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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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