Chapter 8.

General Discussion

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Subclinical Thrombotic Events as a Mechanism for Troponin Release?

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It is projected by reports of the Dutch Ministry of Health that coronary disease in the Netherlands will increase by 20% within a 12-year span, due to ageing and unhealthy lifestyle. ¹ This will put further pressure on the limited resources in healthcare. It is essential to optimise therapeutic decision making and both avoid unnecessary diagnostic testing and interventions and prevent avoidable morbidity and mortality. The data generated in this thesis will help to optimize cardiovascular care in a number of ways; (1) we showed that large scale population screening for cardiovascular risk is possible, (2) we demonstrated the value of high sensitivity troponin T (hs-TnT) levels measured in coronary artery disease (CAD) and (3) provided more evidence on a number of inflammatory biomarkers, which did not show a clinical benefit comparable to hs-TnT.

Large scale risk profiling (Chapter 2)

Last decade prevention is becoming a more important focus in medicine and cardiovascular medicine in particular, and has been adopted in several guidelines. ²⁻³ It is obvious that prevention of full-blown disease is more effective than trying to cure or repair diseases and damage to the heart. ⁴

Therefore, we designed the “Heart Attack Prevention Program for You” (HAPPY). In this program we have set up a large-scale risk profiling program, which allows for mass risk profiling at low cost. Participants fill in an online questionnaire and subscribe themselves via a website to schedule for a “health carousel” for biometrical measurements, consisting of: blood pressure, length, weight, waist circumference and blood samples for lipid profiling and serum sober glucose measurements. With the acquired data the 10-year risk of myocardial was estimated. ⁵

We have many similar programs in different countries, such as India, Nepal, Germany, USA and in the Netherlands. In the report described in this thesis we focused on a program conducted in Maastricht, the Netherlands. One of the striking outcomes was the enthusiasm in the general public to actually undergo these screenings. The HAPPY program demonstrated that it is possible to do mass profiling in up to 250 participants per hour in the Netherlands. A parallel program in India was able to screen 10000 participants in a single day, which all underwent clinical risk profiling using the health carousel as designed by HAPPY.

In the first step of the program we aimed to investigate the effect of risk profiling and follow up lifestyle coaching in 595 participants. The lifestyle
coaching was done in a low-cost manner, using internet and personalised email messages.

The results of the initial assessment and follow-up showed that 50% of the participants had elevated blood pressure (systolic >140 and/or diastolic >90) and after mass intervention this number decreased to 30%. The percentage of hypertensive blood pressure in the initial measurements is high compared to other screening studies in the Netherlands. This may have been increased by stress due to the novelty of the test and maybe the anxiety for the forthcoming blood sample draw. However, during the second test after the intervention, these blood samples were also taken after blood pressure measurements.

The total cholesterol levels decreased from 215 mg/dl to 206 mg/dl (p<.0001) after the internet intervention. The LDL-C decreased from 140 mg/dl to 136 mg/dl (p=.001). Triglycerides levels decreased from 98 mg/dl to 89 mg/dl (p=.001). The mean weight decreased from 77.4kg to 76.1kg (p<.0001).

To calculate the 10-year risk at a myocardial infarction we used the Prospective Cardiovascular Münster (PROCAM) score, a risk score algorithm containing most traditional risk factors. The Framingham risk score is frequently used but originates from a North American cohort and we have chosen for the PROCAM risk score because it is a European study. Another argument to choose for the PROCAM score is that family history is incorporated while it is lacking in the Framingham algorithm. The mean PROCAM risk score decreased from 6.3% at baseline to 5.5% (p=.000) after 3-month intervention. One of the main concerns in this study is the selection bias that could influence the result, only 595 participants of the 900 initial participants took part in the second health test after intervention. These participants could have been the more motivated people while the non-returning participants did not commit themselves to the health advises or measured increased body weight. However, this still underlines the fact that participating in a mass lifestyle intervention can significantly decrease your calculated cardiovascular risk.

These data show that large scale risk profiling and mass lifestyle intervention is possible and leads to a decrease in calculated average 10-year risk in a large cohort of participants. This is in line with a just published key-article reviewing the impact of remote monitoring. A follow up via an e-mail questionnaire 10 years later showed that the PROCAM score prediction of myocardial infarction in a 2007 cohort of 167 voluntary participants is accurate. In the “low risk” group of participants with a calculated PROCAM risk of 0-5% the actual percentage of participants suffering myocardial infarction 10 years later was 2.8%. 11.4% of the participants...
in the calculated “intermediate risk” with a PROCAM score of 5-20% had a myocardial infarction. And finally, 13.3% of Participants who had a high calculated risk of >20% had suffered from myocardial infarction the last 10 years. It is interesting to see if mortality data follows the same predictive pattern, which are currently being requested at the national records and will be investigated in the near future.

