Study Title: ***Time for a DIagnostic paradigm shift from ST-elevation/non-ST-elevation to OCClUsion/non-occLusion myocardial infarcTion?***

Acronym: ***DIFOCCULT-3 Study***

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Abbreviations

ACO, acute coronary occlusion

AI, artificial intelligence

ECG, electrocardiogram

LVEF, left ventricular ejection fraction

MI, myocardial infarction

NSTEMI, non-ST-segment elevation myocardial infarction

PCI, percutaneous coronary intervention

STE, ST-segment elevation

STEMI, ST-segment elevation myocardial infarction

TIMI, Thrombolysis in Myocardial Infarction Study

ULN, upper limit of normal

WMSI, wall motion score index

I. BACKGROUND AND SIGNIFICANCE

The patients with acute coronary occlusion (ACO) or potentially imminent occlusion, with insufficient collateral circulation, have myocardium that is at risk of infarction unless they undergo immediate reperfusion via thrombolytics or percutaneous coronary intervention (PCI). One of the most important tasks in emergency cardiology is to immediately identify acute coronary occlusion (ACO) myocardial infarction (OMI) among all patients who present with symptoms compatible with acute myocardial infarction (MI), and distinguish them from those without MI, and from those with MI that does not have ongoing myocyte loss (Non-OMI, or NOMI) who can be managed with medical therapy and for whom potentially harmful invasive interventions can be deferred. The electrocardiogram (ECG) plays a central role in this process.

The presence or absence of ST-segment elevation (STE) is principally used to define patients who need emergent coronary revascularization, since subgroup analyses of the Fibrinolytic Therapy Trialists' (FTT) meta-analysis indicated that patients with STE on ECG gain a slightly better survival benefit from emergent reperfusion. After fine-tuning of STE cutoffs used in this analysis, universally agreed STEMI criteria became the current guideline-supported ECG paradigm.

However, the evidence accumulated in the past 20 years indicate that there is still room for substantial improvement. Although patients with ACO are the group that is believed to benefit from emergent reperfusion therapy, fibrinolytic studies did not investigate the presence or absence of ACO among enrolled patients. Moreover, they did not specifically focus on ECG findings, including STE; four of the nine trials even did not use ECG for enrollment, and the remaining five defined their version of STE with varying cutoffs, and without specified measurement methods. To reconcile different STE criteria used in various studies, several investigators compared STE in normal subjects and patients with MI. However, none of the studies cited in the current universal definition of MI used ACO on

angiography as an endpoint, so these criteria were actually not designed to differentiate STEMI from non-STEMI.

In the past 20 years, several investigators, including our group, have demonstrated that factors other than STE, including STE of magnitude less than those recommended by the guidelines (but in combination with other features), can help in diagnosing ACO or excluding it. Proportionality, which is unfortunately completely absent in the STEMI criteria, is a common factor in most of these studies: proportionality is the idea that any amount of STE or STD, or T-wave size, must be assessed relative to the QRS amplitude. Many other clues should also be taken into account when differentiating STE due to ACO from other causes of STE, which has been described in detail in recent reviews published by our group.

Studies show current STEMI criteria miss nearly one-third of ACO with the result that this unfortunate group of patients, labeled as non-STEMI, are deprived of emergent reperfusion therapy. Many studies showed that approximately one third to one-fifth of the patients with ACO had equal to or less than 1 mm of STE, hyperacute T-waves, non-contiguous STE patterns, etc. These patients are unfortunately deprived of emergent reperfusion therapy and ACO is only found after rising troponin level identifies them as having MI and they undergo a next-day angiogram. Furthermore, this proportion may be underestimated, since a large percentage of total thrombotic occlusions are spontaneously reperfused by this time; unfortunately, only after a substantial loss of myocardium. These findings are highly relevant and important, as those with unrecognized ACO had higher short and long-term risk of mortality.

It is not clear why a disease of a known pathophysiology (ACO) was named with an inaccurate surrogate ECG sign (Q-wave MI/non-Q-wave MI or STEMI/non-STEMI) instead of the pathologic substrate itself (ACO-MI/non-ACO-MI or OMI for short), but this fundamental mistake created important implications for our current practice. As briefly outlined above, ACO can be reliably recognized with the help of many other ECG findings,

such as minor STE not fulfilling STEMI criteria, STE disproportionate to preceding QRS, unusual patterns with contiguous leads showing opposite ST deviations and some patterns not showing STE at all.

Recently, the DIagnostic accuracy oF electrocardiogram for acute coronary OCClUsion resuLTing in myocardial infarction (DIFOCCULT) study, compared OMI/non-OMI approach with STEMI/non-STEMI paradigm. This is the largest study specifically designed to question the STEMI/non-STEMI paradigm, in which a set of predefined ECG findings in addition to STEMI criteria were used, and the final outcome was a composite ACO endpoint. In accordance with the previous observations, over one-fourth of the patients initially classified as having non-STEMI were re-classified by the ECG reviewers, blinded to all outcome data, as having OMI. This subgroup had a higher frequency of ACO, myocardial damage, and both in-hospital and long-term mortality compared to the non-OMI group. The OMI/non-OMI approach to the ECG had a superior diagnostic accuracy compared to the STE/non-STEMI approach in the prediction of both ACO and long-term mortality.

