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Articles

Temporal Relation Between Ischemic Episodes and Activation of the Coagulation System in Unstable Angina

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Abstract

Background Although a major role of coronary thrombosis in the pathogenesis of unstable angina has been demonstrated, the results of a series of studies have suggested that activation of the hemostatic system may not be confined to ischemic episodes. The purpose of this study was to investigate the temporal relation between ischemic episodes and activation of the coagulation system in unstable angina.

Methods and Results Thrombin–antithrombin III (TAT) and prothrombin fragment 1+2 (F_{1+2}) levels were measured in 13 patients during spontaneous ischemic episodes (time 0, 5, and 15 minutes and 1 hour) to evaluate the time course of the activation of the coagulation system associated with the development of ischemia (protocol A). TAT and F_{1+2} levels were also measured in 28 patients with unstable angina on admission to hospital (every 6 hours for 24 hours and daily for 3 days) to assess their temporal relation with ischemic episodes (protocol B). In protocol A, TAT and F_{1+2} levels were elevated in 10 of 13 patients (77%) in at least 1 sample. The median value of TAT showed a peak at 5 minutes and returned to baseline within 15 minutes ($P<.05$), consistent with its plasma half-life of 5 minutes, whereas the median value of F_{1+2} showed no significant changes, possibly because of its longer half-life, which tends to dampen sudden bursts of thrombin production. In protocol B, activation of the clotting system was found in 10 of 33 samples (30%) temporally related to ischemia and also in 23 of 150 (15%, $P=.07$) of those not temporally related to ischemia.

Conclusions Our study demonstrates that patients with active unstable angina develop frequent bursts of thrombin production not necessarily associated with ischemic episodes and that, conversely, some ischemic episodes are not associated with evidence of thrombin activation.

Key Words:
- ischemia
- coagulation
- angina

A major role of coronary thrombosis in the pathogenesis of unstable angina has been demonstrated by postmortem, angiographic, angioscopic, and biochemical studies.[1 2 3 4] The waxing and waning of symptoms over a period of up to 2 months[5] and the very common multilayered appearance of coronary thrombi at postmortem examination[6] indicate a recurrent episodic activation of the hemostatic system. However, the results of a series of studies suggest that such activation may not be confined to the timing of ischemic episodes. Elevated levels of FPA, TAT, and F_{1+2} for prolonged periods after ischemic episodes have been observed in unstable patients,[3 7 8 9] which, given the short plasma half-life of these components, suggests that activation of the coagulation system, either continuously or intermittently, is not necessarily related to episodes of ischemia. Platelets are very strictly related to the process of thrombus formation,[10 11 12 13] and a temporal dissociation between ischemic episodes and platelet activation in unstable angina was reported by Fitzgerald[14] and confirmed by Vejar et al,[15] who observed that only a minority of episodes of enhanced urinary excretion of 11-dehydrothromboxane B_2 was related to ischemia.

A recurring activation of the clotting system and its possible dissociation from ischemic episodes would have important pathophysiological implications. To investigate their temporal relation with ischemic episodes in patients with unstable angina, we measured two markers of coagulation activation with different plasma half-lives: TAT (plasma half-life, 5 minutes)[16] and F_{1+2} (plasma half-life, 90 minutes).[17] Two different protocols were implemented: protocol A, with frequent sampling during and after spontaneous ischemic episodes, to assess the time course of hemostatic activation associated with the development of ischemia, and protocol B, with a fixed sampling schedule over the first 4 days of hospitalization, to follow the behavior of the coagulation system during the active phase of unstable angina and its temporal association with ischemic episodes.

Our results confirm an ongoing activation of the clotting system in unstable angina and demonstrate that bursts of thrombin production occurring during some but not all spontaneous ischemic episodes and also apparently unrelated to ischemia are a pathophysiological hallmark of this syndrome.

Methods

Patient Population

The original study group was composed of 41 consecutive patients (32 men) 55±12 years old (range, 42 to 79 years) admitted to our CCU for the severity of their unstable angina. However, 3 patients were subsequently withdrawn because of the demonstration of angiographically normal coronary arteries with spasm diagnostic of variant angina. Thus, 38 patients formed the study group. The inclusion criteria were angina at rest, with at least 2 ischemic episodes or 1 episode lasting more than 20 minutes during the previous 24 hours, with diagnostic ST-segment shift and no evidence of myocardial infarction detected by enzymatic techniques. The exclusion criteria were left bundle-branch block, dilated cardiomyopathy, valvular heart disease, previous myocardial infarction with ejection fraction <40% or with evidence of left ventricular aneurysm, previous myocardial infarction within 4 weeks or treatment with heparin within the previous 24 hours, atrial fibrillation, the presence of a pacemaker, or any other ECG abnormality that would invalidate ST-segment analysis. Major surgery or trauma within the previous month, known thrombotic disorders, malignancy, and inflammatory diseases were also excluded to avoid inclusion of patients with increased clotting tendency. Patients with poor peripheral veins were also excluded because of the difficulties in obtaining repeated clear venipuncture. Between October 1992 and October 1993, 138 patients were admitted to our CCU with a diagnosis of unstable angina: 23 patients were excluded because they had experienced no ischemic episodes during the previous 24 hours, 14 were already on heparin, 10 had an increase in total creatine kinase within 6 hours of admission, 20 had suffered a recent myocardial infarction, and 11 had poor peripheral veins. In addition, 19 patients were excluded because of neoplastic disease (2), inflammatory diseases (9), severe anemia (2), left bundle-branch block (3), Wolff-Parkinson-White syndrome (1), abdominal aortic aneurysm (1), and left ventricular pseudoaneurysm with thrombi (1). The onset of instability ranged from 1 to 20 days (5.6±6.3 days). Nineteen of the 38 patients were secondary referrals to our center. All patients were treated with various combinations of calcium antagonists, intavenous nitrates, and/or β-blockers; aspirin (100 mg) was also prescribed, with a

