Metabolic risk markers and relative survival in patients with aortic stenosis requiring surgery.

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2019
Cover art: Drawings by Marianne Gidén; front page and fig 1, 4, 5

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ISBN: 978-91-7855-051-7
ISSN: 0346-6612

Electronic version available at http://umu.diva-portal.org/
Printed by: Umu Tryckservice/Umu Print Service, Umeå University, Sweden, 2019
"The Road Not Taken"

Two roads diverged in a yellow wood,
And sorry I could not travel both
And be one traveler, long I stood
And looked down one as far as I could
To where it bent in the undergrowth;

Then took the other, as just as fair,
And having perhaps the better claim,
Because it was grassy and wanted wear;
Though as for that the passing there
Had worn them really about the same,

And both that morning equally lay
In leaves no step had trodden black.
Oh, I kept the first for another day!
Yet knowing how way leads on to way,
I doubted if I should ever come back.

I shall be telling this with a sigh
Somewhere ages and ages hence:
Two roads diverged in a wood, and I—
I took the one less traveled by,
And that has made all the difference.

Robert Frost (1874-1963)

To Hanna, Mattias, Erik
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Abstract

**Background:** Aortic stenosis (AS) is the most common valve disorder requiring surgery in developed countries. The etiology of AS is only partly known. Identification of new biomarkers in prospective studies could lead to novel insights in the etiology of AS, and possibly lead to improved clinical management. Long term observed survival after aortic valve surgery has improved over the last decades despite an ageing population presenting with more comorbidities. Whether this is reflected in improved relative survival is not known. We evaluated if biomarkers associated prospectively with AS requiring surgery, and if these associations differed between genders, time to surgery and the presence of coronary artery disease (CAD). We also assessed long term observed and relative survival after aortic valve surgery with and without concomitant coronary artery by-pass surgery (CABG).

**Methods and results: Study I:** We prospectively studied the impact of lipoprotein (a) (Lp[a]) and apolipoproteins (Apo) in subgroups of AS. During a 20-year period, 336 patients with prior participation in large population-based surveys in northern Sweden were operated due to AS plus CABG when indicated. For each case two referents were matched. Data from the baseline survey were collected and included data on cardiovascular risk factors, health history, measurements of anthropometry, blood pressure, blood glucose and blood lipid levels were retrieved. Data from pre- and perioperative assessments were also collected. The presence of CAD was determined from the coronary angiogram. Elevated levels of Lp(a) and an elevated Apo B/Apo A 1 ratio were independently associated with future surgery for AS, but only in patients with concomitant CAD (OR 1.29, 95 % CI 1.07-1.55 and 1.43, 95 % CI 1.16-1.76 respectively).

**Study II:** The same patient cohort as in study I was used. A panel of 92 cardiovascular candidate proteins were analysed with the multiplex proximity extension assay in samples obtained at baseline. Six circulating proteins (growth differentiation factor 15 [GDF-15], galectin-4, von Willebrand factor [vWF], interleukin 17 receptor A, transferrin receptor protein 1, and proprotein convertase subtilisin/kexin type 9, [PCSK9]) were associated with future surgery for AS in patients with concurrent CAD (ORs ranged from 1.25 to 1.37 per SD increase in the protein signal). In the validation study with 106 additional cases, the association of all but one, (interleukin 17 receptor A), of these proteins were replicated in patients with AS and concurrent CAD but not in those without concurrent CAD. **Study III:** In the same patient cohort as in study I and II we evaluated if troponin T (TnT) and C-reactive protein (CRP) associated prospectively with future surgery for AS. TnT was independently associated with surgery for AS in patients both with (OR 1.22, 95 % CI 1.02-1.46) and without concomitant CAD (1.39, 95% CI 1.05-1.84). CRP was not associated with surgery for AS (OR 1.06, 95 % CI 0.92-1.23). **Study IV:** 4970 patients between 2005 and 2016 from three Swedish heart surgery centres, undergoing aortic valve replacement (AVR) due to either AS or aortic regurgitation in conjunction with CABG when indicated, were followed up. All-cause mortality, as well as both observed and relative survival, was analysed with focus on age, sex, type of valve prosthesis and the impact of
concomitant CABG. Median follow-up was 4.7 years (2.3-7.6). 30-day mortality was 2.3 %. Long-term survival with 30-day mortality excluded was 96.6 %, 82.7 %, 57.6 % after 1, 5 and 10 years respectively. Relative survival rates (adjusting for the background mortality in the general Swedish population based on age, sex and year) were 99.6 %, 99.5 % and 90.6 % after 1, 5 and 10 years respectively. Age had a negative influence on observed survival (p<0.001) but was associated with better relative survival (relative mortality rate [RMR] 0.74, 95 % CI 0.71 - 0.77). Women had a lower observed mortality than men (p<0.001) but a lower relative survival (RMR 1.17, 95 % CI 1.02-1.35). Combined surgery (AVR+CABG) was not significantly associated with higher mortality (p=0.43) in a multivariable adjusted analysis. The presence of bicuspid morphology was associated with lower observed mortality compared with tricuspid valve, and a relative survival matching that in the general population.

Conclusion: I. Plasma levels of Lp(a) and the Apo B/Apo A 1 ratio were independently associated with future surgery for AS but only in patients with concomitant CAD. This finding suggests that patients with AS have different phenotypes and may open a new avenue of research on targeted risk factor interventions in this population. II. Five circulating proteins – GDF-15, galectin-4, vWF, transferrin receptor protein 1, and PCSK9 – were associated with the need for aortic valve surgery several years later. The role of these proteins should be investigated in future studies. III. Elevated plasma levels of TnT were independently associated with future surgery for AS, irrespective of the presence of concomitant CAD, which could indicate that the myocardium is subject to mechanical stress already in the subclinical stage of AS. This may be used as a clinical tool for identification of patients with subclinical AS who could benefit from early intervention. Elevated CRP levels did not associate with future AVR. IV. Relative survival following AVR was particularly good in the elderly matching that in the general population underlining the benefits of aortic valve surgery in properly selected patients. Women had decreased relative survival compared to men. This should be explored in future studies. Adding CABG to an AVR procedure was not associated with increased risk. Bicuspid valve morphology was associated with lower observed mortality compared with tricuspid valve morphology, and with a relative survival matching that of the general population.

Keywords: Aortic stenosis, aortic valve surgery, coronary artery disease, prospective cohort study, risk markers, lipoprotein (a), apolipoproteins, proteomics, troponin T, C-reactive protein, relative survival, observed survival
Svensk sammanfattning

Metabola riskmarkörer och relativ överlevnad för patienter med aortastenos som genomgått klaffkirurgi.

torer, man ser också mikroskopiska likheter mellan åderförkalkning och den kalk man ser i aortaklaffarna. Trots dessa likheter har de läkemedel som visat sig effektiva vid åderförkalkning, f f a kolesterolläsare, inte visat någon effekt på aortastenosförkalkning trots att man bevisligen erhåll en sänkning av cholesterolnivåerna i blodet. Förutom riskfaktorer kan man även tala om riskmarkörer, dvs något objektivt måtbart (t ex ett ämne i blodet) vars nivåer kan signalera att det föreligger risk för någon speciell sjukdom – eller t o m vara en del av orsaken till sjukdomen – t ex aortastenos. Risken i samband med en operation för aortastenos är inte stor trots att patienterna ofta är gamla och ofta har betydande samsjuklighet. För att avgöra om en behandling, som t ex en hjärtoperation, har god effekt på längre sikt och t o m botar patienten, kan man jämföra långtidsöverlevnaden hos de som genomgått en hjärtoperation (s k observerad överlevnad) med överlevnaden i den generella befolkningen som inte genomgått någon hjärtoperation. Detta kallas för relativ överlevnad och metoden används ofta inom cancervården men har i betydligt mindre omfattning använts inom hjärtkirurgivården. De få studier som finns är, med något undantag, av äldre datum och kan knappast sägas representera dagens situation.

Det här arbetet har haft två huvudmål; dels att undersöka huruvida vissa kända riskmarkörer och vissa presumtiva riskmarkörer för utveckling av aortastenos uppvisar olikartad koppling till aortastenos beroende på om det samtidigt förekommer kramssjuka hos patienten eller ej; dels att utröna huruvida de goda korttidsresultat som föreligger efter aortaklaffbyte avspeglar sig i goda långtidsresultat mått som relativ överlevnad, dvs lever aortaklaffopererade lika länge som de som inte genomgått en aortaklaff-operation.

I de första tre arbetena använde vi oss av 336 patienter som opererats för aortastenos och eventuell samtidig kramssjuka, vid thoraxkliniken i Umeå, Norrlands Universitetssjukhus under åren 1988 - 2014, och som innan operationen även hade medverkat vid någon av de tre stora befolkningsstudierna i Västerbotten och Norrbotten (Västerbotten Intervention Programme – VIP; MONItoring of trends and determinants in CArdiovascular disease – MONICA; Mammary Screening Project – MSP). Vid dessa undersökningar mätas blodtryck, längd och vikt, blodsocker och kolesterol och man får lämna uppgifter om eventuell medicinering och levnadsvanor. Dessutom lämnar man blodprov som sparas för framtida forskning. Som jämförelsegrupp valdes personer som genomgått samma befolkningsstudie men inte opererats för aortastenos, totalt 672 personer dvs två s k kontrollpersoner per opererad patient. Dessa kontrollpersoner ”matchades” mot de klafrerade med avseende på ålder, kön, typ av hälsoundersökning och tidpunkt för hälsoundersökning för att få jämförbara grupper. I genomsnitt hade det gått ca 11 år mellan hälsoundersökning och operation. När vi senare analyserade blodprover (riskmarkörer) fann vi att ett antal av de riskmarkörer som i andra tidigare studier visat koppling till utveckling av aortastenos, inte gjorde det hos våra patienter med mindre än att det samtidigt förelägg kramssjuka. Detta gällde lipoprotein (a) som t o m ansågs orsaka aortastenos, samt ett antal proteiner. Troponin T (ett ämne som utsöndras till blodet vid hjärtmuskelskada) var kopplat till utvecklingen av
aortastenos både hos de med och utan samtidig kranskärlssjuka. C-reaktivt protein ("snabbsänkan") hade ingen koppling till aortastenos oavsett om det förelåg samtidig kranskärlssjuka eller ej.


Våra slutsatser blev följande: Vissa av de riskfaktorer och riskmarkörer vi undersökt (lipoprotein (a), vissa proteiner) verkar vara förknippade med utvecklingen av aortastenos endast om samtidig kranskärlssjukdom föreligger. Troponin T var sammankopplat med utveckling av aortastenos oavsett om det förelåg samtidig kranskärlssjukdom eller inte. C-reaktivt protein verkar inte vara kopplat till utvecklingen av aortastenos. Detta kan tolkas som att det finns flera undergrupper av aortastenos där aortastenos i tredelad respektive tvådelad klaff utgör två av dessa, och aortastenos med samtidig kranskärlssjukdom utgör en tredje. Våra resultat kan tala för att uppkomstmekanismen av aortastenos skiljer sig mellan dessa undergrupper. Detta bör tas i beaktande när man planerar framtida studier som t.ex. riktas in sig på någon identifierbar och angripbar process i förkalkningen. Vidare kan man i förlängningen tänka sig att t.ex. troponin T skulle kunna användas som klinisk riskmarkör för att följa utvecklingen av aortastenos.

Resultaten efter operation för aortastenos mätt som relativ överlevnad var mycket goda och understryker nyttan av klaffkirurgi hos väl utvalda patienter. Den relativa överlevnaden hos äldre patienter t.o.m. överträffade den hos den generella befolkningen vilket sannolikt beror på att dessa patienter är – förutom sin aortastenos – friskare än jämförelsegruppen. Detta kan i sin tur sannolikt förklaras av att man till kirurgi bland f.f.a. de äldre patienterna medvetet eller omedvetet väljer de med så lite sidosjukdom som möjligt för att minimera riskerna. Den sämre relativa överlevnaden hos kvinnor kan inte förklaras i vår studie och är ett fynd som kräver mer forskning. Vidare, att genomgå en större operation med tillägg av kranskärlsoperation till aortaklaffbyte, påverkar inte överlevnaden efter kirurgi. Slutligen fann vi att resultaten efter kirurgi mätt som både observerad och relativ överlevnad var bättre hos de som hade en tvådelad jämfört med de med en tredelad klaff.
Original papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numbers:

I  Johan Ljungberg, Anders Holmgren, Ingvar A Bergdahl, Johan Hultdin, Margareta Norberg, Ulf Näslund, Bengt Johansson, Stefan Söderberg.
   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;6:e007160

II Johan Ljungberg, Mikael Janiec, Ingvar A Bergdahl, Anders Holmgren, Johan Hultdin, Bengt Johansson, Ulf Näslund, Agneta Siegbahn, Tove Fall, Stefan Söderberg.
   Proteomic biomarkers for incident aortic stenosis requiring valvular replacement.
   *Circulation.* 2018;138:590-599.

III Anders Holmgren, Ingvar A Bergdahl, Johan Hultdin, Bengt Johansson, Johan Ljungberg, Ulf Näslund, Stefan Söderberg.
   Troponin T but not C-reactive protein is associated with future surgery for aortic stenosis - a population based nested-case referent study.
   *In manuscript.*

   Results of surgical aortic valve replacement in a Swedish population: relative survival vs observed mortality.
   *In manuscript.*

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### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ACC</td>
<td>American College of Cardiology</td>
</tr>
<tr>
<td>ACE</td>
<td>angiotensin converting enzyme</td>
</tr>
<tr>
<td>AF</td>
<td>atrial fibrillation</td>
</tr>
<tr>
<td>AHA</td>
<td>American Heart Association</td>
</tr>
<tr>
<td>Apo A1</td>
<td>apolipoprotein A1</td>
</tr>
<tr>
<td>Apo B</td>
<td>apolipoprotein B</td>
</tr>
<tr>
<td>AS</td>
<td>aortic stenosis</td>
</tr>
<tr>
<td>ASTRONOMER</td>
<td>aortic stenosis progression observation: measuring effects of rosuvastatin</td>
</tr>
<tr>
<td>AVA</td>
<td>aortic valve area</td>
</tr>
<tr>
<td>AVR</td>
<td>aortic valve replacement</td>
</tr>
<tr>
<td>BAV</td>
<td>bicuspid aortic valve</td>
</tr>
<tr>
<td>BMI</td>
<td>body mass index</td>
</tr>
<tr>
<td>BNP</td>
<td>B-type natriuretic peptide</td>
</tr>
<tr>
<td>CABG</td>
<td>coronary artery by-pass graft</td>
</tr>
<tr>
<td>CAD</td>
<td>coronary artery disease</td>
</tr>
<tr>
<td>CAD-AS</td>
<td>aortic stenosis with concomitant coronary artery disease</td>
</tr>
<tr>
<td>No CAD-AS</td>
<td>aortic stenosis without concomitant artery disease</td>
</tr>
<tr>
<td>CHF</td>
<td>congestive heart failure</td>
</tr>
<tr>
<td>CKD</td>
<td>chronic kidney disease</td>
</tr>
<tr>
<td>CRP</td>
<td>c-reactive protein</td>
</tr>
<tr>
<td>CV</td>
<td>cardiovascular</td>
</tr>
<tr>
<td>CVD</td>
<td>cardiovascular disease</td>
</tr>
<tr>
<td>DM</td>
<td>diabetes mellitus</td>
</tr>
<tr>
<td>DSE</td>
<td>(low) dose dobutamine stress echocardiography</td>
</tr>
<tr>
<td>EAS</td>
<td>European Atherosclerosis Society</td>
</tr>
<tr>
<td>EBF</td>
<td>Enheten för Biobanksforskning; (Department of Biobank Research)</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>GDF-15</td>
<td>growth differentiation factor 15</td>
</tr>
<tr>
<td>HDL</td>
<td>high-density lipoprotein</td>
</tr>
<tr>
<td>HMD</td>
<td>Human Mortality Database</td>
</tr>
<tr>
<td>LDL</td>
<td>low-density lipoprotein</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>lipoprotein (a)</td>
</tr>
<tr>
<td>LV</td>
<td>left ventricular</td>
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X
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
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<tbody>
<tr>
<td>MI</td>
<td>myocardial infarction</td>
</tr>
<tr>
<td>MONICA</td>
<td>MONItoring of trends and determinants in CArdiovascular disease</td>
</tr>
<tr>
<td>NCR</td>
<td>nested case-referent study</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>NYHA</td>
<td>New York Heart Association</td>
</tr>
<tr>
<td>OGTT</td>
<td>oral glucose tolerance test</td>
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<tr>
<td>OR</td>
<td>odds ratio</td>
</tr>
<tr>
<td>OxPL</td>
<td>oxidized phospholipids</td>
</tr>
<tr>
<td>PARTNER</td>
<td>Placement of AoRtic TraNscathetER valves trial</td>
</tr>
<tr>
<td>PCSK9</td>
<td>proprotein convertase subtilisin/kexin type 9</td>
</tr>
<tr>
<td>RCT</td>
<td>randomized controlled trial</td>
</tr>
<tr>
<td>SALTIRE</td>
<td>Scottish aortic stenosis and lipid lowering trial, impact on regression</td>
</tr>
<tr>
<td>Saltire</td>
<td>St Andrews cross, name of the Scottish flag</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SEAS</td>
<td>Simvastatin and Ezetimibe in Aortic Stenosis</td>
</tr>
<tr>
<td>SNP</td>
<td>single nucleotide polymorphism</td>
</tr>
<tr>
<td>ST2</td>
<td>soluble interleukin-1 receptor-like 1</td>
</tr>
<tr>
<td>SVD</td>
<td>structural valve deterioration</td>
</tr>
<tr>
<td>TAVI</td>
<td>transcatheter aortic valve implantation</td>
</tr>
<tr>
<td>TG</td>
<td>triglycerides</td>
</tr>
<tr>
<td>TnT</td>
<td>troponin T</td>
</tr>
<tr>
<td>VHD</td>
<td>valvular heart disease</td>
</tr>
<tr>
<td>VIP</td>
<td>Västerbotten Intervention Programme</td>
</tr>
<tr>
<td>vWF</td>
<td>von Willebrand factor</td>
</tr>
<tr>
<td>WHO</td>
<td>The World Health Organization</td>
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</tbody>
</table>
Introduction

Besides coronary artery disease (CAD), aortic stenosis (AS) is the most common cardiac disorder and the prevalence increases with age. Consequently, along with expected increased longevity the prevalence will increase, as will the number of patients in need of aortic valve surgery. Heart surgery is resource-consuming, especially among the elderly and in those with comorbidities, i.e., characteristic for many of the patients with AS. Any effort to reduce costs and improve management of AS patients, either by refining and developing surgical techniques or contribute to the understanding of AS etiology in order to provide for the development of pharmacological strategies to prevent, reduce or halt the progression of calcification in AS, should be encouraged. The University of Umeå, and the University hospital of Umeå, harbor a substantial amount of information in databases from large population-based health surveys, heart surgery registries, and biobanks, thus providing for excellent research prerequisites. The etiology in AS is only partly known. The identification of new biomarkers for the development of AS in prospective studies could lead to novel insights in the etiology of AS and eventually find a place in guiding clinical management.

