



Research paper

Heparinase treatment of heparin-contaminated plasma from coronary artery bypass grafting patients enables reliable quantification of microRNAs



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ABSTRACT

Background: microRNAs have recently been identified as powerful biomarkers of human disease. Reliable polymerase chain reaction (PCR)-based quantification of nucleic acids in clinical samples contaminated with polymerase inhibitor heparin requires deheparinization. However, the effects of deheparinization procedure on quantification of nucleic acids remain largely unknown. The aim of this study was to determine whether the deheparinization procedure completely eliminates the inhibition of amplification, while maintaining RNA integrity and technical variability of the measured microRNA levels.

Methods: Heparinized plasma from 9 patients undergoing coronary artery bypass grafting (CABG) and the heparin-free plasma from 58 rats were spiked with a synthetic RNA oligonucleotide and total RNA was extracted. The RNA solutions were then treated with heparinase I to remove contaminating heparin prior to reverse transcription. Levels of synthetic spike-in RNA oligonucleotide, as well as endogenous hsa-miR-1-3p and hsa-miR-208a-3p, were measured using quantitative reverse transcription PCR (RT-qPCR). The amplification efficiency and presence of inhibitors in individual samples were directly determined using calibration curves.

Results: In contrast to RNA samples from rat plasma, RNA samples derived from the CABG patient plasma contained inhibitors, which were completely eliminated by treatment with heparinase. The procedure caused a decrease in the amount of detected RNA; however, the technical variability of the measured targets did not change, allowing for the quantification of circulating endogenous hsa-miR-1-3p and hsa-miR-208a-3p in the plasma of CABG patients.

Conclusions: The heparinase treatment procedure enables utilization of RT-qPCR for reliable microRNA quantification in heparinized plasma.

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Abbreviations: PCR, polymerase chain reaction; CABG, coronary artery bypass grafting; EDTA, ethylenediaminetetraacetic acid; hsa-miR-1-3p, 3p strand of mature *Homo sapiens* microRNA-1; hsa-miR-208a-3p, 3p strand of mature *Homo sapiens* microRNA-208a; RT-qPCR, reverse transcription quantitative real-time PCR; RT, reverse transcription; Cq, quantification cycle; TP, time point; tRNA, transfer RNA; cel-miR-39-3p, 3p strand of mature *Caenorhabditis elegans* microRNA-39; RNase, ribonuclease; RIN, RNA integrity number; qPCR, quantitative real-time PCR.

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1. Introduction

Heparin, a commonly used anticoagulant, seriously complicates polymerase chain reaction (PCR)-based analysis of nucleic acids in clinical samples, since it inhibits both reverse transcription (RT) and PCR amplification steps. The routine methods of RNA isolation, including column purification, do not remove heparin from RNA samples [1,2]. Currently, the only effective and universal approach to remove heparin is to treat RNA samples with heparinase [2].

The effects of treating RNA with heparinase for PCR-based studies have been estimated by several approaches that include

evaluation of the electrophoretic band intensity of the PCR product [3], calculation of the proportion of target-positive samples [4], and monitoring changes in values of quantification cycle (Cq) [5–8]. These approaches are only sufficient to check the suitability of heparinase-treated samples for qualitative analysis of target nucleic acids because they measure reduced inhibition of the RT and PCR assays but do not prove complete elimination of heparin from the sample.

Reverse transcription quantitative PCR (RT-qPCR) is a conventional method for quantitative analysis of absolute and relative levels of target RNAs [9]. However, there are problems regarding the reliability of RT-qPCR analyses of RNA from heparinase-treated samples due to the lack of data on several critical parameters of deheparinized samples. These parameters are as follows: (i) efficiency of enzymatic reactions, which directly influences both accuracy and precision of RT-qPCR-based quantification, (ii) variability of RNA levels after additional pipetting steps, which influence precision, and (iii) changes in RNA integrity, which impede comparison of deheparinized and heparin-free untreated samples, as well as detection of low copy number transcripts. In the present study, we sought to characterize these parameters after heparinase treatment to determine whether the procedure completely removed the heparin, thus ensuring equal efficiencies in downstream enzymatic reactions, and whether the variability of measured RNA levels and integrity of the target RNA was preserved.