These data have resulted in 2 spin-off programs in the Netherlands. First, an online risk carousel that was designed in collaboration with a large group of general practitioners in Utrecht, the Netherlands. One of the goals of the program was to enhance collaboration between first and second line cardiovascular care, which was achieved with success. Over 1200 participants were included in the on-line carousel in one neighbourhood in Utrecht (Utrecht-Oost).

Based on the surprising effect of online coaching via e-mail, a tailor-made lifestyle coaching module was developed, enabling personalised lifestyle coaching online. This tool uses detailed lifestyle profiling in combination with algorithms to optimise the effect of health lifestyle messages in every single individual. This lifestyle coaching tool is currently implemented in a large group of practitioners in Amsterdam (ROHA), delivering care to 350,000 patients.

**Imaging and biomarkers (Chapter 3&4)**

**Hs-TNT and atherosclerotic burden**

One of the fastest expanding cost factors in cardiology is the use of advanced imaging, such as the widely used Cardiac Computed Tomography Angiography (CCTA), Cardiac Magnetic Resonance Imaging (CCMR), and Positron Emission Tomography (PET) imaging. The availability of these technologies allows for a more precise diagnosis of cardiovascular disease but comes with substantial costs. Reports indicate that growing healthcare costs have increasing impact on the overall budgets of families. Cardiovascular costs will progressively increase in the future. An important part is represented by the increasing costs of cardiovascular imaging in the last 10-15 years. Several guidelines that describe the appropriate use of cardiovascular imagine have been published. It is needless to emphasize that this puts a high burden of cost on healthcare as a whole.

Better prediction of cardiac CCTA outcome would not only help to reduce costs, but will also decrease unnecessary exposure of patients to radiation and contrast agents that are commonly used during CCTA imaging. With the
expected increase in burden of cardiovascular disease, a better prediction of CCTA imaging outcome will become even more relevant to reduce the number of false positive high-risk patients. Currently, the decision to refer patients for additional CCTA of the coronary arteries relies on population-based algorithms (e.g. PROCAM, Framingham \cite{5,7}) in combination with history taking and additional diagnostic outcomes from the electrocardiogram, exercise testing, echocardiogram, and ultrasound imaging of the carotid arteries. However, population-based risk assessment by the use of existing risk scores in these screening facilities may need revision, as many patients are referred due to specific other complaints and reflect a different domain. The scores used for population-based risk prediction have not taken these symptomatic individuals into account, as most data were derived from normal populations, asymptomatic individuals, who developed incident CAD later in life. \cite{5,7,22} This will not only benefit our business management, but also improve the care we provide to our patients. In the work presented in this thesis, we aimed to better predict cardiac CTA outcome using blood biomarkers, with promising results.\cite{21}

Our data show in patients without acute coronary syndrome, that even mild coronary artery disease is associated with quantifiable corresponding circulating levels of hs-TnT. This has also been shown in recent other studies \cite{22-25}, highlighting the enormous potential of hs-TnT in this patient category.

Use of hs-TnT in combination with imaging in risk profiling and as independent predictor.

Next, we aimed to investigate the use of the combined diagnostic power of hs-TnT and cardiac CT imaging. These data showed that hs-TnT is a useful prognostic biomarker in patients with non-acute chest discomfort suspected for CAD. In addition, hs-TnT was an independent predictor for cardiac events when corrected for cardiovascular risk profiling, calcium score and CT-angiography results.

The relevance of hs-TnT as a biomarker was recently further substantiated.\cite{23} In a large group of patients (N=19,460) presenting with acute chest pain, was demonstrated that the hazard ratio for both cardiovascular and non-cardiovascular mortality rapidly increases with increasing hs-TnT in the normal range. Aforementioned confirmed the power of hsTnT as a clinical biomarker. The question remains however, what the exact mechanism is of troponin release in the absence of necrosis. The authors suggest that troponin maybe released by ischemia alone, possibly through the creation of transient holes or so-called cell wounds due to ischemia.\cite{26}
In early work on the hs-TnT assays, we observed in 615 patients with non-acute chest pain,\(^1\), that higher levels of hs-TnT in the normal range, are associated with increased coronary artery burden as detected by coronary computed tomographic angiography. Based on studies demonstrating that in 50\% of the cases, organized older thrombi were visible at the site of the culprit lesion in patients with acute myocardial infarction admitted for thrombectomy\(^2\), we hypothesized that dislodgement of these thrombi in small coronary vessels could be a potential cause for micro injury and subsequent troponin release. In addition, plaque erosion may be an important cause of localized thrombus formation and subsequent dislodgement.\(^3\) Therefore, we suggest at least one mechanism explaining the increased risk of death and cardiovascular outcomes in the study published by dr. Roos et al\(^4\) could be a reflection of increased coronary atherosclerotic burden and subclinical thrombotic events.