Long-term outcome following heart surgery is preferably presented in actuarial terms. These analyses do not relate the observed survival of surgically treated patients to the expected survival of that in the general population. Relative survival is defined as the ratio of the proportion of observed survivors in a population with a disease, compared to the proportion of expected survivors in a similar population - matched at least for age and gender - without the disease. The relative survival rate is thus a measurement of to what extent the patient is cured by the treatment.

Aortic valve normal anatomy and histology

A normal aortic valve is typically composed of three avascular leaflets, named according to their location with respect to the coronary arteries, i.e., the left coronary, the right coronary, and the non-coronary leaflets. The leaflets are attached to the aorta via a fibrous annulus. Typically, the leaflets are < 1 mm in thickness and are comprised of an outer layer of valve endothelial cells and three internal layers known as the ventricularis, closest to the left ventricle; the fibrosa, which faces the aorta; and the spongiosa in between. The ventricularis layer is rich in radially orientated elastic fibers. This composition provides more compliance, i.e., the ability to expand under pressure, and allows the apposition of free edge regions of leaflets thus preventing diastolic backward flow of blood into the left ventricle. The aortic and the ventricular surface of the aortic valve is covered by endothelial cells, while valve interstitial cells is the predominant cell population inside the aortic valve. Smooth muscle cells reside at the base of the ventricularis.
Aortic stenosis

**Definition, prevalence**

AS is defined as an obstruction of the left ventricular (LV) outflow tract at the level of the ventriculo-aortic junction. The most common form is calcific valvular AS but the obstruction could also be located above or below the valvar plane though these forms are less common and are described in most cases as congenital anomalies. Usually, Mönckeberg is mentioned as the first to describe AS in terms of detailed pathology. However, already in 1663, Lazare Rivière (Lazarus Riverus), praised professor of Medicine at the University of Montpellier, in France, reported the necropsy findings in a patient with progressive dyspnea and palpitations. In the page 178 of this 1659 edition of Observationum Medicarum one can read: “Aperto cadavere [...] in sinistro cordis ventriculo inventae sunt carunculae rotundae, substantiam pulmonis aemulantes, quorum maior ad avellaneae molem accedebat, et arteriae aortae ostium praecludebat, unde pulsationes defectum in arteriis contigisse existimo” (After the corpse was opened [...] rounded fleshy lumps of lung-like mash resemblance were found in the left ventricle of the heart, the biggest of which reached the size of a hazelnut, and bunged the orifice of the artery aorta; what I do consider is the origin of the impaired pulsation of arteries) (1, 2). Whether this finding represented AS or infectious endocarditis is uncertain.

The origin of AS is either congenital or acquired and the latter is further classified into rheumatic or degenerative. Degenerative valve calcification is by far the most common form of aortic stenosis in developed countries while rheumatic forms due to higher rates of rheumatic fever still constitute a considerable proportion in developing countries. Besides CAD, AS is the most common cardiac disorder and the prevalence increases with age, however, studies describing the prevalence of AS are scarce and report diverging results. A prevalence between 2% to 9% depending upon the population studied, has been estimated in some reports (3, 4). AS is also associated with sex - men more affected than women (5). The geographical distribution of AS is heterogeneous which is probably the consequence of genetic factors (6). Socioeconomic factors are also associated with cardiovascular disease (CVD), and low level of education is the strongest socioeconomic predictor of CVD (7).

AS is usually preceded by a preclinical phase, aortic sclerosis, which is defined as small areas of leaflet calcification and thickening without any impact on blood flow across the aortic valve. Aortic sclerosis is frequently seen among aged population, with a prevalence approaching 30 % in individuals above 65 years of age (8). A recent meta-analysis has shown that the rate of progression of aortic sclerosis to AS varies between 1.7%-1.9 % of patients per year (5, 9). Although aortic sclerosis does not obstruct the LV outflow, it is not a benign finding since it is associated with a ≈50 % increased risk of myocardial infarction (MI) and cardiovascular death (8).
Overall, the global burden of AS is expected to increase over the next decades as a consequence of increased life expectancy together with the lack of prevention strategies and treatment aimed at reducing or halting AS disease progression. The number of patients with AS above 70-75 years of age is expected to increase two- to threefold over the next 50 years in developed countries; estimates are based on current prevalence rates and demographic prognostications (10-12).

**Figure 1.** Cross-section of a human heart. *(Illustration: Marianne Gidén)*

**Natural history**

The natural history of AS is characterized by an initial long asymptomatic period. The gradual narrowing of the valve results in increase in LV pressure which in turn initiates hypertrophic remodeling of the myocardium. As long as the left ventricle is able to overcome this increase in pressure overload, the patient will stay asymptomatic but as soon as there is an imbalance between the two, symptoms will develop. There is usually a gradual onset of symptoms that especially occurs during exercise, but with progressive narrowing of the valve, symptoms at rest will eventually occur. Unless individuals have not been identified with AS earlier, most patients seek medical help once they recognize the progressive worsening of symptoms and limitation of activity. Classical symptoms of AS comprise dyspnea, angina, dizziness and syncope. All these symptoms can be explained by insufficient oxygen delivery to the heart and brain as a consequence of
impaired blood supply caused by significant narrowing of the aortic valve orifice with its effect on the stroke volume. Also, the backward pressure buildup in the ventricle results in increased wall stress and subendocardial ischaemia due to increased coronary vascular resistance, hence the development of angina like chest pain with exertion. If symptoms are ignored, the cardiac status carries poor clinical prognosis with an annual mortality of 25% and an average survival of 2 - 3 years (13). Usually most patients in developed countries are diagnosed before onset of symptoms although a small proportion might remain undiagnosed until a late stage in the disease when they present with signs and symptoms of heart failure (14).

**Figure 2:** Schematic illustration of the pathophysiology in the development of aortic stenosis. LV; left ventricular.

*Progression rate.*

The rate of hemodynamic progression in AS is variable but once even mild obstruction is present, severe AS eventually will occur. Overall the average rate of hemodynamic progression in adults with mild to moderate AS is an increase in trans-aortic velocity of 0.3 m/s per year, an increase in mean gradient of 7 mm Hg per year and a decrease in valve area of 0.1 cm² per year (15). However, the rate of hemodynamic progression varies significantly between patients with progression tending to be more rapid in older patients and in those with more severe valve calcification. Other factors associated
with faster stenosis progression include female sex, smoking, hypertension, obesity, metabolic syndrome, renal failure, diabetes mellitus (DM), and elevated circulating levels of lipoprotein(a) (Lp[a]) (15-18). In particular, elevated levels of plasma Lp(a) (>50 mg/dL ≈ 100-125 nmol/L) is associated with a two-fold faster stenosis progression (16).

**Associated conditions**

A condition associated with AS is Heyde’s syndrome, which is characterized by an increased risk of hemorrhage, especially into the gastrointestinal tract, due to angiodysplasia of the colon. Involvement of an abnormal von Willebrand factor (vWF) in the bleeding expression of the angiodysplasia has been suggested (19-21).

**Etiology, pathobiology**

The etiology of AS is only partly known. Some evidence supports age as a factor, another an inflammatory process and a third opinion relates valve calcification to atherosclerosis since the two entities share similar risk factors, i.e., age, dyslipidemia, smoking, hypertension, DM and obesity (3, 22, 23). The pathological process was earlier regarded a simple, passive degenerative process but is now looked upon as an active multifaceted biological process involving lipoprotein deposition, chronic inflammation, osteoblastic transition of valve interstitial cells with remodeling of the extracellular matrix, leading to bone formation (24).

The hallmarks of the pathobiology in the development of AS are inflammation, valve interstitial cell transformation, neoangiogenesis, extra cellular matrix remodeling, and biomineralization. The cause of the initiation of the valve remodeling is not clear but the fibro-calcific process in the aortic valve is suggested as a response to injury, e.g., shear stress or tensile stress, of the endothelial layer, predominantly on the aortic side (25, 26). The lesion of the endothelium and basal membrane allows for infiltration of the aortic valve by lipoproteins, specifically low-density lipoprotein (LDL) and lipoprotein(a) (Lp[a]). This trigger the recruitment of inflammatory cells like macrophages and T-lymphocytes, and induces an osteogenic program and pathologic mineralization (27). Several apolipoproteins such as Apo B, Apo E, Apo A 1 and Apo(a) are present in surgically removed calcified aortic valves (28). Oxidized LDL have also been identified in calcific aortic valves, possibly as a response to oxidative stress (29, 30). The presence of inflammatory cells along with oxidized LDL and apolipoprotein accumulation, activates several cytokines and angiotensin II. This may promote extracellular matrix proteins secretion at early stages of mineralization and start the processes of bone formation by triggering the differentiation of interstitial cells. There is also evidence of angiogenesis, which is essential for bone growth in stenotic valves. T-lymphocytes aggregates tend to co-localize with sites of neoangiogenesis within ossified valves (31). However, the exact role of neoangiogenesis in driving AS is still largely unknown. Calcification of aortic valve leaflets tends to occur more during the later stages of AS. The process of calcification (and sometimes
ossification) of aortic valve leaflets resembles calcification of atheromatous plaque. The presence of inflammatory cells, fatty streak formation from lipid depositions, cytokine release, metalloproteinases, angiotensin converting enzyme, angiotensin II, Lp(a), C-reactive protein (CRP), together with fibrosis could all possibly contribute to the production of an extracellular matrix, promoting mineralization (26). The more detailed role of Lp(a) in the development of AS is discussed later.

**Figure 3:** Scheme of postulated mechanisms underlying aortic valve lesion formation. An early lesion provides for the invasion of inflammatory cells (macrophages, T-lymphocytes) and lipid accumulation in the leaflet. Induction of an osteogenic program and pathologic mineralization through chemical stimuli and disrupted valvular homeostasis promoting extracellular matrix proteins secretion at early stages of mineralization and start the processes of bone formation by triggering the differentiation of interstitial cells.

TNF-α tumor necrosis factor-α; TGF-β1 transforming growth factor-β1; ACE angiotensin converting enzyme; TXNIP thioredoxin interacting protein; BMP-2 bone morphogenetic protein 2; MGP matrix g1a protein
Diagnosis of aortic stenosis

The typical finding on auscultation of AS patients is a harsh, systolic, crescendo-decrescendo murmur radiating to the neck which in most patients should result in a referral for Doppler echocardiographic examination which is the standard diagnostic tool in patients with aortic valve disease. The diagnosis of AS is confirmed upon the visualization of thickened aortic valve cusps with restricted leaflet movement and raised peak transvalvular velocity and peak and mean transvalvular pressure drop. As the disease progresses with narrowing of the valve, the aortic valve area (AVA) declines which causes significant blood flow acceleration (i.e., a rise in peak aortic jet velocity) and pressure drop across the valve (i.e., increase in mean gradient). The hemodynamic severity of AS could accurately and reliably be measured by the use of aforementioned hemodynamic parameters, using Doppler echocardiography. Together with valve structure findings, as well as assessments of dimensions, geometry and function of the left ventricle, the patients are diagnosed with mild, moderate or severe AS; the latter defined as peak aortic jet velocity > 4 m/s, mean gradient > 40 mm Hg and AVA < 1 cm². Any secondary consequence of AS on left atrial size and the right heart should also be evaluated, with special focus on left atrial pressure rise and pulmonary artery pressure since pulmonary hypertension is associated with increased risk in AS surgery patients (32).

The electrocardiogram (ECG) in patients with AS frequently show signs of LV hypertrophy, most often seen as increased amplitude of the QRS. However, these finding is non-specific for AS. A normal ECG does not exclude the presence of AS.

Low-flow low-gradient AS.

The majority of patients with severe AS have high peak aortic jet velocity and a high mean gradient. However, some patients may have a low peak aortic jet velocity/mean gradient despite the presence of severe AS. The most common cause of this “low gradient AS” is the presence of a low flow state. Low-flow, low-gradient AS are divided into two subtypes; classical low-flow (stroke volume index < 35 ml/m²), low gradient (< 40 mm Hg) AS with reduced LVEF (<50%) (33) and paradoxical low-flow, low-gradient AS with preserved LVEF (> 50%) (34). In the presence of a low flow-low gradient AS, the stroke volume is not sufficient to open the valve which is only mildly or moderately stenotic. In these cases, resting Doppler echocardiography may be unable to discriminate between true severe AS and pseudo-severe AS. A low-dose dobutamine stress echocardiography (DSE) should be used for patients with classical low-flow low-gradient AS in order to confirm stenosis severity. Dobutamine is a positive inotropic agent that stimulates the myocardium and enhances its contractile function and heart rate. Consequently, intra-cavitary pressure rise i.e., mimicking the effect of exercise. If the mean gradient increases to > 40 mm Hg or peak aortic jet velocity increases to > 4m/sec and an AVA of < 1.0 cm² is calculated with the DSE, the patient is considered as having true severe AS.
DSE may also be used in patients with paradoxical low-flow low-gradient AS (35) but results from these tests may be difficult to interpret. In patients with classical or paradoxical low-flow low-gradient AS in whom DSE is not feasible or results are inconclusive, a multidetector computed tomography, which is a high-resolution form of computed tomography, may be used. The aortic calcium valve load can be quantified and the stenosis severity corroborated and further analysis may even help in predicting the risk of rapid stenosis progression and also to predict mortality risk (36). However, multidetector computed tomography is not accurate to quantify valve stenosis qualitatively.

**Figure 4.** Different stages in the development of aortic stenosis (AS).
A, normal valve closed; B, normal valve open
C, moderate AS, closed; D, moderate AS, open
E, severe AS, closed; F, severe AS, open
(*Illustration: Marianne Gidén*)
Discordant calcified aortic valve disease grading.

AS with discordant markers of severity, that is a small AVA, (< 1 cm²), but a low mean gradient (< 40 mm Hg), represents a special problem. Other determinants for mean gradient than AVA and flow have been suggested as an explanation, like arterial compliance and severity of valvular calcification, and a low mean gradient could be the result despite the presence of severe AS. This situation is not uncommon. In a study by Clavel et al almost a third of patients presented with discordant grading of AS by AVA and gradient, which raised uncertainty with regard to actual severity of AS (37). Obviously, these patients need additional diagnostic tests to confirm stenosis severity, like quantification of aortic valve calcification by multidetector computed tomography technique.

Cardiac catheterization may be another option in patients with inconclusive or discordant echocardiography results. However, this technique is associated with increased risk of bleeding and cerebral embolism and is recommended only if the results could better classify stenosis severity and have an impact on therapeutic management.

Left ventricular remodeling

AS is not only a disease of the valve but also of the myocardium. AS causes an increase in pressure afterload and ventricular wall stress that stimulates hypertrophy of the LV myocardium. Myocytes enlarge and wall thickness increases in a response that initially restores wall stress and preserves LV function (38, 39). LV hypertrophy is now considered a marker of an adverse prognosis in a number of cardiac conditions (40, 41). In AS patients, the pattern of the LV response to pressure overload is highly heterogeneous which is of prognostic importance (42). Four patterns of LV remodeling are seen; normal, concentric remodeling, concentric hypertrophy and eccentric hypertrophy. Somewhat surprisingly, the degree of LV hypertrophy in AS patients is only weakly related to the severity of valve obstruction (43, 44). Instead, the magnitude of the hypertrophic response is influenced by other factors including age, sex, genetic factors, metabolic factors and the coexistence of CAD and hypertension (45-48). Women tend to predominantly develop concentric remodeling/hypertrophy, whereas men are more prone to developing eccentric hypertrophy (45). LV concentric remodeling or hypertrophy has been linked to worse myocardial function and increased risk of cardiac events and mortality compared to patients with normal LV geometry or with LV eccentric hypertrophy (42, 49, 50). LV hypertrophy leads to increased transmural pressure, resulting in reduction in coronary flow reserve in patients with AS (51, 52). Repetitive myocardial ischemic events related to the reduced coronary blood flow reserve leads to apoptosis of myocytes and development of replacement myocardial fibrosis, a condition not always reversible after AVR.

Management of AS patients

Patients without symptoms should be followed on a regular basis including repeat Doppler echocardiography according to clinical guidelines (53, 54).
The staging of AS is based on the hemodynamic severity, the response of the LV to the pressure overload, patient symptoms and aortic valve morphology (55, 56). Of note, symptoms do not always comply with severity of AS, with some patients with mild or no symptoms could have severe AS which underlines the importance of repeat objective assessment (15). At present, medical therapy has a limited role in the management of AS. Diuretics to minimize symptoms of congestive heart failure (CHF), and antiarrhythmics for heart frequency regulation in patients with atrial fibrillation (AF), may relieve symptoms but will not change the dismal prognosis of symptomatic AS.

Statins have failed in showing any effect on progression rate of the stenotic process in AS patients (57-59), possibly due to late treatment initiation.

Surgical valve replacement is the conventional treatment for severe AS. As soon as symptoms develop, patients should be referred for surgery, without delay since the risk of sudden death within 3 months after onset of symptoms is substantial (60, 61). Asymptomatic patients with severe AS requires special attention (54, 55). These patients are recommended to undergo an exercise test to confirm their asymptomatic status or unmask their symptoms which may be due to an unconscious adaptation. Approximately one-third of those patients have exercise-limiting symptoms, fall in blood pressure below baseline, or have complex ventricular arrhythmias revealed on the stress test. In these patients, surgical intervention should not be delayed (62, 63). The mean gradient during exercise/stress echocardiography is also important in confirming their hemodynamics and symptoms. An increase of $>18-20$ mm Hg with stress is indicative of a higher risk of short-term cardiac events, even in the absence of symptoms (62, 63). Finally, AS individuals with impaired LV function, irrespective of symptoms, should be considered for surgical intervention in order to prevent further deterioration of left ventricle function, myocardial stiffness, raised filling LV pressures with their effect on patient prognosis (54).