In the present study, RNA samples from rat plasma were used as a control to assess variability of the measured Cq in the absence of both inhibition and heparinase treatment. The inhibitory action of heparin in RNA samples from the plasma of coronary artery bypass grafting (CABG) patients collected at different timepoints during surgery was analyzed and the effect of heparinase treatment on the efficiency of enzymatic reactions was determined. RT-qPCR analysis of either heparinase-treated or untreated aliquots of target RNA aqueous solutions allowed us to determine the effect of heparinase treatment on RNA integrity and Cq variability. Finally, we tested whether treatment with heparinase allows for the detection of endogenous high- and low-abundance microRNAs in plasma samples of CABG patients. Hsa-miR-1-3p was selected as one of the most abundance microRNAs in both skeletal and cardiac muscles and hsa-miR-208a-3p was selected as relatively low-abundance cardiospecific microRNA [10]. Both microRNAs are known to be upregulated in the plasma of patients after acute myocardial infarction [11].

2. Materials and methods

2.1. Study cohort and blood sampling

Written informed consent was obtained from all patients undergoing CABG. The study has been approved by the local Ethics Committee and conforms to the principles outlined in the Declaration of Helsinki. Patients included in this study (n=9) had cardiac troponin I levels less than 0.011 ng/mL before surgery and greater than 1.249 ng/mL within 120 min after aortic declamping. Patients received bolus dose of unfractionated heparin (B. Braun Medical Inc., Melsungen, Germany) approximately 30 min after thoracotomy. Some patients received maintenance dose of heparin during cardiopulmonary bypass. Heparin doses were adjusted to maintain activated clotting time during cardiopulmonary bypass between 300 and 450 s. The average total heparin dose was 312 IU/kg (range 261–450 IU/kg). Whole blood samples were collected in EDTA tubes at the following time points: 40 min before heparin bolus dose administration (time point 1 (TP1)) as well as 20 min (TP2), 140 min (TP3), and 260 min (TP4) after heparin bolus dose administration (Supplementary Fig. 1). Samples at TP1, TP4 and TP2, TP3

were drawn from the peripheral vein and from the coronary sinus, respectively. Clinical data and information on exact heparin doses are provided in the Supplementary Table 1.

2.2. Animals and blood sampling

Fifty eight male Wistar rats (weight 300–350 g) were enrolled in the study. Whole blood was collected in EDTA tubes from the aorta immediately after thoracotomy (control group, n=10) or 120 min after thoracotomy (ischemia-reperfusion group, n=48). All procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication No. 85–23, revised 1996), European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes. The study protocol was approved by the local Ethics Committee. The details on animal care and surgery procedures used to induce myocardial ischemia-reperfusion are provided in the Supplementary methods.

2.3. Plasma preparation

Human and rat whole blood samples were stored at 4°C for a maximum of 30 min after collection. Plasma was isolated using double centrifugation at 1600g for 10 min at 4°C. Samples were aliquoted, snap frozen in liquid nitrogen and stored at –80°C.

2.4. Isolation of total RNA

Total RNA, including small RNA species, was isolated from 200 µL of plasma using 600 µL of TRIzol LS reagent (Life Technologies Co., Carlsbad, USA) that was pre-mixed with 1 µg of *Escherichia coli* tRNA (Sigma-Aldrich Co., Missouri, USA) and 10⁸ molecules of the synthetic RNA oligonucleotide synth-cel-miR-39 (Syntol, Moscow, Russia), which is identical to mature *Caenorhabditis elegans* microRNA cel-miR-39-3p (miRBase accession: [MIMAT0000010](#)). RNA was isolated according to the manufacturer's recommendations. The RNA pellet was air dried, and dissolved in 10 µL of RNase-free water and stored at –80°C. The details on RNA integrity analysis are provided in the Supplementary methods and Supplementary Fig. 2.

