DEPARTMENT OF CLINICAL SCIENCE AND EDUCATION
DIVISION OF CARDIOLOGY
SÖDERSJUKHUSET
Karolinska Institutet, Stockholm, Sweden

DETERMINATION
OF THE ROLE OF OXYGEN IN
ACUTE MYOCARDIAL INFARCTION

Robin Hofmann

Karolinska Institutet

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DETERMINATION OF THE ROLE OF OXYGEN IN ACUTE MYOCARDIAL INFARCTION

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Robin Hofmann

Principal Supervisor:
Nils Witt, M.D., Ph.D.
Karolinska Institutet
Department of Clinical Science
and Education, Division of Cardiology,
Södersjukhuset

Co-supervisor(s):
Leif Svensson, M.D., Ph.D.
Karolinska Institutet
Department of Medicine, Solna
Cardiology Section

Mats Frick, M.D., Ph.D.
Karolinska Institutet
Department of Clinical Science
and Education, Division of Cardiology,
Södersjukhuset

Lennart Nilsson, M.D, Ph.D.
Linköping University
Department of Medical and Health
Sciences, Linköping
Division of Cardiovascular Medicine

Opponent:
Mikael Dellborg, M.D., Ph.D.
University of Gothenburg
Department of Molecular and
Clinical Medicine
Cardiology

Examination Board:
Anna Norhammar, M.D, Ph.D.
Karolinska Institutet
Department of Medicine, Solna
Cardiology Section

Claes Held, M.D., Ph.D.
Uppsala University
Department of Medical Sciences
Cardiology

Ulf Näslund, M.D., Ph.D.
Umeå University
Department of Public Health and
Clinical Medicine
Cardiology

"Love is like oxygen:

You get too much you get too high

Not enough and you're gonna die."

The Sweet, 1978

I dedicate this thesis to my parents, Brigitte and Volker Hofmann, who on top of the unwavering support and love they gave me, taught me two essential things:

My father gave me the confidence to believe in my abilities and taught me perseverance to reach my goals.

My mother coached me to consciously amplify the positive aspects of a situation and see possibilities instead of limitations.

Both aspects have been crucial to me, not only for the completion of this thesis but in the greater perspective of my life.

ABSTRACT

Background

Oxygen therapy has been used routinely in patients with suspected acute myocardial infarction (AMI) for more than a hundred years. Even today, supplemental oxygen is widely recommended in guidelines and implemented in clinical practice, despite limited data supporting a beneficial clinical effect.

The overall objective of the present thesis was to clarify the role of routine oxygen therapy in AMI. After testing logistics, feasibility and safety in a pilot study, a nationwide registry-based randomized clinical trial (RRCT) concept was used to evaluate hard clinical endpoints. In a subgroup of patients, biomarkers were used to get insights on aspects of underlying pathophysiology.

Methods and results

Study I was a pilot study at Södersjukhuset. One hundred twenty-nine normoxemic patients with suspected AMI were randomized 1:1 to either oxygen therapy at 6 L/min delivered by open face mask for 12 hours or ambient air. A total of 81 (63%) patients were diagnosed with AMI. No unexpected logistical or notable medical problems occurred. Oxygen therapy for 12 hours was well tolerated.

Study II was a nationwide, multicenter, prospective, registry-based randomized clinical trial (RRCT) using a public quality registry for coronary care (SWEDEHEART) for trial procedures and evaluating the primary outcome – all-cause mortality at one year – through national health registries. Patients with suspected AMI and oxygen saturation of 90% or above were randomly assigned to either supplemental oxygen at 6 L/min for 6-12 hours delivered by open face mask or ambient air. A total of 6,629 patients were enrolled from April 2013 through December 2015. No patients were lost to follow-up. The primary endpoint death from any cause at 1-year occurred in 5.0% (166 of 3,311) of patients in the oxygen group compared to 5.1% (168 of 3,318) in the ambient-air group (hazard ratio 0.97; 95% confidence interval, 0.79 – 1.21; p=0.8). The results were consistent across all predefined subgroups.

Study III was a prespecified two-center substudy to study II. One hundred forty-four patients were consecutively recruited after randomization and blood samples were secured at randomization and 5-7 hours after. Ninety-two inflammatory biomarkers, using proximity extension assay technology, were analyzed to evaluate the effect of oxygen on the systemic inflammatory response to AMI. The inflammatory response did not differ between the two treatment groups, neither did plasma troponin T levels. After adjustment for increase in

troponin T over time, age, and sex, the release of inflammation-related biomarkers was still similar in the groups.

Summary and conclusions

In summary, study I found the design of the DETO$_2$X-AMI-trial to be robust and feasible. Implemented inclusion criteria identified patients with acute cardiac disease with a high proportion of acute myocardial infarctions among the study population.

Study II demonstrated that the routine use of supplemental oxygen in patients with suspected AMI without hypoxemia at presentation did not reduce 1-year all-cause mortality. Neither did it affect the incidence of rehospitalization with myocardial infarction or the size of myocardial injury as assessed by highly sensitive cardiac troponin T.

Study III showed that the use of supplemental oxygen did not have any impact on the early release of systemic inflammatory markers.

In conclusion, we were able to build up a study system and nationwide network based on the SWEDEHEART registry. Thereby, we managed to recruit many eligible patients within a short time frame, delivering high quality data at relatively low cost. Normoxemic patients with suspected AMI did not benefit from routine oxygen therapy when assessing 1-year all-cause mortality. Although our findings do not support the general use of oxygen in the normoxemic patient with suspected AMI, there nevertheless remains the risk to develop hypoxemia which must be detected and treated immediately. Furthermore, even though we could not demonstrate deleterious effects of routine oxygen treatment, there might be a dose-dependent relationship and inadvertent hyperoxemia should be avoided.

Key words

Myocardial infarction, oxygen, hypoxemia, hyperoxemia, RRCT, inflammation, ROS.

LIST OF SCIENTIFIC PAPERS

The present thesis is based on the following studies, henceforth referred to by their Roman numerals.

I. Hofmann R, James SK, Svensson L, Witt N, Frick M, Lindahl B, Östlund O, Ekelund U, Erlinge D, Herlitz J, Jernberg T.

DETermination of the role of OXygen in suspected Acute Myocardial Infarction trial

Am Heart J 2014; 167:322-8.

II. Hofmann R, James SK, Jernberg J, Lindahl B, Erlinge D, Witt N, Arefalk G, Frick M, Alfredsson J, Nilsson L, Ravn-Fischer A, Omerovic E, Kellerth T, Sparv D, Ekelund U, Linder R, Ekström M, Lauermann J, Haaga U, Pernow J, Östlund O, Herlitz J, Svensson L, for the DETO2X–SWEDEHEART Investigators.

Oxygen Therapy in Suspected Acute Myocardial Infarction

The New England journal of medicine. 2017;377:1240-1249.

III. Hofmann R, Tornvall P, Witt N, Alfredsson J, Svensson L, Jonasson L, Nilsson L.

Supplemental oxygen therapy does not affect the systemic inflammatory response to acute myocardial infarction

Submitted.

CONTENTS

LIST OF ABBREVEATIONS

ACS	Acute coronary syndrome
AMI	Acute myocardial infarction
CABG	Coronary artery bypass grafting
CAD	Coronary artery disease
CMR	Cardiac magnetic resonance imaging
CRF	Case report form
DETO$_2$X	**DET**ermination of the role of **OX**ygen in suspected **A**cute **M**yocardial **I**nfarction
ED	Emergency department
EMS	Emergency Medical Service
IL	Interleukin
I-RI	Ischemia-reperfusion injury
MACE	Major adverse cardiac events
MI	Myocardial infarction
NSTEMI	Non-ST-elevation myocardial infarction
O$_2$	Oxygen
PCI	Percutaneous coronary intervention
PEA	Proximity extension assay
PROBE	Prospective randomized open blinded endpoint assessment
RCT	Randomized controlled trial
RIKS-HIA	National registry of acute cardiac care
ROS	Reactive oxygen species
RRCT	Registry-based Randomized Clinical Trial
SCAAR	Swedish coronary angiography and angioplasty registry
SEPHIA	National registry of secondary prevention
STEMI	ST-elevation myocardial infarction
SWEDEHEART	Swedish Web-system for Enhancement and Development of Evidence-based care in Heart disease Evaluated According to Recommended Therapies
UCR	Uppsala Clinical Research Center

1 RESEARCH QUESTION AND RATIONALE

Coronary artery disease (CAD) with its feared primary presentation as acute myocardial infarction (AMI) remains the prominent cause of morbidity and mortality in the world.[1] AMI is caused by acute coronary syndrome (ACS) which describes plaque rupture and subsequent coronary thrombosis leading to either subtotal coronary occlusion and Non-ST-Elevation Myocardial Infarction (NSTEMI) or acute total occlusion of the vessel and ST-Elevation Myocardial Infarction (STEMI). Modern medical reperfusion strategies and acute percutaneous coronary intervention (PCI) with stent implantation have improved prognosis immensely.[2-5]

Supplemental oxygen has been administered in the setting of suspected AMI in pre-hospital and hospital clinical routine across the world for over a century[6] and is still manifested today in international treatment guidelines.[7,8] This recommendation is based on the belief that reduced coronary blood flow leads to diminished delivery of oxygen to the threatened myocardium and, therefore, an imbalance between myocardial oxygen demand and supply. The administration of supplemental oxygen is intended to optimize oxygen delivery to the ischemic heart muscle with the goal of reducing infarct size[9,10] as well as potential complications such as heart failure and malignant arrhythmias.

However, this common practice has been challenged lately which brings into question the scientific evidence for such a ubiquitous therapy.[11] Considerable data suggest that oxygen therapy may lead to negative cardiovascular hemodynamics and possibly detrimental effects concerning myocardial injury and, ultimately, survival.

In summary, the existing scientific evidence is inconsistent and fails to resolve the role of oxygen therapy in patients with acute ischemic heart disease. The need to clarify this important issue is urgent.

With the DETermination of the role of OXygen in suspected Acute Myocardial Infarction (DETO$_2$X) trial series presented in this thesis, we aimed to add substantial knowledge to determine the role of routine oxygen therapy in AMI. By building up a nationwide trial alliance for a large randomized clinical trial we could evaluate hard clinical endpoints. Underlying pathophysiology was assessed in a biomarker analysis in a subgroup of patients.

2 INTRODUCTION

2.1 CORONARY ARTERY DISEASE AND ACUTE MYOCARDIAL INFARCTION

2.1.1 Pathogenesis and clinical classification

The current understanding of the pathogenesis of CAD and AMI was first established in 1970.[12] It was shown that the formation of blood clots in a coronary artery was caused by the exposure of prothrombotic material through the acute rupture or erosion of a focal calcification or plaque. The subsequent narrowing of the vessel in combination with distal embolization of thrombotic material leads to partial or total obstruction of blood flow to the heart muscle and, ultimately, myocardial infarction.

Hemodynamically, the flow-limiting narrowing of the vessel diameter causes clinical symptoms of angina based on a mismatch of myocardial perfusion and metabolic demand. The degree and duration of ischemia and subsequently the level of myocardial necrosis, is determined by the severity of pre-existing stenosis, extent and persistence of the thrombus, degree of concomitant vasoconstriction, presence of collaterals, and the myocardial perfusion demand.[13,14]

The clinical picture is commonly graded as follows:

1. Stable angina[15]
   Reversible angina symptoms caused by exertion – No myocardial damage

Acute coronary syndrome encompassing:

2. Unstable angina[7]
   New onset or sudden worsening of previously stable symptoms that arise more often with less exertion or even at rest due to temporary thrombi with partial/intermittent occlusion. No myocardial damage.
3. Non-ST-elevation myocardial infarction (NSTEMI)[7]
   Permanent thrombi leading to partial/intermittent occlusion and myocardial damage
4. ST-elevation myocardial infarction (STEMI)[8]
   Permanent thrombi leading to total occlusion of a coronary artery and myocardial damage.

The Universal definition of myocardial infarction classifies myocardial necrosis according to a clinical setting consistent with acute myocardial ischemia.[16] An increase and/or decrease of a cardiac biomarker in combination with typical symptoms of ischemia, or new significant ECG changes, or imaging evidence of myocardial damage, or intracoronary thrombus on angiography or autopsy is required to confirm diagnosis.

Since the early 1990s, this complex clinical picture is known as acute coronary syndrome.[17,18]

According to the pathogenesis, AMIs are thereafter typed into spontaneous AMI due to ACS (type 1), AMI secondary to ischemic imbalance (type 2), AMI resulting in death when biomarkers are not available (type 3), AMI related to PCI (type 4), and AMI related to coronary artery bypass grafting (CABG) (type 5).

2.1.2 The systemic inflammatory response to acute myocardial infarction

Today, it is recognized that atherosclerosis is caused by a chronic inflammation in the vessel wall over a lifetime[19] in response to biological effects of risk factors.[20,21]

The inflammatory response to myocardial injury induced by AMI and the kinetics of associated biomarkers such as interleukin (IL-) 6,[22] IL-8,[23] C-reactive protein and fibrinogen are well known.[24] Recently, underlying inflammatory pathways,[25,26] interleukins associated with prognosis,[27] as well as interleukins and their receptors as therapeutic targets have been explored in greater depth.[28] However, the utility of inflammatory biomarkers in clinical practice remains largely uncertain.

2.1.3 Current treatment strategies in ACS

Modern evidence-based ACS management consists of early reperfusion therapy and revascularization, medical strategies with a combination of multiple drugs targeting platelet function and modifiable risk factors, and in certain cases with severe left ventricular dysfunction, device therapy such as cardiac resynchronization and implantable cardioverter defibrillators.[7,8]

However, strategies for supportive care to relieve pain, breathlessness and anxiety are to date still based on expert opinion only.[8]

2.1.4 Prevalence and prognosis

In Sweden during 2014, 27,000 individuals developed an AMI, of whom 22,500 were hospitalized. Thirty-day mortality after MI was 26% for all patients and 11% for those hospitalized. The number with MI as an underlying or contributory cause of death was 7,000 individuals[29]. The number of patients initially treated for symptoms suggestive of AMI is unknown, but chest pain remains the most common reason for visits to the emergency department, amounting to about 20% of all visits.[30]

Although cardiovascular mortality has declined substantially in recent decades,[31] coronary artery disease remains the leading cause of death both in Sweden[32] and worldwide.[1]

2.2 OXYGEN THERAPY IN SUSPECTED ACUTE MYOCARDIAL INFARCTION

2.2.1 History and rationale

Oxygen as a medicinal substance was discovered independently by Scheele and Priestley 1771-1775 and was henceforth the agent ubiquitous in modern medicine.[33] Commercial oxygen became available surprisingly quickly and was used for all kinds of dubious purposes. A more scientific use of the drug became more common in the late eighteenth century with case reports on patients with pneumonia and tuberculosis. Steele was the first to describe the use of oxygen to relieve angina pectoris in 1900.[6]

The rationale behind the widespread use of oxygen in patients with suspected AMI is based on the belief that inhaled oxygen therapy improves oxygen delivery to the diseased heart muscle, leading to reduced myocardial injury which thereby diminishes the risk for complications such as heart failure and arrhythmias.

2.2.2 Physiological background and definitions

Human life and most physiological processes depend on a continuous supply of oxygen to sustain cell function.[34,35] Hypoxemia – low oxygen content caused by reduced oxygen delivery and/or failure of cellular use of oxygen – leads to organ dysfunction, cell and tissue damage, and ultimately death. To prevent these deleterious effects of hypoxemia is an essential aim of medical therapy.

In contrast to hypoxemia, which is a common situation in disease or even physiologically at high altitudes, hyperoxemia - above-normal oxygen levels in the blood - is a man-made phenomenon which humans by nature are not equipped to handle. Hence, the body only detects unnatural high levels of oxygen by indirect factors in response to the stress of high oxygen tension.[36] Clinically, the risk of inadvertent hyperoxemia is high because the standard monitoring is done non-invasively by pulse oximetry to avoid hypoxemia but does not allow assessment of above-normal levels. Hyperoxemia is feared to cause detrimental cardiocirculatory and metabolic effects (described in detail in 2.4).