Similarly, another retrospective case-control study of 808 patients with suspected ACS symptoms compared the accuracy of STEMI criteria vs. structured expert ECG interpretation which incorporates other findings of OMI including hyperacute T-waves, STD of posterior OMI, STE less than the STEMI criteria cutoffs, etc. STEMI (-) OMI patients had similar infarct size measured by peak troponin but greater delays to angiography compared with the STEMI (+) OMI patients. Of the 808 patients, 49% had AMI (33% OMI; 16% NOMI). Sensitivity, specificity, and accuracy of STEMI criteria vs Expert 1 for OMI among all 808 patients were 41% vs 86%, 94% vs 91%, and 77% vs 89%, and for Expert 2 among 250 patients were 36% vs 80%, 91% vs 92%, and 76% vs 89%. OMIs were correctly diagnosed a median of 1.5 hours (mean 3.0 hours) earlier by structured expert ECG interpretation than by STEMI criteria, or by angiogram if the ECG never met STEMI criteria.

Lastly, the STEMI/NSTEMI vs. OMI/NOMI paradigm were compared in 467 consecutive high/risk acute coronary syndrome patients. Among the 108 patients with OMI, only 60% had any ECG meeting STEMI criteria. STEMI (-) OMI patients had similar peak troponins, wall motion abnormalities, left ventricular ejection fraction (LVEF) and clinical outcomes as compared with the STEMI (+) OMI patients, but were much less likely to receive emergent catheterization.

These data support the notion that the STEMI (-) but OMI (+) patients likely represent a missed opportunity under the STEMI/NSTEMI paradigm. A new OMI/NOMI approach has the potential of being the next significant improvement in modern MI care.

II. THE HYPOTHESIS

Our hypothesis is that the new OMI/NOMI approach will be superior to the established STEMI/NSTEMI paradigm in early detection of ACO, limiting infarct size, reducing rehospitalizations and most important of all, reducing mortality.

III. METHODS

1. Application for Institutional Review Board (IRB)/Ethics board approval

IRB/Ethics board approval is obtained from Marmara University Ethical Board. Each principal investigator at each individual study site will be required to obtain IRB/Ethics board approval from his/her own institution.

2. Study population

The adult patients (age >18 years) who are admitted to the emergency department with a

clinical picture compatible with acute coronary syndrome will be screened for enrollment into the study. patients with an ECG or clinical (see below) diagnosis of acute myocardial infarction will be enrolled into the study.

An ECG will be acquired as soon as possible in all screened patients and serial ECGs will be taken if the first one is not diagnostic. The ECGs will be scanned and digitized via an artificial intelligence (AI)-powered mobile phone application. If the patient gets a STEMI or OMI diagnosis by the ECG or clinical gestalt (refractory pain, hemodynamic instability, arrhythmia, cardiac arrest) they will be included in the study even if the later troponin results turn negative. If the ECG is not diagnostic for OMI or STEMI, a myocardial infarction diagnosis with a positive troponin will be necessary for the inclusion in the study. According to 4th universal definition of MI, the term acute MI will be used when there is *acute myocardial injury* (detection of a rise and/or fall of cTn values with at least one value above the 99th percentile upper range limit) with at least one of the following clinical indicators of acute myocardial ischemia:

- Symptoms of myocardial ischemia;
- New ischemic ECG changes;
- Development of pathological Q waves;
- Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality in a pattern consistent with an ischemic etiology;
- Identification of a coronary thrombus by angiography or autopsy;
- Post-mortem demonstration of acute atherothrombosis in the artery supplying the infarcted myocardium.

All non-procedure related (excluding type 4a and 5 MIs), including type 1 (MI caused by atherothrombotic coronary artery disease which is usually precipitated by atherosclerotic plaque disruption (rupture or erosion)), type 2 (evidence of an imbalance between myocardial oxygen supply and demand unrelated to acute atherothrombosis), type 3 (cardiac death in

patients with symptoms suggestive of myocardial ischemia and presumed new ischemic ECG changes before cTn values become available or abnormal) and type 4b and c (stent/scaffold thrombosis or restenosis associated with percutaneous coronary intervention) will be included in the study. Patients with *myocardial injury* (either acute, as in acute heart failure or myocarditis, or chronic, as in chronic kidney disease or stable increased troponin levels with structural heart disease) without *ischemia* (abovementioned following clinical indicators of acute myocardial ischemia) will be excluded from the study.

The inclusion and exclusion criteria are summarized below.

Inclusion criteria

Age >18 years

ECG and/or clinical diagnosis of acute myocardial infarction

Exclusion criteria

Active pregnancy or a suspicion of pregnancy

Rejection or withdrawal of consent

Failure to acquire any of the pre-participation ECGs

Non-ischemic myocardial injury

Application of thrombolytic therapy instead of primary PCI

Re-occlusion of the culprit lesion after intervention*

New vessel occlusion during hospital stay*

*Exclusion from final analyses.

Randomization

The patients will be randomized to the current STEMI/NSTEMI versus OMI/NOMI approaches using a cluster randomized trial design. Although the STEMI/NSTEMI approach

is the current norm (a diagnosis of STEMI requires emergent catheterization, whereas the patients with NSTEMI are stabilized first and then electively undergo catheterization unless there are high-risk features), it would be unethical for a ECG reviewer, who is trained in recognizing the signs of ACO not fulfilling the current STEMI criteria, to suspend emergent reperfusion therapy after an OMI diagnosis has been made. Therefore, the ECG interpreters who are trained in OMI diagnosis cannot be randomized to STEMI/NSTEMI versus OMI/NOMI approaches. Hence, we will randomize the groups in the following fashion: In each center, a STEMI/NSTEMI and an OMI/NOMI intervention group will be formed. After these two groups are formed, the patients will be block-randomized into STEMI/NSTEMI and OMI/NOMI cohorts according to the team on-duty, i.e., the approach that center will follow on a certain day will be defined by the team on duty. The interventional cardiologists in both groups will be ensured to have a similar experience level (in terms of years of training, and angiography and primary PCI counts in the past year).