loading dose of 325 mg when necessary. Heparin was not used as a first-step treatment and was added to the standard therapy only in cases of worsening symptoms, in which case blood sampling was discontinued. All admission samples were taken under aspirin cover.

The study was approved by the Ethics Committee of the Catholic University, and all patients gave their signed informed consent.

Study Design

To assess the temporal relation between activation of the hemostatic system and ischemic episodes, we designed two different protocols. Protocol A, with frequent sampling during spontaneous ischemic episodes, was designed to assess the time course of hemostatic activation during and after an ischemic episode. This was assessed in 13 patients by taking blood samples as soon as possible after the onset of ST-segment changes, with or without chest pain, and subsequently at 5, 15, and 60 minutes from the onset. At the onset of ST-segment changes, a diagnostic 12-lead ECG was recorded and the amount of ST-segment shift measured. Protocol B, with a fixed sampling schedule over the first 4 days of hospitalization in the CCU, was designed to follow the behavior of the coagulation system during the active phase of unstable angina and to evaluate its temporal relation with ischemia. Twenty-eight patients were assessed by blood samples, taken from separate clean venipunctures, as soon as possible after admission and subsequently every 6 hours for the first 24 hours, between 8 and 10 AM on days 2 (48 hours), 3 (72 hours), and 4 (96 hours), and before hospital discharge. Sampling was discontinued when the addition of heparin or urgent revascularization (coronary angioplasty or bypass) was clinically indicated. All patients had Holter monitoring for 24 hours and remained in the CCU, under ECG monitoring of the lead with the most striking ischemic ST changes, until completion of the study. The nurses were instructed to recognize and annotate each ST-segment change from the monitors. The results of samples taken in the absence of ischemia were analyzed separately from those taken within 3 half-lives of an ischemic episode for each of the investigated markers (ie, a period during which TAT and F_{1+2} could reasonably still be elevated, as defined in the study): within 15 minutes for TAT (n=17 episodes) and within 4.5 hours for F_{1+2} (n=33 episodes).

Coronary angiography was performed within 5 days of admission in 26 patients because of the severity of symptoms and within days 5 to 9 in 7 other patients. In 5 patients, angiography was not performed: in 3 because of waning of symptoms and a negative exercise stress test and in 2 because of death. The angiograms were reviewed by an expert angiographer (G.S.) who was unaware of the patients' clinical and analytical data.

Blood Sampling and Laboratory Assays

Blood was always withdrawn through a clean venipuncture with minimal venostasis via a 19-gauge needle. Repeated venipunctures were always performed in different veins or in different and progressively more distal segments of the same vein. A 4.5-mL sample was immediately transferred into precooled tubes containing 0.5 mL citrate, theophylline, adenosine, and dipyridamole (CTAD tubes, Behring Werke). The tubes were centrifuged at 2000*g* and at 4°C for 20 minutes. Plasma aliquots of 500 μL were pipetted into appropriate tubes, snap-frozen, and stored at −80°C within 1 hour of venipuncture, according to the method previously described by our group and by others.[16 17 18] All samples were taken by the investigators only. The aliquots were assayed for TAT and F_{1+2} by use of commercially available ELISAs (Enzygnost thrombin-antithrombin III complex and Enzygnost Micro F_{1+2} ELISA test kit, Behring Werke).[16 17] The assays were performed at the end of each protocol by one of the investigators (W.v.d.G.), who was unaware of the patients' clinical data.

Since measurement of products of thrombin generation can be biased by in vivo and in vitro artifacts and since our control group consisted of only normal subjects who had low baseline levels of TAT and F_{1+2},[3] we considered it reasonable to adopt rather conservative limits for the definition of abnormal elevation in our study. We arbitrarily chose as a value of definitely elevated TAT and F_{1+2} the maximum level in normal subjects (volunteer staff members) +2 SDs, ie, 6 μg/L and 1.3 nmol/L, respectively. In addition, we excluded from further analysis TAT and F_{1+2} values above the detection limit of the assays (ie, 60 μg/L and 8 nmol/L), because they were considered to be likely artifacts. Of a total of 291 samples, 6 (2%) were excluded.