In the human body, oxygen is mainly bound to hemoglobin, a protein in the red blood cells. The relationship between the partial pressure of oxygen and the saturation of hemoglobin in the bloodstream is shown in the oxygen-hemoglobin dissociation curve. At levels above 6 kPa, the standard dissociation curve is relatively flat, and the total oxygen content of the red blood cells does not change significantly despite large amounts of oxygen given (figure 1).[37]

Figure 1: Oxygen-hemoglobin dissociation curve adapted with $DETO_2X$ inclusion level of ≥90% of blood oxygen saturation
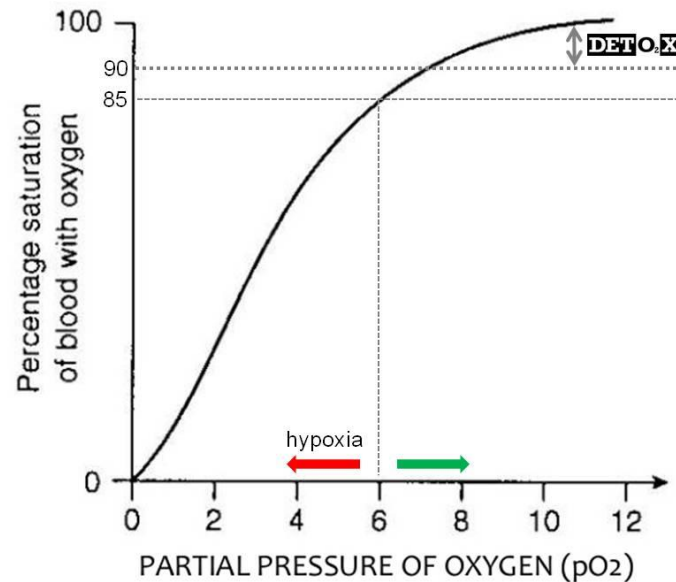
Table 1: Definitions

Term	Definition
SaO_2	Oxygen content in an arterial blood gas
SpO_2	Peripheral oxygen saturation (measured by pulse oximetry). An estimate of arterial oxygen saturation that refers to the amount of oxygenated hemoglobin in the blood
PaO_2	Partial pressure of oxygen
FiO_2	Fraction of inspired oxygen, meaning the amount of oxygen a patient breathes in (21% = room air, 30-40% = 6 L/min by open face mask)
Hypoxemia	Lack of oxygen at the level of the organ, tissue, or compartment. Refers to levels <85-90% or PaO_2 <60 mmHg
Hyperoxia	High oxygen content (either excess O_2 or higher than normal physiological pO_2)
Hyperoxemia	Supranormal oxygen tension in the blood
ROS	Reactive oxygen species; chemically reactive molecules containing oxygen, such as superoxide, peroxide, hydroxyl and peroxyl radicals which are generated by enzymatic and non-enzymatic catalysis

Modified with permission from Sepehrvand and Ezekowitz[38]

2.2.3 Experimental data supporting the use of oxygen in AMI

In two studies on anesthetized dogs, inhalation of 40-100% oxygen after coronary artery occlusion reduced myocardial infarct size and improved left ventricular ejection fraction as compared to room air.[39,40] In a small study in humans, 17 patients with anterior AMI received 100% oxygen and a reduction in ST-segment elevation was seen on precordial ECG-mapping.[41]

Inspired by the hypothesis that a higher arterial oxygen tension reduces myocardial injury, other modalities have been evaluated. In a multicenter trial, 112 patients with STEMI were randomized to either hyperbaric oxygen or routine oxygen therapy during thrombolysis for anterior STEMI.[42] No significant improvement was found with this technique. Another randomized multicenter trial used an even more advanced approach. A total of 269 STEMI patients were randomly assigned to either intracoronary hyperoxemic reperfusion after PCI of the diseased vessel or normoxemic reperfusion. At 30 days, there was no significant difference between the groups concerning infarct size, ST-segment resolution or wall motion score index. Later, a post-hoc analysis on a subgroup of patients (N=98) with anterior infarctions and early reperfusion proposed a benefit of hyperoxemic reperfusion on cardiac function.[43] Based on the latter findings, a follow-up RCT directed at this group of patients was performed in 269 patients. Here, the investigators reported a significant reduction in infarct size with non-inferior rates of major adverse cardiovascular events at 30 days.[44]

However, a meta-analysis performed in 2009 that pooled all available data on hyperoxic myocardial reperfusion therapy could not confirm positive findings.[45] On the contrary it showed a significant decrease in coronary blood flow, an increase in coronary vascular resistance, and a significant reduction in myocardial oxygen consumption, suggesting possible harmful effects of hyperoxemic oxygen therapy.

Using an updated device, a new RRCT is currently enrolling patients to confirm the safety and effectiveness of hyperoxemic reperfusion therapy (Evaluation of Intracoronary Hyperoxemic Oxygen Therapy in Anterior Acute Myocardial Infarction Patients, NCT02603835).

2.2.4 Experimental data against the use of oxygen in AMI

Already as early as 1950 Russek and colleagues warned that the indiscriminate use of oxygen in normooxic ACS patients might be harmful.[46] Even more so today, concerns have been raised that the omnipresent, routine use of oxygen might be based more on tradition than solid scientific evidence.[11,47-50] Proclaimed harmful effects are mainly based on two phenomena:

1) hyperoxemia-induced vasoconstriction in the cardiac vasculature;[51] and

2) increased endothelial production of reactive oxygen species (ROS).[38,52,53]

Hyperoxemia-induced vasoconstriction deteriorates cardio-circulatory parameters such as coronary and systemic oxygen delivery,[54] left ventricular perfusion,[54] coronary blood flow,[55-58] it leads to increase in coronary vascular resistance[55,56,59-61] and ultimately to reduced cardiac output.[54,57,59,60,62] Several underlying mechanisms have been proposed: closure of K^+_{ATP} channels,[63] activation of the angiotensin I - angiotensin II - endothelin 1 axis,[64] direct effects on L-type Ca^{2+} channels,[65] and increased production of 20-HETE,[66] a powerful vasoconstrictor.

Furthermore, the generation of ROS leading to a reduced bioavailability of nitric oxide seems to be of great importance in the pathophysiology of vasoconstriction.[53,61,67] High levels of ROS overpower the body's antioxidant capacity, resulting in a cascade of adverse reactions such as: increased oxidative stress, activation of apoptosis, creation of a prothrombotic environment, intracellular calcium overload and aggravated inflammatory response, all of which contributes to tissue damage, which determines the final infarct size.[68,69]

Some experimental studies propose increased systemic inflammation to be the leading mechanism behind hyperoxemia-related observations[70-72] whereas other studies could not support these findings.[73,74] To the best of our knowledge, the effect of hyperoxemia on the inflammatory response to AMI has not been previously studied in humans.

Independently, ROS activation can directly induce electro-physiological changes which can heighten the risk of malignant arrhythmias.[75] Clinically, vasoconstriction mediated by oxygen therapy may result in underestimation of vessel size during PCI, potentially increasing the risk of subsequent stent thrombosis, a powerful predictor of adverse events.[76,77]

Figure 2: Schematic illustration of proposed hyperoxemia-induced adverse effects in AMI
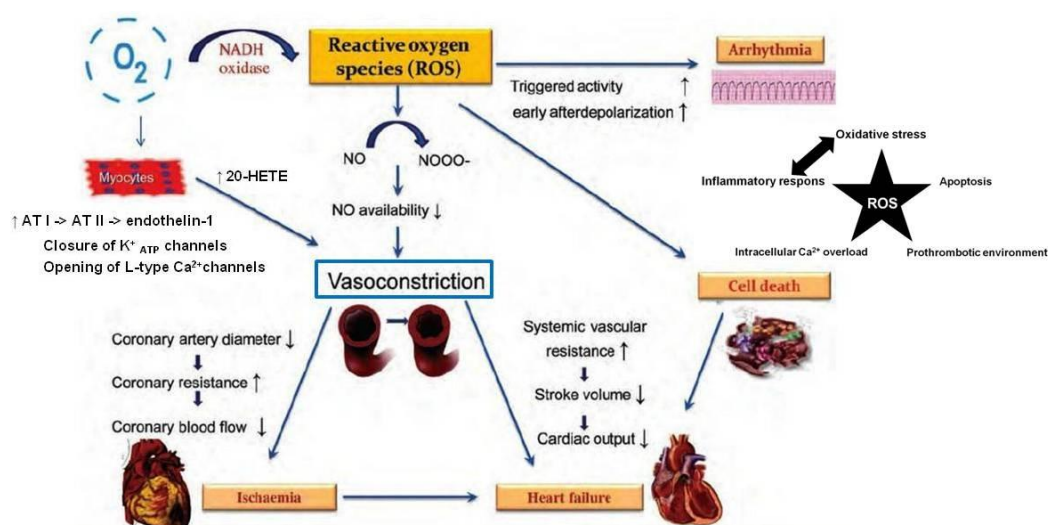
Modified with permission from Shuvy et. al.[11]

2.2.5 Clinical trial data

An updated 2016 report from the COCHRANE library reviewed the data available from randomized controlled trials (RCT) on oxygen therapy for AMI.[47] Five studies were included: Rawles and Kenmure from 1976,[78] Wilson and Channer from 1995,[79] Ukholkina et. al. from 2005,[80] Ranchord from 2012[81] and Stub from 2015[82] adding up to a total of 1,173 patients in the meta-analysis. No significant difference was found between supplemental oxygen and ambient air concerning all-cause death or pain relief.

In summary, the authors conclude: *"There is no evidence from randomized controlled trials to support the routine use of inhaled oxygen in people with AMI, and we cannot rule out a harmful effect. Given the uncertainty surrounding the effect of oxygen therapy on all-cause mortality and on other outcomes critical for clinical decision, well-conducted, high quality randomized controlled trials are urgently required to inform guidelines in order to give definitive recommendations about the routine use of oxygen in AMI"* (cited with permission from the authors).

Scrutinizing the most recent studies in greater detail, the Australian Air Versus Oxygen in myocarDial infarction (AVOID) trial included 441 STEMI patients who were randomized to receive oxygen (8 L/min) or no oxygen and followed for 6 months. Mean peak creatine kinase was elevated in the patient on oxygen, whereas no differences in mean peak troponin I were detected. Follow-up data indicated an increased risk of recurrent myocardial infarction and a larger infarct size on cardiac magnetic resonance imaging (CMR) at 6 months in the oxygen treated group.[82,83] In a post hoc analysis, high oxygen exposure was associated with clinically significant increase in CK and troponin I.[84] However, the validity of these conclusions has been questioned.[85] The Swedish Supplemental Oxygen in Catheterized Coronary Emergency Reperfusion (SOCCER) trial included 95 STEMI patients randomized to oxygen (10 L/min) or no oxygen and assessed myocardial damage by modern CMR parameters. No significant differences in myocardial salvage index, myocardium at risk or infarct size was found.[86,87]

Both trials were underpowered to assess effects on cardiovascular morbidity and mortality, so the conclusion from the Cochrane report persisted: A definite RCT was still urgently required.

3 AIMS

The overall aim of the $DETO_2X$ trial series was to illuminate the role of routine oxygen therapy in AMI patients by building a trial concept and nationwide alliance to evaluate hard clinical endpoints in a large, randomized, registry-based clinical trial and assess possible underlying pathophysiological mechanisms in a biomarker substudy.

3.1 SPECIFIC AIMS

- To develop a study concept that enables a nationwide clinical trial using established AMI treatment routines without influences from the medical industry; to implement a study organization and logistics to perform the trial; to test the concept concerning logistics, safety and feasibility in a three months' pilot study at Södersjukhuset.

- To study the long-term effect of oxygen therapy on mortality in patients with suspected AMI in a prospective, registry-based randomized clinical trial (RRCT) across Sweden based on the SWEDEHEART registry.

- To assess, in a substudy from the $DETO_2X$ trial, if supplemental oxygen given in the clinical ACS setting increases the release of inflammation-related biomarkers.

4 METHODS

4.1 ETHICAL CONSIDERATIONS AND RISK-BENEFIT ANALYSIS

The studies were conducted in accordance with the Declaration of Helsinki[88] and Good Clinical Practice Guidelines[89] in the latest version. Approval was given by the ethics committee as well as from the Swedish Medical Products Agency.

Apart from the assignment to the study group, patients enrolled in the DETO$_2$X-AMI study followed routine AMI treatment. Oxygen therapy at a flow rate of 6 L/min for 6-12 hours given by open face mask is commonly used in clinical practice and is considered safe and well-tolerated by patients. The study protocol ensured that if hypoxemia developed in either group, supplemental oxygen was provided immediately.

The possible benefit of the trial was to provide new evidence of the effects of oxygen therapy on cardiovascular morbidity and mortality in patients with suspected AMI. If these patients would either be put at risk of harm or not be found to gain from this therapy, it should consequently be removed from the treatment guidelines.

Overall, we considered the risk of participation in the present trial to be very low and greatly exceeded by possible benefits of gaining new knowledge.

4.2 PATIENTS

The DETO$_2$X trial series was constituted of 129 patients in the pilot study (study I) recruited at Södersjukhuset, Stockholm; 6,629 patients in the main national multicenter trial (study II) enrolled at 35 sites across Sweden, and 144 patients in the biomarker substudy (study III) recruited at Södersjukhuset, Stockholm and at Linköping University hospital, Linköping.

4.3   PILOT STUDY (STUDY I)

4.3.1   Trial organization, logistics, communication and education

From the start the DETO$_2$X-AMI trial was planned as a two-center RCT at Södersjukhuset in Stockholm and at Sahlgrenska University Hospital in Gothenburg. When the study board realized that the necessary sample size could not be achieved this way, SWEDEHEART (Swedish Web-system for Enhancement and Development of Evidence-based care in Heart disease Evaluated According to Recommended Therapies), a national quality of care registry for patients with ischemic heart disease, was approached for cooperation, suggesting a nationwide RRCT design (details below). SWEDEHEARTs leadership was interested but wished for a pilot study to establish a model trial organization and test logistics and communication between participating units. Södersjukhuset in Stockholm was chosen as the primary site.

In patients with suspected AMI, oxygen therapy is usually initiated at first medical contact when the patient is treated by ambulance staff or at the emergency department (ED). According to established clinical practice, these units immediately contact the coronary care unit (CCU) to discuss treatment options such as acute coronary angiography or medication. To optimize the cooperation and logistics between these units – usually belonging to different departments or even care providers – was of utmost importance for the success of the trial. Therefore, the local study organization was constituted of a local primary investigator (PI) who led a group of designated representatives from the ambulance service, ED, cath lab and the CCU. Several "DETO$_2$X ambassadors" promoted trial procedures under their guidance.

Figure 3: Schematic illustration of model trial organization
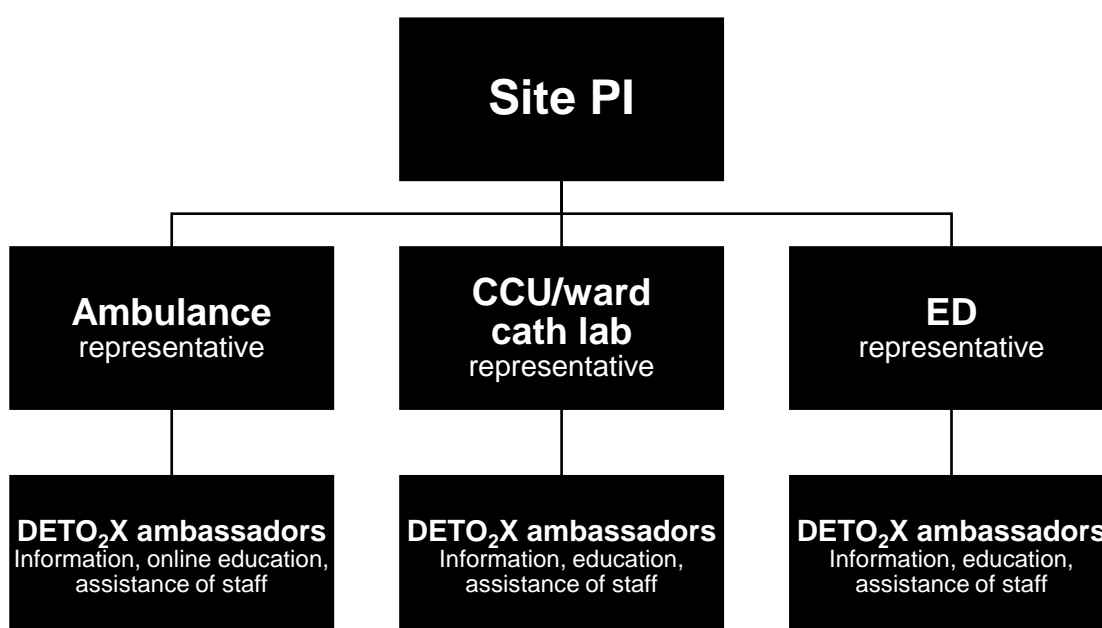
To simplify logistics, communication and education, a webpage (www.deto2x.se) was launched to spread information, updates and news, supply forms and education material, and to enable networking and facilitate cooperation.