2.5. Heparin elimination procedure

Heparin was eliminated from RNA isolated from CABG patient plasma samples using the protocol described by Izraeli et al. [2] with some modifications as described in the Supplementary methods. To test the effect of heparinase treatment on Cq variability and RNA integrity, we compared the amplification of synth-cel-miR-39 from heparinase-treated with that of a control synth-cel-miR-39 water solution. The details of these methodologies are provided in the Supplementary methods.

2.6. RT-qPCR quantification

Target RNAs were detected using hydrolysis probe-based TaqMan MicroRNA Assays. Reverse transcription was performed using TaqMan MicroRNA Reverse Transcription Kit (Life Technologies), amplification was performed using TaqMan Universal PCR Master Mix (Life Technologies) according to the manufacturer's recommendations. The details of these assays are provided in the Supplementary.

2.7. Data analysis

The Shapiro-Wilk test was used to check the normality of data. The Student's *t*-test (for normally distributed data and

independent samples) or nonparametric Wilcoxon signed-rank test (for non-normally distributed data and paired samples) were used to assess the differences between the means of two groups. The *F*-test was used to compare *C_q* variances between two groups. Two-sided *P* values less than 0.05 were considered statistically significant. R-package (version 2.12.0) was used to perform statistical analysis and visualizations.

3. Results

3.1. Efficiency of RT-qPCR enzymatic reactions can be analyzed by amplification of spike-in synth-cel-miR-39 from serially diluted individual RNA samples

In the present study, we analyzed calibration curves to directly assess inhibition and efficiency of RT-qPCR enzymatic reactions. In a validation experiment, 10-fold serial dilutions of synth-cel-miR-39 in water were used as templates. The dependence of *C_q* on the logarithm of the synth-cel-miR-39 concentration was linear ($R^2 = 0.996$, efficiency = 91%) throughout the entire range of concentrations tested (10^4 – 10^9 molecules in 5 μ L reverse transcription reaction) (Supplementary Fig. 3A). This range includes a value of 0.83×10^7 molecules in a 5 μ L reverse transcription reaction, which is the maximum concentration of synth-cel-miR-39 oligonucleotide that can be achieved in RNA samples isolated from plasma (see RT-qPCR quantification section of Supplementary methods for detailed calculations). Thus, the set of reagents used for RT and PCR amplification of synth-cel-miR-39 has sufficient sensitivity and capacity for accurate detection of levels of this spike-in oligonucleotide in RNA samples from patient plasma and therefore it can be used to control for the presence of inhibitors by monitoring the deviations from linearity of synth-cel-miR-39 calibration curves at high template concentrations.

3.2. RNA samples from CABG patients plasma collected before, during, and after surgery contain various amounts of RT-qPCR inhibitors

No inhibition of the RT-qPCR enzymatic reaction was observed when synth-cel-miR-39 was amplified from serial dilutions of RNA samples obtained from rat plasma (Fig. 1C). In contrast, calibration curves for RNA samples from CABG patient plasma were non-linear. The linear relationship between *C_q* and the logarithm of synth-cel-miR-39 concentration was restored when RNA samples from CABG patient were diluted from 10- to 1000-fold (Figs. 1A and B; 2).

Contamination of RNA samples from CABG patient plasma with various amounts of RT-qPCR inhibitors was confirmed by observation of considerable *C_q* variability (SD = 2.97) when synth-cel-miR-39 was amplified from undiluted RNA from plasma samples collected before surgery (TP1) (Fig. 1D) and the absence of synth-cel-miR-39 amplification from undiluted RNA from plasma samples collected 20 min after administration of the bolus heparin dose (TP2) (data not shown).

