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Endothelial Function and Hemorheological Parameters Modulate Coronary Blood Flow in Patients without Significant Coronary Artery Disease

Tommaso Gori[1,2], Ana Damaske[1,2], Selina Muxel[1,2], Marie-Christine Radmacher[1], Federica Fasola[1,] Sarina Schaefer[1], Massimo Fineschi[3], Sandro Forconi[4], Friedrich Jung[5], Thomas Münzel[1*], John D Parker[6*]

[1]Department of Cardiology, and [2]Center for Thrombosis and Hemostasis, University Medical Center Mainz, Germany,[3] Department of Cardiology, University Medical Hospital of Siena, Italy, [4] Siena-Toronto Center, University of Siena, Italy, [5]Center for Biomaterial Development and Berlin-Brandenburg Centre for Regenerative Therapies, Helmholtz-ZentrumGeesthacht, Institute of Polymer Research, Teltow, Germany, and [6]Department of Cardiology Mount Sinai and University Health Network Hospitals. *Equally contributed to the study.

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Address for correspondence:

TommasoGori, MD PhD

Department of Cardiology

UniversityMedicalCenterMainz

Mainz, Germany

Email: tommaso.gori@unimedizin-mainz.de

Abstract

Background: coronary (micro)vascular resistance is regulated by the complex interplay of several factors. Two potentially important determinants include endothelial function and the rheological properties of blood. However, their impact on the control of the coronary resistance vasculature is poorly understood.

Methods: The corrected Thrombolysis In Myocardial Infarction frame count (TIMIfc, an index of coronary flow velocity), conduit artery endothelial function, intima-media thickness of the common carotid artery and complete blood counts were measured in 145 patients undergoing elective coronary angiography. Patients with obstructive coronary artery disease or systemic conditions thought to be associated with microvascular disease were excluded from the analysis.

Results: There was a strong correlation between the TIMIfc measured in the three main coronary artery distributions (R values between 0.71 and 0.85, $P<0.00001$). The TIMIfc was higher in males ($P<0.05$), but there was no association with traditional risk factors for coronary artery disease (all $P>0.1$). There was a correlation between TIMIfc and L-FMC, a parameter of resting endothelial function ($R=0.33$, $P<0.0005$). TIMIfc also correlated with mean platelet volume (a marker of platelet activation, $R=0.33$, $P<0.001$), and hematocrit ($R=0.33$, $P=0.0002$). There was no correlation between TIMIfc and carotid intima-media thickness and the degree of coronary atherosclerosis. Logistic regression analysis showed that L-FMC and hemorheological variables may explain as much as 19% of the variability in TIMIfc.

Conclusions: Resting peripheral endothelial function, as well as parameters of platelet function, correlate with coronary TIMIfc. These data emphasize the existence of an association between endothelial function, hemorheological variables and coronary blood flow velocity.

Keywords: endothelial function; coronary microcirculation; platelets

Introduction

Beyond epicardial atherosclerosis, a number of factors affect the control of coronary vascular resistances at the micro- and macrovascular level. Endothelial function and the interaction between the vessel wall and the blood column are both felt to have a central importance, but their role remains incompletely understood. Particularly, the contribution of hemorheological variables and endothelial function to resting coronary blood flow remains poorly investigated in humans.

The thrombolysis in myocardial infarction frame count (TIMIfc) has been used as a simple and reproducible method to provide quantitative information concerning coronary blood flow (velocity) in patient populations [11, 12]. Although the clinical importance of abnormalities in the TIMIfc has been confirmed in a number of studies, the mechanism(s) determining these abnormalities remain incompletely understood. In the setting of acute myocardial ischemia and after percutaneous coronary interventions, distal embolism, acute endothelial ischemic damage and neurohormonal changes are felt to play a role[12]. By contrast, in the absence of an acute coronary syndrome, the mechanism(s) leading to abnormal TIMIfc are less understood. Slow epicardial flow and high TIMIfc have been associated with a number of conditions including coronary ectasia, coronary (microvascular) spasm, subclinical atherosclerosis, myocardial muscular bridges, increased adrenergic activity, increased left ventricular end-diastolic pressure (in the setting of heart failure, valvular disease, cardiomyopathies or hypertension), as well as liver, kidney and inflammatory conditions[4, 5, 23, 24]. Even after excluding these conditions, however, a very high interindividual variability in the TIMIfc remains, reflecting important differences in tissue perfusion in resting conditions. Endothelial dysfunction and/or hemorheological factors have been proposed, but not demonstrated, as possible mechanisms to explain this variability. We set out to explore whether there is a systematic relationship between systemic endothelial function, hemorheological variables, platelet activation and coronary blood flow velocity.

