Platelets function in patients with coronary artery diseases

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Abstract: The knowledge of platelet function has improved substantially over the past decade, particularly in the setting of CVD. Studies have defined a role for transcripts in anucleate platelets, which contain a broad number of miRNAs that seem to have functional relevance. The triad of functional activity of CD40L in atherosclerotic models, high content in platelets, and mobilization during platelet thrombosis provides a readily testable hypothesis and places platelet-derived CD40L squarely in the forefront as an important, mitigating factor in this disease. Still, several questions arise.

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Introduction

Platelets are small, anucleated cytoplasmic bodies circulating in blood stream. Theses cellular fragments are derived from megakaryocytes in the bone marrow.[1] Platelets play a povital role in the development of atherosclerotic lesions, plaque destabilization, and atherothrombosis.[2] Platelets have an increasingly well- defined critical role in coronary artery thrombosis, and in other common cardiovascular diseases, including stroke, peripheral vascular disease, and diabetes mellitus.[3] Although the role of platelets in thrombosis is well characterized, platelets may also have a role in the pathogenesis of underlying atherosclerotic process. Platelet function tests have been studied in cardiovascular disease as a means to predict clinical outcomes. [4]Mean platelet volume (MPV) is a marker of platelet size, activity, and a predictor of cardiovascular risk. [5]It is quantified on automated hemograms routinely measured before coronary revascularization. In the setting of PCI, increased mortality due to rise in MPV during the postoperative period has been reported. [6]Large platelets have enhanced reactivity with normal sized platelets.[7] MPV has been associated with clinical and angiographic outcomes. [8]Patients with a high MPV before balloon angioplasty have been more likely to develop restenosis.[9] In patients undergoing primary PCI high MPV has been associated with impaired angiographic reperfusion and increased six months mortality. It aims to evaluate the effect of the pre-procedural MPV level on the long term clinical outcome in patients undergoing elective percutaneus coronary intervention (PCI). [10]

Physiology of platelets

Platelet activation:

The adhesion of platelets to the subendothelial matrix is the initial step in primary hemostasis. Platelets interact with extracellular matrix proteins via specific adhesive glycoproteins (GP). Binding of biochemical agonists to their receptors, receptor cross-linking, or changes in the plasma membrane induce a complex cascade of signals, transuded from the membrane into the cytoplasm, which results in platelet activation (outside-in signaling). [11]

Platelet Adhesion

In certain conditions of flow, platelets have to slow down to stop at sites of vascular damage. The high molecular weight (1-10 MDa) multimeric plasma protein von Willebrand factor (vWF) associates with the major matrix protein collagen on the surface of the subendothelium and serves as a substrate for platelet adhesion, predominantly under high shear. The multiple binding sites of vWF multimers enables first contacts to the GPIb/V/IX complex on platelets leading to formation of firm bonds and platelet capture. In contrast to vWF monomers, only dimers and multimers are able to cross-link and to activate the GPIb/V/IX complex. Conformational changes in the GPIb/V/IX or vWF molecule are thought to modulate these interactions. Under physiological conditions it is supposed that binding of vWF to collagen enables binding to GPIb/V/IX.[12]

Platelet Secretion

Activated platelets release several granule components which modulate functions of interacting platelets and blood and vascular cells. Several secretion products of immobilized platelets stimulate additional circulating platelets which are recruited to form aggregates. The dense bodies of platelets contain important secondary agonists like ADP or serotonin. About 50% of platelet ADP is stored in the dense bodies (storage pool), which is released after platelet activation but cannot be refilled. In contrast, the metabolic pool of adenine nucleotides, localized in the

cytoplasm but not connected to the dense bodies, is able to synthesize new ADP but cannot be released.[13]

Platelet aggregation

The aggregation of platelets is characterized by the accumulation of platelets into a haemostatic plug. The central platelet receptor in this process is the GPIIb/IIIa ($\alpha_{IIb}\beta_3$-integrin) linking activated platelets through fibrinogen bridges. A resting platelet presents 40,000 to 50,000 GPIIb/IIIa complexes on its surface. In its nonactive state this integrin cannot bind soluble ligands like plasma fibrinogen, vWF, TSP, fibronectin, or vitronectin. Only stimulation of a platelet leads to an increase in GPIIb/IIIa molecules, via α-granule exocytosis, and to activation of surface-exposed GPIIb/IIIa, enabling binding of soluble ligands. On the other hand, immobilized fibrinogen on stimulated platelets serves as an adhesive substrate for resting platelets through GPIIb/IIIa that leads to amplification of primary aggregation.[14]