3.3. Heparinase treatment of RNA samples from CABG patient plasma completely eliminates inhibition of RT-qPCR enzymatic reactions

Since heparin is a known inhibitor of both the RT and PCR reactions, we tested whether heparinase treatment would alter the efficacy of the enzymatic reaction. We found that heparinase treatment of RNA samples prior to reverse transcription decreased *C_q* variability in TP1 samples (SD = 0.35) and enabled detection of synth-cel-miR-39 in TP2 samples (SD = 0.48) (Fig. 1D), suggesting that heparin did indeed inhibit target detection via RT-qPCR. Analysis of data variance showed statistically significant difference

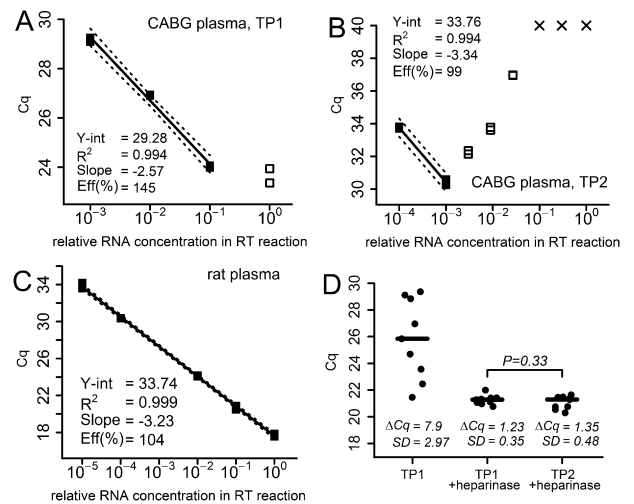


Fig. 1. RNA samples isolated from CABG patient plasma contain polymerase inhibitors, which are sensitive to heparinase treatment.

Standard curves were generated using 10-fold serial dilutions of RNA samples isolated from CABG patient plasma collected at TP1 (A) and TP2 (B) or from rat plasma (C). Y-intercepts (Y-int), coefficients of determination (R^2) for linear regression models, slopes and reactions efficiencies (Eff) were determined and are indicated. The black and open squares represent data points within and outside the linear range of dependence of *C_q* on the logarithm of the synth-cel-miR-39 concentration, respectively. Crosses represent data points, which *C_q* values were arbitrary assigned to 40, since corresponding sample dilutions show no reporter fluorescence until cycle 40. The dotted lines indicate 95% confidence band of the best fit lines.

(D) Scatter-plots show the variability of *C_q* values obtained after synth-cel-miR-39 amplification from RNA samples isolated from CABG patient plasma collected at TP1 ($n=9$) and TP2 ($n=9$). RNA samples were either untreated or treated with heparinase prior to reverse transcription as indicated. The horizontal lines show the median *C_q* values. SD represent standard deviation, ΔC_q represent difference between the highest and the lowest *C_q*s for each group, *P* value is for Student's *t*-test.

between *C_q* variances of TP1 samples before and after treatment (*F*-test, $P = 2 \times 10^{-6}$). There were no statistically significant differences between *C_q* variances (*F*-test, $P = 0.382$) and *C_q* values (Student's *t*-test, $P = 0.33$) of TP1 and TP2 samples after treatment.

To evaluate the extent of inhibitor removal by heparinase treatment, synth-cel-miR-39 was amplified from serial dilutions of several RNA samples isolated from CABG patients, which were either treated or untreated with heparinase. Analysis of the resulting calibration curves demonstrated that, despite a variable degree of inhibition at different time points in the untreated samples, addition of heparinase resulted in the complete elimination of inhibition and equal efficiency of the enzymatic amplification reaction in all samples (Fig. 2).

3.4. Heparinase treatment reduced the target RNA yield but had no effect on *C_q* variability

The heparin elimination procedure includes additional sample handling steps and requires treatment of RNA for an extra 3 h at 25 °C with heparinase. To assess the cumulative impact of this extra handling on *C_q* variability and RNA integrity, we compared synth-cel-miR-39 amplification from three groups of aliquots of aqueous synth-cel-miR-39 solutions. The first group of aliquots contained no heparin and was not treated with heparinase (control), the second contained no heparin but was treated with heparinase (heparinase), and the third group of aliquots was supplemented with heparin and was treated with heparinase (heparinase + heparin). Without heparinase treatment, the concentration of heparin in the reverse transcription reactions with RNA from the third group of aliquots would be similar to that with RNA from CABG patients plasma collected a short time after administration of bolus dose of heparin (see Heparin elimination procedure section of