Materials and methods

145 consecutive patients undergoing coronary angiography between November 2009 and June 2010 for the clinical suspicion of coronary artery disease were enrolled in a prospective registry. Patients were excluded from this analysis if they had any of the following:epicardial stenosis ≥ 60%; coronary artery ectasia (diameter >4mm); known connective tissue or systemic inflammatory disease; serious infective disease within the last 3 months; recent or ongoing acute coronary syndrome; history of myocardial infarction; prior coronary artery by-pass surgery; left ventricular ejection fraction <40%; elevated left ventricular end-diastolic pressure; uncontrolled hypertension; significant left ventricular hypertrophy by two-dimensional echocardiography; clinically relevant valvular disease and, finally, those with ongoing malignancy. None of the patients was on oral anticoagulant therapy. All patients gave informed consent to participate in the study, and the protocol was approved by the institution's human research committee (Ethic committee of the LandesärztekammerRheinlandPfalz; approval number: 837.401.10).

Fasting blood samples were drawn from all patients and analyzed for complete blood count, creatinine, blood urea nitrogen, liver function tests, parameters of coagulation, fibrinogen and c-reactive protein. The following cardiovascular risk factors were documented: obesity (body mass index >30Kg/m^2); age; smoking (or previous smoking); hyperlipidemia (current or previous finding of total serum cholesterol >220 mg/dL and/or serum triglycerides >200 mg/dL); hypertension (systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg on two consecutive seated measurements or therapy with antihypertensive medication); family history (first degree relatives with cardiovascular disease); diabetes mellitus (fasting serum glucose levels >126 mg/dL or therapy with oral hypoglycemic agents or insulin). The use of cardiovascular medications and antiplatelet agents was recorded.

Assessment of endothelial function and carotid intima-media thickness

The methods employed for endothelial function analysis in our laboratory have been previously described in detail, along with repeatability and reproducibility data[3, 16]. Briefly, the diameter of the radial artery is measured in resting conditions, during a 4.5 minutes suprasystolic inflation of a pneumatic cuff placed distal to the measurement site, and for the 4.5 minutes after deflation of the cuff. Low-flow mediated vasoconstriction (L-FMC) is calculated as the % reduction in diameter observed during cuff occlusion; this parameter is thought to reflect endothelium-dependent vasomotor tone in resting conditions[16]. Flow-mediated dilation (FMD) is expressed as the maximum % dilation upon cuff deflation, a parameter that reflects endothelium-dependent responsiveness to the increase in shear stress caused by reactive hyperaemia. Thus, the two parameters reflect two different but complementary aspects of endothelial function. Vascular ultrasound data were analyzed in a blinded fashion prior to coronary angiography by an investigator not aware of the clinical status of the patient. All patients were asked to refrain from physical activity for 60 minutes and to discontinue vasoactive medications for 12 hours before peripheral vascular function measurements. Carotid artery intima-media thickness was assessed as previously described[3]. Briefly, ECG-gated images (at least 3 measures) of the posterior wall of the common carotid artery were acquired for both carotid arteries, and the average value of the intima-media thickness was calculated using validated software.

Assessment of TIMI frame count and coronary artery disease

Coronary angiography was performed in all patients 1-7 days after assessment of endothelial function using a standard Judkins technique. Two study investigators, blinded to clinical and other study data, independently interpreted the angiograms. The presence of any epicardial stenosis >30% at angiography and/or of coronary artery stents in1, 2 or all 3-vessels was recorded. Patients with stenoses >60% and >20mm-long were excluded because of the potential impact of such anatomical features on TIMIfc. The TIMIfc was determined for each major coronary artery in each patient and

control subject according to the method first described by Gibson et al[11]. Briefly, the number of cineangiographic frames, recorded at 30 frames per second, required for the leading edge of the column of radiographic contrast to reach a predetermined landmark, was counted. The TIMIfc was calculated for each of the three major coronaries (in the left anterior descending the number of frames was divided by 1.7 to compensate for the longer length of the vessel) and as a mean value across the three vessels. Patients who had received nitroglycerin or other vasoactive drugs during angiography were not included in the analysis. The Syntax Score (www.syntaxscore.org), which takes into account the number, position and anatomical characteristics of coronary lesions, was calculated in all patients.

Laboratory variables

A sample (ca. 20 mL) was taken from an antecubital vein, and processed within one hour. Hematological parameters were determined with absorption spectroscopy (Advia, Siemens). CRP (immune-torbidometry, Latex Antibodies), GOT and GPT (NADH Oxidation), creatinine (Jaffé), cholesterol (enzymatic), triglycerides (GPO-PAP), HDL (chromogenic enzymatic color-test) and LDL (selective dilution) were assessed with a Architect C-Module (Abbott). PT and aPTT (coagulometry with photooptical detection) were assessed with a BCS II (Siemens) machine.

