Cardiovascular risk factors in
Aortic Stenosis

Johan Ljungberg
To Christina

“This've had my ups and downs, my fair share of dumpy roads and heavy winds. That's what made me what I am today.”

Jean-Claude Camille Francois Van Varenberg (1960-)
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Abstract

Introduction
Aortic stenosis is the most common hemodynamic significant valvular heart disease and affects about 1-2% of the population. The incidence increases with age and about 3.5% of persons above 75 years of age are affected. When symptoms of the stenotic aortic valve disease eventually occur (fatigue, angina or syncope), the 2-year mortality exceeds 50%. The only known treatment is replacement of the valve by surgery or by catheter intervention. The causes of aortic stenosis are only partly known, despite that the disease has been known since the beginning of 17th century. In younger individuals, a bicuspid valve is present in about 80% of the cases.

The histology of the stenotic aortic valve and the atherosclerotic plaques shares several features such as inflammation, lipid deposition and calcification. Also, traditional cardiovascular risk factors for ischemic heart disease such as hypertension, diabetes and hypercholesterolemia have been associated with aortic stenosis. However, many of these studies have been cross-sectional and the presence of different phenotypes with or without concomitant coronary artery disease (CAD) has often not been taken into account.

Lipoprotein(a) [Lp(a)] has been linked to atherosclerosis, and in large prospective cohort studies, elevated Lp(a) levels have been associated with aortic stenosis; where causality was supported by Mandelian randomisation techniques. The previous findings were partly based on International Statistical Classification of Diseases and Related Health Problems (ICD)-coding and obtained no information about concomitant CAD. End-stage renal disease is associated with increased risk of aortic stenosis, and it increases the progression rate, but it is not known if early impairment increases the risk. Recently, it has been suggested that a discrepancy between estimated glomerular filtration rates from creatinine and cystatin C (the so called “shrunken pore syndrome”) could indicate early impairment and possibly endothelial dysfunction.

Due to the natural history of the disease with slow progression over decades without symptoms, it is extremely challenging to perform clinical trials with the aim to halt the progression of the stenotic process; further, randomised controlled trials with statin treatment have not shown any effect, probably due to late initiation of treatment and an already advanced pathological process in the affected valves.
Hypotheses and aims
The hypothesis has been that traditional cardiovascular risk factors associate with valvular disease differentially depending on the phenotype of the disease including the presence or absence of concomitant CAD. Furthermore, it has been hypothesised that circulating substances in plasma prior to surgery for valvular disease could be identified, not least those related to early metabolic disturbances and impaired endothelial function.

1. To evaluate if the traditional cardiovascular risk factors are associated with developing valvular heart disease and with special focus on aortic stenosis, and if any associations differ between those with or without concomitant CAD.

2. To study the impact of arterial hypertension on the risk for surgery for aortic stenosis after careful stratification for age and presence of CAD.

3. To study the impact of dyslipidaemia as measured by apolipoproteins and Lp(a) on the future risk for surgery for aortic stenosis.

4. To use new methodology for determining early renal impairment, explore if the “shrunken pore syndrome” is associated to future risk for surgery for aortic stenosis.

Material and methods
There were 873 patients identified with surgery for valvular heart disease and/or disease of the ascending aorta with a prior participation in one of three large population based health surveys in northern Sweden (Västerbotten Intervention Program [VIP], MONItoring Of trends and Determinants in CArdivascular Disease survey [MONICA], and the Mammary Screening Project [MSP]). The period between survey and surgery was approximately 10 years. For each case, two to four referents matched by age, gender, type and date of survey, and geographical location were randomly selected. From the health surveys, data on cardiovascular risk-factors and health history as well as measurements of anthropometry, blood pressure, glucose and cholesterol levels were retrieved. Each case was carefully validated and data from pre- and perioperative assessments were collected, such as results from echocardiographic and electrocardiographic (ECG) examinations, and the coronary angiogram, and the surgeons reports on type of valvular disease and surgical procedures performed. Apolipoproteins B and A1, Lp(a), creatinine and cystatin C were analysed in samples obtained at the initial survey which were stored in -80°C until analysis in 2017.
Since this is a matched case-referent study where cases and referents had the same follow-up duration within strata, logistic regression using the conditional maximum likelihood routine designed for matched analysis was used to estimate odds ratios (ORs) with 95% confidence intervals. Studied variables were tested in uni- and multivariable models.

Results

Paper I: Of the identified 873 cases were 62 excluded due to DNA and 12 due to no questionnaire. 322 were primarily operated for aortic stenosis, 91 for aortic regurgitation, 181 for mitral regurgitation, 131 for disease of ascending aorta, and 52 for CAD (and for concomitant valvular or aortic disease) and had questionnaire. The remaining 22 had various indications for valvular heart surgery, i.e. mitral stenosis, pulmonary stenosis etc., and were excluded. Altogether 38% of patients were women. The median time (interquartile range) from health survey to surgery was 10.5 (9.1) years, and the median age at surgery was 65.9 (13.9) years.

Aortic stenosis: In the multivariable model, hypertension (OR 1.87 [1.37–2.54]), diabetes (OR 1.78 [1.01–3.11]) and total cholesterol (OR 1.64 [1.07–2.49]) were associated with aortic stenosis demanding surgery. After exclusion of those with concomitant CAD, none of the cardiovascular risk factors remained significant.

Aortic regurgitation: None of the cardiovascular risk factors were associated with increased risk for aortic regurgitation demanding surgery, whereas high levels of cholesterol were associated with reduced risk for surgery (OR 0.29 [0.12–0.71]).

Mitral regurgitation: High levels of cholesterol associated with surgery for mitral regurgitation (OR 1.74 [1.01–3.00]), but not in those without CAD. None of the other risk factors were associated with surgery, regardless of concomitant CAD or not.

Disease of the ascending aorta: Hypertension (OR 2.42 [1.44–4.06]) and previous smoking (OR 1.97 [1.12–3.49]) were associated with surgery for disease of the ascending aorta, whereas diabetes was inversely associated with surgery (OR 0.09 [0.01–0.73]). When excluding those with CAD, only diabetes remained protective (OR 0.24 [0.07–0.81]).

Paper II: Altogether 322 patients (median age at survey 69.2 [13.1] years, median age at surgery 59.8 [10.3] years, 47% females) had surgery for aortic stenosis as their primary indication for heart surgery and had questionnaire. Of them, 70 had surgery before the age of 60 years and 252 had surgery after 60 years of age. After exclusion of those with CAD, 49 and 82 patients remained in these age groups.
Arterial hypertension associated with surgery in those operated before the age of 60 years regardless of concomitant CAD or not (OR 3.40 [1.45–7.93] and OR 5.88 [1.46–23.72], respectively). A high diastolic blood pressure associated also with surgery in those younger than 60 years of age and without CAD (OR 1.60 [1.09–2.37]), but not in those with CAD. In those older than 60 years at surgery and with concomitant CAD, all traditional cardiovascular risk factors associated with surgery, but in those without concomitant CAD, only impaired fasting glucose (IFG) was associated with surgery (OR 3.22 [1.19–8.76]).

Paper III: Altogether 336 patients (median age at survey 59.4 (10.3) years, median age at surgery 68.3 (12.7) years, 48% females) having surgery for aortic stenosis had stored blood samples from the initial health survey. Lp(a) was independently associated with future surgery in those with concomitant CAD (OR 1.29 [1.07–1.55]), but not in those without CAD. Similarly, a high Apo B/A1 ratio was associated with surgery in patients with CAD (OR 1.43 [1.16–1.76]), but not in those without.

Paper IV: The same cohort was studied as in paper 3. Renal function was estimated by the ratio between glomerular filtration rates (GFR) obtained from cystatin C and creatinine, and a low ratio indicates early impairment of renal function. A high ratio associated with lower risk for aortic stenosis requiring surgery (OR 0.85 [0.73–0.98]). After stratification for sex, the protective effect was seen in women but not in men (0.75 [0.60–0.94] and 0.94 [0.77–1.15], respectively). After further stratification for CAD, the association remained significant in women with CAD but not in men with CAD (0.56 [0.40–0.80] and 0.96 [0.75–1.24], respectively). A low ratio was also associated with shorter survival irrespective of case-status.

**Conclusion**

The traditional cardiovascular risk factors were associated with future surgery for valvular heart disease and for surgery of the ascending aorta, however with a clear difference if there was concomitant CAD or not. Notably, irrespective of concomitant CAD, high levels of cholesterol were associated with reduced risk for surgery due to aortic regurgitation and a diagnosis of diabetes was protective against surgery of the ascending aorta.

Arterial hypertension and a high diastolic blood pressure were major risk factors in separate models, for surgery for aortic stenosis in younger patients without CAD, whereas IFG was associated with surgery in elderly patients without CAD.

High levels of Lp(a) and an atherogenic lipid profile expressed as a high Apo B/A1 ratio were independently associated with future surgery for aortic stenosis only
in patients with concomitant CAD. Similarly, early renal impairment expressed as low ratio of estimated GFR by cystatin C and by creatinine was associated with future surgery for aortic stenosis.

In summary, the aortic stenosis disease should be carefully evaluated as different phenotypes, e.g. with and without CAD, and may have different risk factors and different pathophysiological mechanisms that should be differentially targeted in future trials of prevention. Notably, arterial hypertension and increased blood pressure should be addressed at an early age. Furthermore, the aortic stenotic process is linked to dyslipidaemia and early endothelial dysfunction which merits future mechanistic studies.
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ACE</td>
<td>Angiotensin-Converting-Enzyme</td>
</tr>
<tr>
<td>Apo A1</td>
<td>Apolipoprotein A1</td>
</tr>
<tr>
<td>Apo (a)</td>
<td>Apolipoprotein(a)</td>
</tr>
<tr>
<td>Apo B</td>
<td>Apolipoprotein B</td>
</tr>
<tr>
<td>ASTRONOMER</td>
<td>Aortic Stenosis Progression Observation: Measuring Effects of Rosuvastatin</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>CAD</td>
<td>Coronary Artery Disease</td>
</tr>
<tr>
<td>CAPA</td>
<td>Caucasian and Asian Pediatric and Adult subjects</td>
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<tr>
<td>CI</td>
<td>Confidence Interval</td>
</tr>
<tr>
<td>CRP</td>
<td>C-Reactive Protein</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
</tr>
<tr>
<td>eGFR</td>
<td>Estimated Glomerular Filtration Rate</td>
</tr>
<tr>
<td>ESC</td>
<td>European Society of Cardiology</td>
</tr>
<tr>
<td>FADS</td>
<td>Fatty Acid Desaturase</td>
</tr>
<tr>
<td>GFR</td>
<td>Glomerular Filtration Fate</td>
</tr>
<tr>
<td>GWAS</td>
<td>Genome-Wide Association Study</td>
</tr>
<tr>
<td>HDL</td>
<td>High-Density Lipoprotein</td>
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<tr>
<td>ICD</td>
<td>International Statistical Classification of Diseases and Related Health Problems</td>
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<tr>
<td>IFG</td>
<td>Impaired Fasting Glucose</td>
</tr>
<tr>
<td>IGT</td>
<td>Impaired Glucose Tolerance</td>
</tr>
<tr>
<td>LDL</td>
<td>Low-Density Lipoprotein</td>
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<tr>
<td>Lp(a)</td>
<td>Lipoprotein (a)</td>
</tr>
<tr>
<td>LPA</td>
<td>Lipoprotein (a) gene</td>
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<tr>
<td>MMA</td>
<td>Matrix Metalloproteinases</td>
</tr>
<tr>
<td>MONICA</td>
<td>Monitoring Of trends and Determinants in Cardiovascular Disease</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>MSP</td>
<td>Mammary Screening Project</td>
</tr>
<tr>
<td>NSHDS</td>
<td>Northern Sweden Health and Disease Study</td>
</tr>
<tr>
<td>OGTT</td>
<td>Oral Glucose Tolerance Test</td>
</tr>
<tr>
<td>OR</td>
<td>Odds Ratio</td>
</tr>
<tr>
<td>PARTNER</td>
<td>Placement of Aortic Transcatheter Valves</td>
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<tr>
<td>PCSK9</td>
<td>Proproteinconvertase subtilisin/kexin type 9</td>
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<tr>
<td>PTMV</td>
<td>Percutaneous Transluminal Mitral Valvuloplasty</td>
</tr>
<tr>
<td>SALTIER</td>
<td>Scottish Aortic Stenosis and Lipid Lowering Trial, Impact on Regression</td>
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<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SEAS</td>
<td>Simvastatin and Ezetimibe in Aortic Stenosis</td>
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<tr>
<td>SNP</td>
<td>Single-nucleotide polymorphism</td>
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<tr>
<td>T1DM</td>
<td>Type 1 Diabetes Mellitus</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>T2DM</td>
<td>Type 2 Diabetes Mellitus</td>
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<tr>
<td>TNF</td>
<td>Tumor Necrosis Factor</td>
</tr>
<tr>
<td>VIP</td>
<td>Västerbotten Intervention Program</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organisation</td>
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Original Papers

This thesis is based on the following four articles. They will be referred in the following text by the corresponding Roman numerals (Paper I-IV).


II. **Ljungberg J**, Johansson B, Engström KG, Norberg M, Bergdahl IA, Söderberg S. Arterial hypertension and elevated diastolic blood pressure is associated with developing aortic stenosis requiring surgery in persons less than 60 years of age. Submitted.

III. **Ljungberg J**, Holmgren A, Bergdahl IA, Hultdin J, Norberg M, Näslund U, Johansson B, Söderberg S. Lipoprotein(a) and the Apolipoprotein B/A1 Ratio Independently Associate with Surgery for Aortic Stenosis Only in Patients with Concomitant Coronary Artery Disease. *J Am Heart Assoc*. 2017 Dec 15;6(12).

Enkel sammanfattning på svenska

Bakgrund


Orsaken till att man drabbas av aortastenos är inte känd men man vet att en tvådelad (bicuspid) aortaklaff i stället för en tredelad (tricuspid) ökar risken. Den bicuspid aortaklaffen är vanligt förekommande hos de som opereras för aortastenos före 60 års ålder. Tidigare var reumatisk feber en vanlig orsak men är i dag i västvärlden mycket ovanlig. I utvecklingsländer är däremot är reumatisk feber fortfarande den vanligaste orsaken till aortastenos. Tidigare studier har sammankopplat utvecklandet av aortastenos med de klassiska riskfaktorerna för hjärtkärlsjukdomar, högt blodtryck, diabetes, övervikt, rökning och högt kolesterol. Man har också vid mikroskopiska studier av aortaklaffar och åderförkalkning sett mikroskopiska likheter, dvs fett och kalkinlagring, mellan de två sjukdomarna. Tyvärr har många av de tidigare studierna varit retrospektiva, dvs tillbakablickande, och inte tagit hänsyn till om det har förekommit åderförkalkning i kranskärlen eller inte. Under hösten 2017 presenterades en kanadensisk studie med totalt 1,12 miljoner deltagare över 65 år och med 20 995 individer som utvecklade aortastenos. I denna studie fann man att högt blodtryck,
diabetes och höga blodfetter var associerat med att utveckla aortastenos. Även i
denna studie har men inte tagit hänsyn till i fall det förekommer samtidig
kranskärlssjukdom eller inte. Under de senaste åren har man undersökt en av
fettmolekylerna, lipoprotein a, Lp(a), om den bidrar till utveckling av
aortastenos. Den är känd bl.a. för att vara associerad med att utveckla
åderförkalkning. Nivån av Lp(a) i blodet är genetiskt styrd (LPA-genen) och
påverkas inte i större utsträckning av kost, motion eller ålder. Hög cirkulerande
nivå av Lp(a) samt en specifik variant av LPA-genen som ger höga cirkulerande
nivå av Lp(a), ökar risken för att utveckla aortastenos. Detta stöder ett
orsakssamband mellan Lp(a) och aortastenos (s.k. Mandelisk randomisering).