All possible first responders in the network of a study center (who contact the patient first, according to the center this can be either a referring physician, an emergency physician or a cardiologist) will be provided with an AI-powered application for ECG diagnosis. These responders will receive diagnostic prompts from the application according to the center's on-duty team. If an OMI team member is on duty, the ECG interpretation will be OMI or not-OMI. If a STEMI team member is on-duty, the ECG interpretation will be disabled and will read "follow standard care". The patient will thus be elected to go for catheterization based on this approach and, whether that is by OMI or STEMI paradigm, the patient will be enrolled accordingly and the reason for proceeding to the catheterization laboratory will be written on the study form (or electronic sheet on the dedicated website).

In the STEMI/NSTEMI arm, the contributors will blindly continue their usual practice, the ECG interpretation and decision to activate the catheterization laboratory will be done as usual. The STEMI/NSTEMI group will use the following criteria for the diagnosis of

STEMI: (1) New ST-segment elevation at the J-point in two contiguous leads with the cut-point: ≥ 1 mm in all leads other than leads V2–V3 where the following cut-points apply: ≥2 mm in men ≥40 years; ≥2.5 mm in men <40 years, or ≥1.5 mm in women regardless of age, and (2) a peak troponin level above 99th percentile with a characteristic rapid rise and fall (retrospectively) and (3) a clinical picture compatible with acute coronary syndrome. If the decision to proceed to the cath lab was done only with the first criterion, the participant will remain in the study, even if the second criterion is not met. The patients meeting only criteria (2) and (3) will be classified as NSTEMI.

In the OMI/NOMI group, the diagnosis of OMI can be based on clinical gestalt, ECG findings, and adjunct modalities. Clinical gestalt includes hallmark presentations such as almost pathognomonic chest pain, and ischemic arrhythmias, hemodynamic instability, or cardiac arrest following typical symptoms. ECG diagnosis, whether interpreted by physicians or aided by an AI-powered smartphone application, incorporates static or serial changes for ACO using the DIFOCCULT-1 study algorithm (Aslanger et al. In J Cardiol Heart Vasc, 2020; Aslanger et al. J Electrocardiol, 2021; Aslanger et al. Arch Turk Soc Cardiol, 2021). On OMI/NOMI days, the smartphone application is activated and available to all first responders associated with this center. This application assists diagnosis, but the final decision is left to the interventionalist on duty. Adjunct modalities include bedside echocardiography demonstrating new or presumed new wall motion abnormalities in patients with ongoing or recurrent chest pain, and significantly elevated initial troponin levels. For high-sensitive cardiac troponin (hs-cTn) T, it has been shown that a level exceeding 1000 ng.mL^{-1} is highly specific for major epicardial coronary artery occlusion. Similarly, a hs-cTn I >200 times the upper limit of normal (e.g., Architect, Abbott Diagnostics, Illinois, USA: 5000 ng/L; ADVIA Centaur, Siemens Healthcare, Tarrytown, USA: 5000 ng/L; Access, Beckman Coulter, Brea, USA: 2400 ng/L) is defined as a marker for OMI in patients with ongoing or fluctuating chest pain. In patients diagnosed with OMI, immediate catheterization laboratory activation with

the intent to perform PCI is pursued. In NOMI patients, initial medical stabilization is prioritized, followed by elective catheterization per the NSTEMI pathway unless high-risk features are identified.

STEMI and OMI patients (will be taken as STEMI equivalents for therapeutic purposes) will be managed according to the current STEMI guidelines, whereas NSTEMI and NOMI patients are managed according to the current NSTEMI guidelines. A separate diagnostic group with 'probable OMI' and 'high-risk STEMI' is also allowed for patients who do not fulfil STEMI/OMI criteria but need urgent catheterization for other high-risk features or high clinical suspicion for having an ACO. These patients will also be managed according to the current guidelines. However, patients will be excluded from analysis if their early catheterization is based solely on social or logistical considerations, and not based on the medical need. For example, a patient would be excluded if he/she is brought to the cath lab early based on the immediate availability of cath lab or because the patient is already scheduled for elective coronary angiography. The patients who have alternative diagnoses (myocarditis, pericarditis, pulmonary embolism etc.) but were not included due to a clinical or ECG diagnosis of STEMI/NSTEMI or OMI/NOMI will be excluded from the study. Similarly, the patients without a characteristic troponin kinetics who were not included due to a clinical or ECG diagnosis of STEMI/NSTEMI or OMI/NOMI will be excluded from the study.

Endpoints

The primary composite endpoint is all-cause mortality and all-cause re-hospitalization during follow-up. The co-primary endpoint is all-cause mortality and all-cause re-hospitalization in OMI (+) STEMI (-) patient subgroups. The secondary comparisons will be done for the presence of ACO on angiogram, false positive catheterization laboratory activation rate, the infarct size as defined by 24-72 hour peak troponin, wall motion score index (WMSI), left

ventricular ejection fraction (LVEF), in-hospital CPR, intubation and mortality. These will be analyzed both with intention to treat and per protocol approaches.

To define this subgroup in the OMI/NOMI arm, all ECGs diagnosed as OMI during the study will be randomly assigned to researchers in the STEMI/NSTEMI arm after study completion. The researchers will then assess whether the ECG is compatible with STEMI or NSTEMI. Patients diagnosed as NSTEMI within the OMI group will be classified as the OMI (+) NSTEMI subgroup in the OMI/NOMI arm. In the STEMI/NSTEMI arm, patients diagnosed with NSTEMI will have their ECGs scanned and interpreted by the AI-powered application. If the ECG is interpreted as OMI, these patients will be included in the OMI (+) NSTEMI subgroup within the STEMI/NSTEMI arm. The OMI diagnosis also includes clinical variables, such as clinical gestalt and very high first troponin levels. However, clinical gestalt cannot be acted upon retrospectively (e.g., bedside echocardiography or serial ECGs). Nevertheless, if a patient is recorded with ongoing chest pain and a very high first troponin level (based on center-specific and troponin kit-specific values), this will be included in the OMI (+) NSTEMI subgroup in the STEMI/NSTEMI arm, even if the ECG is not interpreted as OMI by the AI-powered application.

