Biomarkers for Risk Prediction in Cardiac Surgical Patients

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“Biomarkers will make a bad doctor worse and a good doctor better”

- Dr. Alan S. Maisel

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Preface

Ancient surgery can be traced back to 6500 Before the Common Era when trepanning was used in France to cure diseases with intracranial origin. [1] On September 4th 1895, the Norwegian surgeon Axel Hermansen Cappelen was credited with the first surgery on a human heart, after a patient was stabbed in the chest resulting in a damaged left ventricle. [2] In 1925 the first open heart surgery was conducted with correctional surgery on a damaged mitral valve, [3] and in 1964 the first successful saphenous vein coronary artery bypass surgery was performed. [4] Three years later, in 1967 the first successful heart transplant was performed in Cape Town, South Africa, by doctor Christian Barnard. [5, 6] These are extracts of the cardiac surgical evolution, and in the years to come, rapid developments in cardiac surgery and anesthesia have enabled surgical procedures assumed impossible only decades earlier.

The developments in cardiac surgery have had remarkable impact on the life of a great number of patients. However, reward always needs to outweigh risk, and perioperative mortality has always been a great concern with cardiac surgery. To assist in preoperative risk calculations and to prevent unnecessary deaths, several models for assessing the risk of death have been developed. [7-11] After their implementation in cardiac surgery during the last three decades, these risk stratification models have contributed to lower perioperative mortality by enabling early preventive measures in high-risk patients. [8] The European System for Cardiac Operative Risk Evaluation (EuroSCORE) II model is widely used, especially in Europe, and it can give valuable information together with clinical assessment.

A biological marker, or a biomarker, is an objectively measured indicator of the presence or the severity of a medical state. [12] Biomarkers have been found to reflect specific pathophysiology, [13, 14] thereby providing additional information to clinical information and examination. Biomarkers that are measurable in blood, referred to as circulating biomarkers, are the topic of my Ph.D. thesis and will be discussed later in the thesis. The circulating biomarkers are a fairly new addition to the medical adventure and started to evolve in the 1950s. Aspartate Transaminase was the first biomarker to diagnose acute myocardial infarction (AMI) in the 1950s. [15] In 1954 an assay for C-reactive protein (CRP) became available, and it was reported that CRP was detectable in patients with chronic heart failure (HF). [16] In 1991 cardiac troponin T (cTnT) was introduced to diagnose AMI as it was a more sensitive protein with higher specificity to cardiac muscle than the previously used circulating cardiac biomarkers. [17] In the same year, B-type natriuretic peptide (BNP) was recognized for its origin in the cardiac ventricle and increased concentrations in patients with HF. [18] The performance of N-terminal pro-B-type natriuretic peptide (NT-proBNP) as a

biomarker in patients with cardiac impairment and decompensation was recognized in 1997. [19]

Old and seasoned biomarkers are improved with new and more sensitive assays, which make them highly valuable also today. High-sensitivity cardiac troponin T (hs-cTnT) and I (hs-cTnI) have improved early detection of AMI,[20] and dynamic troponin elevation is an obligate criterion for the diagnosis of AMI today. [14] BNP and NT-proBNP are equally good as the clinicians' interpretation in the diagnosis of HF, [21, 22] also in small general hospitals. [23] New biomarkers, like secretoneurin (SN), evolve rapidly and new biomarkers are almost daily introduced in research articles. However, very few new biomarkers will be introduced into clinical practice. I believe the biomarker adventure has just begun, and that biomarkers will be more important and have a principal role, both for diagnosis and prognosis, in future medicine. Together with risk stratification models and good clinical knowledge, biomarkers can be valuable assets and give important information when used correctly.

Acknowledgements

Some say things in life happen for a reason, and I´m not going to challenge their assertion. However, I believe life evolves as you go. I didn't have a childhood dream of becoming a doctor, nor a researcher. I wanted to become a pilot in the Norwegian Air Force for as long as I can remember. Before Top Gun came to the movie theaters and everyone started to wear jeans, white t-shirts, leather jackets, and Ray-Ban Aviators I had flown countless F-16 missions in a plastic box propelled by my parents, while I was wearing a helmet and ski goggles. The dream of becoming a pilot was broken with the blink of an eye by an ophthalmologist halfway through second round of admission tests in the Norwegian Air Force. So, what to do next?

A couple of years later the officer candidate school opened my eyes to medical service, and in the continuation of this, medical school. I never planned on going into research after medical school. As a matter of fact, before starting on my student project in medical school I was looking for a way to do it that didn't involve research. But that was before I met **Helge Røsjø**, and this meeting changed my medical path. As an eager up and coming research fellow and the teacher of my problem-based learning group in 2010, Helge "tricked" me to write my research paper in the Cardiovascular Research Group with **Professor Torbjørn Omland** and himself as my supervisors. I have not regretted this decision. I have been very fortunate to have Associate Professor Helge Røsjø and Professor Torbjørn Omland as my supervisors for this Ph.D.-project. They are both highly recognized and merited in their fields of research and have published their work in leading journals [21, 24-31]. Helge has been the leader of the Department of Research at Akershus University Hospital (Ahus) and is now the new Director of Research and Innovation at Ahus. Torbjørn was in 2019 awarded with the Heart Research Award from the National Association of Public Health handed by his Majesty the King of Norway. Helge is a member of the editorial board of the highly ranged Circulation Journal, and Torbjørn is an associate editor in the same journal, which together with their other merits reflect their high-quality work and achievements. Together with their staff and Ph.D.-students they have brought the Cardiovascular Research Group to outstanding achievements in a rich blend of research questions and modalities. [26, 31-35] I am grateful and honored to have them as my supervisors, and very thankful for everything they have taught me so far during my time in their group.

The Cardiovascular Research Group would not have been what it is today without the study personnel that perform the core operations every day, and their hard work and contributions are greatly appreciated. I would like to give a special attention to the study nurses **Annika Lorentzen** and **Vigdis Bakkelund**, and bioengineer **Marit H. Pedersen** for everything they have taught me in the lab and for their warm welcome on my arrival to the group in January 2013.

This Ph.D.-project would not have been possible without my collaborators in Finland. Their work on collecting and organizing data is impressive, and I owe my sincere gratitude to all of the

involved personnel in the FINNish Acute Kidney Injury (FINNAKI) Study and the FINNish Acute Lung Injury (FINNALI) Study. I especially want to acknowledge **Liisa Petäjä** and **Professor Ville Pettilä** for all their help and constructive feedback.

To the people involved in the aortic stenosis study at Oslo University Hospital, Rikshospitalet, I owe you my greatest gratitude for the study you have performed, and that I have had the opportunity to use this as part of my Ph.D. project. I would especially like to thank the leader of the group and Chief of Cardiology at Rikshospitalet, **Professor Thor Edvardsen**, for letting me use the material and for valuable feedback on my work.

Without the work of **Mats Stridsberg**, Uppsala, Sweden this Ph.D. project would not have been feasible. The SN-/granin analyses done by him have been essential for this project and I am forever grateful. **Anett H. Ottesen** has been a pioneer in the field of SN with publications in high-ranked international journals. Her basic research work has been crucial for my SN papers and I am sincerely grateful for the work she has done and the help she has given me.

To my fellow Ph.D.-students, I am forever grateful for our discussions, both on and off topic. Our prolonged lunches with statistical FAQs, and especially the camaraderie at "the cabin" have been greatly appreciated. Because of all of you, a long day of writing and staring into the light of the MacBook has been feasible. A special thanks goes to **Magnus N. Lyngbakken** and **Peder L. Myhre** for all the help with editing and proof-reading manuscripts and valuable statistical discussions.

Finally, and most importantly, to those who have made me to who I am, and gotten me to where I am, my dearest family. A sincere thanks to **my family** for backing me through school, pushing me to achieve top grades, dreaming big, and getting past broken fighter pilot dreams. Allowing me move to New York as an 18-year-old boy from the Norwegian countryside to get new experiences other than tractors and chainsaws, and to seek new opportunities. From the day I met my wife **Kristine**, she has been my steady rock. She deserves all the gratitude a person can get for standing by my side. Being the person that drags me back to earth when my ideas and adventures get too far-fetched, but also supporting me through everything I do. Our daughter **Theoline** for always spreading joy with her happy personality, getting me up early in the morning, not missing a ray of sunrise in the past five years, for our calm mornings watching cartoons, and early morning trips to the bakery every Saturday. And finally, our always smiling, giggling, and exploring son **Kristian**, for joining this crazy family of three and making us complete.

Jon Brynildsen
Bjørkelangen, Norway, December 6, 2019

Abbreviations

Ahus	Akershus University Hospital
AMI	acute myocardial infarction
ANP	atrial natriuretic peptide
AS	aortic stenosis
AUC	area under the curve
BNP	B-type natriuretic peptide
CABG	coronary artery bypass graft
CAD	coronary artery disease
CgA	chromogranin A
CgB	chromogranin B
CNP	C-type natriuretic peptide
COPD	chronic obstructive pulmonary disease
CRP	C-reactive protein
CT	computed tomography
cTn	cardiac Troponin
cTnT	cardiac Troponin T
CV	coefficient of variation
CVD	cardiovascular disease
DNP	dendroaspis natriuretic peptide
ECG	electrocardiogram
EuroSCORE	European System for Cardiac Operative Risk Evaluation
FINNAKI	FINNish Acute Kidney Injury
FINNALI	FINNish Acute Lung Injury
HF	heart failure
HR	hazard ratio
hs-cTnT	high-sensitivity cardiac Troponin T
hs-TnT	high-sensitivity Troponin T
ICU	intensive care unit
LAD	left anterior descending
LIMA	left internal mammary artery
LoB	limit of blank
LoD	limit of detection
LV	left ventricular
MRI	magnetic resonance imaging
NSTEMI	non-ST-elevation myocardial infarction
NT-proBNP	N-terminal pro B-type natriuretic peptide

NYHA	New York Heart Association
PCI	percutaneous coronary intervention
RAAS	renin-angiotensin-aldosterone system
RIA	radioimmunoassay
ROC	receiver operating characteristics
SAVR	surgical aortic valve replacement
Sg	secretogranin
SN	secretoneurin
STEMI	ST-elevation myocardial infarction
SYNTAX	the Synergy between Percutaneous Coronary Intervention with TAXUS and Cardiac Surgery
TAVR	transcatheter aortic valve replacement

List of scientific papers

I **The predictive value of NT-proBNP and hs-TnT for risk of death in cardiac surgical patients**

Jon Brynildsen, Liisa Petäjä, Ville Pettilä, Ståle Nygård, Suvi T. Vaara, Rita Linko, Marjatta Okkonen, Tor-Arne Hagve, Leena Soininen, Raili Suojaranta-Ylinen, Magnus Nakrem Lyngbakken, Torbjørn Omland, and Helge Røsjø.

Clin Biochem. 2018;53:65-71. doi:10.1016/j.clinbiochem.2018.01.012

II **Circulating secretoneurin concentrations after cardiac surgery: data from the FINNAKI Heart Study**

Jon Brynildsen, Liisa Petäjä, Peder L. Myhre, Magnus N. Lyngbakken, Ståle Nygård, Mats Stridsberg, Geir Christensen, Anett H. Ottesen, Ville Pettilä, Torbjørn Omland, and Helge Røsjø.

Crit Care Med. 2019; May;47(5):e412-e419. doi: 10.1097/CCM.0000000000003670

III **Circulating secretoneurin concentrations in patients with moderate to severe aortic stenosis**

Jon Brynildsen, Peder L. Myhre, Magnus N. Lyngbakken, Ståle Nygård, Mats Stridsberg, Geir Christensen, Thor Edvardsen, Torbjørn Omland, Helge Røsjø.

Clin Biochem. 2019 Jun 19. pii: S0009-9120(19)30324-8. doi: 10.1016/j.clinbiochem.2019.06.008

Summary

Patients who undergo cardiac surgery constitute a heterogeneous group with increased short- and long-term mortality compared to the general population. [36-38] With an aging population and more elderly undergoing high-risk cardiac surgery, [39, 40] a demand for accurate risk prediction is emerging. Biomarkers have been shown to predict risk of mortality in cardiac surgery, [41-43] and there are many models available for risk prediction in cardiac surgery that combine a variety of individual risk factors. [7-9]

In order to investigate an accurate way of predicting risk in patients undergoing cardiac surgery, we tested our hypotheses in cardiac surgical cohorts. In **paper I**, we tested the hypothesis that the established cardiac biomarkers cTnT and NT-proBNP would improve the EuroSCORE II risk model. We also wanted to develop a parsimonious risk model of biomarkers and clinical variables that would provide comparable prognostic information as EuroSCORE II. In **paper II**, we explored the novel cardiac biomarker SN in the same cohort from the FINNAKI Heart Study and in **paper III** we tested SN in a cohort of patients with aortic stenosis.

Through the work presented in the three papers we demonstrate substantial additional prognostic information by circulating cardiac biomarkers to established risk models and circulating cardiac biomarkers in patients either undergoing or being evaluated for cardiac surgery. We also show that it is possible to simplify risk models by adding biomarker measurements without losing prognostic information in cardiac surgical patients.

1 Introduction

1.1 Coronary artery disease

The heart consists of three main layers from the inside to the outside; the endocardium, the myocardium, and the epicardium, and is encapsulated by a fibrous sac called the pericardium with a small amount of fluid between the two pericardial layers. The myocardium is the muscle responsible for the contractions of the heart. The coronary arteries supply the myocardium with blood, and normally the coronary arteries start as two main arteries, originating from the basis of the aorta, branching into a network of smaller arteries as it enfolds the heart. As early as 1876, Adam Hammer described the pathophysiology of coronary artery disease (CAD) as interrupted blood flow in coronary arteries, with myocardial infarction occurring if a minimum of one coronary artery was occluded. [44]

CAD is also known as ischemic heart disease, and includes stable angina pectoris, unstable angina pectoris, non-ST-elevation myocardial infarction (NSTEMI), and ST-elevation myocardial infarction (STEMI). Chest pain is the principal symptom in patients with CAD, but the disease may also present with other symptoms like dyspnea, fatigue, dizziness, nausea, vomiting, and rapid heartbeat. Moreover, some patients experience ventricular arrhythmia and sudden cardiac death as the first manifestation of CAD. [45] The formation of atherosclerotic plaque will over time lead to narrowing and potentially occlusion of one or more segments of the coronary arteries, which again will result in decreased blood flow, myocyte ischemia, and symptoms (Figure 1). [46]

CAD can be chronic like stable angina pectoris, which recently was re-named to chronic coronary syndrome, [47] with chest discomfort during exercise or physiological stress. Normally these patients have stable obstructive atherosclerotic plaques in wall of the coronary arteries. [46] CAD can also be acute with sudden chest pain due to plaque rupture, erosion or intraplaque hemorrhage and subsequent acute thrombosis formation, which results in total or subtotal occlusion of a coronary artery with subsequent myocyte necrosis. [48] Patients with acute exacerbation are referred to as having acute coronary syndrome. Today, cardiac troponin T or I measurements in peripheral blood can detect myocyte necrosis, and therefore cardiac biomarker measurements are used all over the world to diagnose acute myocardial infarction. [14]

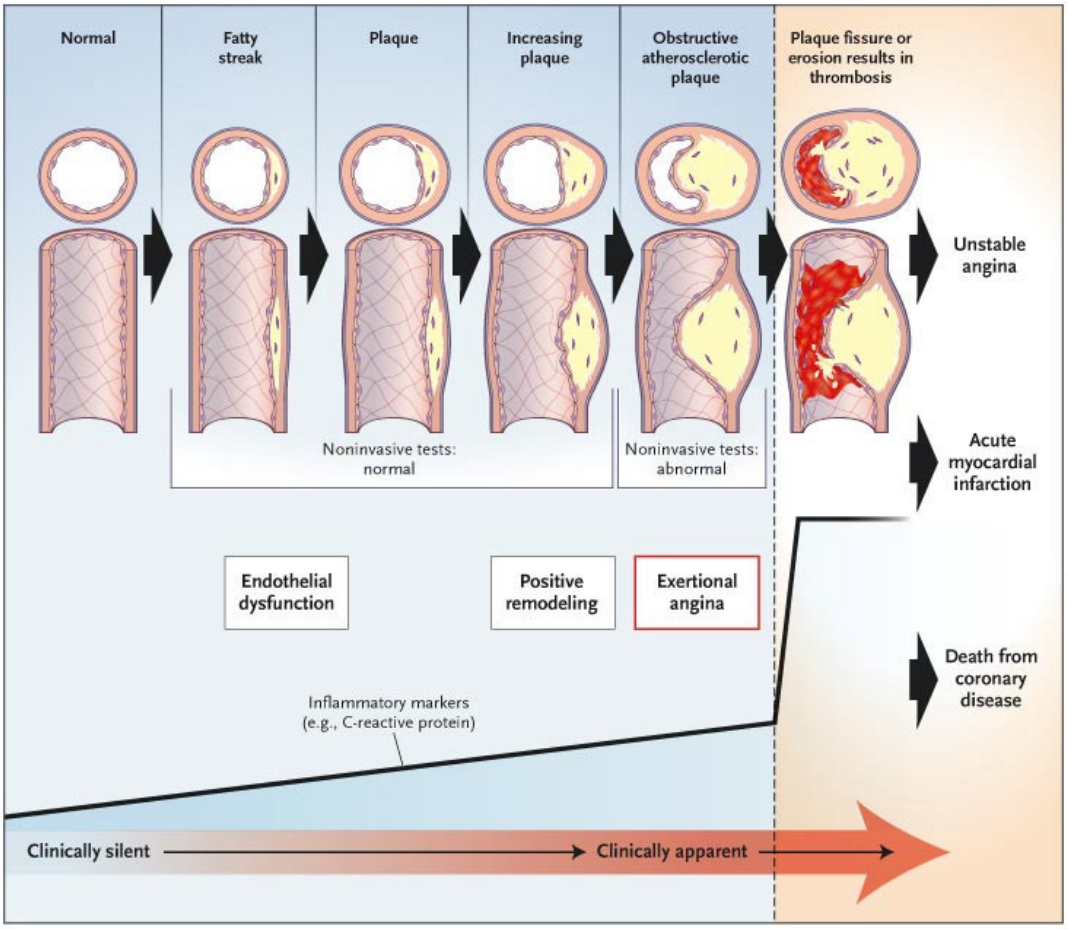

Figure 1. *Typical progression of coronary atherosclerosis. Reproduced with permission from Abrams.* [46] *Copyright Massachusetts Medical Society.*

Risk factors for CAD can be nonmodifiable like age, sex, genetics, ethnicity, and race, and modifiable like high blood pressure, high blood cholesterol, smoking, type 2 diabetes mellitus, obesity, excessive alcohol consumption, lack of exercise, and poor diet. [48, 49]

CAD can be prevented and/or stabilized with preventive medication and opposing risk factors by lifestyle changes, and treated with medications and invasive procedures such as percutaneous coronary intervention (PCI) and coronary artery bypass grafting (CABG). [49, 50] Newly released results from the ISCHEMIA trial at the American Heart Association conference in November 2019 show that there is no difference in heart attack and death when comparing state-of-the-art pharmacological therapy with PCI in a population with stable CAD. This has also previously been shown in the COURAGE trial comparing PCI and optimal medical treatment to optimal medical treatment alone, [51] and in the ORBITA trial when comparing PCI with sham-PCI and optimal medical treatment. [52]

1.1.1 Treatment options for coronary artery disease

In patients with symptoms of CAD, non-invasive (e.g. computed tomography (CT) coronary angiography) or invasive examinations like coronary angiography are performed to confirm or rule out the diagnosis of CAD, and identify the culprit lesion(s). [53] Coronary angiography can also be used as an aid to decide treatment; CABG surgery, PCI, or medical treatment. [53, 54] Images obtained during the angiography will help the surgical team prior to and during cardiac surgery. [55] Coronary angiography is an invasive procedure with preferably radial access, alternatively femoral access, where a catheter is moved into the coronary arteries to visualize the coronary arteries by the use of X-ray visible dye. [56]

To make the correct decision regarding treatment in patients with CAD, physicians and patients should weight risk over benefit for the different options; CABG surgery, PCI, or medical treatment. [50] Patients with persisting symptoms while on medical treatment for stable CAD have indication for revascularization. [50] Invasive treatment have traditionally also been considered to save lives in selected patients with chronic coronary syndrome, including patients with proximal lesions in important coronary arteries, patients with multi-vessel disease, and patients with high total risk (due to comorbidities or HF). [47] However, some recent articles have challenged whether this assumption is correct for all high-risk groups, including the recent ISCHEMIA study that did not find benefit by invasive therapy over state-of-the-art pharmacological therapy in patients with stable CAD. Of note, the ISCHEMIA study did not include patients with left main CAD, and for patients with left main disease there is consensus that invasive therapy is indicated, and that CABG seems to reduce mortality over PCI. [57] Hence, invasive therapy reduces mortality in patients with chronic coronary syndrome and left main coronary artery disease, but the impact on mortality is less clear for patients with stenosis outside of the left main coronary artery. In contrast, revascularization is clearly indicated in patients with acute coronary syndrome due to acute plaque rupture or hemorrhage. [50] In patients with STEMI, primary PCI is the preferred reperfusion strategy if possible to perform within 90 minutes, otherwise thrombolysis is the preferred option in STEMI. [50] Patients with NSTEMI and selected patients with unstable angina pectoris should also be treated with invasive coronary artery revascularization, and the urgency for revascularization should be decided based on the diagnosis and the total risk of the patient.[50] Of note, approximately 20% of patients with total occlusion of the left circumflex artery will present without ST elevation on the ECG, [58] and these patients should receive immediate invasive treatment. Patients with established bundle branch block (especially left bundle branch block) and patients who are totally dependent on cardiac pacing, i.e. pacemaker with 100% ventricular pacing, are also difficult to assess concerning the need for immediate revascularization as the ECG normally cannot be used for diagnosis in these patients.