Reproducibility of Repeated Venipuncture

We tested the long-term reproducibility of our measurements during serial venipunctures by determining TAT levels in 5 normal volunteers in whom blood was collected every 6 hours for 24 hours and then once a day for 3 days. TAT levels remained stable throughout the study, and no significant increases were observed with repeated venipuncture, with an average intersample CV of 25%. In 4 volunteers, samples at times 0, 5, 15, and 60 minutes, as in protocol A, were taken, with a CV of 27%. To exclude interoperator or technical variabilities, single samples were taken from 5 patients at the same time, from different veins, by two different investigators. The average CV between different arms was 22%. Interassay and intra-assay CVs were 3% and 6%, respectively.

Statistical Analysis

Since F_{1+2} and TAT were not distributed normally, nonparametric tests were used. The results are expressed as median and range; the Mann-Whitney *U* test was used to evaluate differences between individual groups. Discontinuous variables were tested by contingency χ^2 test. Continuous variables containing clinical data are expressed as mean±SD and were evaluated by unpaired *t* test. A probability of $P<.05$ was assumed to be significant. All tests are two-tailed.

Results

In our laboratory, normal values of TAT and F_{1+2} ranged from 1 to 4.2 μg/L and from 0.4 to 0.9 nmol/L, respectively. We considered arbitrarily a cutoff point of 6 μg/L for TAT and of 1.3 nmol/L for F_{1+2} (ie, the maximum of normal values +2 SDs) as indicative of a detectable activation of the clotting system.

Protocol A

Clinical characteristics are reported in Table 1⇓. TAT and F_{1+2} levels were found to be elevated in 10 of 13 patients (77%) in at least 1 of the 4 samples taken over a period of 1 hour after the onset of the spontaneous ischemic episodes. In all episodes, ECG changes diagnostic of ischemia were observed on the 12-lead ECG: ST-segment depression in 6 patients, ST-segment elevation in 3, and pseudonormalization of previously negative T waves in 4. No significant increases in heart rate or blood pressure were observed during any of these episodes. Signs of ischemia never lasted longer than 15 minutes and were always relieved promptly by nitrates (Table 1⇓).

Table 1.

Clinical and Angiographic Characteristics of Patients in Protocol A

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In 9 patients, TAT levels were >6 μg/L in at least 1 sample (16 of 52, 31% of the samples taken), and the elevation exhibited a characteristic time course (Fig 1⇓). At time 0, all but 1 patient had normal TAT levels; at 5 minutes, values had increased in 6 patients, in 4 of whom it represented the peak (median, 22.8 μg/L and range, 6.1 to 41.3 for the 6 patients; median, 6.1 μg/L and range, 0.3 to 41.3 for all 13 patients). In other patients, the peak was reached at 15 minutes, at which time 3 patients continued to have elevated levels. In all but 2 patients, TAT levels returned to baseline at 1 hour; in one patient, levels increased further, and in another, TAT levels increased weakly only at 1 hour (Fig 1⇓). The median value of TAT in the 13 patients showed a typical dome-shaped curve with a peak increase at 5 minutes and a return to baseline at 15 minutes (P<.05). The same curve is more sharply defined when only the patients with elevated values of TAT are considered (Fig 2⇓).

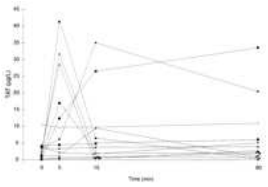

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Figure 1.

Time course of individual TAT levels during spontaneous ischemia (protocol A). At time 0, all but 1 patient had normal TAT levels; at 5 minutes, values had increased in 6 patients, in 4 of whom it represented the peak value (median, 22.8 μg/L and range, 6.1 to 41.3 for the 6 patients; median, 6.1 and range, 0.3 to 41.3 for all 13 patients). In 2 other patients, the peak was reached at 15 minutes, at which time 3 patients continued to have elevated levels. In all but 2 patients, TAT levels returned to baseline at 1 hour; in one patient, levels increased further, and in another, TAT levels increased weakly only at 1 hour.

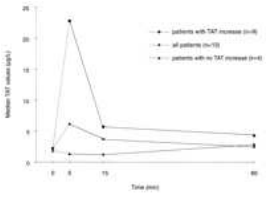

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Figure 2.

Time course of median TAT values during spontaneous ischemia (protocol A). Median values of TAT in the whole group of 13 patients (◆), in the 9 patients with elevated levels of TAT (▪), and in the 4 patients without any increase in TAT (▴): a typical dome-shaped curve with a significant peak increase at 5 minutes (P=.05) and a return to baseline at 15 minutes can be observed in the overall population and, more clearly, in patients with elevated values of TAT. This pattern is consistent with the 5-minute half-life of TAT.

F_{1+2} levels were higher than 1.3 nmol/L in 10 of 13 patients in at least 1 sample (28 of 52, 54% of the samples taken), but the elevation exhibited a time course different from that of TAT: F_{1+2} was elevated in 5 patients at time 0, in 7 at 5 minutes (peak median value), in 5 at 15 minutes, and after 1 hour in 7 patients (Fig 3⇓). The median value of F_{1+2} for the 13 patients showed a "flat" curve, which exhibited no significant differences over the 60 minutes (Fig 4⇓).

