4.2±0.4	3.9±0.3	0.063

Table (7): Illustrates the results of routine labs in different studied groups.

Results of lipid profile in different studied groups:

There was no significant difference between the different studied groups regarding their lipid profile: total cholesterol, LDL, HDL & TGs. Table (8) and figure (6)

	Group 1	Group 2	Group 3	P value
Total cholesterol	180.1±36.9	189.2±29.2	186±15.2	0.5
LDL	103.2±23.9	112.1±19.7	98.4±14.1	0.066
HDL	38.8±3.9	39.5±4.3	39.0±3.1	0.77
TGs	162.9±40.9	171.2±28	154.4±24.6	0.22

Table (8): Illustrates the lipid profile in different studied groups.

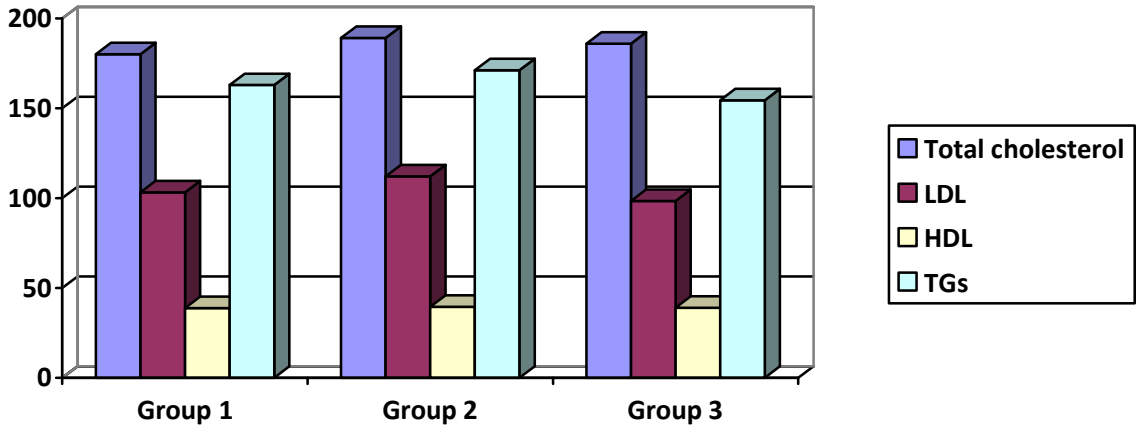

Figure (6): Illustrates the lipid profile in different studied groups.

Comparison between group 1 & group 2 as regards LV systolic function determined by echocardiographic indices

Although the LV systolic function namely FS% & FS% were lower in group 2 compared to group 1, yet this finding was statistically insignificant with P value >0.05. Table (9) and figure (7)

	Group 1	*Group 2*	*P value*
FS%	29.3±6	26.9±5.5	0.12
EF%	56.8±8	53±9.7	0.14

Table (9): Illustrate the comparison between group 1 & group 2 as regards FS & EF

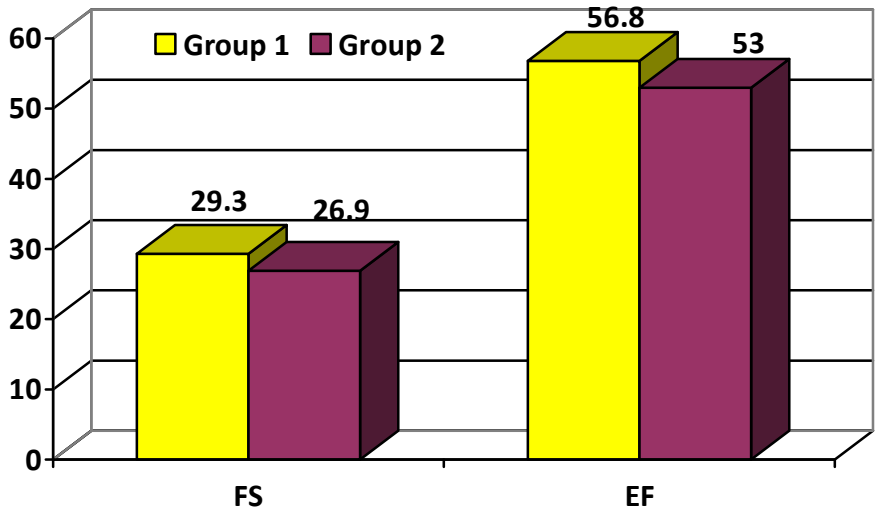

Figure (7): Illustrates the comparison between group 1 & group 2 as regards FS% & EF%

Comparison between different studied groups as regards the level of VEGF

There was statistical significant difference between the studied groups as regards their serum VEGF level. Group 1A followed by group 2A had the least VEGF level, 43.2± 47.5 and 60.9± 3.3 pg/L respectively. On the other hand Group 1B followed by group 2B had the highest VEGF level, 357.5± 142.8 and 257 ± 146.7 pg/L respectively. The VEGF serum level in the control group was74.6 ±53.3 pg/L. Table (10) and figure (8)

Group	Mean	SD
1A	60.9	53.3
1B	357.5	142.8
2A	43.2	47.5
2B	257.0	146.7
3	74.6	53.3

P value <0.001

Table (10): Illustrates comparison between different studied groups regards their serum VEGF level.

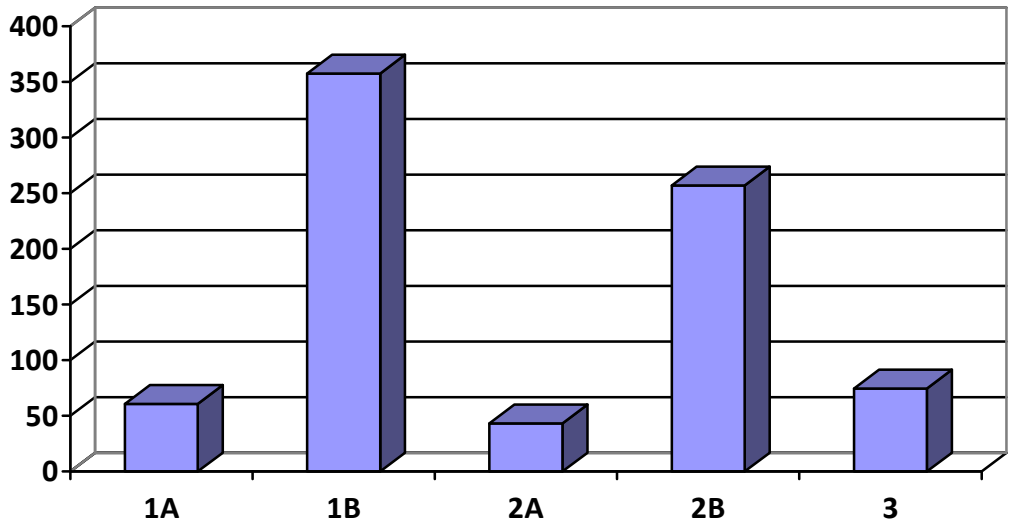

Figure (8): Illustrates comparison between different studied groups as regards their serum VEGF level.

Comparing patients in groups 1A & 2A with patients in control group, patients in control group had a higher serum level of VEGF compared with patients in group 1A & 2A with significant P value (74.6±53.3 vs 60.9±53.3& 43.2±47.5 pg/L respectively, P value: 0.009). Figure (9)

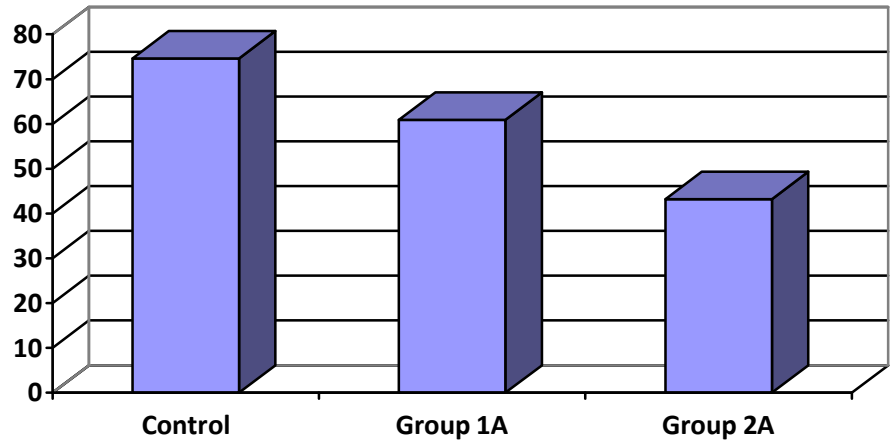

Figure (9): Illustrates the comparison between group 1A, 2A and 3 as regards their serum VEGF level.

On the other hand when patients in group 1B & 2B compared with patients in control group regarding their serum level of VEGF, patients in group 1B & 2B were found to have a higher serum level of VEGF than patients in control group with significant P value (357.5±142.8 & 257.0±146.7 vs 74.6±53.3 pg/L respectively, P value < 0.001). Figure (10)

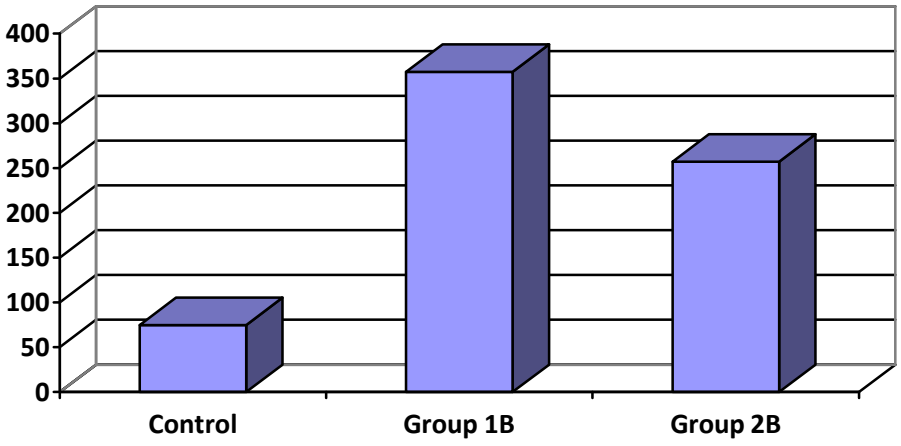

Figure (10): Illustrates the comparison between group 1B , 2B and 3 as regards their serum VEGF level.

These findings denotes that patients presented with either unstable angina or NSTEMI& were not previously treated with statin had a higher serum level of VEGF compared with patients in control group. However when patients in group 1A & 1B compared with patients in group 2A & 2B respectively. There was no statistical significant difference in these groups (60.9±53.3 & 357.5±142.8 vs 43.2±47.5 & 257.0±146.7, pg/L, P value: 0.914 & 0.065) ie that VEGF could not be used as a marker for myocardial injury. Figure (11) and Figure (12)

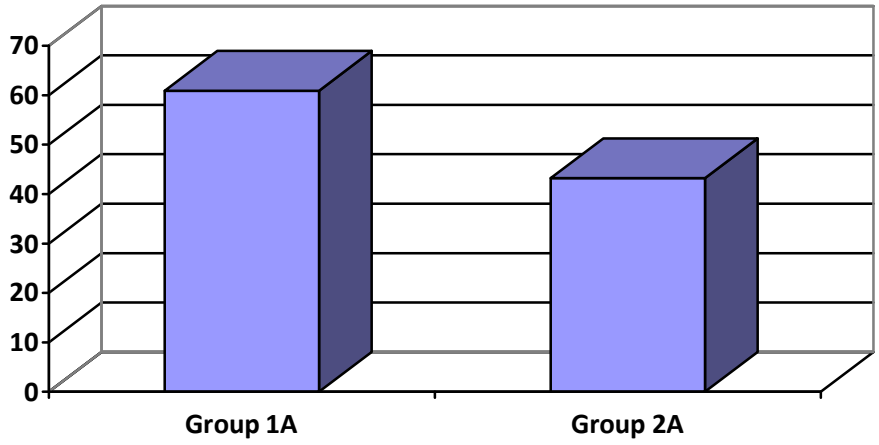

Figure (11): Illustrates the comparison between group 1A and 2A as regards their serum VEGF level.