For CABG the risks of periprocedural complications, as assessed by EuroSCORE II,[8] the Society of Thoracic Surgeons' cardiac surgery risk model,[10] or other models should be weighed against improvements in quality of life and anticipated reductions in morbidity and mortality. [50] Whether to choose CABG or PCI should be based on the localization and number of vessels diseased, the anticipated completeness of revascularization, and comorbidities like diabetes mellitus who favor CABG and other, serious illnesses that may favor PCI in patients with high assumed perioperative surgical risk. [50] Hence, a tool to help guide decision making between CABG and PCI is the Synergy between Percutaneous Coronary Intervention with TAXUS and Cardiac Surgery (SYNTAX) II score, which is based on anatomical and clinical factors and is useful to predict 4-year mortality. [59] Patients with left main coronary artery stenosis have generally been considered to have lower 4-year mortality prediction with PCI in the low and intermediate SYNTAX score tertiles and with CABG in the high SYNTAX score tertile. [59] For patients with three-vessel disease, lower 4-year mortality is seen with CABG in all three tertiles compared to PCI. [59] Current guideline on treatment of stenosis on the left main coronary artery and three-vessel disease with and without diabetes mellitus in stable CAD recommends CABG as a class I level A recommendation for all SYNTAX score tertiles, while left main coronary artery stenosis and three-vessel disease without diabetes for the lowest SYNTAX score tertile also has a class I level A recommendation for PCI. [50] The EXCEL trial did not find significant difference between PCI and CABG with regard to death, stroke, or myocardial infarction at 5-year follow-up in patients with left main CAD and low or intermediate anatomical complexity defined by the SYNTAX score, however, CABG seemed to reduce mortality over PCI. [57] In the EXCEL trial, patients with BNP concentrations below the cutoff of 100 ng/L seemed to benefit from PCI treatment, while patients with BNP concentrations above this cutoff seemed to benefit from CABG. [60] As they did not find significant difference between PCI and CABG in this trial, this intriguing result for BNP show the possible use of circulating biomarkers in personalized medicine. [61]

In other studies and meta-analyses, CABG and PCI have been found to be associated with similar 1-year mortality in patients with multi-vessel CAD and with 5-year mortality in patients with left main coronary artery disease. [62, 63] PCI has shown increased 5-year mortality compared to CABG in patients with multi-vessel disease. [62] However, patients undergoing PCI have less bleeding, blood transfusion, wound infections, and arrhythmias compared to patients being treated with CABG, [64] which should be taken into consideration. Patients treated with PCI also experience less cerebrovascular incidents, [62] but have increased need for repeat revascularization compared to patients treated with CABG. [62, 63] Diabetic patients with multivessel CAD have increased 5-year mortality and risk of AMI

when treated with PCI compared to CABG, [62, 65] and these differences between subgroups reflect the importance of multidisciplinary evaluation by a Heart Team of cardiologists and cardiac surgeons before deciding treatment and revascularization strategy.

With increasing lesion complexity, the mortality benefit of CABG over PCI increases. [65] Today, patients with stenosis in the left main artery and 3-vessel disease are normally considered for CABG as recommended by current guidelines [50] and these patients are normally discussed in the multidisciplinary Heart Team prior to the selection of therapy. Other factors that favor CABG over PCI are highly complex lesion as these patients often need repeat revascularization due to unsuccessful revascularization and therefore residual angina after PCI, while patients with assumed high stroke risk or high age normally are treated with PCI as this procedure has less serious early complications like arrhythmias, stroke, bleeding, and wound complications, as well as faster recovery time. [66]

1.1.2 Medical treatment

Lifestyle changes and medical treatment to reduce risk factors constitute the basis for all treatment of CAD. Lifestyle changes include smoking cessation, eating healthy and having a physical active lifestyle. Medical treatment for CAD can be initiated prior to symptoms (primary prophylactic) in patients considered at high risk of developing CAD or secondary prophylactic in patients who already have established CAD. [67] Targets for treatment include lowering of total cholesterol and low-density lipoproteins with statins and other lipid lowering agents, preventing formation of blood cloths with single or dual antiplatelet therapy, and blood pressure lowering with medications like beta-blockers, calcium antagonists, angiotensin-converting-enzyme inhibitors, and angiotensin II receptor blockers. [49] Symptomatic patients should also use nitroglycerin to alleviate chest pain as nitroglycerin will dilate the coronary arteries. [68] Treatment for CAD also includes optimization of therapy for associated comorbidities, and especially outcome for patients with CAD and type 2 diabetes mellitus treatment has recently been improved with the development of new drugs. [69, 70]

1.1.3 Percutaneous Coronary Intervention

PCI is a catheter-based method used to treat arterial stenosis or occlusions, including in the coronary arteries of the heart when a patient suffers from CAD. [50] Using the Seldinger technique, after local anesthesia, the artery is first punctured with a needle and later a guidewire is introduced through the lumen of the needle. [71] The needle is then removed, a skin incision is done, and the tract is dilated prior to the introduction of a sheath or blunt cannula into the artery by treading it over the guidewire. With the introducer in place, the guidewire is removed and the sheath or blunt cannula is secured to the skin. The physician

then has access to the artery and can move a catheter into the artery (radial or femoral). For coronary angiography radial access is now recommended in most situations to reduce the risk of major bleeding and later arterial pseudo aneurism. [50, 72] During coronary angiography the physician moves the catheter to the proximal aorta and identifies the orifices of the coronary arteries by the use of contrast media and X-ray visualization (coronary angiogram). If the angiogram identifies a stenosis that is deemed significant (≥50% diameter obstruction of the lumen), the physician can inflate a balloon at the tip of the catheter to directly open the blocked area of the artery. Atherectomy is also an option, removing plague at the narrow site, however, this procedure has not been found to improve 30-day or 1-year outcome over angioplasty with a balloon alone. [73, 74] To ensure that the narrow area stays open, a stent is normally expanded after balloon angioplasty. There are two types of stents; bare metal stents and drug-eluting stents that are coated with an anti-proliferative drug to prevent restenosis. Drug-eluting stents require longer double anti-platelet therapy to avoid early stent thrombosis, but has lower prevalence of restenosis and is therefore the preferred choice in most angiography laboratories today. [50]

1.1.4 Coronary Artery Bypass Grafting

CABG is an open-heart surgery performed through a midline incision entering the thorax cavity through the sternum (sternotomy). [55] Normally, uncomplicated CABG procedures last three to four hours. [55] The purpose of CABG surgery is to bypass an occluded segment of the coronary artery by one or more *in vivo* conduits. The saphenous veins and the left internal thoracic artery, also known as the left internal mammary artery or LIMA, are the most commonly used conduits (Figure 2). LIMA has longer patency and is used for stenosis in the left main coronary artery and the left anterior descending artery. [75] The in-situ graft like LIMA will only be attached to the coronary artery distal of the stenosis with an end-to-side anastomosis, while the proximal end of the graft will have its native origin and blood flow (Figure 2). Saphenous veins are also used as grafts to bypass occluded segments and are harvested during surgery from the patient's leg or thigh. [76] The bypass graft is then sewed on with an end-to-side anastomosis before attached with a similar method to the proximal ascending aorta distal to the stenosis (Figure 2).

To optimize the working conditions during surgery, the beating heart is arrested with a cold cardioplegia solution containing high concentrations of potassium. [55] A body with an arrested heart needs perfusion pressure and oxygen to keep the body alive during cardiac arrest. The need for perfusion and oxygen is supplied by a cardiopulmonary-bypass machine that pumps oxygenated blood throughout the sedated body, so called "on-pump" CABG. [55]

Despite CABG surgery being performed for many decades, it is still a high-risk procedure with increased risk of peri- and postoperative complications and peri-and postoperative mortality. [77] Frequently seen complications are arrhythmias, cardiac tamponade, pulmonary embolism, pneumonia, wound infection, paralytic ileus, stroke, acute renal failure, and also death. [77]

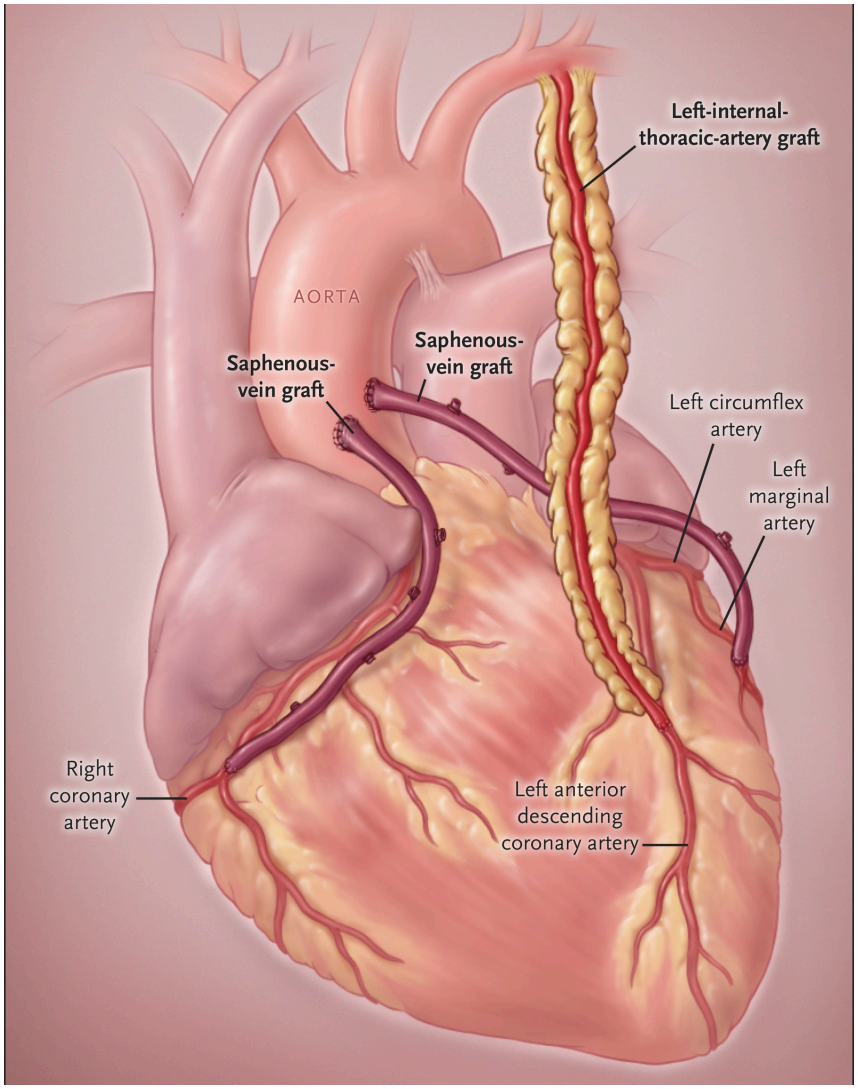

Figure 2. *Coronary-Artery Bypass Grafting. Shown are a left-internal-thoracic-artery graft to the left anterior descending coronary artery and saphenous-vein grafts to the left marginal and right coronary arteries. Reproduced with permission from Alexander.* [55] *Copyright Massachusetts Medical Society.*

However, so-called off-pump coronary artery bypass is an alternative to "on-pump" CABG, and this technique is considered to have less harmful effects on the body. A Cochrane review from 2012 [78] concluded with increased mortality in patients with off-pump coronary artery bypass surgery compared to on-pump CABG. Other large randomized trials have concluded with no difference in 30 day [79] or 1-year [80] outcomes regarding death, nonfatal stroke, nonfatal AMI, or nonfatal renal failure requiring dialysis. There were no significant benefits of off-pump coronary artery bypass regarding risk of stroke and myocardial infarction, while

there was less atrial fibrillation among off-pump coronary artery bypass patients after surgery. [78]

CABG surgery was previously the preferred method in CAD revascularization, while newer guidelines recommend PCI in one- and two-vessel CAD, and in patients with low SYNTAX score without diabetes mellitus. [50] This change in guideline recommendations can account for the decrease in CABG surgeries and the 60% increase in PCI in Ontario, Canada, from 2001 to 2005. Similar trends are reported also elsewhere, including more recent data like the decrease in CABG surgery and the increase in PCI for NSTEMI patients between 2001 and 2009 in Denmark. [53, 81] With the evolution in surgical techniques, this trend will likely increase as catheter-based cardiology and minimally invasive cardiac procedures become more available. [82]

1.1.5 Minimally invasive and hybrid procedures

Minimally invasive coronary surgery and hybrid coronary revascularization are less invasive methods than CABG, [83] and may be better options for selected patients with multi-vessel coronary artery disease. [84] Minimally invasive coronary surgery by using the LIMA as a graft to bypass a stenosis or block in the left anterior descending (LAD) artery and the harvested right radial artery to bypass stenosis in smaller coronary arteries without sternotomy, is as safe and effective as CABG, both on- and off-pump. [85, 86] This minimally invasive coronary surgery reduces length of post-operative stay, [86] gives an early quality of life benefit, [87] and less postoperative wound infections, [86] however, increased pain the first postoperative days due to spreading of ribs is common.[87] Hybrid coronary revascularization is the combination of minimally invasive coronary surgery with PCI on non-LAD vessels. [88] Hybrid coronary revascularization can be done consecutively, or on separate occasions from hours to weeks apart. [83] Hybrid coronary revascularization does not have significant lower 1- and 5-year mortality, nor AMI, stroke, major bleeding, or repeated revascularization compared to CABG. [85, 88, 89] Minimally invasive and hybrid procedures are less comprehensive surgeries with few complications and shorter recovery time, which might give patients with high comorbidities an option to perform heart surgery.

1.2 Valvular heart disease

The heart has four valves; the tricuspid and the pulmonal valve on the right side, and the bicuspid (mitral) and the aortic valve on the left side (Figure 3). Valvular heart disease can be congenital or acquired, and it can be due to physiological processes in pregnancy or diseases such as rheumatic disease (mitral stenosis), hypertension (e.g. aortic stenosis (AS)), infections (e.g. endocarditis), myocardial infarction (e.g. mitral regurgitation) and more. [90]

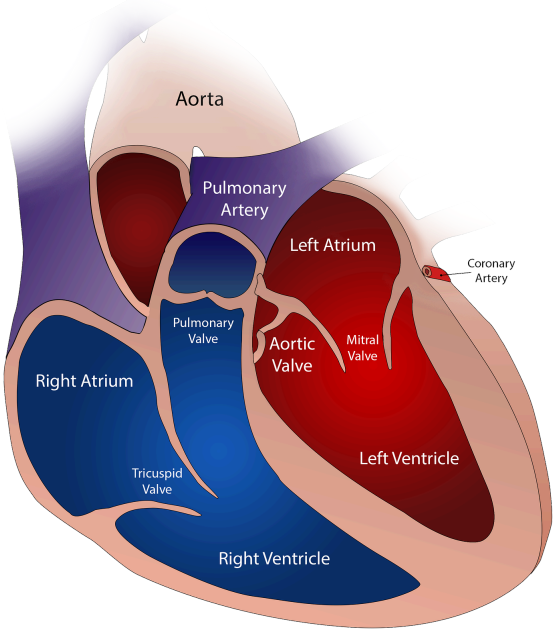

Figure 3. *Illustration of chambers and valves of the human heart. Licensed under CC0 on Pixabay.*

Symptoms from valvular heart disease is often recognized late in the course of the disease due to slow progression and gradually limitations on the patients' daily activities. [91] Symptoms and clinical signs of valvular heart disease are related to HF, such as fatigue, shortness of breath during exercise and when lying down, swelling of ankles and legs, dizziness, fainting, and irregular heartbeat. Valvular heart disease can be diagnosed by the heart murmur, which is detectable during auscultation, although the diagnosis needs to be verified by appropriate imaging methods. [90]

Many modalities are involved in diagnosing and assessing the severity of valvular heart disease. Investigation with electrocardiogram (ECG) to diagnose cardiac arrhythmias and chest-X-ray to assess lung pathology are helpful in the initial stages. [91] Standard transthoracic echocardiography constitutes the most widely used imaging method to diagnose valvular heart disease [85] but cardiac CT, and magnetic resonance imaging (MRI) [91, 92] can also be part of the diagnostic work up, especially CT of the proximal aorta and stenotic aortic valve in patients referred for Transcatheter Aortic Valve Replacement (TAVR). [93] In

elderly patients, impairment in functional capacity is assessed by aerobic exercise testing or a 6-minute walk test. [92] Clearly reduced functional capacity together with oxygen dependency indicate a poor prognosis and are associated with increased mortality after valvular surgery, including after TAVR. [92]

Serum concentrations of BNP and NT-proBNP can be helpful to evaluate left ventricular burden caused by AS and/or mitral regurgitation, [94] and to follow the development regarding the need for surgery, as increased concentrations have been found to correlate with the severity of AS. [95] Elevated concentrations of BNPs are also associated with mortality in valvular heart disease and patients with high concentrations should be monitored closely before, during, and after surgery. [94] Increasing concentrations of NT-proBNP are a useful marker to anticipate need for aortic valve replacement surgery, [96] and is a poor prognostic sign in patients treated conservatively. [95] NT-proBNP could therefore be a biomarker for monitoring disease severity and progression before surgery and during follow-up, especially in asymptomatic patients. [95] After surgery, concentrations of NT-proBNP decreases, [95] reflecting reduced stress on the left ventricle. AS will be the only valvular heart disease further discussed in this thesis.

1.2.1 Aortic stenosis

AS occurs when the aortic valve cannot fully open due to stenosis and narrowing, caused by calcification of a congenitally malformed valve (i.e. bicuspid valve) or a normal tricuspid valve. [97] AS is a progressive disease and symptomatic AS is the result of decades with pathology that starts with endothelial damage from mechanical stress or chronic inflammation from rheumatic fever, later atherosclerotic build up on valvular leaflets, and finally calcification of the valve. [98, 99] In embryonically normal valves it is postulated that the initial process of AS is micro damage to the valve with subsequent subclinical inflammation and that these processes therefore are the origin for aortic valve calcification and AS. [99] An embryonically normal aortic valve consists of three leaflets, while 1-2% are born with a bicuspid valve, with a 2:1 male:female ratio. Subjects with a bicuspid valve are at increased risk of AS and develop AS at a younger age than subjects with a tricuspid aortic valve. [100]

Valve calcification is a major reason for AS and some clinical factors associated with aortic stenosis are equivalent to risk factors for atherosclerotic cardiovascular disease (CVD); age, smoking, hypertension, high LDL-cholesterol concentrations, and diabetes mellitus, [101, 102] Bicuspid aortic valve and previous rheumatic fever are also associated with AS. [99] Radiation therapy towards the mediastinum as part of treatment for cancer is also a risk factor for later AS. [103] With lower cancer-related mortality for breast cancer and lymphomas, an

increasing number of symptomatic AS cases due to prior radiation therapy is expected during the next decades.

