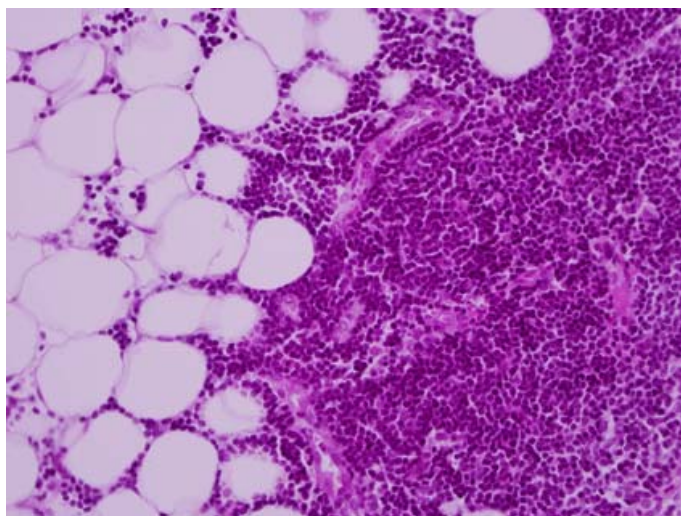


# **Vascular Inflammation in Rheumatic and Non-rheumatic Patients: A Controlled Study of Biopsy Specimens Obtained During Coronary Artery Surgery (Feiring Heart Biopsy Study)**

**Doctoral thesis by  
Ivana Hollan**

Hospital for Rheumatic Diseases, Lillehammer, Norway  
The Feiring Heart Clinic, Feiring, Norway  
Rikshospitalet-Radiumhospitalet University Hospital, Oslo, Norway



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Imagination is more important than knowledge.  
For knowledge is limited to all we now know and  
understand, while imagination embraces the entire  
world, and all there ever will be to know and  
understand.

*Albert Einstein (1879-1955)*

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## Abbreviations

ANCA	Anti-neutrophil cytoplasmic antibody
CABG	Coronary artery bypass grafting
CAD	Coronary artery disease
CRP	C-reactive protein
ESR	Erythrocyte sedimentation rate
FDG-PET	Fluorodeoxyglucose-positron emission tomography
GCA	Giant cell arteritis
IMA	Internal mammary artery
IRD	Inflammatory rheumatic disease
MCI	Mononuclear cell infiltrate
NSAIDs	Non-steroidal anti-inflammatory drugs
NYHA	New York Heart Association Functional Classification
RA	Rheumatoid arthritis
SLE	Systemic lupus erythematosus
TNF alpha	Tumor Necrosis Factor – alpha



# List of papers

The present thesis is based on following papers, referred to by their Roman numerals:

## **Paper I**

Inflammatory Rheumatic Disease and Smoking Are Predictors of Aortic Inflammation: a Controlled Study of Biopsy Specimens Obtained at Coronary Artery Surgery. Hollan I., Scott H., Saatvedt K., Prayson R., Mikkelsen K., Nossent H.C., Kvelstad I.L., Liang M.H., Forre O.T. *Arthritis Rheum* 2007; 56(6):2072-9.

## **Paper II**

Inflammatory Cell Infiltrates in Vessels with Different Susceptibility to Atherosclerosis in Rheumatic and Non-Rheumatic Patients. A controlled study of biopsy specimens obtained at coronary artery surgery. Hollan I., Prayson R., Saatvedt K., Almdahl S.M., Nossent H.C., Mikkelsen K., Liang M.H., Kvelstad I.L., Aamodt G., Forre O.T. *Circ J* 2008; 72(12):1986-92.