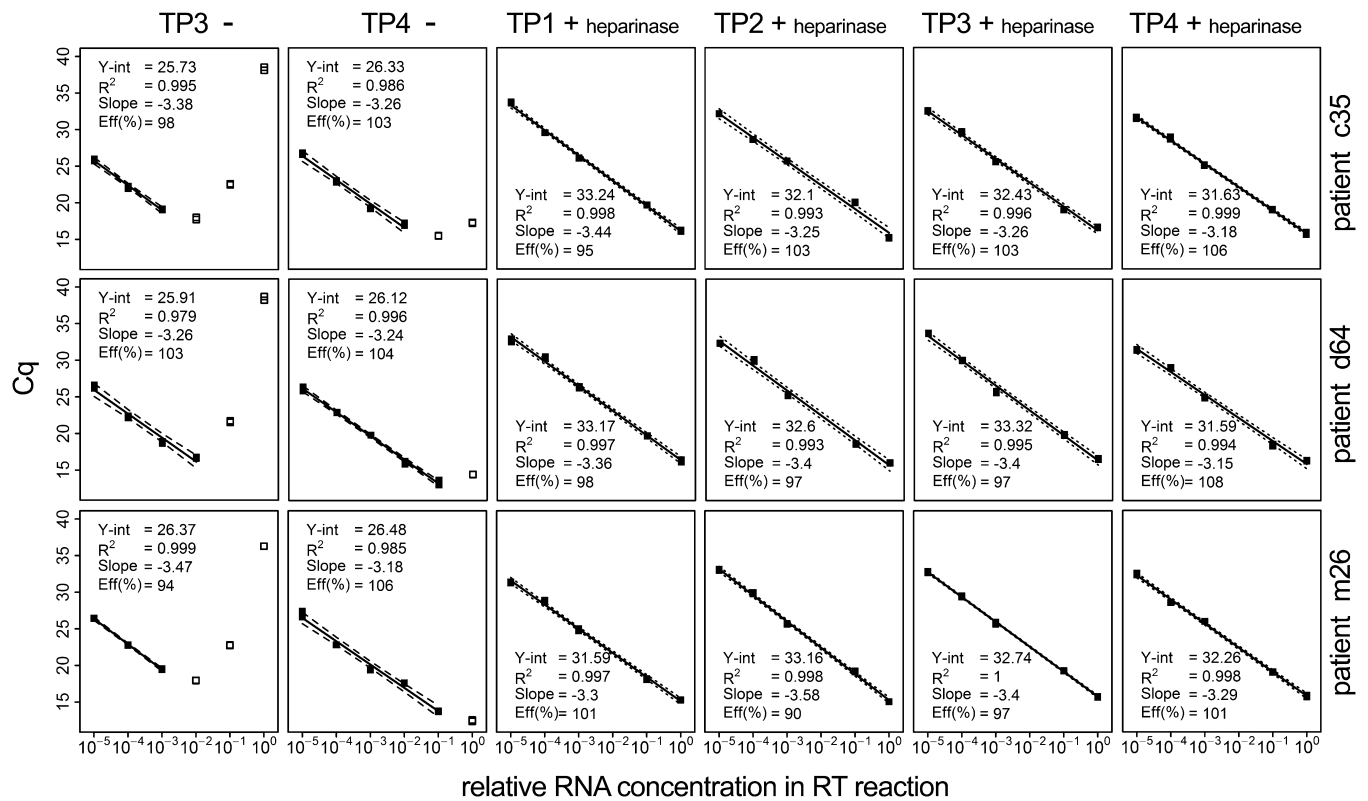


Fig. 2. Heparinase treatment of RNA samples from CABG patient plasma completely abolished inhibition of PCR amplification. Calibration curves generated after synth-cel-miR-39 amplification from 10-fold serial dilutions of RNA samples isolated from plasma of selected CABG patients (c35, d64, m26), collected at indicated time points. RNA samples were either untreated (-) or treated (+heparinase) with heparinase prior to dilution and reverse transcription. Y-intercepts (Y-int), coefficients of determination (R^2) for linear regression models, slopes and reactions efficiencies (Eff) were determined and are indicated. The black and open squares represent data points within and outside the linear range of dependence of Cq on the logarithm of the synth-cel-miR-39 concentration, respectively. The dotted lines indicate 95% confidence band of the best fit lines.