AS induces a pressure-overload of the left ventricle, which results in increased left ventricle pressure with subsequent increased filling pressure also of the left atrium and pulmonary hypertension. [98] Exertional chest pain, syncope, and dyspnea due to HF are the three cardinal symptoms of AS. [104] Symptoms and clinical signs like fatigue, shortness of breath, dizziness, swelling of ankles, murmurs on auscultation, tachycardia are also often found in moderate-to-severe AS. [90] The normal opening of a healthy aortic valve is 3-4 cm^2, while symptom onset is usually when the area is below 1 cm^2, and the first symptom is often a small decrease in exercise capacity. [99] First clinical sign of AS is often a loud grade (≥3/6) systolic murmur with radiation to the carotid arteries on cardiac auscultation. [91] The New York Heart Association (NYHA) functional class is a subjective grading of HF patients' symptoms and functional ability from I to IV, and can also be used to classify patients with AS (Table 1). [105]

Table 1. *NYHA Functional Class. Adopted from jointcommission.org.* [105]

NYHA Functional Class	Symptoms/limitations
I	No symptoms and no limitations in ordinary physical activity.
II	Mild symptoms (mild shortness of breath and/or angina) and slight limitation during ordinary activity.
III	Marked limitation in activity due to symptoms, even during less-than-ordinary activity. Comfortable only at rest.
IV	Severe limitations. Experiences symptoms even while at rest. Mostly bedbound patients.

NYHA indicates New York Heart Association

AS is divided in four stages; A through D with subgroups, and the severity of AS is based on aortic maximum velocity of blood flow through the aortic valve orifice, the aortic valve area (typically ≤1cm^2), and the mean delta pressure (Table 2). [91] Symptomatic patients with calcified aortic valve and an aortic valve area between 0.8 cm^2 and 1.0 cm^2 should be evaluated for benefit of valve intervention. [91]

Table 2. *Stages of valvular AS. Adopted and simplified from Nishimura et al.* [91]

Stage	Definition	Valve Anatomy	Valve Hemodynamics	Symptoms
A	At risk of AS	- Bicuspid aortic valve - Aortic valve sclerosis	-Aortic V_{max} <2 m/s	- None
B	Progressive AS	- Mild-to-moderate leaflet calcification of a bicuspid or tricuspid valve with some reduction in systolic motion	- Mild AS: Aortic V_{max} 2.0-2.9 m/s or mean ΔP <20 mmHg - Moderate AS: Aortic V_{max} 3.0-3.9 m/s or mean ΔP 20-39 mmHg	- None
C	Asymptomatic severe AS			
C1	Asymptomatic severe AS	- Severe leaflet calcification with severely reduced leaflet opening	- Severe: Aortic V_{max} ≥4 m/s or mean ΔP ≥40 mmHg - Very severe: Aortic V_{max} ≥5 m/s or mean ΔP ≥60 mmHg	- None: Exercise testing is reasonable to confirm symptom status
C2	Asymptomatic severe AS with LV dysfunction	- Severe leaflet calcification with severely reduced leaflet opening	- Aortic V_{max} ≥4 m/s or mean ΔP ≥40 mmHg	- None
D	Symptomatic severe AS			
D1	Symptomatic severe high-gradient AS	- Severe leaflet calcification with severely reduced leaflet opening	- Aortic V_{max} ≥4 m/s or mean ΔP ≥40 mmHg	- Exertional dyspnea or decreased exercise tolerance - Exertional angina - Exertional syncope or presyncope
D2	Symptomatic low-flow/low-gradient AS with reduced LVEF	- Severe leaflet calcification with severely reduced leaflet motion	- Aortic V_{max} <4 m/s or mean ΔP <40 mmHg	- HF - Angina -Syncope or presyncope
D3	Symptomatic severe low-gradient AS with normal LVEF or paradoxical low-flow severe AS	- Severe leaflet calcification with severely reduced leaflet motion	- Aortic V_{max} <4 m/s or mean ΔP <40 mmHg	- HF - Angina -Syncope or presyncope

Aortic V_{max} indicates maximum aortic velocity; AS, aortic stenosis; HF, heart failure LV, left ventricular; LVEF, left ventricular ejection fraction; ΔP, pressure gradient.

1.2.2 Treatment options for aortic stenosis

Medical treatment is a part of the treatment in patients with AS prior to surgery, or in patients not eligible for surgery. [92] Medical treatment alone cannot cure AS, and intervention is the only definitive therapy for symptomatic AS. [92] Surgical intervention includes balloon aortic valvuloplasty, surgical aortic valve replacement (SAVR), and TAVR. [97]

1.2.3 Medical treatment

There is no medical treatment that can cure AS, but still medical therapy has a place prior to surgery to reduce risk factors and symptoms. Normally, all patients with echocardiographic severe AS will receive a small dose of β-blockers to reduce the risk of ventricular arrhythmias. Echocardiography is used to follow the development, and medical treatment is also indicated to reduce risk factors like hypertension and diabetes mellitus. [92] Moreover, in later stages, HF symptoms will develop and medication that inhibit the renin-angiotensin-aldosterone axis and loop diuretics are indicated. [92, 106] Patients with severe AS are also at risk for other cardiac diseases, including CAD and atrial fibrillation and should receive optimal therapy for these conditions. [92] In severe AS, medications that reduce preload and decrease afterload should be used with extreme caution as they reduce mean arterial pressure and these medications should be introduced slowly when indicated. [99] In some centers, digoxin and long-acting nitroglycerin are used as symptomatic therapy in patients not eligible for intervention, but the evidence base for this therapy is poor.

1.2.4 Balloon valvuloplasty

Balloon aortic valvuloplasty is a percutaneous procedure where a deflated balloon is placed in the aortic valve orifice and inflated to fracture calcific deposits in the valve leaflets and to dilate the stenotic valve. [91, 107] Balloon aortic valvuloplasty is not a substitute for SAVR, but is used for palliation in patients who cannot undergo surgery and as a bridge in unstable patients waiting for AS surgery. [107] Balloon aortic valvuloplasty has a short-lasting effect and in most patients, serious acute complications as severe aortic regurgitation, restenosis, and clinical deterioration occur within 6-12 months, [91] therefore this procedure is very seldom used today as TAVR can be performed in most of these patients.

1.2.5 Indications for intervention

In patients with symptomatic AS, intervention has a class I recommendation in patients with severe, high-gradient stenosis (mean gradient ≥40 mmHg or peak velocity ≥4.0 m/s) and in patients with severe low-flow, low-gradient stenosis (<40 mmHg) with reduced ejection fraction according to guidelines. [92] In asymptomatic patients, intervention is recommended as a class I indication in a subgroup of patients meeting specific criteria (discussed later). [92] Increased concentrations of BNPs have been discussed as a tool to determine early aortic valve intervention in patients usually not offered surgery due to mild symptoms or with

comorbidities masking symptoms of severe AS. [108] Risk stratification models like the EuroSCORE II model [8] and the Society of Thoracic Surgeons cardiac surgery risk model [10] are used prior to surgery to predict postoperative outcomes, and the Society of Thoracic Surgeons cardiac surgery risk model also have a model for isolated valve surgery. [109]

1.2.6 Surgical aortic valve replacement

SAVR is performed as open heart surgery with surgical replacement of the original aortic valve with a new aortic valve. There are two types of valves available, mechanical and bioprosthetic, the latter being bovine or porcine. [97] In symptomatic AS patients with indication for surgery, SAVR is a class I recommendation in patients at low surgical risk by Society of Thoracic Surgeons or EuroSCORE II and no other risk factors not included in these risk scores (i.e. porcelain aorta and sequelae of chest radiation). [92] Patients at increased surgical risk according to these risk scores should be discussed in a Heart Team consisting of cardiac surgeons, cardiologists, and radiologists for choice of SAVR or TAVR. [92] Patients with severe AS undergoing CABG or surgery on the ascending aorta or other valves have a class I recommendation for concomitant SAVR. [92] There are also clinical and anatomical aspects to consider when choosing intervention. Age <75 years and suspicion of endocarditis are both recommended for SAVR. Difficult access for TAVR and other unfavorable anatomical aspects for TAVR will also favor SAVR. [86] In patients considered asymptomatic, intervention is a class I recommendation in patients with severe AS and systolic left ventricular dysfunction or AS related symptoms on exercise test. [92] Currently, asymptomatic patients without risk factors are recommended close follow-up instead of early surgery as the risk-benefit ratio has been considered unfavorable. [92] However; this may change over time with the development of TAVR, which seem to offer similar benefit as SAVR and with much less post-operative morbidity as valves and techniques improve over time.

There is an increased risk of stroke with bioprosthetic valves, especially the first 90 days after valve replacement, and anticoagulation is necessary in this period. [91, 99] Mechanical valves have better long-term durability, leave a greater opening for blood flow than bioprosthetic valves, but require lifelong anticoagulation. [97, 99] Both mechanical and bioprosthetic valves have a greater opening for blood flow than TAVR, but with progress of techniques and valves for TAVR these differences are expected to diminish over time. SAVR compared to TAVR have higher incidence of major bleedings that require blood transfusions, which is associated with mortality, infection, morbidity, and length of stay. [97] New-onset atrial fibrillation is more frequently associated with SAVR than TAVR, [97] while AV block III and the need for pacemaker has been more common until now with TAVR. [110]

Also, worth mentioning is a more advanced procedure called the Ross procedure. It is a relocation of the pulmonary valve to the aortic position, and is done mostly in children and young adults. This procedure is favorable in young age because of the durability of the native valve and no need for anticoagulation. [99]

1.2.7 Transcatheter aortic valve replacement

TAVR is a percutaneous procedure to insert a new aortic valve in the orifice of a stenotic aortic valve without open heart surgery as in SAVR. The most common access point, and also the safest, is through the femoral artery. Other access points are transapical, transaxillery, transcarotid, transcaval, and transaortic. [97, 111] TAVR has a class I recommendation in patients who are fount not suitable for SAVR by the Heart Team. [92] TAVR is recommended in patients ≥75 years, in patients with increased risk as estimated by established risk scores, in patients with severe comorbidities, and in patients that previously have been subjected to cardiac surgery or not suited for SAVR due to anatomical difficulties. [92] If the vascular anatomy is suitable, TAVR is a surgical option with similar risk of mortality and vascular complications as SAVR. [97, 111] Complications associated with TAVR are stroke, bleeding from vascular access-site, paravalvular leak, and need for pacemaker due to AV block III. [97, 110] A higher incidence of major stroke within 30 days has been shown in high-risk patients treated with TAVR compared to SAVR. [97] Compared to standard treatment in inoperable patients, patients undergone TAVR has better survival and functional status. [112] Minimalist TAVR has been introduced and uses moderate sedation compared to regular general anesthesia in TAVR, with profitable results on mortality, stroke, and hospitalization. [113] Techniques and valves for TAVR are continuously being improved and recent data have indicated similar or superior outcome for TAVR as for SAVR also in more low-risk patients; hence, it is likely that the numbers of SAVR will decrease over the next decade. [114, 115]

1.3 Cardiovascular biomarkers

1.3.1 What is a biomarker?

The Biomarkers Definitions Working Group defined a biomarker as *"... a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention."* [12] Biomarkers come in different formats; blood pressure, pulse, ECG, echocardiography, CT, MRI, etc. However, the term biomarker is normally used for circulating biological markers (Figure 4) like CRP, prostate-specific antigen (PSA), NT-proBNP, cardiac troponin (cTn), and SN. In this thesis three groups of circulating biomarkers (BNPs, cTn, and the granin protein family) will be further discussed.

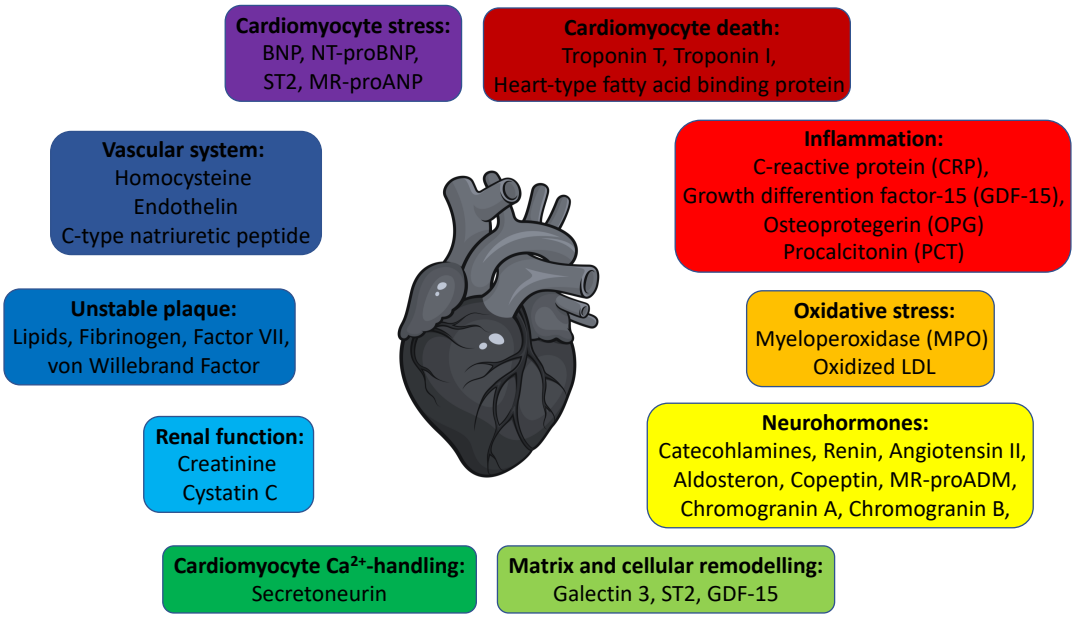

Figure 4. *Cardiovascular biomarkers according to suggested pathophysiology reflected by the biomarker. The list is non-exhaustive and some of the biomarkers are not finally validated regarding their pathophysiology; i.e. secretoneurin. Illustration of the heart: Colourbox.com.*

Biomarkers have many possible clinical applications; (1) early detection of subclinical disease, (2) diagnosis of acute or chronic syndromes, (3) risk stratification/staging of suspected or confirmed diagnosis, (4) selection of therapeutic intervention, and (5) monitoring disease progression or clinical response to an intervention (Figure 5). [12, 116]

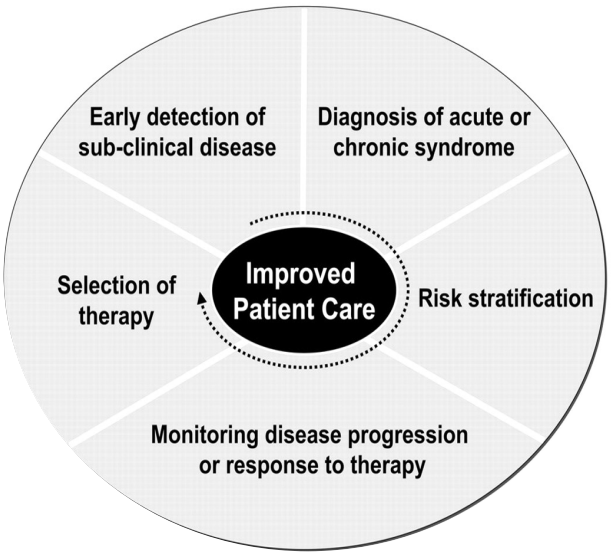

Figure 5. *Possible applications of cardiovascular biomarkers in clinical use. Reproduced from Morrow and de Lemos [116] with permission from Wolters Kluwer Health, Inc.*

Biomarkers have over the past five decades been important for better detection of AMI as circulating cardiac troponin concentrations are used to detect myocardial necrosis. [117] A variety of biomarkers are constantly being tested, however, it is expected that only a few will ever be introduced into clinical medicine. [116] To help evaluate novel biomarkers, stringent criteria have been developed and these includes a need for the biomarker to be easily measurable, to add new information, and to help the clinician better manage the patients (Figure 6). [116]

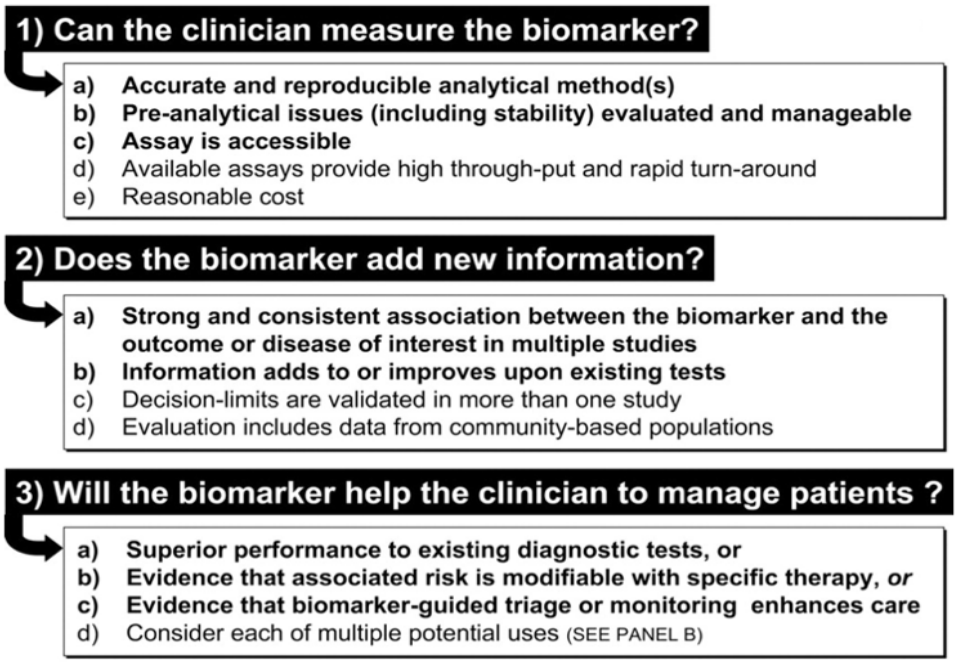

Figure 6. *Criteria for assessment of novel cardiovascular biomarkers for clinical use. Reproduced from Morrow and De Lemos [116] with permission from Wolters Kluwer Health, Inc.*

1.3.2 Natriuretic peptides

A total of five peptides encounter for the natriuretic peptide family, with the three most known being; atrial natriuretic peptide (ANP), B-type (brain) natriuretic peptide (BNP), and C-type natriuretic peptide (CNP). These three peptides all share a common 17-amino-acid ring structure and a common action to protect the cardiovascular system from the effects of volume overload. [13, 118] Dendroaspis natriuretic peptide (DNP) [119] and urodilatin are less mentioned members of the natriuretic family. [120] ANP has its name from experiments with extracts from atrial myocytes injected into rats giving the rats natriuresis and diuresis. [121] In the normal state, ANP is primarily produced in the cardiac atria as a response to increased atrial wall tension and increased intravascular volume. [118] BNP has its original name "brain natriuretic peptide" from an experiment demonstrating a natriuretic peptide similar to ANP in porcine brain. [122] However, BNP was later re-named to B-type natriuretic peptide as the majority of circulating BNP molecules in the peripheral circulation are produced in myocardial tissue, and especially the left ventricle during HF. [118] The main stimulus for BNP production is cardiomyocyte stretch, but BNP can also be produced as a response to other processes, like ventricular hypertrophy and increased wall pressure. [123] CNP is a peptide homologous to ANP and BNP, however, it is produced in brain and endothelium, and not in cardiac myocytes as ANP and BNP. [124] The less prominent members are DNP and urodilatin. DNP was first isolated from the green mamba snake's venom, [119] while urodilatin was discovered when isolated from human urine and might be important for the local regulation of sodium and water in the kidneys. [118, 120] Only BNP will be discussed in detail in this thesis.