**Treatment of patients with AS**

As mentioned above, there is no medical treatment for AS for either preventing or halting disease progression, valve leaflet calcification and orifice narrowing. Hence, aortic valve replacement (AVR) is the only option for treating patients with symptomatic AS. With the use of a mechanical ball valve prosthesis, the first successful surgical AVR was performed in 1960 by Dwight Harken and colleagues (64, 65) and by 1965 the first stented biological valve (bioprosthesis) was implanted by Binet (66). Since then, tremendous advances in operative management, cardiopulmonary by-pass techniques and valve design have been achieved and dramatically improved outcome for AS patients. However, there is still an ongoing search for the optimal valve prosthesis without need for anticoagulation, lifelong durability, no reduction in the aortic orifice area and that can be lenient implanted. In Sweden, a total of approximately 1300 patients per year
undergo AVR due to AS or aortic regurgitation. Out of these 1300, approximately 900 patients undergo isolated AVR and 400 undergo a combination of AVR and other surgery mainly coronary artery by-pass surgery (CABG), with a 30-day mortality of \( \approx 2 \% \) and \( \approx 2.5 \% \) respectively (SwedeHeart 2018). Reports from USA (the Society of Thoracic Surgeons) and Germany (German Aortic Valve Registry) show comparable results. In fact, AVR related mortality has significantly fallen during the past decades in spite of patients being older and probably with more comorbidities (67, 68). In Sweden, the total number of performed surgical AVR have remained stable over the last years, whereas the number of TAVI procedures has increased substantially and for the moment constitutes approximately 50 % of all aortic valve interventions performed.

Today there are five different therapeutic options for the treatment of AS, surgical aortic valve replacement, transcatheter aortic valve implantation, (TAVI), the Ross procedure, the implantation of an aortic allograft (homograft) and balloon aortic valvuloplasty. A conventional surgical AVR with either a mechanical or a biological prosthesis remains the predominant method.

*Balloon aortic valvuloplasty* was developed 30 years ago and offered an alternative to surgical AVR in elderly and frail patients. Initially there was hemodynamic and symptomatic benefits, however only lasting for a limited time and eventually patients had the same poor outcome as those with untreated AS (69). Despite this, a small increase in the utilization of the method have been recognized in recent years mainly as a bridge to definitive treatment with TAVI or surgical AVR. Balloon aortic valvuloplasty is also an option in pediatric cardiac surgery in children with congenital AS.

*The Ross procedure.*

The implantation of a pulmonary autograft into the aortic root was first proposed by Donald Ross in 1962, who then performed it for the first time in 1967 (70). A diseased aortic valve is replaced using the patient’s own pulmonary valve. A pulmonary donor allograft is then used to replace the patient’s own pulmonary valve. This procedure offers several advantages. The most important is that the valve grows as the patient grows. This makes it especially applicable in pediatric and youth aortic valve surgery. Other advantages include minimal risk of thromboembolic events without the need of anticoagulation, excellent hemodynamics and the absence of foreign material in the valve. However, the procedure is technically challenging and the need for late autograft reintervention remains a concern (71). Furthermore, a single valve disease is treated with a two-valve procedure which increases surgical risk.

*Homograft implantation.*

The first homograft replacement of the aortic root was reported in 1962 by Donald Ross. The advantages with this technique are the same as those mentioned above for the Ross procedure. Currently, the use of homografts in surgery for aortic valve disease is decreasing. Instead it has been suggested
as the best substitute for AVR in cases with infective endocarditis, due to their resistance to infection with a very low risk of relapsing endocarditis (72). As with the Ross procedure, there is also a concern for structural deterioration over time (73). Homografts carry the limitation with the need for valve bank that requires specific regulations.

Aortic valve replacement.

The implantation of a mechanical or a biological valve prosthesis is the standard treatment in patients suffering from severe and symptomatic AS and according to guidelines it is considered a Class I indication (53, 54). This procedure is usually accompanied by an improvement in quality of life due to relief of symptoms, and an overall excellent surgical outcome with relatively low perioperative mortality and morbidity even among the elderly (74, 75). In fact, survival following surgical AVR matches and eventually surpasses survival in the general population with increasing age of patients (76, 77). The choice between the type of prosthesis (i.e., a mechanical or a biological prosthesis) is usually based on patient age. In general, bioprostheses are recommended for older patients (with comorbidities) and a limited expected survival, whereas mechanical prostheses are recommended for younger patients with a longer life expectancy. Current European Guidelines state that a bioprosthesis should be considered in patients >65 years of age and a mechanical prosthesis in patients < 60 years of age. In patients aged 60-65 years, both valve types are considered acceptable options (54). According to American guidelines a mechanical prosthesis should be considered in patients below the age of 60, and a bioprosthesis in patients above 70 years of age. Both mechanical and biological prostheses are considered reasonable options in patients between 60 and 70 years of age (53). However, in USA there has been a shift towards a greater use of bioprostheses in patients between 60 and 70 years of age (67). Younger patients increasingly opt for a bioprosthesis to avoid anticoagulation, despite its shorter durability. The option of a later TAVI procedure as an alternative to aortic valve reoperation in the case of a valve prosthesis deterioration may have contributed to this shift (78). In women who wish to become pregnant, the high risk of thromboembolic complications with a mechanical prosthesis during pregnancy and the low risk of elective reoperation are incentives to consider a bioprosthesis, despite the rapid occurrence of structural valve deterioration (SVD) in this age group (54). Furthermore, mechanical valve prostheses demand anticoagulation therapy with the risk of teratogenic effects. In a Swedish population with patients aged 50-69 years who had undergone first time isolated AVR there was a better long-term survival in those who had received mechanical prostheses compared to those who had received bioprostheses. The risk of stroke was similar in the two groups. Patients who had received a bioprosthesis had a higher risk of aortic valve reoperation and a lower risk of major bleeding than those who had received a mechanical valve prosthesis (79).
**Advantages and disadvantages of valve substitutes.**

**Mechanical valve prostheses.**

The most commonly used mechanical prosthesis is of a bileaflet design with two semicircular leaflets made from pyrolytic carbon mounted on a stent which is covered with a polyester knit fabric. The major advantage with a mechanical prosthesis is the long-term durability. The main disadvantage is the need of life-long anticoagulation therapy with consequential risk of bleeding and limitation of lifestyle. Another disadvantage is the clicking sound resulting from the opening and closing of the valve leaflets which can be disturbing for some patients. However, the design of the majority of modern mechanical valve prostheses provide for less prominent clicking sound. Mechanical valve prostheses have slightly better hemodynamic properties compared to bioprostheses, e.g., lower transvalvular gradients.

**Biological valve prostheses.**

Bioprostheses are usually made either from porcine aortic valve tissue or bovine pericardial tissue. The three leaflets are mounted on a stent covered by a polyester fabric. During the last decade sutureless balloon-expanded aortic valve bioprostheses have been introduced in order to shorten aortic cross clamp time during surgery and to facilitate implantation. Bioprostheses have a limited durability and do not require anticoagulation therapy. However, longevity of bioprostheses has improved during the last decades with reports of > 90% freedom from SVD after 15-20 years in elderly patients (80). Of note is the fact that the durability of bioprostheses increases with age of the patient, and vice versa; in younger patients SVD occurs earlier (80, 81). Other risks of SVD than younger age include renal failure, hypertension, metabolic syndrome and disturbed calcium metabolism. The predicted 15-year risk of SVD is approximately 20% for patients 50 years of age and 30% for patients 40 years of age (82).

**Transcatheter aortic valve implantation.**

TAVI is a minimally invasive procedure with an implantation of a biological valve prosthesis inside the native calcific aortic valve with the use of a catheter. There are several approaches. The catheter could be placed either via the femoral artery, via the subclavian artery, transaortical, or directly into the apex of the heart through a small thoracotomy. Even access through the carotid artery and retrograde via the inferior vena cava has been described (83). The valve prosthesis is mounted in a shrinked form on a catheter and is either deployed by balloon inflation or in a self-expandable way. The TAVI technique was first described in animal models in the late 1980s by Henning Ruud Andersen, a cardiologist from Denmark (84). However, his early papers describing the idea were rejected several times. Not until 1992 his research was accepted and published in a paper in European Heart Journal but it was yet another 10 years until the first TAVI procedure in man was performed by Alain Cribier and co-workers in Rouen, France, (85). The patient was a 57-year old male with severe AS together with multiple comorbidities and severe LV dysfunction. He had been declined surgery by several heart surgery teams. The TAVI procedure was
preceded by a balloon aortic valvuloplasty from which he sustained initial hemodynamic improvement but only days later his condition deteriorated with recurrence of cardiogenic shock. Under these circumstances a potentially life saving TAVI procedure was undertaken. After the implantation, there was a dramatic hemodynamic improvement but due to severe noncardiac complications the patient died 17 weeks after the operation.

Initially patients considered as not suitable for surgical AVR due to unacceptable high risk of operative mortality, were scheduled for TAVI. Also, patients with contraindications for surgical AVR due to technical difficulties like porcelain aorta were regarded as potential candidates for TAVI. The superiority of the outcome in TAVI patients compared to optimal medical treatment in this group of patients, was first described in the Placement of AoRtic Transcatheter valves trial (PARTNER) (86) in which patients with high surgical risk were randomized to TAVI or optimal medical treatment. Later the PARTNER 2 trial was undertaken. Patients with severe AS and an intermediate surgical risk were randomized to either TAVI or surgical AVR. The study showed similar rates for mortality and neurological events in the TAVI and surgical AVR groups. The TAVI group was found to have a lower incidence of acute kidney injury, bleeding events, and atrial fibrillation (AF). Conversely, the surgical AVR group experienced fewer vascular complications and lower rates of paravalvular regurgitation (87). Recently, results from two large randomized trials (RCT) in low surgical risk AS patients were published (88, 89). The two trials provide strong evidence that TAVI is noninferior, and even superior, to surgery over 1-year and 2-year time frames. In addition, in the TAVI group there was fewer strokes, less bleeding, and less AF than in the surgery group, as well as a shorter hospital stay and faster recovery. This could imply that it is time for a paradigm shift in how we approach decisions about valve type in patients with aortic stenosis (90).

Although TAVI has evolved as a successful therapy in patients with severe AS who earlier where doomed a fatal outcome, there is a risk of several complications. Of these complications, moderate or severe paravalvular aortic regurgitation has been associated with increased mortality (91). Other complications are major vascular injury and the need of a permanent pacemaker due to ativoventricular block (92). As of today, more than 100,000 patients have been treated worldwide and TAVI is now, according to guidelines, the default option for patients with higher surgical risk (53, 54). Along with increased operator experience and enhanced transcatheter valve systems, outcome is improving and the frequency of complications is declining (93). Long-term valve durability after TAVI remains a concern, though there are some reports on excellent long-term transcatheter aortic valve function (94). Finally, TAVI is becoming more and more common as a valve-in-valve procedure in patients with failed bioprostheses as an alternative to a redo surgical AVR procedure (95).
Risk factors for AS

The pathogenesis of AS is determined by clinical factors, genetics and valve anatomy. The three main risk factors for developing AS are congenital leaflet abnormality, e.g., bicuspid valve, older age and rheumatic fever. The latter is mainly a problem in developing countries. The other clinical risk factors associated with AS are similar to those associated with atherosclerosis; DM, hypertension, renal failure, smoking, metabolic syndrome/obesity, increased LDL levels, elevated Lp(a) (3, 96-98), and genetic factors. Male sex is also associated with increased risk for AS. Specific populations at increased risk for AS include patients with a history of mediastinal radiation, renal failure, familial hypercholesterolemia, or disorders of calcium metabolism.

Bicuspid aortic valve

Bicuspid aortic valve (BAV) is the most common congenital cardiac anomaly with a prevalence of 1-2 % in the general population and with a 2-4:1 predilection for men (99).

The abnormal bicuspid morphology of the aortic valve results in valvular dysfunction and subsequent hemodynamic derangements. The exact cause and mechanism responsible for the development of the BAV is uncertain. During valvulogenesis, two instead of three leaflets occur, with a remnant raphe on the larger, “fusioned” leaflet. (100). Instead of three leaflets, two usually asymmetrical leaflets of different sizes develop. As a consequence of this asymmetry, aortic cusps often do not fully open (101) and coaptation is often eccentric (102). Together, these abnormalities can produce an elliptical orifice area and flow turbulence. This may predispose early valve degeneration and calcification leading to clinically significant AS. However, even in healthy tricuspid valves, the three leaflets are rarely of equal area, with large variations in leaflet size. It is possible that this variation also could lead to aortic valve calcification (103).

Calcification occurs in many patients with a normal trileaflet aortic valve, however the presence of a bileaflet morphology accounts for 60% of AVRs under the age of 70 and 40% of those >70 years of age (104). The lifetime risk of AVR is at least 50 % in individuals with a bicuspid valve (105). In fact, in Europe and the USA, bicuspid aortic valve disease accounts for 50% of all valve replacements for AS (104). The mean age for patients with BAV AS undergoing AVR is around 50 years of age, thus they are approximately 15-20 years younger than individuals with tricuspid AVR (106).

BAV is also associated with ascending aortic aneurysms, aortic dissections and other cardiovascular (CV) malformations like coarctation of the aorta and ventricular septal defects. The risk of endocarditis in BAV has also been found to be much higher than in individuals with tricuspid AS (107). Furthermore, BAV is associated with aortic incompetence but this condition is much less common than BAV with AS (108). As in tricuspid AS, atherosclerotic risk factors of hypertension and dyslipidemia have also been linked to the development of AS in those with a BAV (109).
There is a known high inheritability of BAV and familial clustering has also been described with a BAV prevalence of up to 24% in families with more than one family member carrying the valve malformation (110). The valve malformation has an autosomal dominant inheritance with reduced penetrance and variable expressivity (111). Gene mutations as in the NOTCH1 and GATA binding protein 5 have been associated with aortic valve abnormalities such as BAV and also seem to induce aortic valve calcium deposition suggesting their potential role in cardiac disease in humans (111-114).

Because many of the BAV-related complications can be predicted or prevented, the identification of BAV inheritability supports the recommendation that echocardiographic screening of first-degree relatives of patients with BAV is warranted in order to identify persons with structural cardiac abnormalities (110). Despite the increased risk of AS development and the association with infective endocarditis, ascending aortic aneurysms and aortic dissection, the survival of patients with BAV was found to be similar to that of a matched general population, probably due to current aggressive surveillance and prophylactic surgical interventions (115).

Finally, why a BAV becomes stenotic, another regurgitant, another is associated with aortic dilatation, and yet another is functioning throughout a lifetime, remains unknown and unpredictable.

**Diabetes mellitus**

There are two types of DM, type 1 DM (T1DM) due to pancreas failure to produce enough insulin, and type 2 DM (T2DM) characterized by insulin resistance, most common caused by overweight and insufficient exercise. T2DM is often preceded by a disorder of glucose metabolism, *i.e.*, prediabetes, (116) which consists of two different glycemic states, impaired fasting glucose, and impaired glucose tolerance.

DM has been shown to associate with aortic valve calcification assessed by electron-beam computed tomography, and with faster AS progression (97, 117). In a large population-based prospective study from Larsson et al, the incidence of AS in patients with T2DM during long-term follow-up was 3.42% versus 1.68% in non-diabetic patients (*p < 0.05*) (118). In the Canheart study DM among other risk factors, was also significantly associated with increased risk of developing severe AS (22). The mediating mechanism between DM and AS is not clear but chronic inflammation has been suggested as an important contributor to the pathophysiology. Further data from larger epidemiological and controlled studies are required.

It has been shown that diabetic patients have elevated postoperative morbidity and mortality compared to non-diabetic patients after AVR. Renal failure and deep sternal wound infection occurred more commonly in patients with diabetes, and hospital length of stay was longer (119).
Hypertension

Hypertension is one of the most important risk factors for atherosclerosis and CVD and affects about 20-25% of adults worldwide. It affects men slightly more than women and becomes more common with age. The prevalence varies substantially among different populations and ethnicities. Together with smoking and alcohol abuse, hypertension constitutes the three major risk factors of global burden disease (120). Hypertension is also a risk factor for AS (5, 121), and has been found present in 30-70% of patients with AS (122, 123). However, most of these studies were cross-sectional and before the Canheart AS study, only limited longitudinal data from large, representative, population-wide studies regarding the association between hypertension and AS existed (124, 125). In the Canheart AS study the relationship between conventional CV risk factors and incident severe AS during extended follow-up was examined in a large, unselected, elderly population. In addition, an assessment whether a dose-response relationship existed was made by evaluating the relationship between the number and duration of cardiac risk factors with the development of severe AS. An independent and dose-response relationship between conventional CV risk factors such as hypertension, DM, and dyslipidemia and the risk of developing severe AS was found and hypertension had the highest attributed risk (22).

The mechanism how hypertension induces AS is unclear. Potential explanations include the possibility that hypertension results in abnormally high tensile stress on aortic leaflets (126, 127). Alternatively, turbulent flow patterns associated with high volume flow rates may lead to shear stress, endothelial injury and disruption, as seen in atherosclerotic lesions (126, 127).

Antonini-Canterin et al demonstrated that in hypertensive symptomatic AS patients, symptoms developed at a relative earlier stage of the disease, with larger valve areas and lower stroke loss, probably because of the additional LV overload due to the hypertension itself. These findings could suggest that in these patients, hypertension should be treated more aggressively, and this group of patients should also be followed-up more closely (128). The coexistence of AS and hypertension presents a therapeutic challenge. There has been a reluctance to adequately treat hypertension in patients with AS due to concerns that antihypertensive medications might reduce the cardiac output (129). However, this has not been corroborated in clinical studies, likely because AS does not result in “fixed” valve obstruction until late in the disease process (53). There are no studies addressing specific antihypertensive medications in patients with AS, and according to ACC and AHA guidelines medical therapy for hypertension in patients at risk for developing AS and in patients with asymptomatic AS should follow standard guidelines, emphasizing the importance of starting at a low dose with a gradually uptitrating to achieve optimum blood pressure control. Vasodilators should be avoided because of concern that they may precipitate life-threatening hypotension (53). In summary, arterial hypertension and AS are common diseases, and the prognosis is worse when
these disorders coexist (122, 130) underlining the importance of blood pressure control in these patients.