Procoagulant activity-model of receptor-mediated thrombin generation of platelets

The formation of a stable platelet plug during secondary hemostasis is characterized by thrombin-mediated conversion of fibrinogen to fibrin. Thrombin is generated on surfaces of blood and vascular cells. However, the platelet membrane contains a specific lipid assembly and receptors with high-affinity binding sites for clotting factors, a favored preferential and specialized locus to induce and modulate secondary haemostatic processes.[15]

Microvascular release /shedding of adhesion molecules

Strong agonists like collagen in combination with thrombin or complement (C5b-9) induce shedding of microvesicles from the platelet surface. This "budding" process is due to Ca^{2+}-mediated activation of calpain and leads to vesicles containing exclusively intracytoplasmatic substances. [16]

Signal transduction- stimulatory signaling

Stimulatory platelet signaling as a result of receptor ligation and receptor cross-linking leads to production and release of several intracellular messenger molecules: Ca^{2+}, products of the phospholipase C (PLC)-mediated phosphoinositol hydrolysis, diacylgycerol, inosit-1,4,5-triphosphat (IP3), and thromboxane A_2 (TxA_2). Platelet agonists like ADP, TxA_2, epinephrine, serotonin, and thrombin interact with seven specific transmembrane receptors that are coupled by GTP-binding heterotrimeric G-proteins, initiating several signaling pathways. Signaling through receptors coupled to the Gq-family of G-proteins (PAR1, PAR4, TxA_2-receptor, 5-HT2A-receptor) leads to activation of PLC. PLC catalyzes the hydrolysis of phosphatidyl inositolbisphosphate (PIP2) to IP3, which induces the mobilization of Ca^{2+} from the dense tubula system. An increase in intracellular Ca^{2+} is associated with a phosphorylation of the myosin-light-chain by myosin-light-chain kinase, a process that is necessary for shape change. In addition, receptor signaling through Ga12/13-proteins (PAR1, PAR4), contributes to shape change, too.[17]

Signal transduction- inhibitory signaling

For regulation and limitation of collagen-induced thrombus formation, platelets express platelet-endothelial cell adhesion molecule-1 (CD31), a member of the inhibitory receptor family. Cross-linking of CD31 includes phosphorylation of immunoreceptor tyrosine-based inhibition motifs (ITIMS), inhibiting the actions of ITAMS.[18].

Acute Myocardial Infarction

Myocardial infarction (MI) is the irreversible necrosis of heart muscle secondary to prolonged ischemia. This usually results from an imbalance of oxygen supply and demand. The appearance of cardiac enzymes in the circulation generally indicates myocardial necrosis.[19]

Pathology:

Myocardial infarction can be subcategorized on the basis of anatomic, morphologic, and diagnostic clinical information.

From an anatomic or morphologic standpoint:

- A transmural MI is characterized by ischemic necrosis of the full thickness of the affected muscle segment(s), extending from the endocardium through the myocardium to the epicardium.
- A nontransmural MI is defined as an area of ischemic necrosis that does not extend through the full thickness of myocardial wall segment(s). In a nontransmural MI, the area of ischemic necrosis is limited to either the endocardium or the endocardium and myocardium. It is the endocardial and subendocardial zones of the myocardial wall segment that are the least perfused regions of the heart and are most vulnerable to conditions of ischemia.

Based on clinical diagnostic criteria :

determined by the presence or absence of Q waves on an electrocardiogram (ECG). However, the presence or absence of Q waves does not distinguish a transmural from a non-transmural MI as determined by pathology.[20]

Pathogenesis of AMI

Acute coronary syndrome are initiated by rupture of a coronary artery atherosclerotic plaque, atherosclerotic plaque are (a fibromuscular cap and an underlying lipid-rich core). Plaque erosion may occur due to the actions of metalloproteases which results in activation of circulating platelets, thrombosis, and a secondary cascade of prothrombotic, procoagulant, and vasoconstrictor mechanisms that promote further coronary artery occlusion. The degree of lumen compromise largely determines the resulting form of ACS. [21]