1.3.2.1 B-type Natriuretic Peptide

The BNP gene is located on chromosome 1. [124] Factors that increases wall stress, like volume expansion, pressure overload, or myocardial ischemia, will induce pre-proBNP production in the ventricular myocardium. Pre-proBNP is thereafter cleaved to the 108-amino acid long prehormone proBNP. [13] The proBNP peptide is further enzymatically cleaved by the proteolytic enzyme furin into equal amounts of the biologically active 32-amino acid long peptide BNP and the inactive 76-amino acid long peptide NT-proBNP (Figure 7). [13, 118, 125] Unprocessed proBNP is also found in plasma and processed in the circulation, especially in heart failure patients. [126, 127] Of note, glycosylation of proBNP may influence the processing of proBNP to BNP and NT-proBNP. [128]

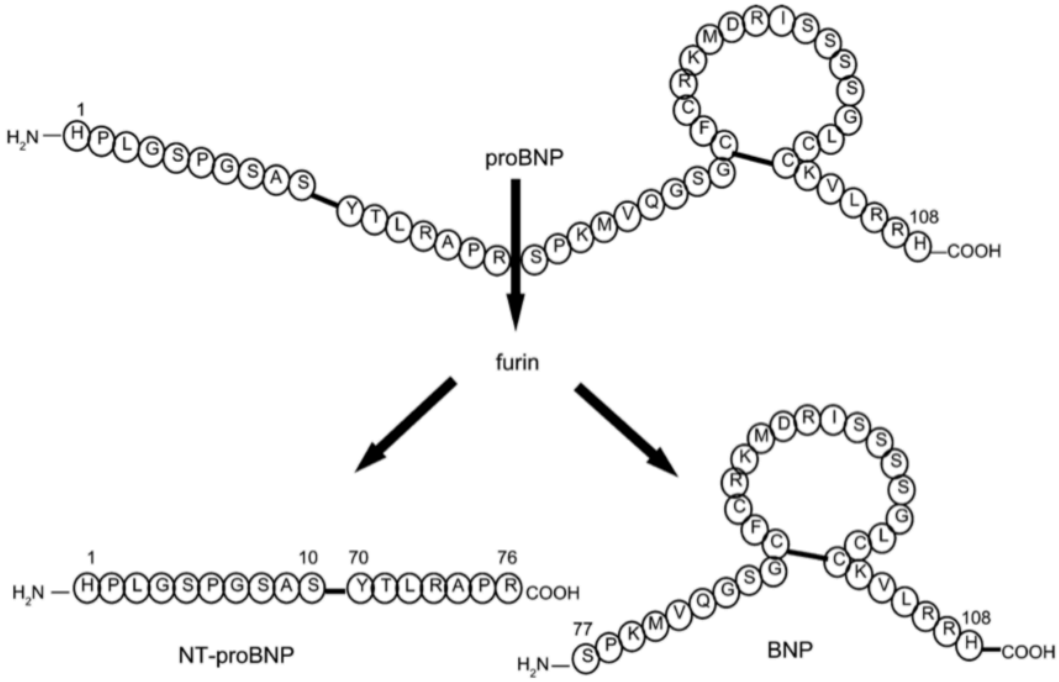

Figure 7. *Schematic drawing of proBNP showing enzymatic cleavage to the biologically active BNP and the inactive NT-proBNP. Reproduced from Hall [124] with permission from John Wiley and Sons.*

The biological actions of BNP are many and almost exclusively beneficial; inhibition of the renin-angiotensin-aldosterone system (RAAS) and the sympathetic nervous system, balancing vasodilation, and increment in natriuresis (Figure 8). All of these processes lead to reduced blood pressure, which again is beneficial to reduce ventricular wall stress (Figure 8). [118, 129] RAAS is an intricate hormone system that regulates blood pressure and fluid balance by electrolyte and water retention, as well as vasoconstriction to counteract low blood pressure and is activated by a decrease in renal perfusion. [130] Blocking the RAAS system is beneficial to treat hypertension and to stop inappropriate remodeling of the heart after myocardial infarction and in patients with HF with reduced ejection fraction. [130]

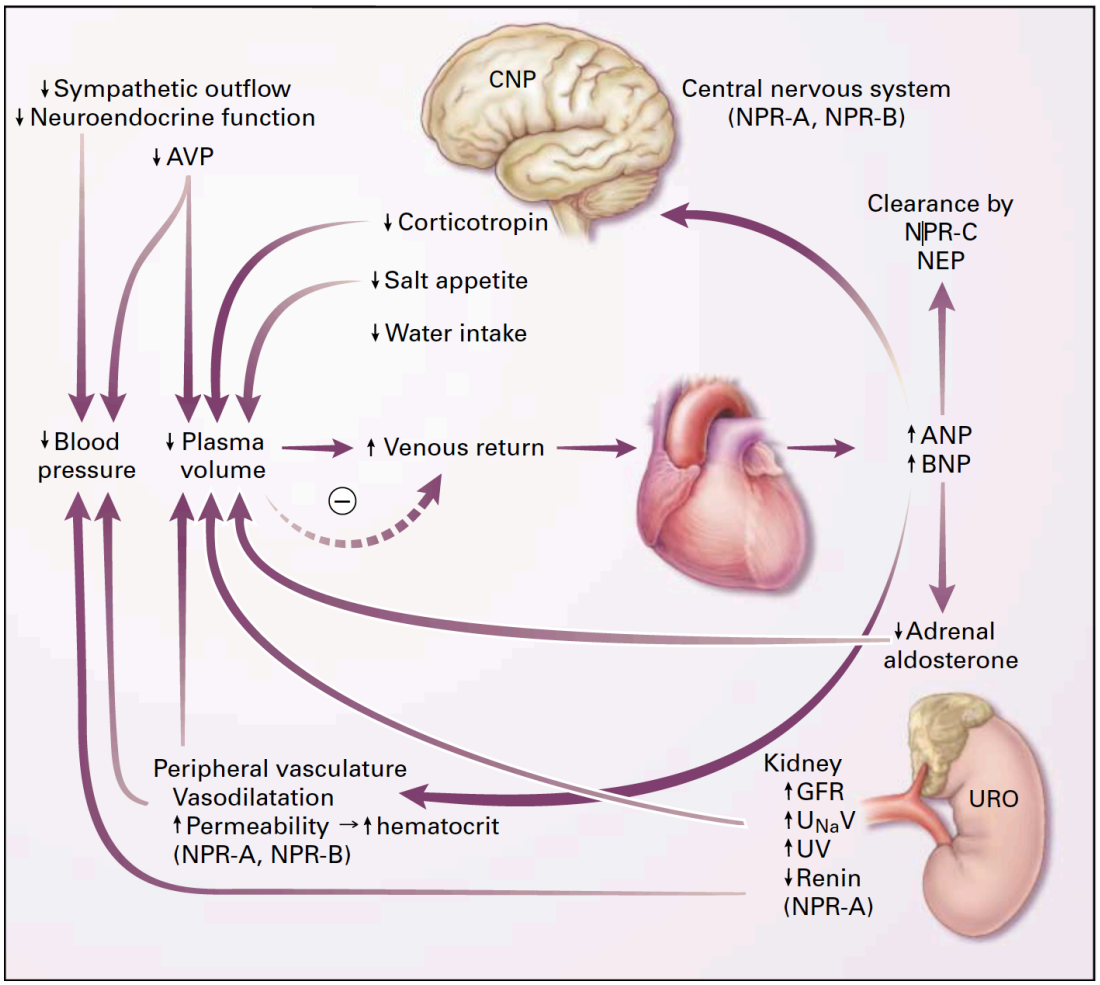

Figure 8. *Physiologic effects of natriuretic peptides released from the heart when venous return is increased. Increased secretion of the natriuretic peptides reduces blood pressure and plasma volume through coordinated actions in the brain, adrenal gland, kidney, and vasculature. The minus sign indicates that a decrease in plasma volume leads to a decrease in venous return, which in turn decreases the secretion of the natriuretic peptides. URO denotes urodilatin; NEP, neutral endopeptidase; CNP, C-type natriuretic peptide; NPR-A, NPR-B, and NPR-C, natriuretic peptide receptors A, B, and C, respectively; AVP, arginine vasopressin; ANP and BNP, atrial and brain natriuretic peptides, respectively; GFR, glomerular filtration rate; $U_{Na}V$, urinary sodium excretion; UV, urinary volume; BP, blood pressure. The receptors that mediate the functions of the natriuretic peptides are indicated in parentheses. Reproduced with permission from Levin.* [118] *Copyright Massachusetts Medical Society.*

The inactive NT-proBNP peptide has a six-time longer half-life (120 minutes vs 20 minutes) than the active BNP peptide, [131] resulting in a higher serum concentration, despite 1:1 secretion. [13] Being a peptide that is produced in situations of increased myocardial wall stress, [13] BNP and NT-proBNP are ideal biomarkers for detecting and monitoring HF, [21, 132] and therefore these peptides are also included in the European Society of Cardiology´s HF definition in the 2016 Guidelines. [133] Studies have also shown that BNP and NT-proBNP are useful in unselected patients presenting with dyspnea as these peptides improve diagnostic accuracy for HF and provides prognostic information in patients with HF and [21, 22, 106] in patients with acute coronary syndrome. [27, 134] BNP and NT-proBNP are also useful to predict short-term mortality in acute HF [132] and to assess risk of postoperative mortality after

cardiac surgery. [135-137] Preoperative measurements of BNP have previously been shown to predict long-term outcome in cardiac surgery, [138-140] and more recently postoperative NT-proBNP concentrations were found also to improve long-term risk assessment. [141]

There are several factors that can influence the concentration of NT-proBNP besides pressure/volume overload on the heart. Decreased renal function results in elevated concentrations of natriuretic peptides, and it seems that renal function is more closely associated with NT-proBNP concentrations than with BNP concentrations. [142, 143] Studies have also found elevated BNP and NT-proBNP concentrations in anemic patients. [143, 144] Both BNP [145] and NT-proBNP [146] concentrations are elevated in patients with traumatic brain injury. There are also elevated NT-proBNP concentrations with age and in healthy women (compared to healthy men), [147] while there are inverse correlations between BNP and NT-proBNP concentrations and body weight. [148]

1.3.3 Cardiac troponins

Troponin was discovered and first described in 1965. [149] Troponin can be found in striated skeletal muscle and in cardiac muscle, more specifically in the cardiomyocytes. [150] The troponin complex consists of three components, troponin I, troponin T, and troponin C, which again is attached to tropomyosin. [150, 151] Troponin C is identical in striated muscle cells and in cardiomyocytes, [152] while troponin I [153] and T [154, 155] are genetically and immunologically different in striated muscle cells and cardiomyocytes. This difference in troponin I and T makes them highly specific to cardiomyocytes and therefore these peptides are referred to as cardiac troponins (cTn). [156, 157] The troponin complex is bound to the actin filament, which together with calcium interacts with myosin to create and control the sliding motion that is responsible for muscle contractions (Figure 9). [156] The troponin molecules have individual tasks; troponin C binds intracellular calcium, [151] troponin I is the inhibitor and blocks interaction between actin and myosin until calcium binds to troponin C, [158] while troponin T binds to tropomyosin, troponin C, troponin I, and to the actin filament. [159]

There are six proposed mechanisms to explain troponin release into the bloodstream; normal cell turnover, myocyte necrosis, programmed cell death, proteolytic fragmentation, increased permeability of the cell membrane, and membranous blebs. [150, 160]

Troponin can be found two places in the cardiac muscle; 4-8% is free in the myocyte cytoplasm and make up the unbound pool which is thought to be immediately released, regardless of type of myocyte injury, and immediately cleared assuming a normal renal function. In contrast, the remaining 92-96% of cardiac troponin is attached to the actin

filaments and degrades over several days giving a stable and gradual release of troponin after myocyte injury and necrosis. [150, 160, 161]

The half-lives of troponin I and troponin T are about 2 hours, and a quick rise and fall in troponin concentrations within 24 hours is thought to be caused by release from the unbound pool and may reflect reversible myocyte damage rather than myocyte necrosis. [160, 161] In contrast, a rise and fall pattern lasting several days due to gradual degradation of myofibrils and accompanying troponin release is believed to be caused by myocyte necrosis. [160, 161] A large number of cardiac diseases other than AMI and also non-cardiac diseases can give elevated troponin concentrations. [150] There are also differences in acute vs chronic release of troponin, where acute release has a characteristic rise and fall curve seen in AMI, while chronic release has more stable elevation of troponin. Stable cardiac troponin elevations are common with structural heart disease and ventricular hypertrophy and in conditions such as chronic HF and valvular heart disease. [150, 162]

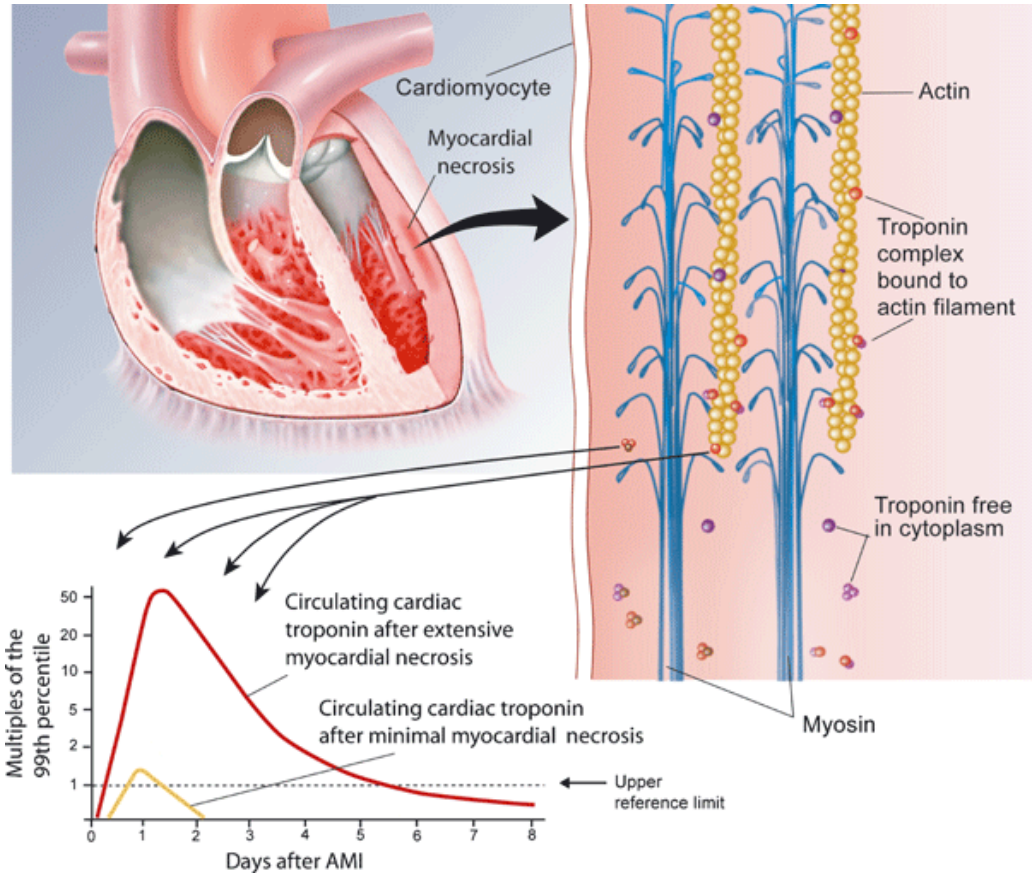

Figure 9. *Release of cardiac troponins in acute myocardial infarction. With courtesy from Omland.* [163] *Reproduced with permission from Antman.* [164] *Copyright Massachusetts Medical Society.*

In clinical settings, elevated troponin concentrations measured with high-sensitivity cTn assays are less specific for AMI when the 99th percentile is used as a single cutoff level. [165]

Clinical context and examination will therefore give valuable information together with serial sampling of cTn concentrations. Biological factors can also affect the cTn concentrations, and we find higher concentrations in men, older individuals, black race, and in patients with chronic kidney disease. [166, 167]

Assays for the detection of cTn have improved over the last decades with improving analytical sensitivity and accuracy in the lower range of detection (Figure 10). [150]

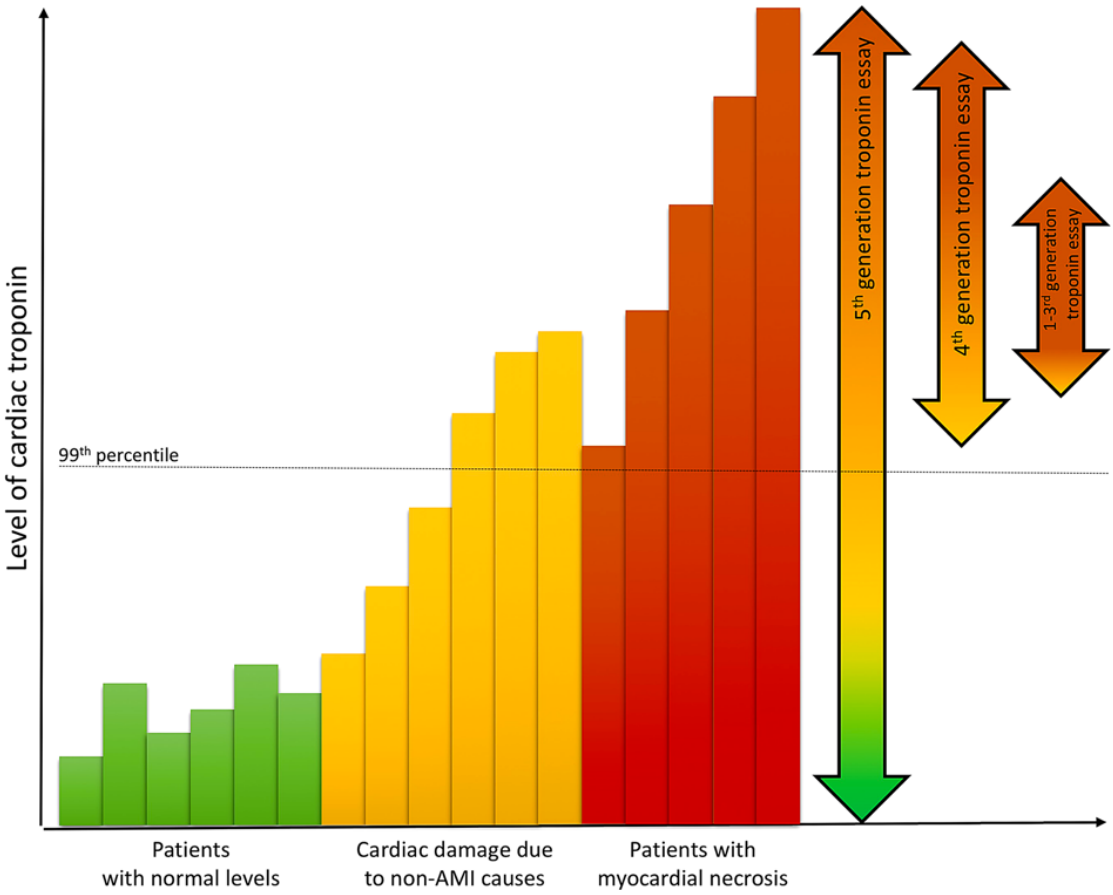

Figure 10. *Detection range of different troponin assays. The green bars represent the normal turnover range of troponin in healthy individuals. With the onset of myocardial infarction, a slight rise in cardiac troponin can be seen that represents either ischemia-induced release of cytosolic troponin or micronecrosis (orange-bars). Between 2 and 6 h, a steep increase in concentrations of cardiac troponin can be seen that represents extensive myocardial necrosis (red-bars). Only this major increase of cardiac troponin can be detected by first to fourth generation troponin assays. High-sensitivity cardiac troponin assays (5th generation troponin assay) can also detect lower concentrations of troponin including ischemia/micronecrosis and even the normal turnover. Reproduced from Garg [150] under the Creative Commons Attribution 4.0 International (CC BY 4.0).*

With the development of high-sensitivity, fifth-generation, troponin assays we now reliably can quantify troponin concentrations in more than 50% (ideally >95%) of the healthy population. [168] This has enabled determination of the 99th percentile in the normal population with better confidence [168] and contributed to earlier diagnosis of patients with

AMI by the use of strategies with shorter time intervals between serial cardiac troponin measurements. [150, 169, 170] Earlier and more correct diagnosis should also transcend into improved patient management, although direct evidence for this hypothesis is lacking. [171] The new high-sensitivity cardiac troponin assays have also given the opportunity to explore cardiac troponins as biomarkers of risk in the general population. With more reports from the general population it is also clear that the 99th percentile will vary across cohorts based on age distribution in the population, sex, ethnicity, and race. [166]

Cardiac surgery comprises many different surgical modalities, and there is an expected elevation in troponin concentration after surgery. [172] Due to this common finding, and historical use of creatinine kinase (CK)-MB in surgical patients, many centers also use CK-MB to assess perioperative myocardial injury during cardiac surgery. However, it is clear that cardiac troponins are excellent risk markers also in cardiac surgical patients with complicated postoperative course being more common in patients with high troponin T concentrations after surgery. [172] In CABG surgery without cardio-pulmonary bypass, the expected release is significantly lower, and preoperative concentrations could be a better prognostic marker. [173, 174] In a Finnish study, elevated postoperative troponin T concentrations in patients who also had elevated preoperative troponin T concentrations were associated with mortality, including after adjustment for established risk scores and NT-proBNP. [175] For most patients undergoing cardiac surgery, postoperative measurement of cardiac troponin T is thought to give superior prognostic information to pre-operative measurements. [176] Many patients undergoing cardiac surgery have also had previous AMI, some only hours to days prior to surgery. In this setting, preoperative cTn concentrations can be temporarily elevated due to AMI and may not necessarily reflect the cardiac troponin concentration in a steady-state situation for the patient.