Statistics

Continuous variables are presented as mean ± standard deviation. Discrete variables are presented as count, percentage. The Student's t or the Chi-square test was used to compare variables between subjects with TIMIfc above and below the median. The prevalence of each risk factor was compared using Chi-square statistics. Linear and logistic regression analyses were used to determine the association between continuous variables. Receiver operating characteristic analysis was used to determine the predictive power of the variables of interest. Analyses were performed

with and without adjustment for the above risk factors. Analysis was performed with Statview (SAS) and Medcalc (Mariakerk, Belgium) and $P<0.05$ was taken as the threshold for significance.

Results

Patient characteristics (Table 1)

The 145 subjects included in this analysis had a mean age of 65±10 years; 76 (52%) were males. Thirty-six (25%) of the patients suffered from diabetes mellitus, 106 (79%) from hypertension, 50 (34%) had a family history of CAD, 53 (37%) had a body mass index >30 and 39 (27%) had neither a stenosis >30% at angiography nor a history of coronary stenting. There was a correlation of the TIMIfc between the 3 major coronary arteries (R values ranged from 0.85 to 0.71, $P<0.0001$ for all comparisons, Figure 1). 68% of the patients used aspirin. The median TIMIfc was 26.

The relationship between cardiac risk factors, CAD and carotid artery intima-media thickness and the TIMIfc

Clinical characteristics of patients divided by the median value of TIMIfc are presented in Table 1. Of the traditional cardiovascular risk factors, only male gender was associated with a higher mean TIMIfc (mean TIMIfc in men: 30.0±9.3; in women: 26.3±9.0; $P<0.05$). In contrast, age, hypercholesterolemia, smoking, diabetes, hyperlipidemia, family history of cardiac disease, hypertension and obesity were not associated with differences in the TIMIfc ($P>0.1$ for all). The presence or absence of angiographic evidence of CAD had no impact on TIMIfc (patients with at least one lesion >30%, mean TIMIfc: 29.2±9.2; patients with no CAD: 27.8±9.7, $P=0.4$). Furthermore, there was no correlation between TIMIfc and the syntax score ($P>0.1$ for all vessels). When patients were divided based on the median of the TIMIfc (26 frames), there was no difference across groups in the prevalence of any of the traditional risk factors (Table 1).There was no relationship between carotid intima-media thickness and TIMIfc ($R=0.008$, $P=0.9$). Finally, there

was no relationship between use of cardiovascular medications, including antiaggregant therapies, and TIMIfc (Table 2).

The relationship between TIMI frame count and endothelial function parameters

Data are presented in Figure 2. No correlation was found between TIMIfc and FMD ($P>0.4$ for all vessels). In contrast, in univariate linear regression analysis, L-FMC showed a positive correlation with TIMIfc in all 3 coronary distributions ($R = 0.3$ for all, $P<0.0005$ for all vessels). When only subjects with a TIMIfc smaller than the median were included, the independent contribution of L-FMC to a multivariate model including cardiovascular risk factors, FMD and laboratory variables was maintained ($P=0.004$). A ROC curve analysis demonstrated that an L-FMC>2.4% identified patients with a TIMIfc greater than the median with a sensitivity of 62% and a specificity of 71% (area under the ROC curve 0.67, $P<0.001$).

The relationship between TIMI frame count and hematologic parameters

A positive correlation was shown between TIMIfc and hematocrit (Figure 3, upper panels), total red blood cell count ($R=0.25$, $P=0.001$) and hemoglobin ($R=0.25$, $P=0.0002$); similarly, there was a positive correlation between TIMIfc and mean platelet volume (MPV, $R=0.32$, $P<0.0001$, Figure 4, lower panels) as well as between TIMIfc and total platelet count ($R=0.20$, $P=0.01$). There was an inverse relationship between platelet count and MPV ($R=0.44$, $P<0.0001$). In a multivariate regression model hematocrit, platelet count and mean platelet volume were predictors of TIMIfc. There was no correlation between TIMIfc and total white blood cell counts ($P>0.5$).

There was also no correlation between any hemorheological parameter and either L-FMC and FMD responses (all $P>0.5$).

The ROC analysis demonstrated that an MPV>8.7fl identified patients with a mean TIMIfc greater than the median with a sensitivity of 53% and a specificity of 65% (area under the ROC curve 0.62, P<0.001, Figure 3). A hematocrit >42.6% identified a TIMIfc> median with a sensitivity of 49% and a specificity of 87% (area under the ROC curve 0.68, P<0.001, Figure 3).