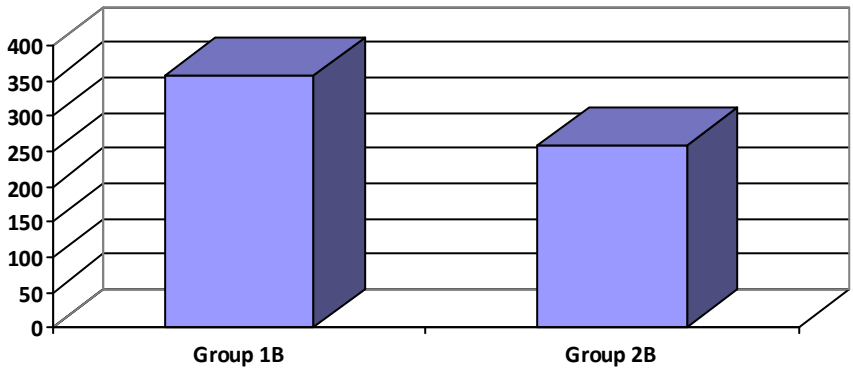

Figure (12): Illustrates the comparison between group 1B and 2B as regards their serum VEGF level.

Angiographic findings in relation to the serum level of VEGF among the groups 1& 2 patients

Patients in both groups 1 & 2 were stratified into 3 groups according to their serum level of VEGF as the following:

VEGF 1 group: included patients in both groups who had VEGF level ≤100 pg/L. This group included 25 patients represented 43.9% of the whole patients in both groups.

VEGF 2 group: included patients in both groups who had VEGF level > 100 pg/L & ≤200 pg/L. This group included 13 patients represented 22.8% of the whole patients in both groups.

VEGF 3 group: included patients in both groups who had VEGF level >200 pg/L. This group included 19 patients represented 33.3% of the whole patients in both groups.

Patients in these 3 groups were compared to each other as regards the severity and the extent of the coronary artery disease that assessed angiographically, using modified Gensini score, severity score (the number of vessels affected) and presence of fresh thrombus. Figure (13) and Table (11)

As shown in table (11), (12) and figure (13) there was no statistical significant difference between the modified Gensini score, severity score or presence of fresh thrombus in different VEGF groups.

	VEGF1	VEGF2	VEGF3
Modified Ginsini score: Mean±SD	9.16±4.81	10.31±4.27	9.79±5.02
Number of vessels affected: Mean±SD	1.92±0.76	2.15±0.89	2.21±1.03

P value: 0.617

Table (11): Illustrates the severity of CAD at different serum VEGF level

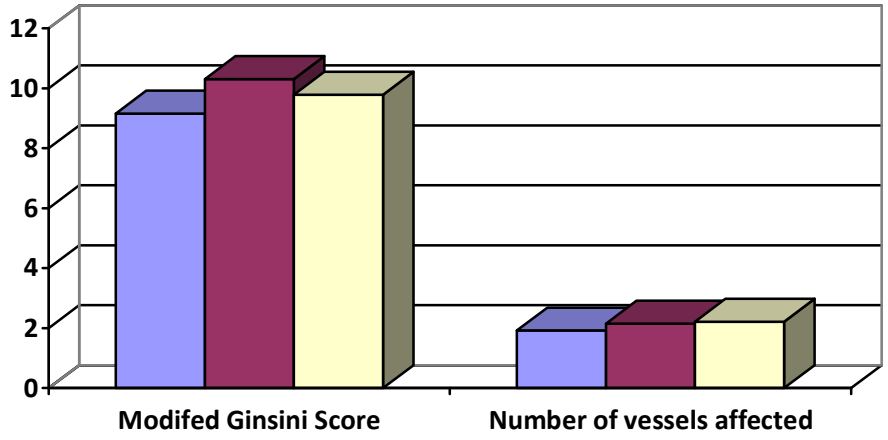

Figure (13) Illustrates the severity of CAD at different serum VEGF level

Presence of fresh thrombus		VEGF1	VEGF2	VEGF3	Total
No	Number	21	13	18	52
	%	84.0	100.0	94.7	91.2
Yes	Number	4	0	1	5
	%	16.0	0.0	5.3	8.8
Total	Number	25	13	19	57
	%	100.0	100.0	100.0	100.0

P value: 0.204

Table (12): Illustrate the incidence of presence of fresh thrombus at different serum VEGF level

Hospital course:

When the following 5 major adverse cardiovascular were assessed:

1. Recurrence of ischemic episodes.
2. Development of heart failure.
3. Development of cardiogenic shock.
4. Occurrence of arrhythmia.
5. Death.

It was found that these complications occurred more in groups 1B & 2B (i.e. in patients who had higher serum level VEGF) compared to patients in group 1A & 2A (i.e. in patients who had lower serum level VEGF). Table (13) and Figure (14)

	Group 1		**Group 2**		***Total***
	1A	1B	2A	2B	
Complicated patients					
N	5	12	4	11	32
%	31.3	80.0	30.8	84.6	56.1
Non complicated patients					
N	1	3	9	2	25
%	68.8	20.9	69.2	15.4	43.9

P value: 0.001

Table (13): Illustrates the number & percentage of complicated & non complicated patients in different studied groups.

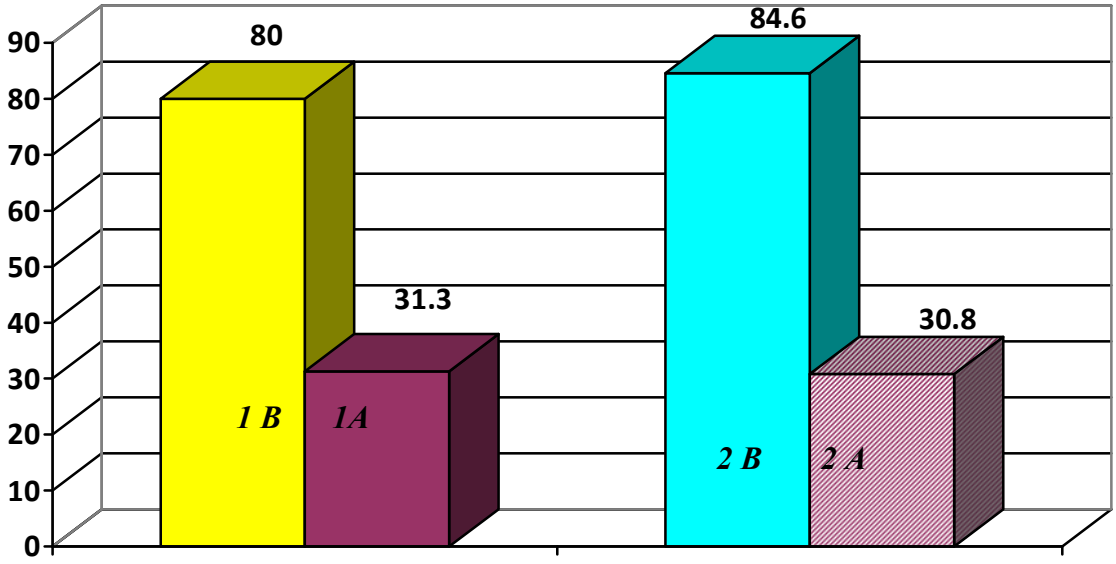

Figure (14): Illustrates the % of patients who had any of 5 adverse cardiovascular events among the different studied groups

When the complicated patients were compared with non complicated patients regarding the severity & the extent of CAD that assessed angiographically as well as regarding the serum level of VEGF. It was found that the complicated patients had a more severe disease & a higher serum level of VEGF. P value was < 0.05 denoting that there was a statistical significant difference between the 2 groups regarding these variables. Table (14)

	Complicated patients	*Non complicated patients*	*P value*
VEGF Mean±SD	254.34±171.63	84.0±114.14	0.001
Modified Gensini score Mean±SD	10.97±4.82	7.92±4.03	0.02
Severity score Mean±SD	2.34±0.9	1.72±0.73	0.009

Table (14): Illustrates the comparison between the complicated & non complicated patients as regards their VEGF level, modified Gensini score & number of vessels affected.

When each of the 5 adverse cardiac complications assessed separately, it was found that the recurrence of ischemic episodes was the most frequent complication & it was occurred in 21 patients that represent 36.8% of the studied patients. Arrhythmia came in the 2nd category after recurrence of ischemic episodes as it occurred in 19 patients that represent 33.3% of the studied patients, in these patients medical treatment either with B-blockers, Ca channel blockers or aminodarone was sufficient & none of them needed an electrical intervention. Heart failure & cardiogenic shock were the least encountered complications as they occurred in 12 & 4 patients represent 21.1% & 7% respectively. None of the studied patients died during the hospital admission & till they underwent coronary angiogram. Figure (15)

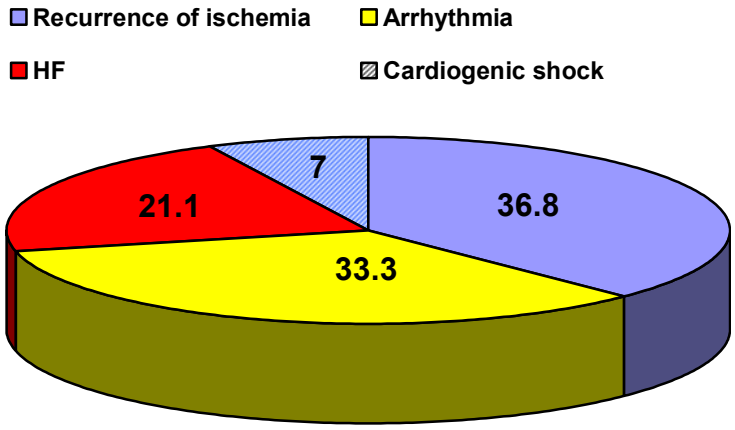

Figure (15): Illustrates the % of each of the adverse cardiac events that occurred among the studied groups

The studied patients stratified according to their VEGF level as mentioned before:

VEGF1: Who had VEGF level ≤100

VEGF2: Who had VEGF level >100 & less than or equal 200.

VEGF 3: Who had VEGF level > 200

Patients in these 3 groups were compared to each other as regards the incidence of different adverse cardiac events. It was found that the incidence of recurrent anginal episodes was significantly higher in VEGF 3 group. Nonetheless there was a trend toward increasing incidence of heart failure in patients with high serum VEGF level (12.0 vs 15.4 vs 36.8 % in patients with low, moderate and high VEGF level respectively, p value: 0.052). On the other hand there were no significant correlation between the serum VEGF level and arrhythmias or cardiogenic shock. Table (15) and Figure (16).

	VEGF1	VEGF2	VEGF3	Total	P value
Recurrent of ischemic					<0.001
N	3	5	13	21	
%	12.	38.5	68.4	36.8	
Arrhythmia					
N	8	3	8	19	0.523
%	32.0	23.1	42.1	33.3	
Heart failure					
N	3	2	7	12	0.052
%	12.0	15.4	36.8	21.2	
Cardiogenic					
N	2	1	1	4	0.73
%	8.0	7.7	5.3	7.0	

Table (15): Illustrates the number & percentage of each of adverse cardiac events in relation to the level of VEGF

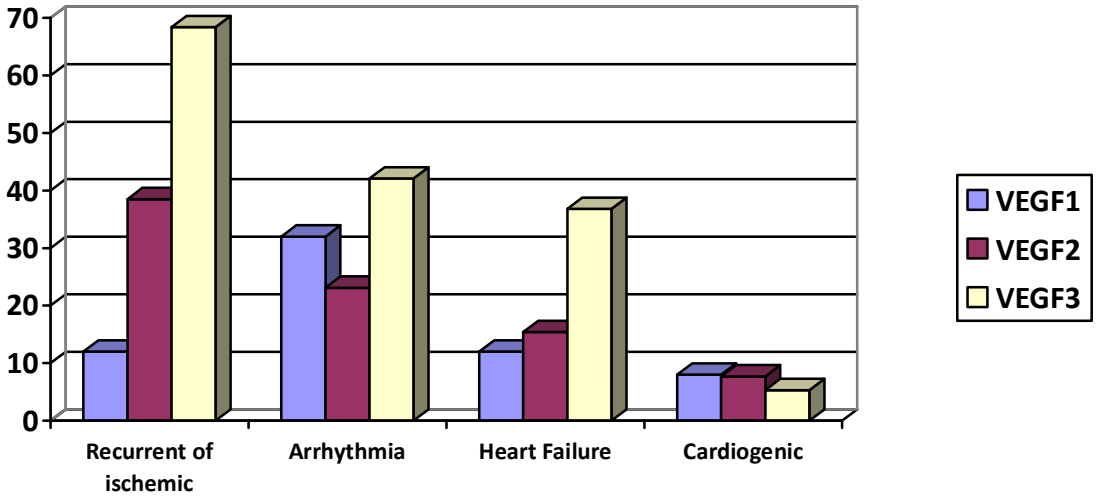

Figure (16) Illustrates the number & percentage of each of adverse cardiac events in relation to the level of VEGF

Discussion

The role of angiogenesis in atherosclerosis and other cardiovascular diseases is a major unresolved issue. Angiogenesis has attracted interest from opposite perspectives. A variety of studies suggest that neovascularization contributes to the growth of atherosclerotic lesions and is a key factor in plaque destabilization leading to rupture. Conversely, Angiogenic cytokine therapy has been widely regarded as an attractive approach for treating ischemic heart disease. So it remains a debate in the literature as to whether angiogenesis either is a key causative factor in the pathogenesis of coronary atherosclerotic plaque formation or is a way to treat coronary heart disease. This debate reflects the complexity of the underlying disease process.