Consider nonatherosclerotic causes of acute MI in younger patients or if no evidence of atherosclerosis is noted. Such causes include coronary emboli from sources such as an infected cardiac valve, coronary occlusion secondary to vasculitis, primary coronary vasospasm (variant angina), cocaine use, or other factors leading to mismatch of oxygen supply and demand, as may occur with a significant gastro intestinal bleed. [22]

Precipitating factors

Accelerating angina and rest angina, two patterns of unstable angina may climinate in MI on cardiac surgical procedures have been noted as precursors of MI. Other factors reported as predisposing to MI include respiratory infections, hypoxemia of any cause, pulmonary embolism, hypoglycemia, administration of ergot preparations, use of cocaine, sympathomimetics, serum sickness, allergy.[23]

Risk factors

*Nonmodifiable risk factors for atherosclerosis

- Age
- Sex
- Family history of premature coronary heart disease

*Modifiable risk factors for atherosclerosis

- Smoking or other tobacco use
- Diabetes mellitus
- Hypertension
- Dyslipidemia
- Obesity

*New and other risk factors for atherosclerosis

- Elevated homocysteine levels
- Male pattern baldness
- Sedentary lifestyle and/or lack of exercise
- Psychosocial stress
- Presence of peripheral vascular disease
- Poor oral hygiene[24]

Diagnosis

I- Chest pain

Is the most common symptom of acute myocardial infarction (MI) and is often described as a sensation of tightness, pressure, or squeezing. Pain radiates most often to the left arm, but may also radiate to the jaw, neck, right arm, back, and epigastrium. [25] Women often experience different symptoms than men the most common symptoms of MI in women include dyspnea, weakness, and fatigue. Fatigue, sleep disturbances, and dyspnea have been reported as frequently occurring prodromal symptoms which may manifest as long as one month before the actual clinically manifested ischemic event. In women, chest pain may be less predictive of coronary ischemia than in men. [26] Approximately one third of all myocardial infarctions are silent, without chest pain or other symptoms. This happens more often in elderly patients and patients with diabetes mellitus. [27]

II-Laboratory finding:

Table (1)Biochemical markers of cardiac injury

Marker	Time to raised plasma value	Peak	Duration of elevation
AST	**8 -12h**	**1-2 days**	**3-6 days**
LDH	**8-12h**	**2-3 days**	**7-10 days**
CK	**4-6h**	**12-36h**	**3-4 days**
CK-MB(activity)	**4-6h**	**12-24h**	**2-3days**
CK-MB(mass)	**4-6h**	**12-24h**	**2-3 days**
CK isomers	**1-3 h**	**8-12h**	**18-30h**
Hydroxy-butyrate dehydrogenase	**8-12h**	**2-3 days**	**7-14 days**
Myoglobin	**2-3h**	**6-12h**	
Glycogen phosphorylase BB	**2-4h**	**8-12h**	**24-36h**
Heart fatty acid binding protein	**2-3h**	**8-12h**	**18-30h**
Myosin light chain	**3-6h**	**4 days**	**10-14 days**
Troponin T	**4-6h**	**12-24 h**	**7-10 days**
Troponin I	**4-6h**	**12-24h**	**6-8 days**

[28]

III-Electrocardiography:

The ECG may show ischemic ST-segment abnormalities or T-wave abnormalities, or both. [29]

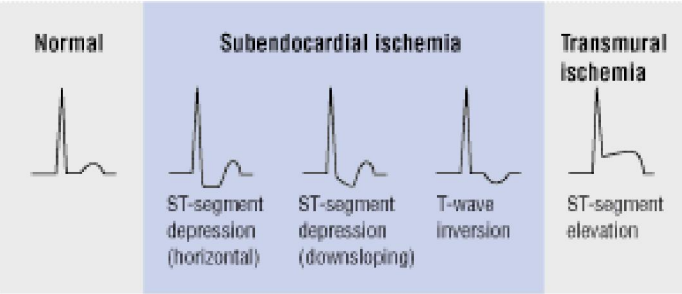

Figure 1: Electrocardiographic ST segment changes of ischemia & injury. Unless ST segment elevation is brief, transmural ischemia evolves into MI typically reflected by pathological Q waves.