Figure 4: Schematic illustration of logistics, communications and education
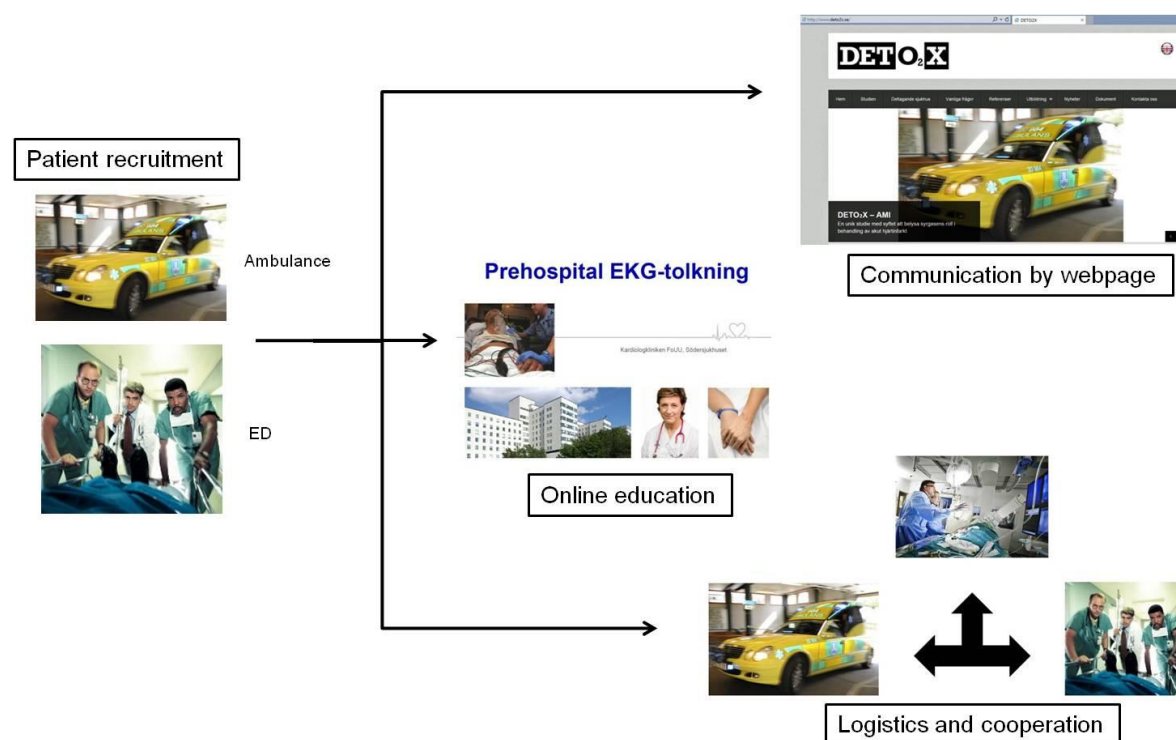
4.3.2 Patient recruitment and flow chart

Eligible patients presenting with symptoms indicative of ACS (chest pain and/or typical dyspnea), $SpO_2 \geq 90\%$, age ≥ 30 years, ECG changes indicating ischemia (ambulance/ED) and/or elevated cardiac troponin levels (ED) were asked to participate in the trial by ambulance staff or ED personnel. Patients were excluded if they were not willing to participate, were unable to provide informed consent, had on-going oxygen therapy or had experienced cardiac arrest before inclusion. After giving oral consent, patients were randomized assisted by CCU staff to receive either 6 L/min of oxygen by open face mask for 12 hours or ambient air. Other therapies were left to the discretion of the treating physician. Oxygen saturation was documented at the beginning and at the end of the study period. If hypoxemia developed, patients could receive supplemental oxygen outside the trial protocol which was reported separately. To minimize unintentional crossover, stickers and patient bracelets were used that indicated the randomized group. Patients received comprehensive written study information directly after being admitted to a ward and were requested to confirm informed consent by signature. Data regarding baseline characteristics, presentation, in-hospital course and treatments were obtained from the SWEDEHEART registry.

Figure 5: Flow chart study I
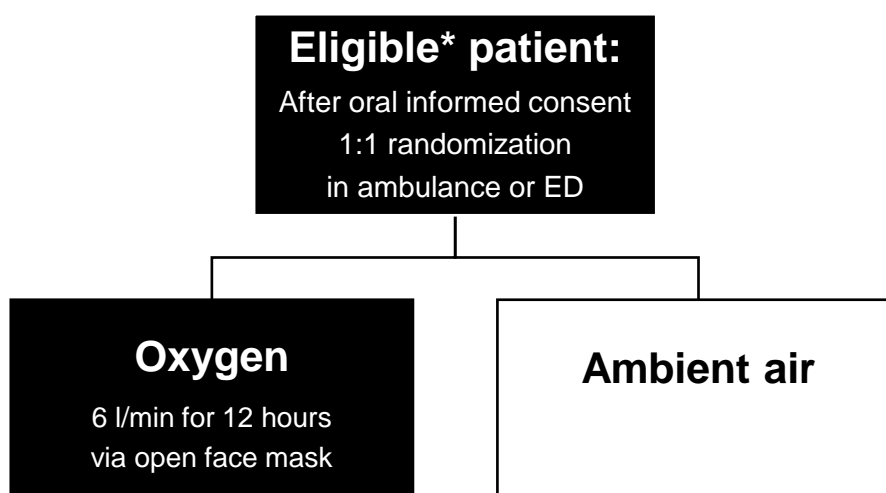
* Inclusion criteria: symptoms suggestive of AMI within 6 hours, SpO2 $\geq$ 90%, $\geq$ 30 years, ECG changes indicating ischemia and/or elevated troponin levels.

* Exclusion criteria: unwillingness to participate, inability to provide informed consent, continuous oxygen treatment or cardiac arrest prior to enrollment.

4.4   REGISTRY-BASED RANDOMIZED CLINICAL TRIAL (STUDY II)

4.4.1   SWEDEHEART and the RRCT trial concept

After successfully completing the pilot study, the DETO$_2$X-AMI trial was launched as a multicenter, prospective, registry-based, randomized clinical trial (RRCT). This trial concept was established in the Thrombus Aspiration in ST-Elevation myocardial infarction in Scandinavia (TASTE) trial.[90,91] This trial design utilizes the setting in Sweden where most patients with coronary artery disease are recorded in the SWEDEHEART registry, a national quality of care registry which includes RIKS-HIA (national registry of acute coronary care), SCAAR (national registry of angiography and angioplasty), the Swedish heart surgery registry and SEPHIA (national registry of secondary prevention).[92]

The registry is web-based with all data documented online directly by the user. The platform is linked to the Swedish National Population Registry for direct access to personal data and vital statistics.[93] For hospitalized patients with symptoms suggestive of ACS, data are gathered prospectively for 106 variables and include: patient demographics, admission logistics, risk factors, past medical history, medical treatment prior to admission, electrocardiographic changes, biochemical markers, other clinical features and investigations, medical treatment in hospital, interventions, hospital outcome, discharge diagnoses and discharge medications.

SWEDEHEART provides manuals, education and technical advice, including a telephone help desk for all users. To ensure the correctness of the data, designated monitors visit the hospitals regularly. The agreement between key variables in the registry and medical records has repeatedly been 95-96%.[92]

In the DETO$_2$X-AMI trial, randomization was performed by means of an online randomization module imbedded in SWEDEHEART. Inclusion and exclusion criteria needed to be confirmed before the patient was assigned to one of the treatment arms. At the same time, enrolled patients were automatically recorded in the SWEDEHEART registry, enabling direct access to relevant clinical and outcome data. No further documentation was needed.

Mortality data after discharge was obtained by merging with the Swedish population registry, which includes information on the vital statistics of all Swedish citizens. Due to the unique personal identification number of all Swedish citizens, virtually complete follow-up was achieved as deficiencies in reporting deaths and emigration is assumed to be less than 0.5 percent.[93]

4.4.2 National trial organization

To coordinate participating sites and optimize cooperation and logistics, a stringent national trial organization was implemented. The executive group met regularly for trial management and carried out all decisions and trial procedures with assistance of the steering group. For the ambulance service, two national coordinators facilitated contacts and assisted if needed. The local operative units were implemented as tested in the pilot study and were continuously aided by SWEDEHEART staff. The academic research organization with trial management, monitoring and statistical assistance was provided by the Uppsala Clinical Research Center (UCR), Uppsala, Sweden.

Figure 6: Schematic illustration of national trial organization
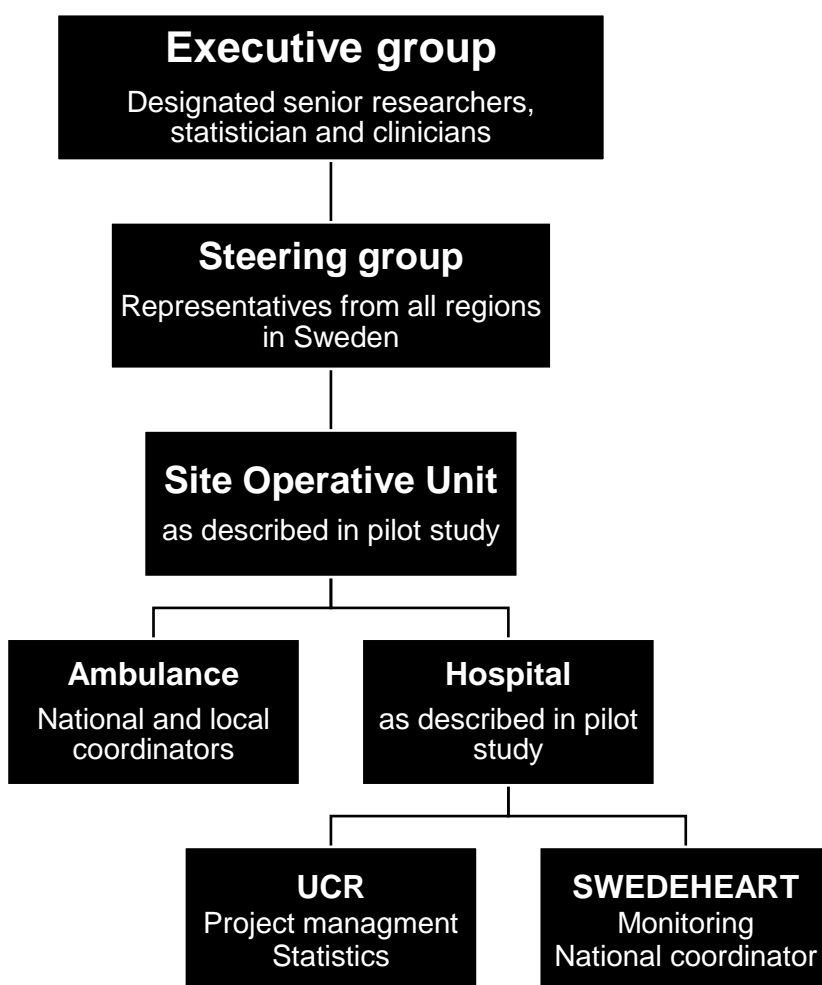
4.4.3 Flow chart (enrollment, allocation, follow-up, analysis)

Patient contact with ambulance service or emergency department

DETO₂X — Inclusion criteria

1. Chest pain or dyspnea suggestive of AMI
2. SpO₂ ≥ 90 %
3. Age ≥ 30 år
4. ECG changes and/or elevated troponin levels

- *For inclusion, all 4 criteria must be fullfilled!*
- *Treatment group receives 6L/min O₂ on Oxymask for (6-)12 hours*

Questions? Call DETO₂X personel: 7462 or CCU on-call 3035

DETO₂X — Exclusion criteria

1. Unwillingness to participate
2. Inability to understand given info
3. Continuous O₂ treatment at home
4. Cardiac arrest prior to inclusion

- *Do NOT start O₂ therapy prior to inclusion assessment!*
- *Ongoing O₂ treatment prior to inclusion: contact doctor!*

Questions? Call DETO₂X personel: 7462 or CCU on-call 3035

Unrestricted online 1:1 randomization on CCU using SWEDEHEART

Oxygen

Delivered by open face mask at 6 L/min continuously for 6-12 hours

Standard ACS treatment

Ambient air

No oxygen given if O₂ saturation ≥ 90%.

Standard ACS treatment

Primary endpoint: 1-year all-cause mortality

Additional secondary endpoints

Follow-up based on Swedish Population Registry and SWEDEHEART

4.5 SUBSTUDY ASSESSING EFFECTS OF SUPPLEMENTAL OXYGEN ON THE SYSTEMIC INFLAMMATORY RESPONSE TO AMI (STUDY III)

The DETermination of the role of OXygen in acute myocardial infarction by biomarkers (DETO$_2$X-biomarkers) was a pre-specified multicenter substudy to the DETO$_2$X-AMI trial with Södersjukhuset and Linköping University Hospital as participating units.

Patients recruited in the main trial were asked to participate in the DETO$_2$X-biomarkers substudy whenever qualified research staff was available for lab assistance. Baseline blood samples where obtained as soon as possible after randomization, and a follow-up blood sample was secured 5-7 hours later. Apart from that, patients followed the regular DETO$_2$X-AMI study protocol as well as standard care but were registered on a separate CRF.

The primary outcome was the effect of oxygen on systemic inflammation. A multiplex panel with 92 markers was analyzed by the Clinical Biomarkers facility, Science for Life Laboratory, Uppsala University, Uppsala. Personnel conducting the analysis were blinded to the allocated therapy. Analyses were carried out with a high-throughput technique on the Olink Proseek® Multiplex Inflammation I 96*96 kit (Olink Bioscience AB, Uppsala, Sweden), which measures 92 selected inflammatory disease related proteins simultaneously in plasma samples. A proximity extension assay (PEA) technology was used, where 92 oligonucleotide-labeled antibody probe pairs can bind to their respective targets present in the sample.[94,95] The kit has been shown to have high reproducibility and repeatability.[95] The platform supplies normalized protein expression (NPX) data where a high DNA amplicon value equals a high protein concentration but does not enable an absolute quantification.

The protein markers included on the Inflammation I 96*96 assay are given in the supplement to study III.

To allow adjustment for the systemic inflammation caused by the size of the myocardial injury, plasma levels of highly sensitive cardiac troponin T were analyzed at the same time points using an electrochemiluminescence immunoassay (Troponin T hs STAT, Roche Diagnostics, Mannheim, Germany) on the Elecsys 2010 immunoassay analyzer (Roche Diagnostics).

4.6 STATISTICAL CONSIDERATIONS

4.6.1 General

Baseline characteristics are tabulated by randomized treatment group. Categorical data are depicted as total number and percentage. Numerical data is described using number of patients with data and median with interquartile range or arithmetic mean with standard deviations where applicable. Statistical testing comparing the randomized treatment groups was performed using chi-square tests or Fisher's exact test for categorical variables and Wilcoxon's signed-rank test for non-parametric numerical data. The results are presented as p-values if statistical testing was performed. A p-value < 0.05 was considered statistically significant.

Because the groups are randomized, all perceived differences are expected to be due to chance.

4.6.2 Study II

There are basically three methods of analysis available in clinical research when comparing two therapies: 1) superiority (A is better than B or B is better than A); 2) equivalence (A is as good as B); 3) non-inferiority (A is not worse than B).

Equivalence and non-inferiority trials depend on certain assumptions. The basic demand is that superior efficacy of the standard treatment over placebo has been conclusively shown for a certain indication in earlier trials.[96] As described in 2.5, no high quality clinical data on oxygen versus ambient air was available, resulting in a superiority efficacy analysis being the only available option for the DETO$_2$X-AMI trial. Furthermore, choosing a two-tailed superiority design enabled both potential benefit and harm to be shown in a two-sided analysis.

The primary endpoint was death of all causes within one year. The time-to-event analysis of all-cause death within 365 days after randomization is presented as Kaplan–Meier curves. Hazard ratios were calculated using Cox proportional-hazards model, with adjustment for age in years (as a linear covariate on the log-hazard scale) and sex.[97] Estimates of differences between the study groups are presented with two-tailed 95% confidence intervals and associated p-values. A two-tailed p-value < 0.05 was considered statistically significant. Subgroup analyses were performed with the use of proportional-hazards models with adjustment for age and sex and formal tests for interaction.

4.6.3 Study III

The primary endpoint was the effect of oxygen on the systemic inflammatory response to AMI assessed by 92 inflammatory biomarkers provided as normalized protein expression (NPX) data. The NPX are on log2 scale. Biomarkers with values below the lower limit of detection (LLOD) were set at LLOD/2. Twenty-seven proteins with less than 85% valid measurements of that protein were excluded from further analysis. Thus, 65 proteins were analyzed (complete list in Supplementary Table).