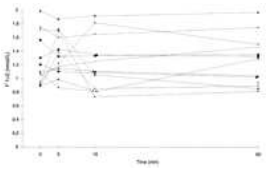

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Figure 3.

Time course of individual F_{1+2} levels during spontaneous ischemia (protocol A), P=NS. There is not a clear increase in F_{1+2} during the study. F_{1+2} was elevated in 5 patients at time 0, in 7 at 5 minutes (peak median value), in 5 at 15 minutes, and after 1 hour in 7 patients.

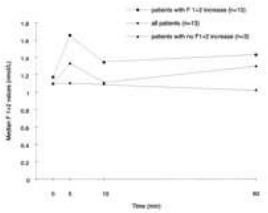

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Figure 4.

Time course of median F_{1+2} values during spontaneous ischemia (protocol A), P=NS. Median value of F_{1+2} for all 13 patients (◆), the 10 patients with elevated levels of F_{1+2} in at least one sample (▪), and the 3 patients without any elevation (▴). No significant differences over the 60 minutes were observed.

In 1 patient, only F_{1+2} levels were elevated, despite the presence of chest pain with ischemic ECG changes. No increases in either TAT or F_{1+2} were detected in 3 patients, 2 of whom presented ST-segment elevation during the ischemic event (Table 1⇑).

Protocol B

Episodic elevation of TAT and F_{1+2} associated with ischemic episodes. During the study, 87 ischemic episodes were observed (67 symptomatic, 20 asymptomatic), and blood samples were taken within 4.5 hours (3 half-lives of F_{1+2}) in 33 episodes (38%) and within 3 half-lives of TAT (15 minutes) in 17 episodes. Twenty-seven of the 33 episodes were observed in the first 24 hours during Holter monitoring. F_{1+2} levels were >1.3 nmol/L in 8 of 33 episodes (24%; median, 1.77 nmol/L; range, 1.33 to 6.42) but were <1.3 nmol/L in 25 episodes (median, 0.85 nmol/L; range, 0.24 to 1.28; Fig 5⇓).

TAT levels were >6 μg/L in 9 of the 17 episodes (53%; median, 17.9 μg/L; range, 12 to 51) but in 8 were <6 μg/L (47%; median, 2.3 μL; range, 1.3 to 4.1; Fig 6⇓). Overall, 10 of 33 (30%) were associated with activation of the clotting system. The mean time from onset of ischemia to blood sampling was 8 minutes (range, 2 to 15 minutes) for TAT and 47 minutes (range, 2 to 270 minutes) for F_{1+2}. The mean duration of ischemia was 20±18 minutes (range, 5 to 60 minutes; Table 2⇓).

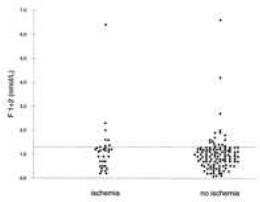

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Figure 5.

Individual levels of F_{1+2} associated (ischemia) and not associated (no ischemia) with ischemic episodes (protocol B). Left, F_{1+2} levels associated with ischemia. Only 8 of 33 samples taken during ischemia had elevated levels of F_{1+2}; conversely (right), 17 of 150 samples taken in the absence of detectable ischemia had elevated levels of F_{1+2}. The values higher than the chosen upper limit are not significantly different in the ischemia and no ischemia groups.

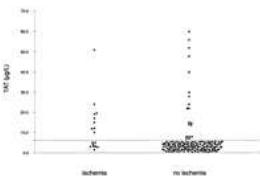

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Figure 6.

Individual levels of TAT associated (ischemia) and not associated (no ischemia) with ischemic episodes (protocol B). Left, TAT levels associated with ischemia. Only 9 of 17 samples taken during ischemia had elevated levels of TAT; conversely (right), 19 of 150 samples taken in the absence of detectable ischemia had elevated levels of TAT. The values higher than the chosen upper limit are not significantly different in the ischemia and no ischemia groups.

Table 2.

Clinical Findings of Patients in Protocol B

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Episodic elevation of TAT and F_{1+2} not associated with ischemia. An episodic activation of the clotting system was observed in most but not all patients: in 22 of 28 patients (80%), at least one increase in either TAT or F_{1+2} levels was detected, but in 6 of 28 patients, no such activation was observed. During the study, 150 samples were taken during periods free of ischemia (ie, not taken within 3 half-lives of F_{1+2} from an ischemic episode), but in 23 samples (15%), activation of the clotting system was observed: In 17 samples (11%) and in 19 samples (13%), levels of F_{1+2} and TAT, respectively, were elevated (Figs 5⇑ and 6⇑). Median levels of TAT were 2.3 μg/L (range, 0.1 to 60) in the 150 samples and 23.6 μg/L (range, 6 to 60, *P*=NS versus elevated TAT during ischemia) in the 19 samples with elevated levels. Median levels of F_{1+2} were 0.9 nmol/L in the 150 samples (range, 0.1 to 6.48) and 1.7 nmol/L (range, 1.3 to 6.48, *P*=NS versus elevated F_{1+2} during ischemia) in the 17 samples with elevated levels.

