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Development of a Translational Autologous Microthrombi-Induced MINOCA Pig Model

Nikola Cesarovic*, Miriam Weisskopf*, Thorald Stolte, Nina Trimmel, Melanie M. Hierweger, Tobias Hoh, Jasper Iske, Conny Waschkies, Jia Lu Chen, Eva van Gelder, Andrea Leuthardt, Lukas Glaus, Yannick Rösch, Christian T. Stoeck, Petra Wolint, Dominik Obrist, Sebastian Kozerke, Volkmar Falk, Maximilian Y. Emmert

Myocardial infarction with nonobstructive coronary arteries (MINOCA) is defined as ischemic myocardial damage without flow-limiting coronary artery stenosis (>50%).[1] It is present in 15% of patients presenting with acute myocardial infarction. Causes include coronary artery dissection, spasm, or plaque disruption. Although clinical presentation is similar to classical myocardial infarction, MINOCA usually shows more heterogenous characteristics such as moderately elevated cardiac Troponin, unspecific ischemia-associated ECG-changes w/o ST-segment elevation; patchy pattern of late gadolinium enhancement in cardiac magnetic resonance imaging.[2] Its diagnosis is therefore often delayed and patient management is challenging. Therefore, we developed a novel, clinically relevant pig model of autologous coronary microthrombi (MT)-induced MINOCA to enable in-depth study of the underlying pathophysiology for the development of new diagnostic and therapeutic strategies for affected patients.

First, we determined the number of MT required to reproducibly induce MINOCA (development study). Thereafter, we validated our MINOCA-model according to the clinically relevant criteria: (1) MT morphology; (2) angiographic appearance; (3) ECG patterns; (4) biomarker profile; (5) cardiac function and size/pattern of ischemic-injury; and (6) MINOCA-induced microvascular pathology and inflammatory profile (validation study).

In 15 female pigs (82±5 kg; 4–5 months) autologous arterial thrombi were created by multiple carotid artery crushes with a surgical clamp. After 60 minutes, the formed thrombus was dissected into multiple MT and passed through various-sized (300–150 µm) filters. The created MT (<200 µm) were injected via an occlusive through lumen balloon catheter into either LAD or LCX (development study; n=6) or into LAD only (validation study; n=9). Angiography and FFR/IMR-evaluation were used to assess vessel-patency. ECG, hemodynamics and cardiac Troponin-measurements were taken for 5 hours before cardiac magnetic resonance imaging (1.5T MR-system) was performed to assess heart function and late gadolinium enhancement. Thereafter, hearts were harvested for histopathological analysis. Animal studies were approved by the Veterinary Office (License ZH213/2019).

Testing different amounts of MT (40–200) showed that a number of 200 MT is required to reliably induce MINOCA according to the predefined criteria and was therefore used in the validation study.

Corresponding to the findings in patients,[3] scanning electron-microscopy of MT revealed a heterogeneous structure with high corpuscular density-areas containing activated platelets, erythrocytes, and only occasional leukocytes adjacent to loosely interconnected fibrin-mesh and a tight fibrin-layer on surface (criterion 1).

No flow-limiting stenosis nor overt changes in thrombolysis in myocardial infarction flow grades and corrected thrombolysis in myocardial infarction frame-count were detected at 5 hours following MT-injection. Progressive QT-prolongation with ST-segment elevation was found in 6 of 9, and T-wave inversion in 4 of 9 of pigs (criterion 2 and 3; Figure [A]).

At 5 hours post MT-injection, FFR/IMR assessment (n=3) confirmed the absence of coronary artery stenosis (FFR: 0.97±0.2) and showed unaffected microvascular function (IMR: resting: 28±15; hyperemic: 9±4).

Key Words: angiography ■ biomarkers ■ catheter ■ hemodynamics ■ myocardial infarction

Correspondence to: Nikola Cesarovic, Leopold-Ruzicka-Weg 4, HCP H 12.1, 8092 Zürich, Switzerland. Email nikola.cesarovic@hest.ethz.ch
*N. Cesarovic and M. Weisskopf contributed equally.
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Nonstandard Abbreviations and Acronyms

MINOCA	myocardial infarction with nonobstructive coronary arteries
MT	microthrombi

All animals remained hemodynamically stable but displayed overt cardiac Troponin-elevation >99th percentile threshold (14 ng/L), with the maximum at 5 hours at an average of 671 ng/L (140–1423 ng/L).