1.3.4 The granin protein family

The chromogranin-secretogranin (granin) protein family includes nine acidic prohormones with intracellular effects on the regulated secretory pathway and extracellular effects via multiple shorter peptide fragments with different biological actions. [177, 178] These peptides have also been tested as biomarkers across different diseases and conditions. Chromogranin A (CgA), chromogranin B (CgB/SgI), and secretogranin (Sg) II (chromogranin C) are often called the "classic" granin proteins (Figure 11), [178, 179] but, six other proteins are suggested to be a part of the granin protein family; SgIII (1B1075), [180] SgIV (HISL-19), [181] SgV (7B2), [182] SgVI (NESP55), [183] SgVII (VGF), [184, 185] and SgVIII (proSAAS). [186]

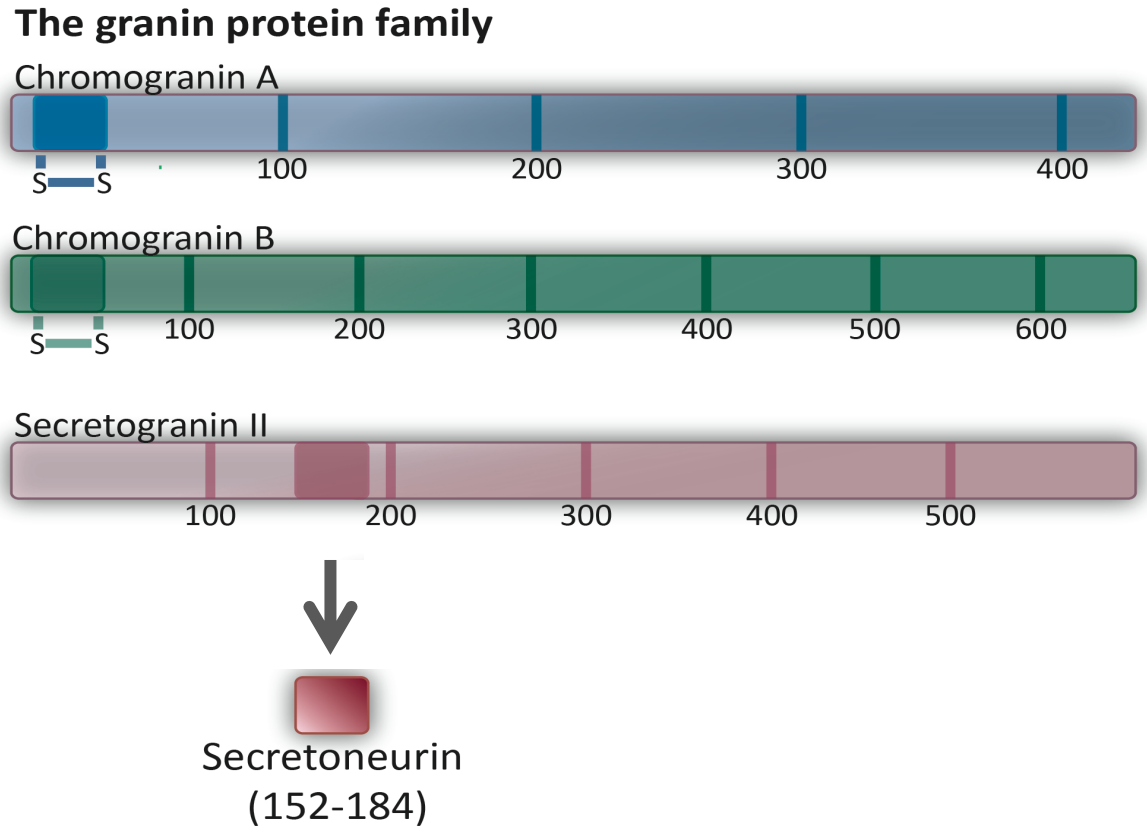

Figure 11. *The "classic" granins of the granin protein family. Adapted with changes from Bartolomucci [187] by Anett H. Ottesen, Ph.D.. Reproduced with permission from Oxford University Press and Anett H. Ottesen.*

Granins are synthesized in most organs with exocytosis, but are found in the highest concentrations in organs with endocrine cells like the adrenal gland and the pituitary, the central and peripheral nervous system, and the gastrointestinal tract. [178] Synthesis also occurs in non-neuroendocrine cells, including the heart, liver, and kidneys. [29, 188, 189] Production in these organs seems to increase with pathology and as these cells attain a

paracrine/endocrine phenotype; including cells in the myocardium, [190, 191] the prostate, [192, 193] and lungs. [194]

Common to all granin proteins is a single-polypeptide chain of 180-700 amino acids connected to an N-terminal signal peptide that have multiple dibasic cleavage sites. These cleavage sites produce numerous short amino acid peptide fragments, which is a hallmark of the granin proteins (Figure 11). [178, 187] Chromogranins include CgA and CgB and can be distinguished from the secretogranins (SgII-SgVIII) by having a disulfide bonded loop at the N-terminal end (Figure 11). [195] The principal proteases for cleavage of granins to multiple shorter peptides are prohormone convertase (PC) 1 (also known as PC3) [196, 197] and PC2. [198] Most other prohormones have one cleavage site and produces only two shorter fragments; i.e. proBNP being cleaved into BNP and NT-proBNP (Figure 7). [187] The granin proteins are larger than 24 kDa with the majority of peptides having size greater than 50 kDa, which is higher than other classical neuropeptide prohormones. [187] They also have an isoelectric point (the pH where the molecule is electrically neutral) with a mean of 4.9, while other neuropeptide prohormones have isoelectric points around 7.1. [187]

1.3.4.1 Intracellular physiology of granins

The regulated secretory pathway in the human body is found in specialized secretory cells (i.e. endocrine and neuronal tissue) where soluble proteins and other substances are concentrated and stored in secretory granules, which are called large dense-core granules. These large dense-core granules are ready for later release by exocytosis and contain hormones, neurotransmitters (neuropeptides), and digestive enzymes. [199, 200] The storage of these proteins and substances intracellularly in secretory granules make them ready for rapid release on demand with the right signal, which can be an electrical nerve impulse or a circulating signal substance (hormone). [199]

The rough endoplasmatic reticulum synthesizes granins and other proteins that are targeted for extracellular release via the Golgi apparatus. [199] Granins stabilize intracellular granula, which together with sorting and packing, make these peptides key elements in the sequestration of hormones/proteins and the formation of secretory granules for regulated secretion (granulogenesis) (Figure 12). [201, 202]

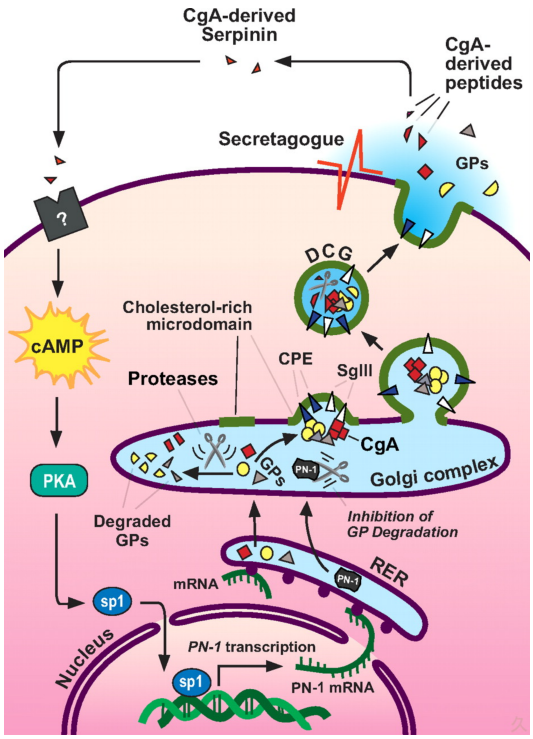

Figure 12: *Granins are an important factor in the granulogenesis in specialized secretory cells of the regulated secretory pathway. Granins are key elements in the sequestration of hormones/proteins and the formation of the secretory granules, as well as a CgA positive feedback-loop. Reproduced from Bartolomucci* [187] *with permission from The Endocrine Society.*

1.3.4.2 Extracellular physiology of granins

Since granins are part of granulogenesis, these peptides will be secreted and therefore be possible to measure in the peripheral circulation with concentrations correlated with the degree of exocytotic activity. [203] Granins can also have extracellular properties and these functions are related to extensive proteolytic processing to smaller peptides, which occurs both intracellularly and extracellularly. [204] The actions of granin-derived peptides are numerous and widespread throughout the body [178] as granin peptide fragments exert autocrine, paracrine, and endocrine effects. [178]

1.3.4.3 Granins as biomarkers

Granins are highly relevant for diagnosis, prognosis, and prediction of therapeutic outcome in patients with neuroendocrine tumors due to the increased production in malignant cells. [205] Non-neuroendocrine cells can be transformed to secretory cells at late stages of disease; i.e. tumor cells in lungs, [194] prostate cancer, [192, 193] and in cardiomyocytes, [31, 188, 191] which seems associated with increased synthesis of granins and granin-derived peptides.

The properties of granin proteins and the most relevant peptide fragments will be discussed for CgA, CgB, SgII individually.

1.3.4.4 Chromogranin A

CgA was first discovered in the adrenal medulla [206] and is synthesized in both the neuroendocrine system [207] and in non-neuroendocrine cells. [208-210] CgA is synthesized as a 439 amino acid protein and CgA has ten dibasic cleavage sites that can produce functionally active peptide fragments after proteolytic processing. [211] CgA has an N-terminal loop due to a disulfide bridge, which influences the 3-dimensional form of CgA. [212] Circulating CgA is the only granin protein that currently is used in clinical medicine, where CgA is used as a diagnostic biomarker for neuroendocrine tumors, [205] especially pheochromocytoma. [205, 213] The measurement of intact CgA has been shown to have greater sensitivity for neuroendocrine tumors than the measurements of several CgA peptide fragments. [214] CgA could also be useful as a marker of adrenergic tone since circulating concentrations of CgA are correlated with catecholamine concentrations. [215]. As epinephrine and norepinephrine are difficult to measure in a routine clinical setting due to rapid degradation, [216] CgA could be an attractive alternative as CgA is stable under ambient incubation and repeated freezing and thawing cycles, [217] which results in a high signal-to-noise ratio for CgA. [217] Still, more studies are needed before CgA may be used for this purpose.

CgA is found in the ventricular myocardium, co-stored and co-released with BNP into the circulation. [191] The release of CgA from the heart is postulated to contribute to circulating CgA concentrations and to influence cardiac function, [191] but the impact is uncertain, especially since CgA is hyperglycosylated during HF with subsequent reduction in CgA processing to functional peptide fragments. [189] Some of the peptides that originate from CgA have documented cardiovascular effects, including vasostatin I, vasostatin II, and catestatin. [218]

1.3.4.5 Chromogranin B

CgB is also synthesized in the neuroendocrine system and is 677 amino acids long with 16 dibasic cleavage sites. [219] Like CgA, CgB has a N-terminal loop due to a disulfide bridge. [187] Both CgB and CgA are expressed in high concentrations in the adrenal medulla. [220] In various neuroendocrine tumors, immunoreactivity for CgB has been found; i.e. prolactin-producing tumors of the anterior pituitary gland. [178] Elevated concentrations of circulating CgB derived peptides have also been found in patients with pancreatic tumors [221] and bronchial tumors. [222]

1.3.4.6 Secretogranin II and secretoneurin

SgII is found in high concentrations in the pituitary gland, [220, 223, 224] as well as in the granules of endocrine cells, in neurons, and neuroendocrine tissues; e.g. pituitary gland, C-cells of the thyroid, the gastrointestinal tract, and peripheral nerves. [225, 226] The highest concentrations of SgII is found in the gastrointestinal tract, and it is possible that a high amount of SgII in the

circulation originate from this pool. [227] SgII consists of 617 amino-acids and has nine pairs of dibasic cleavage sites [187] but lacks the N-terminal disulfide loop of the chromogranins. More than 90% of SgII is cleaved into shorter peptides, [228] thus giving the opportunity for a wide range of peptides. However, so far, only SN ($SgII_{154-186}$) has been identified to carry biologically active functions. [179] SN is 33-amino-acid long [228] and SN concentrations exceed concentrations of CgA, CgB, and other neuropeptides in cerebrospinal fluid. [227] SN is a highly conserved peptide through evolution with >90% identical amino acid sequences between mammals. [187, 229] Conservation across species, high concentrations in the developing hypothalamus, and functional properties like cell proliferative effects, support the model of SN as an important peptide for human physiology. [226, 229-231]

SN has also been related to functions in the immune system as it increases migration of monocytes, eosinophils, and endothelial cells. [227] SN is also found in endocrine cells in the enteric neuroendocrine system, exerting an important role in the modulation of gastrointestinal motility. [232] In patients with endocrine tumors, SN concentrations in serum are elevated, more in young children as SN concentrations have been found to decline with age. [233] SN also exerts other effects like positive regulation of the luteinizing hormone in the anterior pituitary gland. [230] SN is, as most other peptides, eliminated from the body through the kidneys. [233]

There have been a number of recent reports on a possible role for SN as a prognostic biomarker across different cohorts, including patients with acute HF, [31] after cardiac arrest, [31, 234] severe infections [235] and sepsis, [236] and in patients with acute respiratory failure. [237] SN also seems to influence cardiovascular pathophysiology, and the putative role of SN as a biomarker and functional peptide in cardiovascular disease will be discussed later.

1.3.4.7 Secretoneurin and cardiovascular pathophysiology

SN is an important inhibitor of endothelial and cardiomyocyte apoptosis [188] and a stimulator of vascular endothelium chemotaxis and proliferation. SN also induces angiogenesis [238] and vascularization [239] after ischemic injury. [240] These effects of SN are important to prevent ischemic injury and SN has shown to attenuate ischemia/reperfusion injury in the myocardium, [188] skeletal muscle, [241] and the brain.[242] SN gene therapy also improved cardiac function after myocardial infarction in mice. [243] In patients with cardiac disease, increased SN concentrations are thought to origin from both neuroendocrine tissue and myocardial cells, and SN is thought to play a cardioprotective role in the myocardium. [31]

One possible cardioprotective effect of SN is the role of SN in cardiomyocyte Ca^{2+} handling where SN reduces diastolic Ca^{2+} leak through direct protein-protein interactions in

cardiomyocytes (Figure 13). [31] The reduction in diastolic Ca^{2+} leak by SN is considered beneficial during acute or chronic injury.

Patients undergone cardiac arrest have increased SN concentrations, [31] and in patients with ventricular fibrillation SN is a dynamic biomarker returning to normal concentrations within 24 hours of cardioversion. [31] Our group has also shown that HF patients have increased concentrations of circulating SN and that SN concentrations measured on admission for acute HF improve risk assessment over established risk indices and biomarkers. [31, 188] Patients undergoing cardiac surgery often have acute and/or chronic myocardial injury and experience arrhythmias due to peri- and postoperative cardiac stress. A biomarker like SN could therefore potentially give valuable information regarding cardiac status in cardiac surgical patients.

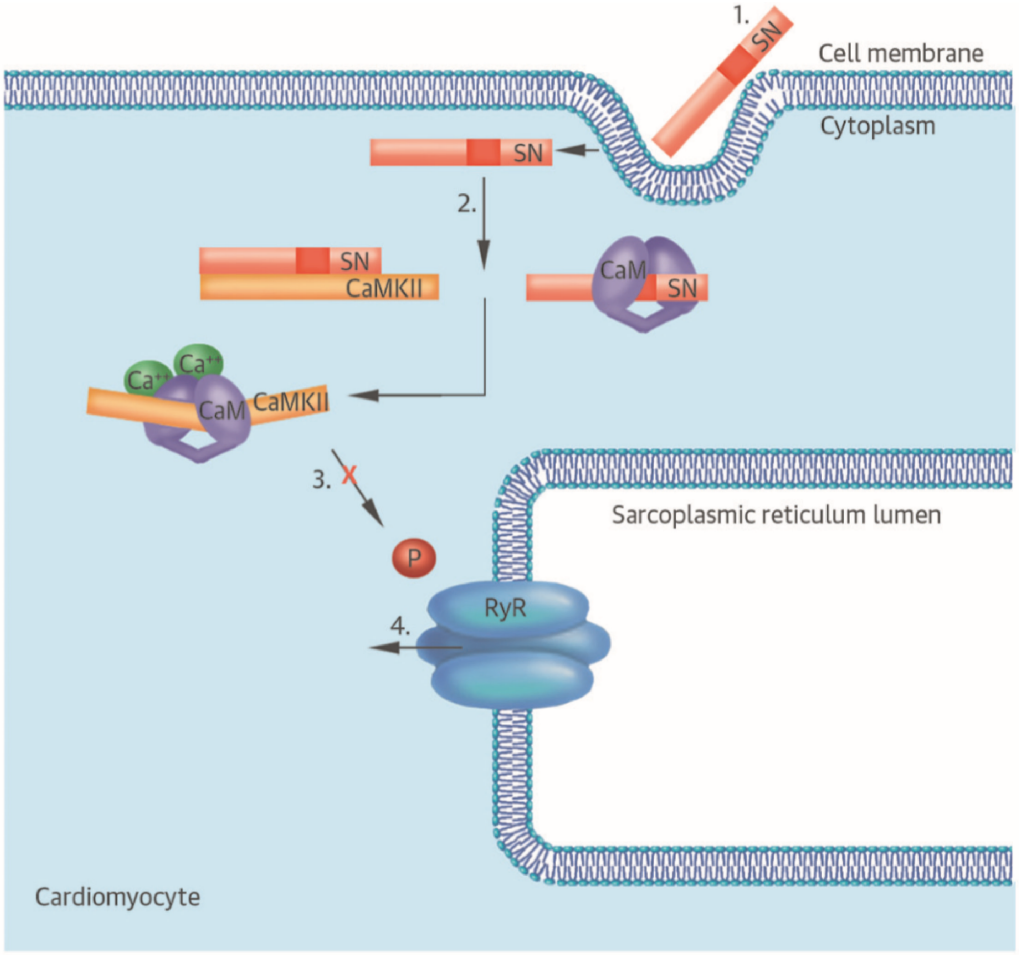

Figure 13. *Experimental studies of SN in isolated cells and explanted hearts demonstrate that (1) SN is internalized into cardiomyocytes and the intact heart by endocytosis, (2) SN binds directly to calmodulin (CaM) and CaM dependent protein kinase II δ (CaMKIIδ), and inhibits CaMKIIδ activity, (3) this leads to reduced ryanodine receptor 2 (RyR2) phosphorylation, and (4) improved Ca^{2+} homeostasis. Reproduced from AH. Ottesen [31] with permission from Elsevier Inc.*

1.4 Models for risk stratification

1.4.1 The demand for risk stratification models

Cardiac surgery is high-risk surgery associated with perioperative death. [244, 245] Still, although an increasing number of elderly patients are undergoing cardiac surgery, mortality rates are declining. [82, 246] One reason for this reduction in mortality is believed to be the use of good clinical preoperative evaluation and quality improvement programs. [246, 247]

Risk stratification models are designed to objectively predict the probability of a certain outcome for a patient. [10, 248] In clinical use, risk stratification models are an important part of preoperative evaluation of patients. The risk stratification models aid clinicians in patient counseling and decision support, for example to choose between cardiac surgery and other therapeutic modalities, to choose between different intervention methods, e.g. CABG vs. PCI, or to choose between surgery or no surgery [10, 249-251] The preoperative status of a patient is an important factor to postulate how the patient will tolerate high-risk surgery, and wrong conclusions can be made by not adding the right variables into the models. [252]