Renal failure

Mild impairment in renal function does not seem to be associated with the development of AS (131), while previous small studies have demonstrated an association between end-stage renal disease and AS (132-136). Thoracic surgery database studies and percutaneous valve intervention trials have shown that among patients undergoing valve surgery or intervention, the presence of chronic kidney disease (CKD) is associated with a greater risk of death (137, 138). In a large observational study Samad et al, demonstrated that the prevalence of at least mild AS is substantially higher and is associated with significantly lower survival among patients with versus without CKD. There is significant interaction among CKD, AS severity, and mortality, with increasingly worse outcomes for CKD patients with increasing AS severity (139).

The higher prevalence of AS in patients with end-stage renal disease is thought to be related to progressive valve calcification of the cardiac skeleton and valve leaflets—a result of altered or deranged calcium–phosphate metabolism and hypertension in end-stage renal disease (140). End-stage renal disease is associated with faster stenosis progression in aortic valves (18). Despite that, recent evidence has shown a relationship between the extent of coronary calcification and aortic root calcification, rather than leaflet calcification, thus suggesting an arterial calcification disease rather than valve leaflet disease (141).

Smoking

Previous studies of degenerative AS have found associations with atherosclerosis risk factors, but the significant factors have varied among studies. Usually smoking, is mentioned as one of the main risk factors for AS as well as for CAD. However, the literature does not provide many studies supporting smoking as an independent risk factor of AS, and some even report a lack of association between smoking and AS (126).

In a study by Stewart et al, smoking was found to be independently associated with a 35% increase in risk of AS (3). Smoking intensity also had an impact on risk, with increased risk with increasing smoking intensity. On the other hand, former smokers who had quit smoking 10 or more years previously had similar risk for AS as non-smokers (142).

Smoking may increase the risk for aortic valve disease through mechanisms analogous to those postulated for atherosclerosis (143).

Metabolic syndrome, obesity

Metabolic syndrome

The criteria for metabolic syndrome include obesity, dyslipidemia (low high-density lipoprotein and/or elevated triglycerides), elevated blood
pressure, and alterations in glucose metabolism (144). However, there are several definitions. It is associated with T2DM, lipid disorders, CVD and hepatic steatosis. A large population-based multiethnic cohort (MESA), was the first study to present an independently association between the metabolic syndrome and AS (97). This study also concluded that the AS prevalence is increased with increasing number of metabolic syndrome components. In another study, Briand et al showed that the metabolic syndrome was associated with faster AS progression and with a higher rate of adverse events (i.e., AVR, death, or both) (145). Other retrospective or cross-sectional studies also revealed independent associations between metabolic syndrome and increased prevalence of aortic valve calcification (97) or faster degeneration of bioprostheses (146). A substudy of the ASTRONOMER trial confirmed previous results with more pronounced impact in younger patients (17). The mechanisms by which metabolic syndrome might mediate increased valvular calcification are not known. However, metabolic syndrome is associated with inflammation and increased oxidative stress. Both oxidized cholesterol and inflammatory cytokines, each of which is present in human aortic valve lesions (30, 147), have been shown to increase calcific nodule formation by valvular fibroblasts in vitro (148).

**Obesity**

Isolated obesity is considered one of the classical risk factors in CVD (149). Studies exploring the impact of obesity on the risk of AS development have been divergent. In a large Swedish cohort, an association between obesity and increased risk of AS was revealed. They also concluded that a large proportion of the cases might be prevented if the population maintained a healthy BMI (150). Some studies have shown better survival after CABG and AVR in patients with obesity, a phenomenon referred to as the “obesity paradox” (151, 152). This concept has been criticized on the grounds of being an error arising from biases in observational studies. Strong confounding by smoking has been noted by several researchers (153, 154).

**Lipids**

Elevated levels of cholesterol and LDL have in cross sectional and in retrospective studies been found to be a risk marker for developing aortic valve calcification and AS (3, 121, 155, 156). Familial hypercholesterolemia also has been shown to increase the risk of AS (157) and LDL has been found in calcified aortic valves (158). Based on this knowledge, three randomized, double blind, placebo-controlled trials, SALTIRE, SEAS and ASTRONOMER, was performed with the aim to reduce the progression rate of calcification in AS with statins (57-59). However, in all these three randomized trials, there was no effect on progression of AS measured as peak jet velocity and/or AVA or AVR, even though the levels of LDL-cholesterol were reduced with at least 53%.
Apolipoprotein A1 and apolipoprotein B

Apolipoprotein A1 (Apo A1) is the major protein component of high-density lipoproteins (HDL). The protein, as a component of HDL particles, enables efflux of fat molecules for transport back to the liver for excretion; protective “fat removal” particles.

Apolipoprotein B (Apo B) is the primary apolipoprotein of LDL particles. Since each particle harbors only one Apo B, measurements of Apo B reflect the number of particles, in contrast to LDL-cholesterol, which measures the amount of cholesterol in LDL-particles (159). At a given LDL-cholesterol concentration, the presence of a few, and thus larger, particles are more favorable than the presence of a large number of small particles. Thus, the Apo B/Apo A1 ratio, representing the balance of proatherogenic and antiatherogenic lipoproteins, may be a better risk predictor of atherosclerotic disease than the LDL/HDL ratio (160). In the INTERHEART cross-sectional case-control study a high Apo B/Apo A1 ratio was strongly associated with myocardial infarction (161).

Triglycerides

Although the role of triglycerides (TG) as a risk factor for CVD has been strongly debated, recent data favor the role of TG-rich lipoproteins as a risk factor for CVD (162).

Recent data from genetic studies utilizing a Mendelian randomization design have consistently linked non-fasting TG levels to increased risk of CVD events and all-cause mortality (163, 164). The association between TG and development of AS is not known.

Lipoprotein (a)

Lp(a) was discovered in 1963 by Kaare Berg in Norway, and elevated levels were considered a CV risk factor (165), later confirmed by Dahlén et al (166).

Lp(a) is a well-known independent risk factor for CVD and AS (167-174). Causality is suggested between Lp(a) and AS (16, 172, 173, 175). Notably, most of these studies did not consider the potential influence of concomitant CAD.

Serum Lp(a), synthesized in the liver, is basically a LDL particle with an extra protein, apolipoprotein(a) (Apo A), covalently bound to apolipoprotein B-100 (176) (Figure 5). Circulating levels of Lp(a) in humans vary significantly inter-individually from 0.1 mg/dL to 300 mg/dL, but are markedly stable over time intra-individually and levels are not much affected by ageing, diet and physical activity (177). In women, menopause is related to increasing levels of LP(a) that have been related to increased risk for CV disease (178). The Apo A component is encoded by the LPA gene, and levels of Lp(a) are almost entirely explained by genetics. Indeed, elevated Lp(a) is the most common genetic dyslipidemia, but levels can also be affected by liver and kidney disease (179, 180). The size of the particle varies markedly
depending on the number of kringle repeats which are genetically determined, and small isoforms relate to higher plasma levels due to faster clearance from the hepatocytes. Several single nucleotide polymorphisms (SNP) within the LPA gene on chromosome 6 may also determine plasma levels of Lp(a) and rs10455872 and rs3798220 are both associated with small isoforms and high Lp(a) levels. A causal role for Lp(a) and the development of atherosclerotic disease has been suggested.

Genetically determined small isoforms of Lp(a) due to either kringle repeats or the above-mentioned SNPs are associated with CV events, such as MI and stroke, that is Mendelian randomization studies have supported causality (167, 181-183). Further findings supporting this concept is that genetically determined low levels of Lp(a) due to loss of function of the proprotein convertase subtilisin/kexin type 9 (PCSK9) protein is associated with lower risk for both ischemic MI and AS (184). The atherogenic properties of Lp(a) could be due to Lp(a)-derived entrapment of cholesterol in the intima, via inflammatory cell recruitment and binding of oxidized phospholipids (OxPL), and via interruption of fibrinolytic processes (24, 170). The physiological function of Lp(a) is unclear but Lp(a) has been associated with ischemic atherosclerotic disease such as MI and stroke (167-171). Recently genomwide association studies and Mendelian randomization studies suggest causality between Lp(a) and clinical AS (16, 172, 173, 175). The levels of Lp(a) were strongly associated with the rs10455872 SNP, and they were also associated with aortic valve calcification. Another study showed that progression rate of aortic stenosis and the need for AVR was increased in the the top tertiles of Lp(a) and OxPL on apolipoprotein B-100 in a cohort of patients with mild-to-moderate AS (16). However, in these studies only the one by Thanassoulis et al., the presence of CAD was taken into account.
Figure 5. Lp(a)
Lipoprotein(a) consists of an LDL-like particle to which apolipoprotein(a) is covalently linked. The central core of the LDL-like moiety consists of cholesteryl esters and triglycerides, and is surrounded by phospholipids, free cholesterol, and a single molecule of apolipoprotein B. Apolipoprotein(a) contains 10 different types of plasminogen kringle 4-like repeats as well as regions homologous to the kringle 5 and protease (P) regions of plasminogen. The kringle 4 type 2 domain (42) is present in multiply repeated copies from 2 to >40 that differ in number between apolipoprotein(a) isoforms. Apolipoprotein(a) is linked to apolipoprotein B100 by a single disulfide bond.

LDL, low-density lipoprotein; CE, cholesteryl esters; TG, triglycerides; PL, phospholipids; FC, free cholesterol; apoB, apolipoprotein B; P, protease.

(Illustration: Marianne Gidén)
How elevated Lp(a) levels lead to AVS is not completely clear but there are several proposed mechanisms (185):

1. Lp(a), consisting in part of LDL, is transferred from the bloodstream into the aortic valve and leads to cholesterol deposition.
2. Lp(a), resembling plasminogen, may promote thrombosis by competing with plasminogen and inhibit the role of plasmin in dissolving fibrin clots. Via fibrin deposition this could lead to progressive AS.
3. Lp(a) may be important in wound healing process. It is possible that Lp(a) could bind to fibrin and be transported to and accumulated at sites of injury, thereby delivering cholesterol via its LDL component to sites of tissue healing, i.e., minor injuries in the valve of early AS.
4. OxPL can lead to AS through pro-inflammatory and procalcifying effects. Elevated levels of Lp(a) and OxPL-apoB are associated with increased progression of AS, more pronounced in younger individuals. As OxPL are carried mainly by Lp(a) (186) it is speculated that Lp(a) as an independent predictor of aortic valve stenosis could be explained by OxPL(16).

Treatment options of elevated Lp(a).

As Lp(a) is a well-known independent risk factor for both CVD and AS, and since approximately 20% of the population have elevated levels of Lp(a), i.e., >50mg/dL (~100-125 nmol/L) (80th percentile), it is important to target therapies against this (170). Consequently, much effort has been put into the development of an effective drug targeting elevated Lp(a) levels.

Pharmacological strategies include PCSK9 inhibitors, antisense oligonucleotide treatment and lipid apherese, which lower Lp(a) levels between 24% to 90% (187, 188). Recently, alirocumab, a PCSK9 inhibitor, in addition to high-intensity statin therapy, has been shown to improve CV outcomes in patients after an acute coronary syndrome (189). Niacin reduces levels of both LDL-cholesterol and Lp(a) with reduction in CV events (190, 191). However, the effect of niacin on progression rate and development of AS is not known (170) and the use of niacin has been abandoned due to detrimental adverse effects.

Observational studies have shown a reduction in CV events on Lp(a) lowering therapies, (188, 192-195). Treatment goal for CVD patients is first to lower LDL aggressively with statins and/or ezetimibe and subsequently lower Lp(a) levels below 50 mg/dl (~100-125 nmol/L). Apheresis treatment may be added in patients who, despite treatment, have signs of progressing atherosclerosis/CAD (196).

However, to date no RCT has directly evaluated CV outcome for Lp(a) lowering in individuals with elevated Lp(a) levels. In general, most interventional studies, in particular those that include the use of drugs, do not target the lowering of Lp(a) concentrations alone. It is therefore hard to evaluate if an effect on outcome is only attributed to the lowering of Lp(a) levels or whether other concomitant factors such as lowering of LDL levels or improvement of other risk factors also play a role. The 2016 ESC/EAS...
Guidelines for the management of dyslipidemias do not provide specifically recommendations for the management of patients with elevated Lp(a) levels other than an intensified treatment of the modifiable risk factors, including LDL (159). In addition, no trial has shown any outcome effect on the calcific progression rate of AS of Lp(a) lowering strategies. There is a need to confirm the causality of Lp(a) on the development of AS. This can only be achieved by clinical trials with treatment strategies showing decreased levels of Lp(a) together with a clear effect on the progression rate of AS. The effect of antisense oligonucleotide treatment, with the ability to lower Lp(a) levels with 90%, is currently under investigation in a phase 2 study.

**Genetic risk factors in AS**

Several studies suggest that a genetic component is involved in the development of calcific AS associated with bicuspid or tricuspid aortic valves (6, 110, 175, 197). As mentioned above, variants of NOTCH1 and GATA binding protein 5 have been associated with BAV in humans. As mentioned earlier, there is also a known high inheritability of BAV and familial clustering has also been described with a BAV prevalence of up to 24% in families with more than one family member carrying the valve malformation (110).

In 2013, a large study using Mendelian randomization identified that the single SNP rs 10455872 in the LPA gene was associated with development of AS (175), see also Lp(a) above. Furthermore, a mutation in the fatty acid desaturase locus was found to associate with the development of AS, a discovery that implicates fatty acid metabolism in the development of AS (198). Large-scale genome-wide analyses have also identified robust associations for PALMD, and TEX41 with aortic stenosis (199).

Recently, a study demonstrated that a sibling history of clinically diagnosed AS was associated with increased risk of AS. Having at least one sibling with AS was associated with a hazard ratio of 3.41 (95% CI 2.23–5.21) to be diagnosed with AS in an adjusted model. Individuals with more than one sibling with AS had an exceptionally high risk (hazard ratio=32.84) but were uncommon (200).

**Biomarkers in AS**

In patients with AS there is often a mismatch between onset of symptoms and the hemodynamic severity of valvular stenosis making the management of asymptomatic patients controversial (54). This discrepancy might in part be explained by heterogeneity in the hypertrophic response to AS, which itself is an independent marker of an adverse prognosis (42, 201). Despite otherwise successful valve surgery, critical CV events such as fatal arrhythmias and CHF remains problematic after AVR. Therefore, a simple and stable biomarker is needed in order to predict the prognosis of AS patients and ideally prove useful for earlier identification of patients with subclinical AS, a potential subgroup who may need early therapeutic intervention. An ideal biomarker should be a prognostic indicator, should assist in the early diagnosis, should reflect the therapeutic response, and
help grading the risk associated with each stage of the disease (202-204). Optimally, a biomarker of valvular heart disease (VHD) could also contribute to the understanding of the pathogenesis (203). Biomarkers of VHD could either reflect valve pathology itself, or myocardial manifestations secondary to the valve disorder – myocardial stress, hypertrophy, fibrosis or myocardial cell damage/necrosis. Several biomarkers of VHD have been suggested, most of them are of myocardial origin since outcome after heart valve surgery is mainly related to myocardial dysfunction. Specific biomarkers of valve leaflet pathology in AS though, are lacking, possibly due to poor understanding of the underlying pathobiology. So far, biomarkers have not played a significant role in the management of AS patients except for natriuretic peptides. Natriuretic peptides are secreted from cardiac myocytes in response to ventricular wall stress (205) and B-type natriuretic peptide (BNP) serum levels are related to New York Heart Association (NYHA) functional class and prognosis, particularly in AS and mitral regurgitation (206). BNP has been found to be of value for risk stratification and timing of intervention, particularly in asymptomatic patients with AS (54).

**C-reactive protein (CRP)**

CRP is a plasma protein that participates in the systemic response to inflammation. The plasma concentration increases during inflammatory conditions, a feature that has long been employed for clinical purposes. Elevated levels of CRP indicate unspecific inflammation and elevated levels of CRP are associated with the atherosclerotic process (207, 208). CRP levels are known to be significantly influenced by infection and tissue damage, as well as obesity, old age, hypertension, DM, and smoking. The exact function of CRP is not yet fully understood, it is believed to be a part of the innate immune system (209). The first study showing increased levels of CRP in patients with AS was published by Galante et al, in 2001 in patients with no CAD (210). Since then further studies have confirmed (211) and expanded these observations (212-215) and also reported reduced levels of CRP from before to 6 months after AVR (216). On the other hand, several studies have shown a week association of CRP and AS (217-219). In summary, results from previous cross-sectional studies regarding the association of CRP to AS have been divergent.

**Troponin T (TnT)**

Cardiac TnT is a protein secreted from cardiac myocytes as a result of myocardial injury. Recent advances in assay technology have greatly improved sensitivity, now allowing quantification of troponin with a high degree of precision at very low plasma concentrations (220). TnT has been documented as an independent marker of increased risk of CVD in the general population (221) and has also been proved a reliable marker of prognosis after AVR but in most cases in close conjunction with surgery (222-225). There is less knowledge about its reliability in subclinical patients with AS.
Other blood biomarkers for VHD - galectin-3, growth differentiation factor 15 (GDF-15), soluble interleukin-1 receptor-like 1 (ST2), microDNA and DNA profiling - hold promise for risk stratification in AS (226). However, as stated earlier, none of these have yet been clinically established.

Given the multiple processes involved in myocardial degeneration, a combination of biomarkers is more likely to prove beneficial than any single marker and have been successfully used in other disease areas but only to a limited extent in VHD. There are few existing studies of multiple biomarkers in VHD. One study examined three biomarkers in patients with severe AS and suggested a combination of ST2, GDF-15 and N-terminal pro-BNP in addition to troponins as useful biomarkers for predicting prognosis in AS (227).

