

Transition to an Unstable Coronary Syndrome is Marked by Hypercoagulability, Platelet Activation, Heightened Platelet Reactivity, and Inflammation: Results of the Thrombotic Risk Progression (TRIP) Study

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ABSTRACT

Background: It is well known from various independent studies that inflammation, high platelet reactivity, and hypercoagulability play important roles leading to the development of atherothrombotic events. However, to date, no single study prospectively evaluated all of these pathophysiological processes together in patients with stable and unstable coronary artery disease (CAD).

Methods: Thrombin-induced platelet-fibrin clot strength (MA), time to thrombin generation (R), CRP, prothrombotic factors, platelet reactivity to ADP, and platelet activation (ADP-stimulated and unstimulated GPIIb/IIIa receptor expression) were studied in patients with asymptomatic stable CAD (AS), and in patients undergoing PCI for stable angina (SA), unstable angina (UA), and myocardial infarction (MI). MA and R were measured by thrombelastography, GPIIb/IIIa expression by flow cytometry, and all other parameters by fluorokine multi-analyte profiling assays.

Results: Results and statistical significance are shown in the table. There was an incremental increase in all parameters from a clinically stable to an unstable disease state (Table).

Conclusion: A uniform and distinct pathophysiological state of heightened platelet reactivity to ADP, platelet activation, hypercoagulability, and inflammation marks the development of unstable cardiovascular disease from chronic stable disease. Further studies are required to investigate the mechanisms activating the prothrombotic state that destabilizes the disease.

	AS (n=71)	SA (n=84)	UA (n=26)	MI (n=7)	p-value (AS/SA)	p-value (SA/UA)
CRP (µg/mL)	4±8	9±15	15±23	11±5	0.006	0.2
PAl-1 (ng/mL)	69±28	81±39	88±30	130±70	0.01	0.28
Fibrinogen (mg/mL)	3.7±1	4.0±2	4.8±3	3.7±1	0.15	0.22
vWF (µg/mL)	21±9	24±26	39±54	30±13	0.34	0.17
MA	65±5	67±10	70±6	69±6	0.002	0.053
R (min)	6.5±2	4.5±1	4.1±2	5.5±1	<0.0001	0.98
GPIIb/IIIa-unstimulated (MFI)	13±9	17±15	38±25	-	0.051	<0.0001
GPIIb/IIIa-stimulated (MFI)	129±50	150±55	180±161	-	0.002	0.14

BACKGROUND

Coronary atherosclerosis is a progressive disease influenced by endothelial dysfunction, inflammation, hypercoagulability and heightened platelet function, ultimately leading to catastrophic events after plaque rupture.

Independent studies in patients with coronary artery disease (CAD) have demonstrated that:

- Hypercoagulability, defined by an increase in fibrinogen, von Willebrand factor (vWF) and high platelet-fibrin clot strength is linked to ischemic events (1,2).
- Ex vivo measurements of platelet activation and high platelet reactivity to adenosine diphosphate (PR-ADP) are linked to ischemic events (3).
- Elevated inflammation markers, especially C-reactive protein (CRP) are associated with ischemic events (4,5).

However, to date, no single study prospectively evaluated all of these markers of pathophysiological processes together in patients with various stages of coronary artery disease [asymptomatic CAD (AS), stable angina (SA), unstable angina (UA), and acute myocardial infarction (MI)].

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OBJECTIVE

To simultaneously study hypercoagulability, platelet function, and inflammation at various clinical stages of CAD.

To determine whether changes in these markers indicate a transition in clinical disease state.

METHODS

Subjects

84 patients with stable angina, 26 patients with unstable angina and 7 patients with acute myocardial infarction undergoing percutaneous coronary intervention; and 71 patients with asymptomatic CAD.

Asymptomatic patients had CAD documented by prior coronary angiography at least 6 months prior to enrollment.

Stable angina patients were undergoing elective PCI of at least one lesion. All lesions had $\geq 50\%$ luminal diameter stenosis.

Unstable angina was defined as: typical symptoms associated with ECG changes, requiring emergent hospitalization and a $\geq 75\%$ luminal diameter stenosis.