Elevated TAT or F_{1+2} levels showed no correlation with the interval between sampling and occurrence of the last ischemic episode or with the duration of the episode.

Thus, overall, 33 episodes of activation of the clotting system were detected in protocol B, but only 10 (30%) were temporally related to ischemia (Figs 5⇑ and 6⇑). Moreover, peak values of TAT and F_{1+2} associated with ischemic episodes did not differ significantly from peak values not associated with ischemia.

Had we considered TAT and F_{1+2} levels of 4 μg/L and 1 nmol/L (mean+2 SDs), respectively, as cutoff points, activation of the coagulation system would have been observed in 19 of 33 samples temporally related to ischemia (58% instead of 30%) and in 57 of 150 samples not temporally related to ischemic episodes (38% instead of 15%, *P*=.06).

Discussion

Our findings demonstrate the occurrence of bursts of thrombin production in unstable angina consistent with an ongoing waxing and waning activation of the coagulation system, as suggested by previous studies.[3,19] We have also demonstrated that this activation is not necessarily temporally related to detectable ischemic episodes, because about one fourth of ischemic episodes in protocol A were not associated with detectable activation of the clotting system and, conversely, from about one half to two thirds of all episodes of activation in protocol B were unrelated to detectable ischemic episodes.

Temporal Relation Between Thrombin Formation and Ischemic Episodes

An ongoing activation of the clotting system in unstable angina was suggested by Neri Serneri et al[20] and Theroux et al[8] on the basis of elevated plasma levels of FPA. Elevated levels of FPA and F_{1+2} on admission to hospital and of F_{1+2} at 6 months after discharge have recently been reported in unstable angina,[3] and raised levels of FPA have been described in association with angiographic evidence of thrombosis[21] and related to ST-segment shifts,[22] although the latter was not confirmed in a recent study.[23] Because of the short half-life of FPA and F_{1+2}, it is not clear whether such elevated levels of FPA and F_{1+2} represent the tail of acute bursts of thrombin generation or a persistent low-grade activation of thrombin. A dissociation between platelet activation and spontaneous myocardial ischemia was reported by Vejar et al,[15] who reported that 60% of episodes with enhanced urinary excretion of 11-dehydrothromboxane B_2 in patients with unstable angina occurred in the absence of ST-segment changes or chest pain. A similar finding was also observed by Fitzgerald,[14] who interpreted the dissociation as being due, most likely, to episodes of silent ischemia, although Holter monitoring was not included in their study. Thus, the results of previous studies based on products of platelet activation show a dissociation between ischemic episodes and platelet activation. This similarity to our findings is not surprising, since platelets are a fundamental component of the

clotting cascade (especially at the site of a stenosis),[10] are activated by thrombin, enhance the process of thrombin formation, and lead to vasoconstriction by the release of such products as thromboxane A_2, serotonin, ADP, and platelet-activating factor.[12 13 24 25]

At variance with previous studies that considered only one[22] or two[3 20] time points (entry plus discharge or follow-up), we systematically monitored the activation of the coagulation system during 4 days of hospitalization in the CCU under ECG cover. Such a protocol allowed us to investigate specifically the temporal relation between activation of the coagulation system and ischemic episodes. Thus, we were able to demonstrate a temporal relation between bursts of thrombin formation and at least some ischemic episodes in both protocols. We also observed bursts of thrombin generation that were unrelated to ischemic episodes. In protocol A, 3 of 13 patients (23%) had no increase in the levels of TAT or F_{1+2} associated with ischemia during 1 hour of follow-up, and in protocol B, activation of the coagulation system, temporally related to ischemia, was observed in 9 of 17 samples (53%) for TAT and in 8 of 33 samples (24%) for F_{1+2}. In protocol A, the sharp increase in TAT over 5 minutes and its return to normal median values within 15 minutes suggest that a very brief burst of thrombin production is often associated with an ischemic episode; however, we were unable to assess whether these bursts are a cause or a consequence of ischemia. In protocol B, an episodic activation of the coagulation system was observed in 18% of samples (33 of 183) taken over 4 days; in 6 of 28 patients (21%), no activation of the coagulation system was observed, but, intriguingly, activation was also observed in 15% of samples (23 of 150) taken during periods free of ischemia, suggesting a dissociation between thrombin formation and ischemic events. Although in vivo and in vitro artifacts represent a possible bias in studies involving proteases of the coagulation system, our findings are unlikely to be due to artifacts, because great care was taken over sampling procedures. Moreover, to reduce the possibility of artifacts and to increase specificity, we also excluded very high levels of TAT and F_{1+2} and accepted as elevated only those that were higher than the maximum levels in normal subjects +2 SDs. Had we had adopted lower values of TAT and F_{1+2} as cutoff points for activation of the coagulation system, we would have detected not only more episodes of activation associated with ischemic episodes (30% with cutoff at 6 μg/mL and 58% with cutoff at 4 μg/mL) but also more episodes unrelated to ischemic events (15% and 38%, respectively).