The main limitation for biomarkers of LV function is that they are non-specific and may be altered by other concomitant diseases. BNP levels, for example, may be elevated in various other conditions, including renal failure, chronic obstructive airways disease, obesity, liver cirrhosis, MI and vary according to age, exercise and fluid status (228). Therefore, these biomarkers should always be interpreted in caution together with standard parameters of AS severity. Furthermore, the majority of the existing biomarkers are useful only in the end stages of the disease where few successful intervention options exist. Biomarkers that can detect earlier stages of the valvular disease are still lacking.

**Proteins**

Several proteins have been associated with CAD in previous cross-sectional studies, e.g., GDF-15, proprotein PCSK9, vWF and galectin-3 (229-232). However, only a few proteins have been associated with AS, for example elevated levels of GDF-15 have been found to predict outcome in patients undergoing TAVI (233) and were superior to NT-proBNP for TAVI risk stratification. Galectin-3 has been associated with AS in cross-sectional studies (234).

**Mendelian randomization**

RCTs are the ‘gold standard’ for inferring the causal role of a biomarker in the development of disease. However, RCTs are expensive. In the absence of RCT evidence, as an alternative, non-interventional approaches have been increasingly used in clinical research, especially a particular type of analysis where a genetic variant is used to conduct ‘Mendelian randomization’. In simple terms, a Mendelian randomization study is one in which genetic variants are used to investigate the causal relationship of a biomarker on risk of disease (235-237).

An example of Mendelian randomization is the studies on the association between Lp(a) levels and CVD/AS. Three pieces of data is needed to help provide evidence for a causal link between elevated plasma Lp(a) levels and CVD/AS. First, elevated plasma Lp(a) levels should be associated with increased CVD/AS risk. Secondly, genetic variation should exist in human
populations that can explain a large fraction of the variation in plasma Lp(a) levels. Thirdly, such genetic variation should be linked directly with CVD/AS risk (170).

**Survival analysis**

Long-term results after cardiac surgery are usually presented in actuarial terms and risk factors for adverse outcome have been identified by further statistical analyses. Consequently, the prospects for this patient population are well established and provides for the identification of high-risk patients. However, these analyses do not relate the observed survival of these surgical patients to the expected survival of the general population. Moreover, much of our knowledge concerning survival of cardiac surgery patients rely on results from RCTs which usually recruit relatively selected patients that are followed during a limited time. The findings of these trials may not be generalizable to the general population.

**Relative survival**

Relative survival is defined as the ratio of the proportion of observed survivors in a population with a disease, compared to the proportion of expected survivors in a similar population - matched at least for age and sex - without the disease. Usually this is a comparison over a certain period of time. The relative survival rate could also be described as a measurement of escaping the excess risk of dying from the disease under study, or to what extent the patient is cured by the treatment; or whether the disease shortens life. Obviously, the main limitation of cause specific survival analysis is its dependence upon reliable coding of information on the cause of death. This problem is eliminated in relative survival while information on individual cause of death is not required. One limitation in the use of relative survival analysis is that calculation of relative survival is based on the assumption that the survival in the general population is unaffected by deaths related to the disease being studied. We cannot tell for sure that this is the case in our study, albeit death from aortic valve disease is uncommon in the general population at least among the younger. Moreover, due to the relative rarity of undiagnosed aortic valve disease, the influence on survival in the general population should be only marginal. In other words, if the prevalence of the disease is low, this will have little impact on the estimates. The method has been widely used in cancer epidemiology but in cardiac surgery only a few studies have been reported (76, 77, 238, 239).

**The human mortality database (HMD)**

The Human Mortality Database (HMD) was created to provide detailed mortality and population data to researchers, students, journalists, policy analysts, and others interested in the history of human longevity. The project began in 2000 as an outgrowth of earlier projects in the Department of Demography at the University of California, Berkeley, USA, and at the Max Planck Institute for Demographic Research in Rostock, Germany. The Human Mortality Database project team seek to provide open, international
access to these data. At present the database contains detailed population and mortality data for 40 countries or areas and it includes the majority of European countries (240).
Aims of the thesis

The aim of this thesis was to evaluate if biomarkers were associated with prospective aortic stenosis requiring surgery, and if these associations differed between genders, time to surgery and the presence of coronary artery disease. We also assessed long-term observed and relative survival after aortic valve surgery with and without concomitant coronary artery bypass grafting in a large Swedish register cohort.

The specific aims of individual studies were:

**Study I:** to analyze whether lipoprotein (a) and apolipoproteins predict future surgery for aortic stenosis and if any found association was affected by any concomitant presence of coronary artery disease.

**Study II:** to identify novel protein biomarkers for incident aortic stenosis and evaluate whether any detected association was affected by any concomitant presence of coronary artery disease.

**Study III:** to analyze if troponin T and C-reactive protein associate prospectively with the need for surgery for aortic stenosis in patients with and without concomitant coronary artery disease.

**Study IV:** to assess long-term observed and relative survival after first time aortic valve replacement in a large Swedish population.
Patients and methods

Study populations (study I-III)

All cases and controls were recruited from the Northern Sweden Health and Disease Study (NSHDS) which in turn consists of three sub-cohorts; the Västerbotten Intervention Programme (VIP), the multinational MONItoring of trends and determinants in CArdiovascular diseases (MONICA) project, and the Mammary Screening Project. In total, 336 of them had an AVR due to AS with concomitant CABG if indicated, and 237 had previously participated in VIP, 37 in MONICA and 62 in MSP.

-VIP

In Sweden, mortality related to CVD steadily increased during the 20th century with the highest rates in the county of Västerbotten in the 1970s and early 1980s. Therefore, a community intervention program, VIP, was launched. The aim of this still ongoing program, is to reduce mortality and morbidity from CVD and diabetes (241). The start was in 1985 in the Västerbotten municipality of Norsjö, and thereafter it has successively expanded and been implemented in the whole county of Västerbotten and by today it is integrated into ordinary primary care routines. All county residents, at the ages of 30 (until 1995), 40, 50 and 60 years, are asked to participate in a health survey and receive counselling regarding lifestyle modification at their primary health care center. Participants are invited to complete a questionnaire that covers socioeconomic and psychosocial conditions, self-rated health, personal health history and family history of CVD and diabetes, quality of life, social network and support, working conditions, physical activity, alcohol and tobacco consumption, eating habits and a food frequency questionnaire. Measurements of blood pressure and anthropometry are undertaken, and blood samples are obtained for lipid and glucose measurements including a 2-hour OGTT. Participants are also asked to donate blood for future research. 6,500 - 7,000 examinations were annually made and by the end of 2014 a total of 99,268 unique individuals had participated in VIP.

Initially, the VIP was characterized as a project, and health surveys could be discontinued for periods of time due to decisions at each health centre. This contributed to decreased participation rates, i.e., 48-57% during 1991 - 1995. In order to give priority to prevention efforts, the VIP was integrated into ordinary primary care routines in 1995. Thereafter, participation rates increased and have remained at 66-67% since 2005. A dropout rate analysis in 1998 indicated only a small social selection bias (242).

-MONICA

The World Health Organization (WHO) MONICA project was established in the early 1980s to monitor trends in CVD and to relate these trends to risk factor changes in the population over a 10-year period (243, 244). The project was set up by the WHO to explain the diverse trends in CVD
mortality which was observed from the 1970s and onwards. It was a major international collaboration project with 38 centers in 21 countries involved and the ten-year data collection was completed in the late 1990s. The total population aged 25-64 years monitored was ten million men and women. The first participants in the Northern Sweden MONICA were included in 1986. The study has maintained and continued with registrations of incident stroke and MI and with repeated population based surveys even after the completeness of the WHO study in the late 1990s (245). The randomization was stratified for age. In the first two surveys (1986 and 1990) 2,000 inhabitants aged 25 – 64 years were invited, and in the following five surveys (1994, 1999, 2004, 2009 and 2014) 2,500 inhabitants in the age of 25-74 years were invited. In total, MONICA enrolment involved asking randomly selected individuals in the counties of Västerbotten and Norrbotten (530,000 inhabitants), to participate in this health survey and at the end of 2014, 12,368 unique individuals had participated. The participation rate was 81 % in 1986, and 63% in 2014. In the Northern Sweden MONICA study, similar procedures as in the VIP study were used concerning blood glucose and blood lipids, OGTT, measurements of blood pressure and anthropometry, and questionnaires on lifestyle and eating habits. Participants were also asked to donate blood for future research.

-MSP

The MSP was initiated in 1995 and completed in 2006 (246). Women were asked to donate blood and to have their blood pressure and anthropometry measured when they were attending their regularly mammography examinations. Every woman within the age of 40 to 74 years was invited every two or three years for breast cancer screening. Altogether 28,778 women have participated in the project and the participation rate was approximately 57 %.

Taken together, these three surveys (VIP, MONICA, MSP) included 140,414 participants up to December 2014, which reflected participation rates of 65 to 75%.

Study population – (study IV)

Between 2005 and 2016, 4970 consecutively patients from three Swedish cardiac surgery centres (Linköping, Umeå and Örebro) were subject to aortic valve surgery, due to either aortic stenosis or aortic regurgitation. Additional concomitant CABG where performed when indicated. All cardiac surgery procedures are recorded in a database ("Carath"). This database was established in January 2005 in collaboration between four cardiac surgery centres in Sweden; Umeå, Linköping, Örebro and Karlskrona. It comprises procedural- and patient related characteristics, intra- and postoperative events and also laboratory results, in total more than 400 variables for each patient. All cardiac surgery patients have been registered consecutively since the start. Register data have been validated twice. Coverage was very high with 99 % of the performed procedures found in the registry. There was also a high reliability of the variables in the registry (247). Consensus on
definitions of variables was obtained by a standardization committee with participants from each center.

**Selection of cases**

Figure 6. Flowchart of selection of cases.

**Study I-III.**

The only cardiothoracic surgery center in the northern part of Sweden is located in Umeå. The area from which patients are referred constitutes 53% of the surface area of Sweden and the total number of inhabitants is approximately 910,000. All kinds of general thoracic and cardiothoracic procedures are performed at the center except heart and lung transplants, congenital heart surgery and pediatric general thoracic surgery. During the last 5 years, approximately 700 cases of open-heart surgery have been performed each year. Between March 1988 and December 31, 2014, a total of 6691 valvular procedures were performed at this center. Of these, 336 who underwent surgery because of AS, had also before surgery participated in one of three above mentioned health surveys. These 336 patients constituted the study cases; in study I, all 336 were included; in study II, 334 of them were included; and in study III, all 336 patients were included. Each case was thoroughly evaluated and data carefully registered. Cases were classified according to the primary indication for surgery. If there was any uncertainty regarding classification, consensus was achieved after discussion with co-
workers. In 84% of patients the primary indication for surgery was AS; the remaining 16% had an AVR performed with another primary indication procedure such as CABG (10%) and surgery for ascending aorta disease (5%).

In study II a separate set of 106 additional cases with 212 matched controls was used in a validation study. These additional 106 cases were identified within the same cohorts; 70 from VIP (66%), 16 from MONICA (15%) and 20 from the MSP (19%). The majority of surgical procedures in this set of cases were performed between April 2012 and December 2014.

Study IV.

During a 12-year period (2005-2016) 5544 patients were subject to aortic valve surgery with or without concomitant coronary artery surgery, in three Swedish heart surgery centres (Linköping, Umeå and Örebro). After exclusion of TAVI-patients (n=456), patients with mortality within 30 days (n=117), patients with double inclusion (n=60) and patients undergoing homograft surgery (n=1), 4970 pts remained – 1479 from Linköping, 1448 from Örebro and 2043 from Umeå.

Selection of referents

Study I-III.

Referents were recruited from the same cohorts as the cases, i.e., the VIP, MONICA and MSP surveys. For each case two randomly selected referents were allocated. The referents were matched for sex, type of health survey, age (± 2 years), date for inclusion in health survey (± 4 months) and geographical area. Participants in the MSP did not provide blood samples for future research. The number of referents in study I was 671, in study II, 662, in study III, 671.

Study IV.

The survival rates of the study population, i.e., AVR patients, was compared with the survival of the general Swedish population using the HMD stratifying for age, sex and calendar year (240). This enabled assessment of relative survival.

Nested case-referent study

The study design was identical in the first three papers, i.e., nested case-referent (NCR) study design. The accurate description of this study design is “a case-control study within a cohort”.

In this study, cases of a disease that occur in a defined cohort are identified and, for each, a specified number, usually 2-4, of matched controls is selected from among those in the cohort who have not developed the disease by the time of disease occurrence in the case. A NCR analysis with good matching procedures yields estimates that are as efficient and unbiased
as estimates from the full cohort study. For many research questions, the nested case-control design potentially offers reductions in costs and efforts of data collection and analysis compared with the full cohort approach, with relatively minor loss in statistical efficiency. The nested case-control design is particularly advantageous for studies of biologic precursors of a disease (248). In this way, the NCR is a truly prospective study since blood samples are obtained before onset of a disease (or before surgery as in our studies). One limitation of the design is that the matching procedure hinders us to evaluate the impact of matched factors on the future risk for AVR.

Examinations and definitions at baseline (health survey)

**Anthropometry, BMI**

Height and weight in light clothing and without shoes were measured and body mass index (BMI) in kilogram per square meter was calculated. Height and weight were measured in all three surveys.

**Blood pressure**

In all surveys, blood pressure was measured after 5 minutes of rest, by a mercury sphygmomanometer prior to 2004, and using semi-automatic devices since 2004 (Omron M7, Omron Corp., Kyoto, Japan). Patients were seated for blood pressure measurement throughout the MONICA and MSP surveys, whereas in the VIP survey, blood pressure was measured with participants in the recumbent position until September 2009 and thereafter in the sitting position. Measurements obtained with participants in the recumbent position were adjusted using a sex- and age-specific formula (242). Hypertension was defined as systolic blood pressure of ≥140 mmHg, and/or diastolic blood pressure of ≥90 mmHg, and/or use of anti-hypertensive medication.

**Diabetes mellitus and oral glucose tolerance test**

An oral glucose tolerance test (OGTT), including measurements of fasting and post-load glucose levels, was routinely performed in the VIP, was performed in 60% of MONICA participants, but was not performed in the MSP. The test was done with a 75-g oral glucose load after at least 4 hours of fasting following WHO standards (249). Subjects with previously known DM or with fasting glucose exceeding the criterion for diabetes did not undergo OGTT. DM was determined by self-reported usage of anti-diabetic medication, fasting plasma glucose levels ≥7.0 mmol/L, and/or post-load plasma glucose levels ≥11.1 mmol/L (≥12.2 mmol/L based on capillary plasma in the VIP). Impaired fasting glucose was defined as a fasting glucose level of ≥6.1 and <7.0 mmol/L. Impaired glucose tolerance was defined as a post-load glucose level of ≥7.8 and <11.1 (≥8.9 and <12.2 in the VIP) combined with a non-diabetic fasting glucose level. Glucose intolerance was defined as the
presence of any of the categories impaired fasting glucose, impaired glucose
tolerance, and DM.

**Smoking**

Participants were asked about their smoking habits and were categorized
as smokers (including both current smokers and ex-smokers) or never-
smokers.

**Questionnaires**

VIP and MONICA have similar questionnaire that covers socioeconomic
and psychosocial conditions, self-rated health, personal health history and
family history of CVD and diabetes, quality of life, social network and
support, working conditions, physical activity, alcohol and tobacco
consumption, eating habits and a food frequency questionnaire. The
questionnaire has gradually expanded and modified. The MSP questionnaire
covered reproductive factors, smoking habits and medication.

**Blood sampling and analysis**

At the health survey blood samples from participants are obtained for
lipid and glucose measurements. Participants are also asked to donate blood
for non-specific future research. These plasma samples were obtained after
fasting for a minimum of 4 hours (extended to 8 hours 1992) and kept stored
in a deep-freeze blood bank at –80°C until analysis.

**Cholesterol**

Total serum cholesterol was measured at the time of the health survey using a
bench-top analyser (Reflotron®, Boehringer Mannheim GmbH Diagnostica,
Mannheim, Germany) in the VIP until September 2009, or at a central
laboratory using an enzymatic method (Boehringer Mannheim GmbH
Diagnostica, Mannheim, Germany) in MONICA and the VIP after September
2009. Cholesterol values obtained using the bench-top method were adjusted to
the results measured at the central laboratory. VIP and MONICA, but not MSP,
participants had their cholesterol samples analysed at survey.

**ApoB/ApoA1**

In 2017, analysis of Apo A1 and Apo B was performed on a Coabs 8000
modular analyzer, c502 module (Roche Diagnostics, Basel, Switzerland).
The reagents employed were Tina-quant apolipoprotein A1 and B (catalog
Nos. 03032566122 and 03032574122, respectively, both version 2). Apo A1
and Apo B were standardized to reference standards, The International
Federation of Clinical Chemistry and Laboratory Medicine SP1-01 and SP3-
07, respectively. The Apo B/Apo A1 ratio was calculated. To adjust measured
Apo B levels for the amount of Apo B in the Lp(a) particle, Apo B levels were
converted from g/L to nmol/L and the number of Apo B-containing particles
not related to Lp(a) was calculated, as described by Enkhma et al (177).
adjusted Apo B level was used to calculate a Lp(a)-independent Apo B/Apo A1 ratio. The total coefficients of variation were Apo A1 3.42% and 2.18% at levels of 0.86 and 1.45 mg/L, respectively; and Apo B 1.93% and 2.19% at levels of 1.0 and 1.8 mg/L, respectively.

**Lp (a)**

In 2017, analysis of Lp(a) were analysed on a Cobas® 8000 modular analyser, c502 module using reagents Tina-quant Lp (a) Generation 2, cat no 05852625190 (Roche Diagnostics). For Lp(a) the lowest level of detection was 7 nmol/L. Lp(a) was standardized to reference material IFCC SRM2B for SI-unit nmol/L. Total coefficients of variation (CV%) were 2.4% and 3.2% at levels 34 and 115 nmol/L respectively.

**C-reactive protein (CRP)**

In 2017, analysis of hs-CRP was performed on a Cobas 8000 modular analyzer. The reagents employed were CRPL3 (catalog No. 05172373190; Roche Diagnostics). Lowest level of detection was 0.3 mg/L. CRP is traceable to CRM 470 (CRPL3 2011-01, V3). The total coefficients of variation were 1.5% and 1.9% at levels of 8 and 47 mg/L, respectively.