The primary source of outcome data will be the Turkish national electronic database (*e-nabız*), which provides comprehensive, real-time updates on all deaths and hospitalizations nationwide. To ensure the completeness and accuracy of data, direct phone contact with participants or their families will be conducted as a secondary measure. All collected outcomes will be reviewed by an independent outcome adjudication board blinded to the study arms.

Estimated number of subjects to be submitted:

We estimated that the enrollment of 3472 participants would provide the study with a statistical power of 95% to detect a 4% absolute decrease in combined primary endpoint in OMI/NOMI

approach compared to STEMI/NSTEMI approach (from 25% to 21%) with the use of a two-sided test at the 0.05 level, with a prediction of an additional 30% OMI in NSTEMI cohort. Similarly, 2351 participants would be necessary to detect a 5% difference in area under curve (AUC, from 0.750 to 0.700) for the comparison of the predictive accuracy of two approaches for primary outcomes. For the co-primary outcome, a total of 880 OMI (+) STEMI (-) participants would provide the study with a statistical power of 95% to detect a 50% relative decrease in combined primary endpoint in OMI/NOMI approach compared to STEMI/NSTEMI approach (from 20% to 13%) with the use of a two-sided test at the 0.05 level. This corresponds to an approximate total sample size of 4000 patients, with a prediction of 1:1.5-2 STEMI/NSTEMI ratio and a frequency of 25% OMI in NSTEMI cohort. However, given the planned one-year enrollment duration and uncertainties regarding the treatment effect in OMI patients, we decided to target the enrollment of 6,000 patients for the study. This total number is also expected to provide the study with a statistical power of 95% to detect at least a 10% relative decrease in infarct size, 10% better left ventricular ejection fraction and 10% better wall-motion score index STEMI (-) OMI (+) patients who underwent early revascularization in OMI arm compared to those who underwent revascularization with standard timing in STEMI/NSTEMI approach, with a prediction of an additional 25% OMI in NSTEMI cohort.

3. Centers and Personnel

The patient enrollment will take place in the following centers:

- ***Ankara Etlik City Hospital***

  Chief investigator: Belma Kalaycı

- ***Antalya City Hospital***

  Chief investigator: Dursun Akaslan

- ***Bagcilar Training and Research Hospital***

  Chief investigator: Esra Dönmez

- ***Basaksehir Pine and Sakura City Hospital***

  Chief investigator: Emre Aslanger

- ***Dr. Siyami Ersek Thoracic and Cardiovascular Surgery Training and Research Hospital***

  Chief investigator: Can Yücel Karabay

- ***Erzurum Atatürk University, Faculty of Medicine, Research Hospital***

  Chief investigator: Oğuzhan Birdal

- ***Eskisehir Osmangazi University Hospital***

  Chief investigator: Kadir Uğur Mert

- ***Eskisehir City Hospital***

  Chief investigator: Ezgi Çamlı Babayiğit

- ***Kartal Kosuyolu Training and Research Hospital***

  Chief investigator: Sedat Kalkan

- ***Kutahya Health Sciences University***

  Chief investigator: Taner Şen

- ***Marmara University, Pendik Training and Research Hospital***

  Chief investigator: Zekeriya Doğan

- ***Mehmet Akif Ersoy Cardiovascular and Throacic Training and Research Hospital***

  Chief investigator: Ümit Bulut

- ***Mugla Sitki Kocman University***

  Chief investigator: Özcan Başaran

- ***Necmettin Erbakan Meram Faculty of Medicine Hospital***

  Chief investigator: Mehmet Akif Düzenli

- ***Sirnak State Hospital***

  Chief investigator: Barış Güven

- ***Tokat Gazi Osman Paşa University Hospital***

  Chief investigator: Çağrı Zorlu

- ***Van Training and Research Hospital***

  Chief investigator: Remzi Sarıkaya

- ***Yuzuncu Yil University Hospital***

  Chief investigator: Yüksel Kaya

4. Data Collection

From September 1, 2024, AI-powered ECG App will be distributed to the referring hospitals by the participating centers. The study will start at all participating centers on October 1, 2024. A dedicated website (difoccult.org) will be used for data entry and storage. Study data is collected and managed using REDCap (Research Electronic Data Capture) tool hosted at a dedicated server. REDCap is a secure, web-based software platform designed to support data capture for research studies, providing (1) an intuitive interface for validated data capture; (2) audit trails for tracking data manipulation and export procedures; (3) automated export procedures for seamless data downloads to common statistical packages; and (4) procedures for data integration and interoperability with external sources. The researchers were instructed that the entry of collected data into the REDCap system within three days of acquisition is mandatory. A *data monitoring board* ensures the completeness, integrity, and accuracy of the entries, providing feedback to the data entry team and requesting explanations or modifications as needed.

Baseline variables

Collected baseline variables and their definitions are listed in the REDCap printout in the supplemental file. In brief, the following baseline variables will be entered to the study spreadsheet: Center specific enrollment no., protocol no., cohort, age, gender, prior history of

MI, prior history of PCI or CABG, presence or absence of diabetes (medication use or HbA1c>6.5), dyslipidemia (LDL ≥160 mg/dL or anti-lipid medication use), hypertension (medication use or average blood pressure over 140/90 mmHg during hospital stay), smoking (current smoker or past smoker with in 1 year), chronic kidney disease (baseline creatinine level greater than the upper limit of normal), admission systolic blood pressure, admission heart rate, admission creatinine level, admission hemoglobin level, Killip class on admission, admission troponin T level (from a blood sample collected before, during or just after coronary angiography or at admission to the emergency department), 24, 48 and 72h troponin level (the maximum troponin level during hospital stay), ejection fraction, wall motion score index, time from constant pain to ECG, time from ECG to PCI (will be calculated from system recordings), infarct related artery, number of diseased vessels, the number of additional lesions treated, in-hospital mortality, in-hospital resuscitation, in-hospital intubation, long term mortality. From these parameters, baseline GRACE risk score, the change within troponin value in the first 24 hours will be calculated.