Linear regression was used for the primary endpoint. The treatment effect on each biomarker (at 5-7 hours post randomization) was estimated in a model with the baseline value of each biomarker as covariate (i.e. adjusting for the baseline value). Results are in table format with beta estimate, 95% confidence interval and p-value and are also shown as a forest plot. Descriptive values shown are median with interquartile range (IQR). The statistical test for the comparison between treatment and baseline levels, irrespective of randomized group, was a paired t-test.

Two thresholds of significance were used: A nominal significance level of $p < 0.05$ and a threshold calculated for the primary endpoint using permutation tests. The permutation procedure gave the result that $p < 0.001$ was needed for significance at the 5% level.

4.6.4 Power calculations

Being a pilot study for feasibility, no power calculations were performed for study I.

For study II, sample size calculations were based on observational clinical trials[98,99] and historical data from SWEDEHEART between 2005-2010. The one-year total mortality among patients with confirmed myocardial infarction was estimated to be around 12%. A clinically relevant effect of supplemental oxygen was defined as a 20% relative risk reduction. We expected that before 2010 the great majority of patients were according to guidelines generally treated with oxygen. Thus, assuming a significant benefit of oxygen treatment, the mortality rate in the ambient-air group would come up to 14.4%. With the chi-square test, to be able to reject the null hypothesis at a significance level of 0.05 (two-tailed) with a power of 0.90, a total of 2,856 patients per group were needed. To control for patients crossing over or not completing the trial, the planned sample size was increased to 3,300 patients per group, which resulted in a total of 6,600 patients.

Sample size calculations for study III were based on previous published data.[100] To be able to reject the null hypothesis at a significance level of 0.05, and with a power of 0.80, an estimated number of 140 patients (70 O_2, 70 ambient air) would suffice to show significant differences in biomarkers levels.

5 RESULTS

5.1 STUDY I

We performed a single center pilot study at Södersjukhuset, Stockholm, between October 2012 and January 2013. A total of 129 normooxic patients were enrolled by the ambulance service or at the emergency department and randomized in a 1:1 ratio to either oxygen at 6 L/min for 12 hours delivered via open face mask or ambient air.

Except for being younger, baseline characteristics were similar to those seen in the overall SWEDEHEART population (table 2). A total of 81 (63%) patients were diagnosed with AMI (53% NSTEMI and 47% STEMI). Of those that remained, 32 (25%) patients were diagnosed with other acute cardiac conditions such as angina pectoris, myocarditis, heart failure, Takotsubo cardiomyopathy, or valvular disease. Sixteen (12%) patients received unspecified chest pain as primary diagnosis. No substantial logistical or medical problems occurred. Oxygen treatment for 12 hours was well accepted. Crossover from ambient air to oxygen occurred in two patients who developed hypoxemia due to pulmonary edema. There was no crossover from oxygen to ambient air. At 30 days, there were 3 (4.6%) deaths in the ambient-air group and no deaths in the oxygen group (p=0.12, Fisher´s Exact test).

Figure 8: Schematic illustration of patient distribution and final diagnoses
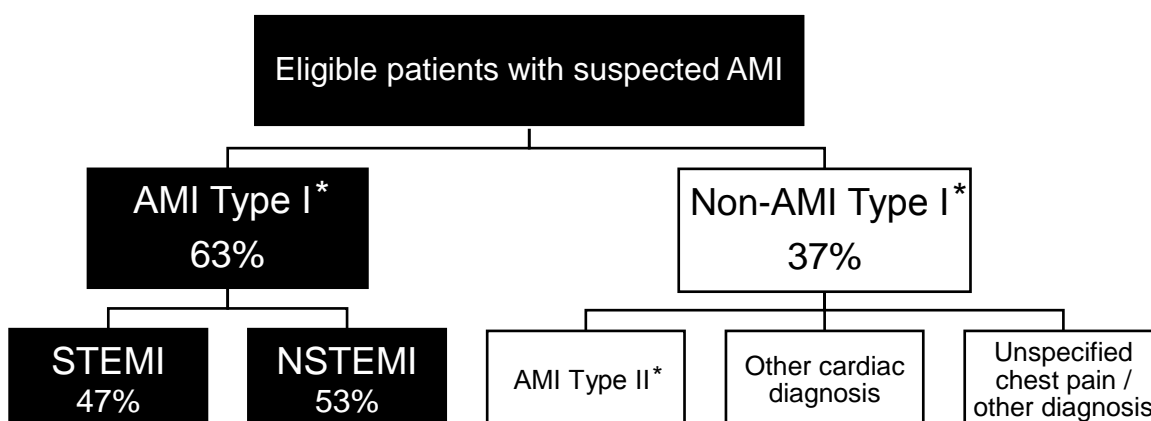
*According to the universal definition of myocardial infarction, 3rd edition[101]

Table 2: Baseline characteristics and final diagnoses in the DETO$_2$X-AMI pilot study

	All (N=129)	Oxygen (N=65)	Ambient air (N=64)
Demographics – no. (%)			
Age, median (IQR)	68 (58-78)	69 (58-78)	65 (58-76)
Men	87 (67)	40 (62)	47 (73)
Risk factors – no. (%)			
Current smoking	26 (20)	13 (20)	13 (20)
Diabetes Mellitus	21 (16)	16 (25)	5 (8)
Hypertension	51 (40)	27 (42)	24 (38)
Previous CV disease – no. (%)			
Myocardial infarction	26 (20)	11 (17)	15 (23)
Percutaneous coronary intervention	25 (19)	9 (14)	16 (25)
Coronary artery by-pass graft	4 (3)	2 (3)	2 (3)
Stroke	3 (2)	1 (2)	2 (3)
Medication on admission – no. (%)			
Aspirin	39 (30)	20 (31)	19 (30)
Clopidogrel	2 (1.6)	1 (1.5)	1 (1.6)
Beta-blocker	41 (32)	23 (35)	18 (28)
Statin	34 (26)	18 (28)	16 (25)
ACE-inhibitors or ATII-blockers	37 (29)	21 (32)	16 (25)
Presentation – no. (%)			
Ambulance transportation	82 (64)	40 (62)	42 (66)
Systolic blood pressure – mmHg	150 (135-170)	150 (133-168)	150 (136-191)
Heart rate - beats/min (IQR)	80 (67-93)	81 (67-92)	80 (67-93)
Electrocardiography – no. (%)			
ST-elevation	48 (37)	21 (32)	27 (42)
ST-depression	28 (22)	15 (23)	13 (20)
T-wave inversion	18 (14)	10 (15)	8 (13)
Normal or other	35 (27)	19 (29)	16 (25)
Final diagnosis – no. (%)			
Myocardial infarction	81 (63)	42 (65)	39 (61)
Unstable or stable angina	17 (13)	6 (9)	11 (17)
Other heart disease	15 (12)	9 (14)	6 (9)
Unknown/other non-cardiac cause	16 (12)	8 (12)	8 (13)

5.2   STUDY II

Trial Population

Of the 69 hospitals in Sweden with acute cardiac care facilities, 35 participated in the trial. Between April 13, 2013, and December 30, 2015, a total of 6,629 patients with suspected myocardial infarction were enrolled and included in the intention-to-treat analysis (figure 9).

Figure 9: Enrollment, randomization, and analysis
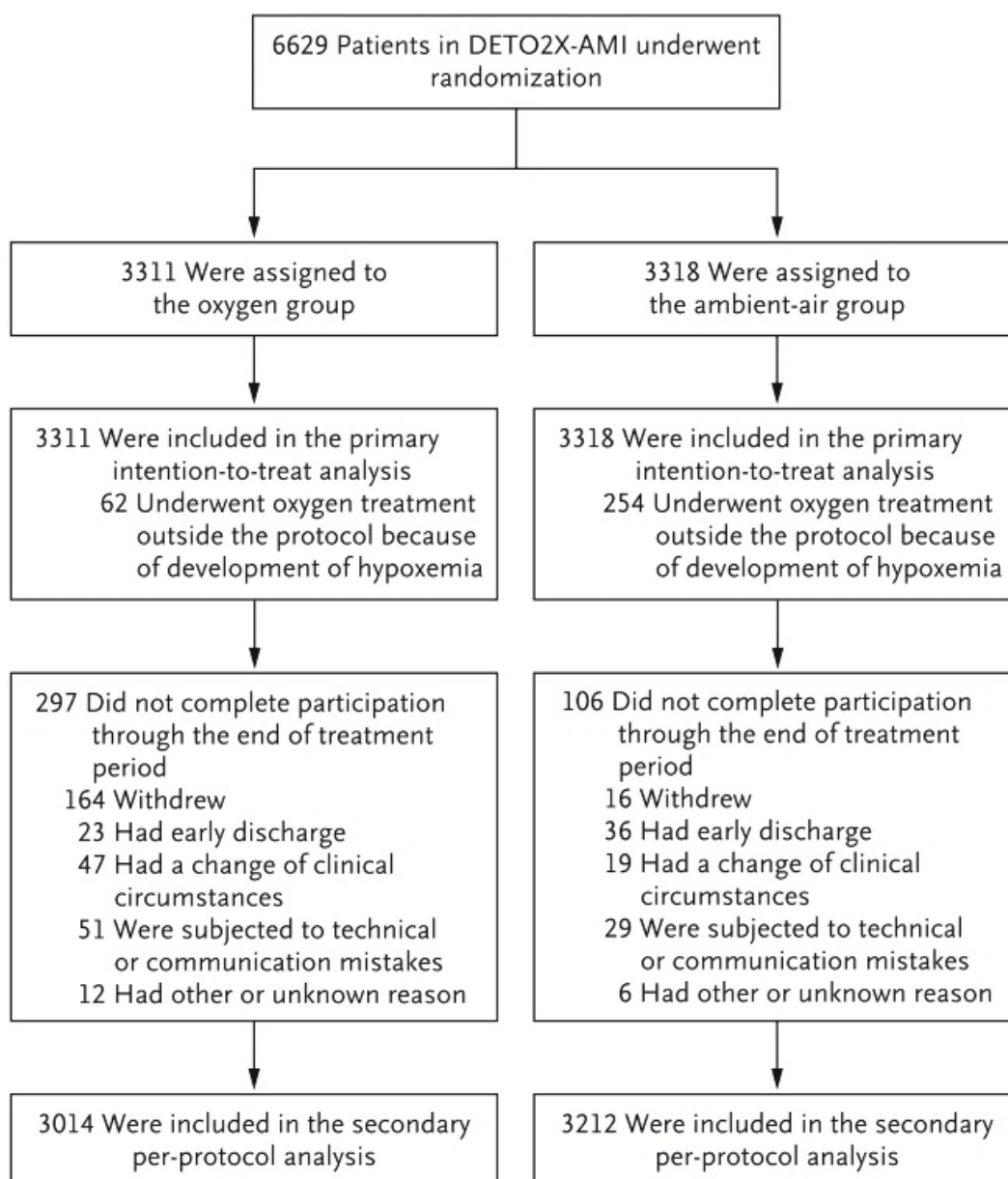
Adapted with permission from NEJM[102]

The baseline characteristics and clinical presentation of all the patients, as well as the final diagnoses, were similar in both groups (table 3).

Table 3: Baseline characteristics, clinical presentation, and discharge diagnoses

	Oxygen (N=3,311)	Ambient air (N=3,318)
Demographics – no. (%)		
Age – years, median (IQR)	68.0 (59.0-76.0)	68.0 (59.0-76.0)
Male sex	2,264 (68.4)	2,342 (70.6)
Risk factors – no. (%)		
Body-mass index	27.1±4.4	27.2±4.4
Current smoking	704 (21.3)	721 (21.7)
Hypertension	1,575 (47.6)	1,559 (47.0)
Diabetes mellitus	589 (17.8)	644 (19.4)
Previous CV disease – no. (%)		
MI	682 (20.6)	667 (20.1)
PCI	525 (15.9)	549 (16.5)
CABG	208 (6.3)	206 (6.2)
Causes of admission		
Chest pain	3,123 (94.3)	3,120 (94.0)
Dyspnea	63 (1.9)	77 (2.3)
Cardiac arrest	1 (0.0)	1 (0.0)
Medication on admission – no. (%)		
Aspirin	904 (27.3)	961 (29.0)
P2Y12 Receptor Inhibitors	177 (5.4)	173 (5.2)
Beta-blockers	1,030 (31.1)	1,052 (31.7)
Statins	884 (26.7)	895 (27.0)
ACE-inhibitors or AT II-blocker	1,186 (35.8)	1,237 (37.3)
Calcium-blockers	617 (18.6)	615 (18.5)
Diuretics	543 (16.4)	525 (15.8)
Presentation		
Time from symptom onset to randomization minutes, median (IQR)	245.0 (135.0-450.0)	250 (134.0-458.0)
Ambulance transportation – no. (%)	2,215 (66.9)	2,218 (66.8)
Systolic blood pressure – mmHg	150.3±27.8	148.7±28.0
Heart rate – beats/min	78.6±19.3	78.1±19.5
Oxygen saturation – %, median (IQR)	97 (95-98)	97 (95-98)

	Oxygen (N=3,311)	Ambient air (N=3,318)
Discharge diagnosis		
MI (I.21+I.22)	2,485 (75.1)	2,525 (76.1)
STEMI	1,431 (43.2)	1,521 (45.8)
Angina pectoris (code I.20)	189 (5.7)	185 (5.6)
Other cardiac diagnosis	254 (7.7)	257 (7.7)
Atrial fibrillation (I.48)	52 (1.6)	44 (1.3)
Heart failure (I.50)	43 (1.3)	40 (1.2)
Cardiomyopathy (I.42)	48 (1.4)	46 (1.4)
Peri-myocarditis (I.30+I.40)	32 (1.0)	43 (1.3)
Pulmonary embolism (I.26)	7 (0.2)	9 (0.3)
Pulmonary disease	17 (0.5)	15 (0.5)
Pneumonia (J.15+J.16)	8 (0.2)	7 (0.2)
COPD/asthma (J44+J45)	2 (0.1)	2 (0.1)
Unspecified chest pain (R.07)	258 (7.8)	234 (7.1)
Other non-CV diagnosis	108 (3.3)	102 (3.1)
Musculoskeletal pain (M.54+M.79)	7 (0.2)	14 (0.4)

Procedural Data

The data on procedures, medication, and complications during the hospitalization period were similar in both groups except for the rate of patients developing hypoxemia, the oxygen saturation at the end of the treatment period and the use of iv inotropes (table 4).

