ONLINE SUPPLEMENT

METHODS

Assessment of miR-122-5p levels

Extraction and quantitative RT-PCR. Total RNA was extracted from serum samples using the miRNeasy serum/plasma kit and the semi-automated QIAcube system (Qiagen) according to the manufacturer' instructions. 200µL of serum was mixed with 1 mL of QIAzol. After 5 min, 3.5µL of spike-in working solution (Qiagen) containing $1.6x10^8$ copies of cel-miR-39 per µL was added to the sample for correction of extraction efficiency. 200µL of chloroform was added during 3 minutes before a centrifugation step of 20 min at 12,000xg and 4°C. The aqueous phase was introduced into the QIAcube. RNA elution was performed using 18µL of water. Reverse transcription of RNA was performed on 12µL of RNA eluate with the miScript reverse transcription II kit and miScript HiFlex buffer (Qiagen). The resulting cDNA was diluted 10-fold before quantitative PCR with the miScript SYBR-green PCR kit (Qiagen). miRNA-specific miScript primer sets were obtained from Qiagen. Amplifications were performed in a CFX96 thermocycler (Bio-Rad). An inter-run calibrator was used in each PCR to correct technical variations between PCR runs.

Absolute quantification of miR-122-5p. Synthetic miR-122-5p mimics (1nmol, Qiagen) were dissolved into 50µL of water to obtain a 20µM stock solution ($1.204x10^{13}$ copies/µL). A working solution was prepared from this stock solution to obtain 10^{10} copies/µL. A 10-fold serial dilution

of miR-122-5p mimics was performed from 10^{10} copies/µL to 10^4 copies/µL. 5µL of each miR-122-5p mimic dilution was reverse transcribed using the HiFlex miScript reverse transcription II kit in presence of 50ng of carrier RNA (Qiagen). Negative reverse transcription controls were obtained with 50ng of carrier RNA only. Quantitative PCR was performed with 4µL of each cDNA after a 10-fold dilution. Each assay was run in duplicate and a standard curve from 10^9 to 10^3 copies per well was produced. The slope of the standard curve (b=-3.244) and the Y-axis intercept (m=41.644) were determined using the CFX96 Manager 3.1 software (Bio-Rad) (Figure S1). The amount of miR-122-5p per well of PCR plate was obtained with the formula: $10^{((Ct\ miR\text{-}122\text{-}5p\ -b)/m)}$. The amount of miR-122-5p per sample was obtained by multiplying this last value with a correction factor of 75 to account for dilutions during the process. The number of copies of miR-122-5p per µL of serum was obtained by dividing this corrected value by the starting volume of serum (200µL).

Normalization. The number of copies of miR-122-5p per µL of serum was normalized by multiplication with a normalization factor, as described by Mitchell and colleagues [1]. This factor was calculated for each serum sample with the following formula: $1/[2^{(\text{median cel-miR-39 Ct value})-(\text{cel-miR-39 Ct value of the given sample})}]$, where the median cel-miR-39 Ct value was obtained by calculating the median of cel-miR-39 Ct values of all samples to be compared.

Overall, the normalized miR-122-5p values expressed in number of copies of miR-122-5p per µL of serum was obtained using the formula:

$10^{((Ct\ miR\text{-}122\text{-}5p\ -b)/m)}$ x dilution factor x normalization factor

where dilution factor is 75 / 200

and normalization factor is $1/[2^{(\text{median cel-miR-39 Ct value})-(\text{cel-miR-39 Ct value of the given sample})}]$.

TABLES

Table S1. Comparison of the demographic and clinical features of the miRNA cohort and the TTM cohort.

Characteristic	miRNA cohort (n=590)	TTM cohort (n=939)	P value
Age, years	64 (20-94)	65 (20-94)	0.39
Gender			
Male	473 (80%)	761 (81%)	0.72
Co-morbidities			
Hypertension	233 (39%)	374 (40%)	0.95
Diabetes mellitus	83 (14%)	141 (15%)	0.86
Known IHD	154 (26%)	260 (27.7%)	0.67
Previous MI	109 (18%)	193 (21%)	0.004
Heart failure	33 (6%)	61 (6%)	0.77
COPD	48 (8%)	97 (10%)	0.26
Renal failure	5 (1%)	6 (1%)	0.65
Previous cerebral stroke	47 (8%)	73 (8%)	0.96
Alcohol abuse	17 (3%)	37 (4%)	0.54
First monitored rhythm			0.60
VF or non-perfusing VT	475 (80%)	729 (78%)	
Asystole or PEA	97 (16%)	178 (19%)	
ROSC after bystander defibrillation	8 (1%)	13 (1%)	
Unknown	10 (2%)	19 (2%)	
Witnessed arrest	525 (89%)	838 (89%)	0.71
Bystander CPR	431 (73%)	683 (73%)	0.53
Time from cardiac arrest to ROSC (min)	25 (0-170)	25 (0-170)	0.26
Initial serum lactate (mmol/l)	5.7 (0.5-25)	6.0 (0.5-24)	0.41
Circulatory shock on admission	72 (12%)	137 (15%)	0.22