## **Paper III**

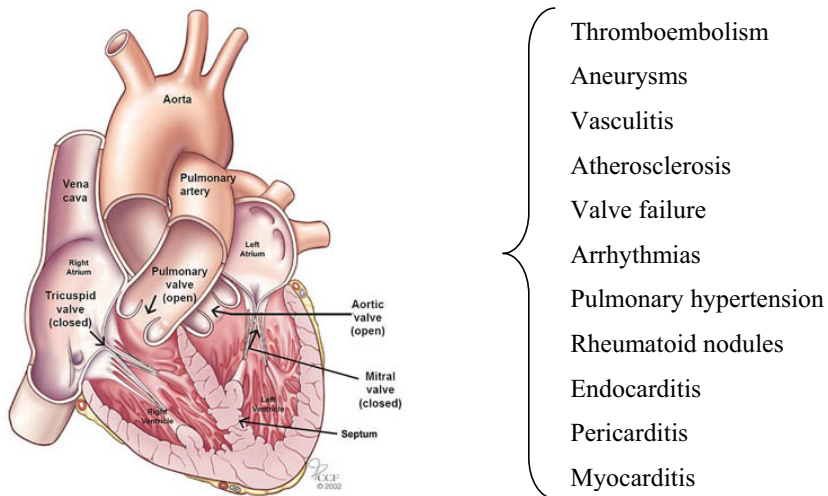
Spondyloarthritis: a Strong Predictor of Early Coronary Artery Bypass Grafting  
Hollan I., Saatvedt K., Almdahl S.M., Mikkelsen K., Moer R., Halvorsen P., Veel T., Forre O.T. *Scand J Rheumatol* 2008; 37(1):18-22.

# 1. General introduction

## 1.1 Cardiovascular complications in inflammatory rheumatic diseases

Inflammatory rheumatic diseases (IRD) are associated with various cardiovascular manifestations, including peri-, myo-, and endocarditis, valvular abnormalities, heart failure, arrhythmias, aortic aneurysms, arterial and pulmonary hypertension, thromboembolism, amyloidosis, vasculitis, and atherosclerosis (Figure 1) (1;2).

**Figure 1. Cardiac and vascular manifestations in rheumatic diseases**



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### 1.1.1 Coronary artery disease in inflammatory rheumatic diseases

Coronary artery disease (CAD), predominantly caused by atherothrombosis, is a leading cause of mortality in both rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) (3-10). In RA, that affects 0.5% of population, most of the observed standardized mortality ratios for cardiovascular death range between 1.13 and 5.15, depending on the selected patient population (10). The cardiovascular risk is increased also in young individuals; Bjornadal et al. found a 5.5 –fold increase in the risk for cardiovascular death in 20-39 year old women with RA compared to women without RA (11). In RA, CAD is often unrecognized and thus, undertreated (12-15). Acute coronary syndromes in RA are more frequent and more severe, associated with higher relapse and mortality rates, than observed in the general population (12;16). Patients with SLE are 5-10 times more likely to experience a coronary event than the average individual in the healthy general population (7). Esdaile et al. estimated a 17-fold higher risk of mortality secondary to CAD in SLE compared to the general population (17).

Manzi et al. demonstrated a 50-fold higher risk of a myocardial infarction in women 35 to 44 years old with SLE compared to women without SLE (18).

Although cardiovascular research in rheumatology has focused mainly on RA and SLE, there is increasing evidence that other IRDs, including primary vasculitides, antiphospholipid syndrome, and gout, may be associated with accelerated atherothrombosis (4;19-21). In a study on Pima Indians with unspecified arthritis, a 2-fold increase in cardiovascular mortality was found in those with two or more swollen joints compared to those with no swollen joints (22).

Cardiovascular morbidity in IRD is related to traditional cardiovascular risk factors, medication side effects, and the activity and severity of the rheumatic disease (8;9;23;24). There are indications that effective antirheumatic treatment may lower cardiovascular risk (4;25-29).