Table 4: Data on procedures, medication, and complications during hospitalization

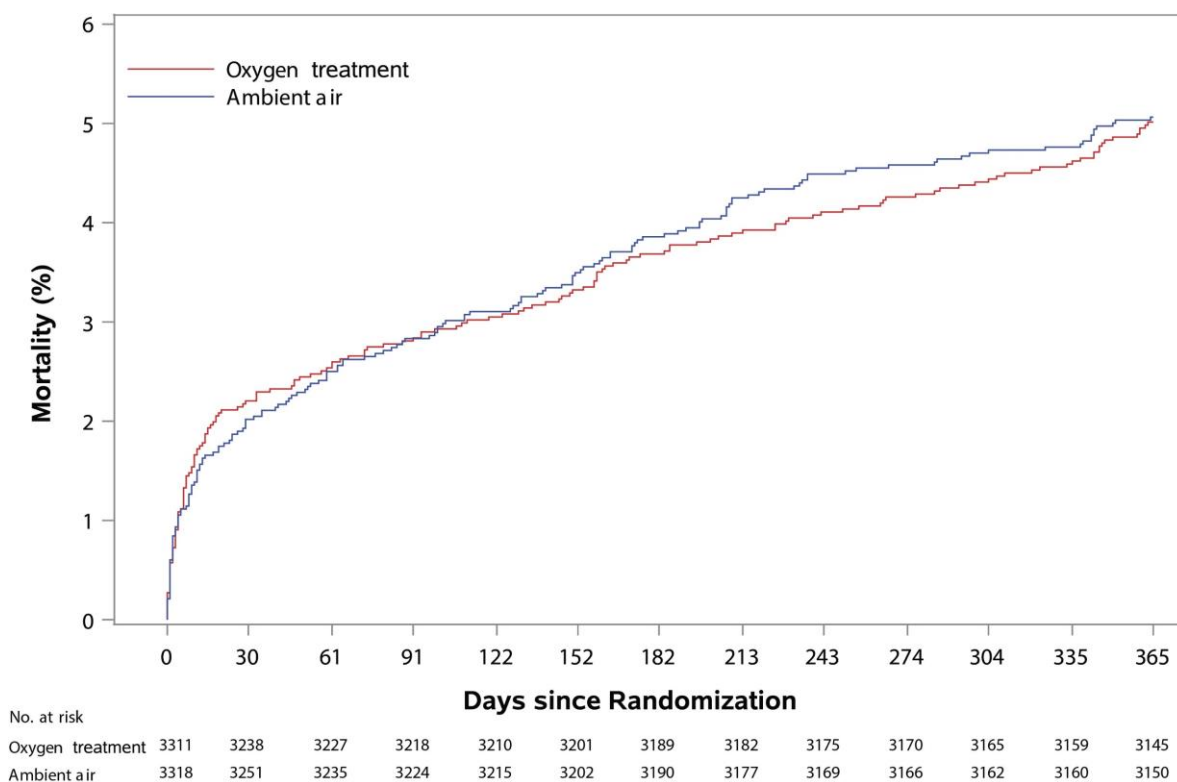|	Oxygen (N=3,311)	Ambient Air (N=3,318)	p value
Trial procedural data			
Duration of oxygen therapy hours, median (IQR)	11.6 (6.0-12.0)		
Received oxygen due to the development of hypoxemia	62 (1.9)	254 (7.7)	<0.001
Oxygen saturation at end of treatment period – %, median (IQR)	99 (97-100)	97 (95-98)	<0.001
Procedures – no. (%)			
Coronary angiography	2,797 (84.5)	2,836 (85.5)	0.26
PCI	2,183 (65.9)	2,246 (67.7)	0.13
CABG	96 (2.9)	110 (3.3)	0.51
Hospital stay – days, median	3.0 (0-68)	3.0 (0-95)	0.87
Medication – no. (%)			
Iv diuretics	309 (9.3)	322 (9.7)	0.58
Iv inotropes	46 (1.4)	70 (2.1)	0.02
Iv nitroglycerin	252 (7.6)	221 (6.7)	0.14
Aspirin	2,758 (83.3)	2,803 (84.5)	0.16
P2Y12 Receptor Inhibitors	2,445 (73.8)	2,463 (74.2)	0.62
Beta-blockers	2,702 (81.6)	2,752 (82.9)	0.13
Statins	2,782 (84.0)	2,765 (83.3)	0.46
ACE-inhibitors or AT II-blockers	2,586 (78.1)	2,557 (77.1)	0.32
Calcium-blockers	519 (15.7)	547 (16.5)	0.36
Diuretics	607 (18.3)	615 (18.5)	0.82
Complications – no. (%)			
Reinfarction	17 (0.5)	15 (0.5)	0.72
New-onset atrial fibrillation	94 (2.8)	103 (3.1)	0.53
AV-block II or III	46 (1.4)	58 (1.7)	0.24
Cardiogenic shock	32 (1.0)	37 (1.1)	0.54
Cardiac arrest	79 (2.4)	63 (1.9)	0.17
Death	53 (1.6)	44 (1.3)	0.35

Clinical Outcomes

Follow-up data on mortality were obtained for all patients from the records of the Swedish National Population Registry. All other variables were obtained from SWEDEHEART (table 5).

The primary endpoint of death from any cause within 1 year after randomization occurred in 5.0% of patients (166 of 3311) assigned to oxygen and in 5.1% of patients (168 of 3318) assigned to ambient air (hazard ratio, 0.97; 95% confidence interval, 0.79 to 1.21; P = 0.80) (figure 10).

Figure 10: Kaplan-Meier curves for death from any cause

Kaplan-Meier curves are shown for the cumulative probability of death from any cause up to 365 days after randomization among patients assigned to oxygen or ambient air. The proportional-hazards assumption was subjected to post hoc testing by inserting a linear treatment–time interaction in the Cox proportional-hazards model, which did not noticeably improve the model fit (P = 0.61). The inset shows the same data on an expanded y-axis.

No. at risk

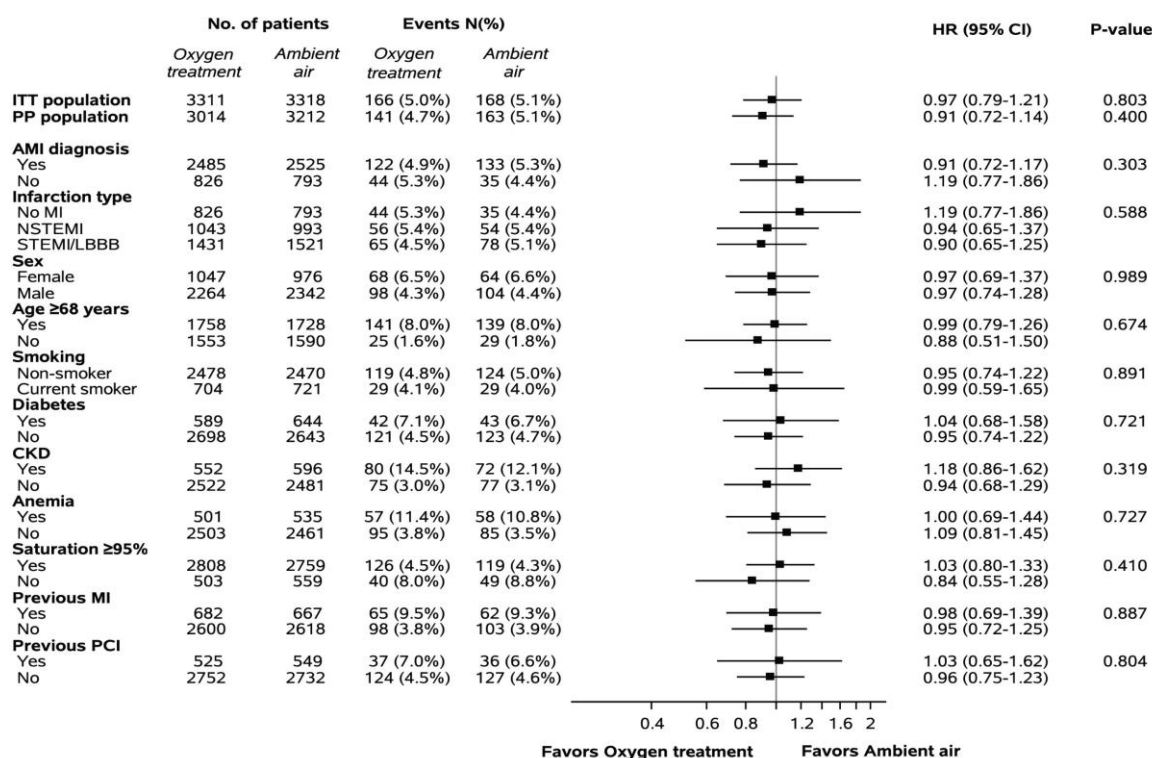|												
Oxygen treatment	3311	3238	3227	3218	3210	3201	3189	3182	3175	3170	3165	3159	3145
Ambient air	3318	3251	3235	3224	3215	3202	3190	3177	3169	3166	3162	3160	3150

Adapted with permission from NEJM[102]

The corresponding one-year mortality in the per-protocol population was 4.7% (141 of 3,014) and 5.1% (163 of 3,212), respectively (hazard ratio, 0.91; 95% confidence interval, 0.72 to 1.14; P = 0.40). The findings for the primary endpoint were consistent across all prespecified subgroups.

Figure 11: Prespecified subgroup analyses

Hazard ratios (HR) are shown for the primary endpoint of mortality within 365 days after randomization in the intention-to-treat population, the per-protocol population and in subgroups of patients. ITT denotes intention-to-treat; PP per-protocol, AMI acute myocardial infarction, MI myocardial infarction, NSTEMI non-ST-segment elevation myocardial infarction, STEMI ST-segment elevation myocardial infarction, CKD chronic kidney disease, and PCI percutaneous coronary intervention.

	No. of patients		Events N(%)			HR (95% CI)	P-value
	Oxygen treatment	Ambient air	Oxygen treatment	Ambient air			
ITT population	3311	3318	166 (5.0%)	168 (5.1%)		0.97 (0.79-1.21)	0.803
PP population	3014	3212	141 (4.7%)	163 (5.1%)		0.91 (0.72-1.14)	0.400
AMI diagnosis							
Yes	2485	2525	122 (4.9%)	133 (5.3%)		0.91 (0.72-1.17)	0.303
No	826	793	44 (5.3%)	35 (4.4%)		1.19 (0.77-1.86)	
Infarction type							
No MI	826	793	44 (5.3%)	35 (4.4%)		1.19 (0.77-1.86)	0.588
NSTEMI	1043	993	56 (5.4%)	54 (5.4%)		0.94 (0.65-1.37)	
STEMI/LBBB	1431	1521	65 (4.5%)	78 (5.1%)		0.90 (0.65-1.25)	
Sex							
Female	1047	976	68 (6.5%)	64 (6.6%)		0.97 (0.69-1.37)	0.989
Male	2264	2342	98 (4.3%)	104 (4.4%)		0.97 (0.74-1.28)	
Age ≥68 years							
Yes	1758	1728	141 (8.0%)	139 (8.0%)		0.99 (0.79-1.26)	0.674
No	1553	1590	25 (1.6%)	29 (1.8%)		0.88 (0.51-1.50)	
Smoking							
Non-smoker	2478	2470	119 (4.8%)	124 (5.0%)		0.95 (0.74-1.22)	0.891
Current smoker	704	721	29 (4.1%)	29 (4.0%)		0.99 (0.59-1.65)	
Diabetes							
Yes	589	644	42 (7.1%)	43 (6.7%)		1.04 (0.68-1.58)	0.721
No	2698	2643	121 (4.5%)	123 (4.7%)		0.95 (0.74-1.22)	
CKD							
Yes	552	596	80 (14.5%)	72 (12.1%)		1.18 (0.86-1.62)	0.319
No	2522	2481	75 (3.0%)	77 (3.1%)		0.94 (0.68-1.29)	
Anemia							
Yes	501	535	57 (11.4%)	58 (10.8%)		1.00 (0.69-1.44)	0.727
No	2503	2461	95 (3.8%)	85 (3.5%)		1.09 (0.81-1.45)	
Saturation ≥95%							
Yes	2808	2759	126 (4.5%)	119 (4.3%)		1.03 (0.80-1.33)	0.410
No	503	559	40 (8.0%)	49 (8.8%)		0.84 (0.55-1.28)	
Previous MI							
Yes	682	667	65 (9.5%)	62 (9.3%)		0.98 (0.69-1.39)	0.887
No	2600	2618	98 (3.8%)	103 (3.9%)		0.95 (0.72-1.25)	
Previous PCI							
Yes	525	549	37 (7.0%)	36 (6.6%)		1.03 (0.65-1.62)	0.804
No	2752	2732	124 (4.5%)	127 (4.6%)		0.96 (0.75-1.23)	

0.4 0.6 0.8   1.2 1.6 2

Favors Oxygen treatment   Favors Ambient air
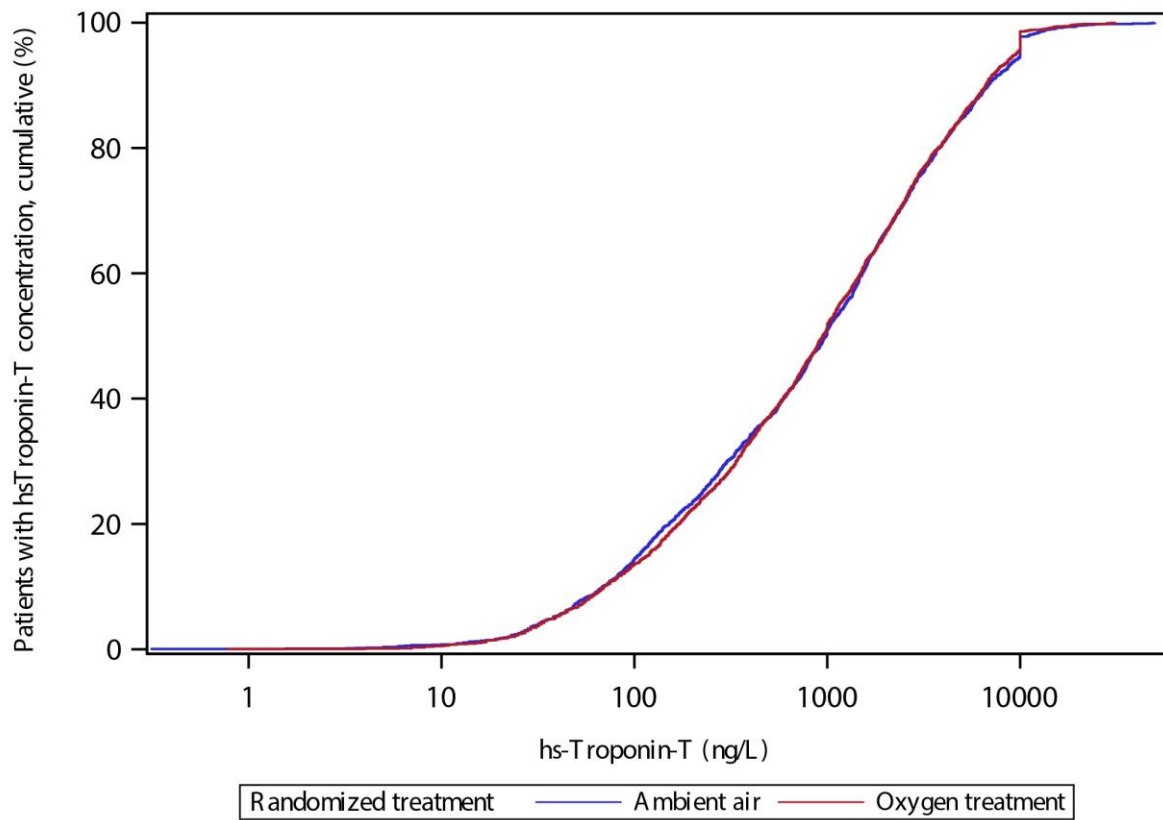
Adapted with permission from NEJM[102]

Secondary endpoints included rehospitalization with myocardial infarction within and the composite endpoint of death from any cause or rehospitalization with myocardial infarction at one year. No significant difference between the two groups was detected at 30 days regarding death, rehospitalization with myocardial infarction, or the composite of these two endpoints. Data on the highest measured level of highly sensitive cardiac troponin T during hospitalization were available for 3,976 of 5,010 patients (79.4%) with confirmed myocardial infarction, and this measure did not differ significantly between the study groups (table 5).

Table 5: Endpoints during and after hospitalization

Endpoint	Oxygen (N=3311)	Ambient Air (N=3318)	Hazard Ratio (95% CI)	P Value
365 days – no. (%)				
All-cause death	166 (5.0)	168 (5.1)	0.97 (0.79-1.21)	0.80
Rehospitalization with MI	126 (3.8)	111 (3.3)	1.13 (0.88-1.46)	0.33
Composite of all-cause death or rehospitalization with MI	275 (8.3)	264 (8.0)	1.03 (0.87-1.22)	0.70
30 days – no. (%)				
All-cause death	73 (2.2)	67 (2.0)	1.07 (0.77-1.50)	0.67
Rehospitalization with MI	45 (1.4)	31 (0.9)	1.46 (0.92-2.31)	0.11
Composite of all-cause death or rehospitalization with MI	114 (3.4)	95 (2.9)	1.19 (0.91-1.56)	0.21
During hospital stay				
hs-troponin T – no. (%)	1998 (80.4)	1978 (78.3)		
median	946.5	983.0		0.97
IQR	(243.0-2884.0)	(225.0-2931.0)		

Figure 12: Cumulative distribution of highly sensitive cardiac troponin T per treatment group

For each troponin value displayed, the graph shows the proportion of patients below that cut-point

Adapted with permission from NEJM[102]

Outcomes among Patients Not Enrolled in the Trial

During the trial period, a total of 22,872 patients with confirmed myocardial infarction were reported in the SWEDEHEART registry at participating sites, of whom 5,010 (21.9%) were enrolled in the DETO$_2$X-AMI trial. The remaining 17,862 patients with confirmed myocardial infarction who did not undergo randomization were at higher risk for all the endpoints we considered, were more often admitted with dyspnea and after cardiac arrest (table 6), and had considerably worse outcomes than those with confirmed myocardial infarction who were enrolled in the trial (table 7). Patients with suspected but not confirmed myocardial infarction who were not enrolled in the trial are not recorded in the SWEDEHEART registry, and, therefore, data for such patients were not available for comparison.