Continuous characteristics are indicated as median (range) and categorical characteristics are indicated as number (frequency). COPD: chronic obstructive pulmonary disease; CPR: cardio-pulmonary resuscitation; IHD: ischemic heart disease; MI: myocardial infarction; PEA: pulseless

electric activity; ROSC: return of spontaneously circulation; VF: ventricular fibrillation; VT: ventricular tachycardia.

Figure S1. Quantitative PCR results for the generation of standard curves for miR-122-5p. Amplification curve (left panel), melting curve (middle panel) and standard curve (right panel) are shown. Negative control (pink color) is composed of carrier RNA only.

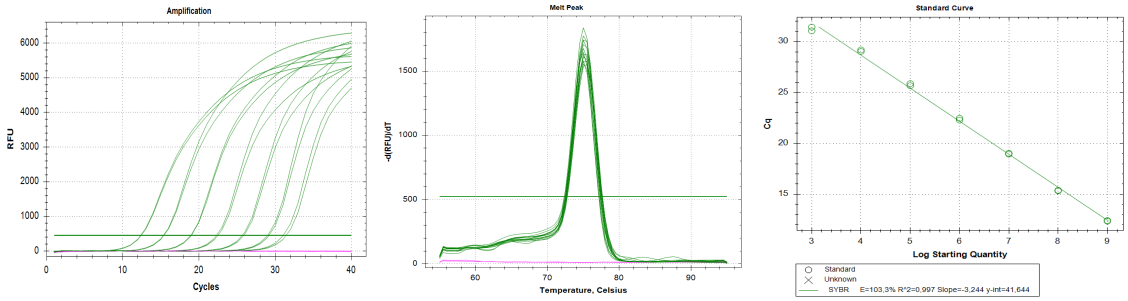


Figure S2. Study flow chart. 590 of the 939 patients of the TTM trial were included in the present sub-study.