IV-Echocardiography

A— Two dimensional echocardiography:

In patients with chest pain compatible with MI but with a nondiagnostic ECG, the finding of echocardiography of a distinct region of disordered contraction can be helpful diagnostically because it supports the diagnosis of myocardial ischemia. [30]

B- Doppler echocardiography

This technique allows assessment of blood flow in the cardiac chambers and across cardiac valves. Used in conjunction with two dimensional echocardiography, it is helpful in detecting and assessing the severity of mitral or tricuspid regurgitation after STEMI.[31]

V-cross-sectional imaging of the coronary arteries

A- Computed tomographic (CT) angiography

Permits evaluation of the arterial wall (not just the lumen), has shown that the characteristics of plaque associated with acute coronary syndromes include low attenuation (i.e., little or no calcification) and outward expansion of the artery wall, a process that tends to accommodate the growth of plaque while minimizing luminal encroachment. [32]

B- Intravascular ultrasonography

It has shown that in acute coronary syndromes, the culprits often lie proximal to the sites of maximal stenosis — the traditional targets of revascularization therapies. [33].

Platelets and cardiovascular diseases

Introduction

Platelets have a central role in CVD, particularly in acute coronary syndrome and stroke, contributing to the development of acute thrombotic events. [34]

Platelets become activated via soluble receptor-mediated stimulants, such as thrombin, ADP, and thromboxane A2, thereby enhancing the thrombogenicity of activated platelets. Platelet aggregation occurs when the glycoprotein IIb/IIIa receptor binds adhesive proteins. Platelets can release many cytokines and signaling molecules that can lead to a cascade of further platelet activation, thrombosis, and vasoconstriction. Activated platelets form a growing coronary thrombus with concomitant propagation of fibrin. [35]

Platelets and transcripts

Platelets are anucleate, and their cytoplasmic RNA has long been considered to be residual transcripts of their forming cell, the mega-karyocyte.[36][37] Investigators in several studies have used microarray and RNA sequencing to define the transcripts contained in platelets. Current estimates of the number of transcripts in platelets range from 1,500 to 9,500 in healthy human platelets, [38][39]Importantly, human platelets contain several forms of small RNAs, with miRNAs being the most abundant and comprising 80% of all small RNAs in platelets.[40]These miRNA–mRNAs can be co expressed.[41]

miRNAs

Small RNAs, including miRNAs, are important components of an evolutionarily conserved system of RNA-based gene regulation in eukaryotes. [42]These miRNAs are diverse in sequence and expression patterns, and are evolutionarily widespread, suggesting that they might participate in a wide range of critical genetic regulatory pathways across species. miRNAs interfere with the expression of mRNA encoding factors that control developmental timing, stem-cell maintenance, and other physiological processes in animals.[43] miRNAs are initially transcribed as long primary miRNAs (pri-miRNAs), which are processed by the RNase III enzyme Drosha (also known as ribonuclease 3) to generate stem–loop precursor miRNAs (pre-miRNAs) of ~70 nucleotides in length.[44]

miRNAs as biomarkers

Some miRNAs are expressed in a cell-specific manner. miRNAs are detectable in plasma, where they can be contained in microparticles or transported by lipoproteins. [45] They are also found in circulating blood cells, and release of miRNAs into the circulation has been linked to cellular damage, such as cardiomyocyte injury, or active processes, including secretion. miRNAs seem to be stable in the cir-culation, and quantifiable with a high degree of

accuracy. Even at low abundance, miRNAs are comprised of nucleic acids with sequences that can often be easily amplified, making them ideal biomarkers of disease. [46]

miRNAs in cardiovascular disease

miRNAs have important roles in cardiac development, including stem-cell differentiation and proliferation of cardiomyocytes in the embryo; however, they are also important in susceptibility to CVD. [47] miRNAs in cardiac tissue show dynamic changes in several forms of heart disease, suggesting that they have important roles in regulating an acute injury response. For example, animal and human studies have clearly demonstrated that cardiac miRNAs have critical roles in regulating important pathophysiological processes underlying heart diseases, including electrical remodeling (miR-1, miR-21, miR-133, and miR-328), cardiac fibrosis (miR-21 and miR-29), hypertrophic structural remodeling (miR-208), and cardiac ischaemia–reperfusion injury (miR-150 and miR-320). [48]