The relationship between TIMI frame count and other laboratory parameters

The TIMIfc showed no correlation with parameters of renal or hepatic function, markers of inflammation, coagulation or with blood lipids (P>0.2 for all). When patients were divided based on the median value of TIMIfc, all laboratory parameters were similar in the 2 groups (Table 2).

Logistic regression analysis

Logistic regression analysis was used to determine the impact of L-FMC, MPV, hematocrit on TIMIfc. This analysis yielded a R^2=0.19 (P=0.0002), demonstrating that these parameters collectively explain approximately 19% of the variability of coronary blood flow velocity.

Discussion

The calculation of the corrected TIMIfc provides a simple, and reproducible quantification of the rate of flow through the conduit coronary arteries allowing quantitative insight into global (macro+microvascular) coronary resistances. In their original paper, Gibson et al. assessed TIMIfc in 78 patients with normal coronary arteries[11], and the mean corrected TIMIfc in this sample was 21±3. However, even in the absence of epicardial stenoses, there is a very wide variability in this parameter when subjects undergoing routine angiography are compared (Figure 1). The mechanisms leading to this variability in (micro)vascular resistances and myocardial perfusion, as well as their clinical implications, remain incompletely understood[21]. Beyond local factors (e.g. myocardial oxygen consumption), endothelial function and hemorheological properties are thought,

but have never demonstrated, to play a role[18]. Furthermore, while extreme levels of hemoconcentration or hemodilution are known to limit myocardial perfusion [26], it is unclear whether changes in hematocrit or platelet function within the "normal" reference values have an impact on coronary perfusion.

Endothelial function and coronary flow

Elevated levels of plasma endothelin-1, asymmetric dimethylarginine, adhesion molecules, homocysteine and decreased levels of nitric oxide have been described in patients with elevated TIMI frame counts, suggesting that abnormalities in systemic endothelial function may be associated with (and determine) increased coronary resistance[24, 27]. The data in the current study support the existence of a relationship between (systemic) endothelial function and coronary resistance. Notably, while there was the relationship between TIMIfc and L-FMC was clear, there was no relationship with FMD. Importantly, L-FMC and FMD are felt to reflect different aspects of endothelial and vascular function: while "traditional" FMD measures the endothelial reactivity to a specific stimulus (a sudden increase in shear stress), L-FMC quantifies the vasoconstriction that is observed when local shear stress is reduced from physiological values to near zero. As such, L-FMC is a measure of endothelial tone in resting conditions, and is expression of resting (micro)vascular tone rather than reactivity. In line with this concept, L-FMC (but not FMD) showed a strong correlation with the TIMIfc, which reflects *resting* coronary vascular tone. The present data provide evidence in support of our previous observation that resting vascular tone may be impaired even though arterial responsiveness is preserved[15, 22]. As such, the current data suggest the existence of a relationship between a (novel) parameter of peripheral endothelial function and central (coronary) blood flow.

The impact of hemorheological parameters

Along with vascular cross sectional area, hemorheological parameters are important determinants of vascular resistance and blood flow[18]. Platelets and their bioactive functions are widely known to play a critical role in the physiopathology, morbidity and mortality of cardiovascular diseases. Although a number of parameters can be used to assess platelet function, MPV is a readily available marker that complements measures of platelet numbers since it takes into account platelet size, density, age, the density of adhesion receptors as well as the intracellular content of granules (and therefore platelet "coagulability"). The biologic importance of platelet volume is supported by the observation that increased MPV is associated with an increased risk of acute myocardial infarction, ischemic stroke, hypertension, pre-eclampsia and overall cardiovascular mortality [6, 17, 25, 29]. Importantly, recent studies have reported that increased MPV is associated with increased blood viscosity, an observation that is consistent with our findings[28]. We provide the first evidence of a linear relationship between MPV and TIMIfc across a large spectrum of patients.

Similar considerations can be made for hematocrit, which, along with plasma proteins and red blood cell aggregability/deformability, is the most important determinant of whole blood viscosity. Previous studies have shown an increased viscosity in patients with coronary slow flow at angiography[8], and the existence of a strong correlation between blood viscosity and MPV[28]. In our dataset, hematocrit was strongly associated with TIMIfc and was independent of endothelial function and platelet parameters across a wide range of TIMIfc.