The variables included in the risk stratification models are carefully selected by using different statistical metrics. In most models, mortality is defined as death within 30 days of surgery. [9, 36, 109, 250, 253] However, some models are based on analysis with in-hospital mortality as the endpoint, regardless of the duration after cardiac surgery. [36, 109, 250, 253]

The utilization of risk stratification models is widespread and not only used preoperatively to calculate mortality risk. The use of risk-adjusted results after CABG surgery have been important in many areas like economic-, clinical-, research-, and quality improvement questions. [250] They are used as a basis for pay-for-performance reimbursement systems, [10] which gives economic benefits to the provider based on type of surgery. [10] Benchmark comparison among hospitals and surgeons can be done on the basis of risk scores, as well as public reporting. [10] Surgeons and hospitals operating on high-risk patients will likely experience more adverse events (due to the case mix) than other surgeons and hospitals, which may influence the number of referrals to the hospital. Hence, it is important that public reporting of results for surgeons and hospitals consider differences in baseline risk, which can be adjusted for with risk models. Providing adjusted data is important before the data is used to alter resources, spread discourage among staff treating high-risk patients, and give the public false impressions of poor results. [7, 254] Hence, no such data should be reported unless corrected for the underlying differences in patients´ preoperative status between hospitals. [10]

A limitation to the risk stratification models is that they predict risk of mortality at a group level and do not have the ability to predict mortality of specific individuals, [249] and it is not always best to use large-group-data to adapt on an individual level. [82] The risk scores neither take in evaluation the surgical skills, knowledge, or experience of the performing surgeon, nor issues regarding the institution, and just a handful comorbid diseases. [82] High-risk patients, that would maybe benefit the most from surgery, might therefore be in danger of not getting surgery due to the emphasis on mortality in risk models, so-called "high-risk case avoidance". [249, 255]

1.5 European System for Cardiac Operative Risk Evaluation

1.5.1 EuroSCORE II

The European System for Cardiac Operative Risk Evaluation (EuroSCORE) II is an updated version of the first EuroSCORE model. [8] EuroSCORE performed well when validated in the Society of Thoracic Surgeons database, and was a robust risk score seen as the gold standard in Europe, and was recommended for use also in North-America. [82, 256] As the EuroSCORE model aged, there was evidence that it over-predicted risk since cardiac surgery had improved over the past years and, accordingly, risk-adjusted mortality has been reduced. [8] The updated EuroSCORE II was released in 2012 based on 22381 patients undergoing major cardiac surgery from May to July 2010 in 154 hospitals in 43 countries. [8] The EuroSCORE II model is better calibrated to meet the composition of today´s population. The risk-adjusted mortality has fallen dramatically compared to the 1990s, leaving the old EuroSCORE behind and outdated, giving a strong acknowledgement to the improvement in quality of care in cardiac surgery. [8]According to Enger et al. in 2017, the EuroSCORE II is the largest and most used risk stratification model in the world. [257]

The new and improved risk stratification model has eighteen variables distributed across the same three categories as before (Table 3), whereas there have been some changes and additions of variables from the original seventeen. [8] The variables were selected on the background of availability, objectivity, resistance to falsification, and credibility to users. [8]

Table 3. *Illustration of EuroSCORE II with categories, variables, and the different options. Adapted with changes in design from euroscore.org/calc.html*

Categories	Variables	Options
Patient related factors		
	Age	Years
	Gender	Male/Female
	Renal impairment †	Normal (CC >85ml/min)
		Moderate (CC >50/ <85ml/min)
		Severe (CC < 50ml/min)
		Dialysis (regardless of CC)
	Extracardiac arteriopathy	Yes/No
	Poor mobility *	Yes/No
	Previous cardiac surgery	Yes/No
	Chronic lung disease	Yes/No
	Active endocarditis	Yes/No
	Critical preoperative state	Yes/No
	Diabetes on insulin *	Yes/No
Cardiac related factors		
	NYHA *	I
		II
		III
		IV
	CCS class 4 angina †	Yes/No
	LV function †	Good (LVEF > 50%)
		Moderate (LVEF 31-50%)
		Poor (LVEF 21-30%)
		Very poor (20% or less)
	Recent AMI	Yes/No
	Pulmonary hypertension †	No
		Moderate (31-55 mmHg)
		Severe (>55 mmHg)
Operation related factor		
	Urgency †	Elective
		Urgent
		Emergency
		Salvage
	Weight of intervention †	CABG
		Single non-CABG
		Two procedures
		Three procedures
	Surgery on thoracic aorta	Yes/No

AMI indicates acute myocardial infarction; CABG, coronary artery bypass graft; CC, creatinine clearance; CCS, Canadian Cardiovascular Society; LV, left ventricular; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association.

** new variable in the EuroSCORE II, † variable changed from the original EuroSCORE*

The EuroSCORE II model does not include circulating biomarkers. Recent studies have shown that high-sensitivity troponin T [175] add information to the model regarding major adverse events and mortality, while NT-proBNP [258] add information to the model regarding mortality. Cardiac troponin was not added to the EuroSCORE II model due to multiple assays available in different hospitals for troponin I and T. Because of this there would be different troponins measured and different ranges of "normal". [8]

The EuroSCORE II model is well calibrated, newly improved, and the largest and most used risk stratification model in the world. [8, 257] Overall performance for EuroSCORE II is comparable to the Society of Thoracic Surgeons cardiac surgery risk score. [259, 260] Nevertheless, EuroSCORE II does have some limitations. The model is not validated for patients above 95 years of age, and there were only 21 patients above the age of 90, making the model weak in this patient group. [8] There might also be a self-selection bias since all participating units were volunteers, however, this might be the best way to collect high-quality data since units that volunteer are eager to commit and do their best. [8] On the other hand, units that volunteers with outcome data might do so because they have better results than those that do not want to attend. The study group tried to eliminate this bias by encourage all types of units to attend. [8] Some argue that the model has too many variables, and has shown that a simpler model would give similar results, [260, 261] though without biomarkers included.

1.6 Study design

The two most common approaches for answering research questions and for hypothesis testing in clinical medicine and epidemiology are observational and experimental studies, each with different study designs. [262] It is important to know the strengths and limitations of each type of study design as individual designs provide unique characteristics and not all designs are suitable for all research questions. The best fit is always desirable, but can be obstructed by practical obstacles or ethical considerations. [263] How you choose to execute the study after you have chosen the design can also influence the quality of the study by altering its internal and external validity. [262] Internal validity can be determined on how well alternative explanations between exposure and outcome can be ruled out. The less the chance of confounding, the higher is the internal validity of the study. A confounder is a non-studied factor which is associated with the exposure/independent variable and is causally related to the outcome/dependent variable, [264] altering the true effect of the exposure on the outcome; [265] e.g. does having chronic obstructive pulmonary disease (COPD) increase risk of CAD (Figure 14)?

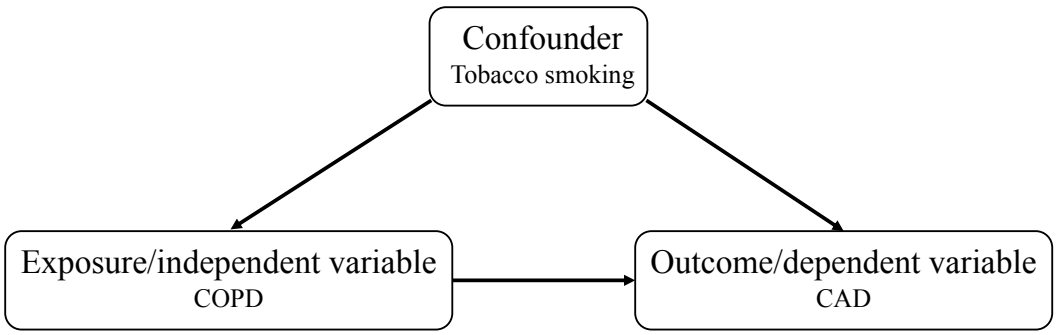

Figure 14. The exposure/independent variable affects the outcome/dependent variable. A confounding variable have a hidden effect on the dependent variable/outcome. In this case a confounding variable is tobacco smoking, since tobacco smoking increases both the risk of COPD and CAD.

External validation refers to generalization; i.e. how well the results can be applied to other populations. [264] The internal validity can be improved by alterations and close monitoring of the study population; e.g. adding groups for comparison and randomization. [262] External validation can be improved by representative samples and equal intervention in all groups. However, efforts to improve internal validity can make the observed population too specialized, reducing its generalizability, i.e. reducing the external validity. [262] Confounders can be prevented or corrected by restrictions in the study population, by matching with controls, by stratification in placebo and intervention groups, and by using statistical procedures. [265] Randomization is the only of the four mentioned techniques with ability to adjust for unknown confounders. [264]

Bias is another factor that can undermine the internal validity of a study. Unlike confounders, once bias occurs, irreparable damage has been done and cannot be corrected. There are many types of bias, and they can occur in all studies. Most observational studies have some bias, and also poor randomized controlled trials have bias. Perhaps the two most important sources of bias in observational studies are selection bias and information bias. Selection bias refers to the similarity of the groups being studied, while information bias refers to how the information is gathered in the compared groups, also involving recall bias in case-control studies. [265]

1.6.1 Observational Studies

In observational studies the investigator(s) assess(es) a population without doing any alterations to it. The time-point for when the risk factor is measured in relation to the outcome is the major difference between different types of observational studies; cross-sectional, cohort, and case-control. [264] We will discuss cohort studies here as this is the study design used in the patient cohorts of this thesis.

1.6.1.1 Cohort study

In prospective cohort studies, subjects without the studied outcome are enrolled and assessed for outcome during a follow-up time. Strengths of prospective cohort studies are minimized recall bias and reverse causality, and the opportunity to calculate incidence rate. [264] The most famous prospective cohort study, the Framingham Study, [266] has followed patients since 1948, and is still going. However, the possible long duration of cohort studies is their greatest disadvantage, especially if the disease takes long time to develop. [264] Long time equals less cost efficiency, ineffectiveness, and bias due to subjects lost to follow-up. Also, new treatment might be invented, or others may have answered your research question by the time your study is finished.

2 Aim and research questions

2.1 General aim

The overarching aim of this thesis was to examine established and novel cardiac biomarkers and their prognostic value during long-term follow-up after cardiac surgery. More specifically; we wanted to see if NT-proBNP, hs-cTnT, and SN could improve the established EuroSCORE II risk model for cardiac surgery, and whether SN would provide independent prognostic information in patients with AS.

2.2 Main research question

2.2.1 Paper I

Can NT-proBNP and hs-cTnT improve the EuroSCORE II risk model in cardiac surgical patients? Can a risk model with fewer key clinical variables, but with biomarker measurements included, provide comparable or better prognostic information to the tedious EuroSCORE II risk model?

2.2.2 Paper II

Can SN provide additional prognostic information EuroSCORE II and the established cardiac biomarkers NT-proBNP and hs-cTnT in cardiac surgical patients?

2.2.3 Paper III

What pathophysiology is associated with circulating SN concentrations in AS patients? And, can SN concentrations provide additional prognostic information to established circulating cardiac biomarker concentrations?

3 Materials and methods

3.1 In brief

In **paper I** we measured preoperative and postoperative concentrations of NT-proBNP and cTnT with a high-sensitivity assay in a Finnish cohort of patients undergoing composite cardiac surgery. We also explored the same variables in a second Finnish cohort of cardiac surgical patients requiring ≥6 hours of ventilatory support. In **paper II** we measured SN together with NT-proBNP and hs-cTnT in the same cohort as in **paper I**. In **paper III** we measured preoperative concentrations of SN, NT-proBNP, and hs-cTnT in a Norwegian cohort of patients with moderate to severe aortic stenosis (AS). Patients in **paper III** were extensively characterized regarding cardiac structure and function and this was done at presurgical evaluation at Oslo University Hospital, Rikshospitalet. In this study, we also compared SN directly to the other main granin proteins; CgA and CgB, and we also compared biomarker concentrations in the AS patients with concentrations in 10 healthy age- and gender-matched control subjects. The endpoints were all-cause mortality during long-term follow-up for all cohorts.

3.2 Cohorts

3.2.1 FINNAKI Heart Study

The FINNish Acute Kidney Injury (FINNAKI) Heart Study was utilized for **paper I** and p**aper II.** The FINNAKI Study is a prospective observational study comprising 17 of 25 Finnish ICUs (intensive care units) lasting from September 1st 2011 to February 1st 2012. [267] All emergency ICU admissions and all elective patients hospitalized in ICU longer than 24 hours were included, except some pre-specified groups according to the study protocol. The Ethics Committee of the Department of Surgery in Helsinki University Hospital approved the study and a delayed consent from a patient or a next of kin was allowed. Data collection from medical records was approved by The Finnish National Institute of Health and Welfare. The FINNAKI Heart Study is a prospective observational single center sub-study of the multicenter FINNAKI Study with an extended inclusion making the total period range from September 1st 2011 to June 20th 2012. [175] It consists of two cohorts of cardiac surgical patients; (1) consecutive coronary artery bypass graft (CABG) surgery patients from September 1st 2011 to June 20th 2012, and (2) consecutive other cardiac surgical patients with some exceptions from September 1st 2011 to January 31st 2012. The total follow-up was 961 days. Mortality data for endpoints were obtained from the Finnish Population Register Center. Each patient or a next of kin provided informed consent, and the study was approved by the Ethics Committee in Helsinki University Hospital, Finland.

3.2.2 FINNALI Study

This study was also included in **paper I**. The FINNish Acute Lung Injury (FINNALI) Study was a prospective observational multicenter cohort study with 25 participating ICUs. [268] The study was conducted from April 16th to June 10th 2007. The aim of the study was to define overall incidence and mortality of acute respiratory failure. All patients 16 years or older admitted to the ICUs were screened for their need of respiratory support. Both patients who required invasive and non-invasive treatment were eligible for the study. Only patients who required more than 6 hours of ventilatory support were included in the study, which resulted in 958 patients included in the study. Of the 958 included patients, 90 patients (9.5%) had undergone cardiac surgery prior to developing acute respiratory failure. Maximal follow-up was 365 days, and data on all-cause mortality was obtained from Statistics Finland. Informed consent was given by all patients or a close relative before they were included in the study. Ethical consent covering all hospitals was obtained from the Helsinki University Ethics Committee and the study was conducted according to the Declaration of Helsinki.

3.2.3 Aortic stenosis cohort

The aortic stenosis study was conducted at Oslo University Hospital, Rikshospitalet, in Oslo, Norway from May 2005 to April 2009. The study included 57 patients with AS recruited at pre-surgical evaluation with echocardiography, and 10 healthy controls matched on age and sex, and no previous history of CVD or diabetes mellitus. The patients had moderate to severe AS and were followed for maximum 1537 days (4.2 years). An adjudication committee of two independent physicians (TE, TO) determined the NYHA functional class of all patients, and we resolved discrepancies (4.7%) by consensus. Written informed consent was acquired from all patients before study commencement. The study was performed according to the Declaration of Helsinki, and it was approved by Oslo University Hospital, Rikshospitalet, and the Norwegian South-Eastern Regional Ethics Committee.

3.3 Biochemical analyses

We used different assays to quantify NT-proBNP in the studies as measurements were performed at different sites and time points. We measured NT-proBNP by a commercially available immunometric assay (proBNP, Roche Diagnostics) on an Elecsys 2010 auto-analyzer (Roche Diagnostics) in the FINNALI Study, while we used the same assay but the Cobas 8000 e801 analyzer (Roche Diagnostics) for NT-proBNP quantification in the FINNAKI Heart Study. In the aortic stenosis study, NT-proBNP concentrations were measured by the pro-BNP II assay (Roche Diagnostics) on an auto-analyzer with a coefficient of variation (CV; see Table 4 for definition) of 4.5 at 120 ng/L and 4.0 at 580 ng/L in the laboratory at Akershus University Hospital.

Table 4. *Definitions*

Term	Definition
Coefficient of variation (CV)	A measure of the dispersion of data around the mean defined as the ratio of the standard deviation to the mean
Limit of blank (LoB)	The highest concentration expected to be found in a sample without the analyte [269]
Limit of detection (LoD)	The lowest concentration that can be detected and distinguished from a sample without the analyte and from the LoB with the analytical method used [269]

Concentrations of cTnT were measured with an auto-analyzer (Cobas 8000 e801, Roche Diagnostics) by a commercial assay (troponin T hs STAT, Roche Diagnostics) in the FINNAKI Heart Study. The limit of detection (LoD; see Table 4) of this assay is 5 ng/L, limit of blank (LoB; see Table 4) is 3 ng/L, and the 99th percentile value in healthy subjects is 14 ng/L with a CV of 8%. [168, 270] In the aortic stenosis study, cTnT concentrations were measured with an auto-analyzer (Cobas e411, Roche Diagnostics) with a high-sensitivity cardiac troponin T assay with an LoD of 5 ng/L and a CV of 10% at 13 ng/L and 99th percentile 14 ng/L.

SN concentrations were measured with an in-house radioimmunoassay (RIA) at the Department of Medical Sciences at Uppsala University, Sweden in all cohorts. [188] The SN assay has an LoD of 50 pmol/L, and a CV of 9% in the lower range (110 pmol/L) and 4% in the upper range (380 pmol/L).

We measured CgA concentrations with a commercially available RIA (EuroDiagnostica AB, Malmö, Sweden). [271] The assay has an LoD of 0.80 nmol/L with a CV of 13% in the lower range (3.1 nmol/L) and 9% in the upper range (17.0 nmol/L).

CgB concentrations were measured with an in-house RIA at Uppsala University Hospital, Sweden. [29] The LoD is ≥0.80 nmol/L with a CV of 17% in the lower range (1.40 nmol/L) and 8% in the upper range (6.40 nmol/L).

The Cockcroft-Gault formula was used to calculate creatinine clearance. [272]

3.4 Statistical analyses

In the papers of this thesis the statistical analyses were performed with different statistical programs, including IBM SPSS Statistics 22.0, 24.0, and 25.0 (IBM Corp, Armonk, NY, USA), MedCalc Statistical Software version 14.10.2 (MedCalc Software, Ostend, Belgium), Stata Statistical Software (StataCorp. 2017. Stata Statistical Software: Release 15 College Station, TX: StataCorp LLC), and the statistical programming language R (R Foundation for Statistical Computing, Vienna, Austria).

The same set of basic statistical analyses were performed in all papers to achieve equal statistical handling throughout the papers.

3.4.1 Baseline analyses

The focus on circulating biomarkers in this thesis has influenced the choice of statistics chosen for continuous variables in the baseline analyses. Distributions of biomarkers are often skewed due to their natural characteristics of low mean value with even lower median, large variance, and only positive values. [273] Parametric statistics presented as mean and standard deviation can only be applied to normally distributed data, [273] and as a consequence we have only used non-parametric statistics for biomarkers and other continuous variables for consistency throughout all three papers. The non-parametric statistical methods; the Mann-Whitney *U*-test and the Exact test have been used as appropriate for continuous variables. We have transformed the biomarkers by the natural logarithm to deal with skewness, and they are, as the other continuous variables, presented as median and quartile 1-3. Outliers do not influence the median value as much as the mean value, and by also performing transformation of skewed data with the natural logarithm, we have minimized skewness and the outliers' effect on the results and presentation of data.