**Use of inflammatory biomarkers in risk profiling (Chapter 5)**

We investigated the prognostic and predictive value of circulating Annexin A5, elastase and myeloperoxidase (MPO) levels in patients with stable coronary artery disease (CAD) during long-term follow-up and their relation to plaque burden assessed by non-invasive imaging.

206 patients with non-acute chest pain who underwent cardiac computed tomographic angiography (CCTA) with a median follow-up of 9 years. The combined outcome was coronary revascularization, acute coronary syndrome (ACS), cardiovascular death. Plasma biomarkers Annexin A5, elastase and MPO plasma levels were measured at baseline. In addition, clinical biomarkers hs-TnT, high sensitivity C-reactive protein and N-terminal pro-B-type natriuretic peptide were also assessed. Coronary atherosclerotic burden was assessed by CCTA.

Our analysis demonstrated that circulating levels of Annexin A5, elastase and MPO were not significantly correlated to extent of CAD on CCTA. Kaplan-Meier analysis further revealed that Annexin A5, elastase and MPO levels were not predictive of significant coronary stenosis or acute coronary events during 9-year follow-up.

This study suggests that Annexin A5, elastase and myeloperoxidase do not provide incremental prognostic value as biomarker for the identification of patients at risk of cardiovascular events after 9 years, while clinically
established biomarkers hs-TnT and NT-proBNP confirmed correlation between burden on CCTA. These results suggest, that there is no clinical added value for the examined biomarkers. Although the results are negative in the presented study, these markers could still have value in strategic decision making combined with other markers and cardiovascular risk features. This seems an interesting subject for future research.

**Vitamin K antagonist and coronary calcium (Chapter 6)**

We used CT outcome as a measure to investigate the potential effect of Vitamin K antagonists (VKA) in the generation of coronary calcification. VKA are widely used in patients with thromboembolic risks. By antagonizing vitamin K, the vitamin K-dependent blood coagulation factors are blocked, resulting in a prolonged bleeding time. However, matrix Gla-protein is also dependent on vitamin K. Matrix Gla-protein down regulates calcification and is negatively affected by VKA, resulting in an increase in calcium formation.

Coronary calcium can be measured using a non-contrast-enhanced, CCTA. Post-processing using the Agatston method results in a quantifiable calcium score. This gave us the ability to compare the amount of coronary calcium in different patients and patient groups. We compared the amount of coronary calcium in patients using VKA for different periods of time and compared the coronary calcium in VKA naïve patients. We concluded that the amount of coronary calcium is increased in patients using VKA. Furthermore, in ApoE-/- mice VKA changes the plaque morphology, such as positive remodeling and inflammation in the plaque “shoulders” which are features of plaque instability. These findings underscore the need for alternative anticoagulants that do not interfere with the vitamin K cycle. Interestingly, newer oral anticoagulants (like apixaban, rivaroxaban and dabigatran) show reduced plaque phenotypes when used in the ApoE-/- mice. Based on these phenomena, clinical trials comparing new oral anticoagulants and VKA for their effect on coronary artery disease have already been started.
**Figure 1. Added value to cardiovascular risk profiling**
The colours red, orange and green represent the patients at respectively high, intermediate and low risk for coronary disease.

**Molecular imaging in cardiovascular disease (Chapter 7)**
Non-invasive identification of pathophysiological processes may be a key in revealing the patient at risk for myocardial infarction. Using molecular imaging the biological features of tissue can be highlighted. The main idea behind molecular imaging is using proteins or other substances that have a high affinity to accumulate in the area of the pathological process. By labeling these proteins with a radioactive element or fluorescent label, it is possible to determine the location of these labeled proteins or tracers. Radiolabeled Annexin A5 showed to be promising in detecting carotid artery plaque apoptosis and may be clinical useful in detecting instable carotid plaques, responsible for recent TIA’s. And more recent carotid plaques showed uptake of Fluoro18 labeled glucose at the ipsilateral side in cryptogenic stroke patients. Molecular imaging therapy is progressively used to detect and differentiate the patients at risk. Interesting is that the same molecular profiling is also used to develop probes that can act as navigators in targeted therapy and targeted drug delivery.
Future directions

One of the major findings in the work presented in the thesis is that large scale risk profiling and mass lifestyle intervention is a possibility. In addition, the thesis shows that the development of novel serum biomarkers has added value to standard risk profiling using Framingham and SCORE algorithms. The power of hs-TnT has been demonstrated in various studies thereafter. How should we proceed from here?