Collectively, these data demonstrate the important role of hemorheological properties in coronary physiology. Importantly, the relationship between viscosity, endothelial function and blood flow is complex[9, 10]: on one hand, increased viscosity may increase wall shear stress[26], which in turn determines endothelial activation, thus reducing vascular resistances; on the other, Poiseuille's law establishes that flow resistance is directly proportional to the viscosity of a fluid[18]. Our data

provide support for the importance of blood viscosity as a determinant of flow, and our ROC analyses suggest that there is a "threshold" blood viscosity (in the range of MPV>8.7fL, hematocrit >42.6%) beyond which coronary microvascular perfusion is negatively affected. This concept is in partial agreement with recent findings by Jung et al reporting a correlation between blood viscosity correlates and maximum postischemic (but not resting) capillary erythrocyte velocity in a large cohort study (1256 healthy volunteers and patients)[19]. Beyond their effects on blood flow, hemorheological factors have also been suggested to be involved in the progression of atherosclerosis[20], although findings from our group recently reviewed in[14] appear to contrast this hypothesis.

An association between inflammatory markers and microvascular endothelial dysfunction has been observed in previous studies, but was not associated with TIMIfc in our database[1]. The reason for this discrepancy is unclear.

Limitations

Several limitations need to be acknowledged. First, the relatively large sample size did not allow a diagnosis of CAD with intravascular ultrasound. It has been previously reported that slow coronary blood flow may be associated with atherosclerotic plaque burden in patients without visible angiographic abnormalities[24], and the role of subclinical atherosclerosis as a determinant of TIMIfc is acknowledged. Catheter size and the pressure of contrast injection may also influence TIMIfc. In order to avoid these limitations, all angiograms were performed using standard 6F catheters and an automatic injection pump. Further, blood samples were processed immediately after collection in order to limit the impact of storing on hemorheological parameters. Finally, the role of platelet, erythrocyte and leukocyte aggregability has been previously demonstrated[8, 13] and was not studied here.

Conclusions

The control of coronary resistances and myocardial perfusion is complex, with both local and systemic phenomena playing important roles. While stenoses at the level of epicardial coronaries are of clear importance, evidence of a prolonged TIMIfc in the absence of coronary stenoses has also been associated with acute coronary syndromes and life-threatening arrhythmias[7]. The large interindividual variability in TIMIfc in the absence of epicardial stenoses emphasizes the importance of other factors (including at the microvascular and hemorheological level) in regulating myocardial perfusion and determining ischemia. Our data provide emphasize the role (and possible clinical importance) of systemic alterations in parameters such as platelet activation, hematocrit and resting endothelial tone in the regulation of coronary blood flow.

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Figures:

Figure 1

Correlation of TIMIfc in the three major coronary arteries. There was a strong association between the results measured in the three vessels.

Figure 2

The impact of endothelial function on coronary flow velocity: while a correlation was shown between corrected TIMIfc and L-FMC(resting endothelial function, upper panels), no correlation at all was shown with FMD (endothelial recruitability).

Figure 3

ROC analysis for the diagnosis of slow (TIMIfc>26) versus normal flow. Upon logistic regression analysis, three variables (L-FMC, hematocrit and MPV) were associated with TIMIfc (P=0.0003).The R^2 for this regression analysis was = 0.19, suggesting that these three parameters represent 19% of the total contribution to the regulation of coronary blood flow.

Figure 4

The impact of hemorheological parameters on coronary flow velocity: both mean platelet volume (MPV, a marker of thrombocyte activation, lower panels)and hematocrit (upper panels) were positively associated with TIMIfc.

Table 1. The impact of traditional cardiac risk factors and cardiovascular therapies on TIMI frame count

	TIMI frame count<26	TIMI frame count>26	P value
Condition	**n=74 (51%)**	**n=71 (49%)**	
Diabetes	18 (24%)	18 (25%)	0.6
Hypertension	59 (78%)	57 (80%)	1
Hyperlipidemia	44 (59%)	49 (69%)	0.13
Family History of CAD	29 (39%)	21 (30%)	0.3
Age >65 years	39 (53%)	36 (51%)	1
Obesity	26 (35%)	27 (38%)	0.8
Male gender	45 (61%)	54 (76%)	0.06
Drug Therapy			
β-blockers	47 (64%)	42 (59%)	0.4
ACE inhibitors/ATR blockers	50 (68%)	46 (65%)	0.5
Acetylsalicylic Acid	51 (69%)	48 (68%)	0.7
Organic nitrates	12 (16%)	9 (13%)	0.3
Statins	40 (54%)	44 (62%)	0.14
Ca^{2+} antagonists	7 (9%)	9(13%)	0.4

CAD: Coronary artery disease; ACE: angiotensin-converting enzyme; ATR: Angiotensin receptor.