miRNAs and megakaryopoiesis

Megakaryocytes are derived from common myeloid progenitors, with platelets formed in turn from megakaryocytes. Most platelet-derived miRNAs are presumed to have been formed in the megakaryocyte. Of note, each mRNA has multiple miRNAs that bind and, given that each miRNA binds multiple mRNA targets, many miRNAs will regulate pathways in a 'rheostat-like' manner. miR-155 is a common small RNA found in hematopoietic stem cells and implicated in platelet development. Specifically, up regulation of miR-155 inhibits hematopoietic stem-cell differentiation into megakaryocytes, as shown by the transplantation of hematopoietic stem cells over expressing miR-155 into mice whose bone marrow had been depleted, which resulted in reduced numbers of megakaryocytes.[49]

miRNAs in platelets

Platelets are known to have miRNAs as well as Dicer and Argonaute protein complexes, thereby enabling the processing of pre-RNAs to miRNAs and control of reporter transcripts. On the basis of transcriptomic profiling, the vast majority of common small RNAs in the platelet are miRNAs. Profiling from healthy human platelets has characterized 492 platelet miRNAs and 40 novel miRNAs, with the most abundant being members of the let-7 family.[50]

Effects on platelet function

Transcripts are present in platelets in variable quantities and are associated with specific human phenotypes and disease states, [51] but whether the platelet miRNA signature reflects or influences platelet function remains unknown. Such an association is certainly suggested by the distinct platelet miRNA profiles linked with age and sex as well as diseases.[52]

Roles in cardiovascular disease

The platelet miRNA profile that is associated with myocardial infarction is distinct from that of other cell types and plasma. Moreover, platelet miRNA signatures differ between patients with ST-segment elevation myocardial infarction (STEMI), typically a more thrombosis-dependent form of myocardial infarction, and those with non-STEMI, suggesting that platelet levels of important miRNAs might influence platelet thrombogenicity and type of infarction.[53]

Role of inflammation and oxidative stress in platelet activation

Involvement of inflammation in cardiovascular disease is well defined. Circulating platelets are affected by this metabolic disruption and by inflammatory mediators synthesized and/or released on contact with inflammatory signals. Platelets are known to play a major role in this process and have been identified as targets and players in inflammation-induced cardiovascular disease. [54]

Platelet-Derived CD40L

The Switch-Hitting Player of Cardiovascular Disease

Emerging data suggest that CD40L may be at the heart of the atherosclerotic process. What makes CD40L so unique? Its localization and its multifunctionality. CD40L is a surprisingly abundant protein in platelets and may have roles in the inflammatory aspects of atherosclerotic lesion progression, and thrombosis**.** [55]

CD40L

CD40L is a trimeric, transmembrane protein of the tumor necrosis factor family that was originally identified on cells of the immune system (activated CD4 cells, mast cells,

basophils, eosinophils, and natural killer cells). The role of CD40L in the immune response involves binding to its receptor on B cells, CD40, to induce B-cell proliferation, generate memory B cells, block B-cell apoptosis, and mediate antibody class switching. However, it was subsequently shown that CD40L and CD40 are also present on several cells of the vasculature, including endothelial cells, smooth muscle cells, monocytes, and macrophages. [56]

The Platelet–CD40L Axis

It suggests that chronic activation of the vessel wall contributes to the recruitment of platelets, which in turn allows further endothelium damage. The theory of platelet recruitment on a physically intact but functionally dysregulated endothelium seems even more relevant, because activated endothelial cells support platelet rolling, their translocation, and occasionally their adherence. Pioneering studies of **Dr**

E.J. Bowie and **coworkers** also established that the lack of von Willebrand Factor (the principal ligand mediating platelet aggregation under high shear rates) affected atherosclerotic lesion progression, which further validated this hypothesis. Because platelets in their granules possess a large range of proinflammatory molecules, such as transforming growth factor-_, platelet factor-4, RANTES, and P-selectin, a direct link between platelets, inflammation, and atherosclerosis was far from being speculative. [57]

Mean Platelet Volume in Patients with Acute Coronary Syndromes

Coronary artery disease is the most important cause of mortality and morbidity in industrialized as well as in developing countries. Both endogenous and exogenous risk factors such as smoking, hypercholesterolemia, DM, and hypertension increase the risk of ACS [58].MPV is a parameter which states platelet size and indirectly proves its activity. It is known that larger platelets are more reactive due to higher concentration of active substances in micro granules (e.g. thromboxane A2 and B2, platelet factor 4, P-selectin, platelet-derived growth factor) and expression of adhesive receptors (glycoprotein IIb/IIIa) [59].