The difference between the behavior of TAT and that of F_{1+2} may be explained on the basis of the nearly 20 times longer half-life of F_{1+2}, which tends to dampen the peaks and troughs related to episodic bursts of activation and to give persistently slightly elevated levels of F_{1+2}. Conversely, the sharp rise and fall in TAT are in agreement with its short half-life.

Pathophysiological Implications

The lack of evidence of thrombin formation in one fourth of the ischemic episodes cannot be explained by episodic increases in myocardial demand (which was not detectable in our study); it may, therefore, be explained by coronary constriction. Conversely, the bursts of thrombin formation observed during periods free of ischemia may be explained either by antithrombotic mechanisms, which may have prevented the formation of a coronary flow-limiting thrombus, or by episodic thrombin formation unrelated to coronary thrombosis.

F_{1+2} and TAT complexes are related to the amount of formed and circulating thrombin, respectively, and provide indications of the activity of factor Xa but not of actual fibrin formation. Since our study addressed the mechanisms underlying the activation of the coagulation system in unstable angina rather than the mechanisms responsible for ischemia, markers of fibrin production, such as FPA, were not measured. Another potential limitation of our study is that the roles of tissue factor[26] and of platelet activation,[10 11 12 13 24 25 26] which may be responsible for thrombin activation, were not assessed.

The commonly held hypothesis that coronary thrombosis is just the result of a purely mechanical plaque fissure does not, by itself, explain the recurrent activation of the coagulation system that we recorded even in patients with a 2-week history of unstable angina.

Given the short plasma half-life of TAT and F_{1+2}, the fact that 30% of all samples taken during the 4 days of the study had elevated levels of these markers suggests a very frequent activation of the coagulation system. However, the presence of powerful stimuli that maintain the system either activated or in a hyperreactive state for prolonged periods of time, rather than during occasional bursts, cannot be excluded.

The finding of elevated levels of TAT and F_{1+2} in the peripheral blood in response to a localized coronary thrombotic process is also intriguing, because a critical mass of thrombus may be necessary to detect activation of the coagulation system in the peripheral blood.[27] Although we have no definite explanation for this enigma, we must stress that a number of authors have also reported this finding[3 7 8 9 20 21 22 23] and that, intriguingly, increased levels of FPA have been reported within 5 minutes of the start of episodes of vasospastic angina.[28 29] Explanations for this latter finding include the possibility that the coagulation cascade may hyperreact to localized stimuli, producing either a detectable response in the periphery or a washout of thrombin formation products from the site of a thrombus after restoration of flow. The peak values of TAT and F_{1+2} associated with ischemic episodes were comparable to those unrelated to ischemic episodes and also to those found in cases of deep vein thrombosis and pulmonary embolism, when the actual amount of thrombus is several times greater than that present in the coronary arteries.[30]

Alternatively, activation of the coagulation cascade in unstable angina may not necessarily be localized at the site of a single unstable coronary atherosclerotic plaque, because occasional bursts of systemic production of thrombin might also be caused by circulating activated monocytes[31] or be diffused within the coronary tree.[32 33] This possibility would be compatible with the increased urinary excretion of 11-dehydrothromboxane B_2 unrelated to ischemic episodes,[15] even in the presence of platelet cyclooxygenase 1 blockade.[34] The growing evidence of an important inflammatory component in unstable angina,[33 35 36 37 38 39] together with the multiple links between inflammation and the coagulation system, might help explain the persistent recurrence of bursts of activation of the hemostatic system over periods of days and weeks. The relation between inflammation and activation of coagulation in unstable angina, therefore, deserves further study.

Conclusions

Our study demonstrates ongoing bursts of thrombin production in unstable angina not necessarily related to ischemic episodes. While confirming the frequently increased generation of thrombin in unstable angina, our findings also suggest that the mechanisms responsible for instability may be more complex than a simple mechanical fissure of an atherosclerotic plaque and may possibly be related to recurrent production of inflammatory cytokines.

Selected Abbreviations and Acronyms

CCU=coronary care unit
CV =coefficient of variation
F_{1+2}=prothrombin fragment 1+2
FPA =fibrinopeptide A
TAT =thrombin-antithrombin III complexes