Relevant studies have focused on the role of vascular endothelial growth factor, a highly specific mitogen for endothelial cells, as a key regulator of angiogenesis. An ever-growing interest has been focused to this growth factor because it is thought to be implicated not only in the pathogenesis of atherosclerosis but also in atherosclerotic plaque neovascularization and thus promotes its infiltration by inflammatory cells. These events, through a complex mechanism, may trigger plaque destabilization. ***[Efstathios Papalambros, et al, 2004]***

Therefore the acute problem in the case of coronary artery disease is therefore vascular insufficiency, but this is the outcome of a complex pathophysiological process in which angiogenesis may itself play a vital role.

In 2001, Celletti et al. have shown that injection of recombinant VEGF enhanced atherosclerosis in hypercholesterolemic mice and rabbits, thereby supporting the possible role of VEGF in plaque progression.

On the basis of these reported data, it is expected that there is a relationship between manifestation of complicated atherosclerotic coronary artery disease and the serum level of VEGF.

This study aims at investigating the potential alteration of the serum VEGF level in patients presenting with non ST elevation acute coronary syndrome and to determine whether this level is higher than in the control group and to determine whether this level is positively correlated to the severity and the short in-hospital prognosis of the disease or not.

In order to achieve this aim, 78 patients were included in the study, 57 of these patients were admitted with non ST elevation acute coronary syndrome and were divided into 2 groups. Group 1 included 31 patients who were presented by unstable angina, the other 26 patients represent a group 2 who presented by non ST segment elevation infarction. Patients in group 1 and 2 were furtherly divided according to the prior statin use into: Group 1A: include statin treated patients in group 1, this group included 16 patients, Group 2A: included statin treated patients in group 2, and this group include 13 patients, Group 1B: included non statin treated patients in group 1, and this group include 15 patients and Group

2B: included non statin treated patients in group 2, and this group include 13 patients.

Twenty one patients without any known myocardial disease who referred to the cath. Lab for chest pain evaluation and their results revealed normal coronaries were served as a control group (Group 3).

All groups shows no significant difference as regards the different risk factors of ischemic heart disease, ECG findings, results of routine labs (except for TLC and AST) and LV systolic function assessed by echocardiography namely FS% & EF%.

This study shows that the serum level of VEGF was significantly higher in patients presented with either unstable angina or NSTEMI & were not previously treated with statin in comparison to control patients (357.5$\pm$142.8 & 257.0$\pm$146.7 vs 74.6$\pm$53.3 pg/L respectively, P value < 0.001).

This finding was in agreement with the study conducted by N A Chung, et al. in 2003 who studied the plasma concentrations of von Willebrand factor (a marker of endothelial damage/dysfunction), vascular endothelial growth factor, soluble VEGF receptor Flt-1, and tissue factor (a key component of coagulation) in 111 patients attending for coronary angiography, and he found that all of these indices were raised in the patients compared with 34 healthy controls except sFlt-1, which was lower in the patients group.

In 2004 Kaeng W. Lee, et al. reported the same findings in their study. They demonstrated the increased level of plasma VEGF in patients presented with acute coronary syndrome compared with patients with stable angina and healthy controls (in acute MI the VEGF ranged between 83 and 217 with mean 126, in unstable angina ranged between 48 and 118 with mean 86, in stable angina ranged between 28 and 67 with mean 46 and in control group ranged between 21 and 30 with mean 27 pg/ml, these data were significantly different between groups, $P<0.001$).

Results published by M. Slevin, et al. in 2000, Andrew j Makin, et al. in 2003 and Nouran Elghandour, et al. in 2006 also reported elevated VEGF level in patients presented with either cerebrovascular stroke or peripheral vascular disease. In M. Slevin, et al. study, they serially (at days 0, 1, 3, 7, and 14) measured the serum levels of VEGF in 29 patients with acute ischemic stroke and 26 age-matched healthy subjects were used as controls. In their study they found that the mean concentration of VEGF in the serum of patients with stroke was significantly higher than that of the controls at all time points (days 0, 1, 3, 7, and 14; $P<0.05$). Moreover comparison of the subgroups of stroke patients revealed the highest expression of VEGF in the patients with large infarcts compared with moderate infarcts, whereas patients with small infarcts had the lowest expression.

Andrew j Makin, et al. and Nouran Elghandour, et al. measured the plasma levels of VEGF and its soluble receptor sFlt-1 in patients with proven PAD (ankle brachial pressure index<0.8) and compared them with

healthy controls. They found that patients suffering from proven PAD have higher plasma levels of TF and VEGF compared with controls. These findings imply that VEGF increased in any of atherothrombotic disease.

In contrast to our result H F Alber, et al. in 2005 reported that coronary artery disease patients and controls had similar VEGF level. The discrepancy between our results in comparison to his result may be related to different patient populations as patients with recent acute coronary syndrome were excluded from their study, but were selectively included in our study.

Our results also showed that the previously statin treated patients either in unstable angina or in NSTEMI groups had lower serum level of VEGF than controls (60.9$\pm$53.3 & 43.2$\pm$47.5 vs 74.6$\pm$53.3 pg/L respectively, P value: .009). If we consider the possible proatherogenic effect of VEGF to human atherosclerosis, the reduction of VEGF by statin could represent an atheroprotective property of this this type of treatment.

In accordance with our data, Blann, et al., 2001, demonstrated that lipid-lowering therapy with fluvastatin or fenofibrate decreased the VEGF plasma levels in hypercholesterolemic patients with/without peripheral or carotid atherosclerosis.

In 2002, Hannes Franz Alber, et al. reported that atorvastatin may lower the plasma level of VEGF in CAD patients, when they studied 14

male patients with angiographically confirmed CAD (defined as >30% lumen stenosis in at least one major coronary artery branch) and with hypercholesterolemia requiring lipid-lowering therapy, after two months of atorvastatin therapy VEGF plasma concentration was significantly decreased. H F Alber, et al, in 2005 confirmed his previous results regarding the effect of statin treatment on the level of VEGF when they studied the effect of atorvastatin on the VEGF level in CAD patients.

In 2006, Yasushi Kadoma, et al. published that atorvastatin treatment induced an increase in sFlt-1 levels and a reciprocal decrease in free VEGF and free PlGF levels at 6 months after MI compared with placebo treatment. Nonetheless the increase in sFlt-1 levels and the decrease in VEGF and PlGF levels were correlated with improvement of left ventricular ejection fraction during the follow-up period.

In contrast, Vasa et al., 2001, have shown that atorvastatin therapy (40 mg) for four weeks did not change VEGF levels in the serum of patients with stable CAD. Potential explanations for the latter study compared to our and the other mentioned studies maybe related to short duration of statin treatment (4 weeks) in their study.

The present study revealed that the serum VEGF level did not differ between unstable angina patients and NSTEMI patients either in previously statin treated patients or in non previously statin treated patients (60.9 ± 53.3 & 357.5 ± 142.8 vs 43.2 ± 47.5 & 257.0 ± 146.7, pg/L, P value: 0.914 & 0.065) respectively. In contrast to these results Kaeng W. Lee, et al., 2004, reported that the VEGF level is higher in patients

with acute MI is higher than that of unstable angina. This discrepancy is possibly related to the type of patients included in their study, as only 27 of 82 patients had NSTEMI while the remainder had STEMI, such type of patients were excluded from the present study.

In the present study when patients in both groups 1& 2 were stratified into 3 groups according to the serum level of VEGF. Although there was a trend toward increase of number of coronary vessels affected in high VEGF level groups (1.92±0.76 vs 2.15±0.89 vs 2.21±1.03 in low, moderate and high VEGF groups respectively, P value: NS), there was no significant correlation between the serum level of VEGF and the coronary artery disease severity that assessed angiographicaly using modified Gensini score (9.16±4.81 vs 10.31±4.27 vs 9.79±5.02 in low, moderate and high VEGF groups respectively, P value: NS) or severity score (1.92±0.76 vs 2.15±0.89 vs 2.21±1.03 in low, moderate and high VEGF groups respectively, P value: NS).

This lack of correlation between the serum level of VEGF and coronary artery disease severity is possibly related to the more generalised nature of thrombogenesis, angiogenesis, and endothelial disturbance, rather than that occurring in specific vascular beds.

Nonetheless when the serum level of VEGF was correlated with the presence of fresh thrombus that detected during coronary angiogram, it was found that there was no significant correlation between these two variables (16% vs 0.0% vs 5.3% of patients had visible fresh thrombus in low, moderate and high VEGF groups respectively, P value: 0.204).

In accordance to these data N A Chung, et al., 2003, reported similar results. In their study they found that there were no significant correlations found between the coronary atheroma score or number of coronary vessels with significant stenoses.

In contrast to our data Christopher Heeschen, et al., 2003, reported that there was a trend toward a more severe atherosclerotic coronary disease in patients had higher level of VEGF as they found that TIMI flow ≤ 1 was documented for 10.3% in patients with high VEGF levels compared with 7.6% for patients with low VEGF levels (*P*=0.15), and thrombus was visible in 7.8% of patients with high VEGF levels compared with 5.9% for patients with low VEGF levels (*P*=0.28).

In addition in 1999 Fleisch and colleagues found a trend toward higher concentrations of intracoronary VEGF with more extensive coronary artery disease, as assessed by the number of diseased coronary arteries (stenosis > 50%) on coronary angiography.

In 2004, Nakajima K, et al. examined the plasma level of VEGF concentration in 73 patients who underwent coronary angiography and 70 apparently healthy control subjects. According to the number of the three major coronary vessels with significant (> or = 75%) stenosis, they divided the patients into two groups: the mild stenosis group (0- and single-vessel disease, n = 36) and the severe stenosis group (double- and triple-vessel disease, n = 37). They found that VEGF value of the severe stenosis group was significantly higher than that of the mild stenosis ($p < 0.05$) and control groups ($p < 0.05$). Furthermore, there was a significant

positive trend in the VEGF value according to the number of vessels with significant stenosis (p = 0.016).

As mentioned before patients in groups 1 and 2 were stratified into 3 groups according to the serum level of VEGF, it was found that the incidence of cardiac adverse events-recurrence of myocardial ischemia events, arrhythmia, development of heart failure or cardiogenic shock was higher in patients with higher VEGF level compared with patients with low VEGF level. Recurrent ischemic attacks were significantly higher in patients with higher serum VEGF level compared with patients with low serum VEGF level (12.0 vs 38.5 vs 68.4% in patients with low, moderate and high VEGF respectively, p value: <0.001). Regarding development of heart failure, it occurred more in patients with higher serum VEGF level compared with patients with low serum VEGF level (12.0 vs 15.4 vs 36.8% in patients with low, moderate and high VEGF respectively, p value: 0.052). On the other hand there was no significant correlation between arrhythmias or development of cardiogenic shock and the serum level of VEGF. These results were in agreement with Christopher Heeschen, et al, 2003, who published that VEGF was significantly and independently associated with the patients' outcome.

Summary

The neovascular network in coronary atherosclerotic plaques may be prone to rupture leading to acute coronary syndromes. In addition chronic inflammation, a key component of atherogenesis, requires the recruitment of circulating leukocytes into atherosclerotic plaques. This leukocytic infiltration is probably enhanced by plaque neovascularization.

With regard to these potentially harmful effects of neoangiogenesis, the main angiogenic growth factor, vascular endothelial growth factor, may be associated with atherosclerosis progression and lesion destabilisation. through its ability to enhance plaque inflammatory infiltration and neovascularisation.

The aim of this study is to investigate the level of the vascular endothelial growth factor in patients presented with non ST elevation acute coronary syndrome and to determine whether this level is higher in these patients in comparison to control group and to determine whether this level is positively correlated with the severity of the disease and the short in-hospital prognosis or not.