Behandling av de klassiska riskfaktorerna har visat sig reducera risken för att
utveckla hjärtkärlsjukdomar, såsom hjärtinfarkt och stroke. Med denna kunskap
har man försökt att reducera hastigheten med vilken aortastenosen försämras. I
tre randomiserade kliniska studier har man med hjälp av kolesterolsänkande
mediciner, s.k. statiner, sänkt nivån på kolesterol i blodet hos de med måttlig till
svår aortastenos och utan symptom. Trots att kolesterolvärdena i blodet sjönk
signifikant kunde man inte påverka utvecklingen av aortastenos. Varför man
inte hade någon effekt av kolesterolsänkning är inte klar men det spekuleras att
insättandet av behandlingen skedde i ett sent stadium och att
förkalkningsprocessen inte gick att påverka.

Trots omfattande studier är orsaken till aortastenos i stor utsträckning
fortfarande okänd.

**Hypoteser och mål**

Hypotesen har varit att traditionella riskfaktorer för hjärtkärlsjukdomar är
sammankopplade med att utveckla aortastenos och även andra
hjärtklaffsjukdomar men att förekomsten av åderförkalkning i kranskärlen eller
inte påverkar riskfaktorprofilen. Hypotesen har också varit att markörer i blodet
är associerade till utvecklandet av aortastenos.

1. Att utvärdera ifall traditionella hjärtkärlriskfaktorer är associerade
   med utvecklandet av hjärtklaffsjukdom och då särskilt aortastenos
   och om det skiljer ifall det förekommer samtidig åderförkalkning i
   kranskärlen.

2. Att studera associationen mellan blodtryck och utveckling av
   aortastenos med avseende på ålder och förekomst av åderförkalkning
   i kranskärlen.
3. Att studera associationen mellan höga blodfetter och särskilt Lp(a) och utveckling av aortastenos.


Material och metoder
Sammanlagt hittades 873 patienter som hade genomgått hjärtklaffkirurgi eller opererat uppåtstigande delen av aorta och som före operationen deltagit i tre större befolkningsstudier i norra Sverige, Västerbotten Intervention Programme (VIP), Mammography Screening Project (MSP), och MONItoring Of trends and Determinants in CArdivascular Disease survey (MONICA). I genomsnitt var tiden mellan deltagande i studie och operation ca 10 år. Till varje fall matchades fyra kontroller. Fallen och kontrollerna var matchade på kön, ålder, bostadsort, typ av och tidpunkt för deltagande i studie. Från studierna hämtades information om förekomsten av riskfaktorer, sjukdomshistoria, aktuell medicinering och uppmätta värden på längd, vikt, blodtryck, kolesterol och blodsocker. För fallens del kompletterades databasen med uppgifter från utredningen inför operationen. De uppgifter som samlades in var resultat från hjärtultraljudsundersökningar, kranskärlsröntgen, EKG och information från operationsberättelsen, såsom utförd operation och ev. information om den opererade kllffen. Frista blodprover tinades och analyserades med avseende på diverse blodfetter, kreatinin och cystatin C.

Resultat
Papper I: Av de 873 fallen hade 799 ifyllt frågeformulär. Av dem var 322 primärt opererade för aortastenos, 91 för aortainsufficiens, 181 för mitralinsufficiens, 131 för sjukdom på uppåtstigande aorta och 52 för kranskärlssjukdom. 22 stycken var opererade för andra klaffsjukdomar eller var inte opererade. Av de 799 fallen var 308 kvinnor (38%).

Aortastenos: Högt blodtryck, diabetes och högt kolesterol var förknippat med att utveckla aortastenos, men endast i de fall det förekom samtidig kranskärlssjukdom.

Aortainsufficiens: Ingen av de klassiska riskfaktorerna var associerade med aortainsufficiens men höga kolesterolvärdet minskade risken för att opereras för aortainsufficiens.
Mitralinsufficiens: Endast högt kolesterol var förknippat med operation för mitralinsufficiens men endast om det förekom samtidig kranskärlssjukdom.

Uppåtstigande aorta: Hög blodtryck och tidigare rökning var båda förknippat med operation av uppåtstigande aorta så länge det förekom samtidig kranskärlssjukdom. Diabetes å andra sidan, hade en skyddande effekt, d.v.s. risken för att behöva operera uppåtstigande aorta minskade med förekomst av diabetes.

Papper II: Sammanlagt 322 fall var primärt opererade för aortastenos och med ifyllt frågeformulär, och av dem var 70 opererade före 60 årsålder och 252 efter 60 års ålder. Efter uteslutande av de med kranskärlssjukdom var det 49 under 60 år och 82 över 60 år. Oavsett om det förelåg kranskärlssjukdom eller inte var högt blodtryck sammankopplat med att utveckla operationskrävande aortastenos hos de som opererades före 60 års ålder. In den äldre gruppen utan kranskärlssjukdom var endast högt fasteblodsocker förknippat med aortastenos. Hos de med kranskärlssjukdom var alla de traditionella riskfaktorerna associerat med utveckling av aortastenos.

Papper III: Totalt 336 fall som var opererade för aortastenos och som hade blodprover sparade från hälsoundersökningen. Lp(a) och en hög kvot mellan blodfetternas lipoprotein B och lipoprotein A1 var båda associerade med att utveckla aortastenos men endast om det förelåg samtidig kranskärlssjukdom.


Sammanfattning
De traditionella riskfaktorerna för hjärtkärlsjukdom verkar vara förknippade med utveckling av aortastenos, mitralinsufficiens och sjukdom i uppåtstigande aorta så länge det föreligger samtidig kranskärlssjukdom. Likartad bild ses för Lp(a). Om det inte föreligger kranskärlssjukdom är inte Lp(a) associerat med att utveckla aortastenos. Hos yngre som opereras för aortastenos är högt blodtryck förknippat med att utveckla sjukdomen oavsett om det föreligger kranskärlssjukdom eller inte. Tidig förändring i njurfunktionen hos kvinnor med
samtidig kranskärlssjukdom indikerar ökad risk för operation av aortastenos, vilket inte ses hos män eller hos kvinnor utan kranskärlssjukdom.

Förekomsten av kranskärlssjukdom verkar vara en vattendelare vad gäller riskfaktorer för att utveckla operationskrävande klaffsjukdomar eller inte. Förekommer kranskärlssjukdom verkar de traditionella riskfaktorerna, inklusive Lp(a), vara riskfaktorer för att utveckla operationskrävande aortastenoser och borde också då kunna vara möjliga att behandla. Om det inte förekommer samtidig kranskärlssjukdom är det troligen andra faktorer som bidrar till att utveckla aortastenos men dessa är ännu inte kända. Ett undantag är de som är opererade före 60 års ålder där blodtryckssjukdomen associerar med utveckling av aortastenos oavsett om kranskärlssjukdom föreligger eller ej.
Introduction

Definition of valvular heart disease

The heart has four valves – the aortic and the mitral valves on the left side and the pulmonary and the tricuspid valves on the right side (Figure 1). The aortic valve has its position between the aorta and the left chamber and the pulmonary valve is located between the pulmonary artery and the right chamber. The mitral and the tricuspid valves are located between the atria and the chamber on each side. As part of the circulation and acting as back flow valves the function of the valves is crucial for maintain adequate circulatory status. If the valves are damaged or altered the function of the heart and the circulation will be affected with associated morbidity and even mortality (1).

Alterations of the valves can be results from congenital malformation, infections or degeneration. There are mainly two different types of valvular heart disease – stenosis and regurgitation. In stenosis, the valves are narrowed due to calcification, deposition in the valve leaflets or congenital malformation. The obstruction of the outflow of the ventricles imposes a pressure overload on the ventricle, which sequentially causes hypertrophy of the myocardium. In regurgitation, the ventricle enlarges progressively to compensate for the regurgitant volume i.e. volume overload, with increasing end-diastolic pressure and thereby preserving the cardiac output (1).

Figure 1. Anatomy of the heart. Copyright Encyclopaedia Britannica, Inc.
The global burden of valvular heart disease

The burden of valvular heart diseases globally is not well known, since many patients are asymptomatic, and differences in health care systems and health care funding generate different possibilities to diagnosis, investigate and treat the disease. However, the aetiology of valvular heart disease is shifting from the traditional rheumatic valvular heart disease to other not well-known aetiologies. The rheumatic valvular heart disease is caused by rheumatic fever which is a complication to Streptococcus A infections, an infection that is the leading cause of valvular heart disease in developing countries. With an ageing population in the western countries, and with better access to health care and higher socioeconomic standard, degenerative valvular heart disease becomes more common (2). In the 1960’, rheumatic valvular heart disease was still the most common cause of valvular heart disease even in the developed countries (3). The prevalence of valvular heart disease in developing countries is not known, but in developed countries the prevalence has been estimated in a few studies to be about 2.5% in the population older than 18 years of age and in the population older than 65 years of age 6.4% (2, 4). If those with mild valvular heart disease are included the prevalence rises dramatically to 51% in the population older than 65 years of age. In a population with an increasing proportion of older people, the total prevalence will increase and the demand on the health care system will grow with a higher number of out-patients follows-ups and valve interventions in the elderly population. In Sweden, the total number of valvular interventions were 4,282 (44/100,000 inhabitants) in 2015 compared with 2,773 (31 /100,000 inhabitants) in 2005; an increase by almost 54% (Swedish National Board of Health and Welfare, The statistical database).

Aortic stenosis

Prevalence and natural history

Aortic stenosis was first described in detailed by J.G. Mönckeberg 1904, as an uncommon disease (5), but already in 1663 Lazare Rivière described the necropsy findings from a patient with palpitations, progressive dyspnoea and loss of peripheral pulses (6). He noted left ventricular enlargement and identified large caruncle-like masses obstructing the left ventricular outflow to the aorta. Before the development of cardiac catheterization and ultrasound of the heart the only way to diagnose the disease was by physical examination, preferably with a stethoscope and findings of calcification of the aortic valve on chest x-ray.

The natural history of aortic stenosis disease is characterized by a long asymptomatic phase followed by a short symptomatic phase (7). It starts with aortic sclerosis, a limited alteration of the valve with focal valve thickening and...
small foci of calcification (8). The hemodynamics are, however, not yet altered. The process with increasing fibrosis and calcification leads sooner or later to a stenotic aortic valve with impaired flexibility and a stiffness of the valve with obstruction of the left ventricular outflow (9). About 1.8-1.9% per year of the sclerotic valves will progress into an aortic stenosis (10). The prevalences of aortic sclerosis and aortic stenosis are low in the general population but rise sharply after 65 years of age (10). In the US, the prevalence of aortic stenosis is estimated to 0.4% in the general population. The prevalence of aortic stenosis between 65 and 75 years of age is about 1.3% and after 75 years of age is 2.8% (11). Aortic sclerosis is much more common and the prevalence is estimated to 26% in the population older than 65 years of age and increase to 48% in those older than 85 years (11). Even though, aortic sclerosis has a good clinical prognosis it is associated to other potentially harmful diseases. There is about a 50% increased risk of myocardial infarction and cardiovascular death in patients with sclerotic aortic valves (12).

The initial phase in developing aortic stenosis lasts for decades and is asymptomatic. The left ventricle has capacity to handle the increasing obstruction of the left ventricular outflow. During this phase the left ventricle adjusts to the increasing workload with hypertrophy and remodelling of the myocardium (3). As long as the aortic stenosis is asymptomatic there is usually no indication for intervention. On the other hand, when symptoms occur there is an indication for prompt replacement of the aortic valve (7). In this situation the two-year survival rate is 50% without valve intervention (Figure 2). However, if the patient is over 70 years of age the survival rates is even worse with two- and three-years survival rate of 37% and 26%, respectively (13).

Figure 2. The natural history of aortic stenosis, emphasizing a long pre-symptomatic period and the dismal outcome once symptoms begin. AV indicates average (14). Copyright Wolters Kluwer Health, Inc.
Symptoms that may be associated with a severe aortic stenosis are the classical triad – angina pectoris, syncope and congestive heart failure. Angina pectoris is present in approximately 35% of the patients with a severe aortic stenosis. The underlying mechanisms are believed to be increased oxygen demand in the hypertrophic myocardium and to a limited blood flow in the coronary arteries due to increased left ventricular diastolic pressure. During exercise the peripheral resistance is reduced and there is an increased demand on cardiac output but the left ventricle is incapable to increase the cardiac output, due to the fixed high resistance of the aortic valve. Therefore, syncope during exercise is caused by hypotension or reduced coronary blood flow. The last of the three symptoms of aortic stenosis is left ventricular failure. The ventricular failure is probably caused by impaired left ventricular function due to prolonged myocardial hypertrophy and persistent pressure overload (13). Patients with ventricular failure have the worst prognosis with a mean survival of one year.

The calcification process of the aortic valve

The calcification of the aortic valve is a process evolving over years and decades and is characterized in its end stage by fibrotic thickening, inflammation, calcification, neoangionesis and ectopic mesenchymal tissues in the valve (15). To understand the pathophysiological process, the histological structure of the valve is necessary to understand. The valve consists of three layers – the fibrosa, closest to the aorta, the ventricularis, closest to the ventricle and the left ventricular outflow tract, and between these two layers, the spongiosa. The surface of the valve is covered with a monolayer of endothelial cells. The three layers are populated with valvular interstitial cells, with most of the interstitial cells in the spongiosa layer. About 5% of the interstitial cells consist of myofibroblast or smooth muscle cells (16).

It is not known when the calcification process starts, but is believed to be due to damage of the endothelial cell layer, disruption of the basal membrane of the valve and/or hemodynamic stress on the interstitial cells, which thereby shift them to a greater proportion of active myofibroblast- and osteoblast-like cells (17). The damage to the basal membrane triggers an inflammatory response with macrophages and T-lymphocytes and the entrance of different lipid molecules into the valve. The lipids found in early valve lesions are Low-Density-Lipoprotein (LDL), oxidized-LDL, apolipoproteins B, (a) and E. With the inflammatory response and with the entrance of lipids, a cascade of cytokines and cellular signalling starts, and the damage of valve evolves, especially of the fibrosa layer. In sclerotic and in stenotic aortic valves elevated levels of C-Reactive Protein (CRP), matrix metalloproteinases (MMA), tumour necrosis factor-α (TNF-α) and other cytokines are found, and these triggers the differentiation of interstitial cells. The presence of activated osteoblastic ventricular interstitial
cells, T-lymphocytes and macrophages leads to activation of pro-fibrotic/pro-calcific cascades and contributes to the fibrosis and the calcification of the leaflets, and in the long run to the hemodynamic changes of the aortic valve (18). The process is complex and multiple pathways of calcification and ossification of the valve are described. In fact, 13% of the explanted human aortic valves have been identified with laminar bone and even bone marrow (9).

In stenotic aortic valves, raised levels of angiotensin converting enzyme (ACE) and chymases are identified (19). Both of these enzymes are involved in the production of angiotensin II, which is a strong activator of one of the pathway of fibrosis and is a promotor of inflammation and oxidative stress.