Electrocardiogram

ECGs will be acquired using standard conventions. If the first ECG is non-diagnostic, serial ECGs will be acquired every 15 minutes for an hour and the first diagnostic ECG will be used in the analyses. If all of them are non-diagnostic the physician may still use additional tools such as the clinical picture, bedside echocardiogram, troponin results to diagnose 'high-risk NSTEMI' or 'possible OMI'. All pre-intervention ECGs and at least one pre-discharge ECG will be uploaded to a central cloud database to be retrospectively reviewed by core lab investigators. The absence of a technically adequate pre-cath ECG will be a reason for the exclusion of the participant. If ECG diagnosis is not compatible with the inclusion criteria for the assigned group, this will be noted and the patient will be excluded from the per-protocol analyses. Following coding will be used for ECGs:

Type 1 EGGs: New ST-segment elevation at the J-point in two contiguous leads with the cut-point: ≥ 1 mm in all leads other than leads V2–V3 where the following cut-points apply: ≥ 2mm in men ≥ 40 years; ≥ 2.5 mm in men < 40 years, or ≥ 1.5 mm in women regardless of age.

Type 1a: The amplitude, morphology, extent of STE and the presence of additional findings (hyperacute T waves, Q-waves, terminal QRS distortion) make ECG highly obvious for MI presumably due to acute, thrombotic occlusion. These ECGs will be included in both STEMI and OMI definitions.

Type 1b: There is ST-segment elevation that meets criteria for STEMI, but it is uncertain whether it is due to MI or to another condition, such as benign variant STE, left ventricular hypertrophy, left bundle branch block, prior MI, pericarditis, etc. These ECGs will be included in the STEMI definition but not in the OMI definition, unless there are additional findings suspicious for acute coronary occlusion as follows: Differential diagnosis for benign variant STE: Type 1b, if fulfills STEMI criteria. But re-classified as Type 2b, if the Aslanger/Smith formula is positive. Aslanger's formula: (R-wave amplitude in lead V4 + QRS amplitude in V2) – (QT interval in millimeters + STE60 in V3) <12 (Aslanger E Am J Cardiol, 2018). Differential diagnosis for left ventricular hypertrophy: Type 1b unless ST segment to R-S-wave magnitude is equal or greater than 15% (then indicates OMI, Type 2b) (Armstrong EJ et al. Am J Cardiol, 2012, Aslanger et al. Arch Turk Soc Cardiol, 2021). Differential diagnosis for isolated left bundle branch block: Will be coded as Type 1b, unless one of the modified Sgarbossa criteria is positive (then indicates OMI, Type 2b): ≥ 1 lead with ≥1 mm of concordant ST elevation, ≥ 1 lead of V1-V3 with ≥ 1 mm of concordant ST depression, ≥ 1 lead anywhere with ≥ 1 mm STE and proportionally excessive discordant STE, as defined by ≥ 25% of the depth of the preceding S-wave

(Smith SW et al. Ann Emerg Med 2012). Differential diagnosis for prior MI: Type 1b, unless Smith's rule is positive (then indicates OMI, Type 2b): Smith's rule: If any 1 lead between V1-V4 has a T-wave amplitude to QRS amplitude ratio greater than or equal to 0.36 (Klein LR et al. Am J Emerg Med 2015). Differential diagnosis for pericarditis: Type 1b, unless there is ST-depression in aVL (then indicates OMI, Type 2b) (Bischof JE et al. Am J Emerg Med. 2016).

***Type 1c*:** There is ST-segment elevation that meets criteria for STEMI, but there is also T-wave inversion and pathologic Q waves indicative of subacute MI. These ECGs will be excluded from per-protocol analyses, since these patients have ACO on angiogram and higher long-term mortality but gain little, if not any, benefit from reperfusion with both approaches. Patients with preserved QRS complexes (Wellens' pattern) will be included in type 2c ECGs.

Type 2 EGGs: ECG that meets acute myocardial ischemia criteria recommended by fourth universal definition of MI.

***Type 2a*:** The ECG has "primary", i.e. cannot be completely explained as secondary to a depolarization disorder, ST-segment depression or T-wave inversion that is nondiagnostic of STEMI but is diagnostic of myocardial ischemia.

Type 2b: Does not meet recommended criteria for STEMI, but highly suggestive for ACO, despite being subtle and difficult. Possible findings are minor STE with or without minor reciprocal ST-depression not fulfilling STEMI criteria, hyperacute T-waves or DeWinter's pattern, subtle anterior STE hard to differentiate from benign variant STE and nonconsecutive STE. These ECGs will be included in the OMI definition but not in the STEMI definition. The detailed algorithm defined in the DIFOCCULT trial (Aslanger et al. In J Cardiol Heart Vasc, 2020; Aslanger et al. J Electrocardiol, 2021; Aslanger et al. Arch Turk Soc Cardiol, 2021) will be used for recognizing these ECGs.

Type 2c: Patients with preserved QRS complexes (Wellens' pattern), with or without some STE, but with significant T wave negativity will be included in type 2c ECGs. These ECGs will be excluded from per-protocol analyses, since these patients may not gain benefit from emergent reperfusion in both approaches.