In order to achieve this aim, 78 patients were included in the study, 57 of these patients were admitted with non ST elevation acute coronary syndrome and were divided into 2 groups. Group 1 included 31 patients who presented by unstable angina, the other 26 patients represent a group 2 who presented by non ST segment elevation infarction. Patients in group 1 and 2 were furtherly divided according to the prior use of statin

into: Group 1A: included statin treated patients in group 1, this group included 16 patients, Group 2A: included statin treated patients in group 2, and this group included 13 patients, Group 1B: included non statin treated patients in group 1, and this group included 15 patients and Group 2B: included non statin treated patients in group 2, and this group included 13 patients. Twenty one patients without any known myocardial disease who referred to the cath. Lab for chest pain evaluation and their results revealed normal coronaries were served as a control group (Group 3).

All patients were subjected to full history taking, routine general and systemic examination, 12-lead ECG, echocardiography, routine labs including cardiac biomarkers and troponin, Lipid profile, measurement of the serum VEGF by quantitative enzyme linked immunosorbant assay and coronary angiograms that were scored visually into: a severity score (0–3) that defined the number of vessels with a luminal stenosis ≥50% (for right, left anterior descending, and circumflex arteries or its main branch eg 1st diagonal branch of left anterior descending or obtuse marginal of left circumflex). The severity and extent of CAD was graded using a modified Gensini score. The most severe stenosis in each of the three coronaries and in the left main was graded from 0 to 6 (0, no stenosis; 1, 1–29% stenosis; 2, 30–49% stenosis; 3, 50–69% stenosis; 4, 70–89% stenosis; 5, 90–99%; 6, 100% occlusion) and summed to yield a score of 0–24. During hospitalization, the 5 major cardiovascular complications assessed were the following: 1) Recurring myocardial ischemia. 2) Congestive heart failure. 3) Cardiogenic shock. 4) Arrhythmias 5) Death.

This study showed that the serum level of VEGF was significantly higher in patients presented with either unstable angina or NSTEMI & were not previously treated with statin in comparison to control patients (357.5±142.8 & 257.0±146.7 vs 74.6±53.3 pg/L respectively, P value < 0.001). Our result also showed that the previously statin treated patients either in unstable angina or in NSTEMI groups had lower serum level of VEGF than controls (60.9±53.3 & 43.2±47.5 vs 74.6±53.3 pg/L respectively, P value: 0.009). This finding could represent an athero-protective property of this this type of treatment. The present study also revealed that of the serum VEGF level did not differ between unstable angina patients and NSTEMI patients either in previously statin treated patients or in non previously statin treated patients (60.9±53.3 & 357.5±142.8 vs 43.2±47.5 & 257.0±146.7, pg/L, P value: 0.914 & 0.065) respectively.

When patients in both groups 1& 2 were stratified into 3 groups according to the serum level of VEGF , although there was a trend toward increase of number of coronary vessels affected in high VEGF level groups (1.92±0.76 vs 2.15±0.89 vs 2.21±1.03 in low, moderate and high VEGF groups respectively, P value: NS), yet this finding was statistically insignificant. Also there was no significant correlation between the serum level of VEGF and the coronary artery disease severity that assessed angiographicaly using modified Gensini score (9.16±4.81 vs 10.31±4.27 vs 9.79±5.02 in low, moderate and high VEGF groups respectively, P value: NS). In addition the serum level of VEGF did not correlate with the presence of fresh thrombus that detected during coronary angiogram

(16% vs 0.0% vs 5.3% of patients had visible fresh thrombus in low, moderate and high VEGF groups respectively, P value: 0.204)

Regarding the short in hospital prognosis, recurrent ischemic attacks were significantly higher in patients with higher serum VEGF level compared with patients with low serum VEGF level (12.0 vs 38.5 vs 68.4% in patients with low, moderate and high VEGF respectively, p value: <0.001). Heart failure occurred more in patients with higher serum VEGF level compared with patients with low serum VEGF level (12.0 vs 15.4 vs 36.8% in patients with low, moderate and high VEGF respectively, p value: 0.052). However there was no significant correlation between arrhythmias or development of cardiogenic shock and the serum level of VEGF.

Conclusions

- VEGF serum level is higher in non statin treated patients presenting with non ST elevation acute coronary syndrome compared to control group.
- Satin therapy lowers the VEGF level in patients with ischemic heart disease.
- No correlation between VEGF serum level and angiographically defined disease severity.
- Elevated VEGF serum levels may be associated with worse short in hospital prognosis.

References

- Aase K, Lymboussaki A, Kaipainen A et al. Localisation of VEGF-B in the mouse embryo suggests a paracrine role of the growth factor in the developing vasculature. Dev Dyn. 1999;215:12–25.

- Achen MG, Jeltsch M, Kukk E et al. Vascular endothelial growth factor D (VEGF-D) is a ligand for tyrosine kinases VEGF receptor 2 (Flk1) and VEGF receptor 3 (Flt-4). Proc Natl Acad Sci USA. 1998;95:548–553.

- Adamis AP, Shima DT, Tolentino MJ et al. Inhibition of vascular endothelial growth factor prevents retinal ischemia-associated iris neovascularisation in non-human primate. Arch Ophthalmol. 1996;144:66–71.

- Ahmed, WH, Bittl, JA, Braunwald, E. Relation between clinical presentation and angiographic findings in unstable angina pectoris, and comparison with that in stable angina. Am J Cardiol 1993; 72:544.

- Aicher A, Zeiher AM, Dimmeler S. Mobilizing endothelial progenitor cells. Hypertension. 2005; 45: 321–325.

- Aiello LP, Pierce EA, Foley ED et al. Suppression of retinal neovascularisation in vivo by inhibition of vascular endothelial growth factor (VEGF) using soluble VEGF-receptor chimeric proteins. Proc Natl Sci USA. 1995;92:10457–10461.

- Akkerhuis, KM, Alexander, JH, Tardiff, BE, et al. Minor myocardial damage and prognosis: are spontaneous and percutaneous coronary intervention-related events different? Circulation 2002; 105:554.

- Alber HF, M Frick, J Dulak, et al.Vascular endothelial growth factor (VEGF) plasma concentrations in coronary artey disease. Heart 2005;91: 365-366.

- Alpert, JS, Thygesen, K, Antman, E, Bassand, JP. Myocardial infarction redefined--a consensus document of The Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction. J Am Coll Cardiol 2000; 36:959.

- Andreakos E, Foxwell B, Feldmann M. Is targeting Toll-like receptors and their signaling pathway a useful therapeutic approach to modulating cytokine-driven inflammation? Immunol Rev. 2004; 202: 250–265.

- Andrew J Makin, Natalia AY Chung, Stanley H Silverman and Grgory YH Lip. Vascular endothelial growth factor in patients with established peripheral artery disease: A link between angiogenesis and thrombogenesis. Clinical science 2003; 104,397-404.

- Antman, EM, Cohen, M, Bernink, PJ, et al. The TIMI risk score for unstable angina/non-ST elevation MI: A method for prognostication and therapeutic decision making. JAMA 2000; 284:835.

- Armstrong, PW, Fu, Y, Chang, W-C, et al, for the GUSTO-IIb Investigators. Acute coronary syndromes in the GUSTO-IIb trial: Prognostic insights and impact of recurrent ischemia. Circulation 1998; 98:1860.

- Asahara T, Bauters C, Pastore C, Kearney M, Rossow S, Bunting S, Ferrara N, Symes JF, Isner JM. Local delivery of vascular endothelial growth factor accelerates reendothelialization and attenuates intimal hyperplasia in balloon-injured rat carotid artery. Circulation. 1995; 91: 2793–2801.

- Asahara T, Chen D, Takahashi T et al. Tie2 receptor ligands, angiopoietin-1 and angiopoietin-2, modulate VEGF-induced postnatal neovascularisation. Circ Res. 1998;83:233–240.

- Ausprunk DH, Folkman J. Migration and proliferation of endothelial cells in preformed and newly formed blood vessels during angiogenesis. Microvasc Res. 1997;14:53–65.

- Barker SG, Tilling LC, Miller GC, Beesley JE, Fleetwood G, Stavri GT, Baskerville PA, Martin JF. The adventitia and atherogenesis: removal initiates intimal proliferation in the rabbit which regresses on generation of a "neoadventitia." Atherosclerosis. 1994; 105: 131–144.

- Barleon B, Sozzani S, Zhou D et al. Migration of human monocytes in response to vascular endothelial growthfactor (VEGF) is mediated via the VEGF receptor flt-1. Blood. 1996;87:3336–3343.

- Belgore F, Blann A, Neil D, Ahmed AS, Lip GY. Localisation of members of the vascular endothelial growth factor (VEGF) family and their receptors in human atherosclerotic arteries. J Clin Pathol. 2004; 57: 266–272.

- Belgore F, Blann AD, Li-Saw-Hee FL et al. Plasma levels of vascular endothelial growth factor and its soluble receptor (sFlt-1) in essential hypertension. Am J Cardiol. 2001;87: 805–807.

- Belgore FM, Blann AD, Lip GYH. Measurement of free and complexed soluble vascular endothelial growth factor receptor, Flt-1, in fluid samples: development and application of two new immunoassays. Clin Sci 2001;100:567–75.

- Belgore FM, Lip GYH, Wadley M et al. Plasma levelsof vascular endothelial cell growth factor (VEGF) andits receptor (sFlt-1) in haematological cancers: a comparison with breast cancer. Am J Haematol. 2001;66: 59–61.

- Bergers G, Brekken R, McMahon G, Vu TH, Itoh T, Tamaki K, Tanzawa K, Thorpe P, Itohara S, Werb Z, Hanahan D. Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. Nat Cell Biol. 2000; 2: 737–744.

- Betriu, A, Califf, RM, Bosch, X, et. al, for the GUSTO-I Investigators. Recurrent ischemia after thrombolysis: Importance of associated clinical findings. J Am Coll Cardiol 1998; 31:94.

- Blakytny R, Ludlow A, Martin GE, Ireland G, Lund LR, Ferguson MW, Brunner G. Latent TGF-beta1 activation by platelets. J Cell Physiol. 2004; 199: 67–76.

- Blann AD, Belgore FM, McCollum CN et al. Vascular endothelial growth factor and its receptor Flt-1 in the plasma of patients with coronary and peripheral atherosclerosis and type II diabetes. Clin Sci. 2002;102:187–194.

- Bobryshev YV, Farnsworth AE, Lord RS. Expression of vascular endothelial growth factor in aortocoronary saphenous vein bypass grafts. Cardiovasc Surg. 2001;9:492–498.

- Boersma E, Mercado N, Poldermans D, Gardien M, Vos J, Simoons ML. Acute myocardial infarction. Lancet. 2003; 361: 847–858.

- Braunwald, E. Unstable angina. A classification. Circulation 1989; 80:410.

- Brener, SJ, Lytle, BW, Schneider, JP, et al. Association between Ck-MB elevation after percutaneous or surgical revascularization and three-year mortality. J Am Coll Cardiol 2002; 40:1961.

- Brogi E, Schatteman G, Wu T, Kim EA, Varticovski L, Keyt B, Isner JM. Hypoxia-induced paracrine regulation of vascular endothelial growth factor receptor expression. J Clin Invest. 1996; 97: 469–476.

- Brooks PC, Clark RA, Cheresh DA. Requirement of vascular integrin alpha v beta 3 for angiogenesis. Science. 1994; 264: 569–571.

- Burke AP, Virmani R, Galis Z, Haudenschild CC, Muller JE. 34th Bethesda Conference: Task force #2—What is the pathologic basis for new atherosclerosis imaging techniques? J Am Coll Cardiol. 2003; 41: 1874–1886.

- Burton PBJ, Owen VJ, Hafizi S et al. Vascular endothelial growth factor release following coronary artery bypass surgery: extracorporeal circulation versus 'beating heart' surgery. Eur Heart J. 2000;21:1708–1713.

- Califf, RM, Abdelmeguid, AA, Kuntz, RE, et al. Myonecrosis after revascularization procedures. J Am Coll Cardiol 1998; 31:241.

- Calvin, JE, Klein, LW, Vanden Berg, BJ, et al. Risk stratification in unstable angina. Prospective validation of Braunwald classification. JAMA 1995; 273:136.

- Cannon, CP, Weintraub, WS, Demopoulos, LA, et al. Comparison of early invasive and conservative strategies in patients with unstable coronary syndromes treated with the glycoprotein IIb/IIIa inhibitor tirofiban. N Engl J Med 2001; 344:1879.

- Cao Y, Ji WR, Qi P et al. Placenta growth factor: identification and characterization of a novel isoform generated by RNA alternative splicing. Biochem Biophys Res Commun. 1997;235:493–498.

- Cao Y, Linden P, Shima D et al. In vivo angiogenesis and hypoxia induction of heterodimer of placenta growth factor/vascular endothelial growth factor. J Clin Invest. 1996;98:2507–2511.