Table 2. Hematologic parameters

Parameter	TIMI frame count<26	TIMI frame count >26	P value
Leukocytes (10^3/μl)	7.2±2.5	7.0±1.9	0.8
Erythrocytes (10^6/μl)	4.5±0.5	4.7±0.5	<0.001
Hemoglobin (g/l)	13±1	14±1	=0.001
Hematocrit (%)	40±4	42±4	<0.0001
Platelets (10^3/μl)	268±56	254±69	0.2
MPV (fL)	8.6±1.0	9.1±1.1	<0.01
Creatinine (mg/dl)	1.0±0.2	1.0±0.1	0.7
Blood urea (mg/dl)	18±6	18±7	0.1
Uric acid (mg/dl)	40±20	42±20	0.3
SGOT(IU/l)	27±7	28±9	0.7
SGPT(IU/l)	44±56	47±64	0.2
γ–GT (IU/l)	44±56	47±65	0.7
CK(IU/l)	106±66	121±70	0.2
CRP (mg/dl)	5.9±14.1	4.8±7.2	0.6
Triglycerides (mg/dl)	156±96	154±92	0.9
Total cholesterol (mg/dl)	183±48	181±43	0.8
HDL Cholesterol (mg/dl)	49±12	49±16	0.7
LDL cholesterol (mg/dl)	103±39	103±35	0.9
a PTT	30±4	31±4	0.4
aPT	102±17	97±18	0.09
Fibrinogen (mg/dl)	354±97	359±103	0.8
TSHμIU/ml	1.3±0.9	1.4±0.9	0.9

CRP: C-reactive protein; TSH: thyroid-stimulating hormone; sGOT: glutamic-oxaloacetictrasaminase; s-GPT: glutamic-pyruvic transaminase; γGT: gamma glutamyltransferase; CK: creatinin-phosphokinase. aPTT: activated partial tromboplastin time. aPT: activated protrombin time.

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Figure 1

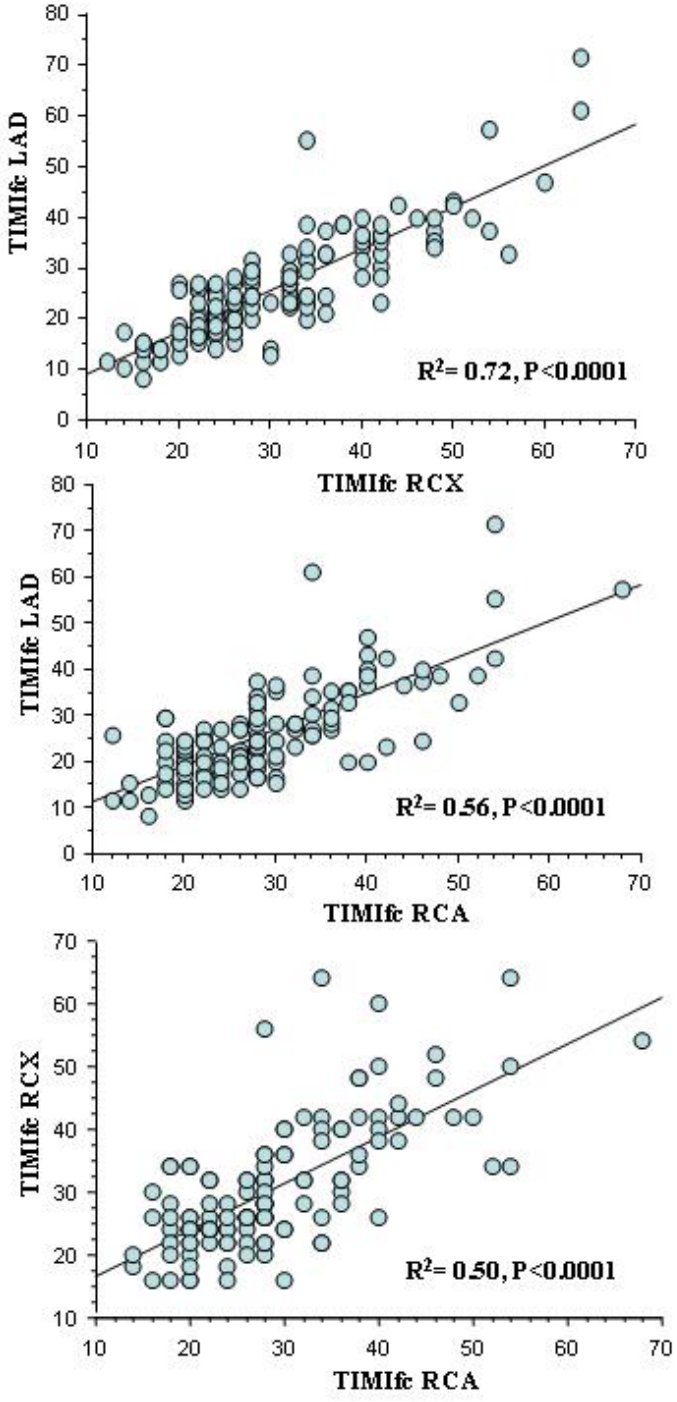

TIMIfc LAD
TIMIfc RCX
R²= 0.72, P<0.0001
TIMIfc LAD
TIMIfc RCA
R²= 0.56, P<0.0001
TIMIfc RCX
TIMIfc RCA
R²= 0.50, P<0.0001

