Supplementary material

Vichova T, Waldauf P, Karpisek M, et al. Oxidative stress markers, thioredoxin 1 and 8-isoprostane, in relation to ischemic time in patients with ST-segment elevation myocardial infarction treated by primary percutaneous coronary intervention. Pol Arch Intern Med. 2021; 131: 755-758. doi:10.20452/pamw.16057

Laboratory analysis

8-isoprostane (8-ISO) assay

The 8-ISO assay (Cayman Chemical, Ann Arbor, USA) is based on the competition between 8-ISO from sample and 8-ISO labeled with acetylcholinesterase for a limited number of binding sites represented by 8-ISO specific rabbit antiserum coated on microtitre plate. Ellman's reagent is used for the detection, and resulting signal is inversely proportional to the concentration of 8-ISO. The assay has a calibration range from 0,8 – 500 pg/ml, sensitivity (80% B/B0) of approximately 3 pg/ml.

SOD3 assay

SOD3 levels were assayed with sandwich ELISA kit from Abfrontier (Seoul, Republic of Korea). Due to low sensitivity of the assay, optical density (OD450 nm) was taken as the reference unit and used for data analysis. The measuring range specified by the manufacturer is 2-128 ng/ml and sensitivity 2 ng/ml.

8-hydroxy-2-deoxyguanosine (8-OHdG) assay

Highly sensitive 8-OHdG Check kit from Japan Institute for the Control of Aging (Fukuroi, Japan) was used for determination of 8-OHdG. The 8-OHdG monoclonal antibody and the sample or standard are added to the microtiter plate which has been precoated with 8-OHdG. The 8-OHdG monoclonal antibody reacts competitively with the 8-OHdG bound on the plate and the 8-OHdG in samples solution. An enzyme labeled secondary antibody against monoclonal antibody is used for the detection. The measuring range was 0,125 – 10ng/ml, sensitivity of the assay was 0.05 ng/ml and intraassay or interassay coefficient of variation (CV) were 7.2% or 10.6 %, respectively.

Cytochrome c assay

Concentrations of cytochrome c in serum samples were determined by commercially available colorimetric sandwich ELISA kits (Biovendor-Laboratorni medicina, Brno, Czech Republic) according to the manufacturer's protocol. Performance characteristics were tested to evaluate the assay. Serial dilution linearity testing was performed in two-time diluted samples for evaluation. Mean recovery was within the range +/-15% of expected value. Recombinant protein cytochrome c was used as the standard prepared at the concentrations 0.08 - 5 ng/ml, the sensitivity of the assay was 40 pg/ml and intraassay or interassay coefficient of variation (CV) were always less than 5%. Briefly, serum samples were diluted 1:2 with assay buffer and incubated for 2 hours at room temperature in the plate with coated monoclonal anti-human cytochrome c antibody together with anti-human cytochrome c biotin labelled antibody. Streptavidin-HRP were added for detection in a consequent step. Recombinant protein cytochrome c was used as the standard prepared at the concentrations 0.08 - 5 ng/ml and the solution PBS- 0,05%Tw20-0,05%BSA-0,1% mouse IgG was used as the assay buffer. The sensitivity of the assay was 40 pg/ml and intraassay or interassay coefficient of variation (CV) were 4,1% and 6,4% respectively.

3- nitrotyrosine assay

Nitrotyrosine was determined with the sandwich ELISA kit from Hycult Biotech (Uden, Netherlands). Serum samples were diluted 1:10 with assay buffer and incubated for 1 hour at room temperature in the plate with coated specific polyclonal antibody. After washing steps, biotin labelled antibody and consequently streptavidin-HRP were added for detection. Nitrotyrosine was used as the standard prepared at the concentrations 2 - 1500 nmol/l. The sensitivity of the assay was 0.2 nmol/l and intraassay or interassay coefficient of variation (CV) were 9% or 13% respectively.

Thioredoxin assay

Concentration of thioredoxin was determined by commercially available colorimetric sandwich ELISA (Biovendor-Laboratorni medicina) according to the manufacturer's protocol. Briefly, serum samples were diluted 1:3 with assay buffer and incubated for 2 hours at room temperature in the plate with coated monoclonal anti-human TRX antibody. After washing steps, biotin labelled monoclonal antibody and consequently avidin-HRP were added for detection. The developed color was determined by reading the plate on the microplate reader Biotek EL808 at a wavelength of 450nm. The measuring range was 0.39 – 25ng/ml, the sensitivity 0.13ng/ml and intraassay or interassay coefficient of variation (CV) were 4.8 and 6.4%.