### 1.1.2 Cardiac involvement in spondyloarthritis

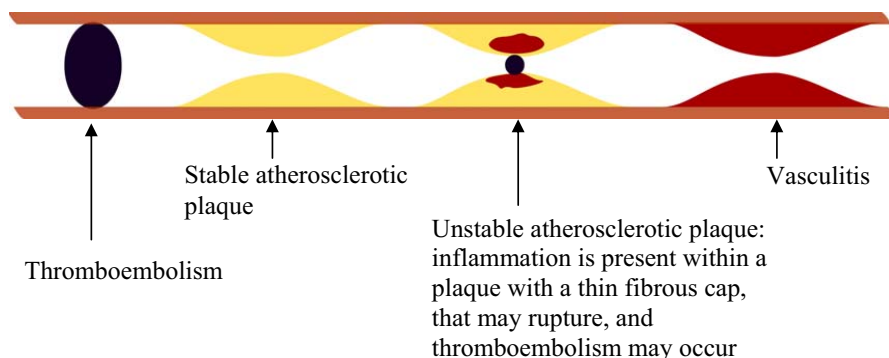
It has long been known that spondyloarthritis are associated with cardiovascular comorbidities related to HLA-B27 genotype, inflammation, and disease duration (30;31). The most characteristic complications are aortic root and valve disease and conduction disorders caused by a sclerosing inflammatory process involving small arteries (obliterative endarteritis) (31-33). Aortic valve and root involvement have been detected in ankylosing spondylitis in more than 50% of patients by echocardiography, and in up to 100% of patients in autopsy studies (31;34). The most common valvular disorder in spondyloarthritis is aortic insufficiency, caused by aortic root dilatation and/or cusp pathology.

Although CAD morbidity is increased in autoimmune diseases, only a few studies have addressed this topic in the spondyloarthritis (35). However, some studies indicate a proatherogenic risk profile and an excess of endothelial damage and/or atherosclerosis in patients with ankylosing spondylitis and psoriasis/psoriatic arthritis (30;35;36;36;37;37-41;41-44). Caliskan et al. found a reduction in the coronary flow reserve in spondyloarthritis, indicating impaired macro- and/or microvascular cardiac circulation (45). The coronary flow reserve was independently related to systemic inflammatory biomarkers.

## 1.2 Causes of vascular lumen narrowing

CAD is mainly caused by a narrowing of a vascular lumen, usually due to atherosclerosis. The narrowing may be also caused by other pathologies, including thromboembolism, vasospasm, aneurysms, and vasculitis, which imply different follow up and treatment procedures (Figure 2). However, due to limited diagnostic tools, it may be difficult or impossible to distinguish these conditions from each other.

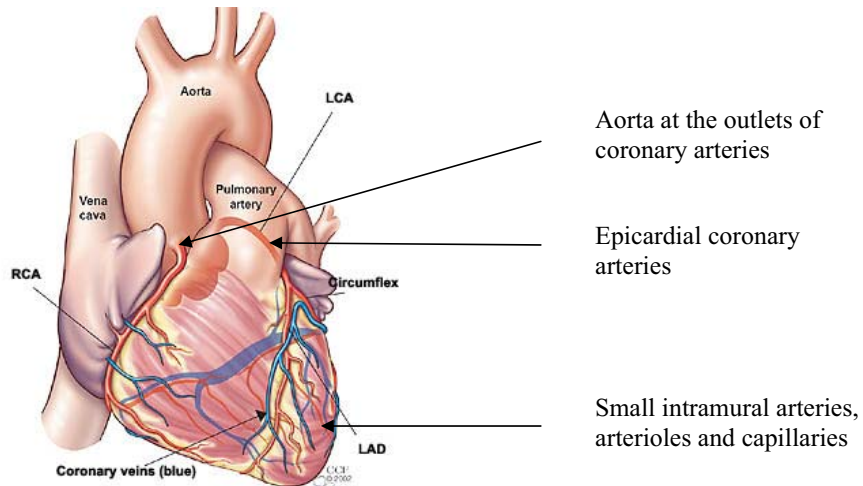
**Figure 2. Causes of vascular lumen narrowing**



### 1.2.1 Aortic and cardiac vasculitis

Cardiac ischemia may be caused by vasculitis in the epicardial coronary arteries, in small cardiac vessels, and/or in the aorta, when the inflammation affects the outlets of coronary arteries (Figure 3). An aortic inflammation may also result in formation of aortic aneurysms or stenoses, and aortic valve failure.