Type 3 ECGs: Nonspecific ECG that is abnormal but nondiagnostic of any kind of acute coronary syndrome. Minor abnormalities including left ventricular hypertrophy without ST-T changes, arrhythmias, impulse generation and conduction diseases etc.

Type 4 ECGs: Completely normal ECG.

AI-Powered ECG Application

In OMI/NOMI arm ECGs can be digitized and interpreted by AI-powered ECG application prospectively. In STEMI/NSTEMI arm, interpretation will be done retrospectively.

All first responders will be equipped with a dedicated AI-powered smartphone application (Powerful Medical, Samorin, Slovakia), trained by expert ECG interpreters and validated in a large cohort. The application will be provided one month prior to the first patient enrollment, and each responder's log-in status was verified through an online system. First responders will be linked exclusively to a single participating center, ensuring no overlap between centers.

The application's functionality will vary based on the study arm determined by the team on duty. On OMI/NOMI days, the AI application will be fully activated and accessible to all first responders associated with that center. When a user captures a photo of an ECG, the application will digitalize the image, interpret the data, and display one of two messages: "OMI" or "Not-OMI." First responders will be instructed to promptly inform the interventionalist on

duty for potential catheterization laboratory activation if result shows "OMI". On STEMI/NSTEMI days, the AI-supported application will be deactivated for that center. If a first responder attempts to capture a photo of an ECG, a warning message will be displayed: "We are now following the standard STEMI/NSTEMI approach. Please continue your usual practice."

A commercial version of the same smartphone application by the same company is also available on the market. During the study, if a network address is detected accessing both the commercial and study-specific applications, the commercial version will be deactivated by the company, and a notification mail will be sent explaining that the commercial smartphone application will not be available to users in Türkiye for the duration of the study. Additionally, all ECGs stored in the study database will be cross-referenced with the commercial smartphone application's ECG history. If any matches are identified, the corresponding patient will be excluded from the study.

After the study completion, ECGs in both study arms will be reviewed and coded as defined above for intention-to-treat and per-protocol analyses. This will be done by two separate ECG interpreter. Should there be any discrepancy between these interpreters, a third interpreter (from data monitoring board) will be consulted.

Type 1a, 1b and 1c ECGs will be deemed as compatible with STEMI. Type 1a, 2b and 2c ECGs will be deemed compatible with OMI diagnosis.

Troponins

The troponin levels will be measured at admission, hourly if needed for the diagnosis, every 6 hours until it peaks after an MI diagnosis is made, and then daily. The 24-72 hour peak troponin level (usually 48h) will be used as a surrogate for infarct size.

Angiograms

Coronary angiography will be undertaken according to the standard conventions. Each angiogram will be reviewed by two interventionalist. Should there be any discrepancy between these interpreters, a third interpreter (from data monitoring board) will be consulted.

Following points will be noted for the presence of an ACO: (1) the Thrombolysis in Myocardial Infarction Study (TIMI) flow level in the infarct-related vessel. The presence of well-developed collaterals to the distal vessel, appearance of the total occlusion, easiness of guidewire crossing will also be assessed to determine if the total occlusion is acute in nature. If necessary, the primary operator will also be contacted. (2) The presence of an acute lesion with definitive culprit features, which was defined based on several angiographic properties including critical stenosis, irregular lesion borders, presence of angiographic thrombus or staining.

ACO Adjudication

Because the infarct-related artery may spontaneously open by the time of the angiogram or total occlusion may be chronic in nature, we defined a composite ACO using following criteria:

(1) An acute culprit lesion with TIMI 0-2 flow

PLUS

a peak troponin level equal to or greater 5 than five times the ULN

PLUS

at least 20% rise within the first 24 hours

(2) A highly elevated peak (for troponin T>1000 ng/mL and for troponin I 200 times of the average of ULN (known to be highly correlated with ACO)) without an obvious alternative diagnosis or with supporting evidence (ECG evolution, culprit-looking

lesion on angiogram in a coronary territory compatible with ECG/echocardiographic area at concern)

(3) cardiac arrest before any troponin rise has been documented with supporting clinical evidence of possible ACO.

Follow-up

The last participant in the study will be followed up to one year. The survival status and re-hospitalization will be checked from the national database and a phone call, if required.

Statistical Analysis

Baseline characteristics will be summarized using standard descriptive statistics. Comparisons of relevant parameters between groups will be performed by chi-square, Fisher's exact test, Mann-Whitney U, and student t-test, as appropriate. Patients with missing values will be excluded pairwise from analyses. A Cohen's κ test will be used for determination of the intra- and inter-observer agreement for ECG classifications and ACO adjudication.

Kaplan-Meier analysis will be performed to determine the cumulative long-term mortality rates in different ECG subgroups. The mortality across groups will be compared using a log-rank test. A Cox-regression model will be used to perform a survival analysis according to basal GRACE risk score, intervention timing and treatment status. Baseline characteristics with a P value of 0.05 or less in the univariate analysis will be included and a step-down procedure will be applied for selection of final covariates.

The sensitivity, specificity and diagnostic accuracy of STEMI/NSTEMI or OMI/NOMI ECG approaches will be calculated using receiver operating characteristics analysis. As these parameters are highly dependent on the pre-test probability of the disease and pre-test probability of ACO and long-term mortality are closely associated with the presentation type,

we will also repeat these analyses after weighing cases for the total number of hospital admissions in the study period.

To address potential variability in outcomes due to interventionist or center-related factors, we will incorporate a random effects (frailty) term into the Cox model.
The *calibration cohort* (the patients with type 1a ECGs, who are getting the same treatment in both study arms) will be used to estimate variability attributable to interventionist practices. The random effect variance (σ^2) calculated from this cohort will inform the frailty term in the full Cox model, ensuring that differences in outcomes due to interventionist-related variability are appropriately adjusted. The final model will include patient-level covariates, random effects for interventionists or centers, and calibration adjustments based on the calibration cohort.