- Carmeliet P and Collen D.Molecular basis of angiogenesis. Role of VEGF and VE-cadherin.Ann NY Acad Sci 2000;902:249-263.

- Carmeliet P, Collen D. Genetic analysis of blood vessel formation. Role of endothelial versus smooth muscle cells. Trends Cardiovasc Med. 1997;7:271–281.

- Carmeliet P. Angiogenesis in health and disease. Nat Med. 2003; 9: 653–660.

- Celletti FL, Waugh JM, Amabile PG, Brendolan A, Hilfiker PR, Dake MD. Vascular endothelial growth factor enhances atherosclerotic plaque progression. Nat Med. 2001; 7: 425–429.

- Celletti FL, Waugh JM, Amabile PG, Kao EY, Boroumand S, Dake MD. Inhibition of vascular endothelial growth factor-mediated neointima progression with angiostatin or paclitaxel. J Vasc Interv Radiol. 2002; 13: 703–707.

- Cha DR, Kim NH, Yoon JW et al. Role of vascular endothelial growth factor in diabetic nephropathy. Kidney Int. 2000;(Suppl 77):S104–S112.

- Chandrasekar, B, Bourassa, MG. Incidence and risk factors predictive of unstable angina resulting from restenosis after percutaneous angioplasty of saphenous vein grafts. Am Heart J 2000; 140:827

- Charnock-Jones D, Sharkey A, Boocock C et al. Vascular endothelial growth factor receptor localization and activation in human trophoblast and choriocarcinoma cells. Biol Reprod. 1994;51:524–530.

- Chen YX, Nakashima Y, Tenaka K et al. Immunohistochemical expression of vascular endothelial growth factor/vascular permeability factor in atherosclerosis of human coronary arteries. Arterioscler Thromb Vasc Biol. 1999;19:131–139.

- Chilov D, Kukk E, Taira S et al. Genomic organisation of human and mouse genes for vascular endothelial growth factor C. J Biol Chem. 1997;272:25176–25183.

- Christopher Heeschen, Stefanie Dimmeler, Christian W Hamm, et al. Prognostic significance of angiogenic growth factor serum levels in patients with acute coronary syndromes. Circulation; 2003,107:524.

- Chung N A, C Lydakis , et al.Angiogenesis, thrombogenesis, endothelial dysfunction and angiographic severity of coronary artery disease .Heart 2003;89:1411-1415.

- Couffinhal T, Kearney M, Witzenbichler B et al. Vascular endothelial growth factor (VEGF/VPF) in normal and atherosclerotic human arteries. Am J Pathol. 1997;150:1673–1685.

- Cutlip, DE, Leon, MB, Ho, KK, et al. Acute and nine-month clinical outcomes after "suboptimal" coronary stenting: results from the STent Anti-thrombotic Regimen Study (STARS) registry. J Am Coll Cardiol 1999; 34:698.

- Dangas, G, Mehran, R, Wallenstein, S, et al. Correlation of angiographic morphology and clinical presentation in unstable angina. J Am Coll Cardiol 1997; 29:519.

- Davis S, Aldrich T, Jones PF et al. Isolation of angiopoietin-1, a ligand for the angiogenic TIE-2 receptor, by secretion-trap expression cloning. Cell. 1996; 87:1161–1169.

- De Boer OJ, van der Wal AC, Teeling P, Becker AE. Leucocyte recruitment in rupture prone regions of lipid-rich plaques: a prominent role for neovascularization? Cardiovasc Res. 1999; 41: 443–449.

- De Servi, S, Arbustini, E, Marsico, F, et al. Correlation between clinical and morphologic findings in unstable angina. Am J Cardiol 1996; 77:128.

- Detmar M, Brown LF, Berse B et al. Hypoxia regulates the expression of vascular permeability factor/vascular endothelial growth factor (VPF/VEGF) and its receptors inhuman skin. J Invest Derm. 1997;108:263–268.

- Diderholm, E, Andren, B, Frostfeldt, G, et al. The prognostic and therapeutic implications of increased troponin T levels and ST depression in unstable coronary artery disease: the FRISC II invasive troponin T electrocardiogram substudy. Am Heart J 2002; 143:760.

- Diver, DJ, Bier, JD, Ferreira, PE, et al. Clinical and arteriographic characterization of patients with unstable angina without critical coronary arterial narrowing (from the TIMI-IIIA trial). Am J Cardiol 1994; 74:531.

- Dove DE, Su YR, Zhang W, Jerome WG, Swift LL, Linton MF, Fazio S. ACAT1 deficiency disrupts cholesterol efflux and alters cellular morphology in macrophages. Arterioscler Thromb Vasc Biol. 2005; 25: 128–134.

- Edfeldt K, Swedenborg J, Hansson GK, Yan ZQ. Expression of toll-like receptors in human atherosclerotic lesions: a possible pathway for plaque activation. Circulation. 2002; 105: 1158–1161.

- Efstathios papalambros, Sotiris Georopopoulos, et al. Changes in circulating levels of vascular endothelial growth factor and vascular endothelial growth factor receptor 2after carotid endarterectomy. International journal of molecular medicine,2004, 14: 133-136.

- Enholm B, Jussila L, Karkkainen M et al. Vascular endothelial growth factor-C: a growth factor for lymphatic and blood vascular endothelial cells. Trends Cardiovasc Med. 1998;8:292–297.

- Eppler SM, Combs DL, Henry TD, Lopez JJ, Ellis SG, Yi JH, Annex BH, McCluskey ER, Zioncheck TF. A target-mediated model to describe the pharmacokinetics and hemodynamic effects of recombinant human vascular endothelial growth factor in humans. Clin Pharmacol Ther. 2002; 72: 20–32.

- Epstein SE, Stabile E, Kinnaird T, Lee CW, Clavijo L, Burnett MS. Janus phenomenon: the interrelated tradeoffs inherent in therapies designed to enhance collateral formation and those designed to inhibit atherogenesis. Circulation. 2004; 109: 2826–2831.

- Faller DV. Endothelial cell responses to hypoxic stress. Clin Exp Pharmacol Physiol. 1999;26:74–84.

- Felton CV, Crook D, Davies MJ, Oliver MF. Relation of plaque lipid composition and morphology to the stability of human aortic plaques. Arterioscler Thromb Vasc Biol. 1997; 17: 1337–1345.

- Ferrara N, Houck K, Jakeman L et al. Molecular and biological properties of vascular endothelial growth factor family of protein. Endocr Rev. 1992;13:18–32.

- Fleiner M, Kummer M, Mirlacher M, Sauter G, Cathomas G, Krapf R, Biedermann BC. Arterial neovascularization and inflammation in vulnerable patients: early and late signs of symptomatic atherosclerosis. Circulation. 2004; 110: 2843–2850.

- Fleisch M, Billinger M, Eberli FR et al. Physiologically assessed coronary collateral flow and intra-coronary growth factor concentrations in patients with 1- to 3-vessel coronary artery disease. Circulation. 1999;100:1945–1950.

- Folkman J, Shing Y. Angiogenesis. J Biol Chem. 1992;267:10931–10934.

- Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. Nat Med. 1995;1:27–31.

- Folkman J. Clinical applications of research on angiogenesis. N Engl J Med. 1995;333:1757–1763.

- Fong G, Rossant J, Gartsenstein M et al. Role of the Flt-1 receptor tyrosine kinase in regulating the assembly ofvascular endothelium. Nature. 1995;376:67–70.

- Friesel RE, Maciag T. Molecular mechanism of angiogenesis: fibroblast growth factor signal transducing. FASEB J. 1995;9:919–925.

- Fukumoto Y, Libby P, Rabkin E, Hill CC, Enomoto M, Hirouchi Y, Shiomi M, Aikawa M. Statins alter smooth muscle cell accumulation and collagen content in established atheroma of watanabe heritable hyperlipidemic rabbits. Circulation. 2001; 103: 993–999.

- Galis ZS, Khatri JJ. Matrix metalloproteinases in vascular remodeling and atherogenesis: the good, the bad, and the ugly. Circ Res. 2002; 90: 251–262

- Gerber HP, Condorelli F, Park J et al. Differential transcriptional regulation of the two vascular endothelial growth factor receptor

genes. Flt-1, but not Flk-1/KDR,is up-regulated by hypoxia. J Biol Chem. 1997;272:23659–23667.

- Gibson, CM, Murphy, SA, Marble, SJ, et al. Relationship of creatine kinase-myocardial band release to Thrombolysis in Myocardial Infarction perfusion grade after intracoronary stent placement: an ESPRIT substudy. Am Heart J 2002; 143:106.

- Gille H, Kowalski J, et al.Analysis of biological effects and signaling prioperities of Flt-1(VEGFR-1) and KDR(VEGFR-2).A reassessment using novel receptor – specific vascular endothelial growth factor mutants. 2001;276:3222-3230.

- Gitay-Goren H, Cohen T, Tessler S et al. Selective binding of VEGF 121 to one of the three vascular endothelial growth factor receptors of vascular endothelial cells. J Biol Chem. 1996;271:5519–5523.

- Glaser, R, Herrmann, HC, Murphy, SA, et al. Benefit of an early invasive management strategy in women with acute coronary syndromes. JAMA 2002; 288:3124.

- Grosskreutz CL, Anand-Apte B, Duplaa C, Quinn TP, Terman BI, Zetter B, D'Amore PA. Vascular endothelial growth factor-induced migration of vascular smooth muscle cells in vitro. Microvasc Res. 1999; 58: 128–136.

- Gu JW, Adair TH. Hypoxia-induced expression of VEGF is reversible in myocardial vascular smooth muscle cells. Am J Physiol. 1997;273: 628–633.

- Guyton JR. Phospholipid hydrolytic enzymes in a 'cesspool' of arterial intimal lipoproteins: a mechanism for atherogenic lipid accumulation. Arterioscler Thromb Vasc Biol. 2001; 21: 884–886.

- Hamm, CW, Braunwald, E. A classification of unstable angina revisited. Circulation 2000; 102:118.

- Hanes Franz Alber, Josef Dulak, Matthias Frick, et al. Atorvastatin decreases vascular endothelial growth factor in patients with coronary artery disaese. J Am coll cardiol; 2002; 39:1951-1955.

- Hansson GK, Libby P, Schonbeck U, Yan ZQ. Innate and adaptive immunity in the pathogenesis of atherosclerosis. Circ Res. 2002; 91: 281–291.

- Hansson GK. Immune mechanisms in atherosclerosis. Arterioscler Thromb Vasc Biol. 2001; 21: 1876–1890.

- Hashimoto E, Ogita T, Nakaoka T, Matsuoka R, Takao A, Kira Y. Rapid induction of vascular endothelial growth factor expression by transient ischemia in rat heart. Am J Physiol. 1994; 267: 1948–1954.

- Heeschen C, Jang JJ, Weis M, Pathak A, Kaji S, Hu RS, Tsao PS, Johnson FL, Cooke JP. Nicotine stimulates angiogenesis and promotes tumor growth and atherosclerosis. Nat Med. 2001; 7: 833–839.

- Heeschen, C, van den Brand MJ, Hamm, CW, Simoons, ML. Angiographic findings in patients with refractory unstable angina according to troponin T status. Circulation 1999; 100:1509.

- Helisch A, Schaper W. Arteriogenesis: the development and growth of collateral arteries. Microcirculation. 2003; 10: 83–97.

- Hillebrands JL, Klatter FA, van den Hurk BM, Popa ER, Nieuwenhuis P, Rozing J. Origin of neointimal endothelium and alpha-actin-positive smooth muscle cells in transplant arteriosclerosis. J Clin Invest. 2001; 107: 1411–1422.

- Hojo Y, Ikeda U, Zhu Y et al. Expression of vascular endothelial growth factor in patients with acute myocardial infarction. J Am Coll Cardiol. 2000;35:968–973.

- Hornig C, Barleon B, Ahmad S et al. Release and complex formation of soluble VEGFR-1 from endothelial cells and biological fluids. Lab Invest. 2000;80:443–454.

- Hoshi S, Nomoto Ki K, Kuromitsu J. High glucose induced VEGF expression via PKC and ERK in glomerular podocytes. Biochem Biophys Res Commun. 2002;290:177–184.

- Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, ainsworth J, Heim W, Berlin J, Baron A, Griffing S, Holmgren E, Ferrara N, Fyfe G, Rogers B, Ross R, Kabbinavar F. Bevacizumab plus irinotecan,

fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med. 2004; 350: 2335–2342.

- Hutter R, Carrick FE, Valdiviezo C, Wolinsky C, Rudge JS, Wiegand SJ, Fuster V, Badimon JJ, Sauter BV. Vascular endothelial growth factor regulates reendothelialization and neointima formation in a mouse model of arterial injury. Circulation. 2004; 110: 2430–2435.