**Figure 3. Sites of vascular stenoses that may cause myocardial ischemia**



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The true prevalence of aortitis and cardiac vasculitis is uncertain due to the limited diagnostic tools and the small number of studies that have addressed the topic. Furthermore, the criteria for histological diagnosis of vasculitis and their interpretation may vary between the studies, and contribute to variations in the results.

Aortitis and cardiac vasculitis occur in various rheumatic and other conditions (32;46-54). Aortitis has been described in primary vasculitides (Takayasu's arteritis, GCA [giant cell arteritis], Kawasaki disease, ANCA associated vasculitides, hypersensitivity vasculitis, Behcet's disease, Cogan's disease), RA, spondyloarthritides, juvenile idiopathic arthritis, polymyalgia rheumatica, SLE, relapsing polychondritis, infections (e.g. syphilis), sarcoidosis, and malignancies. In addition, aortitis may manifest as an idiopathic, single-organ condition. The clinical significance of isolated aortitis, and therefore, its optimal treatment and follow up, is not clear (52;55). The pathology of aortitis is frequently non-specific, contributing little to differential diagnosis. Giant cells, for example, that typically occur in GCA and Takayasu's arteritis, can also occur in other rheumatic diseases and non-rheumatic conditions (including idiopathic inflammatory aneurysms and sarcoidosis) (32).

According to available studies, aortic and cardiac vasculitis appear to occur in IRDs more frequently than commonly assumed in clinical practice. For example, an autopsy study revealed cardiac vasculitis in 17 (10%) of 169 consecutive RA subjects, and 10 of those 17 experienced myocardial infarctions (56). Another autopsy series of 188 RA patients identified 10 patients with aortitis, of which three died as a direct result of aortitis and four others of a

myocardial infarction secondary to vasculitis. Typically, the diagnosis was not made before death (57). Up to 30-50% of RA patients have subclinical aortic abnormalities on echocardiography, but the clinical significance of this finding is unclear (32). In an X-ray study of RA patients, enlargement of the aortic root predicted a higher risk of mortality (58).

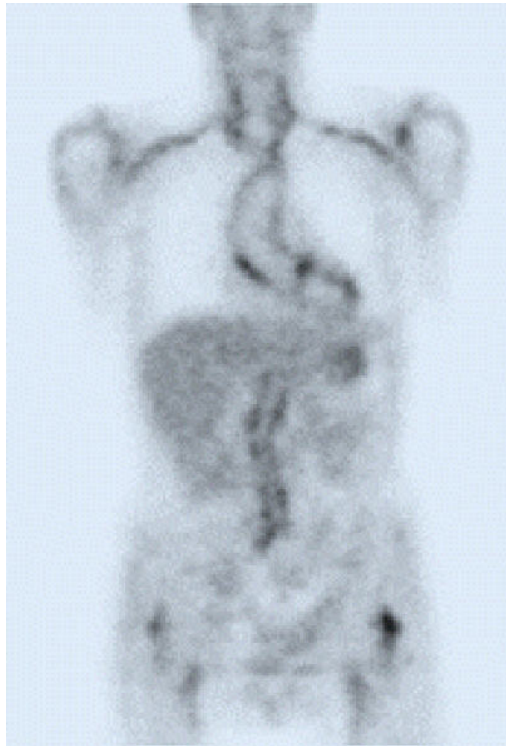
Vasculitis in the aorta and its large branches is increasingly considered to be a feature of GCA (59) (Figure 4). A study using fluorodeoxyglucose-positron emission tomography (FDG-PET) revealed aortic inflammation in >50% of GCA patients (60).