Figure 2

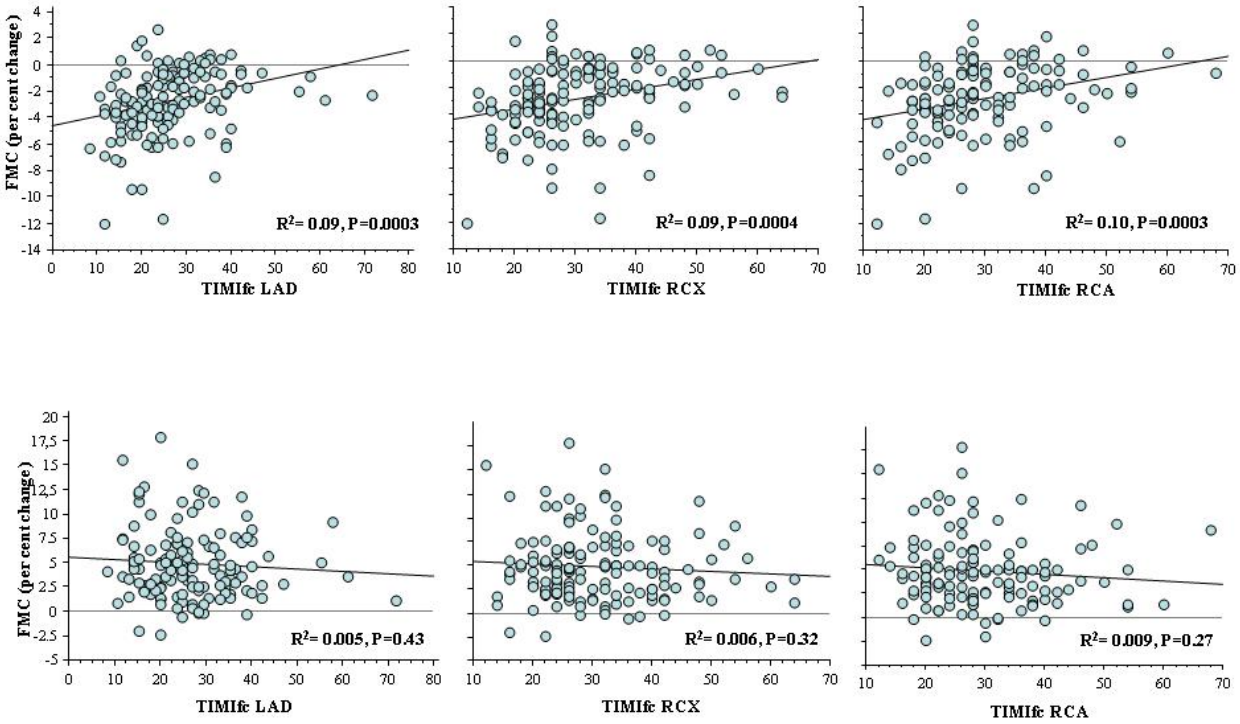

FMC (per cent change)
TIMIfc LAD
TIMIfc RCX
TIMIfc RCA
R²= 0.09, P=0.0003
R²= 0.09, P=0.0004
R²= 0.10, P=0.0003
FMC (per cent change)
TIMIfc LAD
TIMIfc RCX
TIMIfc RCA
R²= 0.005, P=0.43
R²= 0.006, P=0.32
R²= 0.009, P=0.27