3.4.2 Correlations

Correlation is a statistical method to assess a positive, negative, or no association between two variables, and the strength of the association. [274] Variables may be correlated without one variable being the function of the other as in regression analyses with dependent and independent variables. The Spearman's rank correlation coefficient; rho, is a non-parametric measure of the relationship between the ranking of two variables when one or both variables are skewed (not normally distributed). [274]

3.4.3 Linear regression models

The simplest method to compare the association between two variables is a straight line, and linear regression is used to predict the value of one variable (dependent) based on another variable (independent). By plotting data according to x- and y-values, a scatter of plots will most likely appear, as the data of more than two points rarely forms a perfect line. The line that best fits the plot is the line with the lowest sum of the square of all deviation. The equation for the straight line is set by the interception of the y-axis (a) and the slope/regression coefficient (b);

$$y = a + bx.$$

The regression coefficient provides information on the contribution of the independent variable towards explaining the dependent variable. The dependent variable must be continuous, while the independent variable can be either continuous or categorical. [275] The dependent variable cannot always be explained by one independent variable, and two or more independent variables can be added to a multivariable linear regression model. An R^2 is calculated and explains in percentage how much of the variance in the dependent variable that is related to the variables included in the final regression model. [275]

3.4.4 Logistic regression models

Logistic regression also known as binomial logistic regression, predicts the probability of an observation to be in one of two categories of the dependent variable (dichotomous variable) based on one or more independent variables (continuous or categorical). With a binary outcome, logistic regression is often used to study outcomes in epidemiologic studies; e.g. presence or absence of disease. The effect of the independent variable on the dependent variable is quantified by the odds ratio. [276]

3.4.5 Survival analysis by the Kaplan-Meier method

The Kaplan-Meier method is a nonparametric test, and is used to estimate survival probability past given time points within a population. With the log-rank test, the difference in survival between the exposed groups can be estimated. [277] The survival of the population can be visualized with a Kaplan-Meier plot, with survival probability on the y-axis and time on the x-axis (Figure 15). A limitation to the Kaplan-Meier method is that it cannot provide an estimate of the size of the difference between the groups, nor related confidence interval, just the p-value by the log-rank test. [277] Normally, Kaplan-Meier plots are presented as unadjusted data, and no adjustment for confounding effects of other variables reduces the clinical value of these data. [278]

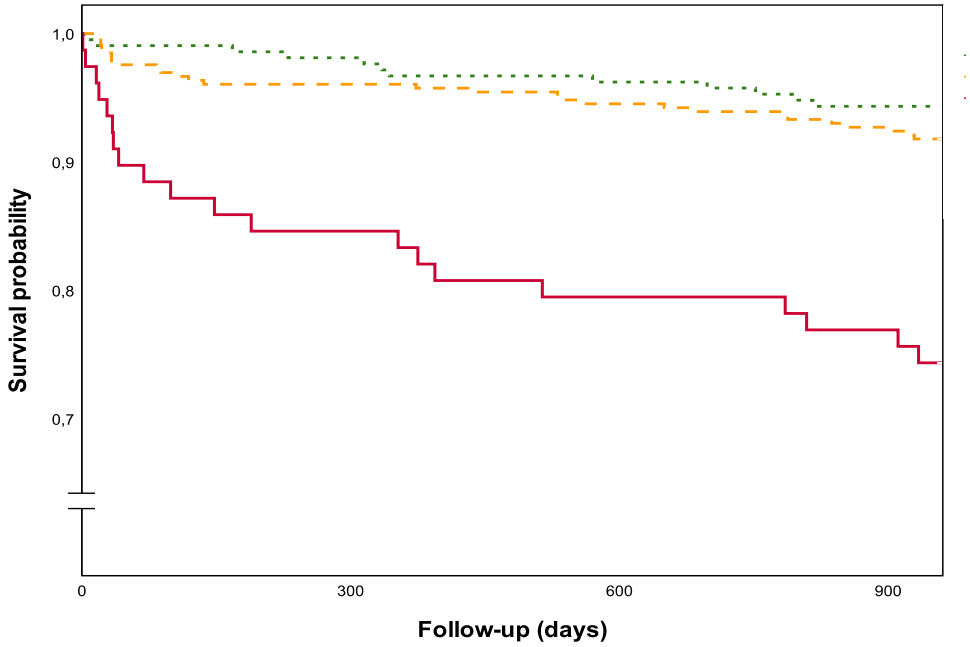

Figure 15. *Kaplan-Meier plot. The survival of a population can be visualized with a Kaplan-Meier plot, with survival probability on the y-axis and time on the x-axis. The variable in this example is divided in three groups represented by the three different lines in the figure (green dotted, yellow striped, and red solid).*

3.4.6 Cox proportional hazard regression models

Cox proportional hazard regression models are useful to analyze survival data. With this method, event and time to event together with one (unadjusted analysis) or more independent variables (adjusted analysis) will provide an effect estimate (hazard ratio [HR]) of the difference in outcome (e.g. survival between the groups if the endpoint of the study is mortality). [277] Other endpoints than mortality are also possible, including the appearance of disease. HR is the ratio of two hazards (e.g. survivors vs. non-survivors), and hazard is an incidence rate of an event within a set timeframe. [277] As HR is only a point estimate of the effect and does not include statistical variation or random error around the estimate, a confidence interval (CI) will help with quantification of the precision of the sample estimate. [279] The strength of Cox proportional hazard regression models is that they can present both unadjusted and adjusted HRs with confidence intervals, which is different from how Kaplan-Meier plots normally are presented. [277]

3.4.7 Receiver operating characteristics

The receiver operating characteristics (ROC) with the area under the curve (AUC), also known as C-statistics for a multivariable model, is a widely used statistical metric to estimate the diagnostic accuracy of a test to distinguish between two outcomes; e.g. disease vs. no disease. The accuracy of a biomarker test provides a characteristic of the test's ability to separate cases from non-cases, and this metric is important to determine the quality of the

biomarker. [280] As many biomarkers are continuous measures, especially circulating cardiac biomarkers, it is important to evaluate a biomarker on a range of values with different sensitivity and specificity, making the ROC curve an illustration of tradeoff between sensitivity and specificity. [280] The ROC curve is a function of the test's sensitivity or true positive rate on the y-axis and 1-specificity or false positive rate on the x-axis (Figure 16). The AUC corresponds to the probability of correctly identifying subjects to be "sick or not sick", or to "die or not die", etc. The closer the line gets to the upper left corner, the closer it is to a perfect fit, or a value of 1. [281] The practical lower limit is 0.5 as this forms a straight diagonal line between the lower left and the upper right corner, the no discrimination line, and represent random guess or flipping a coin instead of using a clinical test. [282]

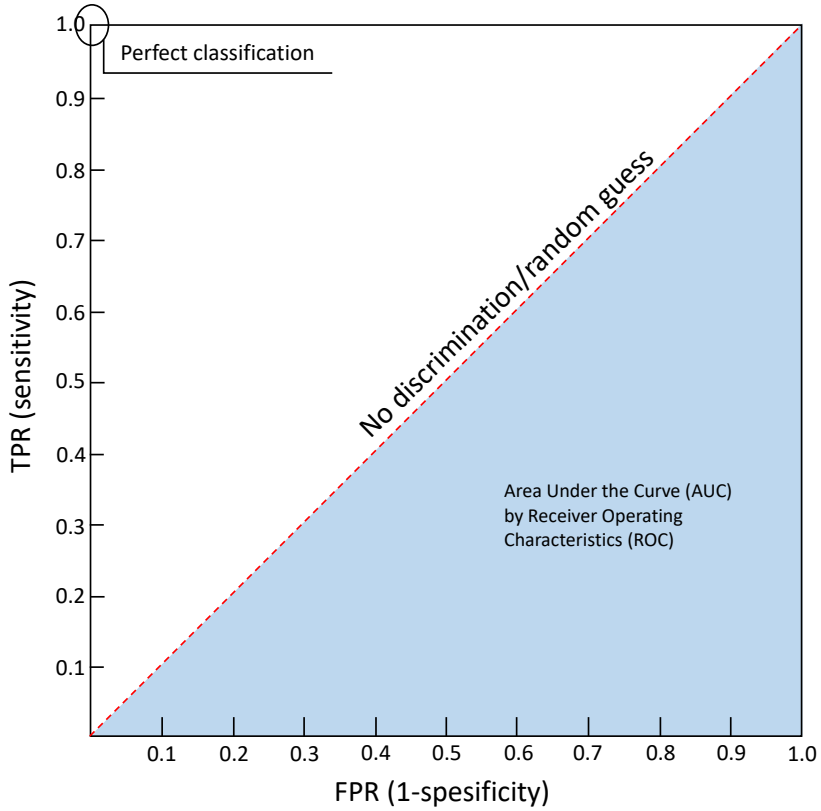

Figure 16. *The reiceiver operating characteristics (ROC) curve with definitions. TPR indicates true positive rate; FPR, false positive rate.*

3.4.8 Youden J index

The Youden J index is a performance measure of a binary classification and is used as a measure of overall diagnostic effectiveness. [283] It combines sensitivity and specificity into one single measure, calculated as sensitivity + specificity – 1. The range of values goes from 0-1, with a perfect test of 1 representing high effectiveness. If a test has no diagnostic value, the value will be 0 and sensitivity=1-specificity. The Youden J score also corresponds to the vertical distance above the no discrimination/random guess line to the ROC curve for a single cut-off. Continuous biomarkers are often dichotomized for diagnostic purposes to

divide healthy and sick patients below and above a cut-off, and the optimal cut-off to separate the two groups can be calculated with the Youden J index. [283] To find the optimal cut-off there has to be a tradeoff between sensitivity and specificity. In the papers of this thesis we used the Youden J index to calculate the optimal cut-off for biomarkers to discriminate between two conditions. We also used it to calculate and define risk-groups in **paper II**; low-risk, intermediate-risk, and high-risk according to SN concentrations.

3.4.9 Net reclassification improvement

The additional information gained from an extended model compared to a baseline model have often been investigated by comparing the models' AUCs. For example, how much does the accuracy of a risk model increase by adding a novel biomarker? An issue identified and problematized by Pencina et al [284] is that the often minor difference (delta) between the two models' AUC does not necessarily incorporate the clinical importance of adding another variable to the original multivariate (basic) model. This is also seen when adding variables to strong and established risk models (e.g. Framingham risk scores). [285] To better quantify this change they proposed that researchers should calculate net reclassification improvement (NRI).

The subjects in the NRI model are divided into two groups, with and without an event, which could be occurrence of a disease or death. By measuring the extended model's reclassification of subjects with and without events (i.e. moving subjects with later event up in risk strata and moving subjects without events down in risk strata), an overall NRI can be calculated. Each groups' score is a percentage ranging from -100% to 100%, where NRI = -100% means that all subjects with later events are (wrongly) classified down and all subjects without later events are (wrongly) classified up. In contrast, NRI = 100% means that all subjects with later events are (correctly) classified up or all subjects without later events are (correctly) classified down. In category-based NRI the event is classified as up/down in categories, while in continuous-based NRI the event is classified as increased/decreased predicted risk. The overall NRI from event NRI and non-event NRI is a rate and the theoretical range is -2 to 2. [286] The integrated discrimination improvement (IDI) is derived from the NRI model and is used for the same purpose as NRI.

In **paper I** we investigated the incremental value by adding NT-proBNP and/or hs-cTnT to the established EuroSCORE II (basic model) by the category-free net reclassification improvement, and in **paper II** we investigated the incremental value SN could add to EuroSCORE II, NT-proBNP, and hs-cTnT by calculating the category-free net reclassification index (NRI) and the integrated discrimination index (IDI). [287]

3.5 Strategy to build a parsimonious risk model

The Cardiac Surgery Biomarker Score is a parsimonious risk model we derived from the FINNAKI Heart cohort through statistical calculation. As EuroSCORE II is a large risk model with many variables and the lack of biomarker information, we wanted to investigate the characteristics of a risk model with less variables and the presence of cardiac biomarker measurements. To identify the statistically strongest variables best suited for this model we first calculated ROC-AUC of all variables included in the EuroSCORE II individually, EuroSCORE II as a model, and all the biomarkers individually. We then arranged all possible combinations of two, three, and four variables, and by using lasso regression we identified the combination with the highest ROC-AUC. A risk score developed from one dataset needs to be validated in an independent external cohort to identify its true performance in cardiac surgery cohorts. Accordingly, we tested also our parsimonious Cardiac Surgery Biomarker Score also in a second cohort of cardiac surgical patients in **paper I**.

3.6 Electrocardiogram

ECG was used in the aortic stenosis cohort in **paper III** to investigate pathology in the heart by recording the electric activity. We recorded ECGs with a standardized 12-lead ECG and the results were interpreted in a standardized way by myself. I was blinded to concentrations of circulating biomarkers when performing the ECG analysis. The ECG was interpreted using criteria from the American Heart Association guidelines [288] for bundle branch block and I used the Sokolow-Lyon Criterion [289] to identify left ventricular (LV) hypertrophy. Automatic reading and validation were done identifying ventricular frequency, PQ-time, and QRS-width. An online calculator [290] was used to calculate QTc by QT-time and ventricular frequency. We did not perform ECG recordings on the control group in **paper III**.

3.7 Echocardiography

Echocardiography was used to investigate cardiac function and structure in patients and control subjects in the aortic stenosis cohort in **paper III**. The full echocardiographic investigation was conducted with a Vivid 7 Ultrasound System (GE Vingmed, Horten, Norway) with the patients in supine left lateral position. A senior physician (TE) reviewed all recording while blinded to concentrations of circulating biomarkers. We determined the structure by calculating LV dimension, mass, and septal and posterior wall thickness as recommended. [291] We calculated LV ejection fraction by using the modified Simpson's rule and also determined fractional shortening to assess LV systolic function. Cardiac index was calculated by dividing cardiac output (stroke volume x heart rate) in L/min with body size in square meters. We also calculated shortening of LV diameter between end-diastole and end-systole to determine fractional shortening. We assessed the narrowing of the aortic valvular orifice by measuring aortic valve velocity and calculating the mean pressure gradient and

aortic valve area. To determine diastolic function, we used pulsed Doppler to measure transmitral peak early (E) and peak late (A) deceleration time. We recorded early diastolic velocity (e') at the base of the septal and lateral mitral annulus and calculated E/e' as a measurement of diastolic function. We also obtained a value for mechanical dispersion from all patients by measuring the time intervals from start Q/R to peak negative strain across 16 segments and calculated the standard deviation from all LV segments to obtain a value for mechanical dispersion.

3.8 Assessment of validity

3.8.1 Internal validity

Internal validity is a measure of how well the study was conducted. Increased sources of bias (e.g. confounding variables, random errors, and systematic errors) will reduce internal validity and such factors should be reduced to a minimum. However, too strict focus on internal validity with broad exclusion criteria will result in inclusion of a selected group of patients, which could reduce external validity.

3.8.2 External validity

The external validity of a study is how well the conclusions can be applied to an unselected environment outside of the study, also known as generalizability. Many variables should be considered to make the study population as identical as possible to the target population outside of the study. To investigate the external validity, an independent validation cohort not associated to the current study is an important asset. Inclusion of patients only from one center also indicate a need for additional validation to ensure stronger external validity.

3.8.3 Sample selection-bias

Sample selection-bias occurs when you try to generalize from a sample population in a trial to the whole population outside the trial. [292] The challenge occurs when the study population is not identical enough to the population of interest in "real life".

4 Results

4.1 Paper I

A total of 640 patients in the FINNAKI Heart Study underwent cardiac surgery and had pre- and postoperative measurements of NT-proBNP and hs-cTnT. During 961 days of follow-up, 61 patients (9.5%) died. In baseline analyses non-survivors had higher concentrations of preoperative and postoperative NT-proBNP, and preoperative hs-cTnT concentrations than survivors. Postoperative NT-proBNP and hs-cTnT concentrations were higher than preoperative concentrations. Preoperative NT-proBNP and preoperative hs-cTnT concentration correlated (rho=0.58; $p<0.001$).

Patients with preoperative NT-proBNP and hs-cTnT concentrations in the 4th quartile had increased mortality during 961 days of follow-up (Figure 17). We found both preoperative NT-proBNP and hs-cTnT to be associated with time to death during the follow-up. However, only preoperative NT-proBNP concentrations gave additional prognostic information to the EuroSCORE II score. The combination of preoperative NT-proBNP and EuroSCORE II yielded a ROC-AUC of 0.762 (0.699-0.826) compared to AUC of 0.740 (0.672-0.807) for EuroSCORE II alone and the addition of preoperative NT-proBNP reclassified a significant proportion of patients when added to EuroSCORE II.

We also identified the Cardiac Surgery Biomarker Score containing three variables from the EuroSCORE II model (age, estimated creatinine clearance, and history of chronic pulmonary disease) and preoperative NT-proBNP concentrations in this work. The parsimonious risk model was derived from the FINNAKI Heart cohort and this new model provided a ROC-AUC of 0.787 (95% CI 0.726-0.848) for all-cause mortality compared to AUC for EuroSCORE II of 0.744 (0.676-0.811). The Cardiac Surgery Biomarker Score also performed well when tested in a second cohort of cardiac surgical patients with respiratory failure from the FINNALI Study.

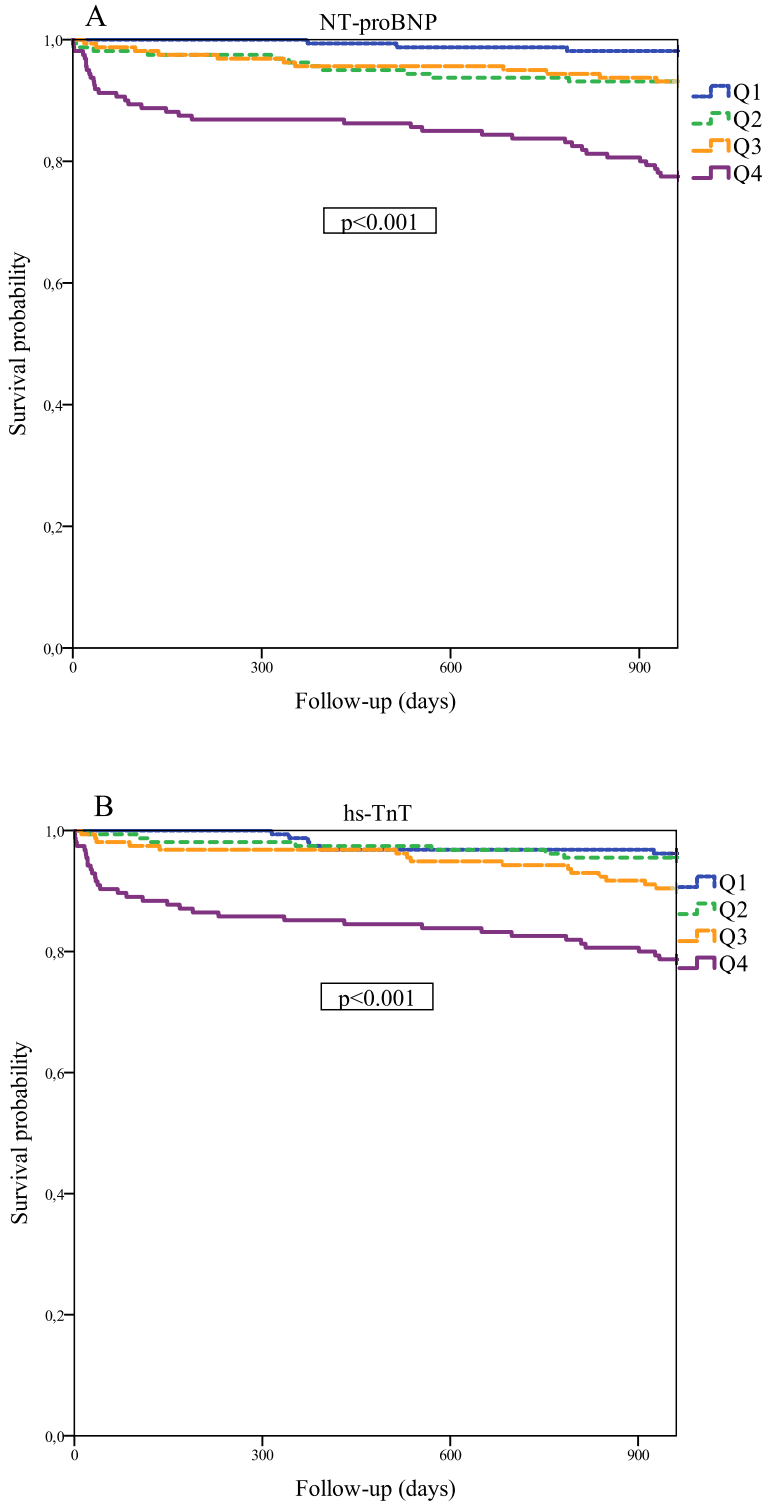

Figure 17. *Kaplan-Meier survival plots divided by quartiles of (A) preoperative NT-proBNP concentrations (ng/L) [Q1<148, Q2 148-438, Q3 439-1574, Q4>1574] and (B) hs-cTnT concentrations (ng/L) [Q1<8.1, Q2 8.1-14.7, Q3 14.7-37.1, Q4>37.1].*

4.2 Paper II

We had 619 cardiac surgical patients available in the FINNAKI Heart Study for SN analyses with 59 patients (9.5%) dying during 961-day follow-up. We found association between higher postoperative SN concentrations and worse prognosis. Postoperative SN concentrations were also associated with time to death in multivariable analysis that adjusted for the established cardiac biomarkers hs-cTnT and NT-proBNP and EuroSCORE II. Adding postoperative SN concentrations to EuroSCORE II improved classification of patients, while the addition of postoperative hs-cTnT and NT-proBNP concentrations to EuroSCORE II did not significantly reclassify patients to their correct risk strata.