Exclusion criteria for the study

The exclusion criteria for patients and controls were following: cancer, current inflammatory disease, advanced chronic kidney disease, diabetes mellitus on insulin, chronic angina

pectoris or exertional dyspnea, symptomatic peripheral artery disease or history of heart failure.

Figures

Figure S1. Flowchart- Patient selection process for the study

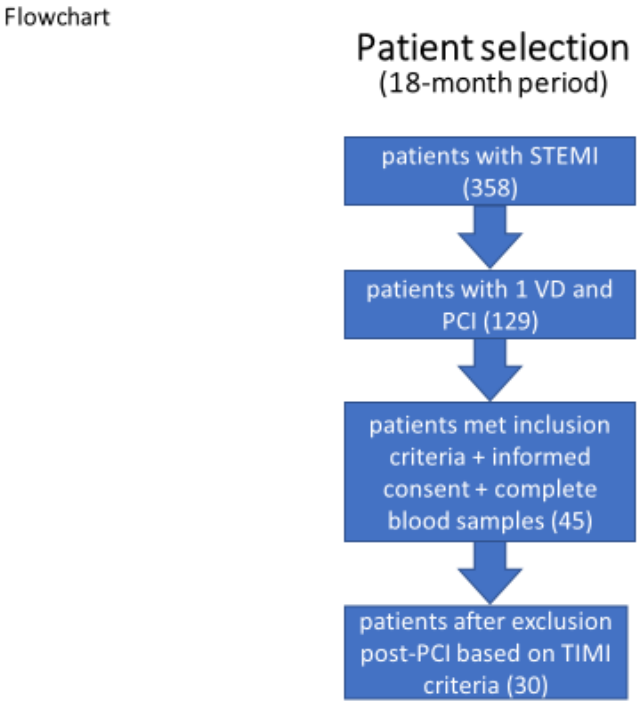


Figure S2. Periprocedural TRX1 levels in patients with AMI (STEMI) and controls

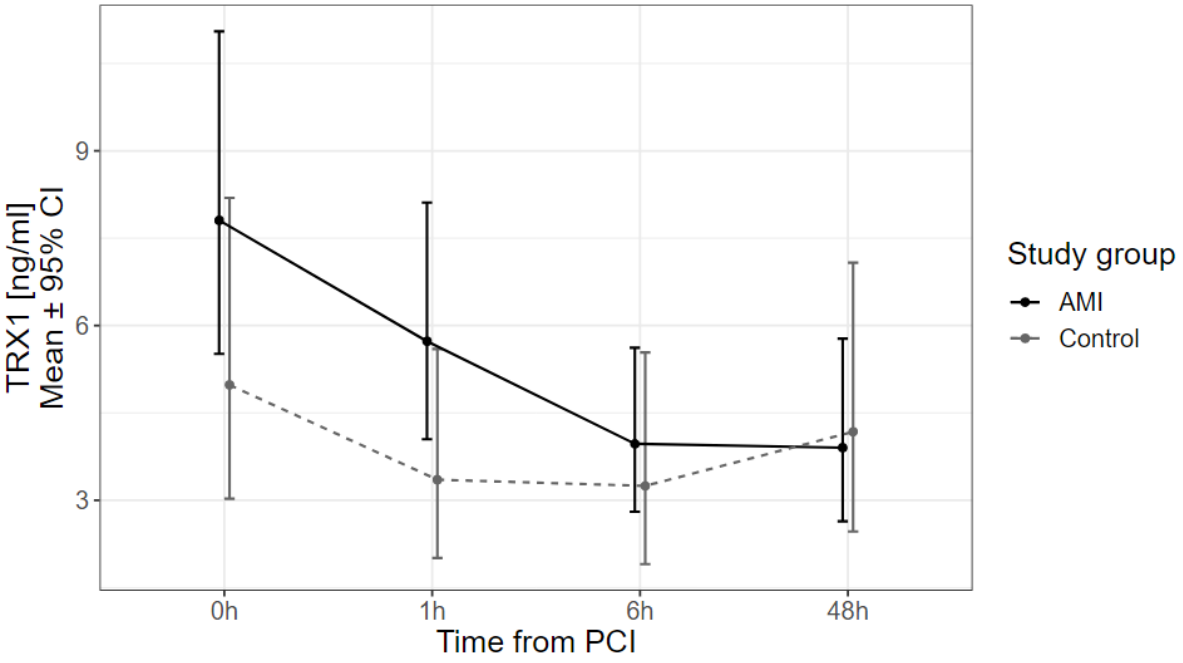


Figure S3. Periprocedural 8-ISO levels in patients with AMI (STEMI) and controls

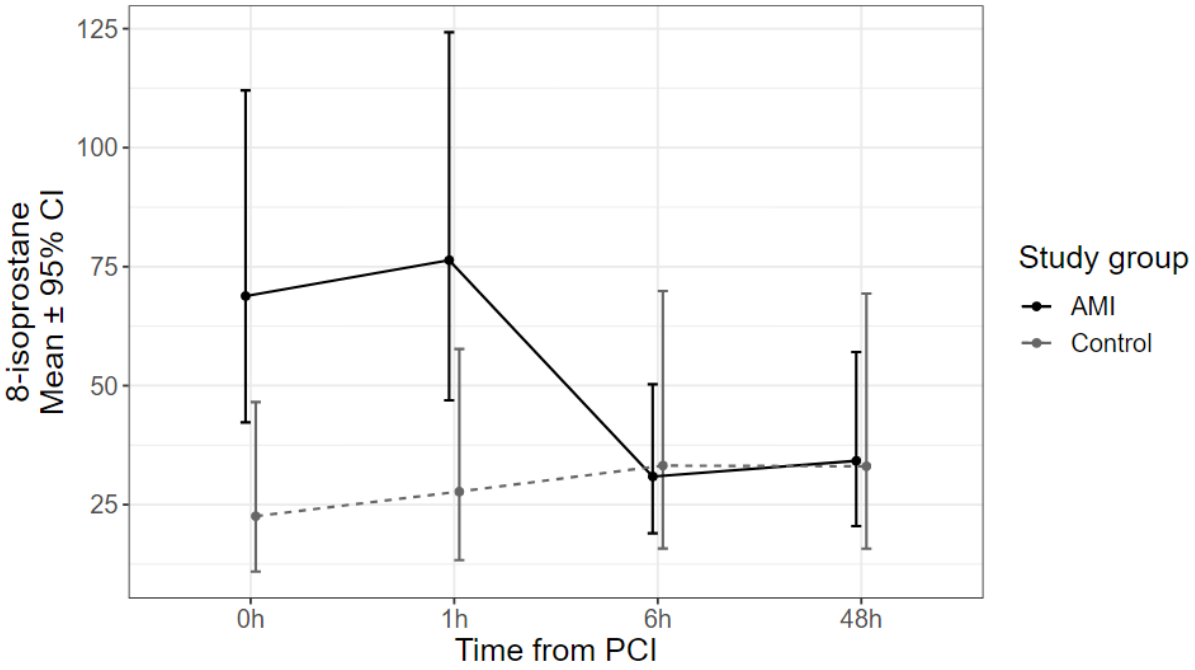


Figure S4. Periprocedural TRX1 levels in patients with AMI (STEMI) in relation to time delay (TD) from symptom onset to PCI