Supplementary methods for detailed calculations). Heparinase treatment of the aliquots led to a 5-fold decrease in measured levels of synth-cel-miR-39 (Student's t -test, $P=0.002$) and did not significantly change the Cq variance as compared to the non-heparinized untreated aliquots (F -test, $P=0.519$). The presence of heparin in samples before heparinase treatment did not influence the measured Cq values or their variances because both parameters did not differ between heparin-free heparinase-treated and heparin-containing heparinase-treated groups (Student's t -test, $P=0.271$; F -test, $P=0.958$) (Fig. 3A). The absence of significant influence of heparinase treatment on Cq variance was confirmed by comparison of the amplification of spike-in synth-cell-miR-39 in heparinase-treated RNA samples from CABG patients plasma ($n=36$) and untreated RNA samples from non-heparinized rat plasma ($n=58$) (F -test, $P=0.96$) (Fig. 3B and C). Coefficients of variation for the concentration of synth-cel-miR-39 were 47.8% in heparinase treated CABG samples and 48% in rat samples. In an additional experiment we analyze influence of heparinase treatment on the integrity of four different microRNAs (synth-cel-miR-39, synth-hsa-miR-16, synth-hsa-miR-21 and synth-hsa-miR-208a) at two different concentrations. Results of this experiment (Supplementary Fig. 4) demonstrate that (i) heparinase treatment has no effect on Cq variability of all investigated targets, both at high and at low concentration, (ii) heparinase treatment causes different degree of degradation of different targets (3- to 4-fold for synth-cel-miR-39, 2-fold for synth-hsa-miR-16, 6- to 6.5-fold for synth-hsa-miR-21 and 1.5- to 1.6-fold for synth-hsa-miR-208a). So deheparinisation procedure introduce bias in gene expression data due to degradation, which is non-reproducible among genes.

3.5. Heparinase treatment enables quantification of both high- and low-abundance muscle-enriched microRNAs in CABG patient samples

Next, we determined whether heparinase-treated RNA isolated from CABG patient plasma is suitable for detection of endogenous high- and low-abundance circulating microRNAs. We used RT-qPCR to measure the absolute levels of endogenous hsa-miR-1-3p and hsa-miR-208a-3p. The highly-abundance hsa-miR-1-3p was reliably detected in all TP1 and TP4 plasma samples. However, levels of low-abundance hsa-miR-208a-3p were below the lower limit of assay linearity in two TP1 samples and two TP4 samples. In the remaining seven TP1 samples no amplification of hsa-miR-208a-3p was detected. We assigned these samples values to the lower limit of assay linearity in order to include them in the study. Analysis of these data revealed statistically significant upregulation in both hsa-miR-1-3p (Wilcoxon signed-rank test, $P=0.008$) and hsa-miR-208a-3p (Wilcoxon signed-rank test, $P=0.023$) in TP4 samples as compared to the TP1 samples (Fig. 4).

4. Discussion

To our knowledge, this is the first study designed to use calibration curves to investigate the presence of inhibitors or RT-qPCR enzymatic reactions in RNA samples isolated from the plasma of CABG patients and to analyze the influence of the deheparinization procedure on circulating microRNAs quantification by RT-qPCR.