We anticipate minimal missing data for the composite endpoint, as death and hospitalization events are covered in the electronic national database (*e-nabız*). For any missing baseline covariates, multiple imputation will be employed to avoid bias and maximize statistical power.

Other predefined secondary analyses include: (1) comparison of OMI/NOMI and STEMI/NSTEMI approaches in in-hospital mortality and infarct size by peak troponin, LVEF and WMSI, (2) a receiver operating characteristics curve analysis for OMI components for ACO; (3) time related benefit analysis in all ECG and MI subtypes; (4) the degree of the adoption of OMI approach in the early and late phase of the study.

Statistical analyses will be performed with SPSS (version 24.0; SPSS Inc., Chicago, IL) and MedCalc Software (version 18.2.1 [Evaluation version]; MedCalc Software, Ostend, Belgium).

5. Safety monitoring and reporting

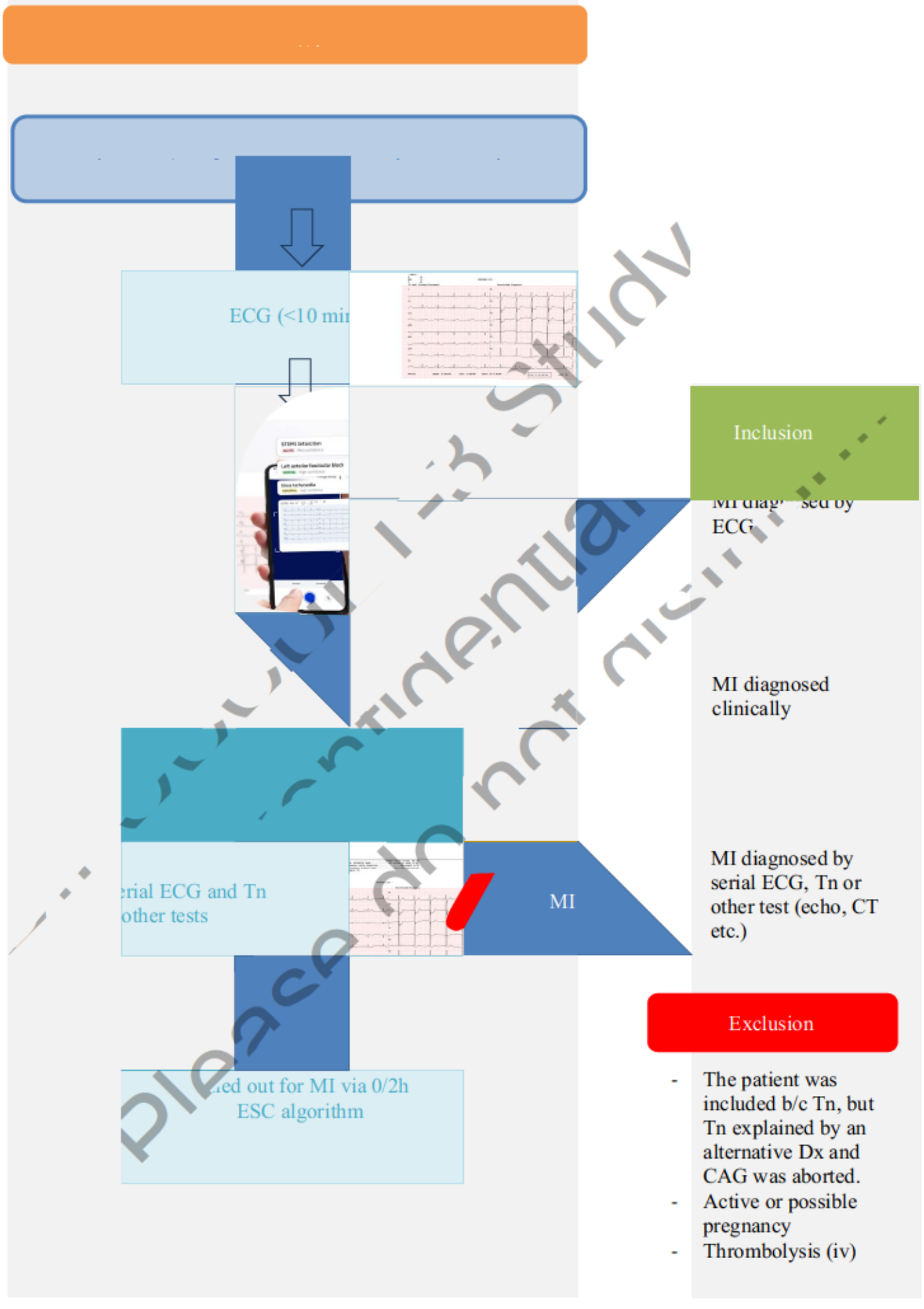
Study REDCap forms necessitate in-hospital adverse events to be actively collected to monitor and report any in-hospital adverse events. An independent Data Safety Monitoring Board (DSMB) has been established to oversee the safety and progress of the trial. The DSMB convened via teleconference during the pretrial period, upon enrollment of 25% of the participant sample size, and will continue to meet after each subsequent 25% enrollment milestone. The primary objective of the DSMB is to monitor enrollment milestones and the safety of the interventions. A three-point combined safety endpoint will be closely monitored: (1) in-hospital cardiopulmonary resuscitation, (2) in-hospital intubation, (3) in-hospital mortality. If a statistically significant increase in this three-point combined safety endpoint is observed in either of the study arms after adjusting for the baseline GRACE risk score, during the enrollment of any 25% of the participant sample size, the DSMB will make a recommendation regarding the revision, rearrangement or potential exclusion of the study participants or the study center.