Figure 3. Disease Progression in Calcific Aortic Stenosis, Showing Changes in Aortic-Valve Histologic Features, Leaflet Opening in Systole, and Doppler Velocities. Reproduced with permission (20). ©Massachusetts Medical Society.
The remodelling of the left ventricle
The calcification and narrowing of the aortic valve is not only a valvular disease, but likewise causes disease of the left ventricle. The obstruction of the left ventricular outflow increases the hemodynamic load on the left ventricle. To maintain the cardiac systolic function in a setting with chronic pressure overload, the result is hypertrophy of the left ventricle (21). The increased pressure in the left ventricle not only initiates hypertrophy of the myocardium but also initiates increased production of the extracellular matrix to support the growing myocytes (22). The extracellular matrix consists mainly of collagen I, and over time, diffuse fibrosis of the myocardium develops. However, hypertrophy and fibrosis of the myocardium are adverse since it increases the risk of heart failure and future death (23). The degree of hypertrophy is related to increased morbidity and mortality (24). After valve replacement, this increased mortality vanishes and mortality, both early postoperative and long-term, is comparable with those without hypertrophic myocardium (25). Furthermore, an ad-hoc study to the Simvastatin and Ezetimibe in Aortic Stenosis study (SEAS-study) has shown increased hypertrophy in relation to concomitant hypertension and obesity (26).

The diagnosis of aortic stenosis
Echocardiography is the golden standard for diagnosis of aortic stenosis and has been so since the 1980s. It is harmless to the patient and the technique is widespread and relatively inexpensive. In the echocardiographic evaluation of the aortic valve, the maximum velocity aortic jet and mean gradient over the aortic valve should be estimated, and the orifice area calculated using the continuity equation. The underlying morphology and the distribution and extent of calcification should also be described (27). In addition to describing the morphology, area, velocity and gradients of the aortic valve, the function and size of the left ventricle and the presence of hypertrophy should be included. Other concomitant valvular pathologies, such as aortic regurgitation, mitral stenosis and regurgitation, are also important to evaluate. In certain cases, the echocardiographic findings are not consistent with a calculated valvular area less than 1.0 cm² and with a low aortic velocity jet and low mean gradient. These evaluations of low-gradient, low-flow aortic stenosis are challenging and occurs mostly in patients with a reduced left ventricular systolic function (ejection fraction <50%); and further investigation with low dose of dobutamin stress during echocardiography is needed. If the transvalvular velocity increases and exceeds 4.0 m/s and the valvular area is still less than 1.0 cm² during dobutamin stress, the findings indicate a severe aortic stenosis. Conversely, if the transvalvular velocity gradient does not exceed 4.0 m/s or the valvular area increase over 1.0 cm², the aortic stenosis is not severe. In the latter case, alternative causes of symptoms and reduced left ventricular function must be sought for and if possible treated (28). High calcium scores in patients with
low-flow low-gradient aortic stenosis with preserved ejection fraction or without a positive response during dobutamin stress also indicate a severe aortic stenosis (29).

Management of patients with aortic stenosis
Most of the patients today with aortic stenosis and aortic sclerosis are discovered by echocardiography performed due to a newly discovered systolic murmur or during an echocardiography performed as a routine examination before planned surgery or other interventions. In the present era, there are only but a few patients who present with a severe stenosis and any of the typical symptoms of aortic stenosis. When aortic sclerosis or aortic stenosis is found, the European Society of Cardiology and the American Heart Association and American College of Cardiology guidelines for valvular heart disease recommend regular follow-ups (30, 31). If only aortic sclerosis is present, the guidelines suggest treatment of cardiovascular risk factors and with clinical examination and medical history annually. When the aortic stenosis is mild to moderate, the patient should be treated and followed by a cardiologist or equivalent and with annually or bi-annually echocardiograms. When the aortic stenosis progress to moderate to severe, the follow-ups should be more frequent with one or two visits per year and at least annual echocardiograms. A patient who develops symptoms of angina, syncope, heart failure, decreased exercise capacity, or shortness of breath with a moderate or severe aortic stenosis should be referred for evaluation for aortic valve replacement. If the patient is asymptomatic but has a severe aortic stenosis, frequent follow-ups are recommended and other examination such as stress-testing should be considered. Stress-testing may unmask symptoms that the patient does not experience in daily life. Asymptomatic patients with reduced left ventricular ejection fraction (<50%) should be considered for aortic valve replacement, mainly to protect the left ventricle from further injury (31).

Treatment of aortic stenosis
The first aortic valve replacement was performed at the Harvard University Hospital by D.E. Harken in September 1960 (32). Since then the techniques and the valves have evolved resulting in very low mortality and complication rates. In Sweden, the total 30-day mortality after all aortic stenosis valve replacement in 2016 was 1.3% (Swedheart, 2016). With the introduction of prosthetic heart valves, the outcome of aortic stenosis has changed dramatically from a highly lethal diagnosis to a curable disease, and millions of lives are being saved (32). Today there are four different alternatives to treat aortic valve stenosis – aortic valve replacement, transcatheter aortic valve replacement, the Ross procedure, and balloon aortic valvoplasty. The latter is only a choice in paediatrics or to stabilise an adult patient prior to definite therapy if other surgery must be done prior to aortic valve replacement, or in palliative care (22). A balloon is inflated
in the stenotic valve and the aortic orifice is widened. The change in aortic orifice area and gradient is only modest, and results will only persist for weeks or months. In young adults and particularly women in childbearing age, the Ross procedure can be an alternative (33). The procedure involves removing a patient’s own pulmonary root and transferring it to the aortic position, and replacing the pulmonary root with a homograft (34). The main advantage of the Ross procedure is the avoidance of warfarin and an adjustment of the neo-aortic root to a growing individual. On the other hand, the procedure is complex and is associated with both later aortic valve disease and particularly homograft failure (33).

**Aortic valve replacement**

Aortic valve replacement with a mechanical or biological prosthesis is still the preferred choice for treatment of aortic stenosis. With the development of techniques and the development of prosthetic valves in combination with overall better cardiovascular care, the survival rate after aortic valve replacement is similar to age-matched adults without aortic stenosis. A mechanical valve is the preferred valve to choose when the patient is younger than 65 years of age and without contradiction to anticoagulation. An exception is women of childbearing age and with plans to have children; then the biological valve can be an alternative due to the teratogenic effect of warfarin and the risk of bleeding complications during pregnancy and delivery (28). The advantages with mechanical valve prosthesis are the long durability of the valve and the low risk of complication. The disadvantages are the need for anticoagulation to avoid valve dysfunction, embolism and limitation in lifestyle and choice of food. A biological valve prosthesis is the choice in the elderly patient. The biological valve does not need anticoagulation but has a limited durability, and therefore carries a potential risk for reoperation. Even though there is a limitation of durability of biological prosthesis, the durability increases with age of the patient and thus the need for reoperation might not be current for the patient. After valve replacement, there is still a need for regular follow-ups, especially with biological prosthesis where a fast deterioration of the valve can occur.

**Transcathether valve replacement**

Transcatheter valve replacement is a fast-growing procedure for treatment of aortic valve stenosis. The first catheter intervention was performed on April 16, 2002 by Cribier in Hôpital Charles Nicolle at the University of Rouen, France (35). It was approved by the Food and Drug Administration in The United States of America in 2011 for use in otherwise inoperable patients, and in 2012 for patients with high surgical risk. The indications for transcatheter implantation of aortic valve prosthesis are in patients with severe aortic stenosis and not suitable for conventional surgical intervention, and with a life expectancy for more than one year and with a likely increase in improvement of life quality, or in patients with high surgical risks where an individual approach, taking account for comorbidity...
and the patients considerations, is an alternative (30, 31). Even though, the results from transcatheter replacement are very promising and has changed the outcome for patients with previously untreatable disease, the morbidity and mortality are still high. In the Placement of AoRTic TraNs catheterER Valves Trial (PARTNER-trial) at the 2-year follow-up, were all-cause mortality in the transcatheter valve replacement arm 43%, compared with 68% in the conservative treated arm, (p<0.001). At the 5-years follow-up, the all-cause mortality was 72% and 94% (p<0.001), respectively (36). The most severe complication is paravalvular aortic regurgitation, and an association between grade of regurgitation and mortality is registered (22). Increased numbers of permanent pacemaker implantation have also been seen in a 3-year follow-up after transcatheter valve replacement compared to regular aortic valve replacement (37). Recent studies have shown that transcatheter valve replacement to be non–inferior to surgical replacement of the valve in patients with intermediate risk (38). Even if the results from transcatheter valve replacement have shown comparable outcome with surgical valve replacement, there should be prudence to substitute surgical replacement with transcatheter replacement due to lack of long–term results and to excellent results from surgical treatment.

**Bicuspid aortic valve**

Bicuspid aortic valve is a congenital heart valve anomaly that affects 0.5-2% of the population and it is more frequent in males with a 3:1 male to female ratio (39). In the younger population (<60 years of age) undergoing aortic valve replacement for aortic stenosis 84%, had a bicuspid valve and in those older than 60 years of age, 48% had a bicuspid aortic valve (40). These numbers deem the bicuspid aortic valve the strongest risk factor for developing aortic stenosis; although not all with a bicuspid aortic valve will develop aortic stenosis. Further, bicuspid aortic valve is also associated with the development of aortic regurgitation. Even though aortic regurgitation and aortic stenosis are the most common complications to bicuspid valves, there are other more severe cardiac and vascular malformations and complications associated with bicuspid valves, e.g. ascending aortic aneurysm, coarctation of the aorta, aortic dissection and other rarer cardiac malformations (41, 42). It is presumed that almost all with a bicuspid aortic valve will sooner or later need surgery for aortic stenosis or aortic regurgitation (43). The reason that bicuspid aortic valves are a risk factor for developing aortic stenosis is not clear, but both increased mechanical stress on the two leaflets and genetic factors have been suggested as triggers for the calcification process (22). The normal aortic valve consist of three equally sized leaflets, whereas the bicuspid aortic valve has two leaflets that are often of different size (42). The structure of the two leaflet makes the rheology over the valve different from the three leaflets structure and the blood flow is more
turbulent with more mechanical stress on the valve (43, 44). Bicuspid aortic valves have also occurred as clusters in families indicating a genetic component (45). Mutation in the NOTCH1 gene was found to be more common in families with bicuspid aortic valves and with aortic stenosis (46). Mutations in the NOTCH1 genes also seem to affect the mineralization process of the aortic valve.

**Other valvular diseases**

**Aortic regurgitation**
Aortic regurgitation accounts for about 12% of all valvular heart disease (2). Aortic regurgitation is associated with bicuspid aortic valve, endocarditis and other malformation of the aortic valve (31, 43). Aortic regurgitation is also seen in conjunction with dilated ascending aorta (31). There are no studies linking traditional cardiovascular risk factors to aortic regurgitation. Aortic valve replacement is the only treatment of the disease and should be performed when symptom of the disease occurs or when the left chamber, dimensions exceed an end-diastolic diameter of 70 mm, or an end-systolic diameter of 50 mm, or when the left ventricular ejection fraction is lower than 50% (47). Replacement of the valve should also be performed when surgery of the ascending aorta is performed.

**Mitral regurgitation**
Mitral regurgitation is the most common valvular heart disease and accounts for almost 50% of all valvular disease (2). Mitral regurgitation is a complex valvular disease with a separation in primary and secondary causes (31). The primary mitral regurgitation is caused by structural defects of the valve and its associated structures, i.e. congenital, degeneration or acquired. Secondary mitral regurgitation is caused by a dilatation of the left ventricle and thereby enlargement of the mitral annulus causing a coaptation defect of the leaflets. Enlargement of the left ventricle can occur in ischemic heart disease or be caused by dilated cardiomyopathy (31). In primary mitral regurgitation, there are no known associations between the disease and traditional cardiovascular risk factors. On the other hand, secondary mitral regurgitation has an association between traditional cardiovascular risk factors, but this association is through the underlying disease that causes dilation of the left ventricle and a coaptation defect.

The treatment of mitral regurgitation depends on whether the defect is a primary or secondary defect. Primary defects should be treated surgically if the regurgitation is large and symptoms occur, or if the chamber exceeds 45 mm in end systolic diameter (31). Further, if atrial fibrillation occurs, pulmonary systolic pressure is more than 50 mmHg or the left ventricular ejection fraction is lower.
than 60%. Treatment of choice is, if possible, a repair of the valve. If repair is not possible, valve replacement should be performed, with biological or mechanical prosthesis. Other procedures are feasible in patients with high surgical risk or if considered non-operable i.e. transcatheter valve replacement or percutaneous edge-to-edge procedure (31). In secondary mitral regurgitation, the evidence for intervention is not as strong as in patients with primary regurgitation and studies also indicate no improvement in survival. Repair of severe secondary mitral regurgitation should be considered in patients undergoing coronary by-pass surgery and in patients with left ventricular ejection fraction more than 30% and in patients who remain symptomatic despite optimal treatment and with a low surgical risk. Percutaneous edge-to-edge procedure may be an alternative in other patients with severe mitral regurgitation (31).

**Mitral stenosis**

Mitral stenosis accounts for about 4% of all valvular heart disease (2). It has traditionally been a late complication to rheumatic fever, but in industrialized countries the degenerative calcific mitral valve is currently the main reason for intervention. Mitral stenosis has been associated with several risk factors such as rheumatic fever and chronic kidney disease but as well to osteoporosis and to the traditional cardiovascular risk factors (48). Treatment with percutaneous transluminal mitral valvuloplasty (PTMV) should be considered if the mitral valve area is less than 1.5 cm², if there are symptoms, and if the morphology of the valves is suitable. If PTMV is not possible, open surgery with valve replacement should be considered (31).

**Tricuspid regurgitation**

Tricuspid regurgitation is mostly secondary to right ventricular dilatation following pressure and/or volume overload. Primary causes of tricuspid regurgitation are endocarditis, Ebstein’s anomaly, carcinoid syndrome and myxomatous disease, among several other causes. Repair of secondary tricuspid regurgitation should be performed liberally during left-sided surgery if there is an indication. It only marginally increases the surgical risk, but it improves the functional status (31). Primary regurgitation of the tricuspid valve is more challenging due to the heterogeneous nature of the underlying valve pathology but the new guidelines from the European Society of Cardiology (ESC) recommend early treatment to avoid irreversible injury of the right ventricle (31). Surgery should be considered even in the absence of symptoms when there is progressive right ventricular dilatation or declining right ventricular function. Repair of the valve with a prosthetic ring, i.e. narrowing the tricuspid annulus, is the preferred method. If the valve is damaged or if there is a severe annulus dilatation, a prosthetic valve is considered.
**Tricuspid stenosis**

Tricuspid stenosis is a very rare valvular disease and guidelines recommend intervention when the mean gradient over the valve is more than 5 mmHg and if left-sided heart surgery is performed (31). Both repair and replacement with a biological prosthesis is recommended, depending on if the leaflets are pliable (31).

**Pulmonary valve disease**

Pulmonary valvular disease is primarily a congenital condition and is often associated with other cardiac malformations, such as tetralogy of Fallot, Williams syndrome, Noonan’s syndrome and transposition of the great arteries. The intervention of choice is pulmonary balloon valvuloplasty and is recommended in asymptomatic patient when peak echo gradient is higher than 60 mmHg or in symptomatic patients when the peak echo gradient is more than 50 mmHg. The success rate exceeds 90%. Replacement with biological or mechanical prostheses or with a homograft are performed when there is a concomitant hypoplastic pulmonary annulus or a large pulmonary regurgitation (49).