Heparin is routinely administered as anticoagulant during cardiovascular interventions, with average bolus dose ranging from 60 IU/kg for cardiac catheterization procedures to 310 IU/kg for

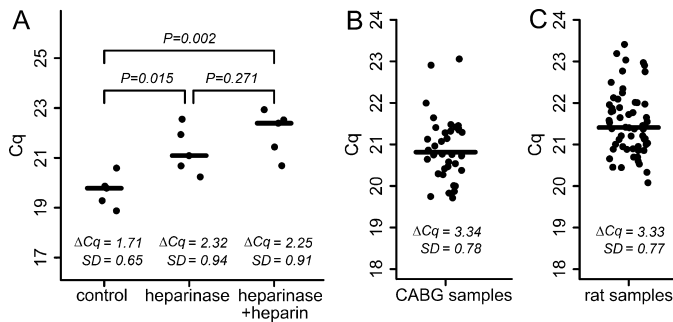


Fig. 3. Heparinase treatment does not increase variability of measured Cq, but does compromise RNA integrity.

(A) Synth-cel-miR-39 was amplified from three groups of aliquots of aqueous synthetic oligonucleotide solution. The aliquot groups were as follows: heparin-free with no heparinase treatment (control, $n=5$), heparin-free treated with heparinase (heparinase, $n=5$), or heparin-containing treated with heparinase (heparinase + heparin, $n=5$). Synth-cel-miR-39 was amplified from RNA samples isolated from (B) CABG patient plasma, treated with heparinase prior to reverse transcription ($n=36$) or (C) non-heparinized rat plasma ($n=58$). Data shown in panels (B) and (C) was collected during different runs of real-time PCR machine. Black circles represent individual samples, horizontal lines show the median Cq values. SD represent standard deviation, ΔCq represent difference between the highest and the lowest Cqs for each group, P values are for Student's *t*-test.

CABG [12,13]. Recent studies have shown that, even in cardiac catheterization patients, within 1 h of dosing, the heparin blood concentration is high enough to interfere with the quantification of circulating microRNA by RT-qPCR methods [6,7]. Effect of partial inhibition can be adjusted by normalization of individual Cqs with the average Cq of multiple targets [7]. Despite these observations, numerous recent publications report the levels of circulating microRNAs in patients undergoing cardiovascular interventions, including transcatheter ablation of septal hypertrophy [14], elective mitral valve surgery [15], and CABG [16,17], and it is unclear whether the effects of heparin were considered in these studies. Moreover, in response to the paper by Libertau et al. [14], Mayr et al. argued that even the traces of heparin can interfere with the results of microRNA measurements in cardiovascular patients [18].

In the present study, we used calibration curves to directly determine the amplification efficiency of RNA targets. Using this method, we were able to detect the presence of inhibitors and to monitor efficacy of inhibitor removal after treatment with heparinase. Moreover, we used the calibration curves to estimate the amount of inhibitor in particular samples by determining the dilution factor required to sufficiently restore a linear relationship between Cq and the logarithm of template concentration. We found that samples collected 20 min after administration of bolus heparin dose (TP2) contained the highest levels of heparin, since amplification of synth-cel-miR-39 failed in all undiluted TP2 samples, but not in undiluted TP1, TP3 and TP4 samples. TP3 samples should be diluted from 100- to 1000-fold, whereas TP4 samples should be diluted only from 10- to 100-fold (Fig. 2) suggesting that the level of heparin in samples rapidly declines after TP2. This temporal inhibitory effect can be explained by the short half-life of unfractionated heparin in circulation and is in agreement with an earlier study that reported the disappearance of heparin inhibition between 4 h and 6 h post administration in plasma samples of cardiac catheterization patients [7]. Unexpectedly, we observed a huge range of measured Cq values ($\Delta Cq = 7.9$, Fig. 1D) in plasma samples from CABG patients collected before surgery (TP1) which indicates the presence of differing amounts of heparin in these samples, possibly from heparin-containing rinse solution left in catheters before they were used for collection of blood samples.

RT-qPCR-based analysis of spike-in synth-cel-miR-39 in plasma from CABG patients clearly showed that samples collected before as well as several minutes to hours after heparin administration had

very different levels of enzymatic assay inhibition. Therefore, it is of great importance to use the heparin elimination protocol to completely remove the heparin regardless of the initial levels in order to avoid inaccurate interpretation of target microRNA expression.

