ranges, and differences were analyzed with the Kruskal-Wallis method. Categorical variables were compared by the chi-square test or Fisher exact test.

A total of 1,414 PPCI for STE-ACS were performed during the study period; in 77 patients, TH was used due to a comatose state after cardiac arrest. The access site for the PPCI was always femoral in the PPCI-TH group and was radial in 59% of the PPCI-only group. Baseline characteristics and in-hospital management are shown in Table 1. All patients received a P2Y$_{12}$ inhibitor. Clopidogrel was more commonly used in the PPCI-only group, whereas the PPCI-TH group received significantly more prasugrel. Aspirin was not administered to 33 (2.5%) patients in the PPCI-only group and 1 (1.3%) patient in the PPCI-TH group, mainly due to allergy or recent gastrointestinal bleeding. Both loading and maintenance doses of aspirin in all of the PPCI-TH–group patients were given intravenously. Glycoprotein IIb/IIIa inhibitors were more commonly used in the PPCI-only group.

A mean of 2 ± 1.2 stents were implanted in both groups, with an overall incidence of ST of 2.3% (n = 32). Among the patients experiencing ST, 30 (2.3%) were in the PPCI-only group: 17 (1.2%) were acute and 13 (1.0%) were subacute. In the PPCI-TH group, there were only 2 (2.7%) ST: 1 definitive acute and 1 probable subacute. Major bleeding, according to GRACE (Global Registry of Acute Coronary Events) definition (4), was observed in 6 (8.0%) patients in the PPCI-TH group as compared with 17 (1.3%) in the PPCI-only group (p < 0.001).

All-cause mortality at 30 days of follow-up was significantly higher in the PPCI-TH group than in the PPCI-only group (n = 33 [44%] vs. 65 [4.9%], p < 0.001). In the PPCI-TH group, 31 patients had a reliable cause of death. Eleven arrived at the hospital in cardiogenic shock that did not improve despite successful revascularization, and died early due to multiple organ failure (MOF) without any acute decompensation; 1 died due to liver rupture related to resuscitation maneuvers; 1 died of unexplained cause (suspected sepsis), and post-mortem examination revealed absence of ST; 1 died of brain death; and 17 died due to the withdrawal of life-sustaining treatment secondary to severe post-anoxic encephalopathy. The other 2 deaths were classified as ST: 1 definite acute ST in a clopidogrel-treated patient complicated with retroperitoneal bleeding after a new PPCI, and 1 subacute probable ST that died on the 25th day of evolution due to MOF.

It has been suggested that TH-treated patients may have an increased risk of ST (2), mainly due to MOF and lower liver metabolism of drugs that inhibit the ADP P2Y$_{12}$ receptors and thromboxane A$_2$ synthesis. In the present observational study, even under the prelude of greater hemodynamic support and a higher frequency of hemorrhagic complications in the PPCI-TH–treated patients, the incidence of ST was almost identical to that of patients not treated with TH. Therefore, and under the need of further prospective trials, we believe that adequate antithrombotic management could be achieved in this population with both the progressive introduction of third-generation P2Y$_{12}$ inhibitors and consideration that the route (intravenous aspirin and crushed nasogastric P2Y$_{12}$) (5) and time (before PPCI) of administration may influence the final result.

In our study, the incidence of ST under the effects of TH is less than that observed in previous series and similar to that expected in standard PPCI-treated patients. The described prothrombotic effects of TH are not clinically relevant in patients treated according to general recommendations.

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Letters to the Editor

Effects of Heparin on Temporal MicroRNA Profiles

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Liebetrau et al. (1) used serial sampling in patients undergoing transcoronary ablation of septal hypertrophy to determine the temporal release of microRNAs (miRNAs) after cardiac injury. This model offers the advantage that the time of onset of myocardial damage is precisely known. However, heparin is routinely administered during intra-arterial coronary interventions, including septal ablation (2). Others (3) and we (4) have recently shown that even a single heparin bolus is sufficient to significantly alter measurements of miRNA by quantitative polymerase chain reaction, in particular the spike-in *C. elegans* control, Cel-miR-39, that was also used for normalization in the study by Liebetrau et al. (1).

In quantitative polymerase chain reaction analysis, the spike-in control is used to adjust for differences in extraction efficiency between samples (5), and the intersample deviation of Cel-miR-39 measurements is usually less than 1 cycle. However, immediately after administration of the heparin bolus, the detectability of Cel-miR-39 decreases by approximately 3 cycles. This effect is confined to the first hours after heparin dosing and directly related to the half-life of heparin in the circulation. Thus, heparin could have interfered with the quantitation of miRNA after transcoronary ablation of septal hypertrophy. The accompanying editorial noted "the stunning precocity of elevation in the peripheral circulation of miR-1 and miR-133: only 15 min" (6). If baseline blood samples were taken before administration of heparin, then the rapid increase may at least in part be explained by the effect of heparin on the normalization control. If the baseline samples were taken after the heparin bolus, then the reference samples are not suitable for measurements of miRNA after the first hour post-dose. Furthermore, a significant increase in plasma miR-21 levels was previously observed after thigh cuff–induced ischemia/reperfusion injury (7). Plasma miR-21 levels are also affected by antiplatelet medication (8). Thus, miR-21 may not be a suitable control in this setting. Describing the nature and timing of treatments administered in miRNA biomarker studies is necessary to facilitate interpretation of data and prevent confounding by treatment effects.

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Reply

Effects of Heparin on Temporal MicroRNA Profiles

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We read with great interest the letter by Dr. Mayr and colleagues regarding our work and the important topic of the effects of heparin on temporal microRNA (miRNA) profiles and the best timing of miRNA measurement after administration of heparin. Dr. Mayr and colleagues were recently able to show that both the timing of blood sampling relative to heparin dosing and the normalization procedure are critical for reliable miRNA measurements in patients receiving intravenous heparin (2,3). At this point, it can clearly be stated that heparin is not the only confounder in the setting of miRNA measurements; several factors, including different isolation protocols, blood sample type (plasma or serum), and inflammation-driven shifts in hematopoietic compartments after myocardial infarction, appear to further affect the detection of cell-free (truly circulating) miRNA (4).

With reference to the letter, the baseline blood samples in our study (1) were taken before administration of heparin. Therefore, we have a true control for the comparison of miRNA concentrations after transcoronary ablation of septal hypertrophy. The transcoronary ablation of septal hypertrophy procedure itself was performed after administration of heparin (bolus 5,000 IU heparin). The miRNAs were isolated from serum samples before miRNA isolation and spiking with native cel-miR-39. The samples were treated with heparinase to minimize the influence of heparin on the miRNA measurements. Of course, we cannot entirely exclude the further existence of an effect of heparin, but pre-study tests using the heparinase protocol showed comparable results for miRNA concentrations with and without heparin. Furthermore, our results clearly show a steep increase in miR-1 and miR-133a levels (approximately a 30-fold change) within 60 min after induction of myocardial infarction. Even if heparin were to cause a 30% difference in miRNA concentrations, the difference compared with miRNA concentrations at 15 min would still be significant.

The patients in our study were without antiplatelet medication. Therefore, we can exclude the interference of this medication with the measurement of miR-21 concentrations. Nevertheless, miR-21 is known to be up-regulated in the presence of cardiac hypertrophy. Thus, an influence on this subset cannot be entirely excluded.

Although methodological issues may slightly interfere with the final miRNA measurements, our results add important information to this new field, and miRNAs may well have a future as biomarkers for myocardial ischemia.

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