Table 6: Baseline characteristics, clinical presentation, and final diagnoses in patients with AMI enrolled and not enrolled in the DETO$_2$X-AMI trial at participating sites during the trial period

|	Enrolled in DETO$_2$X-AMI (N=5,010)	Not enrolled in the trial (N=17,862)	P value
Demographics – no. (%)			
Age – years, median (IQR)	68.0 (60.0-76.0)	72.0 (63.0-82.0)	<0.001
Risk factors – no. (%)			
Hypertension	2,358 (47.1)	9,410 (52.7)	<0.001
Diabetes mellitus	932 (18.6)	4,289 (24.0)	<0.001
Previous CV- disease – no. (%)			
AMI	895 (17.9)	4,667 (26.1)	<0.001
PCI	694 (13.9)	3,187 (17.8)	<0.001
Causes of admission – no. (%)			
Chest pain	4,807 (95.9)	14,011 (78.4)	<0.001
Dyspnea	73 (1.5)	1,574 (8.8)	<0.001
Cardiac arrest	1 (0.0)	445 (2.5)	<0.001
Final diagnoses – no. (%)			
MI (I.21+I.22)	5,010 (100)	17,862 (100)	
STEMI	2,952 (58.9)	5,710 (32.0)	<0.001

Table 7: Endpoints per treatment group and randomization status at 30 days and at 365 days after randomization in patients with AMI enrolled and not enrolled in the DETO$_2$X-AMI trial at participating sites during the trial period

Endpoint	Enrolled in DETO$_2$X-AMI (N=5010)	Not enrolled in the trial (N=17,862)	Hazard Ratio (95% CI)	P value
365 days – no. (%) All-cause death	255 (5.1)	2861 (16.0)	0.30 (0.26-0.34)	<0.001
Rehospitalization with MI	185 (3.7)	989 (5.5)	0.61 (0.52-0.72)	<0.001
Composite of all-cause death or rehospitalization with MI	412 (8.2)	3597 (20.1)	0.38 (0.34-0.42)	<0.001
30 days – no. (%) All-cause death	125 (2.5)	1415 (7.9)	0.31 (0.26-0.37)	<0.001
Rehospitalization with MI	63 (1.3)	286 (1.6)	0.75 (0.57-0.99)	<0.001
Composite of all-cause death or rehospitalization with MI	181 (3.6)	1668 (9.3)	0.38 (0.32-0.44)	<0.001

5.3   STUDY III

Between November 2014 and December 2015, 164 eligible patients were included. Twenty patients were excluded from the analysis (figure 13). Consequently, 144 remained for analysis. At the time of randomization, the median oxygen saturation was 97%. Seventy-six (53%) patients were allocated to oxygen and 68 (47%) patients were allocated to ambient air. The median duration of oxygen therapy was 11.6 hours, with a median oxygen saturation of 99% in patients assigned to oxygen and 97% in patients assigned to ambient air at the end of the treatment period ($p<0.001$). Overall, 74 patients (51%) received the discharge diagnosis ST-segment elevation myocardial infarction (STEMI) whereas 70 patients received the discharge diagnosis non-STEMI (table 8). Seventy-six patients (53%) were randomized to oxygen therapy and 68 patients (47%) to ambient air (figure 13).

Figure 13: Enrollment, randomization and analysis
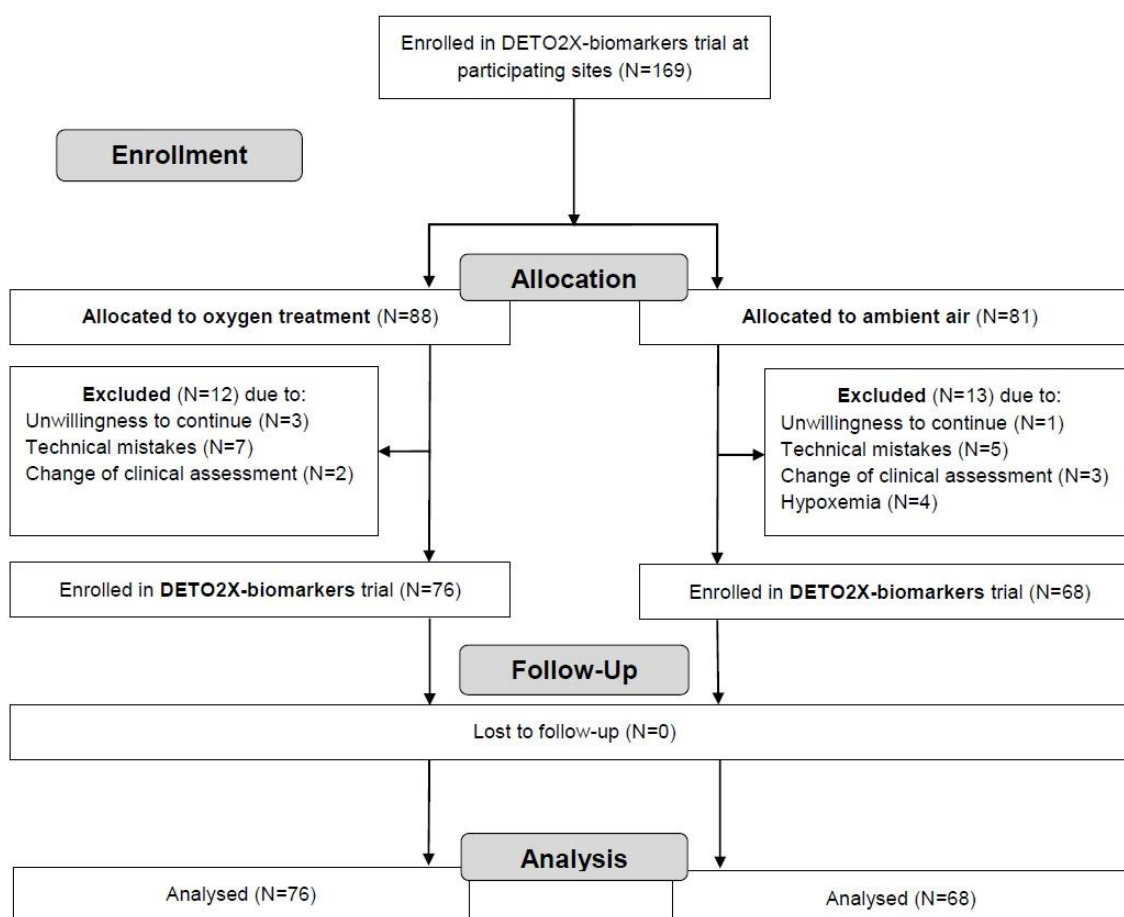
There were no significant differences in baseline characteristics and clinical presentation between the oxygen group and the ambient-air group except for the oxygen saturation at the end of the treatment period (table 8).

Table 8: Baseline characteristics, clinical presentation, study procedural data, and infarction type in the DETO$_2$X-biomarker substudy

	Oxygen (N=76)	Ambient air (N=68)
Demographics – no. (%)		
Age – years, median (IQR)	68.0 (58.5-77.0)	67.0 (58.0-73.5)
Male sex	57 (75.0)	54 (79.4)
Risk factors – no. (%)		
Body-mass index‡	27.6±4.5	27.0±4.3
Current smoking	17 (22.4)	14 (20.6)
Hypertension	41 (53.9)	30 (44.1)
Diabetes mellitus	18 (23.7)	9 (13.2)
Previous cardiovascular disease – no. (%)		
Myocardial infarction	11 (14.5)	10 (14.7)
Percutaneous coronary intervention	8 (10.5)	7 (10.3)
Coronary-artery bypass graft	4 (5.9)	7 (9.2)
Medication on admission – no. (%)		
Aspirin	15 (19.7)	13 (19.1)
P2Y12 receptor Inhibitors	3 (3.9)	2 (3.0)
Beta-blockers	19 (25.0)	19 (27.9)
Statins	21 (27.6)	16 (23.5)
ACE-inhibitors or AT II receptor blockers	33 (43.4)	25 (36.8)
Calcium-blocker	19 (25.0)	14 (20.6)
Diuretics	7 (9.2)	10 (14.7)
Presentation		
Ambulance transportation – no. (%)	58 (76.3)	46 (67.6)
Systolic blood pressure – mmHg	152.4±30.6	151.7±29.2
Heart rate – beats/min	74.7±15.8	79.0±17.5
Oxygen saturation – %, median (IQR)	97 (95-98)	97 (96-98)
Trial procedural data		
Duration of oxygen therapy – hours, median (IQR)	11.7 (6.9-12.0)	-
Oxygen saturation at end of treatment period %, median† (IQR)	100 (99-100)	97 (96-98
Infarction type		
STEMI	51 (67.1)	46 (67.1)
NSTEMI	25 (32.9)	22 (32.3)

Markers of myocardial injury and inflammation

When analyzing the overall NPX levels of the inflammatory biomarkers, eight* increased whereas 13** decreased from baseline to 5-7 hours after randomization (Table 2). At baseline, the median level of cardiac troponin T was 52.9 ng/L in patients assigned to oxygen, and 63.3 ng/L in patients assigned to ambient air. After 5-7 hours, it increased to 1,436 ng/L and 1,574 ng/L, resulting in a median difference of 1,403 ng/L, and 1,238 ng/L in the two groups, respectively (p= 0.696).

Table 9: Overall summary of biomarkers with significant change over study period

Biomarker	Baseline	Treatment	Paired difference	Percent change	P value
IL-6*	2.0 [1.6, 2.7]	3.2 [2.5, 3.8]	1.1 [0.4, 1.9]	114 [34, 265]	7.73e-24
ST1A1*	2.2 [1.6, 3.3]	3.1 [2.0, 4.1]	0.8 [-0.3, 1.5]	74 [-17, 178]	3.64e-08
IL-8*	5.6 [5.3, 6.1]	5.9 [5.5, 6.5]	0.3 [-0.1, 0.8]	26 [-8, 74]	1.85e-06
VEGF-A*	9.5 [9.2, 9.9]	9.7 [9.4, 10.0]	0.2 [0.0, 0.5]	17 [-2, 37]	4.62e-06
CD40*	9.7 [9.4, 10.1]	9.9 [9.5, 10.3]	0.2 [-0.2, 0.6]	16 [-11, 47]	5.26e-05
CCL23*	10.0 [9.6, 10.5]	10.2 [9.8, 10.6]	0.2 [-0.1, 0.5]	15 [-8, 37]	1.64e-04
PD-L1*	3.8 [3.4, 4.1]	3.9 [3.5, 4.3]	0.2 [-0.2, 0.5]	13 [-13, 40]	7.70e-04
CSF-1*	7.3 [7.1, 7.5]	7.4 [7.1, 7.6]	0.1 [-0.1, 0.4]	10 [-9, 28]	3.48e-04
TRAIL**	7.4 [7.2, 7.7]	7.3 [7.0, 7.6]	-0.1 [-0.4, 0.1]	-9 [-24, 8]	1.66e-04
CXCL11**	10.8 [10.2, 11.2]	10.5 [9.9, 11.0]	-0.2 [-0.5, 0.1]	-10 [-31, 8]	1.09e-06
TRANCE**	3.5 [3.1, 4.0]	3.3 [2.9, 3.8]	-0.2 [-0.7, 0.3]	-11 [-40, 20]	7.15e-04
CCL19**	9.5 [8.9, 10.2]	9.3 [8.7, 9.9]	-0.3 [-0.6, 0.1]	-16 [-34, 10]	5.58e-07
Flt3L**	8.2 [7.9, 8.6]	7.9 [7.5, 8.4]	-0.3 [-0.6, 0.0]	-17 [-32, -2]	6.29e-13
CXCL6**	8.6 [8.0, 9.1]	8.4 [7.8, 8.9]	-0.3 [-0.7, 0.2]	-18 [-38, 16.]	7.31e-05
HGF**	7.8 [7.1, 11.4]	7.3 [6.9, 8.0]	-0.3 [-3.9, 0.3]	-18 [-93, 25]	1.10e-07
CCL25**	6.2 [5.7, 6.7]	5.9 [5.5, 6.3]	-0.3 [-0.6, 0.0]	-18 [-34, 0]	1.41e-10
CXCL10**	9.7 [8.9, 10.6]	9.3 [8.6, 10.0]	-0.3 [-1.0, 0.2]	-20 [-50, 12]	3.37e-06
TWEAK**	8.7 [8.3, 11.6]	8.2 [8.0, 8.7]	-0.4 [-3.5, 0.2]	-24 [-91, 13]	6.62e-11
CXCL9**	8.1 [7.0, 9.5]	7.3 [6.6, 8.1]	-0.4 [-1.8, 0.1]	-25 [-72, 6]	1.99e-10
IL-10**	2.7 [1.9, 3.3]	2.0 [1.6, 2.4]	-0.5 [-1.3, 0.1]	-30 [-59, 4]	3.56e-10
OSM**	2.5 [1.9, 3.3]	1.9 [1.4, 2.9]	-0.6 [-1.6, 0.3]	-33 [-67, 25]	1.47e-04

When oxygen therapy was compared with ambient air, no statistically significant difference was detected for any of the inflammatory markers regardless of level of significance. After inclusion of the relative increase in cardiac troponin T levels over time, age, and sex as potential confounders, there were still no significant differences in the inflammatory response to AMI between the two groups (Figure 14).

Figure 14: Forest plot of the adjusted treatment effects on the PEA biomarkers
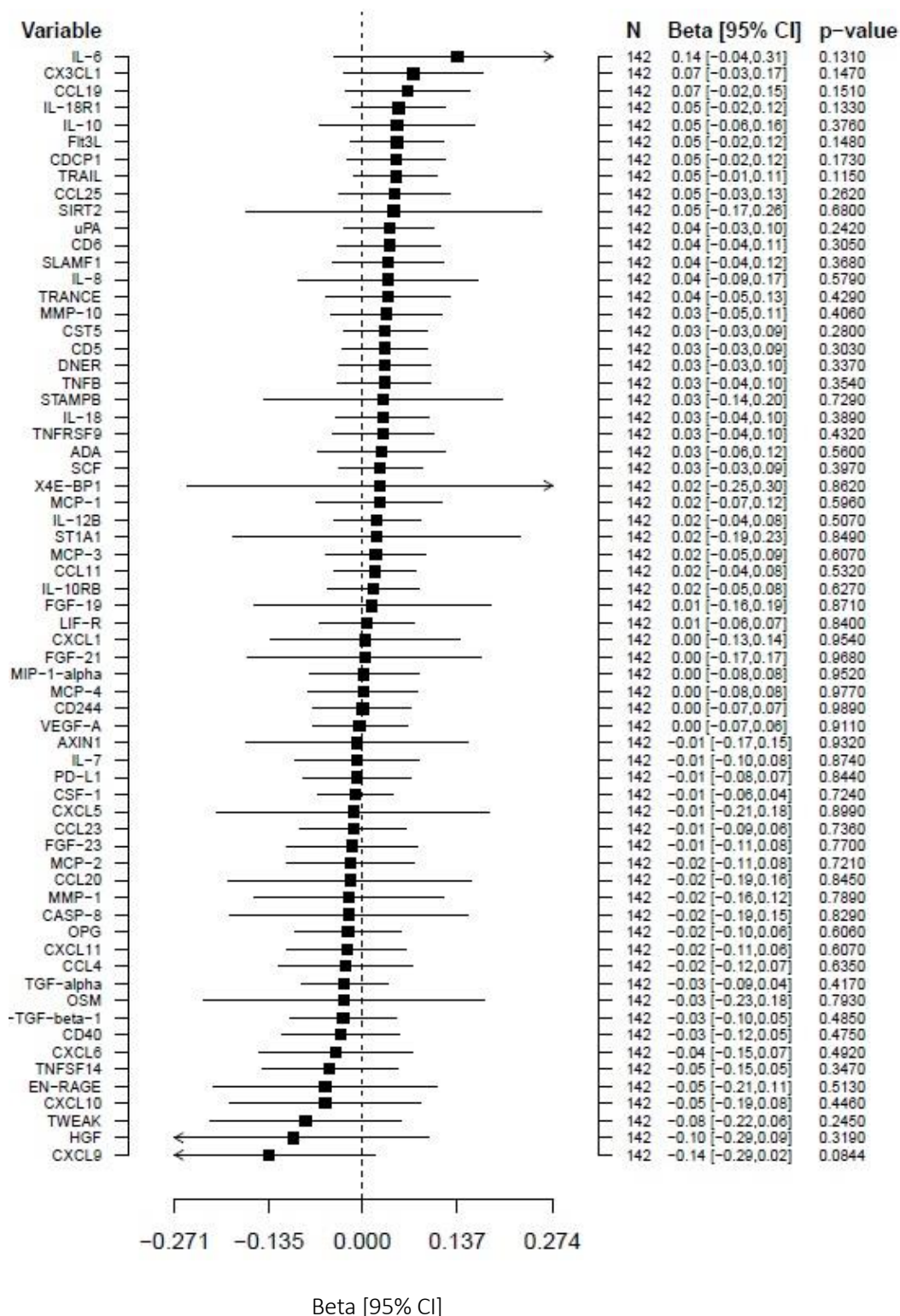
6 DISCUSSION

6.1 MAJOR FINDINGS

The design of the DETO$_2$X-AMI-trial was found to be safe, robust and feasible. Based on the experience of the pilot study, we conducted a pragmatic, registry-based, randomized clinical trial evaluating supplemental oxygen versus ambient air in patients presenting with suspected myocardial infarction who were not hypoxemic at baseline. We found neither a beneficial effect of oxygen treatment nor indication of harm with respect to all-cause mortality, rehospitalization with AMI or the extent of myocardial injury. Furthermore, no impact on the early systemic inflammatory response to AMI was detected.