As sometimes seen in MINOCA patients, heart function was normal at 5 hours (LVEF: 60±13%; LVEDV: 147±20 mL; LVESV: 55±14 mL; LVSV: 88±21 mL). Mild decrease

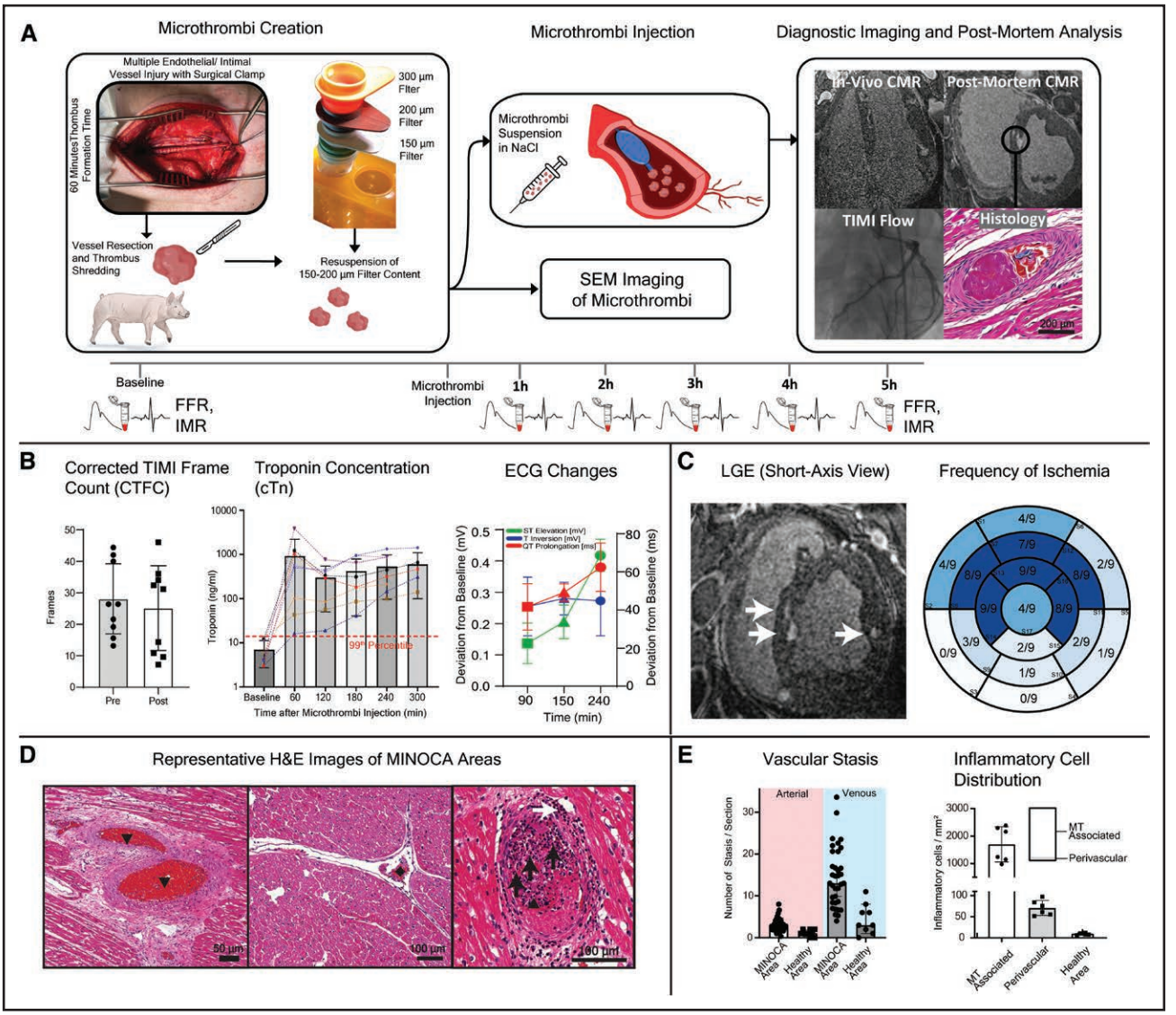

Figure. A, Myocardial infarction with nonobstructive coronary arteries (MINOCA)-induction procedure using autologous microthrombi (MT), multimodal-diagnostics including angiography, cardiac magnetic resonance imaging (CMR), biomarker-analysis, FFR/IMR, ECG, hemodynamics, and postmortem CMR-guided tissue-sampling for histopathology.
B, At 5 hours post MINOCA-induction thrombolysis in myocardial infarction (TIMI)-flow and corrected TIMI frame-count (CTFC) showed no overt changes from baseline indicating the absence of flow-limiting stenosis (>50%). Following an initial peak at 1 hour, cardiac Troponin (cTn) sharply dropped followed by continuous upward trend. ECG changed in all animals (6/9 QT-prolongation and ST-elevation; 4/9 T-inversion). **C**, Representative postmortem late gadolinium enhancement (LGE)-CMR showing patchy, ischemic MINOCA-pattern (white arrows). Number of animals presenting with an ischemic-region in the corresponding heart segment, with highest frequencies detected in mid-ventricular anterior, antero-lateral and antero-septal regions. **D** and **E**, Following CMR-guided sampling, tissue-sections was hematoxylin and eosin stain (H&E) stained and analyzed with ZEN-software (Zeiss). Exemplary H&E-images show arterial (▲) and venous (◆) microthrombi and vascular-stasis (▼) predominantly found in MINOCA-affected regions. MT were predominantly found in arterial vessels in MINOCA-affected regions, frequently causing stasis in adjacent venous vessels. In these regions, myocardial-hemorrhage was more frequent but not more severe than in healthy tissue. Inflammatory cells were mainly present within MT (black arrows) rather than perivascular-tissue (white arrows) suggesting their proinflammatory role. Frequency of congested arterial vessels and especially venous vessels per slide and density of inflammatory cells per millimeter squared was higher in MINOCA-affected areas than in healthy regions. FWT indicates fractional wall thickening.

in segmental wall thickening was observed in mid-ventricular segments (LAD-territory) when compared with adjacent segments. Analysis of in-vivo and postmortem late gadolinium enhancement by thresholding (2 SDs of healthy tissue-intensity) revealed multiple, patchy microinfarctions in the antero-septal, mid-ventricular, and apical-regions, accounting for up to 2% of LV (criterion 4 and 5; Figure [B]).