**Troponin T**

In 2017, analysis of hs-TnT was performed on a Cobas 8000 modular analyzer. The reagents employed were Troponin T hs STAT (catalog No. 05092728190 Roche Diagnostics). Lowest level of detection was 3 ng/L. The total coefficients of variation were 5.4% and 2.0% at levels of 29 and 2362 ng/L, respectively.

**Proteomic profiling**

Plasma samples were analysed at the Clinical Biomarker Facility, Science for Life Laboratory, Uppsala University, with the proximity extension assay (PEA) technique using the Olink Multiplex CVD III 96×96 panel, a high-specificity assay that simultaneously measures concentrations of 92 cardiovascular candidate proteins.

In brief, the assay uses a standard 96-well microplate format, including 90 samples and 6 external quality control standards. Each sample is mixed with 92 pairs of oligonucleotide-labeled antibodies and 4 internal technical controls. When both high-specificity antibodies bind the target protein in the sample, the attached oligonucleotides form a unique DNA reporter sequence that is subsequently amplified and quantified with a standard polymerase chain reaction.

Samples were analysed in individual wells on 12 plates, and each case and its 2 referents were always analysed in the same plate. All samples were randomly distributed in the plates. Polymerase chain reaction values above the fluorescence detection threshold were log2-transformed and corrected for technical variations based on negative and interplate controls. The lower limits of detection were determined with the negative control samples.
In the quality control procedure, 2 proteins (SPON1 and N-terminal pro B-type natriuretic peptide) had >5% missing values, and thus they were removed. For all 996 individuals in the dataset, the measurements were above the limits of detection in >97% of the remaining 90 proteins, and thus they were analysed further.

A principal components analysis indicated that the protein measurements were associated with plate and storage time. In the validation cohort, only results from the 6 proteins identified as associated with AS in the discovery cohort were considered. In the quality control procedure, all 6 proteins had <2% missing values. In both the discovery and validation cohorts, we used standardized residuals from linear regression models for each protein, adjusted for plate and storage time, as the measurement of protein variation.

**Surgical procedure and postoperative management**

Through a median or partial sternotomy access to the heart was obtained. Cardiopulmonary bypass with light to moderate hypothermia was established, and a standard AVR was performed during cardioplegic arrest using cold crystalloid or cold blood cardioplegia delivered antegrade and/or retrograde. Both mechanical and biological prostheses were used according to the surgeon’s assessment and preference. In cases with combined procedures, CABG was usually performed first. Patients from about 70 years of age and above usually received a biological valve prosthesis while younger patients received a mechanical prosthesis with exceptions due to patient’s and/or surgeon’s preferences. Reheparinisation and the initiation of anticoagulation therapy were started within 24 hours after surgery in patients with a mechanical valve prosthesis, while the anticoagulation strategy after bioprosthesis implantation differed between surgeons and between centres. If anticoagulants were initiated, this was usually continued over three months, while other patients did receive aspirin solely. Patients also received perioperative antibiotic prophylaxis for 1-2 days.

**Variables in Carath (study IV)**

Several of the variables in the Carath registry are in concordance with the variables that constitute the EuroScore risk scoring system (250, 251). Carath comprises procedural- and patient-related characteristics, intra- and postoperative events and laboratory results. The following variables were used in study IV.

*Smokers* were categorized as current smoker, past smoker > 1 month ago, or never smoker.

*Chronic obstructive pulmonary disease (COPD):* (yes/no) individuals with long term use of bronchodilators or inhalatory steroids for lung disease were defined as having a diagnosis of COPD.

*NYHA:* Patients were classified according to the New York Heart Association classification for heart failure.
Heart failure (yes/no) was based on objective measurements of LV function together with clinical status and medication.

DM (yes/no) The presence of diabetes was based on self-reported use of antidiabetic medication (insulin; oral antidiabetics; oral antidiabetics + insulin; dietary treated). (yes/no) was classified as yes with a history of either paroxysmal, persistent or permanent.

Endocarditis (yes/no) was based on clinical observation including echocardiography, together with ongoing antibiotic therapy.

Previous cardiac surgery (yes/no) was defined as heart operation with opening of the pericardium

Valve morphology (bicuspid/tricuspid) was assessed perioperatively by the surgeon.

Level of urgency was classified into elective, urgent or acute (within 24 hours after decision)

AS was coded as the primary indication for surgery even in the presence of concomitant AR.

We used the EuroScore I in the study since EuroScore II was not introduced until several years after the first patient inclusion (missing values for EuroScore II was 66.5%).

Examinations at study endpoint (surgery) (study I-III)

In conjunction with surgery, each patient underwent several preoperative examinations including anthropometry, measuring of blood pressure, ECG, chest x-ray, and echocardiography. Results from blood chemistry were retrieved. Hospital files were thoroughly scrutinized and valid CV information like risk factors for CVD, concomitant CVD, comorbidities and current medication was gathered. A coronary angiogram was performed preoperatively in all but one patient. Results were categorized according to established clinical practice. Any degree of coronary atherosclerosis, i.e., even if not reaching significance of diameter reduction (>50%), was regarded as CAD. Perioperative details were recorded such as type of valvular intervention, numbers of coronary by-pass grafts, cross-clamp and by-pass times, length of stay at intensive care unit and at hospital, and outcome. An echocardiogram was performed in 96% of cases as a part of the preoperative assessment. From the echocardiogram protocols LV and left atrium dimensions were determined. Mean and maximum gradient over the aortic valve was estimated and LV function measured as ejection fraction was calculated with the use of Teichholz formula (252).

Classification of cases (study I-III)

Each case with associated data was subjected to close scrutiny in order to determine the primary cause of surgical intervention, and to rank eventually concomitant cardiac surgical procedure. This classification was performed by an experienced cardiologist blinded for baseline survey data and outcomes.
If there was any uncertainty regarding the classification of cases, consensus was obtained after discussion with co-workers.

**Statistics**

**Study I and III**

The same statistical approach was used in study I and III. Continuous data were checked for normal distribution by formal tests and visual inspection, and data were ln-transformed if needed. (ln) z-scores were calculated, separately for men and women, and, as a conservative approach, missing values were replaced with the median value for referents, calculated separately for men and women. The scores with replaced missing values were used in all models and thereby using the entire dataset. Continuous variables were also categorized into quartiles based on the distribution of the referent values, separately for men and women, and missing values were treated as a separate category and are not included in the tables.

Data are presented as (geometric) mean with 95% confidence interval (CI). Student’s t-tests for independent groups were used to analyse differences in the means between cases and referents. Associations between studied variables in referents were tested with partial correlation analysis adjusted for sex and age at survey. Since cases and referents had the same follow-up time within strata in this nested and matched case-referent study, logistic regression analysis (rather than Cox regression) using the conditional maximum likelihood routine designed for matched analysis, was used to estimate odds ratios (OR) and 95% CIs. The influence of studied variables on future surgery for AS was tested in univariable and multivariable models.

**Multivariable analyses**

**Study I:** Two models for multivariable analyses was used. The first model included Lp(a) and Apo B/Apo A 1 ratio; the second model included in addition hypertension (yes/no), glucose intolerance (yes/no), and smoking (present/past or never). In a final model, BMI was added. The analysis was stratified for sex, age at surgery (less than 60 years or 60 years and more), time between survey and surgery (less than 5 years or 5 years and more), and the presence of any visible coronary arteriosclerosis at the preoperative angiogram. Finally, the analysis was repeated after exclusion of the MSP cohort.

**Study III:** Model 1 included hs-CRP and hs-TnT, model 2 included Apo B/Apo A 1 ratio, hypertension (yes/no), glucose intolerance (yes/no), and smoking (present/ past or never), and in the final model, BMI was added to model 2. The analyses were stratified for sex, the time interval between survey and surgery (less than 5 years or 5 years and more), and the presence of any CAD on the preoperative angiogram. Finally, in separate analyses, we excluded the MSP cohort since several cardiovascular risk factors were not registered in MSP. All calculations were performed using the statistical program SPSS version 24 (IBM, Armonk, NY, USA).
**Study II:**

We used a series of nonadjusted conditional logistic regressions in the full discovery cohort to estimate the association between each protein and the case status with the clogit command in STATA. We used a 5% false discovery rate according to the method described by Benjamini and Hochberg (253) to determine significant associations.

For proteins associated with case status at a 5% false discovery rate, we performed a set of adjusted conditional regression models. First, we used the complete-case approach, which excluded individuals with missing data, and the model was adjusted for hypertension status, smoking habits, glucose intolerance, and the plasma Apo B/Apo A1 ratio. We then used multiple imputation by chained equation, with 20 iterations, to impute the missing values in hypertension status, smoking habits, glucose intolerance, Apo B/Apo A1 ratio, and BMI based on the complete-case information for these variables and education status, study center, age, and gender. The imputation thus included some of the matching variables but excluded the identifier of matched pairs, consistent with the method described by Seaman and Keogh (254). Conditional logistic regression models were adjusted with coefficients and standard errors for the variability between imputations, according to the combination rules by Rubin (255). These models were run for each of the six proteins, with adjustment for hypertension status, smoking habits, glucose intolerance, and Apo B. Furthermore, we stratified the analysis based on the presence/absence of atherosclerotic lesions (CAD-AS and non CAD-AS respectively) and based on whether AS was the primary indication for surgery.

In the sensitivity analysis, we restricted the combined sample by excluding the MSP dataset, which contained the majority of cases with missing values. We further restricted the analysis to individuals who had provided samples > 5 years before surgery to avoid confounding with disease severity. We also included models adjusted to BMI.

For those proteins identified as associated with AS status in the discovery cohort, the analyses were repeated in the validation cohort using the identical setting with the exception of the adjustment for the plasma Apo B/Apo A1 ratio, which was replaced with total cholesterol adjustment.

STATA 14.1 were used for statistical analyses and R version 3.1.3 for visualization.

**Study IV:**

Unless otherwise specified, categorical variables are described as n (%); continuous variables as median (lower quartile-upper quartile). Group differences were tested using the $\chi^2$ for categorical data, and Mann-Whitney U and Kruskal-Wallis tests for continuous data. $p$-values <0.05 were considered significant.
**Observed survival**

Observed cumulative survival were calculated using the Kaplan-Meier estimator. Univariate survival analyses, with time since operation as the time variable and death (no/yes) as the event, were performed with the Kaplan-Meier method and log-rank test.

**Relative survival**

Long-term survival and mortality in cardiac surgery patients must be seen in context with that expected in the general population. Relative survival is defined as the ratio between the observed and expected survival rates. Expected survival were calculated from lifetables compiled from the Swedish population stratified on age, sex and calendar year (240).

**Predictors of observed mortality**

Potential predictors of long-term observed mortality were investigated using multivariable Cox proportional hazards (PH) modelling. The selection of candidate predictor variables was guided by clinical knowledge and literature, a method recommended to avoid overfitting and confounders as found with selection based on univariable analysis (256). Violations to the linearity assumption were assessed graphically by categorizing into quantiles, as well as with the linktest. Serum creatinine was not linearly associated with the outcome. Therefore estimated glomerular filtration rate, (eGFR) was calculated with the use of the CKD-EPI equation (257). Estimated GFR showed a linear trend with observed mortality and was therefore concluded to be a more appropriate measure of renal function in our study. Deviations from the proportionality assumption were assessed graphically and by inclusion of interaction terms between the predictors and time. Both age and eGFR violated the proportionality assumption. Thus, we performed time-split analyses (separate analysis of years 0-1, 1-5 and >5) to evaluate time-dependent effects.

Model fit and complexity were compared using log likelihood, the Bayesian and Akaike information criterions. Goodness-of-fit was evaluated with Harrell’s concordance statistic and Somer’s D correlation coefficient, both measures of the concordance of ranked predicted and observed outcomes (258).

**Predictors of relative mortality**

To evaluate factors associated with long-term relative mortality, we applied multiplicative modelling of relative mortality as described by Pohar et al. (259, 260), using the relsurv package in R (261). Differences in relative mortality between patients with different covariate levels are expressed as relative mortality ratios (RMR).

All statistical analyses were performed with Stata (version 14.2, StataCorp LP, Lakeway Drive, USA) and R (version 3.5.2, The R Foundation for Statistical Computing, Vienna, Austria).
Ethical considerations

Study I-III: At the time of survey, written informed consent for future research use of data and blood samples were obtained from all participants. No patient or referent has been contacted during the data collecting procedure or during the data analysis procedure. After data collection and matching procedure, all identifying data was removed from the dataset. The identification key is stored at the Department of Biobank Research (“Enheten för Biobanksforskning” [EBF]) at the University of Umeå.

The use and analysis of stored blood samples requires special ethical considerations. First, the project in which blood samples will be analysed must provide high enough scientific quality to justify efforts and costs, besides the use of stored blood samples per se. The project must have the potential to enrich the scientific knowledge in the area studied and/or contribute to the understanding of a disease and optimally help in improving patient management. Every use of stored blood samples from participants in the NSHDS demands an application to the scientific board of the EBF, thus, as far as possible, ensuring high quality projects. Of course, this procedure is also in respect of survey participants, in order not to waste their donated blood on research projects with doubtable quality. Second, the amount of blood needed for analysis must be estimated and held within reasonable limits. Fortunately, the methods that were used in our studies provided several analyses out of only a small amount of blood. In fact, some blood still remained in samples after extraction for analyses, and samples could be refreezed for future use.

The study protocol was approved by the Regional Ethical Review Board in Umeå (Dnr. 2007-174M and 2014-348-32M) and it complied with the Declaration of Helsinki.

Study IV: At arrival to the hospital for aortic valve surgery, every patient was informed about the collection of decharacterized data for quality registries and their right to refrain to have their data recorded in the registries. There was no intervention in this study and none of the patients were contacted. The study protocol was approved by the Regional Ethical Review Board in Umeå (Dnr. 2016/514-31) and it complied with the Declaration of Helsinki.
Results

Study I

A total of 336 patients were operated due to AS, 48% were women. AS was the primary indication of surgery in 84%. CAD, defined as any detected sign of atherosclerosis on the coronary angiogram, was noticed in 203 cases but not in 132. One case was not classified since no coronary angiogram was performed. Mean age at survey was 56.7 (55.8-57.6) years and mean age at surgery was 67.2 (66.3-68.2) years. The median time (interquartile range) between survey and surgery was 10.9 years (9.3 years). At survey, individuals with future surgery for AS had higher BMI, higher blood pressure, higher Apo B, a higher proportion of hypertension and a higher proportion of glucose intolerance. The Apo B/Apo A 1 ratio was higher in cases than in referents (0.81 [0.79-0.84] vs. 0.77 [0.76-0.79]), p=0.01, especially in women, in patients older than 60 years of age at surgery, and in patients undergoing surgery more than five years after health survey. There were also higher circulating levels of Lp(a) among cases than among referents (23.1 nmol/L [19.8-27.0] vs 17.8 nmol/L [16.2-19.6]), p=0.005, particularly among men, among cases undergoing surgery at ages above 60 years, and among those who underwent surgery more than five years after health survey. After stratification for the presence of CAD the association between Lp(a) and the Apo B/Apo A 1 ratio with future surgery for AS remained in cases with concomitant CAD, but not in those without concomitant CAD.

Study II

The same patient cohort as in study I was used, minus 2 cases. A panel of 92 cardiovascular candidate proteins was analysed with the multiplex proximity extension assay in samples obtained at the baseline survey. In the initial analysis six circulating proteins (GDF-15, galectin-4, von Wf, interleukin 17 receptor A, transferrin receptor protein 1 and PCSK9) were associated with future surgery for AS in patients with concurrent CAD (CAD-AS) (ORs ranged from 1.25 to 1.37 per standard deviation (SD) increase in the protein signal). In the validation cohort of 106 cases, 4 of these proteins, GDF-15, galectin-4, vWF, and PCSK9, remained positively associated with case status (ORs ranged from 1.37-1.68 per SD increase in protein signal).

In the nonstratified analysis with multivariable adjustments for hypertension, smoking habits, glucose intolerance, and the plasma Apo B/Apo A 1 ratio, all six proteins were also associated with AS surgery in the full sample with imputations (ORs were largely unchanged from the initial analysis). When only individuals with complete information on all covariates were included (complete case approach), the sample size was reduced to about 50%. Nevertheless, in adjusted models, 5 of 6 proteins (with the exception of galectin-4) were significantly associated with future AVR. After imputation and adjustments (hypertension status, smoking habits, glucose intolerance, cholesterol levels) GDF-15, galectin-4, vWF, and PCSK9 associated with AVR in the validation group. Using the complete case
approach in the validation group, the sample size was reduced with around 50%. Galectin-4 and PCSK9 remained associated with AVR.

The analysis was then stratified based on the presence/absence of CAD at the time of surgery. In the group with no CAD-AS there was evidence of associations between AS and GDF-15 and galectin-4 in the discovery cohort. Adjusted models yielded similar estimates. However, these proteins (GDF-15 and galectin-4) did not associate with no CAD-AS cases in the validation group. In the CAD-AS group all six proteins were associated with future AVR in adjusted models. Five of them (with the exception of interleukin 17 receptor A) were also found associated with incident AVR in the validation cohort. The exclusion of the MSP cohort with their matched referents yielded similar results as in the main analysis both in the discovery and the validation cohort. Finally, cases with surgery < 5 years after survey were excluded, with 100 cases remaining. In this group GDF-15, galectin-4, vWF, transferrin receptor protein 1 and PCSK9 remained associated with CAD-AS.

Study III

In the same patient cohort as in study I and II we evaluated if TnT and CRP associated prospectively with future surgery for AS with and without CAD. Findings at survey is presented above (study I).

In multivariable analyses, elevated levels of TnT remained associated with surgery for AS after adjustment for CRP, traditional risk factors and BMI in separate models. After stratification for sex and time between survey and surgery, the associations remained in men and in those with surgery <5 years after survey.

After stratification for CAD, elevated levels of TnT remained associated with AVR irrespective of CAD. After further stratification for sex and time between survey and surgery, the association was only seen in men and in those with surgery < 5 years after survey.

Elevated CRP levels did not associate with AVR after adjustments for cardiovascular risk factors. Exclusion of cases from the MSP cohort and their matched referents did not alter the results.