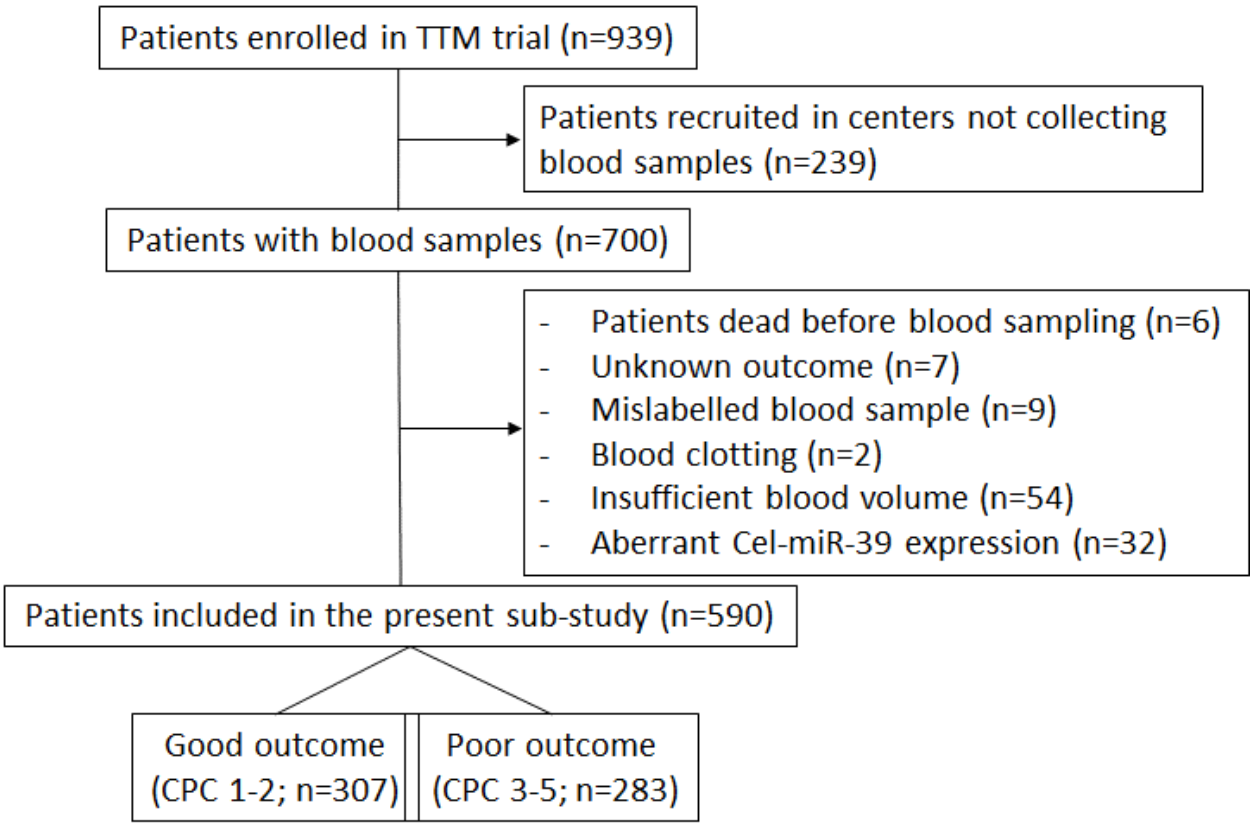


Figure S3. Association between miR-122-5p levels and age. Levels of miR-122-5p were assessed 48 hours after ROSC using quantitative PCR in 590 patients. Correlation coefficient (r) and p value were obtained from Spearman test. Y axis is in log scale.

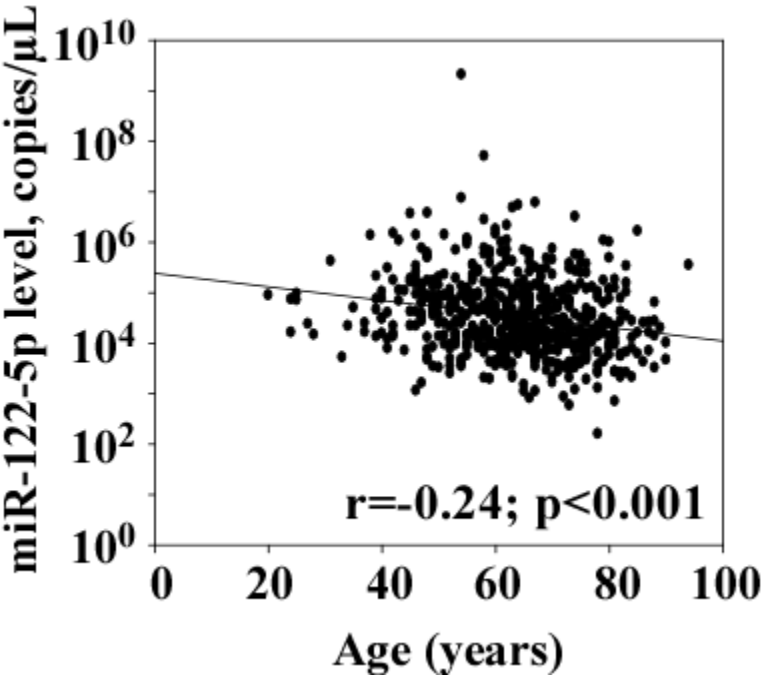


Figure S4. Association between miR-122-5p and miR-124-3p levels. Levels of miR-122-5p were assessed 48 hours after ROSC using quantitative PCR in 590 patients. Levels of miRNAs were assessed in a previous study [2]. Correlation coefficient (r) and p value are from Spearman test. Y axis is in log scale.