Several papers reported heparinase treatment of plasma samples as a method to remove heparin [4,19]. However, it is important to note that heparin in plasma is partially protected from degradation due to interaction with proteins. Therefore, to avoid the protective effect of proteins and also to reduce the volume of enzymatic reaction to consume less heparinase, we treated isolated RNA in the presence of an RNase inhibitor, as described by Izraeli et al. [2]. Moreover, to guarantee complete digestion of heparin, we extended the duration of heparinase treatment from 2 h to 3 h. Analysis of calibration curves demonstrated that, despite initial differences in the degree of inhibition, treatment with heparinase ensured complete removal of the inhibitor. Our data comparing control and heparinase-treated samples revealed that the additional sample handling performed during the heparinase treatment caused a 1.5- to 6.5-fold decrease in the measured target levels but did not increase variability of Cq. These results indicate that heparinase treatment did not reduce the precision of RT-qPCR based assay, but moderately decreased its sensitivity. However, we demonstrated that the heparinase treatment protocol allows for the detection of endogenous muscle-enriched high-abundance hsa-miR-1-3p in TP1 and TP4 samples as well as low-abundance hsa-miR-208a-3p in TP4 samples. Both microRNAs were found to be elevated after surgery. The most likely reason for the decrease of the measured target levels after heparinase treatment is RNA degradation by RNases, which might be present in the heparinase preparation. To minimize the loss of RNA one can try to reduce the time of deheparinisation and/or to increase the concentration of RNase inhibitors. However, it is important to verify whether these changes affect the completeness of heparin removal.

In addition to blood samples collected in heparinized tubes or samples collected from patients receiving heparin regimens, there are other ways in which samples can become contaminated with heparin. For example, our study revealed that patient plasma samples collected in EDTA tubes before systemic heparin administration were contaminated with heparin, probably originating from catheter rinse solutions. Moreover, the spectrum of clinical specimens containing heparin becomes broader if we take into account that injected heparin is partly excreted in urine [20] and retained in organs perfused with heparinized buffers [3]. Therefore, it is recommended to evaluate the presence of inhibitors before starting PCR-based analysis of nucleic acids from all types of clinical samples that can potentially contain heparin. The calibration curve method is a relatively simple and accurate approach to the

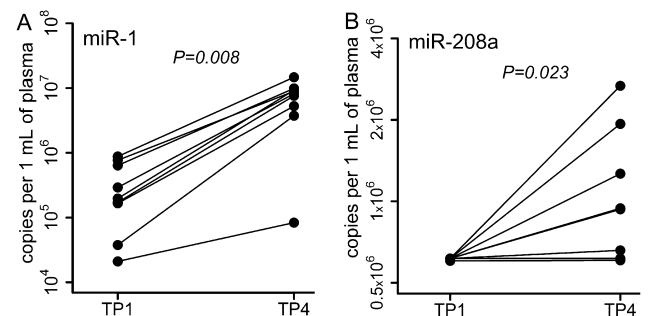


Fig. 4. Heparinase treatment enables quantification of endogenous microRNAs in plasma samples from CABG patients.

Plots show absolute levels of hsa-miR-1-3p (A) and hsa-miR-208a-3p (B) in 1 mL of plasma samples collected before (TP1, $n=9$) and after (TP4, $n=9$) CABG surgery. Black circles represent individual samples. P values are for Wilcoxon signed-rank test.

evaluation of amplification efficiency and presence of inhibitors. To overcome inhibition, samples can be diluted or treated with heparinase depending on the abundance of the target nucleic acid and the dose and timing of heparin administration. We believe that implementing this method will ensure that RT-qPCR can be used to reliably quantify nucleic acids in clinical samples.

In conclusion, this study provides evidence that the heparinase treatment of RNA samples from CABG patient plasma completely reversed heparin-induced inhibition of downstream enzymatic assays and caused different (1.5- to 6.5-fold) reduction of measured RNA levels for different targets without significant changes in Cq variability.

Conflict of interest

The authors declare no conflict of interests.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.bdq.2016.03.001>.

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