Trivial or mild pulmonary regurgitation are common findings in healthy subjects and do not need further investigation or follow-up (50). More severe pulmonary regurgitation should be followed regularly and be assessed by magnetic resonance imaging (MRI). The aetiology is usually iatrogenic after right ventricular outflow tract surgery as in repair of tetralogy of Fallot. Surgery is indicated when the right ventricular end diastolic volumes exceeds 170 mL/m² (51). As in all other valvular regurgitation, intervention should be performed before the onset of symptoms and before the onset of ventricular failure (49).

**Disease of ascending aorta**

Both dilatation and dissection are associated with syndromes such as Marfan, Loeys-Diets, bicuspid aortic valve and Ehlers-Danlos; but most of the dilatations are probably caused by degeneration (52). The upper limits of the normal ascending aorta are dependent of age, sex and body surface area; for men between 41-43 mm and for women 40-42 mm (53). Dissection of aorta is a disruption of the medial layer provoked by intramural bleeding, resulting in separation of the aortic wall layers of the ascending aorta (52). Hypertension and smoking have both been associated with dissection and dilatation of the aorta but the knowledge of risk factors for dissection and dilatation of the ascending aorta is limited. Diabetes has not been shown to be associated with dissection and dilatation, and studies have shown lower frequencies of diabetes in patients with dilatation and dissection compared to healthy controls (54-56).
Dissection of the ascending aorta (Type A dissection) is an acute event with a high mortality and demands advanced surgery in specialized hospitals. The incidence is 6/100,000 (57). Surgery should always be considered although peri- and post-operative mortality is high. Mortality after 1 month with conservative treatment is 90%, compared with 30% after surgery treatment, even in high age-groups (52). Endovascular therapy has been tested, but as yet is not a choice of treatment.

Dilatation of the ascending of aorta is usually detected in screening or imaging for other reasons such as echocardiography or computed tomography of the chest. The progression rate of the dilatation is usually slow but may differ depending on if there is a familial/syndromic disorder or not. In familial thoracic aortic aneurysm, the progression rate is up to 2.1 mm/year and in Marfan syndrome on average 0.5-1.0 mm/year. The extreme is Loyes-Dietz with a progression rate up to 10 mm/year, resulting in death at a mean age of 26 years (52, 58). Surgery of the ascending aorta should be performed when the diameter of the aorta exceeds 55 mm, but during special circumstances surgery should be performed at smaller diameters. Patients with Marfan syndrome should undergo surgery at aortic diameter of ≥50 mm. If there is a family history of early dissection or rupture, systemic hypertension, coarctation of the descending aorta or rapid progress of the dilatation (>3 mm/year), and bicuspid aortic valve surgery should be considered at ≥50 mm. Surgery can also be performed at smaller diameters if aortic valvular surgery is indicated (52).

**Atherosclerosis**

Atherosclerosis and diseases resulting from atherosclerosis are the leading cause of death and account for about 30% of all deaths in the world (59). In Sweden, deaths related to cardiovascular disease are about 35% of all deaths in 2016, and is a reduction from 46% in 2000 (60). Atherosclerosis manifests in all major vascular territories such as the brain, kidney, peripheral arteries and heart thereby causing stroke, renal failure, intermittent claudication, angina and myocardial infarction (61). In Coronary Artery Disease (CAD), the traditional symptom is chest discomfort at exercise, expressed as pain, fatigue, dyspnoea, pressure or equivalent symptoms (62). When symptoms occur or there are reasonable beliefs that there is CAD, the patient should be referred for clinical evaluation that includes identification of risk factors and for cardiac investigation including stress testing and/or coronary imaging (61).

Atherosclerosis is a complex and progressive disorder of the arteries and it is considered as an inflammatory disease in the subendothelial layer of the vessel. LDL-particles invade the endothelium and are accumulated in macrophages. The lipid-laden macrophages promote inflammation in the arterial wall, which leads to multiple pathological consequences such as haemorrhage, rupture, and
calcification. These processes are enhanced by high levels of LDL-particles, smoking, hypertension and diabetes. Even though the mechanism in large has been known for a long time, several steps in the process are still unidentified (63, 64).

Risk factors for cardiovascular disease and aortic stenosis

Lipids
Dyslipidaemia plays a central role in developing atherosclerotic cardiovascular disease and is very well documented in intervention, genetic, pathology and observational studies (65). High levels of LDL-cholesterol increase the risk of mortality and morbidity in atherosclerotic cardiovascular disease. According to studies on LDL reduction by statins, a reduction of LDL-cholesterol with 1.0 mmol/L using statin reduces the risk for cardiovascular mortality and non-fatal myocardial infarction with 20 to 25% (66). Triglycerides are also associated with atherosclerotic cardiovascular disease but the evidence is not as strong as for LDL-cholesterol and there is no randomized trail supporting target levels for triglycerides (65). One of the strongest risk factors for developing cardiovascular disease is the ratio between apolipoprotein B (Apo B) and apolipoprotein A1 (Apo A1), but due to limited access and more expensive analyses than LDL-cholesterol without adding substantially more information, it is not widely in use (67). Apo B is the major apolipoprotein of atherogenic lipoproteins and is similar to LDL-cholesterol as a risk marker, but there is no evidence that it is better (68). Apo A1 is the major apolipoprotein on high-density lipoprotein (HDL) and low levels of HDL-cholesterol are associated with increased risk of cardiovascular disease (65).

High levels of LDL-cholesterol have been the major focus for risk factor intervention in patients with aortic sclerosis and aortic stenosis. Due to the histological similarities, between atherosclerosis and aortic stenosis and findings in studies showing increased levels of LDL-cholesterol, both in blood and in the stenotic aortic valves, and access to effective drugs lowering LDL-cholesterol, LDL-cholesterol has been the number one target for risk factor intervention (69). Other studies have also supported the LDL-cholesterol lowering theory. Familial hypercholesterolemia is characterized by elevated levels of cholesterol and early development of cardiovascular disease. It is a dominant genetic disease and in its rare homozygote form, aortic stenosis is common and always with an atherosclerotic engagement of the aortic root (70). By using plasmaphereses in homozygote familial hypercholesterolemia, regression of the atherosclerosis of the aortic root have been reported (71). Retrospective observational studies and experimental studies are also in support of the LDL-cholesterol lowering therapy (72, 73). There have been three larger randomized clinical trials with the aim to reduce the progression rate of the aortic stenosis – Scottish Aortic Stenosis and
Lipid Lowering Trial, Impact on Regression (SALTIRE), Simvastatin and Ezetimibe in Aortic Stenosis (SEAS), and the Aortic Stenosis Progression Observation: Measuring Effects of Rosuvastatin (ASTRONOMER) (74-76).

**SALTIRE**
In the SALTIRE-study 155 patients with asymptomatic calcific aortic valve and aortic-jet velocity of at least 2.5 m/s were included. Of these, 77 patients received 80 mg of atorvastatin daily and 78 patients received placebo. Patients were followed for a median time of 25 months. The LDL-cholesterol levels in the treatment arm were significantly lowered by 53% to 1.7±0.6 mmol/L compared with the placebo arm where levels were unchanged. The total serum cholesterol levels were also reduced, 3.5±0.7 mmol/L compared with 5.5±0.9 mmol/L. There was however no difference in the aortic–jet velocity at follow-up.

**SEAS**
In the SEAS-study 1,873 patients with mild-to-moderate asymptomatic aortic stenosis were included. The patients received either 40 mg of simvastatin plus 10 mg of ezetimibe daily or placebo. The primary outcome was a composite of major cardiovascular events that included death from a cardiovascular cause, aortic-valve replacement, nonfatal myocardial infarction, hospitalization for unstable angina pectoris, heart failure, coronary-artery-by-pass-grafting, percutaneous coronary intervention (PCI), and non-haemorrhagic stroke. Patients were followed regularly with echocardiogram every 6 months to evaluate the progression of the aortic stenosis and the median followed-up time was 52.2 months. The LDL-cholesterol levels were reduced by 61.3% to 1.36±0.60 mmol/L in the treatment arm after 8 weeks and at the end of study the reduction of LDL-cholesterol was 53.8% in the treatment arm and 3.8% in the placebo arm. There was no significant difference in the outcome and there was no difference in progression of aortic stenosis measured as change in peak aortic velocity–jet. There were, however, a significant reduction of ischemic cardiovascular events not related to the aortic valve stenosis.

**ASTRONOMER**
In the ASTRONOMER-study, 269 patients with mild-to-moderate aortic valve stenosis were recruited and 134 were treated with 40 mg rosuvastatin daily and 135 patients treated with placebo. The objective was to assess the effect on the progression of aortic valve stenosis. Median time of follow-up was 3.5 years. In the rosuvastatin-arm LDL-cholesterol was reduced with 54.5% to 1.45 mmol/L and in the placebo-arm the LDL-cholesterol remained unchanged. HDL-cholesterol increased by 1.8% to 1.61 mmol/L in the rosuvastatin-arm and decreased in the placebo arm with 1.9% to 1.52 mmol/L, a significant difference. The change in peak aortic velocity gradient and change in aortic valve area did not differ between the two arms at follow-up.
In summary, even though there were significant and long lasting effects of the pharmacological treatment on the levels of LDL-cholesterol, the effects on the progression of the aortic stenosis were null. The reasons for the absent effects of statin in aortic stenosis are not clear but late initiation of treatment on an ongoing process might be one and the hypercholesterolemia may be an early initiator of the disease and the hypercholesterolemia is one of many pathways for developing aortic stenosis (77). As an effect of these negative clinical randomized trials, statins are not recommended as pharmacological treatment to prevent or to reduce the progression of aortic stenosis.

**Lipoprotein(a)**

Lipoprotein(a) [Lp(a)], is a well-known risk factor for ischemic cardiovascular disease and has also been associated with aortic stenosis (78-80). Lp(a) levels are progressively associated with atherosclerotic cardiovascular disease without a threshold. The association is independent of high LDL-cholesterol and other cardiovascular risk factors (81).

Lp(a) is a complex molecule and the size of the particles differ between individuals as the composition is genetically determined (80). Consequently, the concentration of Lp(a) is not altered by age, gender and diet (82). Lp(a) consists of a LDL-like molecule and with a covalent bound apolipoprotein(a) [Apo(a)] (Figure 4). The linkage is with a single disulfide bond with the Apo B-100 part of the LDL-like molecule. The Apo (a) part of Lp(a) makes the particle unique compared to other lipoproteins. The Apo (a) consists of two kringles, K-IV and K-V, and an inactive protease domain. K-IV is built by ten different subtypes. Number 1 and numbers 3 to 10 are in one copy each, whereas number 2 appears in 1 to more than 40 copies. Hence the large variety of quantities of number 2 subtypes makes the heterogeneity of the Lp(a) extensive and the concentration in plasma very wide-ranging in different individuals. It can differ more than 1,000-fold (80, 83). In addition, more than 80% of the population have two different sizes of Lp(a), one inherited from each parent. Lp(a) is assembled in the liver cells or in the hepatocytes’ vicinity and the Apo (a) part is synthesized in the liver cells. The LDL-like part of the Lp(a)-particle comes from a newly synthesized Apo B-100. The more repeats of the subtype-2 of K-IV are produced, the longer it takes to make a Lp(a)-particle, and therefore smaller Lp(a), with few repeats of subtype-2, are more common and makes higher concentration of Lp(a) in mmol/L. The clearance of Lp(a) from plasma is not fully understood, but the kidney probably plays a role in the removal of Lp(a) (82).
Figure 4. Figure 1. Lp(a) particle chemical composition. Lp(a) comprises an LDL particle, the Apo B-100 moiety of which is attached to a protein called apolipoprotein(a)—Apo (a)—via a disulfide bond. Apo (a) comprises a protease domain and a series of peptide subunits called “kringles” (K; because of their resemblance to a Danish pastry of the same name). Two K versions are present: 4 and 5 (KIV and KV). The former is subcategorized into 10 subtypes (types 1-10). KIV type 1 (KIV-1) and types 3-10, and KV are identical in all Apo (a) isoforms. Variability in Apo (a) isoform size (molecular mass) is due only to differences in the number of KIV-2 repeats. Apo (a) with 12 KIV-2 repeats is shown here; 3 to 43 repeats have been reported with resulting Apo (a) masses varying from 200 to 800 KDa. Reprinted with permission from Elsevier, Inc.

The plasma levels of Lp(a) were determined to be more than 90% by the variation of the LPA gene. The LPA gene codes for Apo (a), and thus, the number of repeats of subtypes 2 in K-IV (84). In a large-scale single-nucleotide polymorphisms (SNP) analysis, the LPA SNP rs10455872 is associated with calcific aortic stenosis and elevated levels of Lp(a), regardless of ethnicity (85). The findings were further supported in a large Danish cohort study from 2013 using Mendelian randomization that showed a genetic relative risk for aortic stenosis of 1.6% (95% CI: 1.2 to 2.1) for a ten-fold Lp(a) increase, and in a Canadian study from 2015 supporting the causality between elevated Lp(a) levels and calcific aortic stenosis (86, 87). Unfortunately, these studies are mainly based on register and ICD-codes and do not differ between those with or without coronary artery disease.

Pharmacological treatment of elevated Lp(a) would be of great interest but still there is no established treatment to specifically reduce Lp(a). Niacin reduces levels of LDL-cholesterol and of Lp(a) and shows a positive effect on cardiovascular disease but the effect cannot solely be contributed to the lowering of Lp(a). The effect on reducing the progression and development of aortic stenosis is not known (81). For the patient, niacin has intolerable side effects which makes it not as useful as it was once hoped to be. Other available treatments are LDL apheresis, Apo (a) anti-sense and proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors. LDL apheresis reduces Lp(a) levels
with around 60% but the method is expensive and impractical for most patients (88). The Apo (a) antisense therapy reduces the Lp(a) levels by up to 90% but the results from a phase 2 study from 2015 are not yet published (89). The most interesting substances are PCSK9-inhibitors since they are available on the market and were recently shown to be related to reduced risk for cardiovascular disease in a population with previous atherosclerotic disease. Further, levels of Lp(a) were reduced with 27% (90). PCSK9-inhibitors increase the numbers of LDL-cholesterol receptor on the liver cells surface by blocking the degradation of the receptor and thereby increasing the turnover of circulating LDL-cholesterol in plasma. How the PCSK9-inhibitors effects the Lp(a) concentration is under debate (91).

The Apo (a) part of Lp(a) is evolved from plasminogen, a proenzyme that is converted in to plasmin, a fibrinolytic enzyme. Lp(a) exists only in humans, old world monkeys, in primates and in the hedgehog. The Lp(a) in hedgehogs is different from the other spices’ Lp(a) by consisting of the same kind of LDL-like particle but with kringle III instead of kringle IV and V and the inactivated protease domain (92).