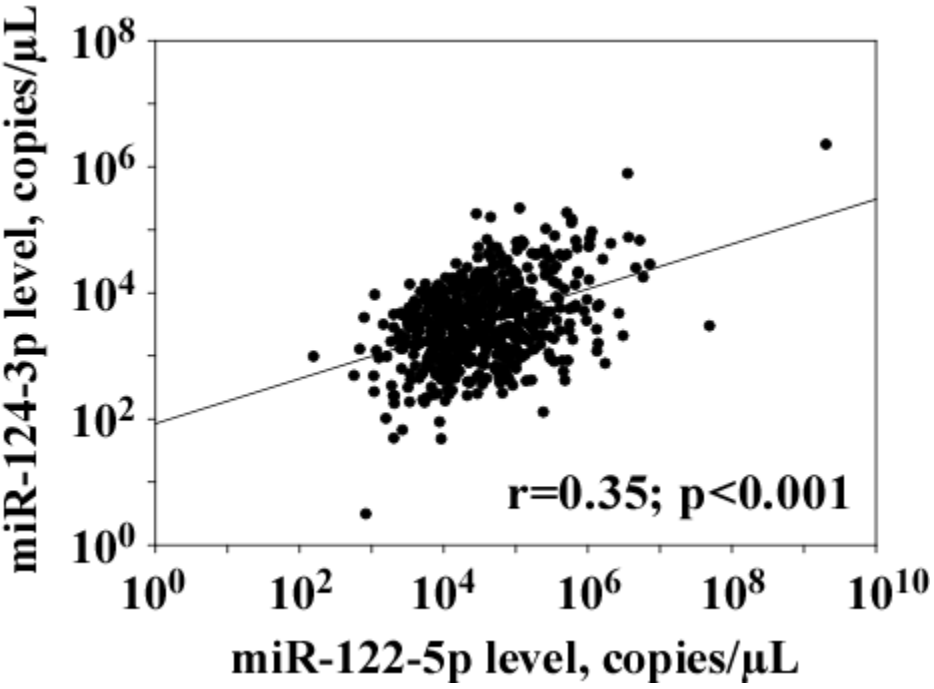
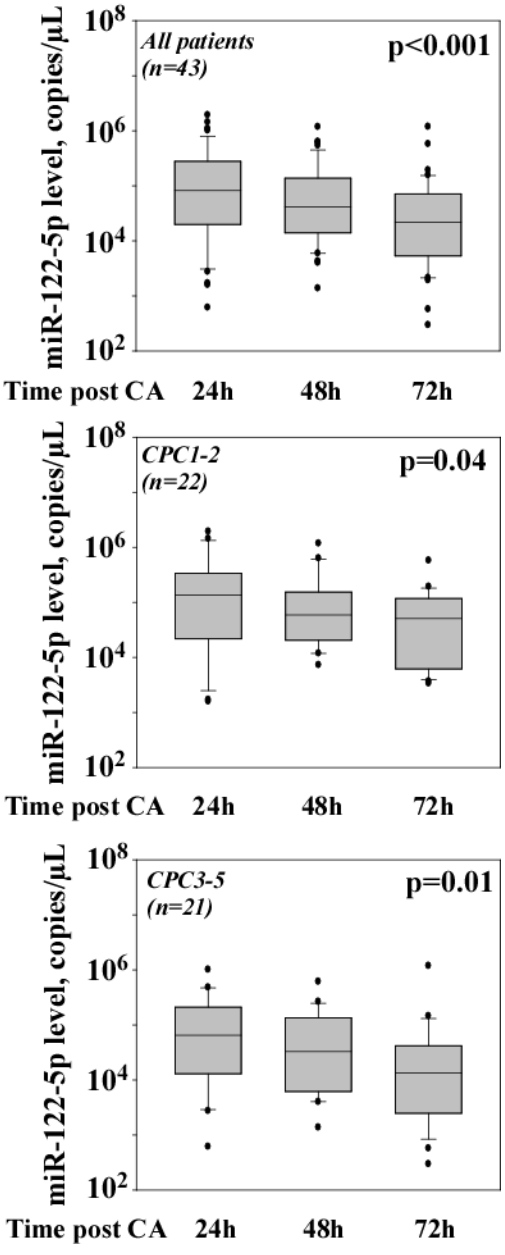


Figure S5. Time-course of circulating levels of miR-122-5p after cardiac arrest. Serum levels of miR-122-5p were assessed by quantitative PCR in 43 patients with blood samples available at 24, 48 and 72 hours after cardiac arrest. Top plot displays data for all patients, middle plot displays data for good outcome patients and bottom plot displays data for poor outcome patients. P values are shown (one-way repeated measures analysis of variance on ranks).



Reference

1. Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, et al. Circulating microRNAs as stable blood-based markers for cancer detection. Proceedings of the National Academy of Sciences of the United States of America. 2008; 105: 10513-8.
2. Devaux Y, Dankiewicz J, Salgado-Somoza A, Stammet P, Collignon O, Gilje P, et al. Association of Circulating MicroRNA-124-3p Levels With Outcomes After Out-of-Hospital Cardiac Arrest: A Substudy of a Randomized Clinical Trial. JAMA Cardiol. 2016; 1: 305-13.