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References

1. Fuster V, Stein B, Ambrose JA, Badimon L, Badimon JJ, Chesebro JH. Atherosclerotic plaque rupture and thrombosis: evolving concepts. *Circulation.* 1990;83(suppl II):II-47–II-59.
2. Mizuno K, Miyamoto A, Satomura K, Kurita A, Arai T, Sakurada M, Yanagida S, Nakamura H. Angioscopic coronary macromorphology in patients with acute coronary disorders. *Lancet.* 1991;337:809–812. CrossRef Medline
3. Merlini PA, Bauer KA, Oltrona L, Ardissino D, Cattaneo M, Belli C, Mannucci PM, Rosenberg RD. Persistent activation of coagulation mechanism in unstable angina and myocardial infarction. *Circulation.* 1994;90:61–68. Abstract/FREE Full Text
4. Fuster V, Frye RL, Connolly DC, Damelson MA, Eweback LR, Kurland LT. Arteriographic patterns early in the onset of coronary syndromes. *Br Heart J.* 1975;37:1250–1259. Abstract/FREE Full Text
5. Braunwald E. Unstable angina: a classification. *Circulation.* 1989;80:410–414. FREE Full Text
6. Falk E. Unstable angina with fatal outcome: dynamic coronary thrombosis leading to infarction and/or sudden death. *Circulation.* 1985;4:699–708.
7. Ardissino D, Gamba G, Merlini PA, Rolla A, Barberis P, Demicheli G, Testa S, Bruno N, Specchia G. Fibrinopeptide A excretion in urine: a marker of the cumulative thrombin activity in stable versus unstable angina patients. *Am J Cardiol.* 1991;68:58B–63B. CrossRef Medline
8. Theroux P, Latour JG, Leger-Gauthier C, De Lara J. Fibrinopeptide A and platelet factor levels in unstable angina pectoris. *Circulation.* 1987;75:156–162. Abstract/FREE Full Text
9. Wilensky RL, Zeller JA, Wish M, Tulchinsky M. Urinary fibrinopeptide A levels in ischemic heart disease. *J Am Coll Cardiol.* 1989;14:597–603. Abstract
10. Folts JD, Crowell EB, Rowe GG. Platelet aggregation in partially obstructed vessels and its elimination with aspirin. *Circulation.* 1976;54:365–370. Abstract/FREE Full Text
11. Willerson JT, Yao SK, McNatt J, Cui K, Anderson HV, Swensen MO, Buja LM. Liposome-bound prostaglandin E1 often prevents cyclic flow variations in stenosed and endothelium-injured canine coronary arteries. *Circulation.* 1994;89:1786–1791. Abstract/FREE Full Text
12. Schmitz JM, Apprill PG, Buja M, Willerson JT, Campbell WB. Vascular prostaglandin and thromboxane production in a canine model of myocardial ischemia. *Circ Res.* 1985;57:223–231. Abstract/FREE Full Text
13. Golino P, Buja M, Ashton JH, Kulkarni P, Taylor A, Willerson JT. Effect of thromboxane and serotonin receptor antagonists of intracoronary platelet deposition in dogs with experimentally stenosed coronary arteries. *Circulation.* 1988;78:701–711. Abstract/FREE Full Text
14. Fitzgerald DJ. Platelet activation in the pathogenesis of unstable angina: importance in determining the response to plasminogen activators. *Am J Cardiol.* 1991;68:51B–57B. CrossRef Medline
15. Veiar M, Fragasso G, Hackett D, Lipkin DP, Maseri A, Born GVR, Ciabattoni G, Patrono C. Dissociation of platelet activation and spontaneous myocardial ischemia in unstable angina. *Thromb Haemost.* 1990;63:163–168. Medline
16. Pelzer H, Schwarz A, Heimburger N. Determination of human thrombin-antithrombin III complex in plasma with an enzyme-linked immunosorbent assay. *Thromb Haemost.* 1988;59:101–106. Medline
17. Pelzer H, Schwarz A, Stuber W. Determination of human prothrombotic activation fragment 1+2 in plasma with an antibody against a synthetic peptide. *Thromb Haemost.* 1991;65:153–159. Medline
18. Biasucci LM, Liuzzo G, Caligiuri G, Monaco C, Quaranta G, Sperti G, van de Greef W, Maseri A. Frequent sampling by clear venipuncture in unstable angina is a reliable method to assess hemostatic system activity. *Fibrinolysis.* 1994;8:142–144.
19. Fuster V, Badimon L, Badimon J, Chesebro JH. The pathogenesis of coronary artery disease and the acute coronary syndromes. *N Engl J Med.* 1992;326:310–318. Medline
20. Neri Serneri GG, Gensini GF, Carnovali M, Prisco D, Rogasi PG, Casolo GC, Fazi A, Abbate R. Association between time of increased fibrinopeptide A levels in plasma and episodes of spontaneous angina: a controlled prospective study. *Am Heart J.* 1987;113:672–678. CrossRef Medline
21. Wilensky RL, Bourdillon PD, Vix VA, Zeller JA. Intracoronary artery thrombus formation in unstable angina: a clinical, biochemical and angiographic correlation. *J Am Coll Cardiol.* 1993;21:692–699. Abstract
22. Eisenberg PR, Kenzora JL, Sobel BE, Ludbrook PA, Jaffe AS. Relation between ST segment shift during ischemia and thrombin activity in patients with unstable angina. *J Am Coll Cardiol.* 1991;18:898–903. Abstract
23. Becker RC, Tracy RP, Bovill EG, Corrao JM, Baker S, Ball SP, Mann KG. Surface 12-lead electrocardiographic findings and plasma markers of thrombin activity and generation in patients with myocardial ischemia at rest. *J Thromb Thrombol.* 1994;1:101–107. Medline
24. Ashton JH, Golino P, McNatt JM, Buja LM, Willerson JT. Serotonin S_2 and thromboxane A_2-prostaglandin H_2 receptor blockade provide protection against epinephrine-induced cyclic flow variations in severely narrowed canine coronary arteries. *J Am Coll Cardiol.* 1989;13:755–763. Abstract
25. Yao SK, Ober J, McNatt J, Benedict CR, Rosolowsky M, Anderson HV, Cui K, Maffrand JP, Campbell WB, Buja LM, Willerson JT. ADP plays an important role in mediating platelet aggregation and cyclic flow variations in vivo in stenosed and endothelium-injured canine coronary arteries. *Circ Res.* 1992;70:39–48. Abstract/FREE Full Text
26. Revilacqua MP, Gimbrone MA Jr. Inducible endothelial functions in inflammation and coagulation. *Semin Thromb Hemost.* 1987;13:425–433. Medline
27. Alexopoulos D, Ambrose JA, Stump D, Borrico S, Gorlin R, Deshmukh P, Fisher EA. Thrombosis related markers in unstable angina pectoris. *J Am Coll Cardiol.* 1991;17:866–871. Abstract
28. Irie T, Imaizumi T, Matuguchi T, Kovanagi S, Kanaide H, Takeshita A, Nakamura M. Increased fibrinopeptide A during anginal attacks in patients with variant angina. *J Am Coll Cardiol.* 1989;14:589–594. Abstract