Acute myocardial infarction was defined as: typical symptoms and elevation of cardiac markers and at least one $\geq 75\%$ luminal diameter stenosis.

All subjects >18 years old. Exclusion criteria: thienopyridine, non-steroidal anti-inflammatory agent or COX-2 inhibitor (<2 wks.), anticoagulant* or glycoprotein (GP) IIb/IIIa antagonist prior to blood sampling, cerebrovascular event (<3 mos.), illicit drug or alcohol abuse, connective tissue disease, malignancy, autoimmune disease, ongoing bacterial or viral infection, prothrombin time $>1.5x$ control, platelet count $<100,000/mm^3$, hematocrit $<30\%$, and creatinine >4.0 mg/dL.

All patients treated with at least 81 mg daily aspirin for ≥ 1 wk before enrollment.

*All patients with MI and 30% of patients with UA treated with heparin prior to blood draw.

DISCLOSURE

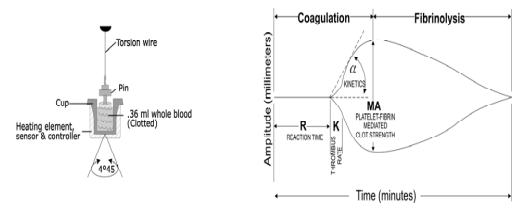
The study was supported by Bayer HealthCare LLC, Morristown, NJ and Sinai Hospital of Baltimore, MD.

Blood Sampling

- In symptomatic patients whole blood samples were drawn from an indwelling femoral vessel sheath in the catheterization laboratory and in asymptomatic patients through venipuncture.
- Blood samples transferred to vacutainer tubes containing 40 USP lithium heparin for thrombelastography (TEG) and 3.2% citrate for flow cytometry and multi-analyte profiling.
- Flow cytometry and TEG measurements conducted immediately after blood collection.
- Multi-analyte profiling plasma samples were stored at -70°C until analysis.

Thrombelastography

- 1 mL heparinized blood transferred to a vial containing kaolin (hydrated aluminum silicate), an intrinsic pathway activator.
- 500 µL activated blood transferred to a heparinase vial to neutralize heparin.
- 360 µL neutralized blood was immediately added to a heparinase-coated cup in the TEG.



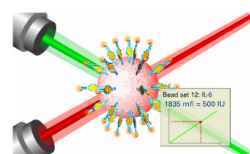
- Platelet-fibrin clot formation measured in a cylindrical cup as it oscillates through an angle of 4° to 45° .
- Pin suspended in the blood by a torsion wire monitored for motion.
- Torque of cup is transmitted to pin only after fibrin and/or fibrin-platelet bonding has linked the cup and pin together.
- Strength and rate of bonds affect magnitude of the pin motion.
- Mechanical-electrical transducer converts pin rotation into electrical signal.
- The resultant thrombotic profile is evaluated by specific parameters:
 - R - a measure of time to initial thrombin-induced platelet-fibrin clot formation, a marker of thrombin generation
 - MA - platelet-fibrin mediated clot strength

Whole Blood Flow Cytometry

- GPIIb/IIIa receptors determined by multicolor analysis before and after stimulating with $5\mu\text{M}$ ADP:
 - FITC (fluorescein isothiocyanate-conjugated) PAC-1 antibody (recognizes active GPIIb/IIIa)
 - R-phycoerythrin (R-PE) conjugated CD41a antibody (recognizes total GPIIb/IIIa)
- Samples fixed with 1% buffered paraformaldehyde and analyzed by a Becton Dickinson FACScan flow cytometer set up to measure fluorescence light scatter.
- After setting the gate around platelets, FL1(FITC)/FL2(R-PE) compensations were adjusted. Data were collected in list mode and then immediately analyzed using CELLQuest Software (BD Biosciences).
- Total and activated GPIIb/IIIa receptor levels expressed as mean fluorescence intensity (MFI).