Platelet density

Human platelets have a density span of 1.04–1.08 kg/L. Over the years the significance of platelet density heterogeneity in human physiology and pathology has been debated. In particular, relationships between age and density have been subject of much controversy. One theory postulates that megakaryocytes release platelets having differing densities. The cells subsequently circulate at a fixed density. [60].

Clinical significance of platelet density

Throughout the years the clinical significance of platelet density heterogeneity has been a topic of scientific interest. **Martin and co-workers** found in 1983 that platelet density and MPV increase in conjunction with acute MI. [61] **Järemo** and his research group have investigated platelet density heterogeneity in various disease states. Active inflammatory bowel disease was associated with small high-density platelets. [62]

Platelet subpopulations

In recent years, interest in platelet subpopulations has increased especially since the discovery of coat platelets About 30% of the human platelet population belongs to this category. Coat platelets have high surface concentrations of procoagulant proteins (examples include factor V, fibrinogen, fibronectin, thrombospondin and vWF) after maximum stimulation with collagen and thrombin. [63]

Surface bound P-Selectin

Soluble P-Selectin

P-Selectin is shed from activated platelets and endothelial cells into the circulation, sP-Selectin. **Fijnheer and co-workers** propose that platelets are the major source of circulating sP-selectin. [64]Most likely, sP-Selectin acts as a regulatory molecule and prevents unsuitable activation of circulating neutrophils. It is possible that sP-Selectin inhibits oxygen release of neutrophils until adhesion and migration occurs at sites of inflammation. [65] P-Selectin (CD62P) was first described on activated platelets. [66]The protein is one of the largest selectins (a group of cell adhesion molecules) with a mass of 140 kDa. In resting platelets, P-Selectin is part of both α-granules and dense granules. [67].

Conclusion

In summary, the knowledge of platelet function has improved substantially over the past decade, particularly in the setting of CVD. Studies have defined a role for transcripts in anucleate platelets, which contain a broad number of miRNAs that seem to have functional relevance. However, important questions remain unanswered, including the origin of the transcriptomic material found in platelets, the specific function of these transcripts in acute and chronic CVDs, how miRNA transfers into and out of platelets, and whether this is a specific or targeted process. An accumulating body of evidence suggests that quantitative differences in the relative abundance of miRNAs contribute to susceptibility to, and prognosis of, human diseases, including atherothrombotic disease. Also intriguing is the role that platelets have in the transfer and distribution of vascular miRNAs. Improved understanding of the importance of platelet miRNAs in the setting of CVD might assist in the diagnosis and treatment of prothrombotic vascular disease.The mechanisms responsible for initiating atherosclerotic lesions are undoubtedly diverse. However, the emerging data on CD40L suggest the evolution of a new paradigm for the role of platelets in inflammation and atherosclerotic lesion progression. The triad of functional activity of CD40L in atherosclerotic models, high content in platelets, and mobilization during platelet thrombosis provides a readily testable hypothesis and places platelet-derived CD40L squarely in the forefront as an important, mitigating factor in this disease. Still, several questions arise. Does the sCD40L systemically generated by activated platelets in circulation or locally by acute thrombosis impact subsequent thrombosis, lesion progression, or restenosis? Will the ability of GP IIb/IIIa antagonists to block sCD40L release in vitro translate into the inhibition of sCD40L release in acute coronary thrombotic indications like

acute coronary syndromes or as result of PCI? Is the activity of antiplatelet agents limited to blocking occlusion and subsequent ischemia, or do these agents have effects that translate into the inhibition of atherosclerotic

lesion progression? Is this the mechanism by which the short-term inhibition of thrombosis with GP IIb/IIIa antagonists eg, (20 hours) in the setting of PCI translates into a prolonged inhibition of the accrual of events eg,(up to a year or longer), as was observed in the Evaluation of c7E3 for the Prevention of Ischemic Complications (EPIC) and Enhanced Suppression of Platelet Receptor GP-IIb/IIIa using Integrilin. Increased MPV is an indicator for larger and more active platelets and an independent risk factor for MI in coronary artery disease. Likewise, increased MPV is a risk factor for platelet activity. High density platelets are functionally more active and have a higher metabolism, than low density cells. They also adhere more rapidly to collagen. In acute MI, with ST elevations, peak platelet density was inversely correlated with the inflammatory response.

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