Study IV

In study IV we assessed long-term observed and relative survival after AVR due to AS (89.3 %) or aortic regurgitation in conjunction with CABG when indicated in 4970 patients. Patients were operated between 2005 and 2016 in three Swedish heart surgery centers.

Median total follow-up was 4.7 years. 28 % died during follow up. 30-day mortality was 2.3 % and observed long term survival with 30-day mortality excluded was 96.6 %, 82.7 %, 57.6 % after 1, 5 and 10 years respectively. Relative survival rates (adjusting for the background mortality in the general Swedish population based on age, gender and year) were 99.0 %, 97.5 % and 89.0 % after 1, 5 and 10 years respectively. There was a clear negative impact of known risk factors as DM, COPD, heart failure, current smoking, and
reduced renal function, on both observed and relative survival. Moreover, there was a reduced observed and relative survival with respect to preoperative atrial fibrillation. Age had a negative influence on observed survival but on the other hand was associated with better relative survival (RMR 0.74, 95 % CI 0.71 - 0.77). Female sex was associated with lower observed mortality compared to men ($p<0.001$) but a lower relative survival (RMR 1.17, 95 % CI 1.02-1.35). Combined surgery (AVR+CABG) was not significantly associated with higher mortality ($p=0.43$) in multivariable analyses adjusting for co-morbidities. Bicuspid morphology was associated with lower observed and relative survival in adjusted models (both $p<0.001$), in fact survival after BAV AVR was similar to that of the general population throughout follow-up. Patients undergoing biological or mechanical valve replacements had significantly different comorbidity and risk factor profiles. When adjusting for these, no difference in neither observed nor relative survival was seen between patients receiving mechanical and bioprostheses respectively. There were significant differences in outcome between centres, but also differences in patient mean age and risk factor profile as measured by the EuroScore risk scoring system, which may account for different survival rates. The aim of the study though was not to compare centres, so consequently no further statistical analyses have been performed regarding this issue.
Discussion

Methodological considerations

Study populations

In the three first studies, the NSHDS with its three sub-cohorts (VIP, MONICA, MSP) constituted the study population. Population health studies are often affected by bias and varying rate of participation. Individuals with a better health than the general population are usually more prone to participate, whereas males and those with an impaired socioeconomic situation, as well as individuals from ethnic minorities, are less likely to participate. Characteristics of nonparticipants have been analysed in the MONICA survey, and the attendance rate of smoking younger persons with lower education has been declining (241). In the VIP study participation rates decreased to 48%-57%, during 1991-1995. Thereafter, participation rates increased and have remained at 66%-67% since 2005. A dropout rate analysis in 1998 indicated only a small social selection bias (242). In total, around 70 % of cases and referents came from the VIP study which has a high participation rate. The VIP study population was found to be representative of the general population (241).

Attempts to contact all individuals who did not participate in the MONICA surveys were made through telephone interviews. Analysis of individuals in the cohorts from 1986, 1990 and 1994, who did not attend the surveys showed that risk factor profile for non-participants had not changed during the study period indicating that the surveys conducted were representative of the population studied (262).

Age criteria for participation differed in all three surveys. Younger and elderly individuals have not been included in these three surveys and, consequently, not in the study population. However, AS is rare in the younger population and the surveys have been ongoing since 1985 which would have enabled most of the today older inhabitants to have participated.

Case Selection

All cases in study I-III were selected from either of the three health surveys. They were operated on between 1988 and 2014. Individuals in study IV were operated during a time period of 12 years. It is possible that surgical techniques, patient peri- and postoperative care changed during these years, possibly affecting results. Furthermore, TAVI was introduced during the study period probably decreasing the number of high risk patients in the study group during the later period. This could possibly have affected results.

All surgical procedures were performed at the department of cardiothoracic surgery, University hospital of Umeå. Surgical AVR was performed in all cases; i.e., no TAVI-procedures are included in the study. Cases with a history of prior aortic valve surgery were excluded. The majority of surgical procedures were elective in nature and cases were preoperatively
thoroughly discussed in multidisciplinary conferences, thus as far as possible ensuring adherence to clinical guidelines. A selection bias could be suspected since many surgeons are prone to accept patients with few comorbidities for AVR, especially among the elderly. Consequently, cases with unacceptable comorbidities, cases with unacceptable operative risk, cases with contraindications for surgery, and very elderly cases were underrepresented in the studies. In study I-III, AS was the primary indication for surgery in 84%, i.e., symptomatic severe AS with echocardiographic findings supportive for surgery. In the remaining 16% aortic valve areas and gradients varied and the indication for AVR were up to the individual surgeon to decide. In the majority of cases AVA were below 1.5 cm², and AVR was performed in order to avoid another heart surgery procedure within not many years ahead when the AS would had become symptomatic. Anyway, these cases represented some degree of AS, not only aortic valve sclerosis.

**Selection of referents**

In study I-III all referents were selected from the same surveys as the cases. Controls were matched for age (±2 years), sex, type of survey (VIP, MONICA, MSP), date of health survey (±4 months), and geographic area. All controls had to be alive at the time point for the surgery of the matched cases.

Referents in study IV were retrieved from the general Swedish population, whereas the study population mainly consisted of individuals from the northern part of Sweden, in particular the county of Västerbotten. The impact of this is difficult to evaluate.

**Data at survey**

Data and blood samples collected at the surveys were gathered during a long period, from 1985 through 2012. Over these years, methods for blood sample analyses as well as methods for blood pressure measurements changed. Blood samples had also been stored for many years in freezers which could have affected levels of analysed compounds.

**Blood pressure**

During the first years, blood pressure in the surveys was measured with a mercury sphygmomanometer until the year 2004. Thereafter, a semiautomatic device was used. In the MONICA and MSP surveys blood pressure was recorded in the sitting position after 5 minutes of rest. In the VIP survey, blood pressure was measured after 5 minutes of rest in the recumbent position until September 1, 2009 and thereafter in the sitting position. Measurements obtained in the horizontal position were adjusted according to a sex- and age-specific formula (242). Due to the study design with referents matched for both type and date of survey, any difference in strata because of recording methods and used devices were attenuated as far as possible. Definition of hypertension was in accordance with the European Society of Cardiology guidelines at the time for the creation of the data file,
2014 (263), i.e., systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg and/or self-reported use of antihypertensive medication.

**BMI**

Cases and referents had their height and weight recorded at the survey and BMI was calculated. In the MONICA study cohort, BMI differences were explored among participants during the years 1986 until 2009. One in five was obese in 2009, which was twice as many as in 1986, and BMI increased by 1.5 kg/ m² corresponding to an increase in weight of 4 kg. There was no further increase in BMI from 2004.

**Diabetes mellitus**

In the MONICA study, the prevalence of DM did not change between 1986 and 2009.

**Smoking**

Data on smoking are often hard to evaluate since smokers usually underreport their smoking habits. However, in the 1994 MONICA cohort, self-reported smoking habits correlated well with biochemical markers of tobacco exposure (264).

**Cholesterol**

The method of cholesterol measurement was changed during the study period. Initially and until September 2009, standard bench-top analysers were used. Thereafter all cholesterol analyses were performed at a central laboratory. Cholesterol values obtained with the former method were adjusted to facilitate comparisons with the results analysed at the central laboratory.

The introduction of statins has markedly affected cholesterol levels and the use of statins has increased markedly since the initiation of the study. In 2014, 14.3% of the Northern Sweden population was on continuous lipid-lowering drug medication (265).

Cholesterol levels decreased by 0.9 mmol/L in the age group 25–64 years between 1986 and 2009 (245) and with 0.7 mmol/L between 1994 -2014 (265). The improvement occurred in all age and gender groups, but more prominently in those at high cardiovascular risk and in women with low education. This change might have affected the results. On the other hand, cases and referents were matched regarding date of survey.

**Apolipoprotein B/Apolipoprotein A 1 ratio**

Plasma samples at survey were obtained after fasting for a minimum of 4 hours (extended to 8 hours 1992) but levels of apolipoprotein are considered not to be affected by fasting (266). We did not have data on statin usage or menopausal status among women, both of which could have affected levels
of Apo B and Apo A 1. However, due to the matching where age was one criterion, a menopausal woman was most probably matched with female referents in menopause. The majority of participants (89%) attended surveys before the year 2000, and 98% did not report any MI prior to survey. Thus, the use of statins was probably very low.

**Lipoprotein (a)**

Lp(a) was analysed with the preferred method in the same laboratory on one occasion during a defined period of time. The assay measured the molar concentrations of Lp(a), (nmol/L), and the results were not affected by the size of the isoforms, in contrast to previous studies that typically determined the mass concentration (mg/dL) of plasma Lp(a) levels instead (267). This latter method does not measure the different Lp(a) isoforms equally, and these measurements are not traceable to international standards and cannot be accurately interconverted. The stability of Lp(a) stored at -70°C has been evaluated (268) and an instability was detected for cases with CVD but not for controls. This instability resulted in lower values for Lp(a). Whether this instability also applies to cases with AS is not known. PCSK9-inhibitors, with documented properties of lowering both LDL and Lp(a) levels were introduced in clinical practice in Sweden in 2018 and could not have affected cases since the last operation was performed in 2014.

**CRP**

Elevated levels of CRP indicate unspecific inflammation. Consequently, since different type of inflammatory conditions is not unusual in the (elderly) population, elevated levels could be expected in any of the cases or referents, but the distribution should be nothing but random.

Samples from cases and their matched referents were analysed in fixed triplets at the same occasion, at the same laboratory and at a defined period of time, thus as far as possible eliminating bias due to methodological reasons.

**TnT**

Modern high-sensitivity assays provide the detection of very low levels of TnT. The majority of patients with stable CVD as well as those with subclinical CVD have detectable TnT levels, and elevated levels are associated with increased risk of CV as well as all-cause death, even in the absence of MI. The analyzing procedure was the same as for other biomarkers.

**Proteins**

The mean age of individuals in the validation cohort was higher than in the discovery cohort, and the interval between survey and surgery was longer compared to that of the discovery cohort. This could have affected the results.
**Relative survival**

In study IV the study population was unselected and consisted of consecutive patients. No information on the cause of death was obtained since all-cause mortality rather than cardiac-related death was analysed. The main limitation of cause-specific survival is its dependence upon reliable coding of information on the cause of death. This reliability is not always well founded, thus, in broad population-based studies, cause of death is often difficult to establish with certainty. This problem is eliminated in relative survival analyses since no information on cause of death is necessary.

Concomitant CABG was performed in 38% of surgical procedures, thus indicating significant atherosclerosis. It may be worth considering alternative comparator groups when using relative survival methods in calcific AS and CAD to derive the expected mortality rates.

For example, patients with both AS and CAD have a high prevalence of co-morbidities and risk factors, which put them at risk of mortality from other causes than AS and CAD, such as stroke or pulmonary disease. In these cases, a comparator group with similar co-morbidities could be selected instead of the general population for assessment of expected mortality. However, this approach would be demanding due to its methodological complexity.

During the preoperative assessment both NYHA class and the Carath variable “heart failure” (yes/no) was recorded. In the final analysis, we decided to use the latter to classify patients. The reason for this was the uncertainty in the NYHA classification procedure as described by others (247, 269). The variable “heart failure” has not been validated but on the other hand is well defined in Carath.

**Data from pre- and perioperative assessments**

Data from the preoperative assessment was retrieved retrospectively and differed in quality. In contrast to the information gathered in the surveys, information on blood pressure, smoking habits and ongoing medication was not collected systematically or were not mentioned at all in hospital files.
Table 1. 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></td>
<td>N (%)</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>116/185</td>
<td>6.0 (1.6–10.4)</td>
<td>6.5 (2.9–10.1)</td>
<td>0.88</td>
</tr>
<tr>
<td>Known renal failure (%)</td>
<td>131/202</td>
<td>4.6 (1.0–8.2)</td>
<td>7.4 (3.8–11.1)</td>
<td>0.28</td>
</tr>
<tr>
<td>Previous MI (%)</td>
<td>126/198</td>
<td>1.6 (0–3.8)</td>
<td>13.1 (8.4–17.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Previous CABG (%)</td>
<td>131/202</td>
<td>0</td>
<td>6.4 (3.0–9.8)</td>
<td>&lt;0.001</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>
<td></td>
<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>0.1</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>13.2 (12.7–13.8)</td>
<td>0.59</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>105/153</td>
<td>247 (230–264)</td>
<td>232 (221–243)</td>
<td>0.13</td>
</tr>
<tr>
<td>EF according to Teichholz (%)</td>
<td>103/147</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>129/193</td>
<td>11.6 (6.0–17.2)</td>
<td>19.7 (14.0–25.3)</td>
<td>0.05</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>0.5</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 for 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 artery by-pass grafting, BP blood pressure, LVESD left ventricular end-systolic diameter, LV left ventricle, VTI velocity time integral, LVOT left ventricle outflow tract, and EF ejection fraction.

**Coronary angiograms**

All but one of 336 cases underwent a coronary angiogram prior to cardiac surgery. Any coronary atherosclerosis affecting lumen dimensions was
regarded as significant CAD. Of note, this should be distinguished from concomitant CABG which reflects surgery in presence of significant CAD, i.e., any coronary artery stenosis of >50%. Coronary atherosclerosis affecting the coronary artery wall but not affecting the coronary lumen was not detectable on an ordinary coronary angiogram.

**Echocardiography**

Standardized echocardiographic examination reports are still not implemented into daily clinical practice in Sweden. However, the echocardiographic evaluation of AS is a fairly straightforward procedure, and in the majority of cases not associated with methodological concerns. However, regardless of echocardiographic findings, all 336 patients were operated on due to some degree of AS and the information from surgical reports in most cases were consistent with preoperative echocardiogram reports.

**General discussion of main findings**

In the first three studies, putative biomarkers for development of AS were explored. We found a difference in association between biomarkers and AS depending on the presence of concomitant CAD. These findings could indicate that AS includes several phenotypes, possibly with different pathobiology, that should be considered when designing interventional trials and it may also be important in designing individual therapies.

Relative survival was very good among the elderly, matching that of the general population, whereas it was lower among younger patients. Women had lower relative survival than men. Classical risk factors, i.e., diabetes mellitus, chronic obstructive pulmonary disease, heart failure, reduced renal function and smoking at the time of surgery, had a clear negative impact on both observed and relative survival. Adding CABG to AVR did not affect relative survival in comparison with isolated AVR. The presence of a bicuspid AS morphology was associated with lower observed mortality compared to tricuspid valve AS, and a relative survival matching that of the general population.

In the first study, we evaluated the impact of Lp(a) and the Apo B/Apo A 1 ratio on the development of AS and found that plasma levels of Lp(a) and the Apo B/Apo A 1 ratio were independently associated with future surgery for AS but only in patients with concomitant CAD. The classical CV risk factors, including Lp(a), have in several studies been associated with the development of AVS requiring surgery, but none of these studies have evaluated the difference in risk factor profile between those with or without CAD. In our study, we found an association between both Lp(a) and the Apo B/Apo A 1 ratio with future surgery for AS but only in the presence of concomitant CAD, an observation not reported previously. Lp(a) has been associated with AS and studies using Mendelian randomization have suggested causality between Lp(a) and the development of AS (16, 172, 173, 175). These studies identified a SNP (rs10455872) in the LPA locus that was strongly associated with Lp(a) levels and also was significantly correlated
with aortic-valve calcification. The presence of coronary artery calcium deposition was considered, but whether this polymorphism is associated with the development of AS in the absence of concomitant CAD is not known. In a population of familial hypercholesterolemia, Lp(a) levels were associated with aortic valve calcification but not with coronary artery calcification (270).

In the second study a panel of 92 CV candidate proteins was analysed in samples obtained at the baseline survey. Six circulating proteins (GDF-15, galectin-4, vWF, interleukin 17 receptor A, transferrin receptor protein 1, and PCSK9) were associated with future surgery for AS in patients with concurrent CAD. In the validation study with 106 additional cases, the association of all but one (interleukin 17 receptor A) of these proteins were replicated in patients with AS and concomitant CAD but not in those without concomitant CAD. Only a few proteins have previously been associated with AS, for example elevated levels of GDF-15 have been found to predict outcome in patients undergoing TAVI (233) and galectin-3 has been associated with AS in cross-sectional studies (234). Previous studies have found association between elevated levels of GDF-15, PCSK9, vWF and CAD (229-231). Therefore, the changes in expression of PCSK9, and vWF could be explained by the presence of CAD and thus they might not be specific predictors of AS. However, using proximity extension assay technique in patients with carotid atherosclerosis, 7 proteins were found associated with case status, and only GDF-15 was overlapping with this study (271).

TnT and CRP was evaluated in the third study. TnT was independently associated with future surgery for AS in patients both with and without concomitant CAD. This could indicate that the progressing stenotic process induces myocardial stress. To our knowledge, the impact of elevated hs-TnT years before surgery for AS has not been studied. Optimally, TnT may be used as a clinical tool and allow for earlier identification of patients with subclinical AS though more studies are warranted exploring if TnT could determine the appropriate timing of AVR. CRP was not associated with surgery for AS and appears to be a poor predictor of subclinical AS.

Several studies have focused on the excellent results achieved following AVR, with survival approaching that of the general population, particularly in the elderly (272, 273). This was also confirmed in our study with survival following AVR matching survival in the general population and in line with previous reports (77, 239). The most likely explanation for this phenomenon is a selection bias for surgery in elderly patients. This could imply that patients who were accepted for surgery on average are healthier than their peers. However, after approximately 6 years there was a decline in relative survival, possibly due to SVD. This underlines the palliative rather than the curative effect of aortic valve surgery in this patient group. Alternative explanations to the decline in relative survival should be explored.

Women had better observed survival than men during the first years following surgery. On the other hand, they had lower relative survival than men the first years following surgery, possibly due to residual confounding in the form of unaccounted comorbidity. This finding is consistent with that of
the study by Enger et al (239). The finding is hard to explain and should be addressed in future studies.