**Hypertension**

Hypertension is one of three leading risk factors of global burden of disease and accounted for 9.4 million deaths globally in 2010. The other two leading risk factors are tobacco smoking and alcohol use (93). Treatment of high blood pressure (according to guidelines; systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg) will decrease the risk of cardiovascular disease, heart failure, chronic kidney disease, cerebrovascular disease, peripheral arterial disease and atrial fibrillation (94). Hypertension is also considered as a risk factor for aortic stenosis (28). In aortic stenosis, most of these considerations are based on cross-sectional studies or on assumptions on trends in cardiovascular disease and in relation with incidence change of aortic stenosis (69, 95). One large prospective population based study has shown a relationship between hypertension and development of aortic stenosis, with a hazard ratio 1.71 (95% Confidence Interval [CI]:1.66-1.76) (96). Although this was based on a large study with 1.12 million participants, it included only those older than 65 years of age; further, the diagnoses of aortic stenosis were based on hospitalization ICD-codes and the extent of coronary artery disease was not known.

**Diabetes Mellitus**

Diabetes is a worldwide disease taking epidemic proportions. In 2011, approximately 360 million had diabetes and diabetes type 2 (T2DM) accounted for 95% (97). The prevalence is believed to be increasing sharply and in 2030, more than 550 million will have the diagnosis of diabetes. Diabetes type 1 (T1DM)
is caused by destruction of pancreatic beta-cells leading to absolute insulin deficiency (97). It usually occurs in earlier ages, but not always (98). In T2DM, a sedentary life style, obesity, insulin resistance and failure of beta-cells characterise the diagnosis. T2DM is also characterized by disorder of glucose metabolism before the development of T2DM, i.e. pre-diabetes (97). Pre-diabetes is a part of the development of T2DM and consists of two different glycaemic states, impaired fasting glucose (IFG) and impaired glucose tolerance (IGT). Individuals with diabetes mellitus have a double risk of cardiovascular disease, and several studies support the importance of strict risk factor control, life style management and good control of glucose levels (65, 99). Also, IGT has an association with increased risk for cardiovascular disease and intervention, with both with lifestyle advice and pharmacological treatment, reduces the risk (100, 101). Pharmacology treatment of diabetes, preferably with metformin, has until recently not shown major improvement in long-term cardiovascular outcome; however, acarbose showed a 49% relative reduction of cardiovascular events in a multicentre randomised clinical trial (102). Data supporting diabetes mellitus as a risk factors for developing aortic stenosis are sparse. Three studies have shown a correlation between aortic stenosis and diabetes but these three studies all have their limitations with population selection and how the diagnoses were retrieved, i.e. ICD-codes or computed tomography scans calculating the amount of calcification of the aortic valves (96, 103, 104).

**Smoking**

Smoking is one of the strongest life-style related risk factors for morbidity and mortality (65). It shortens the smokers’ average life with 10 years and the smoker will have a 50% probability of dying due to smoking-induced conditions (65). About half of the smoking related deaths in smokers are due to cardiovascular diseases (105). Regarding smoking as a risk factor for aortic stenosis the data are even more sparse than for other traditional risk factors, as only two retrospective studies supporting smoking as a risk factor for developing aortic stenosis were found (69, 106).

**Obesity**

Increased body mass index (BMI), as an marker for obesity, is one of the classical cardiovascular risk factors in ischemic heart disease and it is also an overall risk factor for premature death (107). Even though there is support for BMI as an overall cardiovascular risk factor, there are studies showing that overweight is related to better prognosis as has been shown for patients after percutaneous and surgical coronary intervention and in aortic valve replacement, a finding called the “obesity paradox” (108, 109). Also, studies showed a better association between hip and waist circumstances and mortality than BMI (110). Studies
exploring the association between BMI and aortic stenosis have shown mixed results (111, 112).

**Renal function**

Patients with end stage kidney disease have a 50% increased risk of cardiovascular death and a higher incidence of aortic stenosis (113, 114). Even small changes in renal function are associated with increased risk of cardiovascular disease and death (115). An aortic stenosis in patients with end stage kidney disease has a higher progression rate and sooner indication for surgery than in patients with aortic stenosis and normal renal function (116). Renal impairment in earlier stages seems not to be associated with developing aortic stenosis (117). The reason patients with end stage kidney disease develop aortic stenosis is not clear but several factors have been proposed to promote the development. Patients with end stage renal disease and in haemodialysis have an increased cardiac outflow due to anaemia, arteriovenous shunts and/or over-hydration, which increases the mechanical stress on the aortic valve (114). Endothelial dysfunction and disorder of phosphorus and calcium metabolism may also be important factors involved in the process (114, 118, 119).

**Genetics**

There are a few known monogenetic disorders linked to arteriosclerotic cardiovascular disease such as mutations in the LDL-receptor, the Apo B, or in the PCSK9 (120). All these mutations are related to cholesterol and lipid metabolism. As an example, familial hypercholesterolemia is caused by mutations in the LDL-R gene located in chromosome 19. Currently, more than 1,200 mutations in the LDL-R gene have been identified (121). In a larger genome-wide association study (GWAS) more than 152 associated loci and 320 candidate genes have been identified as contributors to increase the genetic risk for arteriosclerotic heart diseases and acute myocardial infarction. Most of these variations have unknown mechanisms and are also affected by environmental factors (120). Two different SNP variants in the LPA gene, rs10455872 and rs3798220, have been associated with the development of atherosclerosis, and as describe above, the rs10455872 with the development of aortic stenosis (85, 122). Some other genetic mutations have been connected with the development of aortic stenosis. Variation in the NOTCH1 is associated with the development of the bicuspid aortic valve and other congenital cardiac malformation (46). The bicuspid aortic valve is a known risk factor for developing aortic stenosis, especially in younger individuals (40). A recent (November 2017) presentation at the American Heart Association Secession 2017 showed an association with mutation in the Lp(a) gene (LPA gene) but also with mutation in the fatty acid desaturase (FADS) locus (123). This is a new finding and indicates fatty acid metabolism as a part in the development of aortic sclerosis and aortic stenosis.
Familiar clustering of aortic stenosis has also been reported although the causing genetic defects have not been found (124).

**Socioeconomic factors**

In several studies, socioeconomic status was inversely related to cardiovascular disease (125). Education is frequently used as an indicator of socioeconomic status and individuals with high education have a significant lower risk for cardiovascular disease and cardiovascular death compared with both medium high education and low education. The same patterns are seen in the presence of cardiovascular risk factors as they are more common in lower levels of socioeconomic status, especially in high income countries (125). Notably, a high socioeconomic status was associated with a higher risk of aortic stenosis, even after adjustment for cofounders, but these findings needs to be confirmed in other studies (126).

**Hill’s criteria for causality and Mendelian randomization**

In January 1965 Bradford Austin Hill had his famous lecture “The Environment and Disease: Association or Causation?” (127). In the speech, he outlined nine different points for evaluating causal relationships between “sickness, injury and conditions of work.”(128)- *Strength, consistency, specificity, temporality, biological gradient, plausibility, coherence, experiment, and analogy*. His theories about causality have been an important part of the epidemiological research since then. *Strength*: A larger effect strengthens the association of causality, but does not exclude small effects. *Consistency*: Same findings or observation in different populations and/or with different methods strengthen the causality. *Specificity*: The effect or the observation has no other obvious explanation and it affects a specific population. *Temporality*: The observation or the effect occurs after the exposure. *Biological gradient*: The effects or the observations are larger or in a higher incidence in a dose-response pattern. *Plausibility*: A plausible mechanism between exposure and effect is described or at least possible. *Coherence*: Findings in epidemiological data should not conflict with generally known facts about the effect or the disease. *Experimental*: If there are experimental data supporting the findings the causality is strongly supported. *Analogy*: If similar findings with similar exposure are known, the thoughts of causality are strengthened.

Even if Hill’s criteria are applied in epidemiological research to support causality and to generate hypothesis they should not be used as strict criteria that have to be fulfilled to support causality. Hill emphasized the use of common sense and the obvious pattern in findings to reveal causation.
Another way to strengthen the causality of a biomarker is to use the Mendelian randomization technique (129). The concept of Mendelian randomization is based on the fact that different genetic variants (alleles) alter the levels of a specific biomarker that is thought to be causal to a disease. The different genetic variants should correlate to both the studied outcome and to the level of the biomarker. Furthermore, the alleles are distributed randomly and resulting levels represent a random allocation to a lifelong exposure. For an example, Lp(a) is correlated to developing aortic stenosis. Lp(a) levels are regulated by a single gene (LPA) and a specific variant of the gene gives high levels of Lp(a) (LPA rs10455872). Studies have shown a two to three-fold increase for aortic valve stenosis when Lp(a) is greater than the 90th percentile. In the same study, LPA rs10455872 is associated with developing aortic valve stenosis and also an increased level of Lp(a) (86). Both of these findings together strengthen the causality of Lp(a) as a risk factor for developing aortic stenosis.
Aims

The hypothesis has been that traditional cardiovascular risk factors associate with valvular disease are differentially depending on the phenotype of the disease including the presence or absence of concomitant CAD. Furthermore, it has been hypothesised that circulating substances could be identified in plasma that are associated with developing aortic stenosis requiring surgery; not least substances related to early metabolic disturbances and impaired endothelial function.

1. To evaluate if the traditional cardiovascular risk factors are associated with developing valvular heart disease and with special focus on aortic stenosis, and if any associations differ between those with or without concomitant CAD.

2. To study the impact of arterial hypertension on the risk for surgery for aortic stenosis after careful stratification for age and presence of CAD.

3. To study the impact of dyslipidaemia as measured by apolipoproteins and Lp(a) on the future risk for surgery for aortic stenosis.

4. To use new methodology for determining early renal impairment, explore if the so called “shrunken pore syndrome” is associated to future risk for surgery for aortic stenosis.
Materials and Methods

Study population
Cases and control were all recruited from the Northern Sweden Health and Disease Study (NSHDS) with its three sub-cohorts; The Northern Sweden MONItroring of trends and determinants in Cardiovascular disease (MONICA) study, the Västerbotten Intervention Programme (VIP), and the Mammary Screening Project (MSP). At the end of year 2014, these studies had surveyed approximately 140,000 unique individuals.

VIP
VIP is a population based ongoing intervention programme (130). It started in 1985 with the intention to reduce morbidity and mortality in cardiovascular disease and diabetes. During the 1970s increasing and high rates of cardiovascular mortality were identified in Sweden and especially in Northern Sweden in the county of Västerbotten. It started on a small scale in the community of Norsjö, with inspiration from the North Karelia Project, and thereafter has expanded to cover the whole county of Västerbotten. All inhabitants of the county are invited to participate in a health survey at their local health care centre the year that they turn 30, 40, 50 and 60 years of age. Currently, those turning 30 years old are no longer invited due to lack of recourses and low attendance rate. By December of 2014, 99,268 unique individuals have participated in the VIP. The ratio of participation was from the beginning was about 55% but has in later years increased to 65%.

All the health survey participants are asked to complete a questionnaire concerning diet and lifestyles factors. Further, blood samples are obtained for lipid and glucose measurements, and for storage in biobank for future research. The health survey also includes a 2-hour oral glucose tolerance test (OGTT) and anthropometry and blood pressure measurements. All measurements and interview are performed by trained nurses.

MONICA
The World Health Organization (WHO) initiated a project in the early 1980s to better understand the changes in morbidity and mortality in ischemic cardiovascular diseases (131). It was then not clear if the changes in mortality were due to changes in risk–factor profile, incidence in coronary events, or if coronary care could be attributed to better outcome. The first participants were included in 1981 in Iceland and in the Northern Sweden MONICA study in 1986. The WHO part of the MONICA study was terminated in 1995. The Northern
Sweden MONICA study has maintained the register and until today has continued however, with registers of incident stroke and myocardial infarction and with repeated population based surveys (132). Altogether seven surveys have been done since 1986 that include randomly selected inhabitants in the counties of Västerbotten and Norrbotten. The randomization is stratified for age, and in the first two surveys (1986 and 1990) 2,000 inhabitants aged 25-64 years were invited, and in the remaining four surveys (1994, 1999, 2004, 2009 and 2014) 2,500 inhabitants aged 25-74 years were invited. Target population in the area has varied from about 306,000 to about 318,000 individuals. At the end of December 2014, 12,368 unique individuals had participated and the overall participation rate was 74%.

In the Northern Sweden MONICA study, similar procedures were used as in the VIP study concerning blood samples donation, OGTT, blood pressure and anthropometry measurements, lifestyle and diet questionnaires completion and cholesterol measurements.

**MSP**

MSP was initiated in 1995 and finished in 2006 (133). Women were asked to donate blood samples and to have their blood pressure and anthropometry measured when they were attending their regular mammography examinations. All women within the age of 40-70 years were invited every two or three years for breast cancer screening. Altogether 28,778 women have participated in the project and the participation rate was about 57%.

**Selection of cases**

The only thoracic surgery department for the Northern healthcare region is in Umeå and almost all thoracic surgery for the Northern healthcare region is performed there except for heart transplantation, paediatric thoracic surgery and advanced congenital thoracic surgery. Between March 1 1988 and December 31 2014, 6,681 patients underwent surgery for valvular heart disease and/or disease of the ascending aorta. Altogether 873 of them had participated in one or more of the three population based health surveys described above (Figure 5).
Figure 5. Flowchart of selection of cases.

140,414 participants in the three health surveys, VIP, MSP or MONICA, December 31, 2014.

6,691 had surgery for valvular heart disease and/or disease of the ascending aorta.

873 had surgery for valvular heart disease and/or disease of the ascending aorta and blood samples and/or questionnaire were found.

62 excluded, only DNA specimen.

811 had surgery for valvular heart disease and/or disease of the ascending aorta.

22 excluded due to surgery for rare valvular diseases and 12 had no questionnaires.

13 excluded due to uncertain diagnosis of aortic stenosis.

Paper I: 777 had primary surgery for aortic stenosis, aortic regurgitation, mitral regurgitation, ascending aorta, and CABG.

Paper II: 322 had surgery for aortic stenosis.

Paper III and IV: 336 had surgery for aortic stenosis and available plasma.

26
Selection of referents

Referents were recruited from the same cohorts as the cases. For each case four referents were randomly selected. The referents were matched for sex, type of health survey, age (± 2 years), geographical area and date for inclusion in health survey (± 4 months). In papers I and II, all four referents were used and in papers III and IV two referents were randomly selected from the four for further biochemical analyses, thus reducing costs while reasonable statistical power was maintained.

Table 1. Compositions of study population

<table>
<thead>
<tr>
<th>Survey</th>
<th>Paper I</th>
<th>Paper II</th>
<th>Paper III</th>
<th>Paper IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIP</td>
<td>600/2,380</td>
<td>54/216</td>
<td>237/713</td>
<td>237/713</td>
</tr>
<tr>
<td>MONICA</td>
<td>78/305</td>
<td>44/173</td>
<td>37/111</td>
<td>37/111</td>
</tr>
<tr>
<td>MSP</td>
<td>99/395</td>
<td>224/1,276</td>
<td>62/186</td>
<td>62/186</td>
</tr>
<tr>
<td>Total</td>
<td>777/3,080</td>
<td>322/1,665</td>
<td>336/1,010</td>
<td>336/1,010</td>
</tr>
</tbody>
</table>

Data show are numbers of cases and referents from the different the surveys used in the papers.