### **1.2.2 Diagnostic challenges in detecting aortic and cardiac vasculitis**

Vasculitis is a potentially life-threatening condition, treatable with immunosuppression. Because immunosuppressive treatment may cause serious side effects, the diagnosis should be accurate. Unfortunately, tools for detecting aortitis are limited, and it is almost impossible to diagnose cardiac small vessel vasculitis and coronaritis antemortem. These conditions have therefore been commonly unrecognized and undertreated, and knowledge about their prognosis and optimal treatment is also limited. Many cases were not diagnosed until performing an autopsy. Ischemic symptoms of aortitis are unspecific, and systemic symptoms of inflammation are not always present (52;53). Elevated levels of acute-phase reactants, including C-reactive protein (CRP) or the erythrocyte sedimentation rate (ESR), are not pathognomonic. In addition, in active vasculitides, these levels may be within a normal range. Angiography provides anatomic information about stenoses and aneurysms, but does not distinguish between etiologies. The types of lumen changes and the fluctuations observed on repeated examinations may evoke a suspicion of vasculitis for some patients, but the specificity is low.

Serial imaging by magnetic resonance angiography with edema-weighted images may reveal wall thickening or “edema”, but these findings do not always indicate active inflammation (49;61). Fluorodeoxyglucose-positron emission tomography (FDG-PET) is a sensitive method for demonstrating large vessel vasculitis, but is not suitable for detecting areas smaller than 3-4 mm in diameter (62) (Figure 4). Also other novel imaging modalities, including intravascular ultrasound, might be useful in detecting vasculitis. However, the usefulness of intravascular ultrasound is limited, because it is an invasive and expensive procedure. The gold standard for diagnosing vasculitis is histology, but obtaining actual specimens from living patients is extremely challenging. Although collecting vascular specimens from coronary endarterectomies is possible, the specimens do not contain the whole vessel wall; thus, their usefulness is primarily limited to studies of the components of atherosclerotic lesions. Myocardial biopsies are associated with a risk for complications, and they likely have low sensitivity due to the small tissue samples. Histological examination of the aorta in living patients has been performed primarily with specimens from surgery for aortic valvular disorders or aneurysms. However, this approach is probably seldom helpful in clinical practice because these surgeries are performed only in a relative small number of patients, and clinicians are likely to overlook this diagnostic opportunity.

**Figure 4. FDG-PET scan in giant cell arteritis**



*FDG-PET scan showing FDG uptake in thoracoabdominal aorta as well as subclavian, axillary, carotid and iliac arteries of a patient with GCA, indicating a widespread vascular inflammation. (Courtesy of Thorsten A. Bley.)*