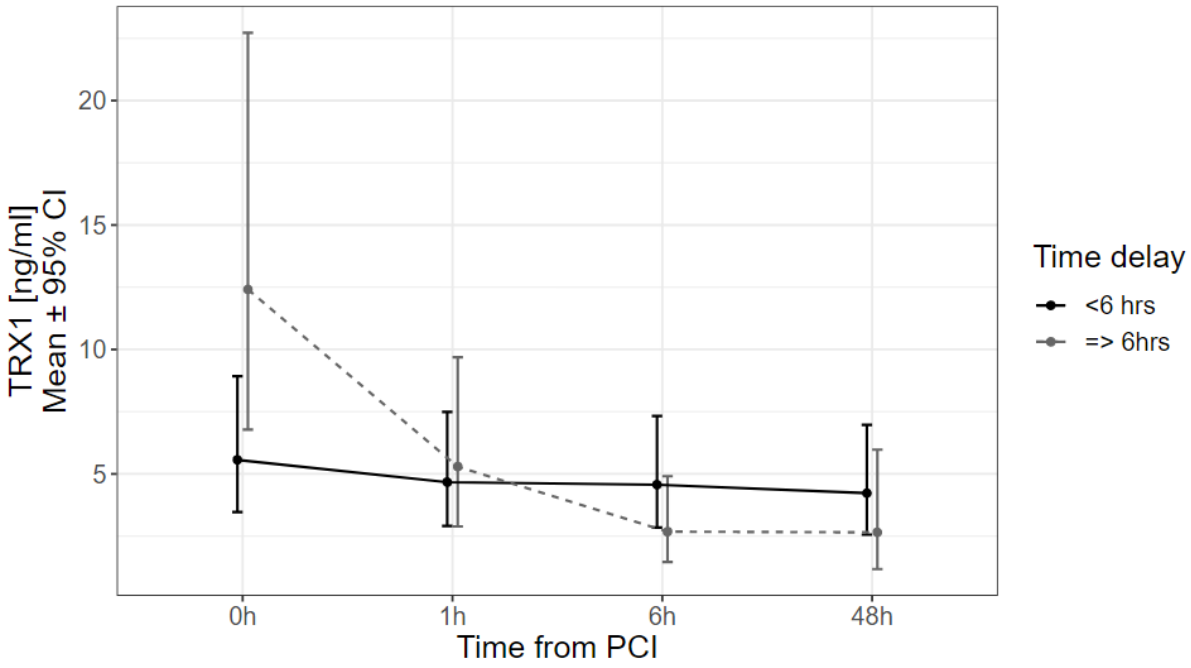
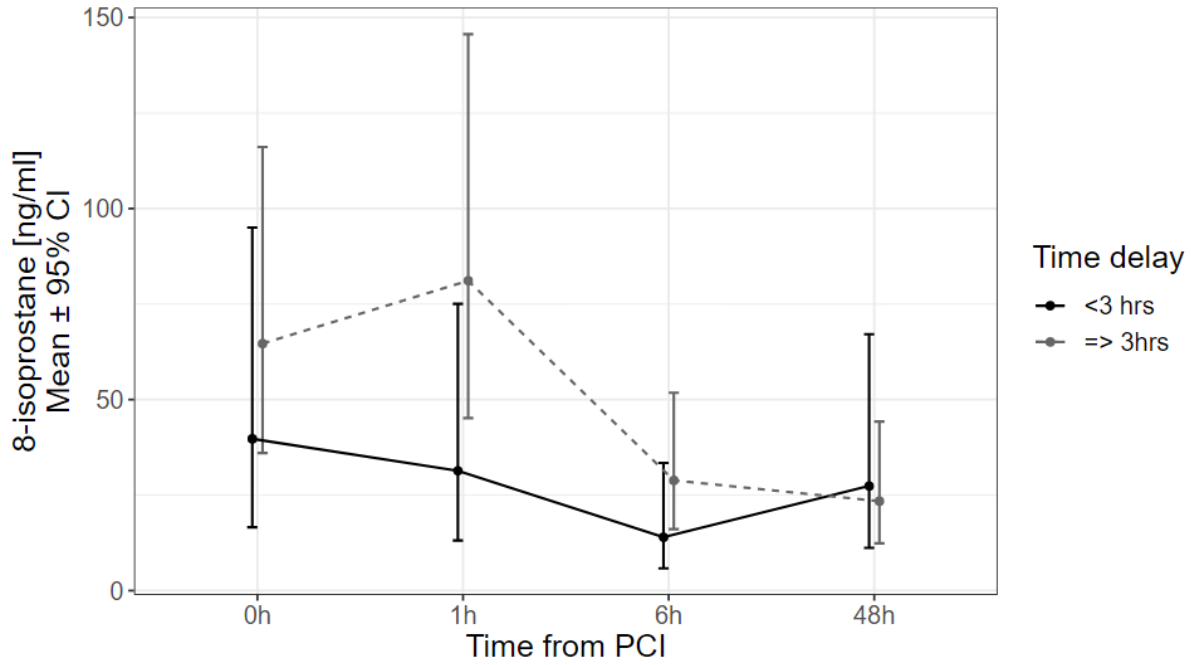


Figure S5. Periprocedural 8-ISO levels in patients with AMI (STEMI) in relation to time delay (TD) from symptom onset to PCI



Abbreviations: STEMI (AMI) - ST-elevation myocardial infarction (acute myocardial infarction); 1 VD- one vessel disease; PCI- primary percutaneous coronary intervention; TIMI- thrombolysis in myocardial infarction; TRX1- thioredoxin 1; 8-ISO- 8- isoprostane; TD – time delay from symptom onset to PCI