Increase in SN concentrations from preoperative to postoperative measures were also associated with time to mortality: hazard ratio 2.53 (95% CI 1.15-5.59; p=0.02). We found no change in SN concentrations from pre- to postoperative measurements for non-survivors while we found a significant decrease in SN concentrations after surgery for survivors (Figure 18). In contrast, NT-proBNP and hs-cTnT concentrations increased after surgery for all patients, both survivors and non-survivors (Figure 18).

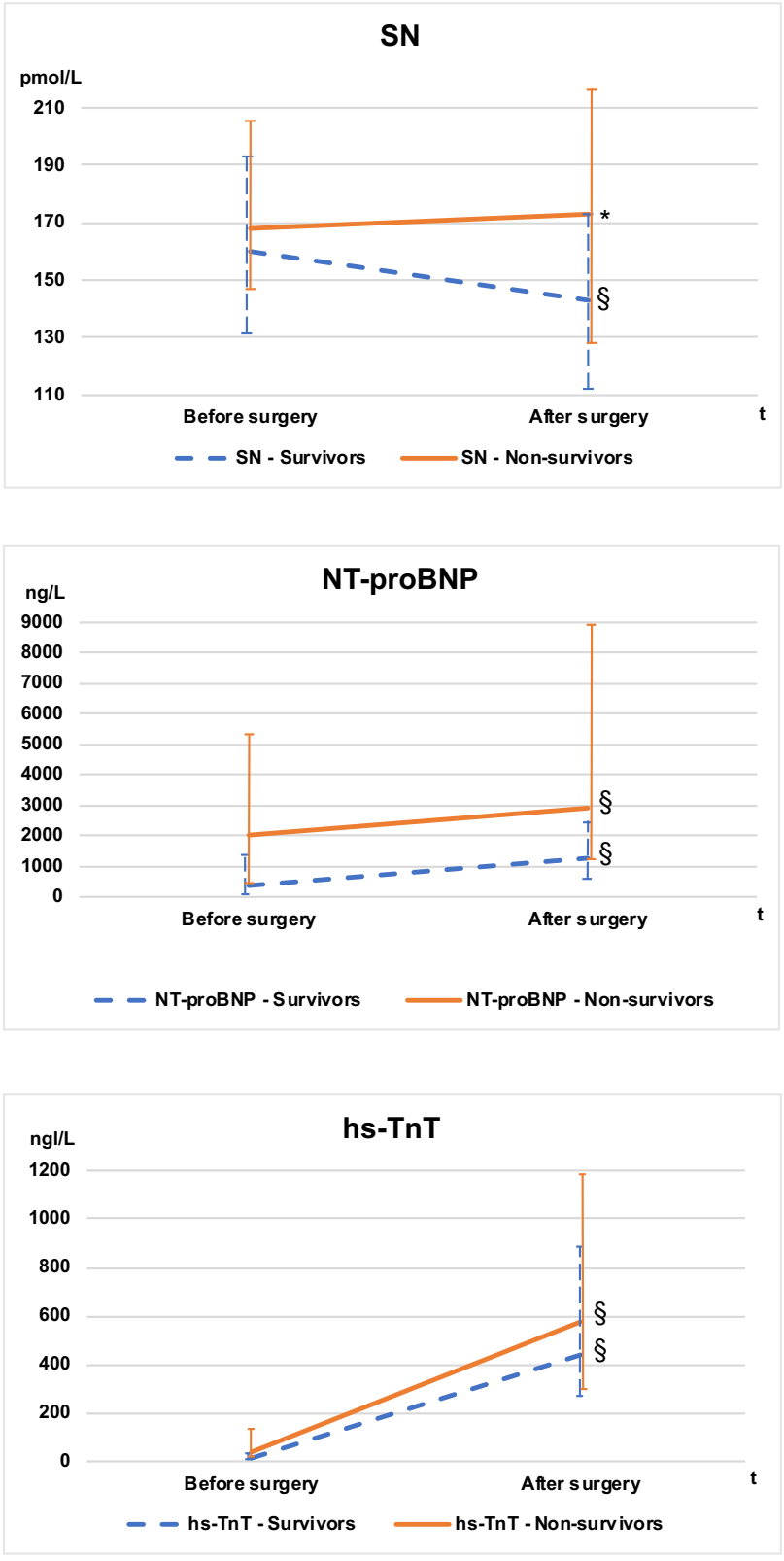

Figure 18. *Changes in biomarker concentrations after cardiac surgery, with vertical lines representing 95% confidence intervals. § p-value <0.001 for preoperative to postoperative change in biomarker concentration. * p-value 0.41 for preoperative to postoperative change in biomarker concentration.*

4.3 Paper III

We included 57 patients with moderate to severe AS and 10 age- and sex-matched controls in the AS study. Median follow-up for the AS patients was 3.5 (Q1-3 2.9-3.8) years and a total of 15 (26.3%) patients died during follow-up. Non-survivors had higher concentrations of SN than survivors (median 156 [133-209] pmol/L vs 140 [116-155] pmol/L; p=0.007). The optimal cut-off for SN concentrations to discriminate long-term survivors from non-survivors was 147 pmol/L, and patients above this cut-off had worse prognosis than patients below the cut-off (Figure 19). We found higher concentrations of SN to be associated with mortality in Cox proportional hazard analysis that adjusted for clinical characteristics, established cardiac biomarkers, and echocardiographic parameters. ROC-AUC to predict mortality was 0.74 (95% CI 0.60-0.88) for SN, 0.73 (0.59-0.87) for hs-cTnT, and 0.67 (0.51-0.82) for NT-proBNP. We found no difference in preoperative SN concentrations between AS patients and the healthy control subjects.

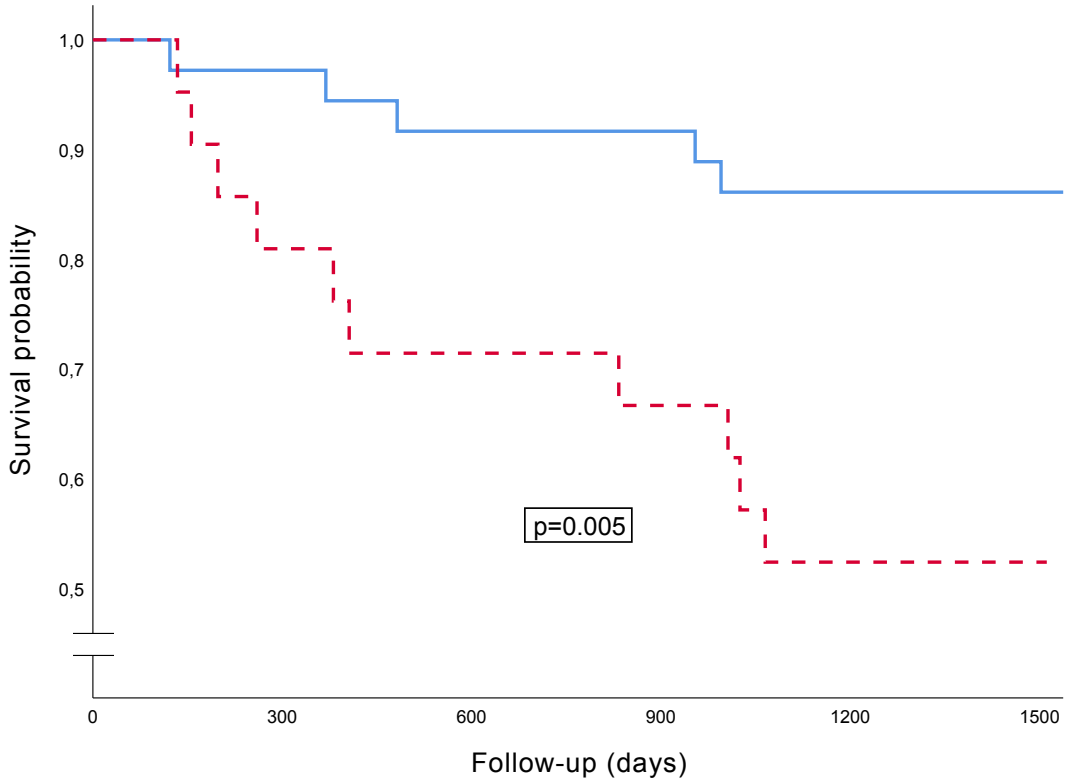

Figure 19. *Cumulative survival in patients with aortic stenosis according to the optimal cut-off concentration (147 pmol/L) for secretoneurin measured prior to aortic valve replacement surgery.*
The event rate in the group above cut-off was compared to the event rate in the group below cut-off by the log-rank test. Below optimal cut-off corresponds to secretoneurin concentrations equal or below 147 pmol/L (blue solid line) and above cut-off corresponds to secretoneurin concentrations above 147 pmol/L (red striped line).

5 Discussion

5.1 General findings

The main findings of this thesis can be divided in two: (1) established cardiac biomarkers add information to EuroSCORE II and may simplify risk prediction, and (2) the novel biomarker SN provides incremental prognostic information to established risk indices and biomarkers in cardiac surgical patients.

5.2 Established cardiac biomarkers add information to EuroSCORE II and may simplify risk prediction

The established cardiac biomarkers BNP/NT-proBNP (BNPs) and hs-cTnT are commonly used as biomarkers reflecting pathophysiology like myocyte stretch, ventricular hypertrophy, and ventricular wall stretch for BNPs and myocyte injury for cardiac troponin. [123, 150] BNPs have previously been shown to assess risk of postoperative mortality, [135-137] and both preoperative and postoperative concentrations predict long-term outcome after cardiac surgery. [138-141] Similarly; the combination of elevated preoperative and postoperative hs-cTnT concentrations have also previously been identified to carry prognostic information in cardiac surgical patients. [175] We now validate these earlier studies in **paper I** by demonstrating that preoperative and postoperative concentrations of NT-proBNP and preoperative concentrations of hs-cTnT are associated with time to death. [135-141, 175] These results may help identify the patients in need of close follow-up, hopefully to prevent development of later heart failure.

In cardiac surgery, preoperative risk assessment has been improved by the introduction of risk scores, and the most commonly used risk score in Europe is EuroSCORE II. [8] Although both NT-proBNP and hs-cTnT have been identified as strong risk markers in cardiac surgical patients, NT-proBNP has only been found to improve EuroSCORE II when dichotomized according to an optimal cut-off and when employed on patients with intermediate EuroSCORE II risk profile. [175, 293] In our work, we find that preoperative and postoperative concentrations of NT-proBNP, included as a continuous variable, give incremental prognostic information to EuroSCORE II regardless of assumed risk status. Hence, our results validate and extends previous knowledge on the use of NT-proBNP in cardiac surgical patients.

As EuroSCORE II is comprehensive and time consuming to calculate, and as EuroSCORE II does not include circulating biomarker measurements, we also created a parsimonious risk score with biomarkers, which we named the Cardiac Surgery Biomarker Score. The Cardiac

Surgery Biomarker Score is a combination of preoperative NT-proBNP concentrations, age, estimated creatinine clearance, and history of chronic pulmonary disease. With only four variables it is easier to use, and it performs well with higher AUC than EuroSCORE II. With increase in cardiac surgery among older individuals and more high-risk patients, [294] we believe there could be clinical need for a parsimonious risk model with the inclusion of a circulating cardiac biomarker. Accordingly; we propose the Cardiac Surgery Biomarker Score to simplify and improve preoperative cardiac surgical evaluations as the variables in the model are all easy to collect.

The strength of NT-proBNP as a prognostic biomarker, individually both pre- and postoperative, and incremental to the EuroSCORE II risk model, positions NT-proBNP as a circulating biomarker also for cardiac surgical patients. As a commercial biomarker known to most physicians, NT-proBNP is available, quick, and easy to use and interpret, which should make NT-proBNP ideal for preoperative evaluation ahead of cardiac surgery. However, there are always possibilities for random errors when using biomarkers both in clinic and in research, thus we ran the NT-proBNP and hs-cTnT analyses from one cohort in one batch to minimize random error between blood samples after they had been thawed. Both NT-proBNP [295, 296] and hs-cTnT [297] have shown long-term stability as frozen samples, also after repeated freeze-thaw cycles, and our measurements should not be source of random error.

The sample size in the FINNAKI Heart cohort is fairly large, counteracting other sources of random errors, and giving it strength in statistical calculations. However, the cohort is from only one center which might give some polarization and need for additional external validation. The Cardiac Surgery Biomarker Score is derived from the material in our cohort and tested towards EuroSCORE II in the same cohort, and therefore we also wanted to test a parsimonious model in a second cohort. We therefore tested the Cardiac Surgery Biomarker Score also in cardiac surgical patients with respiratory failure (FINNALI study), but had to substitute preoperative with postoperative NT-proBNP measurements as no biomarker measurements were performed before surgery in this cohort.

5.3 The novel biomarker SN provides incremental prognostic information to established risk indices and biomarkers in cardiac surgical patients

SN is a novel circulating cardiac biomarker which previously has been investigated in other cohorts by our research group. [31, 235, 237, 298] As SN has shown promising results in other cohorts of critically ill patients, we wanted to investigate the characteristics and prognostic

value of SN in patients undergoing cardiac surgery. We compared the characteristics of SN to the established cardiac biomarkers NT-proBNP and hs-cTnT, the EuroSCORE II risk score, ECG variables, and echocardiographic indices.

In **paper II** we found that postoperative SN measurements provided incremental prognostic information to NT-proBNP, hs-cTnT, and EuroSCORE II in cardiac surgical patients, which have previously not been demonstrated. In both groups, survivors and non-survivors, the concentrations of hs-cTnT and NT-proBNP increased from preoperative to postoperative measurements upon cardiomyocyte stress, stretch, and injury during cardiac surgery. For SN, concentrations for long-term survivors decreased from preoperative to postoperative measurements, while patients later dying during follow-up demonstrated increasing concentrations or no change in SN concentrations after cardiac surgery. We believe this result may have clinical potential as decreasing SN concentrations after cardiac surgery can be used to stratify patients for later events, while such information was not available from changes in NT-proBNP or hs-cTnT concentrations. The difference in delta values between survivors and non-survivors for SN concentrations supports theories of increased SN concentrations in patients with high risk of subsequent mortality. Of note, these results for SN have not been externally validated and further studies are therefore needed to assess whether SN could have a role in cardiac surgical risk assessment.

SN has previously shown to provide additional information to established circulating cardiac biomarkers. [31, 235, 298, 299] In **paper II** postoperative SN was associated with mortality. In **paper III** we also found preoperative SN concentrations to be associated with mortality during follow-up, and SN concentrations were not correlated with established risk indices. We believe that SN may help to identify a subgroup of AS patients with poor prognosis, which is not identified by established circulating cardiac biomarkers, ECG, nor echocardiography. SN concentrations were not higher in patients with moderate-to-severe aortic stenosis compared to healthy control subjects, and we did not find evidence of SN as a biomarker reflective of structural heart disease as there are no association between SN and circulating cardiac biomarkers, ECG, nor echocardiography in **paper III**. This information also supports previous theories of SN as a marker of systemic stress, [31] however, this theory also needs additional research and validation to establish the pathophysiology of SN.

As SN is a novel biomarker not yet commercialized, there are no current standardized cut-offs for use in the general population, however, cut-offs have been identified in previous cohorts. [235, 237] In **paper II** we identified cut-offs for low-risk, intermediate-risk, and high-risk groups in cardiac surgery, which nicely corresponded to previously recorded cut-offs. [235, 237] We also validated that patients in the high-risk group, based on SN measurements.

demonstrated a worse prognosis. Cut-offs simplify the use of biomarkers for clinical use, however, when the cut-off is created from one cohort external validation is important to be able to generalize the findings. In **paper III** previously found cut-offs for SN were validated, [235, 237] and we also validated the cut-off for the high-risk group found in **paper II**.

For the studies in this thesis we want external validity to cardiac surgical patients and patients with moderate to severe AS. One problem for these particular cohorts is the advent and increasing prevalence of TAVR, which suggests that a number of these patients today may have either received TAVR instead of open surgery. Accordingly; a higher proportion of patients in **paper III** may have been accepted for intervention now than during the inclusion period. This could potentially have influenced results and this reduces the external validity of our results for all cardiac surgical patients and patients with moderate to severe AS in 2019.

5.4 How specific do we want to make the risk stratification models?

Risk stratification models are an aid to clinicians in the preoperative evaluation of patients. The risk models vary widely in numbers of risk factors included, from 6 to 41 in some of the most commonly used. [251] Fewer variables make an easier system, but this may come at the cost of lower accuracy for predicting events. Accordingly a simple model with few variables could result in the exclusion of important information for assessing the patients´ preoperative condition. [252] Some models aid in a variety of surgical questions, while others are only used for a specific type of surgery. For valve surgery there has been shown that a specific model is better than for example the original EuroSCORE. [300] However, the most important thing to remember when using risk stratification models is that they should not be used alone or as a substitute for clinical judgement in clinical decision-making. [248, 301, 302] When deciding the risk model to use, the physicians should select the model best calibrated for that specific surgery. [301]

6 Conclusions and perspectives

6.1 Conclusions

We have, through the work in these three papers, demonstrated that both novel and established circulating cardiac biomarkers give incremental prognostic information to the established risk stratification model EuroSCORE II. A parsimonious risk model with NT-proBNP can give comparable prognostic information to EuroSCORE II. The novel circulating cardiac biomarker SN added prognostic information to the established cardiac biomarkers NT-proBNP and hs-cTnT in cardiac surgical patients and patients with moderate to severe AS. Finally, the change in SN concentrations from before to after cardiac surgery provided prognostic information that was not available from change in NT-proBNP and hs-cTnT concentrations.

6.2 Clinical implications

Patients undergoing cardiac surgery are a heterogeneous group and surgery is now performed on an increasingly high-risk population. [294] Better risk stratification with inclusion of a preoperative cardiac biomarker measurements might enable previously unfit patients to undergo cardiac surgery, and prevent surgery in patients who are in risk of an unwanted short- or long-term outcome. By making the risk stratification models less complex, including NT-proBNP measurements, and making the models easy to conduct, will result in increased knowledge and use of this risk models in the clinical setting. Still, such models should also be subjected to external validation.

With measurements of postoperative biomarkers such as NT-proBNP and SN, the strain on patients during surgery can be measured, which might reflect a need for longer ICU stay, closer in-hospital monitoring, and closer discharge follow-up. The addition of SN to other circulating cardiac biomarkers, ECG, and echocardiography in preoperative evaluation can give valuable information on who should and should not undergo cardiac surgery due to increased risk of postoperative mortality. Serial sampling gives extra information as changes in biomarker concentrations can be identified. SN demonstrated a different delta profile for survivors and non-survivors compared to NT-proBNP and hs-cTnT, measuring pre- and postoperative SN concentrations could give valuable prognostic information that cannot be obtained from NT-proBNP or hs-cTnT concentrations. To identify high-risk patients with biomarkers and risk scores and to follow them properly with optimal therapeutic strategies should have the potential to reduce mortality after cardiac surgery.

6.3 Future research

The future is always a very interesting time-period in research, and especially in terms of follow-up of your own research. Based on what we have discovered in the three papers of this thesis, there is a need for new and improved risk stratification models in cardiac surgery. For some of the research following the work in this thesis, the key will be external validation.

We believe NT-proBNP has the potential to improve risk stratification in cardiac surgery. Validation and incorporation to a new EuroSCORE should be pursued. The Cardiac Surgery Biomarker Score should also be externally validated, and with the upbeat pace in modern hospitals, an easy to collect parsimonious risk stratification model should be desired. The Cardiac Surgery Biomarker Score is not more invasive than a blood test, and could be done in primary care to reduce the work load in specialized hospitals.

SN is still a novel biomarker, however, over the last years its prognostic characteristics have positively stood out in several studies of patients with HF, in critically ill patients, and in patients undergoing cardiac surgery. Validation of previous results and more research on the underlying mechanisms of SN should be addressed.

And who knows, maybe there will be individual risk scores and more focus on circulating biomarkers prior to cardiac surgery in the near future.

7 References

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