- Ichimura T, Finch PW, Zhang G et al. Induction of FF-7 after kidney damage—a possible paracrine mechanism for tubule repair. Am J Physiol. 1996;271:F967–F976.

- Jeziorska M, Woolley DE. Local neovascularization and cellular composition within vulnerable regions of atherosclerotic plaques of human carotid arteries. J Pathol. 1999; 188: 189–196.

- Joukov V, Kaipainen A, Jeltsch M et al. Vascular endothelial growth factors VEGF-B and VEGF-C. J Cell Physiol. 1997;173:211–215.

- Kaiser M, Younge B, Bjornsson J, Goronzy JJ, Weyand CM. Formation of new vasa vasorum in vasculitis: production of angiogenic cytokines by multinucleated giant cells. Am J Pathol. 1999; 155: 765–774.

- Kang W lee, Gregory YH Lip, Andrew D Blann. Plasma angiopoietin 1, angiopoietin 2, angiopoietin Tie 2, and vascular endothelial growth factor receptor levels in acute coronary syndrome. Circulation 2004; 110:2355-2360.

- Karkkainen MJ, Petrova TV. Vascular endothelial growth factor receptors in the regulation of angiogenesis and lymphangiogenesis. Oncogene. 2000;19:5598–5605.

- Kendall RL, Wang G, Thomas KA. Identification of a natural soluble form of the vascular endothelial growth factor receptor, FLT-1, and its heterodimerization with KDR. Biochem Biophys Res Commun. 1996;226:324–328.

- Keyt BA, Berleau LT, Nguyen HV et al. The carboxyl-terminal domain (111–165) of vascular endothelial growth factoris critical for its mitogenic potency. J Biol Chem. 1996;271:7788–7795.

- Keyt BA, Nguyen HV, Berleau LT et al. Identification of vascular endothelial growth factor determinants for binding KDR and FLT-1 receptors. Generation of receptor-selective VEGF variants by site-directed mutagenesis. J Biol Chem. 1996b;271:5638–5643.

- Khaliq A, Li XF, Shams M et al. Localisation of placenta growth factor (PIGF) in human term placenta. Growth Factors. 1996;13:243–250.

- Khatri JJ, Johnson C, Magid R, Lessner SM, Laude KM, Dikalov SI, Harrison DG, Sung HJ, Rong Y, Galis ZS. Vascular oxidant stress enhances progression and angiogenesis of experimental atheroma. Circulation. 2004; 109: 520–525.

- Khurana R, Moons L, Shafi S, Luttun A, Collen D, Martin JF, Carmeliet P and Zachary I. Placental growth factor (PlGF) promotes atherosclerotic intimal thickening and macrophage accumulation. Circulation. 2005; 111: 2828–2836.

- Khurana R, Zhuang Z, Bhardwaj S, Murakami M, De Muinck E, Yla-Herttuala S, Ferrara N, Martin JF, Zachary I, Simons M. Angiogenesis-dependent and independent phases of intimal hyperplasia. Circulation. 2004; 110: 2436–2443.

- Kim NH, Jung HH, Cha DR et al. Expression of vascular endothelial growth factor in response to high glucose in rat mesangial cells. J Endocrinol. 2000;165:617–624.

- Kini, AS, Lee, P, Mitre, CA, et al. Postprocedure chest pain after coronary stenting: implications on clinical restenosis. J Am Coll Cardiol 2003; 41:33.

- Kitsukawa T, Shimizu M, Sanbo M et al. Neuropilin-semaphorin III/D-mediated chemorepulsive signals play a crucial role in peripheral nerve projection in mice. Neuron. 1997;19:995–1105.

- Klekamp JG, Jarzecka K, Perkett EA. Exposure to hyperoxia decreases the expression of vascular endothelial growth factor and its receptors in adults rat lungs. Am J Pathol. 1999;154:823–831.

- Koblizek TI, Weiss C, Yancopoulos GD et al. Angiopoietin-1 induces sprouting angiogenesis in vitro. Curr Biol. 1998;8:529–532.

- Kockx MM, Cromheeke KM, Knaapen MW, Bosmans JM, De Meyer GR, Herman AG, Bult H. Phagocytosis and macrophage activation associated with hemorrhagic microvessels in human atherosclerosis. Arterioscler Thromb Vasc Biol. 2003; 23: 440–446.

- Kockx MM, De Meyer GR, Muhring J, Jacob W, Bult H, Herman AG. Apoptosis and related proteins in different stages of human atherosclerotic plaques. Circulation. 1998; 97: 2307–231.

- Kolodgie FD, Burke AP, Farb A, Gold HK, Yuan J, Narula J, Finn AV, Virmani R. The thin-cap fibroatheroma: a type of vulnerable plaque: the major precursor lesion to acute coronary syndromes. Cardiol. 2001; 16: 285–292.

- Kolodgie FD, Gold HK, Burke AP, Fowler DR, Kruth HS, Weber DK, Farb A, Guerrero LJ, Hayase M, Kutys R, Narula J, Finn AV, Virmani R. Intraplaque hemorrhage and progression of coronary atheroma. N Engl J Med. 2003; 349: 2316–2325.

- Kolodgie FD, Virmani R, Burke AP, Farb A, Weber DK, Kutys R, Finn AV, Gold HK. Pathologic assessment of the vulnerable human coronary plaque. Heart. 2004; 90: 1385–1391.

- Koter M, Broncel M, Chojnowska-Jezierska J, Klikczynska K, Franiak I. The effect of atorvastatin on erythrocyte membranes and serum lipids in patients with type-2 hypercholesterolemia. Eur J Clin Pharmacol. 2002; 58: 501–506.

- Kukk E, Lymboussaki ST, Kaipaninen A et al. VEGF-C receptor binding pattern of expression with VEGFR-3 suggestsa role in lymphatic development. Development. 1996;122:3837–3839.

- Kumamoto M, Nakashima Y, Sueishi K. Intimal neovascularization in human coronary atherosclerosis: its origin and pathophysiological significance. Hum Pathol. 1995; 26: 450–456.

- Kuwabara K, Ogawa S, Matsumoto M et al. Hypoxiamediated induction of acidic/basic fibroblast growth factor and platelet-derived growth factor in mononuclear phagocytes stimulates growth of hypoxic endothelial cells. Proc Natl Acad Sci USA. 1995;92 :4606–4610.

- Kwon HM, Sangiorgi G, Ritman EL, Lerman A, McKenna C, Virmani R, Edwards WD, Holmes DR, Schwartz RS. Adventitial vasa vasorum in balloon-injured coronary arteries: visualization and quantitation by a microscopic three-dimensional computed tomography technique. J Am Coll Cardiol. 1998; 32: 2072–2079.

- Kwon HM, Sangiorgi G, Ritman EL, McKenna C, Holmes DR Jr, Schwartz RS, Lerman A. Enhanced coronary vasa vasorum neovascularization in experimental hypercholesterolemia. J Clin Invest. 1998; 101: 1551–1556.

- Labinaz, M, Kilaru, R, Pieper, K, et al. Outcomes of patients with acute coronary syndromes and prior coronary artery bypass grafting: results from the platelet glycoprotein IIb/IIIa in unstable angina: receptor suppression using integrilin therapy (PURSUIT) trial. Circulation 2002; 105:322.

- Lee SH, Wolf PL, Escudero et al. Early expression of angiogenesis factors in acute myocardial ischemia and infarction. N Engl J Med. 2000;342: 626–633.

- Lemstrom KB, Krebs R, Nykanen AI, Tikkanen JM, Sihvola RK, Aaltola EM, Hayry PJ, Wood J, Alitalo K, Yla-Herttuala S, Koskinen PK. Vascular endothelial growth factor enhances cardiac allograft arteriosclerosis. Circulation. 2002; 105: 2524–2530.

- Leon ME, Chavez C, Fyfe B, Nagorsky MJ, Garcia FU. Cholesterol granuloma of the maxillary sinus. Arch Pathol Lab Med. 2002; 126: 217–219.

- Levy AP, Levy NS, Goldberg MA. Identification of 5 hypoxia-inducible RNA-protein binding sites conserved in rat and human vascular endothelial growth factor mRNA. JACC. 1997;90:1–32.

- Levy NS, Goldberg MA, Levy AP. Sequencing of the human vascular endothelial growth factor (VEGF) 3' untranslated region (UTR): conservation of five hypoxia-inducible RNA-protein binding sites. Biochim Biophys Acta. 1997;1352:167–173.

- Libby P, Theroux P. Pathophysiology of coronary artery disease. Circulation. 2005;111(25):3481-3488.

- Libby P. Inflammation in atherosclerosis. Nature. 2002; 420: 868–874.

- Lim HS, Blann AD, Chong AY,et al., Diabetes Care 27:2918-2924, 2004.

- Lip PL, Belgore FM, Blann AD. Plasma vascular endothelial growth factor and soluble VEGF receptor Flt-1 in prolifer-ative retinopathy: a pilot study of the relationship toendothelial dysfunction and laser treatment. Invest Ophthalmol Vis Sci. 2000;41:2115–2119.

- Luttun A, Tjwa M, Moons L, Wu Y, Angelillo-Scherrer A, Liao F, Nagy JA, Hooper A, Priller J, De Klerck B, Compernolle V, Daci E, Bohlen P, Dewerchin M, Herbert JM, Fava R, Matthys P, Carmeliet G, Collen D, Dvorak HF, Hicklin DJ, Carmeliet P. Revascularization of ischemic tissues by PlGF treatment, and inhibition of tumor angiogenesis, arthritis and atherosclerosis by anti-Flt1. Nat Med. 2002; 8: 831–840.

- M Selvin, J Krupinski,, A Slowik, P Kumar, et al. Serial measurment of Vascular endothelial growth factor and transforming growth factor B1 in serum f patients with acute ischemic stoke. Stroke 2000; 31:1863.

- Mach F, Schonbeck U, Fabunmi RP, Murphy C, Atkinson E, Bonnefoy JY, Graber P, Libby P. T lymphocytes induce endothelial cell matrix metalloproteinase expression by a CD40L-dependent mechanism: implications for tubule formation. Am J Pathol. 1999; 154: 229–238.

- Maisonpierre PC, Suri C, Jones PF et al. Angiopoietin-2, a natural antagonist for TIE2 that disrupts in vivo angiogenesis. Science. 1997;277:55–60.

- Matsumoto K. Interleukin 10 inhibits vascular permeability factor release by peripheral blood mononuclear cells in patients with lipoid nephrosis. Nephron. 1997;75:154–159.

- Mehran, R, Dangas, G, Mintz, GS, et al. Atherosclerotic plaque burden and CK-MB enzyme elevation after coronary interventions: intravascular ultrasound study of 2256 patients. Circulation 2000; 101:604.

- Melo LG, Gnecchi M, Pachori AS, Kong D, Wang K, Liu X, Pratt RE, Dzau VJ. Endothelium-targeted gene and cell-based therapies for cardiovascular disease. Arterioscler Thromb Vasc Biol. 2004; 24: 1761–1774.

- Melter M, Reinders ME, Sho M, Pal S, Geehan C, Denton MD, Mukhopadhyay D, Briscoe DM. Ligation of CD40 induces the expression of vascular endothelial growth factor by endothelial cells and monocytes and promotes angiogenesis in vivo. Blood. 2000; 96: 3801–3808.

- Meyer M, Clauss M, Lepple-Wienhues A et al. A novel vascular endothelial growth factor encoded by Orf virus, VEGF-E mediates angiogenesis via signalling through VEGFR-2 (KDR) but not VEGFR-1 (Flt-1) receptor kinases. EMBO J. 1999;18:363–374.

- Millauer B, Longhi MP, Plate KH et al. Dominant-negative inhibition of Flk-1 suppresses the growth of many tumour types in vivo. Cancer Res. 1996;56:1615–1620.

- Millauer B, Wizigmann-Voos S, Schnurch H et al. High affinity VEGF binding and developmental expression flk-1 as a major regulator of vasculogenesis and angiogenesis. Cell. 1993;72:835–846.

- Miyazaki H, Matsuoka H, Cooke JP et al. Endogenous nitric oxide synthase inhibitor: a novel marker of angiogenesis. Circulation. 1999;99:1141–1146.

- Mofidi R, Crotty TB, et al.Association between plaque instability,angiogenesis and symptomatic carotid scclusive disease.Br J surg 2001;88:945-950.

- Molica S, Vitelli G, Levato D et al. Increased serum levels of vascular endothelial growth factor predict risk of progression in early B-cell chronic lymphocytic leukaemia. Br J Haematol. 1999;107:606–610.