Both beneficial and deleterious effects of oxygen therapy in AMI patients have been proposed (discussed in detail in chapter 2.2). The different results compared to our trial may have multiple reasons.

Adverse effects have been shown in experimental studies and smaller clinical trials with high doses of up to 100% oxygen by closed face mask. A dose-dependent correlation between oxygen exposure and risk for hyperoxemia-related myocardial injury might be the underlying reason which has been postulated in experimental animal models[70,103] and was also reported in a post-hoc analysis of a recent clinical study.[84] Our trial used moderate oxygen doses by open face mask thereby avoiding excessive hyperoxemia. Furthermore, in experimental studies, hemodynamic, histologic or biochemical markers were analyzed immediately or within a short period of time in a small number of subjects.[54-56] In contrast, our study had long median follow-up. Hemodynamic changes might be transient with limited sustained long-term risk.

Positive effects were reported in smaller clinical trials evaluating surrogate endpoints, and in some, using a different approach.[44,80,104] Our trial was assessing the routine use of supplemental oxygen in current clinical practice where hyperbaric or superoxygenated ways of oxygen delivery are still not established therapeutic choices. Generally, discrepancies between preclinical studies and smaller clinical trials with surrogate endpoints, and large clinical trials with hard outcomes are common and might explain the difference in our findings.

6.2 THE USE OF OXYGEN IN SUSPECTED AMI - A RELEVANT QUESTION FOR THE CAREGIVER, THE HEALTH CARE SYSTEM OR US?

In the background section of this thesis, I tried to build a scientific case arguing that the widespread use of oxygen in the absence of solid scientific evidence demanded a large, randomized trial clarifying this controversy. But is this quest also relevant for clinicians in

daily routine? Does it matter for health care systems in the larger perspective? And lastly, why did we attempt to resolve this issue and not wait for somebody else to do it for us?

It is difficult to judge how many patients with suspected AMI are treated with oxygen per year in Sweden as this aspect is not routinely recorded. Around 20,000 patients annually receive the primary diagnosis AMI which is only one-fifth of the patients admitted to the ED with chest pain. If patients with dyspnea are included, the numbers increase even further. With this simple estimation, it becomes clear that health care professionals are regularly confronted with the decision of whether to use oxygen or not. On top of that, the belief in the power of the drug is still very strong. In a recent survey of health professionals assessing the reason for using oxygen in suspected AMI, 96% of the respondents generally used oxygen in these patients, 50% of whom did so believing it would reduce mortality.[105] Some others argue that even if it might not do much good, it is at least harmless.[106] This point of view has been argued fiercely,[11,50,107] and today most experts seem to agree that oxygen is a potent vasoactive drug which should be used cautiously.

Health care systems across the world battle with galloping costs. Concerning oxygen, costs are certainly not negligible. Basic calculations for the in-hospital costs of the drug alone come up to $10/day per patient,[11] much more when considering pre-hospital care as well. If adding delivery and storage systems and staff the sum increases substantially to an estimated $100/day per patient,[38] resulting in a significant financial burden. If the use of oxygen therapy was shown to be without effect or even harmful, costs could be reduced significantly.

To perform a high quality RCT when trying to assess a relevant clinical problem is demanding for several reasons. Most importantly, it is expensive and difficult to organize because of the lack of funding and infrastructure from the industry. Furthermore, acceptance from the medical community or the public to challenge a common knowledge is problematic and others have found it difficult to perform such a trial.[105] So why were we able or even obliged to do it? Simply because we could. The Ethical Review Board and the Medical Product Agency believed in the cause and gave us permission for the trial; we received unrestricted study grants from The Swedish Heart-Lung Foundation, the Swedish Research Council and the Swedish Foundation for Strategic Research for the execution of the study; the infrastructure and resources of the SWEDEHEART network permitted logistics and support; and most of all the enthusiasm and unwavering engagement of health care personnel throughout the country enabled us to complete this trial.

6.3 RRCT - POSSIBILTIES AND LIMITATIONS OF A NEW CONCEPT

The possible advantages and disadvantages of RRCTs have been discussed in detail recently.[90,108-113] In short, the RRCT concept comes with many of the features for which regular RCTs became gold standard in clinical research; accounting for the effects of unmeasured confounders and selection bias by indication through randomization. Furthermore, some of the general drawbacks are omitted. A RRCT is not only much less

expensive to perform but also enrolls a broader patient population making the results more applicable to the underlying population than those from a regular RCT with narrow inclusion criteria and multiple exclusion criteria. In the present study, using the RRCT concept enabled us to recruit 6,629 patients from an all-comer, real-life population from all over Sweden in less than 3 years. Where regular large RCTs recruitment per site is generally low (estimated around 1 patient per month)[114] and challenging,[115] we achieved an average inclusion rate of 5.8 patients per month per site optimizing commitment and compliance of participating units. Lastly, using the well-established registry infrastructure of SWEDEHEART, we could reduce costs substantially (roughly 5mkr, a small fraction of the cost of a RCT of similar size)[116] with no loss to follow-up concerning the primary objective.

Another advantage to using a national registry as a trial platform is the ability to follow patients for life by merging with other health registries. A limitation relates to non-adjudicated secondary outcome events. Therefore, total mortality was chosen as the primary objective which does not require adjudication. Any degree of uncertainty in other non-adjudicated secondary outcome variables should be equally distributed over the two randomized study groups. Furthermore, the agreement between key variables in the registry and medical records has repeatedly been found to be 95% to 96% when checked by designated registry monitors in the past.[92]

In summary, RRCTs should be used complementary to regular RCTs and are most suitable for simple, but clinically relevant, issues regarding treatments already used in daily practice for which there are lack of incentives for industry sponsorship due to low potential for payback.[117] A valid hard endpoint with a high degree of completeness should be used as the primary endpoint.

Everything mentioned above considered, the assessment of the routine use of supplemental oxygen in patients with suspected AMI evaluated by mortality was ideal for an RRCT.

6.4 STUDY POPULATION WITH SUSPECTED AMI - WHY INCLUDE SO BROADLY?

In the TASTE trial,[90] only patients with STEMI were included by dedicated interventional cardiologists at the cath lab when diagnosis was confirmed. Even in the most recent trials on oxygen in AMI,[82,86] only STEMI patients were recruited by the ambulance service. There might be various explanations for this selection. Among those may be: STEMI as being considered the most acute presentation of ACS, a more homogenous pathophysiology, well-established logistics and a smaller group of caregivers involved in initial care, easier diagnosis with clear ECG findings and often pronounced symptomatology.

In the present study, we extended the concept by including a broader population with suspected AMI recruited at first medical contact (ambulance service, ED, CCU, cath lab) engaging several different professions (physicians, nurses, paramedics) in patient selection,

randomization and trial procedures. Obviously, this led to much more complicated logistics and demands for education and support of the different participating individuals and units, thus emphasizing the need for a stringent national and local trial organization. On the positive side, this design allowed us to assess patients outside the STEMI group who nowadays are more numerable and often come with a more complicated presentation and more severe long-term prognosis. Hereby, DETO₂X-AMI was intended to mirror the real-world daily situation clinicians face all over the world and allow us to give a more generalizable treatment recommendation.

Furthermore, even the clinical routine of supplying oxygen at first medical contact demanded patient recruitment this way. Otherwise, many patients would have been non-eligible due to early initiated oxygen therapy.

Moreover, it enabled assessment of a real ITT population which we believed was more correct than selecting patients as eligible at cathlab as was done in AVOID,[82,85] thereby excluding 31% of randomized patients and introducing the risk of bias.

On the negative side, we mixed the primary group of interest - AMI patients in general and STEMI patients in particular - with patients who suffered from other conditions than ACS. Planned subgroup analysis will supply more detailed data on all the groups mentioned.

6.5 OXYGEN THERAPY IN SPECIFIC RISK GROUPS

Although oxygen therapy was found to be of neutral effect in the general population with suspected AMI,[102] it is of importance to analyze groups with specific risks separately. As shown in chapter 5.2, we evaluated patients with and without confirmed AMI and different infarction types, males and females, different age groups, patients with known cardiovascular risk factors such as smokers, diabetics, patients with chronic kidney disease or anemia, and patients with higher or lower initial oxygen saturation or previous cardiovascular disease.

When assessing the primary endpoint of death within one year, the neutral results remained unchanged throughout these prespecified subgroups. These findings may suggest that oxygen, although undoubtedly a potent drug, has limited overall cardiovascular effects when administered to normoxemic patients at a moderate dose and for a relatively short time.

The most compelling subgroup is the 1,062 individuals with an initial oxygen saturation below 95% in which there was a non-significant trend to the benefit of oxygen supplementation. However, our trial was not powered to assess the ideal saturation cutoff value around 90%, and therefore, future research must determine the ideal lower limit for the initiation of oxygen therapy.

Another risk group of interest are patients with chronic obstructive pulmonary disease (COPD). As COPD is not a parameter in SWEDEHEART, it must be obtained from the

Swedish National Inpatient and Outpatient Registries with a delay of up to 12 months and is therefore not yet available.

6.6 RESEARCH IN ACUTE CARDIAC CARE: SOME ETHICAL ASPECTS CONCERNING INFORMED CONSENT

There are multiple ethical issues when performing cardiovascular research, especially in the acute setting.[118] Adequate informed consent is a fundamental part of the ethical conduct of research and historically based on the Nuremberg Code.[119] The ideal conditions under which consent should be obtained include a calm environment where thorough information can be given repeatedly, presented in writing and/or other multimedia platforms so that the patient and the responsible physician or investigator can establish a relationship of trust. Time and opportunity for questions and discussion ought to be provided generously. However, the circumstances in acute clinical trials often make it difficult to fulfill these demands.

When full informed consent cannot be obtained, two other forms of consent can be applied: abbreviated consent and delayed consent.

In our case, the ethical committee allowed us to use abbreviated informed consent (oral consent first and written confirmation as soon as possible after admission, described in detail in 4.3.2). Nevertheless, some observers had concerns that patients with ongoing acute myocardial infarction were not able to give any informed consent at all, not even in an abbreviated form, and questioned the validity of a statement given under such conditions. There is a risk that some patients may consent and subsequently withdraw, or vice versa; they may say no initially but after mature reflection may change their mind.

Unfortunately, the complete truth remains unknown as these figures were not all obtained in our trial. We know that in total, 403 (6.1%) patients changed their mind refusing to continue participation, most commonly in the oxygen group declining to continue oxygen therapy.

Further, although unconsciousness was an exclusion criterion in our trial, patients frequently suffered from multiple symptoms like pain, stress, sickness or anxiety, and some investigators might have felt that it was inappropriate to burden a patient in such a situation with even a brief discussion about a research trial on top of all the difficulties they were already facing. The number of patients regretting to have declined participation is unknown. Undoubtedly, this led to a selection of patients who were usually younger, healthier and more vocal than the general AMI population which also explains to some extent the lower event rate discussed in detail in chapter 5.2.

Another option is the delayed consent form where eligible patients are recruited without prior knowledge and asked to provide consent retrospectively during the admission period. Advantages are obvious: A faster and more comprehensive recruitment of a truly unselected study population as close as possible to the general population is achieved, providing data which can be more easily generalized to everyday patient care. On the downside, patients can

be unwilling to participate and refuse consent. A special problem can arise if a patient dies prior to being able to give or withdraw consent. Concerning this issue, some countries like the UK have introduced a special central ethics body where applications for approval for the use of clinical data from patients who died soon after presentation are evaluated. Alternatively, it can be necessary to approach relatives which can be troublesome at a time of grief discussing a complex concept that is difficult for them to grasp.

In summary, research in the field of acute cardiovascular disease is problematic. There is no concept that fits all trials, and a difficult balance between obtaining ideal data and ensuring protection of the patients' ethical demands must be kept. Clinical equipoise, defined by Freedman 1987[120] as the genuine uncertainty in the expert medical community over whether a treatment will be beneficial or not, underlies this decision. If studying two treatments already in routine use, applied within their licensed indication and dosing, both abbreviated consent and delayed consent could be considered. Delayed informed consent is currently not allowed in conscious patients in Sweden, disqualifying it as an option in the DETO$_2$X trial. However, in the latest version of the Declaration of Helsinki,[88] section 30, specific provisions are made for studies in the acute setting which might be used in the line of reasoning when applying for studies in the future. Led by the UK Health Research Authority a framework was developed which can be used as a practical guide for those developing or reviewing research in the emergency setting.[121]

6.7 CLINICAL IMPLICATIONS

When we were planning this trial series in 2010/2011, supplemental oxygen was still an essential part of supportive care to all patients with suspected AMI irrespective of oxygen saturation level. During the course of the trial, attitudes and even guidelines changed gradually to a more cautious approach concerning the use of oxygen, for example by introducing an arbitrary lower limit of <95% for commencing therapy in the ESC STEMI guidelines 2012,[8] and/or adding clinical findings such as respiratory distress.[7] However, these adjustments were still all based on expert opinion (level of evidence C).

With the DETO$_2$X-AMI study we could deliver sound evidence based on good quality data from a large randomized trial that patients with suspected AMI without hypoxemia at baseline do not benefit from routine oxygen therapy.[102] Already, some experts call for a change of clinical practice to reflect this new evidence.[122] The new has already been recognized by the ESC Task force who changed their recommendation for supplemental oxygen in the 2017 STEMI guidelines based on our trial.[123] The interest in our findings across the world has been immense and there is a good chance that other future guidelines and, subsequently, clinical practice might follow ESCs example.

6.8 REMAINING QUESTIONS AND FUTURE RESEARCH

In this thesis, we showed that routine oxygen therapy in non-hypoxemic patients with suspected AMI had no effect on either hard clinical endpoints or infarct size, nor did it affect markers of inflammation in an AMI subgroup. Although our findings do not support the general use of oxygen in the normoxemic patient with suspected AMI, there might be subgroups of patients at risk - both of hypoxemia or hyperoxemia. Further subgroup analysis and substudies from our trial might add additional information on a more detailed level.

For example, in patients with STEMI, the effects of oxygen on PCI associated outcomes are of great interest as hyperoxemia-induced vasoconstriction[45] might enhance the risk of stent undersizing and subsequent complications such as in-stent restenosis and stent thrombosis[76,77] We aim to assess these latter complications in a future subgroup analysis. Adding rehospitalization for heart failure to a composite endpoint will add power to the primary analysis and possibly clarify indications.

Nevertheless, uncertainty remains concerning the ideal cut-off to initiate supplemental oxygen, dosage and duration of therapy. These questions have not been addressed in the published trials including ours, and further hypothesis testing in new trials is warranted. For example, is there a lower threshold that we should accept in certain diagnoses, certain risk groups or accompanying clinical findings? This remains uncertain and the question is an important potential area for new RCTs, as subgroups in the trials mentioned above are unlikely to provide further insight given the enrolment oxygen saturation.