Figure 3

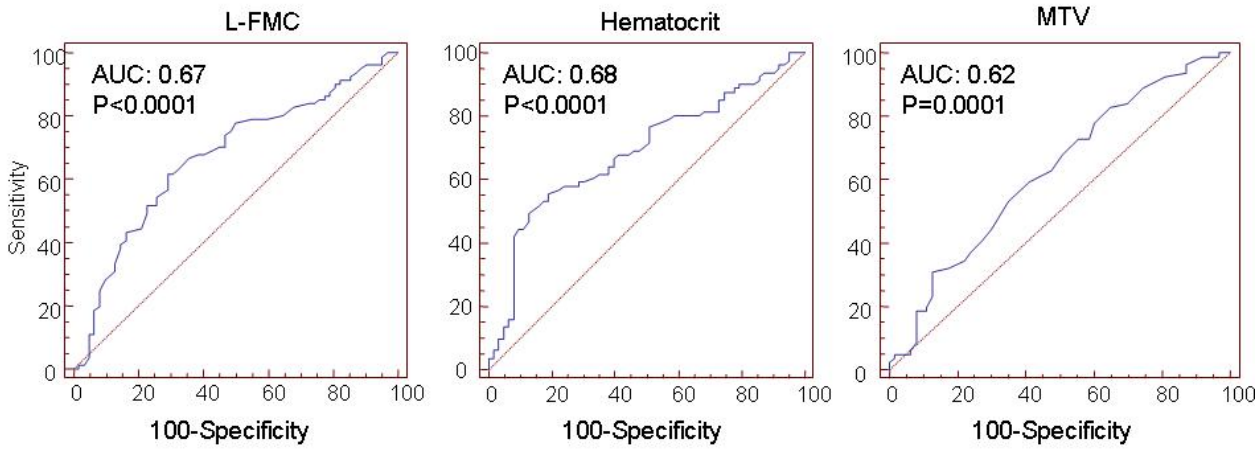
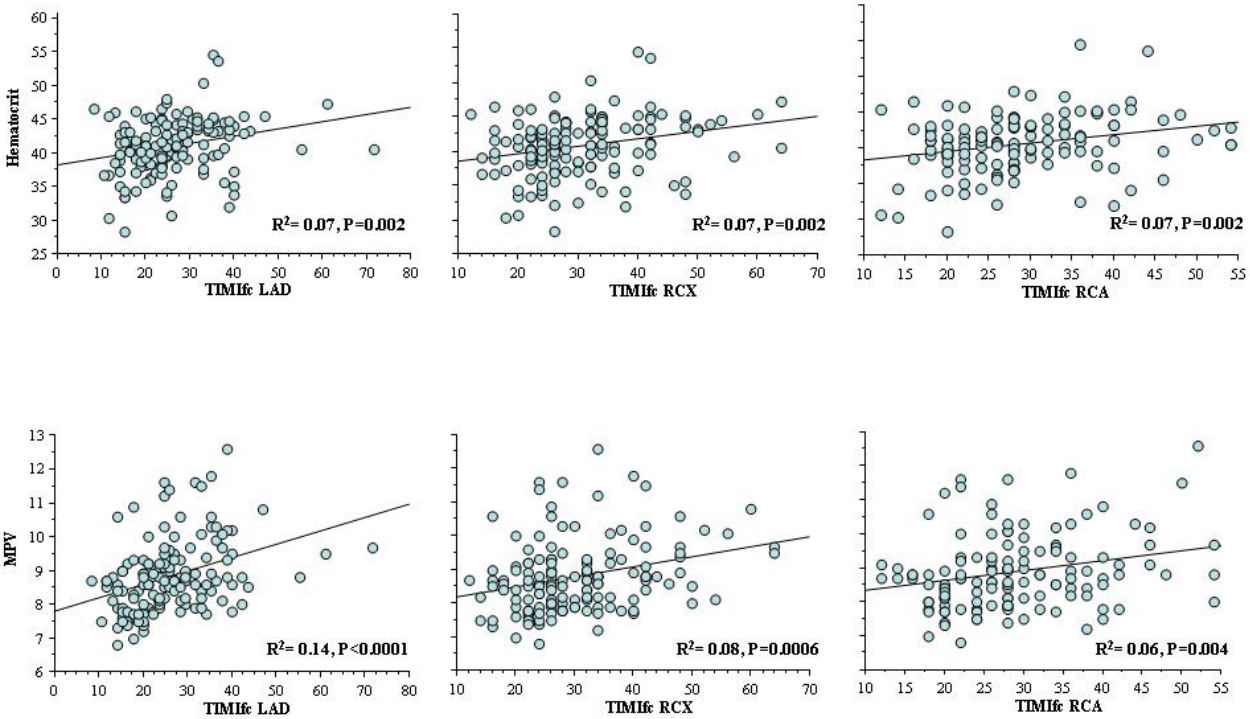

L-FMC
AUC: 0.67
P<0.0001
Sensitivity
100-Specificity
Hematocrit
AUC: 0.68
P<0.0001
100-Specificity
MTV
AUC: 0.62
P=0.0001
100-Specificity

Figure 4


Hematocrit
MPV
TIMIfc LAD
TIMIfc RCX
TIMIfc RCA
R²= 0.07, P=0.002
R²= 0.07, P=0.002
R²= 0.07, P=0.002
R²= 0.14, P<0.0001
R²= 0.08, P=0.0006
R²= 0.06, P=0.004