Examinations at baseline and definitions (health survey)

**OGTT and diabetes**

In VIP and in about 60% of MONICA participants, an OGTT was a part of the survey, but was not in the MSP. The test was performed according to WHO guidelines with 75 g of glucose dissolved in 300 mL water and consumed in 5 minutes after at least 4 hours of fasting (134). Those with known diabetes, on anti-diabetic medication, or had a fastening blood glucose level over 7.0 mmol/L were excluded. Blood glucose levels were measured at the start (fastening) and after 2 hours. According to the World Health Organization guidelines, the presence of diabetes mellitus was based on self-reported usage of antidiabetic medication and/or fasting plasma glucose levels ≥7.0 mmol/L, and/or post load plasma glucose levels ≥11.1 mmol/L (≥12.2 mmol/L in the VIP, based on capillary plasma). Impaired fasting glucose was defined as a fasting glucose level ≥6.1 and <7.0 mmol/L, and impaired glucose tolerance as a post load glucose level ≥7.8 and <11.1 mmol/L (≥8.9 and <12.2 in VIP) in combination with a non-diabetic fasting glucose level.
Blood pressure and hypertension

In the MONICA and in the MSP studies, blood pressure was measured twice in a sitting position after 5 minutes of rest. This was done initially with a mercury sphygmomanometer using the random-zero technique. This was changed to a semiautomatic device in 2004 (Omron M7; Omron Corp, Kyoto, Japan). Agreement between the methods have been assessed (135). In the VIP study, blood pressure measurements were done in a horizontal position until September 2006; and thereafter in a sitting position using the same kind of device as in the MONICA and MSP surveys. The measurements acquired in the horizontal position were adjusted according to an age and sex-specific formula (136). Hypertension was defined according to European guidelines and other large randomized clinical trials (47, 75, 90, 137). Hypertension was defined as systolic BP ≥140 mmHg and/or diastolic BP ≥90 mmHg and/or on anti-hypertensive medication.

Blood sampling and analysis

In the VIP and MONICA survey, cholesterol was measured at the time for survey after an initially 4 hours of fasting; this was extended to 8 hours in 1992. In MONICA, the analysis was performed by an enzymatic method at the same central laboratory (Boehringer Mannheim GmbH Diagnostica, Mannheim, Germany). In VIP, a bench-top analyser (Reflotron®, Boehringer Mannheim GmbH Diagnostica, Mannheim, Germany) was used until September 2009. Thereafter, levels were determined at a central laboratory using the same enzymatic method as in MONICA. The bench-top analyser results were adjusted to the results obtained at the central laboratory. MSP participants did not have their cholesterol values analysed at survey.

In all three studies, participants were informed and signed consent was obtained for blood donation for future, non-specified research. The blood samples were stored at -80° C at the Northern Sweden Medical Biobank at the University Hospital of Umeå.

Lp(a), Apo A1 and Apo B

Lp(a), Apo A1, and Apo B were analysed on a Cobas® 8000 modular analyser, c502 module. The reagents used were: Tina-quant Apo A1 and Apo B (cat. nos. 03032566122 and 03032574122, respectively, both version 2) and Tina-quant Lp(a) Generation 2 (cat. no. 05852625190; Roche Diagnostics, Basel Switzerland). The lowest level of detection was 7 nmol/L for Lp(a) and 0.03 g/L for both Apo B and Apo A1. Apo A1 and Apo B were standardized to reference standards, IFCC SP1-01 and SP3-07, respectively. Lp(a) was standardized to reference material, IFCC SRM2B, to convert into SI-units of nmol/L. The Apo B/A1 ratio was calculated. The total coefficients of variation (CV%) were: Apo A1:
3.42 and 2.18%, at levels of 0.86 and 1.45 mg/L, respectively; Apo B: 1.93 and 2.19%, at levels of 1.0 and 1.8 mg/L, respectively; and Lp(a): 2.4 and 3.2%, at levels of 34 and 115 nmol/L, respectively. The analyses were all performed during the spring of 2017.

_Creatinine, Cystatin C, and CRP_
Creatinine, Cystatin C, and CRP were analysed on a Cobas 8000 modular analyser, c502 module (Roche Diagnostics, Switzerland). The reagents employed were CREP2 (Catalog No. 03263991190), Tina-quant Cystatin C Gen. 2 (catalogue No. 06600239190), and CRPL3 (catalog No. 04956842190), all from Roche Diagnostics, Basel Switzerland. The lowest level of detection was for creatinine 5 μmol/L, Cystatin C 0.4 mg/L, and 3 ng/L for CRP. Creatinine is traceable to Isotope dilution mass spectrometry (IDMS) reference measurement procedure. Cystatin C is traceable to the ERMDA471/IFCC standard. CRP is traceable to CRM 470 (CRPL3 2011-01, V3). The total coefficients of variation were: 3% for Creatinine at both levels of 90 and 500 μmol/L; 1.43% and 0.84% for Cystatin C at levels of 0.99 and 3.95 mg/L, respectively; and CRP 1.5% and 1.9% at levels of 8 and 47 mg/L, respectively.

_Anthropometry and body mass index_
Weight and height were measured in all three surveys. Weight was measured with participants dressed in light indoor clothes and without shoes to the nearest 0.2kg. Height was measured to the nearest centimetre and without shoes. BMI was calculated as weight divided by height squared. Waist and hip circumferences were not routinely measured in VIP during the early phase of the project.

_Questionnaires_
VIP and MONICA have similar questionnaires covering demographic factors, health and disease and its risk factors and factors related to socioeconomic and psychosocial conditions. The questionnaires have expanded since the start in the mid-1980s, with the addition of questions related to health-related quality of life, sleep apnoea and alcohol consumption, etc. However, the questions about diet have been similar during the whole period which enables comparisons over time. In the MSP, there was a questionnaire about reproductive factors, smoking and medication.

_Examinations at study end point (surgery)_
Each case has been thoroughly scrutinized with extracting valid cardiovascular information from hospitals files regarding preoperative assessments and perioperative details. From the preoperative assessments files, information about cardiovascular risk factors, concomitant cardiovascular disease, medication,
anthropometry, blood pressure, electrocardiogram (ECG), recently performed stress testing, coronary angiogram, chest x-ray, echocardiography, and results from blood chemistry were retrieved. Perioperative details that were recorded were kind of valvular disease, nature of valvular malformation (i.e. endocarditis, bicuspid valve, ballooning, calcification etc.), type of valvular intervention (i.e. mechanical or biological valve prosthesis or valvuloplasty), numbers of coronary grafts, cross-clamp time, bypass times, days of postoperative intensive care, the occurrence of atrial fibrillation and outcome (i.e. death during hospitalization or discharged).

**Coronary angiogram**
Results from those who underwent coronary angiogram, as a part of their preoperative assessment, were recorded pursuant to the degree of vascular disease. These were categorized according to established practice – left main stem and 1-, 2-, or 3-vessel disease based on the presence of one or more stenoses with a diameter reduction of at least 50%. The presence of coronary artery engagement with less than 50% stenosis was considered to indicate coronary atherosclerosis. Regardless of the degree of coronary diseases, if there were any sign of atherosclerosis in any of the coronary arteries they were considered as having CAD. Coronary angiograms were performed in 94% of all included cases.

**Echocardiography**
An echocardiogram was performed in 96% of all cases as part of the preoperative assessment. From the echocardiogram protocols, kind and degree of valvular diseases, left ventricular size and function, valvular gradients and velocities and other malformation were recorded. If accessible, kind of valvular pathologies and stroke volumes were also recorded. Ejection fractions were calculated using Teichholz formula (138).

**Electrocardiogram (ECG)**
In 97% of all cases there was an ECG recorded and heart rate and heart rhythm were registered.

**Anthropometry**
Wight and height were routinely registered before the coronary angiogram, in order to estimate renal function.

**Blood pressure and hypertension**
Information about the presence of hypertension and measured blood pressure at preoperative assessment were recorded from hospitals files. There were no notifications of how the blood pressures were measured.
Classification of cases (phenotype)

Each case was thoroughly evaluated in regard to registered data by the author. Cases were classified according to the primary reason for intervention and eventually other surgical interventions were ranked. If there was uncertainty regarding classification of the cases, consensus was obtained after discussion with co-workers.

Statistics

Same statistical approach was used in all four papers. Formal tests and visual inspection were used to check for normal distribution and data were transformed to the natural log (ln) scale when needed. The (ln) z-scores were calculated separately for men and women, and as a conservative approach missing values were replaced with the median value obtained among the referents. Continuous variables were categorised into quartiles, based on the distribution of the referent values, and cut-offs were determined separately for men and women. Missing values were treated as a separate category and were not included in the tables.

Arithmetic and geometric means with 95% confidence intervals were used for descriptive statistics when appropriate, and student’s t-test was used to determine differences between groups. The distribution of categorical data (trend) was tested with a chi-square test.

Within strata, the cases and referents had the same follow-up times in this nested, matched case-referent study. Therefore, we estimated odds ratios (OR) and 95% confidence intervals (CI) with logistic regression analyses (rather than Cox regression) using the conditional maximum likelihood routine designed for matched analysis. The influence of studied variables on future surgery for valvular heart disease and surgery for disease of the ascending aorta were tested in univariate and multivariable models. The analyses were stratified for sex, age at surgery (less than 60 years or 60 years and more), the time between the survey and surgery (< 5 years or ≥5 years), and the presence of any CAD found on the preoperative angiogram. Finally, in separate analyses, MSP cohort was excluded.

Vital status for the entire cohort was checked against population registers on the 24th of August 2017, and the date of death was registered. Survival was calculated from date of surgery or the same date for the referents within the same strata (as by definition the referents were alive until the day of surgery of the corresponding case) until date of death or until date of follow-up, whichever came first. None was lost to follow-up. Long-time survival for the entire cohort stratified for case-status was illustrated by Kaplan-Meier plots, and differences in mean survival were tested with log rank tests. The impact of the ratio between eGFR cystatin C and eGFR creatinine on survival was evaluated using an adjusted Cox model.
In the power calculation, about 500 individuals with valvular heart disease and twice as many controls were needed to identify a significant difference at 5% level in risk factor that occurs in 5% of the population and with an odds ratio of 2-3.

All calculations were performed with the statistical program, SPSS version 22-24 (IBM, Armonk, NY, USA).

**Ethical considerations**

All patients gave their written informed consent at the time of survey for future research use of data and blood samples. None has been contacted as a result of the data collecting procedure or during analysis of data. After data collection and matching, all identifying data were removed from the data-set. The identification key is stored at “Enheten för BiobanksForskning” (EBF) at Umeå University.

The study protocol was approved by the Regional Ethical Review Board in Umeå (Dnr. 07-174M, Dnr. 2010-348-31M, Dnr. 2014-348-32M, Dnr. 2015-326-32M) and complied with the Declaration of Helsinki.

Analysis of stored blood samples and data that has been collected over a long period of time requires ethical consideration. The aim of the study was to find risk factors or risk makers for valvular heart disease and particularly for aortic stenosis. If the analyses of blood samples and data reveals new knowledge about valvular heart disease, it will help to treat future patients. New knowledge will justify the cost of the surveys even if the cases will not benefit from these studies, but their willingness to participate with time, data and blood are fulfilled. Even if our knowledge has increased about different cardiac diseases all the cases should have been treated according to current guidelines and not been withheld adequate treatment.
Results

Paper I

Overall patient characteristics
A total of 777 patients were included in the study and 292 (38%) were women. The median time from survey to surgery was 10.5 (IQ 9.1) years. Primary indication for surgery was as follows: 322 (41%) patients operated for aortic stenosis, 91 patients (12%) for aortic regurgitation, 181 patients (23%) for mitral regurgitation, 131 patients (17%) for disease of the ascending aorta, and 52 patients (7%) for coronary artery bypass. Other forms of cardiac surgery, i.e. concomitant other valvular surgery or coronary artery bypass, were performed in 34-62% of the cases depending of the primary indication for surgery.

Aortic stenosis
There were 322 cases that were operated for aortic stenosis (150 [47%] women) as their primary indication for valvular surgery. In 149 (46%) of the cases other concomitant surgeries were performed that included 109 (34%) for coronary artery bypass. In the multivariable model, diabetes, hypertension, active smoking and high levels of cholesterol were all associated with future surgery for aortic stenosis. After exclusion of those with any sign of arteriosclerosis found in the preoperative coronary angiogram, none of the examined risk factors remained associated with future surgery for aortic stenosis.

Aortic regurgitation
A total of 91 cases were operated for aortic regurgitation as their primary indication, of which 16 (18%) were women. There were 40 (44%) had concomitant surgery of other valves 9 (10%), coronary artery bypass 13 (14%) or disease of ascending aorta 21 (23%). None of the traditional cardiovascular risk factors predicated surgery for aortic regurgitation. However, a high level of cholesterol was associated with a reduced risk for surgery for aortic regurgitation both in the uni- and multivariate models and after exclusion of those with arteriosclerosis in the preoperative coronary angiogram. A diastolic blood pressure above the median was also associated with reduced risk for surgery for aortic regurgitation.

Mitral regurgitation
In the group of mitral regurgitations as primary indication for surgery cases, there were 181 cases, of which 65 (36%) were women. High level of cholesterol was associated with future surgery of mitral regurgitation, but this association was
not persistent after exclusion of those with arteriosclerosis in the preoperative coronary angiogram.

**Disease of the ascending aorta**
A total of 131 cases had underwent surgery of the ascending aorta as their primary indication for intervention, of which 48 (37%) were women. Of the evaluated risk factors, hypertension, elevated diastolic and systolic blood pressure and previous smoking were independently associated with surgery of the ascending aorta. On the other hand, diabetes was associated with a lower risk of surgery of the ascending aorta. None of the evaluated risk factors were associated with future surgery of the ascending aorta after exclusion of cases with arteriosclerosis in the preoperative coronary angiogram, but glucose intolerance was associated with a lower risk for surgery.

**Paper II**

**Patients characteristics**
A total of 322 cases had surgery for aortic stenosis as their primary indication for surgery. In 70 cases, surgery was performed before the age of 60 years, and in 252 cases, surgery was performed after the age of 60 years. After exclusion of those with CAD, 49 and 82 cases remained in respective age groups.

**Younger than 60 years of age**
In those operated for aortic stenosis as primary indication before the age of 60 years, only arterial hypertension and diastolic blood pressure were associated with future surgery for aortic stenosis in separate models. After stratification for CAD, these finding persisted both in those with and without CAD.

**Older than 60 years of age**
In the elderly group of cases, associations differed if there was concomitant CAD or not. In the 82 cases without CAD only IGF was associated with future surgery for aortic stenosis. On the other hand, several of the traditional cardiovascular risk factors were associated with aortic stenosis requiring surgery. Arterial hypertension, high cholesterol levels, diabetes and active smoking were all associated with future surgery for aortic stenosis.
Paper III

**Patient characteristics**
A total of 336 cases had surgery for aortic stenosis regardless if it was the primary indication or not; these patients had donated blood samples for future research at the time they participated in the health survey. The median age at surgery was 68.3 (12.7) years and 161 (48%) were women. The median time from survey to surgery was 10.9 (IQ 9.3) years. CAD was found in 203 cases and no CAD in 132 cases. One case was not classified due to absence of coronary angiogram. Lp(a) was higher in cases than in controls, 23.1 nmol/L (19.8-27.0) vs. 17.8 nmol/L (16.2-19.6), p=0.005 and the ratio between Apo B and Apo A1 was higher in cases than in controls 0.81 (0.79-0.84) vs. 0.77 (0.76-0.79), p=0.01.

**Without CAD**
In cases without CAD, high levels of Lp(a) and high Apo B/A1 ratio did not associate with future surgery for aortic stenosis. These findings were consistent irrespectively of sex, age for surgery, interval between survey and surgery, and BMI.