Most previous reports reported inferior results for AVR with concomitant CABG (272). However, populations of AVR and AVR with concomitant CABG differ significantly in most studies, usually there is a higher mean age, more comorbidities and a higher percentage of men in the AVR + CABG cohorts (272). In relative survival analysis, the design provides for adjustments for age and gender and after further adjustments for comorbidities, combined surgery no longer represented a survival disadvantage in our study. Finally, we found that bicuspid aortic valve disease conveyed a survival advantage compared to tricuspid aortic valve disease in the multivariable analysis in our population with survival rates matching that of the general population regardless whether a mechanical or a biological prosthesis was implanted. This is in line with previous investigations (115). One may speculate that there are differences in these two phenotypes, with tricuspid aortic valve disease representing a marker of disease severity or possibly atherosclerotic burden.

An assumption when using relative survival is that survival in the matched general population is unaffected by deaths related to the disease being studied. Due to the relative low prevalence of aortic valve disease, the influence on survival in the general population should be only marginal, at least in the younger population. However, unnecessary deaths from AS was reported in a study by Olsson et al in 1990 (274). Of 105 (68 surgically treated and 37 deceased) eligible patients with AS, 37 individuals did not receive surgical care in time due to non-referral probably because of insufficient knowledge of the curability of the disease.

**Limitations**

Only cases accepted for surgery were included in our studies. Thus, patients with contraindications for surgery or patients with unacceptable high operative risk, as well as asymptomatic patients, were not included. This is worth to consider when interpreting the results of the studies in this thesis. Furthermore, some patients may have declined an offer of surgery.

CAD was treated as a categorical value (yes/no), meaning that the extent of coronary atherosclerosis was not taken into account and the definition of CAD was based on the findings of any degree of coronary artery stenosis visible on the coronary angiogram. Any presence of atherosclerotic lesions in the coronary artery wall not affecting the vessel lumen was thus not identified.

The matched design precludes us from studying the impact of matching factors.

The studied cohort represents northern Sweden, specifically the county of Västerbotten, thus including mainly individuals of Caucasian ethnicity. Therefore, our findings might not be generalizable to other ethnicities or to the aortic stenosis community as a whole.
Since AS is a slowly progressive disease, some individuals could have harbored some degree of subclinical, undiagnosed AS - since no echocardiography was performed at the time of survey - at the baseline, *i.e.*, the time of blood sampling. In an attempt to adjust for this, cases with provided blood samples > 5 years before surgery were analysed separately in order to avoid confounding with disease severity.

Calculation of relative survival is based on the unverified assumption that survival in the general population is unaffected by deaths related to the disease being studied. We cannot tell for sure that this is the case. However, aortic valve disease is relatively rare in the general population.

Exclusion of early perioperative mortality leads to results in favor of surgery.

Selection bias could be suspected since many surgeons are prone to accept patients with few comorbidities for aortic valve surgery, especially among the elderly.

Diagnosed heart failure was analysed in this study, but LV function, a significant predictor of long-term survival after AVR, and hypertension was not considered in our study.

**Future perspectives, clinical implications**

The goal of AVR in patients with significant AS is to alleviate symptoms, to improve quality of life, and to increase life expectancy. Since the majority of patients with AS are old and often have several comorbidities, one of the major problems is to determine to what extent the patient’s symptoms is due to AS versus other comorbidities and geriatric conditions.

The optimal timing of AVR in patients with severe AS is the day before the patient experience any symptoms. However, this is utopic and not accountable for every individual with severe AS since a number of patients are at increased risk even in the absence of symptoms. Consequently, the goal must be to optimize timing of surgery for AS on a patient-by-patient basis with a minimum of risk and a maximum of benefit. This presumes that individuals with severe AS, even asymptomatic, are identified and this is not always the case. However, in western countries, the majority of patients with AS are identified early, prior to the development of symptoms, thus theoretically ensuring best possible management including timing of intervention.

**Improved postop care, secondary prophylaxis**

Solving the mechanical problem with AS, *i.e.*, replacement of the stenotic valve, does not necessary eliminate risk of cardiac events in the postoperative period. Severe and fatal events occur, even after otherwise successful valve surgery. Thus, attention directed toward strategies and interventions which may improve outcomes after AVR is needed and factors that impair outcome should be identified.
Pathobiology

The pathobiology of AS is complex and includes several pathways, genetic factors, haemodynamic factors, like shear and tensile stress, and ageing. Identification and understanding of causal pathways mediating AS may provide novel targets for earlier therapy before end-stage disease. In addition to pharmacologic interventions to reduce cardiovascular risk, treatment might be targeted to specific molecular pathways at various time points in the disease process. Several intervention studies to retard the progression of AS have been performed but with disappointing results. Since the initial stages of degenerative AS show more similarities with atherosclerosis than the progressive phase, in which fibrosis and calcification are more pronounced, late initiation of treatment has been suggested as a reason for the lack of any effect on AS progression rate in these studies. Causality is a key criterion in evaluating whether circulating biomarkers are possible therapeutic targets, and the evidence in support of Lp(a) appears quite favorable. However, there is a need to confirm the causality of Lp(a) on the development of AS. This can only be achieved by clinical trials with treatment strategies showing decreased levels of Lp(a) together with a clear effect on the progression rate of AS.

Improvement in surgical technique and valve prostheses

Despite older patients probably suffering from more comorbidities, results after AVR have improved over the last decades. 30-day mortality in Sweden in low to medium risk patients is around 1%. Whether there will be further improvement in the future is hard to tell. Long term outcome would certainly improve if the properties of prosthetic heart valves were refined; a valve prosthesis with no need of anticoagulation, lifelong durability, with no reduction in the aortic orifice area and that can be easily implanted. Unfortunately, with most certainty, none of us will experience this improvement in valve design during our lifetime.

Risk markers

So far, biomarkers have not played a significant role in the management of AS patients except for natriuretic peptides. The efforts to elucidate the pathobiology of AS will likely yield novel insights into potential therapeutic targets to reverse or prevent AS. Given the multiple pathobiological processes involved in the development of AS, a combination of biomarkers is more likely to prove beneficial than any single marker. A combination of ST2, GDF-15 and N-terminal pro-BNP in addition to troponins has been suggested as useful biomarkers for predicting prognosis in AS. Biomarkers that can detect earlier stages of the valvular disease have yet to be generated and utilized.

Tailored management strategies

Phenotyping and risk stratification in AS patients will probably become more sophisticated, with more differentiated managements between patients. Several factors, like symptoms, LV response to increased pressure
load, operative risk, and biomarkers will be taken into account together with
the assessment of AS severity in the management strategies of AVR. In
precision medicine, biomarkers will be used to establish signatures from
which therapies can be customized to suit individual patients.

Relative survival

The approach of relative survival in survival analyses in cardiac disease
and after cardiac surgery has not been much utilized. Relative survival gives
an estimate of survival due to the disease of interest without the need for
cause of death information. Estimates of relative survival provide
information of the “curative” effect of AVR. In elderly, excellent relative
survival after AVR is seen during the first years. Thereafter relative survival
decline. Possibly, structural valve deterioration is an explanation but
alternative explanations need to be explored.

Finally, gaps in evidence as listed by Baumgartner et al are given (54).
- The impact of earlier markers of LV dysfunction on postoperative outcome
  requires further research.
- The identification of patients with low-gradient aortic stenosis who have
  severe stenosis and would benefit from intervention requires improvement.
- The criteria for identification of patients who would benefit from early
  elective surgery in asymptomatic severe aortic stenosis requires further
  research.
- Long-term follow-up after TAVI is required; in particular, the long-term
  durability of the valves needs to be studied.
- Criteria for when TAVI should no longer be performed since it would be
  futile need to be further defined.
- Identification of causal pathways mediating AS may provide novel targets
  for earlier therapy before end-stage disease.
Conclusions

- Elevated levels of Lp(a) and a high Apo B/Apo A 1 ratio were associated with future surgery for AS, independent of traditional cardiovascular risk factors. The associations were only seen if concomitant CAD was present. This suggests that AS has different phenotypes, which should be considered in future research on targeted risk factor interventions in this population.

- Alterations in five proteins - GDF-15, galectin-4, PCSK9, transferrin receptor protein 1 and vWF – occurred years before AVR was required. The associations seemed to be driven by concomitant CAD. It remains to be investigated the role of these protein alterations and further validation in external studies are warranted.

- Elevated plasma levels of TnT were independently associated with future surgery for AS, irrespective of the presence of concomitant CAD, which could indicate that the myocardium is subject to mechanical stress already when the stenotic process is subclinical. Optimally, troponin T could be used in clinical management decisions in patients with AS.

- CRP was not associated with the need of future AVR.

- Relative survival following AVR was very good, especially in the elderly with survival matching that of the general population.

- Women had decreased relative survival compared to men. This should be explored in future studies.

- Adding coronary artery surgery to an AVR procedure was not associated with increased risk.

- Bicuspid morphology was associated with lower observed mortality compared with tricuspid valve morphology, and with a relative survival matching that of the general population. One may speculate that tricuspid aortic valve disease represents a marker of disease severity or possibly atherosclerotic burden.
Acknowledgements

To fulfill the work of a thesis on your own is certainly a “Mission Impossible”. You are really in need of support. Many people have in different ways helped and supported me, and many people have inspired and supported me just by their existence.

I would like to express my gratitude to...

Ulf Näslund, my principal supervisor and my good friend since many years, pretty good ornithologist, once young and promising in badminton, now only “and”, for great help and support and an unbelievably ability to, in no time at all, review a manuscript and find any, any, any error or illogicality, and provide for invaluable feed-back. More kayaking will come. And cinema nights!

Stefan Söderberg and Michael Henein, my co-supervisors; two truly gentlemen with really big hearts and a never-ending positivism and optimism during my struggle towards the fulfilling of this thesis. No matter how stupid question I put on the e-mail, you would answer within no time at all.

My main co-authors, Johan Ljungberg, Ingvar Bergdahl, Bengt Johansson, Johan Hultdin, Tone Bull Enger, Alexander Wahba and Vibeke Videm. It has been a pleasure to co-operate.

Jan Hentschel and Göran Johansson, two wizards on computers, absolutely invaluable for a computer moron like me. They finish things on the computer within 5 minutes, things that would take me 5 years to accomplish, if at all.

Eva Jonasson, Elin Lindahl, Kerstin Rosenqvist, Catrin Johansson, Stina Jakobsson, Helena Karlsson, Karin Olsson and Eva Mäkitalo for guiding me in bureaucracy and paper-work that I am not able to handle without help.

All participants in the three health surveys, VIP, MONICA, MSP for voluntary providing data and blood samples for future research. In a way, these people are the true pioneers in the search of new knowledge.

The staff at the Clinical Chemistry Laboratory.

The staff at the Department of Biobank Research, Elin Albertsson, Paul Holmer, Veronica Hellström, Åsa Ägren, Göran Hallmans, Camilla Ring, Mattias Söderberg, Kerstin Enquist, Robert Johansson and Christina Evaldsson.

Fredrik Holmner, my boss, my good friend, a great cardiothoracic surgeon, a lousy ornithologist and without any knowledge at all on mushrooms, for providing time to fulfill this thesis and for countless instances of joy, good laughs and nice AWs and parties.

Per Lindqvist, recently appointed professor in clinical physiology (my congratulations), for friendship and interesting discussions not only
regarding scientific issues but also about life in general and music in special. We shared an unforgettable moment two years ago, the King Crimson concert in Stockholm. I bet everyone envy us.

Magnus “You'll never walk alone” Hedström, former Head of the Heart Centre (now locked up in some dull place at the top management of the Västerbotten County Council; but for sure you will make a difference), for providing resources to fulfill this work, and for inspiring daily discussions on almost any subject. And for countless of good laughs.

Torkel Åberg, my first surgical mentor, for believing in me and not sending me out in nowhere. “Possibly you will make a good enough cardiothoracic surgeon” he stated. His golf swing still needs to be improved and his ability to count score is surprisingly poor considering his skills in healthcare economy. His excellent skills and co-ordination as a cardiothoracic surgeon did not transfer to his game of golf, so to say.

Bengt Andersson, Anna Bohlin, Hanna Forsberg, Anne-Marie Grenholm, Jan Hellström, Hans-Petter Ildgruben, Stefan Jakobsson, Martha Jóhannesdóttir, Mattias Karlsson, Leo Petrini, Louise Stark, my colleagues at the Department of Cardiothoracic Surgery for providing best possible social climate for the daily clinical work, and for a common struggle towards “always the best for the patient” thinking.

All other colleagues and co-workers at the Heart Centre of Umeå University Hospital for making daily clinical work joyful and meaningful.

Britt-Inger Lydig for major daily work support.

Elisabeth Ståhle and Lena Jidéus, my two favorite cardiac surgery colleagues outside of Umeå, for many interesting discussions on research as well as on any other subject, with or without accompanying dinner and/or champagne.

My children, my greatest pride, Hanna, Mattias and Erik. Merely your presence has inspired me to fulfill this work.

My mother, Märta-Lisa, and my siblings, Anna, Magnus, Mats and Erika with families. I probably have the best family possible.

Marianne, my beloved life companion, for the never-ending support and understanding in me being somewhat strange and mentally absent during the first months of this year. The drawings in this thesis are beautiful - and so are you.

Finally, I also would like to acknowledge Terry Kath†, (probably the best ever on guitar), Pat Metheny, Bruce Springsteen, Donald Fagen, Frank Zappa†, David Bowie†, Prince†, Jeanette Lindström, Miles Davis†, Robert Fripp, Kate Bush, Ivan Morrison, Jon Anderson, Chris Squire†, Roy Buchanan†, Annie Lennox, Dmitri Shostakovij†, Candy Dulpher, Peter leMarc, Jon Hassell, Steven Wilson including Bass Communion, Veronica Maggio, Michael Brecker†, Neil Young, Robert Lamm, Walter Parazaider, James Pankow, Joni Mitchell, Lee Loughnane, Daniel Seraphine, Lyle Mays, Steve Rodby, Dicky
Betts, Evert Taube†, Duane Allman†, David Cross, Richard Bona, Ryuichi Sakamoto, The Orb, Ludvig van Beethoven†, Alva Noto, John Williams, Vangelis, Hans Appelquist, Helios, Boards of Canada, Jimmy Page, Mick Jagger, Keith Richards, Charlie Watts, Bill Wyman, John Lennon†, Paul McCartney, George Harrison†, Paul Wertico, Brian Jones†, Ian McDonald, Mark Egan, Jaco Pastorius†, Cuong Vu, Allan Pettersson†, Sergei Rachmaninov†, Snarky Puppy, John Wetton, Antonio Sanchez, Ringo Starr, David Sylvian, Alan Holdsworth†, Walter Becker†, Peter Gabriel, Maurice Ravel†, Rick Wakeman, Steve Howe, Alan White, Bill Bruford, Lou Reed†, Tony Levin, Glenn Gould†, Sergey Prokofiev†, Greg Allman†, Clarence Clemons†, Tommy Bolin†, Rory Gallagher†, Stevie Ray Vaughan†, Jan Akkerman, (could anyone tell me if the guitar solo on “Answers?Questions! - Questions?Answers!” on the album “Focus Live at the Rainbow 1973” is possible to perform; I know that JA did it, but how?), Thijs van Leer, Arvo Pärt, Daniel Federici†, Daryl Hall, Esbjörn Svensson†, Johann Johannsson, Dan Berglund, Jimi Hendrix†, Jeff Beck, Magnus Öström, Paddy McAloon, David Byrne, Johannes Brahms†, Mike Bloomfield, Brian Eno, Laurie Anderson, Steve Reich, Eric Clapton, Bryan Ferry, Steve Winwood, Claude Debussy†, Roy Bittan, Peter Sinfield, Keith Emerson†, Randy Brecker, Al Cooper, Jamie Muir, Peter Thorup, Ian Hunter, Grace Jones, John Martyn†, Mel Collins, John McLaughlin, Mike Scott, Mike Stern, Fryderyk Chopin†, John Adams, Nils Frahm, Paul Simon, Pete Townsend, Harold Budd, Michael Giles, Billy Cobham, Rued Langgaard†, Carlos Santana, Philip Glass, David Gilmour, Lee Ritenour, John Holm, Roger Waters, Jean Sibelius†, Chrissie Hynde, Robbie Robertson, Derek Trucks, Susan Tedeschi, Piotr Tchaikovsky†, Leonard Cohen†, Greg Lake†, Frank Sinatra†, Lars Danielsson, Jonas Knutsson, Jonas Kullhammar, Jan Lundgren, Garland Jeffreys†, Eldkvarn, Ulf Lundell, Dan Hylander, Py Bäckman, Povel Ramel†, Ove Törnyqvist, Richard Strauss†, John Zorn, Aaron Copland†, Tony Joe White, Valentin Silvestrov, Keith Jarrett, Gustav Mahler†, Edvard Grieg†, Steve Gadd, Phil Collins, Ane Brun, Bruce Hornsby, Annika Norlin, Henryk Gorecki†, John Coltrane†, Kent, Sofia Karlsson, Michel Camilo, Biosphere, Yellowjackets, Bob Seger, Stephen Stills, Herbie Hancock, Sven-Bertil Taube, Elvis Costello, Franz Liszt†, Modest Mussorgskij†, Bo Linde†, Jennifer Higdon, Rolf Wikström, Tom Waits, Patti Smith, Jan Johansson†, Dave Holland, Manfred Mann, Chris Thompson, John Lee Hooker, Ray Charles, Kim Carnes, Aretha Franklin, Dave Clemmpson, Ginger Baker, Jack Bruce, JJ Cale, Cab Calloway, Georgie Fame, Robert Plant, Alexis Korner, T Bone Walker, Steven van Zandt, Lang Lang and probably some other great musician or composer that I have forgot, whose music helped me to recover during different phases of this work.
Grants

This work was supported by grants from

Swedish Heart–Lung Foundation (grant numbers 20140799, 20120631 and 20100635),
The County Council of Västerbotten (ALF, VLL-548791),
The Heart Foundation of Northern Sweden and
an unconditional grant from Carl Bennet Ltd, Sweden.


72. Sabik JF, Lytle BW, Blackstone EH, Marullo AG, Pettersson GB, Cosgrove DM. Aortic root replacement with cryopreserved allograft for


221. Daniels LB, Laughlin GA, Clopton P, Maisel AS, Barrett-Connor E. Minimally elevated cardiac troponin T and elevated N-terminal pro-B-


240. Statistics Sweden: Life table by sex and age. Year 1960 – 2018. (Data obtained through the Human Mortality Database, wwwmortalityorg, on 16022018)


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