29. Oshima S, Ogawa H, Yasue H, Okumura K, Matsuyama K, Miyagi H. Increased plasma fibrinopeptide A levels during attacks induced by hyperventilation in patients with coronary vasospastic angina. *J Am Coll Cardiol.* 1989;14:150–154. Abstract

30. Estivals M, Pelzer H, Sie P, Pichon J, Boccalon H, Boneau B. Prothrombin fragment 1+2, thrombin–antithrombin III complexes and D-dimers in acute vein thrombosis: effects of heparin treatment. *Br J Haematol.* 1991;78:421–424. Medline

31. Mazzone A, De Servi S, Ricevuti G, Mazzucchelli I, Fossati G, Pasotti D, Bramucci E, Angoli L, Marsico F, Specchia G, Notario A. Increased expression of neutrophil and monocyte adhesion molecules in unstable coronary artery disease. *Circulation.* 1993;88:358–363. Abstract/FREE Full Text

32. DiSciascio G, Cowley MJ, Goudreau E, Vetrovec GW, Johnson DE. Histopathologic correlates of unstable ischemic syndromes in patients undergoing directional coronary atherectomy: in vivo evidence of thrombosis, ulceration, and inflammation. *Am Heart J.* 1994;128:419–426. CrossRef Medline

33. Van der Wal AC, Becker AE, van der Loos CM, Das PK. Site of intimal rupture or erosion of thrombosed coronary atherosclerotic plaques is characterized by an inflammatory process irrespective of the dominant plaque morphology. *Circulation.* 1994;89:36–44. Abstract/FREE Full Text

34. Ciabattoni G, Liang S, Sritar P, Andreotti F, Davies G, Simonetti BM, Patrono C, Maseri A. Aspirin, but not heparin, suppresses the transient increase in thromboxane biosynthesis associated with cardiac catheterization or coronary angioplasty. *J Am Coll Cardiol.* 1993;21:1377–1381. Abstract

35. Moreno PR, Falk E, Palacios IF, Newell JB, Fuster V, Fallon JT. Macrophage infiltration in acute coronary syndromes: implication for plaque rupture. *Circulation.* 1994;90:775–778. Abstract/FREE Full Text

36. Liuzzo G, Biasucci LM, Gallimore RJ, Grillo RJ, Rebuzzi AG, Pepys MB, Maseri A. Prognostic value of C-reactive protein and serum amyloid a protein in severe unstable angina. *N Engl J Med.* 1994;331:417–425. CrossRef Medline

37. Biasucci LM, D'Onofrio G, Liuzzo G, Zini G, Monaco C, Caligiuri G, Tommasi M, Rebuzzi AG, Maseri A. Intracellular neutrophil myeloperoxidase is reduced in unstable angina and myocardial infarction, but its reduction is not related to ischemia. *J Am Coll Cardiol.* 1996;27:611–616. Abstract

38. Neri Serneri GG, Abbate R, Gori AM, Attanasio M, Martini F, Giusti B, Dabizzi P, Poggesi L, Modesti PA, Trotta F, Rostagno C, Boddi M, Gensini GF. Transient intermittent lymphocyte activation is responsible for the instability of angina. *Circulation.* 1992;86:790–797. Abstract/FREE Full Text

39. Carry M, Korley V, Willerson JT, Weigelt L, Ford-Hutchinson AW, Tagari P. Increased urinary leukotriene excretion in patients with cardiac ischemia: in vivo evidence for 5-lipoxygenase activation. *Circulation.* 1992;85:230–236. Abstract/FREE Full Text

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Abstract Full Text PDF

Activated platelets contribute importantly to myocardial reperfusion injury
Am. J. Physiol. Heart Circ. Physiol.. 2006;290:H692–H699,
Abstract Full Text PDF

Evidence for Antigen-Driven T-Cell Response in Unstable Angina
Circulation. 2000;102:1114–1119,
Abstract Full Text PDF

Procoagulant and proinflammatory activity in acute coronary syndromes
Cardiovasc Res. 1998;40:389–395,
Abstract Full Text PDF