MultiAnalyte Profiling (MAP)

- Fluorokine® MultiAnalyte Profiling (MAP) performed by Rules Based Medicine, Inc. (Austin, Texas) using a Luminex® 100™ analyzer that measures multiple analytes in 50 µL plasma.
- Plasma samples were first incubated with specific fluorokine colored microspheres coated with a specific antibody directed against an analyte.
- Subsequently, samples were washed and incubated with biotinylated antibodies and phycoerythrin.
- Fluorescence was detected by a flow cytometry technique (Luminex® 100™, Luminex® Corporation, Austin, Texas) that analyzes the plasma sample using two laser beams directed onto a file of single microspheres (Figure).
- One laser analyzes the microsphere color (specific for each analyte) whereas the second laser determines the specific analyte concentration by measuring phycoerythrin fluorescence intensity.



- As a microsphere passes through the detection chamber, a red laser excites internal microsphere dyes allowing the proper classification of the microsphere specific for each analyte.
- A green laser excites phycoerythrin fluorescence associated with binding of the biotinylated antibody.

RESULTS

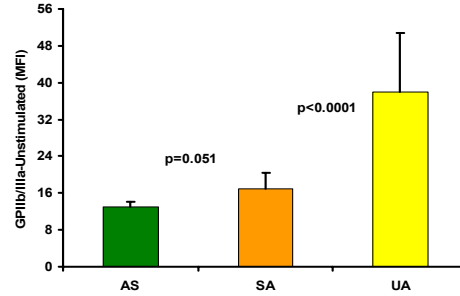
Patient Demographics

	AS (n=71)	SA (n=84)	UA (n=26)	MI (n=7)
Age (yrs)	66±10	67±11	71±11	59±12
Male (%)	68	67	50	67
BMI	30.5±7	29.4±5	29.3±6	29.4±6
Risk Factors/Past Medical Hx (%)				
Current Smoking	10	17	19	57
Former Smoking	31	36	27	29
Family History of Coronary Artery Disease	34	55	31	43
Hypertension	65	71	77	57
Hyperlipidemia	85	76	85	57
Diabetes Mellitus	27	34	69	43
Prior Myocardial Infarction	21	26	42	43
Prior Coronary Artery Bypass Grafting	32	18	31	0
Prior Percutaneous Transluminal Coronary Angioplasty	28	37	38	14
Peripheral Vascular Disease	9	6	12	14
Stroke	7	11	4	0
Medical Therapy (%)				
Lipid Treatment	84	69	81	86
Statins	82	63	73	72
other	2	6	8	14
ACE-inhibitors	58	56	27	71
Beta Blockers	63	83	85	83
Laboratory Values				
White Blood Cell Count ($\times 10^3/mm^3$)	6.5±2	7.8±3	7.3±2	6.9±1
Platelets ($\times 10^3/mm^3$)	234±72	231±67	256±85	247±86
Creatinine (mg/dL)	1.2±0.7	1.1±0.4	1.3±0.5	1.4±0.8

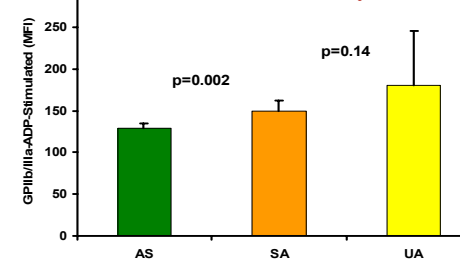
Statistical Analysis

- Values are expressed as mean \pm SD for patient demographics and mean \pm SEM for biomarkers.
- Groups were compared using unpaired t-tests; level of significance, $p < 0.05$ (SigmaStat Software, Point Richmond, CA).