We are currently implementing a lifestyle intervention program in collaboration with a large group of general practitioners in Amsterdam. This program is largely based on the experience gained within the HAPPY program. Prevention strategies and implementation of various lifestyle interventions has gained much attention in our professional community. It is up to us to reach the general population before we see them on our coronary care units after an ACS. By implementing this in our so-called ‘first line’ primary care, we hope to reach patients in the earliest stage possible. Interestingly, education to the general population through mass interventions might be an even earlier step in the creation of awareness for cardiovascular diseases, with specific interests for gender and ethnic differences.

Secondly, one of the promising fields in cardiovascular medicine is Artificial Intelligence and machine learning. Medical algorithms based on large amounts of clinical data will facilitate risk assessment and clinical decision making. This development is here to stay and will certainly influence the future of patient behaviour in search of medical care. Imagine medical algorithms that are capable of monitoring electronic health records and that predict whether a patient in hospital will deteriorate hours in advance and alert physicians about their decline. Although this sounds futuristic, an algorithm developed at the University of Chicago Medical Center, tracks 28 variables like respiratory rate, age, kidney function and length of hospital stay and provides a timeline of the patients’ risk. It detects about 88% of patients who are at risk of deteriorating from organ failure, cardiac arrest, sepsis and other life-threatening conditions within a median 33-hour timeframe. Also tech-giant Google is involved; by using over 50 billion data-points and deep learning algorithms, they showed an incredible predictive capacity for in-hospital mortality and re-admissions, vastly outperforming all traditional models. Webpages offer thousands simplistic (BMI calculations) and complicated computational models and algorithms for diagnosis of a disease and risk prediction. Some predictive analytical medical algorithms claim that they can use real-time data from an intensive care unit to predict events like cardiac arrests 24 hours before they actually occur.
In line with the above, novel algorithms are needed to better predict who should be referred for CCTA, based on large datasets including history, the electrocardiogram, echocardiogram and exercise testing on top of standard risk profiling. Decision support tools using these algorithms would help clinicians to optimise decision making whenever a complex set of patient data is available that surpass the capacity of a single human mind (goal 1). The ultimate use is to build the algorithm in our electronic patient portals to serve as a decision support tool by predicting patient outcome using the real-time patient data (goal 2). This will not only expedite the work of the clinician, but also improve care for patients. The better prediction of outcome will avoid the use of costly unnecessary diagnostics and prevent unnecessary risks. At the same time, high-risk individuals will be better identified through the exclusion of the low risk individuals. For actual implementation however, the efficacy of these tools need to be tested in a randomised fashion in a large population.

Thirdly, as in the past, novel biomarkers will emerge in the future, some of which will have added value in clinical cardiovascular care. Although we were not able to prove clinical benefit from the investigated biomarkers in this thesis, the current research on MicroRNAs, exosomes and other biological ‘carriers’ will certainly shed more light on biological processes and provide measurable substances to quantify diseases as (coronary) atherosclerosis and microvascular disease. But also the use these biomarkers as features in deep-learning algorithms is a promising field in predicting which individual is at risk for certain diseases.

At last, advances in cardiovascular imaging will result in better images with lower patient radiation burden. Iterative reconstruction techniques result in better images and/or lower patient radiation burden and are progressively used by CT manufacturers. Furthermore, in a recently published article the fat attenuation index (FAI) is investigated as an imaging biomarker to highlight inflammation on CCTA images. It turns out that a high FAI is associated with a 5-fold increased risk of cardiac mortality. This study shows that FAI could be promising in detecting vulnerable plaques noninvasively. The advances in estimating the significance of a coronary artery stenosis using computational fluid dynamics modelling, result in so called CT-fractional flow reserve (CT-FFR). This technique looks promising for equally accurately predicting severe stenosis compared to the invasive FFR measurement, which in turn might lead to better non-invasive treatment planning. Furthermore, the field of radiomics (extracting thousands of quantitative parameters from CT images and use algorithms to identify
patterns) is evolving quickly; in the near future we might be able to identify coronary plaques that are vulnerable to plaque rupture, preventing a nearing imminent thrombus formation and myocardial infarction in patients at risk.\(^{43}\)

**Figure 2. Future directions**

The colours red, orange and green represent the patients at respectively high, intermediate and low risk for coronary disease.
References


35. How are algorithms changing healthcare?