- Moreno PR, Purushothaman KR, Fuster V, Echeverri D, Truszczynska H, Sharma SK, Badimon JJ, O'Connor WN. Plaque neovascularization is increased in ruptured atherosclerotic lesions of human aorta: implications for plaque vulnerability. Circulation. 2004; 110: 2032–2038.

- Moulton KS, Heller E, Konerding MA, Flynn E, Palinski W, Folkman J. Angiogenesis inhibitors endostatin or TNP-470 reduce intimal neovascularization and plaque growth in apolipoprotein E–deficient mice. Circulation. 1999; 99: 1726–1732.

- Moulton KS, Vakili K, Zurakowski D, Soliman M, Butterfield C, Sylvin E, Lo KM, Gillies S, Javaherian K, Folkman J. Inhibition of plaque neovascularization reduces macrophage accumulation and progression of advanced atherosclerosis. Proc Natl Acad Sci U S A. 2003; 100: 4736–4741.

- Moulton KS. Plaque angiogenesis and atherosclerosis. Curr Atheroscler Rep 2001;3:225–33.

- Murohara T, Horowitz JR, Silver M et al. Vascular endothelial growth factor/vascular permeability factor enhances vascular permeability via nitric oxide and prostacyclin. Circulation. 1998;97:99–107.

- Neufeld G, Cohen T, et al. Vascular endothelial growth factor and its receptors .FASEB J 1999; 13:9-22.

- Neufeld G, Cohen T, Gengrinovitch S et al. Vascular endothelial growth factor (VEGF) and its receptors. FASEB J. 1999;13:9–22.

- Nouran Elghandour, Hala Aly Gamal, Mervat Eldamarawy, et al. Role of vascular endothelial growth factor and tissue factor in non diabetic egyptian patients with peripheral arterial disease and relation between and angiogenesis and thrombogenesis. Egypt J 58(1):155-163,March 2006.

- O'Brien ER, Garvin MR, Dev R, Stewart DK, Hinohara T, Simpson JB, Schwartz SM. Angiogenesis in human coronary atherosclerotic plaques. Am J Pathol. 1994; 145: 883–894.

- Ogawa H, Suefuji H, Soejima H, et al. Increased blood vascular endothelial growth factor levels in patients with acute myocardial infarction. Cardiology 2000;93:93–9.

- Ohta O, Kusaba A. Development of vasa vasorum in the arterially implanted autovein bypass graft and its anastomosis in the dog. Int Angiol. 1997; 16: 197–203

- Ohtani K, Egashira K, Hiasa K, Zhao Q, Kitamoto S, Ishibashi M, Usui M, Inoue S, Yonemitsu Y, Sueishi K, Sata M, Shibuya M, Sunagawa K. Blockade of vascular endothelial growth factor suppresses experimental restenosis after intraluminal injury by inhibiting recruitment of monocyte lineage cells. Circulation. 2004; 110: 2444–2452.

- Olofsson B, Pajusola K, Kaipainen A et al. Vascular endothelial growth factor B, a novel growth factor for endothelial cells. Proc Natl Acad Sci USA. 1996;93:2576–2581.

- Olofsson B, Pajusola K, von Euler G et al. Genomic organization of the mouse and human genes for vascular endothelial growth factor B (VEGF-B) and characterization of a second splice isoform. J Biol Chem. 1996;271:19310–19317

- Ozawa CR, Banfi A, Glazer NL, Thurston G, Springer ML, Kraft PE, McDonald DM, Blau HM. Microenvironmental VEGF concentration, not total dose, determines a threshold between normal and aberrant angiogenesis. J Clin Invest. 2004; 113: 516–527.

- Park JE, Keller GA, Ferrara N. The vascular endothelial growth factor (VEGF) isoforms: differential deposition into the sub-epithelial extracellular matrix and bioactivity of extracellular matrix-bound VEGF. Mol Biol Cell. 1993;4:1317–1326.

- Park M, Lee ST. The fourth immunoglobulin-like loop in the extracellular domain of FLT-1, a VEGF receptor, includes a major heparin-binding site. Biochem Biophys Res Commun. 1999;264:730–734.

- Park SH, Kim KW, Lee YS et al. Hypoglycemia-induced VEGF expression is mediated by intracellular Ca2+ and protein kinase C signalling pathway in HepG2 human hapatoblastoma cells. Int J Mol Med. 2001;7:91–96.

- Patel, DJ, Gomma, AH, Knight, CJ, et al. Why is recurrent myocardial ischaemia a predictor of adverse outcome in unstable angina?. An observational study of myocardial ischaemia and its relation to coronary anatomy. Eur Heart J 2001; 22:1991.

- Patel, MR, Chen, AY, Peterson, ED, et al. Prevalence, predictors, and outcomes of patients with non-ST-segment elevation myocardial

infarction and insignificant coronary artery disease: results from the Can Rapid risk stratification of Unstable angina patients Suppress ADverse outcomes with Early implementation of the ACC/AHA Guidelines (CRUSADE) initiative. Am Heart J 2006; 152:641.

- Pels K, Deiner C, Coupland SE, Noutsias M, Sutter AP, Schultheiss HP, Yla-Herttuala S, Schwimmbeck PL. Effect of adventitial VEGF(165) gene transfer on vascular thickening after coronary artery balloon injury. Cardiovasc Res. 2003; 60: 664–672.

- Pels K, Labinaz M, Hoffert C, O'Brien ER. Adventitial angiogenesis early after coronary angioplasty: correlation with arterial remodeling. Arterioscler Thromb Vasc Biol. 1999; 19: 229–238.

- Pugh CW, Ratcliffe PJ. Regulation of angiogenesis by hypoxia: role of the HIF system. Nat Med. 2003; 9: 677–684.

- Rajagopalan S, Meng XP, Ramasamy S, Harrison DG, Galis ZS. Reactive oxygen species produced by macrophage-derived foam cells regulate the activity of vascular matrix metalloproteinases in vitro: implications for atherosclerotic plaque stability. J Clin Invest. 1996; 98: 2572–2579.

- Ratner M. Genentech discloses safety concerns over Avastin. Nat Biotechnol. 2004; 22: 1198.

- Reinders ME, Sho M, Robertson SW, Geehan CS, Briscoe DM. Proangiogenic function of CD40 ligand-CD40 interactions. J Immunol. 2003; 171: 1534–1541.

- Ricciardi, MJ, Wu, E, Davidson, CJ, et al. Visualization of discrete microinfarction after percutaneous coronary intervention associated with mild creatine kinase-MB elevation. Circulation 2001; 103:2780.

- Risau W, Flamme I. Vasculogenesis. Annu Rev Cell Dev Biol. 1995;11:73–91.

- Risau W. Mechanisms of angiogenesis. Nature. 1997;386:671–674.

- Robbins, MA, Marso, SP, Wolski, K, et al. Chest pain- A strong predictor of adverse cardiac events following percutaneous interventions (from the Evaluation of Platelet IIb/IIIa Inhibitor for Stenting trial [EPISTENT]). Am J Cardiol 1999; 84:1350.

- Roe, MT, Harrington, RA, Prosper, DM, et al. Clinical and therapeutic profile of patients presenting with acute coronary syndromes who do not have significant coronary artery disease. The Platelet Glycoprotein IIb/IIIa in Unstable Angina: Receptor Suppression Using Integrilin Therapy (PURSUIT) Trial Investigators. Circulation 2000; 102:1101.

- Rogers WJ, Canto JG, Lambrew CT, et al. Temporal trends in the treatment of over 1.5 million patients with myocardial infarction in the US from 1990 through 1999: the National Registry of Myocardial Infarction 1, 2 and 3. J Am Coll Cardiol. 2000;36(7):2056-2063.

- Roller RE, Renner W, Tischler R et al. Vascular endothelial growth factor in plasma of patients undergoing peripheral angioplasty. Thromb Haemost. 2001;85:1119–1120.

- Rosenfeld ME, Polinsky P, Virmani R, Kauser K, Rubanyi G, Schwartz SM. Advanced atherosclerotic lesions in the innominate artery of the ApoE knockout mouse. Arterioscler Thromb Vasc Biol. 2000; 20: 2587–2592.

- Rydberg EK, Krettek A, Ullstrom C, Ekstrom K, Svensson PA, Carlsson LM, Jonsson-Rylander AC, Hansson GI, McPheat W, Wiklund O, Ohlsson BG, Hulten LM. Hypoxia increases LDL oxidation and expression of 15-lipoxygenase-2 in human macrophages. Arterioscler Thromb Vasc Biol. 2004; 24: 2040–2045.

- Satake S, Kuzuya M, Miura H et al. Up-regulation of vascular endothelial growth factor in response to glucose deprivation. Biol Chem. 1998;90:161–168.

- Sato TN, Tozawa Y, Deutsch U et al. Distinct roles of the receptor tyrosine kinases Tie-1 and Tie-2 in blood vessel formation. Nature. 1995;376:70–74.

- Savory LJ, Stacker SA, Flemming SB et al. Viral vascular endothelial growth factor plays a critical role in Orf virus infection. J Virol. 2000;74:10699–10706.

- Sawano A, Takahashi T, Yamaguchi S et al. Flt-1 but not KDR/Flk-1 tyrosine kinase is a receptor for placenta growth factors, which is related to vascular endothelial growth factor. Cell Growth Differ. 1996;7:213–221.

- Seko Y, Imai Y, Suzuki S et al. Serum levels of vascular endothelial growth factor in patients with acute myocardial infarction undergoing reperfusion therapy. Clin Sci. 1997;92:453–454.

- Shanahan CM, Weissberg PL. Smooth muscle cell phenotypes in atherosclerotic lesions. Curr Opin Lipidol. 1999; 10: 507–513.

- Shigematsu K, Yasuhara H, Shigematsu H. Topical application of antiangiogenic agent AGM-1470 suppresses anastomotic intimal hyperplasia after ePTFE grafting in a rabbit model. Surgery. 2001; 129: 220–230.

- Shyu, K-G, Kuan, P-L, Cheng, J-J, et al. Cardiac troponin, creatine kinase, and its isoform release after successful percutaneous transluminal coronary angioplasty with or without stenting. Am Heart J 1998; 135:862.

- Simon M, Grone HJ, Johren O et al. Expression of vascular endothelial growth factor and its receptors in human renal ontogenesis and adult kidney. Am J Physiol. 1995;268:F240–F250.

- Simonini A, Moscucci M, Muller DW, Bates ER, Pagani FD, Burdick MD, Strieter RM. IL-8 is an angiogenic factor in human coronary atherectomy tissue. Circulation. 2000; 101: 1519–1526.

- Simons M, Ware JA. Therapeutic angiogenesis in cardiovascular disease. Nat Rev Drug Discov. 2003; 2: 863–871.

- Simons M. Angiogenesis: where do we stand now? Circulation. 2005; 111: 1556–1566.

- Stabile E, Burnett MS, Watkins C, Kinnaird T, Bachis A, la Sala A, Miller JM, Shou M, Epstein SE, Fuchs S. Impaired arteriogenic response to acute hindlimb ischemia in CD4-knockout mice. Circulation. 2003; 108: 205–210.

- Staker SA, Caesar C, Baldwin ME et al. VEGF-D promotes the metastatic spread of tumor cells via the lymphatics. Nat Med. 2001;7:186–191.

- Staker SA, Caesar C, Baldwin ME et al. VEGF-D promotes the metastatic spread of tumor cells via the lymphatics. Nat Med. 2001;7:186–191. Soker S, Takashima S, Miao HQ et al. Neuropilin-1

is expressed by endothelial and tumour cells as an isoform-specific receptor for vascular endothelial growth factor. Cell. 1998;92:735–745.

- Stary HC, Chandler AB, Glagov S, Guyton JR, Insull W Jr, Rosenfeld ME, Schaffer SA, Schwartz CJ, Wagner WD, Wissler RW. A definition of initial, fatty streak, and intermediate lesions of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. Circulation. 1994; 89: 2462–2478.

- Steinberg D. Atherogenesis in perspective: hypercholesterolemia and inflammation as partners in crime. Nat Med. 2002; 8: 1211–1217.

- Steinhubl, SR, Lauer, MS, Mukherjee, DP, et al. The duration of pretreatment with ticlopidine prior to stenting is associated with the risk of procedure-related non-Q wave myocardial infarction. J Am Coll Cardiol 1998; 32:1366.

- Sueishi K, Yonemitsu Y, Nakagawa K, Kaneda Y, Kumamoto M, Nakashima Y. Atherosclerosis and angiogenesis: its pathophysiological significance in humans as well as in an animal model induced by the gene transfer of vascular endothelial growth factor. Ann N Y Acad Sci. 1997; 811: 311–324.