**With CAD**
Even though high levels of Lp(a) and a high Apo B/A1-ratio were associated with future surgery for aortic stenosis in patients with CAD, these findings were not seen in patients younger than 60 years old or had been operated within 5 years from survey; only a high Apo B/A1 ratio remained significant associated with surgery.

Paper IV

**Patients characteristics**
In paper IV the same patient groups as in paper III were studied. There was no difference between cases and controls regarding levels of cystatin C and creatinine and no significant difference in renal function estimated by plasma levels of cystatin C and creatinine irrespectively of concomitant CAD or not. The ratio between eGFR<sub>cystatin C</sub> and eGFR<sub>creatinine</sub> was lower in cases compared with controls, 1.11 (1.09–1.13) vs. 1.14 (1.13–1.16), p=0.02. After stratification for sex, the finding of low ratios was consistent in women but not in men, (women 1.11 [1.08–1.14] vs. 1.16 [1.14–1.18], p=0.008 and men 1.11 [1.08–1.14] vs. 1.12 [1.11–1.14], p=0.48, respectively).

Vital status was determined in August 2017, 35% of cases and 23% of control had died. The mean survival time from survey was 18.7 (17.9–19.5) and 16.0 (14.7–
17.2) years, respectively (log rank p<0.001), and a high ratio predicted longer survival irrespective of case-status.

**Without CAD**
In those without CAD, a low ratio between $\text{eGFR}_\text{cystatin C}$ and $\text{eGFR}_\text{creatinine}$ did not associate with future surgery for aortic stenosis. After stratification for sex, there were still no associations between the ratio and surgery.

**With CAD**
In the group with CAD, a low ratio between $\text{eGFR}_\text{cystatin C}$ and $\text{eGFR}_\text{creatinine}$ associated with future surgery for aortic stenosis. After stratification for sex, this association remained in women.
Table 2. Summary of risk marker associated with surgery for aortic stenosis

<table>
<thead>
<tr>
<th></th>
<th>Hypertension</th>
<th>Cholesterol</th>
<th>BMI</th>
<th>Glucose intolerance</th>
<th>Smoking</th>
<th>Lp(a)</th>
<th>Apo B/A1</th>
<th>eGFRcystatin</th>
<th>c/eGFRcreatinine</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Men</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Women</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Under 60 year</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Over 60 year</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>n.a.</td>
</tr>
<tr>
<td>Without CAD</td>
<td>All</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Men</td>
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<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
<td>0</td>
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<tr>
<td>Women</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Under 60 year</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Over 60 year</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>n.a.</td>
</tr>
<tr>
<td>With CAD</td>
<td>All</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Men</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>Women</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Under 60 year</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>n.a.</td>
<td></td>
</tr>
<tr>
<td>Over 60 year</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>n.a.</td>
<td></td>
</tr>
</tbody>
</table>

CAD=Coronary Artery Disease, BMI=Body Mass Index. + denotes significant association between risk marker and surgery for aortic stenosis. 0 denotes no association.
Discussion

Methodological considerations

Study populations
In all four papers the studied population came from the Northern Sweden Health and Disease Study (NSHDS) and its three sub-cohorts. Different health studies are often affected by bias. Participants are often healthier than the general population but males and those with lower economic standard tends to participate in a lower rate. However, most of the cases and controls derived from VIP which has a high participation rate and did not alter from the normal population in composition (130). In the MONICA, attempts has been made to contact non-participants by telephone, and analysis of non-participants showed the study to be representative for the population (139). All three health surveys had different age criteria for participation. VIP did initially invite those in Västerbottens county that turned 30, 40, 50 and 60 years of age but after 1995 those turning 30 were excluded due to low attendance rate and budget restriction. In MONICA 2,500 participants in the age range 25-74 years have been randomly invited every 5th year since 1986. In the MSP study females participating in the mammography program during the 1995 to 2006 were invited. The mammography program is eligible to females aged 40-74 years. Due to the age criteria for participating in the surveys, the study populations do not include younger individuals, and the oldest inhabitants have not had the possibility to attend. On the other hand, most of the studied diagnoses are rare in younger ages (2) and the programs have been running since 1985 which enable most of the today older inhabitants to have participated.

Selection of cases
All cases were selected from one of the three health survey- VIP, MONICA or MSP, and all had had surgery for valvular heart disease or disease of the ascending aorta at the department of thoracic surgery at University hospital of Umeå. This is the only thoracic surgery department in northern Sweden. Certain premises had to be fulfilled, i.e. had undergone valvular heart surgery or surgery of the ascending aorta after participating in one of the three surveys and had not undergone cardiac valvular heart surgery previously. If any individual had had heart surgery elsewhere they were not included in the study population. Even though some cases will be missing from the study population, very few inhabitants from northern Sweden have undergone cardiac surgery at places other than at the department of thoracic surgery at the University hospital of Umeå; and those missing may not alter the results. Almost all cases have been
discussed at a multi-professional conference before surgery decision, which ensures decision based on then current guidelines. There were a few patients with acute vital indication for valvular heart surgery or surgery of the ascending aorta that were not discussed in a multi-professional conference prior to surgery due to the acute circumstances. Because all cases were deemed as operable by the thoracic surgeon, the cases with advanced multiple comorbidities, high age or with other reasons for high per- or postoperative risk were underrepresented in the studies. When comparing the studied group of cases with those not included in the study and operated for valvular heart surgery or undergone surgery of the ascending aorta, the studied group were slightly younger than those not included in the study, 64.9 years vs. 67.8 years. A likely reason for this finding could be the age inclusion criteria of the health surveys; where the oldest operated patients are not included, because they have not had the opportunity to participate in the health surveys.

All cases were classified based on their primary indication for surgery. In addition, other concomitant surgeries were ranked according to their severity. The classification was done twice by the same cardiologist at two different time-points without knowledge of the baseline data and outcome; this ensured equivalence in the classification. When comparing echocardiography findings with primary indication for surgery; echocardiography criteria for surgery for valvular heart disease matched the primary indication for surgery, i.e. aortic valve area less than 1.0 cm² for aortic stenosis, enlarged left ventricle for aortic regurgitation, etc.

**Selection of controls**

All controls were selected from the same cohorts as the cases, and were matched for age, sex, time point for participating in survey and geographical area. All controls where alive at the time for the matched cases surgery. The consequence of matching is unfortunately that the matching variables cannot be included in the statistical analysis as for example interaction terms. This was instead handled with stratification and any comparison of effects size between strata should be done with caution. All controls were “forced” to survive until the time for matched cases’ surgery and which indicates that controls probably were healthier than the general population.

**Data at survey**

Measurements and blood samples collected at the health surveys were gathered over a long times span, 1985 to 2012. During this time span, methods and equipment for measurement of blood pressure had changed, methods for measurement of cholesterol had changed and blood samples had been stored for
a long time in a freezer which could have affected levels of analysed compounds, etc.

**Blood pressure**

Blood pressure in the three health surveys had been measured initially with a mercury sphygmomanometer until 2004 and thereafter with a semiautomatic device. In MONICA and MSP, blood pressure had been measured after 5 minutes of rest in a sitting position. In VIP blood pressure was measured in a horizontal position up until September 1 2006, and thereafter as in the two other health surveys. When altering methods of measuring and changing devices, the obtained values are not interchangeable due to different methods and devices. Blood pressures measured in the horizontal position were recalculated according to a sex- and age-specific formula (136). The devices were considered to be compatible. All controls were matched to time point of participation in health survey and in the same health survey, eliminating differences within strata in measuring methods and used device. Hypertension was defined as a measured systolic blood pressure ≥140 mmHg and/or a diastolic blood pressure ≥90 mmHg and/or on anti-hypertensive medication. The blood pressure levels for hypertension were in accordance to current Europeans Society of Cardiology guidelines at the time for creating the study data file, (2014) (47). Those with treatment for hypertension, but with normal blood pressure levels were considered as hypertensive. Before initiation of treatment, they had been exposed to high levels of blood pressure under a certain time frame, and those with high measured blood pressure levels should have been followed-up in accordance to the algorithms in the health survey.

**BMI**

Each case and control had their weight and height measured at the survey (i.e. not based on self-reported height and weight) and BMI was calculated. BMI is not usable as an individual risk marker for cardiovascular disease but as a risk marker for a population (140). Since the start of the first MONICA survey in 1994, the BMI in the population has increased with 1.5 kg/m², which corresponds approximately to 4 kg in total (132).

**Diabetes**

The diagnoses of diabetes and glucose intolerance were based on participants’ self-reported diagnosis of diabetes and treatment with anti-diabetic medication or on the result from the OGTT. OGTT was performed in VIP and in 60% of the MONICA participants, but not in the MSP cohort. Altogether, OGTT was performed in 75% of the studied population. The exact time of fastening was not known but was at least 4 hours before the OGTT, and most participants had had an overnight fast.
**Smoking**
Smoking is a difficult risk factor to evaluate. Usually individuals underreport their smoking habits, but in the 1994 MONICA cohort self-reported smoking habits correlated well to biochemical markers of tobacco exposure (141).

**Cholesterol**
The method to estimate cholesterol levels has changed. Initially standard bench-top analysers were used, but after September 1, 2009 all cholesterol analyses were performed at the central laboratory. The earlier results from the bench-top analyser were adjusted to the values of the central laboratory. In the MSP cohort blood samples were not collected.

Since the start of the surveys the use of statin has increased 10-fold and about 30% of the population older than 64 years of age uses daily statins (132). As an effect the levels of cholesterol have decreased by about 1.0 mmoL/L. The change of the average cholesterol levels over time might affect the results but, cases and controls were selected from the same time period.

**Apolipoprotein B/Apolipoprotein A1 ratio**
The analysed lipoproteins are considered not to be effected by fasting (140), and if they were, this would have had affected controls and cases equally. Unfortunately, there was no information about statin use and no information about menopausal status in women except the age. Thus, one of the matching criteria was age, and women would have been matched with a woman probably in equal menopausal status. The use of statin was considered low due to that most were surveyed before the year 2000 and 98% did not report prior myocardial infarction. All analyses of lipoprotein were performed during 2017 thus eliminating bias in analysis due to methodological reasons.

**Lipoprotein (a)**
The analysis of Lp(a) was performed according to a method traceable to the World Health Organization/International Federation of Clinical Chemistry reference material and was performed on one occasion and at the same laboratory. The used method measures the molar concentration of Lp(a) and is not affected by the size of the isoforms (142). An instability of Lp(a) in stored samples for cases with cardiovascular disease has been identified (143). This instability was shown to significantly reduce the levels of Lp(a), but whether this applies to cases with valvular heart disease is not known. PCSK9-inhibitors are known to lower the levels of Lp(a) (144), but the first PCKS9-inhibitor was approved by the Swedish Medical Product Agency in 2015 and the last of the cases were operated in December 2014.
Renal function
The estimated renal filtration rates were calculated for each case and control by two different methods – CAPA using cystatin C, and Lund-Malmö Revised using creatinine (145, 146). Cystatin C levels can be altered by thyroid disease or steroid administration and creatinine levels are affected by total muscle mass and protein catabolism but the information about muscle mass, thyroid disease and use of steroids was not available.

Data from pre- and peroperative assessment
Data collected from the preoperative assessment were retrospective and differed in quality. Especially blood pressure, current medication and smoking information were not collected systematically or not mentioned in the hospital files. The information about indication for intervention and surgical procedures was collected from surgical reports. The information from the surgical reports matched well with the parameters gathered at the preoperative echocardiogram.

Coronary angiograms
Coronary angiograms were performed in 94% of the cases. Mostly of those who did not undergo coronary angiograms had had emergency surgery performed, and time for coronary angiograms was not available. The coronary angiograms only show visible arteriosclerosis and not intra luminal arteriosclerosis. With the division in non-CAD and CAD, there was a clear difference in predictive ability between the two groups.

Echocardiography
Most of the echocardiograms were performed at the department of clinical physiology at the University hospital of Umeå but with different examiners and not always in a standardised procedure. Even though the echocardiographic results had some bias in assessment, the results from the echocardiographic examinations were a crucial part in the final decision for doing surgery and therefore was considered to be of high quality.

Some clinical and echocardiographic measures at surgery are presented in Table 3, stratified for the presence of CAD (relates to the cases included in Paper III and IV). Patients with concomitant CAD were more often men, and were older both at survey and at surgery. They had more often history of a previous myocardial infarction and coronary bypass surgery. They had higher systolic blood pressure, and their aortic valves were less stenotic with lower gradients.
Table 3. Preoperative characteristics

<table>
<thead>
<tr>
<th></th>
<th>Available no</th>
<th>No CAD</th>
<th>CAD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N (%)</strong></td>
<td></td>
<td>132 (39.4)</td>
<td>203 (60.6)</td>
<td></td>
</tr>
<tr>
<td><strong>History</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female sex (%)</td>
<td>132/203</td>
<td>59.1 (50.6–67.6)</td>
<td>40.9 (34.1–47.7)</td>
<td>0.001</td>
</tr>
<tr>
<td>Age at survey (years)</td>
<td>132/203</td>
<td>54.2 (52.5–55.8)</td>
<td>58.4 (57.3–59.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age at surgery (years)</td>
<td>132/203</td>
<td>63.8 (62.0–65.5)</td>
<td>69.6 (68.6–70.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Known heart failure (%)</td>
<td>132/203</td>
<td>5.3</td>
<td>5.9</td>
<td>0.62</td>
</tr>
<tr>
<td>Known renal failure (%)</td>
<td>132/203</td>
<td>4.5</td>
<td>7.4</td>
<td>0.55</td>
</tr>
<tr>
<td>Previous MI (%)</td>
<td>131/203</td>
<td>1.5</td>
<td>12.8</td>
<td>0.001</td>
</tr>
<tr>
<td>Previous CABG (%)</td>
<td>132/203</td>
<td>0.0</td>
<td>6.4</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Clinical parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>128/195</td>
<td>139 (136–143)</td>
<td>144 (141–147)</td>
<td>0.05</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>127/195</td>
<td>81 (79–83)</td>
<td>82 (80–83)</td>
<td>0.61</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>126/200</td>
<td>71 (69–74)</td>
<td>72 (70–74)</td>
<td>0.83</td>
</tr>
<tr>
<td><strong>Preoperative echocardiography</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Root of aorta (mm)</td>
<td>128/191</td>
<td>34.1 (33.2–35.0)</td>
<td>34.0 (33.3–34.7)</td>
<td>0.83</td>
</tr>
<tr>
<td>Left atrium (mm)</td>
<td>126/189</td>
<td>40.7 (39.4–42.0)</td>
<td>42.1 (41.1–43.2)</td>
<td>0.10</td>
</tr>
<tr>
<td>LVEDD (mm)</td>
<td>130/195</td>
<td>50.2 (48.9–51.5)</td>
<td>49.5 (48.7–50.4)</td>
<td>0.38</td>
</tr>
<tr>
<td>LVESD (mm)</td>
<td>102/147</td>
<td>31.7 (30.2–33.2)</td>
<td>31.5 (30.2–32.8)</td>
<td>0.88</td>
</tr>
<tr>
<td>Intraventricular septum (mm)</td>
<td>117/170</td>
<td>13.0 (12.6–13.5)</td>
<td>13.4 (12.7–13.8)</td>
<td>0.39</td>
</tr>
<tr>
<td>Posterior wall (mm)</td>
<td>107/153</td>
<td>11.4 (11.0–11.8)</td>
<td>11.0 (10.7–11.3)</td>
<td>0.12</td>
</tr>
<tr>
<td>LV mass (g)</td>
<td>106/153</td>
<td>247 (230–264)</td>
<td>232 (221–243)</td>
<td>0.13</td>
</tr>
<tr>
<td>EF according to Teichholz (%)</td>
<td>102/147</td>
<td>66 (64–69)</td>
<td>65 (63–68)</td>
<td>0.62</td>
</tr>
<tr>
<td>LV function (reduced vs normal)</td>
<td>131/201</td>
<td>11.5</td>
<td>18.9</td>
<td>0.07</td>
</tr>
<tr>
<td>Aorta gradient max (mmHg)</td>
<td>132/196</td>
<td>78.9 (74.5–83.4)</td>
<td>68.0 (64.3–71.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Aorta gradient mean (mmHg)</td>
<td>128/194</td>
<td>49.1 (46.0–52.1)</td>
<td>41.8 (39.3–44.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VTI LVOT (cm)</td>
<td>110/162</td>
<td>22.2 (21.3–23.2)</td>
<td>21.7 (20.8–22.7)</td>
<td>0.50</td>
</tr>
<tr>
<td>VTI Aorta (cm)</td>
<td>107/159</td>
<td>105.4 (101.1–109.7)</td>
<td>94.0 (90.4–97.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Aorta valve area (cm²)</td>
<td>127/200</td>
<td>0.78 (0.74–0.81)</td>
<td>0.85 (0.81–0.89)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Data shown are findings from the preoperative assessment, stratified the presence of CAD according to the preoperative coronary angiogram. Available numbers and means with 95% confidence intervals are presented. All cases except one had a preoperative angiogram. CAD indicates coronary artery disease, MI myocardial infarction, CABG coronary by-pass grafting, BP blood pressure, LVEDD left ventricular end–diastolic diameter, LVESD left ventricular end–systolic diameter, LV left ventricle, VTI velocity time integral, LVOT left ventricle outflow tractus, and EF ejection fraction.
General discussion of main findings