6. Study integrity

The study is an investigator-initiated trial conducted under the auspices of the Turkish Society of Cardiology. The Turkish Society of Cardiology supports the investigator team in developing the trial design and organizing the participating centers. The steering committee oversees the processes of recruitment, consent and assent, follow-up, and ensures the validity and integrity of data acquisition. The trial has been approved by the Ethical Board of Marmara University (09.2021.523), any change in protocol or centers will be addressed by this board. The study will be conducted in accordance with Good Clinical Practice guidelines.

We acknowledge that the implementation of the new OMI/NOMI paradigm, even in a randomized controlled trial, may pose challenges due to conceptual, logistical, and individual

or institution-level barriers. To promote adherence, all interventionists in the OMI/NOMI arm participated in a comprehensive training program, including lectures and case simulations, focusing on the OMI/NOMI framework, subtle ECG findings, and the integration of AI-powered diagnostic tools prior to the study's commencement. Additionally, the REDCap system was designed to issue warning messages if patient management deviated from the OMI protocol. During the trial, the data monitoring board oversees adherence, conducts regular audits, and provides feedback on protocol compliance. Any deviations are meticulously documented and analyzed to identify barriers, with adaptive feedback mechanisms employed to address these issues promptly. Investigators also receive ongoing case reviews and targeted discussions to reinforce protocol fidelity. Furthermore, per-protocol and sensitivity analyses will complement the primary intention-to-treat analysis to evaluate the impact of protocol adherence on outcomes. These measures collectively ensure the robustness of the study findings while offering valuable insights into the feasibility of implementing the OMI/NOMI paradigm in real-world clinical practice.



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Informed Consent form for patient.

This Informed Consent Form is for the patients who are being invited to participate in research on ***Time for a DIagnostic paradigm shift From ST-elevation/non-ST-elevation to OCClUsion/non-occLusion myocardial infarcTion?*** (DIFOCCULT 3) Study.

This Informed Consent Form has two parts and four pages:

Information Sheet (to share information about the research with you)

Certificate of Consent (for signatures if you agree to take part)

You will be given a copy of the full Informed Consent Form

PART I: Information Sheet Introduction

I am ……., the chief researcher in the project DIFOCCULT 3 Study at ……. We are doing research on electrocardiogram in myocardial infarction. I am going to give you information and invite you to be part of this research. You do not have to decide today whether or not you will participate in the research. Before you decide, you can talk to anyone you feel comfortable with about the research.

There may be some words that you do not understand. Please ask me to stop as we go through the information and I will take time to explain. If you have questions later, you can ask them of me, the study doctor or the staff.)

Purpose of the research

We believe that current understanding of electrocardiogram in myocardial infarction is below optimal level. Today, we perform emergent coronary angiography in patients with a specific sign on electrocardiogram, called ST-segment elevation, and it is proven effective in this situation. However, many other findings have been proved to show an obstructed coronary artery. We do not know if performing this procedure as an emergency is beneficial or harmful

or neither. If we prove that a general approach to subtleties of electrocardiogram shows a better outcome in patients with myocardial infarction, current treatment standards will change in this direction.

Type of Research Intervention

This research will use your demographic data, blood tests, angiogram results and electrocardiograms stored in the hospital system and no new study will be needed.

Participant selection

We are screening all adults with acute chest pain in the emergency department to participate in the research.

Voluntary Participation

Your participation in this research is entirely voluntary. It is your choice whether to participate or not. Whether you choose to participate or not, all the services you receive at this clinic will continue and nothing will change.

Procedures and Protocol

The data recorded in the hospital system during your stay in the hospital will be used and no new test will be done. After a period your survival status will be checked from the national database.

Duration

The research takes place over 1 to 2 years.

Risks

As there is no new intervention in this study, there is no added risk during the data collection process. The healthcare workers will be looking after you and the other participants very carefully irrespective of your decision on giving consent about your data to be used in the study.

Benefits

There may not be any benefit for you, but your participation is likely to help us find the answer to the research question.

Reimbursements

Your participation is free. You will not be given any other money or gifts to take part in this research, nor will you have to pay any extra expenses

Confidentiality

The information that we collect from this research project will be kept confidential. Information about you that will be collected during the research will be put away and no one but the researchers will be able to see it. Any information about you will have a number on it instead of your name.

Sharing the Results

The knowledge that we get from doing this research will be shared with you through community meetings before it is made widely available to the public. Confidential information will not be shared.

Right to Refuse or Withdraw

You do not have to take part in this research if you do not wish to do so. It is your choice and all of your rights will still be respected.

Whom to Contact

If you have any questions you may ask them now or later, even after the study has started. If you wish to ask questions later, you may contact me or the primary research center (0212 909 60 00).

This proposal has been reviewed and approved by the local ethical committee, which is a committee whose task it is to make sure that research participants are protected from harm.

You can ask me any more questions about any part of the research study, if you wish to. Do you have any questions?

PART II: Certificate of Consent

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate as a participant in this research.

Name of Participant_____

Signature of Participant _____

Date _____ Day/month/year

If illiterate

A literate witness must sign (if possible, this person should be selected by the participant and should have no connection to the research team). Participants who are illiterate should include their thumb-print as well.

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of witness_____ AND

Signature of witness _____

Date _____ Day/month/year

Thumb print of participant

Statement by the researcher/person taking consent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands that the following will be done:

1. I will be provided with the standard therapy if I am in the control group.
2. I will be referred to an earlier angiogram if I am in the active study group and my ECG has signs of acute vessel blockage.
3. Some of my information stored in the electronic hospital database will be used. My survival or rehospitalization status will be confirmed via national electronic database or/and one phone call.

I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this ICF has been provided to the participant.

Print Name of Researcher/person taking the consent_____

Signature of Researcher /person taking the consent_____

Date _____ Day/month/year