- Suri C, Jones PF, Patan S et al. Requisite role of angiopoietin-1, a ligand for the TIE-2 receptor, during embryonic angiogenesis. Cell. 1996;87:1171–1180.

- Tabas I. Cholesterol and phospholipid metabolism in macrophages. Biochim Biophys Acta. 2000; 1529: 164–174.

- Tabibiazar R, Rockson SG. Angiogenesis and the ischaemic heart. Eur Heart J. 2001;22:903–918.

- Takahashi A, Sasaki H, Kim SJ et al. Marked increased amounts of messenger RNAs for vascular endothelial growth factor and placenta growth factor in renal cell carcinoma associated angiogenesis. Cancer Res. 1994;54:4233–4237.

- Takaya N, Yuan C, Chu B, Saam T, Polissar NL, Jarvik GP, Isaac C, McDonough J, Natiello C, Small R, Ferguson MS, Hatsukami TS. Presence of intraplaque hemorrhage stimulates progression of carotid

atherosclerotic plaques: a high-resolution magnetic resonance imaging study. Circulation. 2005; 111: 2768–2775.

- Tenaglia AN, Peters KG, Sketch MH Jr, Annex BH. Neovascularization in atherectomy specimens from patients with unstable angina: implications for pathogenesis of unstable angina. Am Heart J. 1998; 135: 10–14.

- Thom T, Haase N, Rosamond W, et al. Heart disease and stroke statistics—2006 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation. 2006;113(6):85-151.

- Ton J. Rabelink; Hetty C. de Boer; Eelco J.P. de Koning; Anton-Jan van Zonneveld .Arteriosclerosis, Thrombosis, and Vascular Biology. 2004;24:834-838.

- Torres Filho IP, Leunig M, Yuan F, Intaglietta M, Jain RK. Noninvasive measurement of microvascular and interstitial oxygen profiles in a human tumor in SCID mice. Proc Natl Acad Sci U S A. 1994; 91: 2081–2085.

- Valenzuela DM, Griffith JA, Rojass J et al. Angiopoietin 3 and 4: diverging gene counterparts in mice and humans. Proc Natl Acad Sci USA. 1999;96:1904–1909.

- Van Belle E, Maillard L, Tio FO, Isner JM. Accelerated endothelialization by local delivery of recombinant human vascular endothelial growth factor reduces in-stent intimal formation. Biochem Biophys Res Commun. 1997; 235: 311–316.

- van Miltenburg-van Zijl, AJ, Simoons, ML, Veerhoek, RJ, Bossuyt, PM. Incidence and follow-up of Braunwald subgroups in unstable angina pectoris. J Am Coll Cardiol 1995; 25:1286.

- Varnava AM, Mills PG, Davies MJ. Relationship between coronary artery remodeling and plaque vulnerability. Circulation. 2002; 105: 939–943.

- Vasa M, fichtlsherer S, adler K, et al. Increase in circulating endothelial progenitor cells by statin therapy in patients with stable coronary artery disease. Circulation. 2001;103:2885–2890.

- Vikkula M, Boon LM, Carraway KL et al. Vascular dysmorphogenesis caused by an activating mutation in the receptor tyrosine kinase TIE2. Cell. 1996;87:1181–1190.

- Vincenti V, Cassano C, Rocchi M et al. Assignment of the vascular endothelial growth factor gene to human chromosome 6p21.3. Circulation. 1996;93:1493–1495.

- Volz A, Boyle JM, Cann HM et al. Report of the Second International Workshop on Human Chromosome 6. Genomics. 1994;21:464–472.

- Vuckovic M, Ponting J, Terman BI et al. Expression ofthe vascular endothelial growth factor receptor, KDR, in human placenta. J Anatomy. 1996;188:361–366.

- Vuorela P, Helske S, Hornig C et al. Amniotic fluid-soluble vascular endothelial growth factor receptor-1 in preeclampsia. Obstet Gynecol. 2000;95:353–357.

- Wartiovaara U, Salven P, Mikkola H et al. Peripheral blood platelets express VEGF-C and VEGF which are released during platelet activation. Thromb Haemost. 1998;80:171–175.

- Webb NJ, Bottomley MJ, Watson CJ et al. Vascular endothelial growth factor (VEGF) is released from plateletsduring blood clotting: implications for the measurement of circulating VEGF levels in clinical disease. Clin Sci. 1998;94:395–404.

- Weindel K, Moringlane JR, Marme D et al. Detection and quantification of vascular endothelial growth factor/vascular permeability factor in brain tumour tissue and cyst fluid: the key to angiogenesis. Neurosurgery. 1994;35:439–448.

- Westerband A, Gentile AT, Hunter GC, Gooden MA, Aguirre ML, Berman SS, Mills JL. Intimal growth and neovascularization in human stenotic vein grafts. J Am Coll Surg. 2000; 191: 264–271.

- Wiedlocha A, Falnes PO, Madshus IH et al. Dual mode of signal transducing by externally added acidic fibroblast growth factor. Cell. 1994;76:1039–1051.

- Williams B. Factors regulating the expression of vascular permeability/vascular endothelial growth factor by human vascular tissues. Diabetologia. 1997;40:S118–S120.

- Wilting J, Birkenhager R, Eichmann A et al. VEGF 121 induces proliferation of vascular endothelial cells and expression of flk-1 without affecting lymphatic vessels of chorioallantoic membrane. Dev Biol. 1996;176:76–85.

- Winkleby MA, Cubbin C. Changing patterns in health behaviors and risk factors related to chronic diseases, 1990-2000. Am J Health Promot. 2004;19(1):19-27.

- Witzenbichler B, Asahara T, Murohara T et al. Vascular endothelial growth factor-C (VEGF-C/VEGF-2) promotesangiogenesis in the setting of tissue ischemia. Am J Pathol. 1998;153:381–394.

- Xu X, Weinstein M, Li C et al. Fibroblast growth factor receptor (FGFRs) and their role in limb development. Cell Tissue Res. 1999;296:33–43.

- Xu XH, Shah PK, Faure E, Equils O, Thomas L, Fishbein MC, Luthringer D, Xu XP, Rajavashisth TB, Yano J, Kaul S, Arditi M. Toll-like receptor-4 is expressed by macrophages in murine and human lipid-rich atherosclerotic plaques and upregulated by oxidized LDL. Circulation. 2001; 104: 3103–3108.

- Yancopoulos GD, Davis S, Gale NW, Rudge JS, Wiegand SJ, Holash J. Vascular-specific growth factors and blood vessel formation. Nature. 2000; 407: 242–248.

- Yang XJ, Cepko CL. Flk-1, a receptor for vascular endothelial growth factor (VEGF), is expressed by retinalprogenitor cells. J Neurosci. 1996;16:6089–6099.

- Yasushi kadoma, Yashinobu Killa, Takawitsu nakamura, et al. Atorvastatin Increases Plasma Soluble Fms-Like Tyrosine Kinase-1 and DecreasesVascular Endothelial Growth Factor and Placental Growth Factor in Association With Improvement ofVentricular Function in Acute Myocardial Infarction. J Am coll cardiol 2006;48:43-50.

- Yla-Herttuala S, Alitalo K. Gene transfer as a tool to induce therapeutic vascular growth. Nat Med. 2003; 9: 694–701.

- Yue X, Tomanek RJ. Stimulation of coronary vasculogenesis/angiogenesis by hypoxia in cultured embryonic hearts. Dev Dyn. 1999;216:28–36.

- Yusuf S, Hawken S, Ounpuu S, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet. 2004;364(9438):937-952.

- Zachary I, Mathur A, Yla-Herttuala S, Martin J. Vascular protection: a novel nonangiogenic cardiovascular role for vascular endothelial growth factor. Arterioscler Thromb Vasc Biol. 2000; 20: 1512–1520.

- Zhang Y, Cliff WJ, Schoefl GI, Higgins G. Immunohistochemical study of intimal microvessels in coronary atherosclerosis. Am J Pathol. 1993; 143: 164–172.

الملخص العربى

ان الأوعية الدموية الموجودة بداخل أماكن تصلب الشرايين عادة ما تكون عرضة للأنفجار ومن ثم حدوث الأزمات القلبية الحادة. بالأضافة الى ذلك فانها تعتبرمدخلا لكرات الدم البيضاء و العد يد من وسائط الألتهاب الأ خرى .

لذا يفترض أن يكون لمعامل الغشاء المبطن للأوعية الدموية النموى ، المعامل الأساسى لتخليق هذه الأوعية ، دور فى حدوث هذه الأزمات القلبية الحادة.

وقد كان الهدف من الرسالة تحديد مستوى معامل الغشاء المبطن للأوعية الدموية النموى فى حالات الأزمات القلبية الحادة فى الدم وتحديد ما إذا كان هذا المستوى يرتبط بشدة حدوث هذه الأومات وتطورها أوشدة أصابة الشرايين التاجية.

وللصول الى هذا الهدف تم أجراء البحث على 78 مريض تم تقسيمهم الى 3 مجموعات كما يلى:

المجموعة الأولى: وكانت تضم 31 مريض مصابين بذبحة صدرية غير مستقرة

المجموعة الثانية: وكانت تضم 26 مريض مصابين بأحتشاء غير كامل بعضلة القلب.

المجموعة الثالثة: وكانت تضم 21 مريض بغير تاريخ مريضى سابق لأى من أمراض القلب وتم عمل قسطرة قلبية أوضحت سلامة الشرايين التاجية.

وتم أعادة تقسيم المرضى فى المجموعتين الأولى والثانية حسب أستخدام العقاقير المثبطة لنسبة الدهون بالدم إلى:

المجموعة الأولى أ: وتضم 16 مريض من المجموعة الأولى ومن المستخدمين لهذه العقاقير

المجموعة الثانية أ: وتضم 13 مريض من المجموعة الثانية ومن المستخدمين لهذه العقاقير.

المجموعة الأولى ب: وتضم 15 مريض من المجموعة الأولى ومن غير مستخدمى هذه العقاقير.

المجموعة الثانية ب:وتضم 13 مريض من المجموعة الثانية ومن غير مستخدمى هذه العقاقير.

وقد تم أخذ التاريخ المرضى بالكامل لجميع المرضى كما تم عمل فحص أكلينيكى شامل لهم وتم عمل رسم قلب وموجات فوق صوتية على القلب ومعامل كاملة تشمل مستوى الدهون بالدم بالأضافة الى قياس نسبة معامل الغشاء المبطن للأوعية الدموية النموى بالدم. بالأضافة الى ذلك فقد تم عمل قسطرة تشخيصية لتصوير الشرايين القلبية لجميع المرضى لتحديد مدى أصابة هذه الشرايين بالتصلبات.

وقد تمت ملاحظة المرضى أثناء وجودهم بالمستشفى لتحديد ماإذا كان هناك مضاعفات فى صورة تكرار للآلام الصدرية أو أختلال فى ضربات القلب أو هبوط فى عضلة القلب أو الصدمة القلبية.

وقد أوضحت الدراسة أن مستوى معامل الغشاء المبطن للأوعية الدموية النموى يكون أعلى فى مرضى المجموعة الأولى ب والثانية ب أكثر من مرضى المجموعة الأولى أ والمجموعة الثانية أ والمجموعة الثالثة.

بالأضافة الى ذلك فأن المرضى أصحاب المستوى الأعلى لمعامل الغشاء المبطن للأوعية النموى أكثر عرضة لتكرار آلام بالصدر مع هبوط فى عضلة القلب. الا أنه لا توجد علاقة أحصائية بين نسبة مستوى الغشاء المبطن للأوعية الدموية وبين شدة أصابة الشرايين التاجية التى يتم تشخيصها بواسطة القسطرة التشخيصية.

دراسة مقارنة بين معامل الغشاء المبطن للأوعية الدموية النموى وشدة حدوث الجلطات الحادة

رسالة مقدمة من الطبيب

محمد حمدى محمد صالح

توطئه للحصول على درجة الدكتوراة فى طب الحالات الحرجة

تحت إشراف

أ.د. حسام أحمد موافى

أستاذ طب الحالات الحرجة

قسم طب رعاية الحالات الحرجة – جامعة القاهرة

أ.د. طارق الجوهرى

أستاذ م. طب الحالات الحرجة

قسم طب رعاية الحالات الحرجة

جامعة القاهرة

د. عماد عمر

مدرس طب الحالات الحرجة

قسم طب رعاية الحالات الحرجة

جامعة القاهرة

د. أحمد بطاح

مدرس طب الحالات الحرجة

قسم طب رعاية الحالات الحرجة

جامعة القاهرة

جامعة القاهرة

2009