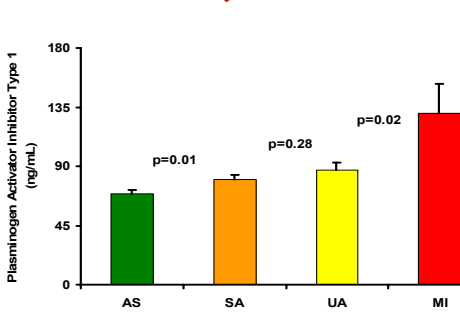
Platelet Activation



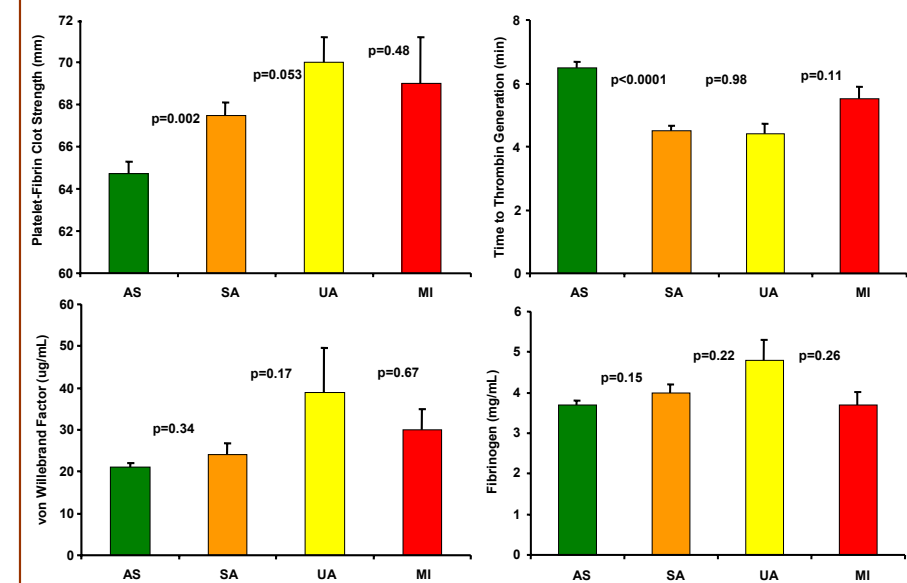
Platelet Reactivity



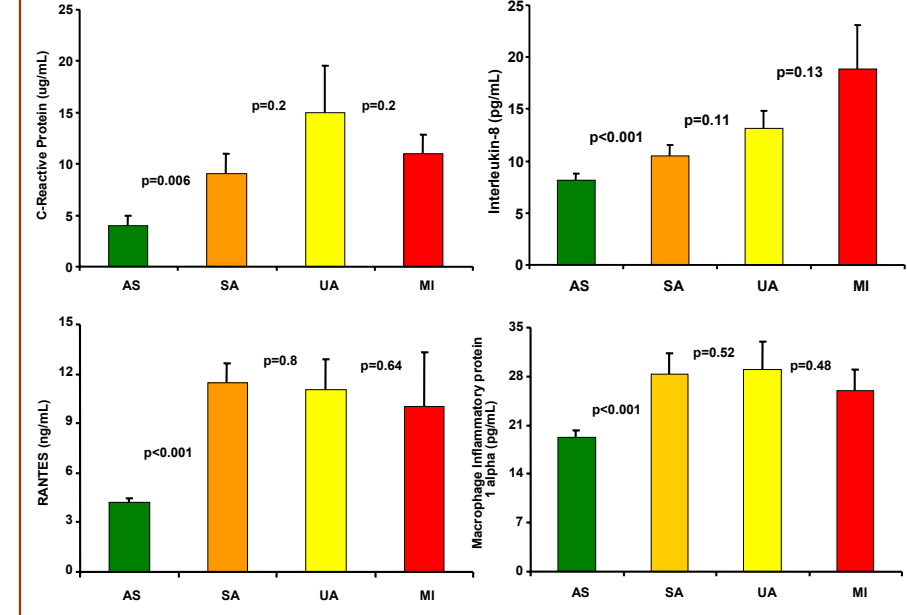
Fibrinolysis Inhibitor



Prothrombotic Markers



Inflammation Markers



CONCLUSIONS

- Our data suggest that:
 - A distinct pathophysiological state of heightened platelet reactivity to ADP, platelet activation, hypercoagulability, and inflammation marks the development of symptomatic cardiovascular disease from chronic stable disease.
 - Ex vivo measurements indicating high tensile platelet-fibrin clot strength highlight the patient vulnerable to thrombosis.
- Our data support the concept that reactive and activated platelets influence inflammation creating a vicious cycle leading to a prothrombotic state culminating in unstable coronary disease.
- Further studies are required to investigate the primary mechanisms activating the prothrombotic state that destabilizes the disease.