Figure 15: Illustration of potential risk-benefit ratio of oxygen treatment in acute cardiovascular conditions, including recently published RCTs and ESC STEMI guidelines
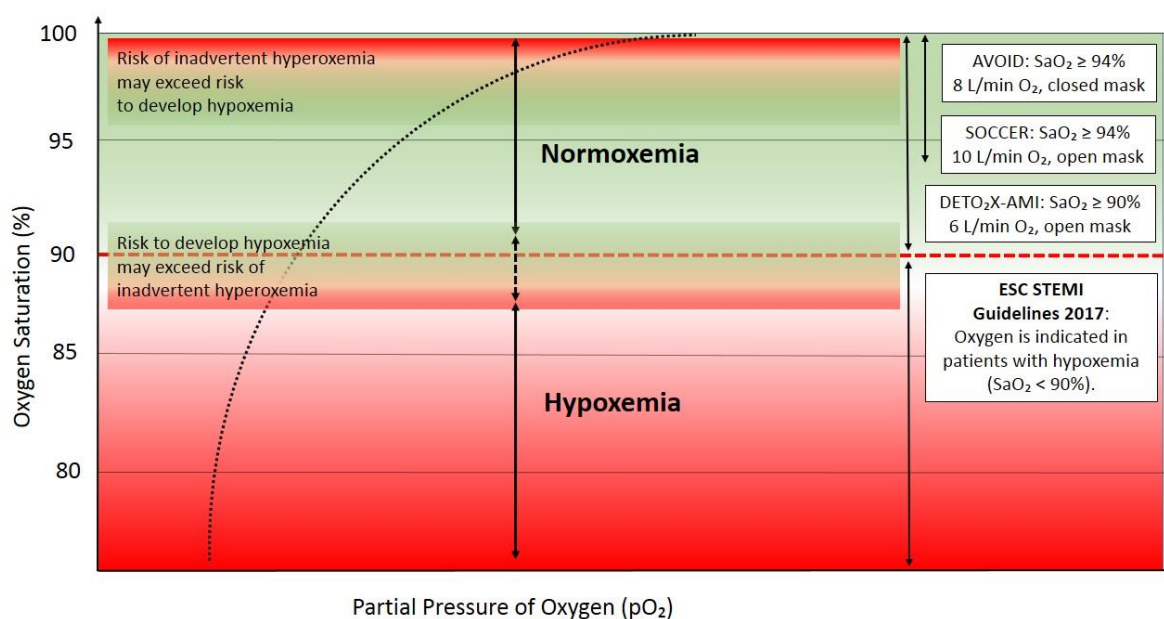
Finally, oxygen saturation is not static so further studies may want to explore a titration above some threshold rather than a fixed dose of oxygen. Automated devices are already available that may alleviate administration and liberate health care personnel but risks such as masking of clinical deterioration with desaturation as a warning sign must be considered.[124] Ranchord and others performed a RCT on titrated versus high flow oxygen but did not have the power to look at hard clinical endpoints.[81] Taking in our results, I would rather compare titrated oxygen versus ambient air. However, considering the expected low effect difference, a very large multicenter (R) RCT would be necessary which will be exceedingly difficult to perform.

Furthermore, our insights could be applied on other groups of patients. Oxygen in heart failure is already frequently discussed,[38] and several research collaborations hope to launch a trial in the near future.

In patients with cerebrovascular disease – ischemic stroke in particular – oxygen therapy has also been widely used without clear evidence. Recently, the results from the multicenter Stroke Oxygen Study from the United Kingdom were published. There, 8003 patients with acute stroke were randomized 1:1:1 to continuous oxygen for 72 hours, nocturnal oxygen for 3 nights, or ambient air. Routine prophylactic low-dose oxygen therapy given via nasal tubes at 3 liters/min did not improve outcome among patients who were not hypoxemic at baseline, regardless if oxygen was given continuously for 72 hours or at night only. This applied to the primary endpoint of 90-day functional outcome and secondary outcome measures, including early neurological recovery, mortality, disability, independence in basic and extended activities of daily living, and quality of life.[125] It is a fair guess that recommendations for oxygen supplementation in patients with acute stroke may change accordingly based on these results.

Even in critically ill patients in postcardiac arrest, sepsis, or traumatic brain injury, the importance of individualized oxygen therapy to avoid hyperoxemia has been proposed[126] and should be evaluated in future trials.

6.9 LIMITATIONS

Several limitations to the concept and our studies should be noted.

General limitations of the RRCT design are discussed above in section 6.3.

Study II used a prospective randomized open blinded endpoint (PROBE) assessment.[127] Generally, the strength of this method is that due to the lack of blinding of investigators and patients the conduct of the trial is simplified and reflects routine medical practice far better. However, this simplification comes at a price; the risk of bias remains.[128] Double blinding was not feasible for practical reasons as there is no pressurized air in Swedish ambulances. Most units use closed Hudson masks which would have put patients at risk of carbon dioxide retention if only used as sham comparator.

When planning the trial, the sources available suggested a 1-year mortality of around 12% in patients with confirmed AMI. A 20% increased risk would have resulted in a mortality of 14.4% in the group assigned to ambient air which was used for power calculations described earlier (chapter 4.6.4). The actual power to detect a 20% reduction in mortality was approximately 55%, clearly lower than the intended 90%. The main explanation for that is that we overestimated the mortality rate in the target population eligible for enrolment. There are various possible explanations for this discrepancy: First, we underestimated the risks involved with hypoxemia at presentation. Unfortunately, since we have no data on oxygen saturation in the SWEDEHEART registry, we had no possibility to estimate in our sample size calculation the proportion of AMI patients who were hypoxemic at presentation, and the mortality rate among those. These patients were excluded from our trial by design, yet they contribute substantially to the total mortality in the unselected AMI population as shown. Second, the informed consent procedure sanctioned by the ethical committee demanded oral agreement prior to study initiation. Therefore, patients with altered conscious state, comprehension difficulties or communication problems were not eligible for the trial; they constitute a larger group than expected posing a significant disease burden. This poses a common challenge as discussed above (discussion, page 43 and 44).

As evident from chapter 5.2, the AMI population not enrolled in the DETO$_2$X study had a high mortality of 16%. In fact, the one-year all-cause mortality in the combined ITT-population and non-DETO$_2$X AMI population was 13%, close to our original estimation in our power calculation. Furthermore, patients were more frequently admitted for dyspnea (8.8% vs 1.5%) or due to cardiac arrest (2.5% vs 0%). Thus, the exclusion of hypoxemic patients and patients otherwise not eligible had greater impact on one-year mortality than we anticipated. Given a one-year mortality rate of 5%, to detect a 20% reduction in mortality with 90% power would have required 16,167 patients which was not feasible to obtain.

Although we consider the point estimate of 0.97, the superimposable mortality curves and the consistent findings in all subgroup analyses indicative of a neutral effect of oxygen on mortality, we cannot with 95% confidence rule-out a positive or negative effect of oxygen on mortality of just above 20%.

The results and conclusions of study III are drawn from a prespecified subgroup analysis and should therefore be interpreted with caution. First, the proximity extension assay technology does not allow absolute quantification of the proteins obtained, and there is a lack of systematic comparison with results from standard cytokine measurement methods, like ELISA[129] or Luminex.[130] Secondly, follow-up blood samples were restricted to 5-7 h. As has been reported from experimental AMI models, an increase in IL-10 is expected to occur at a later stage of the inflammatory response.[131] We cannot exclude that oxygen treatment affects the balance between pro- and anti-inflammatory cytokines at a later stage of the systemic inflammatory response to AMI. Prolonged induction of pro-inflammatory signaling following AMI has been associated with poor prognosis.[27] However, the findings that 6-12 h oxygen therapy did not hamper the 1-year prognosis[102] may counter the assertion that supplemental oxygen has clinically relevant detrimental effects on the inflammatory response at a later time-point.

7 SUMMARY AND CONCLUSION

The design of the DETO$_2$X-AMI-trial was found to be robust, feasible and safe. Inclusion criteria managed to identify high-risk individuals with acute cardiac disease with a high proportion of acute myocardial infarctions among the study population.

Patients with suspected AMI who did not have hypoxemia at baseline did not benefit from routine oxygen therapy when assessing all-cause mortality, rehospitalization with AMI or infarct size by biomarkers. Neither did we find indication of harm when evaluating the same endpoints.

Furthermore, when studying supplemental oxygen with regard to the systemic inflammatory response to AMI, no impact on the early release of systemic inflammatory markers could be shown.

In summary, our findings do not support the general use of oxygen in normoxemic patients with suspected AMI. Nevertheless, the risk to develop hypoxemia remains which must be detected and treated immediately. Although we could not demonstrate deleterious effects of routine oxygen treatment, there might be a dose-dependent relationship, and inadvertent hyperoxemia should be avoided.

8 SVENSK SAMMANFATTNING

Bakgrund

Syrgas är sedan många år en etablerad del i behandlingen av patienter med misstänkt hjärtinfarkt. Aktuell behandlingsrekommendation enligt internationella riktlinjer, är att ge syrgas vid akut koronart syndrom för att korrigera låg syremättnad (<90%). För patienter med syremättnad ≥90% finns inga enhetliga rekommendationer då det här råder brist på vetenskaplig dokumentation. Mot bakgrund av hur utbredd syrgasbehandling är, i Sverige och i övriga världen, finns det ett stort behov att tydliggöra syrgasens roll vid behandling av misstänkt akut hjärtinfarkt.

Den bakomliggande hypotesen är att ökad syretillförsel minskar hjärtinfarktens storlek genom ökad leverans av syre till hotade områden i hjärtmuskeln. Genom begränsning av infarktstorlek minskar risken för utveckling av komplikationer som hjärtsvikt och hjärtrytmrubbningar vilket därmed kan förbättra prognosen. Befintlig vetenskaplig dokumentation ger ett visst stöd för denna hypotes, men den baseras på äldre, mindre studier.

Resultat från experimentella studier har visat att hyperoxi, alltså syremättnad över den normala, kan ha negativa effekter för hjärta och blodkärl. Detta kan t.ex. ske via kärlsammandragning, resulterande i reducerat blodflöde i kranskärlen och produktion av reaktiva syreradikaler. Dessa kan leda till en rubbad balans i kroppens antioxidativa försvar och därigenom resultera i ökad hjärtmuskelskada via cellsvullnad, programmerad celldöd och aktivering av inflammatoriska processer. Huruvida syrgasbehandling och dess effekter har betydelse för det kliniska utfallet vid hjärtinfarkt är inte tidigare studerat.

Målsättning

Syftet med denna avhandling var att bygga upp en nationell studieorganisation med hjälp av ett väletablerat kvalitetsregister inom krankärlssjukdom, SWEDEHEART. Efter att ha testat studiekonceptet i en pilotstudie (delarbete 1) utökades konceptet till hela riket (delarbete 2) för att analysera kliniska utfallsmått såsom mortalitet. I en prespecifierad substudie undersöktes syrgasbehandlingens eventuella effekter på systemisk inflammation med analys av biomarkörer (delarbete 3).

Metod och resultat

Studie I

Studie I är en pilotstudie utförd på Södersjukhuset i Stockholm, där patienter med misstänkt hjärtinfarkt rekryterades i ambulans, på akutmottagningen, på hjärtintensivvårdsavdelningen eller i samband med akut kranskärlsröntgen. Inklusionskriterier var följande: ålder minst 30 år, symtom som ingav misstanke om hjärtinfarkt (bröstsmärta eller andnöd) under kortare tid än sex timmar, en syremättnad på 90% eller mer samt antingen EKG förändringar med tecken på ischemi (syrebrist i hjärtat) eller ett blodprov med förhöjd hjärtskademarkör. Efter muntligt samtycke lottades patienterna till att antingen erhålla syrgas via andningsmask (6 L/min) i 6-12 timmar eller till att andas omgivande luft.

Under tre månader inkluderades 129 patienter med misstänkt hjärtinfarkt och normal syresättning i blodet. Av dessa hade 81 (63%) patienter hjärtinfarkt (53% icke-ST-höjningsinfarkt, 47% ST-höjningsinfarkt). Trettiotvå (25%) patienter diagnosticerades med annan akut hjärtsjukdom. Sexton (12%) patienter fick diagnosen "ospecificerad bröstsmärta".

Inga betydande logistiska eller medicinska problem förekom. Syrgasbehandlingen i 12 timmar tolererades väl. Vid 30 dagars uppföljning hade 3 patienter (4.6%) i luftgruppen avlidit, inga dödsfall hade förekommit i syrgasgruppen (p=0.12, ej signifikant).

Studie II

Med studie I som bas genomfördes studie II som en registerbaserad randomiserad klinisk studie i hela Sverige. Av de 69 sjukhus i landet som har en möjlighet att följa upp patienter med hjärtinfarkt deltog 35. Det nationella kvalitetsregistret SWEDEHEART användes för att ta fram resultat gällande studierelaterade processer. Data för det primära utfallsmåttet, total mortalitet inom ett år, inhämtades ifrån Folkbokföringsregistret.

Mellan april 2013 och december 2015 inkluderades 6629 patienter med misstänkt hjärtinfarkt. Ambulanstransport skedde i 67% av fallen. Tiden från symtomdebut till randomisering var i genomsnitt fyra timmar i båda grupperna. Av samtliga patienter som deltog i studien erhöll 75% diagnosen akut hjärtinfarkt vid utskrivningen från sjukhus.

Dödligheten under ett år var 5.0% bland patienter som behandlades med syrgas och 5.1% bland patienter som fick enbart luft, därmed påvisades ingen signifikant skillnad. Återinläggning på sjukhus på grund av hjärtinfarkt förekom i 3.8% av fallen som behandlades med syrgas och i 3.3% av fallen som behandlades med luft. Inte heller detta är en signifikant skillnad. Blodprovet med hjärtskademarkör troponin T, som är ett mått på hjärtmuskelskadans omfattning, skilde sig inte heller mellan grupperna.

Studie III

Studie III är en substudie till studie II som genomfördes på Södersjukhuset, Stockholm och på Universitetssjukhuset Linköping. Mellan november 2014 och december 2015 inkluderades 144 patienter med bekräftad akut hjärtinfarkt, varav 76 (53%) randomiserades till syrgas och 68 (47%) till omgivande luft. Blodprover togs direkt efter randomisering samt efter 5-7 timmar. Primära utfallsmått var syrgasens effekt på systemisk inflammation mätt som förändring av inflammationsmarkörer mellan de två blodproven. Med en så kallad "proximity extension assay technology", som möjliggör analyser av ett stort antal biomarkörer samtidigt, analyserades 92 olika inflammationsmarkörer.

Vid analysen av förändring mellan blodprov vid baseline och efter 5-7 timmar visade 8 markörer (IL-6, ST1A1, IL-8, VEGF-A, CD40, CCL23, PD-L1, and CSF-1) signifikant ökade nivåer och 13 markörer (TRAIL, CXCL11, TRANCE, CCL19, Flt3L, CXCL6, HGF, CCL25, CXCL10, TWEAK, CXCL9, IL-10, and OSM) signifikant reducerade nivåer.

Medianvärdet för hjärtskademarkören, högkänsligt troponin T, var 52.9 ng/L hos patienter i syrgasgruppen och 63.3 ng/L hos patienter i luftgruppen. Efter 5-7 timmar ökade värdet till 1436 ng/L och 1574 ng/L i respektive grupperna, ledande till en medianskillnad på 1403 ng/L och 1238 ng/L (p= 0.696), en icke-signifikant skillnad.

Efter justering för den relativa ökningen av troponin T över tid samt ålder och kön som potentiella störfaktorer förelåg ingen skillnad i den systemiska inflammationsreaktionen mellan de som fick syrgasbehandling och de som fick omgivande luft.

Sammanfattning och slutsatser

Studie 1 kunde genomföras utan att större logistiska eller medicinska problem uppkom och studiekonceptet bedömdes genomförbart och säkert. Inklusionskriterierna identifierade i hög utsträckning hjärtinfarktpatienter som kunde rekryteras i studien. En välfungerande hemsida som studieplattform för kommunikation och utbildning utvecklades.

Studie 2 genomfördes som en pragmatisk, nationell, registerbaserad, randomiserad klinisk studie. Vi fann inga gynnsamma eller negativa effekter av syrgasbehandling med avseende på dödlighet under ett år, återinläggning för hjärtinfarkt eller utbredning av hjärtmuskelskada.

I studie 3 undersökte vi om syrgasbehandling har effekt på den systemiska inflammationsprocessen utlöst av en akut hjärtinfarkt. Vi fann inga tecken till att syrgasbehandling påverkar denna reaktion.

Sammanfattningsvis finner vi inga belägg för att syrgasbehandling är gynnsam eller skadlig för patienter utan hypoxi (syrebrist) vid akut hjärtinfarkt. Därmed finns det nu underlag för att sluta använda syrgasbehandling i denna patientpopulation vilket redan har lett till en

uppdatering i europeiska behandlingsrekommendationer för patienter med akut ST-höjningsinfarkt.

Behovet av monitorering av syremättnad kvarstår dock då hypoxi kan tillkomma under akutskedet vid hjärtinfarkt och ska då behandlas utan dröjsmål. Även om negativa effekter av hyperoxemi ej kunde påvisas utesluter detta ej att det kan föreligga risker med större mängder syrgas, varför detta bör undvikas.

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