In the first of four prospective studies, using a nested-case design, the impact of traditional cardiovascular risk factors on development disease of the ascending aorta and valvular heart disease, especially aortic stenosis, was studied. Hypertension, hypercholesterolemia, smoking and diabetes were all found to be associated with development of aortic stenosis and disease of the ascending aorta, but after exclusion of those with CAD none of these associations remained. In the second study, the effect of the traditional cardiovascular risk factors on development of aortic stenosis depending on age at surgery was studied. In the younger cases (≤60 years of age) without CAD, hypertension was the only risk factor. For the older cases (≥60 years of age) without CAD, only elevated levels of impaired fasting glucose remained. Lp(a) and Apo B/A1 ratio were evaluated in the third study in developing aortic stenosis. The same patterns as seen in study one were found with a clear association between elevated levels of Lp(a) and Apo B/A1 ratio in developing aortic stenosis in those with concomitant CAD but, the association vanished when CAD were excluded. In the last study, the association between renal function and aortic stenosis was studied. Early impairment of renal function, described as a low ratio between eGFR\text{Creatinine} and eGFR\text{Cystatine C}, was found to be associated with developing aortic stenosis. These finding were not seen in those without concomitant CAD, and not seen in males, regardless of concomitant CAD or not, after stratification for sex.

The difference in risk factor profile in aortic stenosis between those with concomitant CAD and those without concomitant CAD were one of the major findings in these four studies. The traditional cardiovascular risk factors, such as hypertension, hypercholesterolemia, diabetes, smoking and obesity have all been attributed to the development of aortic stenosis (69). Several studies, both prospectively and retrospectively, have evaluated the risk factors in developing aortic stenosis but these studies did not stratify for concomitant CAD (69, 86, 87, 96).

Aortic stenosis with concomitant CAD, from the first two papers, shows an association with the traditional cardiovascular risk factors and confirms findings in other studies (69, 96). Histological examinations of explanted aortic valves have also shown similarities between aortic stenosis and arteriosclerosis (8, 147). Even though there are similarities between the two different diagnoses that strengthen the thoughts about a mutual background, there are some incongruities. In random clinical trials the traditional risk factors have been treated with a clear reduction in morbidity and mortality in cardiovascular disease, i.e. myocardial infarction and stroke (148, 149) but this has not been seen in aortic stenosis. In three different clinical trials, cholesterol as a treatable risk factor for aortic stenosis has not shown a reduction of the progression of the
stenosis (74-76). ACE-inhibitors has retrospectively been studied as a possible treatment for slowing the progression in aortic stenosis but the outcome was not convincing (150, 151).

Those with aortic stenosis without concomitant CAD were younger than those with concomitant CAD, 64.1 years (62.3-65.8) vs. 69.9 years (68.8-71.0), p<0.001. In younger patients, the occurrence of bicuspid aortic valves is more common, and Roberts et al. have shown that as many as 80% are bicuspid, if the valvular replacement needs to be performed before the age of 60 years (40). The bicuspid valve is a known risk factor for developing aortic stenosis in earlier years and is also associated with dilation of the ascending aorta and coarctation of aorta (152). Patients with coarctation of aorta have an increased risk for arterial hypertension, even after the repair of the coarctation, and are associated with several other vascular anomalies (153, 154). In paper II, the patients without CAD and were younger than 60 years of age were associated with an increased risk of hypertension. The increase of hypertension can be a part of a vascular syndrome with the bicuspid valve as the common denominator. Hypertension can induce a mechanical stress on the aortic valve damaging the endothelial layer and stimulating the interstitial cells, shifting them to a greater proportion of active myofibroblast and osteoblast-like cells (17). The patients older than 60 years of age and without concomitant CAD had an association with IFG, indicating a disturbance in glucose metabolism. IFG has been proposed as a pre-diabetic marker and has been associated incidental cardiovascular disease (97, 100, 155). The association with IFG in aortic stenosis in those older than 60 years and without concomitant CAD indicates disturbed glucose metabolism may be a cofounding factor, but this has to be further explored.

In recent years, Lp(a) has advanced as a risk factor for developing aortic stenosis both in traditional prospective studies and in genetic studies using Mandelien randomisation (85, 86). In paper III, Lp(a) is associated with the development of aortic stenosis, but only in those with concomitant CAD. In the absence of CAD there are no association between Lp(a) levels and aortic stenosis. The LPA SNP rs10455872 is associated with elevated levels of Lp(a) and the development of aortic stenosis; but whether LPA SNP rs10455872 is associated with the development of aortic stenosis without concomitant CAD is not known (85). In November 2017 the result from a GWAS for aortic stenosis was presented at the American Heart Association Scientific Session, Anaheim, California, (123). The GWAS showed an association for LPA and FADS which implicates fatty acid metabolism as a part in the development of aortic stenosis. The aortic stenosis cohort from the four papers in this thesis are one of the cohorts in the collaboration, and future studies awaits with main focus on analysing the cohort for correlation for aortic stenosis after stratification for CAD.
In the fourth paper, impaired renal function is associated with the development of aortic stenosis, but only isochronal with CAD and only in women after stratification for sex. The method used to identify impairment in renal function with a ratio between eGFR_{Cystatin C} and eGFR_{Creatinine} is novel and was shown to associate with an increase in both mortality and morbidity in healthy seniors and after elective coronary by-pass (156-158). The reduction in the ratio between eGFR_{Cystatin C} and eGFR_{Creatinine} can be a sign of endothelia dysfunction and vascular damage caused by the other traditional risk factors, and implies the development of aortic stenosis as a part of a general vascular disorder. Why there is a difference between men and women is not clear, but the renal blood flow is particularly complex and with numerous alterations which may explain the difference (159). In an ad-hoc analysis, after adjustment for sex, age at survey and for case–status, a high ratio was associated with longer survival in the entire cohort (HR 0.84 [0.75–0.95]). This indicates the eGFR_{Cystatin C}/eGFR_{Creatinine} ratio can be used as a risk marker for mortality.

Plasma from this study has recently been analysed with the Proximity Extension Assay (PEA) technique, a high-specificity assay that simultaneously measures concentrations of 92 cardiovascular candidate proteins (158). In this analysis, including a validation cohort from the NSHDS, five proteins were associated with the development of aortic stenosis, mainly in patients with CAD. These findings are novel and will be presented in the near future.

**Limitations**

Only patients who were approved for surgery were included in the studies. Patients with severe comorbidities, such as renal failure and chronic obstructive pulmonary disease, patients with high age and those denied surgery due to high peri- and postoperative mortality were therefore not included. The use of age as an inclusion criterion for participation in VIP and MSP exclude younger individuals. In VIP individuals aged 30 (until 1995), 40, 50 and 60 years of age are asked to participate and in MSP; the age span for mammary screening programs are 40-74. Participation in health surveys tends also to be healthier individuals but males, individuals from lower socioeconomic status and ethnic minorities tends to not participate. The participants in the health survey are recruited from northern Sweden and mainly from Västerbotten; whether the results are generalizable to the rest of Sweden or the aortic stenotic community as whole are not known. Due missing data, the detailed description of the valvular morphology was not known and weakens parts of the studies. A better knowledge of valvular morphology could have allowed analysis been done with stratification on valvular morphology, i.e. bicuspid and tricuspid aortic valve and the association to the different cardiovascular risk factors. The inclusion in the health
studies has been running over several decades and guidelines, habits, trends in society and health panorama could have changed during this time.

**Clinical implications**

The findings in the four papers underline the importance of treating cardiovascular risk factors, not only to reduce the risk of traditional cardiovascular morbidity (i.e. stroke, myocardial infarction and renal failure) but also the possibility to reduce the risk for valvular heart disease. Younger individuals with a bicuspid aortic valve, should also be monitored scrupulously in order to avoid unidentified hypertension and thus the development of aortic stenosis and other adverse effect of hypertension.

When evaluating the patients with aortic stenosis, the presence of CAD should carefully be assessed. It seems as if there are two different phenotypes with possibly different pathological mechanism. The different paths of developing aortic stenosis must be taken into account when treating the risk factors and in future clinical trials.
Conclusions

- Surgery for aortic stenosis was associated with the traditional cardiovascular risk factors – hypertension, hypercholesterolemia, diabetes, BMI and smoking, but only in presence of concomitant CAD (Paper I).
- In patients, younger than 60 years of age and without concomitant CAD, surgery for aortic stenosis was associated with hypertension and elevated diastolic blood pressure, however only IFG was associated with patients older than 60 years of age without concomitant CAD (Paper II).
- Elevated levels of Lp(a) and a high Apo B/A1 were independently associated with surgery for aortic stenosis only in presence of concomitant CAD (Paper III).
- Impaired renal function, as measured by a novel approach and defined by the eGFR_{Cystatin C}/eGFR_{Creatinine} ratio, was associated with surgery due to aortic stenosis with concomitant CAD. After stratification for sex, this association remained only in women. (Paper IV)
- The traditional cardiovascular risk factors were associated with surgery of the ascending aorta, but only with concomitant CAD (Paper I).
- Diabetes was associated with a reduced risk for surgery of the ascending aorta (Paper I).
- Irrespective of CAD, high levels of cholesterol were associated with a reduced risk for surgery for aortic regurgitation (Paper I).
Acknowledgement

Under min resa till avhandling och disputation har jag haft hjälp och support av mängder av människor. Alla har hjälpt och stöttat mig på olika sätt i mitt arbete och den hjälpksamhet, uppmuntran, intresse och support är jag oändligt tacksam för och alla som har varit med på resan ska ett stort tack!

Det är dock några som jag särskilt vill tacka för att jag har tagit mig ända hit.

Först vill jag tack min handledare Stefan Söderberg, som med sin entusiasm och hängivenhet för forskningen och aldrig sinande uppmuntran har lyckats få mig ända fram och blivit en god och nära vän.

Jag vill också tacka min biträdande handledare Bengt Johansson som med uppmuntran, kunskap och värme varit ovärderlig för mig under denna resa och har också blivit en god och nära vän.

Ett särskilt tack vill jag ge Anders Waldenström som tog sig an mig i början av min forskarkarriär med vänlighet, erfarenhet och aldrig sinande nyfikenhet.

Mina medarbetare i de fyra artiklarna; Anders Holmgren, Ulf Näslund, Margareta Norberg, Gunnar Engström, Ingvar Bergdahl, Johan Hultdin, Paul Holmer och Elin Albertsson, har alla varit värdefulla i mitt arbete, med delaktighet och synpunkter på artiklarna men också med uppmuntran och stöd. Tack!

Hantering och läsning av journaler, registrering av data, hantering av diverse register, resor, Alf-medel och mycket annat praktiskt har varit en väsentlig del av avhandlingen och mitt innerliga tack till Janne Henschel, Elin Albertsson, Paul Holmer, Camilla Ring, Maja Söderberg, Mattias Söderberg, Anja Isaksson, Eva Jonasson och Stina Jakobsson för ert fantastiska arbete!

Ett speciellt tack till alla de som har deltagit i VIP, MONICA och MSP och ett tack till styrgrupperna som ser till att dessa lysande kohorten fortlever.

Medicinska biobanken och Enheten för Biobankforskning har varit ovärderlig, bland annat för att den finns men också för all hjälp med prover och data. Stort tack, Åsa Ågren, Veronica Hellström, Göran Hallmans, Robert Johansson, Kerstin Enqvist, Christina Evaldsson!

Alla på som på institutionen har hjälpt mig med papper, anmälningar, signaturer, dataprogram etc. är värda ett stort tack; Kerstin Rosenqvist, Elin Jacobsson, Eva
Karlsson, Helena Karlsson, Catrin Johansson, Per Ivarsson, Ulf Näslund och Bo Carlberg!

Utan det arbete med analyser och hantering av prover på Klinisk kemi, Laboratoriemedicin hade inte detta blivit av. Stort tack Johan Hultdin och Eva Samuelsson!

Alla medarbetare på hjärtcentrum som har varit stöd och hjälp under arbetet ska ha ett stort tack!

Mina tidigare och nuvarande chefer, Ulf Näslund, Krister Lindmark, Magnus Hedström och Per Ottander ska också tackas för stöd och uppmuntran!

Särskilt tack till alla vänner och medarbetare på kardiologen!

Mina medarbetare och vänner på klinisk fysiologi är värda ett stort och varmt tack för att ni finns!

All släkt och alla vänner som har uppmuntrat och engagerat sig i avhandlingsarbetet ska också ha ett stort tack!

Vännerna Mats, Sussie, Janne och Stina med familjer ska särskilt tackas för härliga middagar, trevliga träffar och god skidåkning men också för uppmuntran och pep!

Jag är oändligt tacksam för att jag har mina bröder, Erik och Magnus med familjer som stödjer och uppmuntrar och bara att de finns!

Ett varmaste tack till mina föräldrar, Ulla och Ulf som alltid trodde på mig och älskade mig! Jag saknar er oändligt!

Ett största tack till mina barn, Frans och Astrid! Ni är de bästa barn som finns!

Ett sista tack till min evigt älskade och bästa vän, Christina! Jag är evinnerligt tacksam att du står ut med mig och delar livet med mig. Älskar dig!
This work was supported by grants from
Swedish Heart–Lung Foundation (Grant numbers 20140799, 20120631, and 20100635 to Stefan Söderberg)

County Council of Västerbotten ("Centrala ALF": 548791. "Basenhets ALF": 242371, 322051, 401781, 494381, 581261 and 679511)

The Heart Foundation of Northern Sweden
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