Cardiac magnetic resonance imaging-guided LV tissue-sampling and histological analysis (n=6) of ischemic-areas and healthy-areas detected a mean of 5 MT/animal (of the 200 MT injected) with an average size of 100.4±51.3 µm. They were mainly located in small arteries and arterioles, and occasionally in adjacent venules, causing arterial- and venous-stasis in the affected areas. Inflammatory cell-infiltration was detected after MINOCA-induction, which was directly associated with the MT suggesting a MT-induced inflammatory response (criterion 6; Figure [C]).

Our study has several limitations: First, our model mimics MT-induced MINOCA thus limiting its transferability for other causes of MINOCA (eg, vasospasm, dissection, or microvascular-dysfunction). Second, we focused on acute MINOCA with a short observation period, neglecting the influence of ischemia time and thrombus age, which can affect thrombus composition and potentially influence thrombolysis. Therefore, future studies should pursue long-term follow-up, but also advanced hemodynamic-measures (eg, LVEDP). Finally, although we believe that our autologous MT-induced MINOCA-model closely mimics the typical MINOCA-pathophysiology and may therefore be of greater translational relevance, a direct head-to-head comparison against strategies to induce acute microthrombosis (eg, inert microspheres or in vitro created thrombotic particles) is warranted.

Together, we present a novel autologous MT-induced MINOCA pig model mimicking the typical characteristics seen in MINOCA patients. Our new model may therefore be of high clinical relevance, and may represent a valuable tool to (1) study the pathophysiology of MT-induced MINOCA together with its underlying molecular-mechanisms; and (2) support the development of new diagnostic and therapeutic strategies for affected patients.

ARTICLE INFORMATION

Affiliations

Department of Health Sciences and Technology, Swiss Federal Institute of Technology, Zurich, Switzerland (N.C., T.S., E.v.G., P.W., V.F.). Deutsches Herzzentrum der Charite (DHZC), Department of Cardiothoracic and Vascular Surgery, Berlin, Germany (N.C., J.I., V.F., M.Y.E.). Center for Preclinical Development, University Hospital of Zurich, University of Zurich, Switzerland (M.W., N.E.T., M.M.H., A.L., C.T.S.). Institute for Biomedical Engineering, University and ETH Zurich, Switzerland (T.H., C.W., C.T.S., S.K.). Department of Mechanical and Process Engineering, University and ETH Zurich, Switzerland (J.L.C.). ARTORG Center for Biomedical Engineering Research, University of Bern, Switzerland (Y.R., D.O.). Charité-Universitätsmedizin Berlin, Germany (V.F., M.Y.E.). Deutsches Zentrum für Herz-Kreislaufforschung, Partner Site Berlin, Germany (V.F., M.Y.E.). Institute for Regenerative Medicine (IREM), University of Zurich, Switzerland (M.Y.E.).

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Disclosures

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