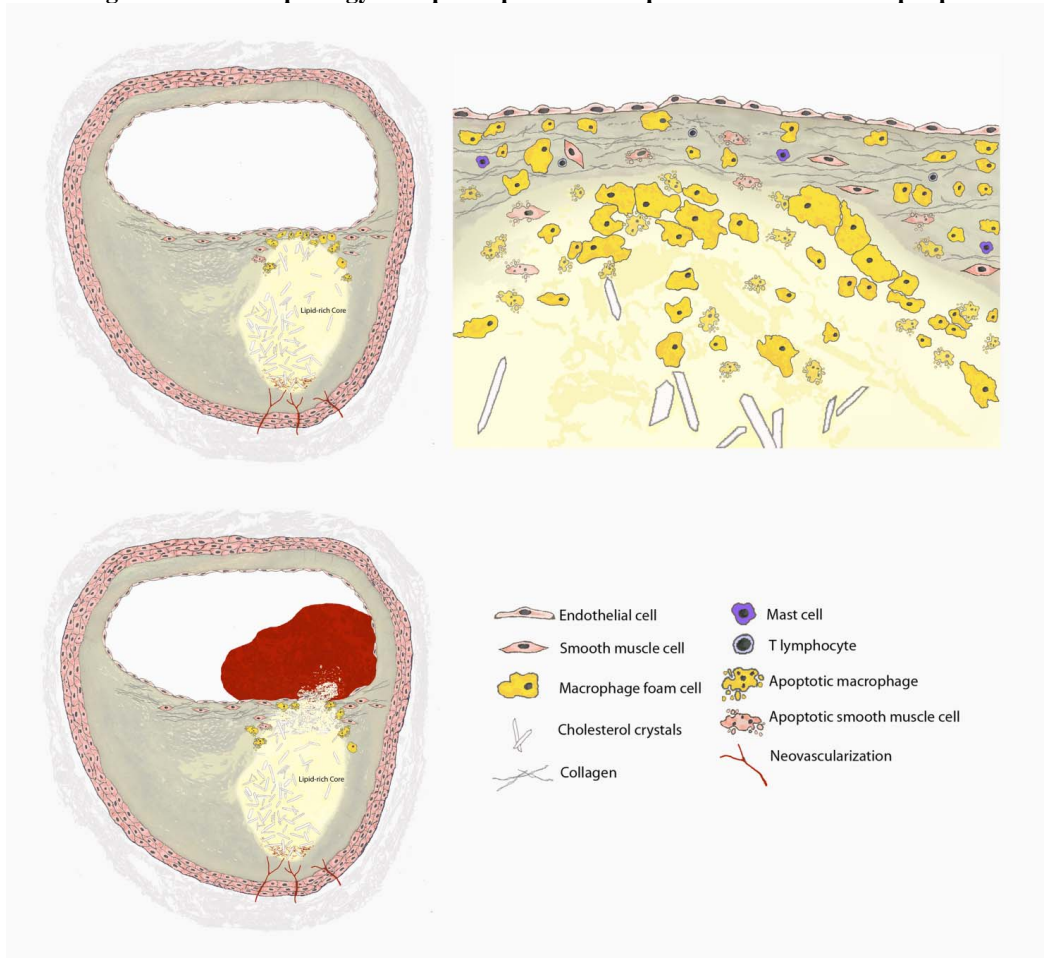
### **1.3 Links between atherosclerosis and inflammation**

Atherosclerosis is an inflammatory disease (63;64). Atherosclerotic lesions contain inflammatory cells (predominantly T cells and macrophages) that appear to contribute to plaque formation and destabilization. Destabilization of plaques leads to their rupture and consequent thrombosis, representing a crucial pathomechanism in acute coronary syndromes (Figures 2 and 5).

In addition to inflammation in vascular lesions, signs of persistent low-grade systemic inflammation are associated with atherosclerosis. Levels of systemic inflammatory biomarkers, including CRP and Tumor Necrosis Factor – alpha (TNF alpha), are related to the severity of cardiovascular disease, and predict future cardiovascular events in patients with cardiovascular disease and in an apparently healthy population (65;66). Even small differences in CRP levels, measured by high-sensitivity assays, rather than conventional methods, appear to be significant (67). The role of systemic inflammation in atherosclerosis has not been fully elucidated. Systemic inflammatory mediators may be secondary to the inflammatory process in atherosclerotic lesions, and/or underlying cardiovascular risk factors (smoking, hypertension, or obesity) (63). Systemic inflammatory biomarkers may also promote atherothrombosis. For example, proinflammatory cytokines, including TNF alpha,

IL-1beta, or IL-6, induce dyslipidemia, insulin resistance, prothrombotic status, endothelial dysfunction, and oxidative stress (enhancing the oxidation of LDL to the highly proatherogenic ox-LDL) (66;67).

**Figure 5. The morphology of rupture-prone and ruptured atherosclerotic plaques**



Top left panel illustrates a rupture-prone plaque. The lipid-rich core occupies approximately 40% of the plaque area and contains multiple cholesterol crystals. The fibrous cap is thin and inflamed with few smooth muscle cells. Plaque microvessels originating from the adventitia extend through the media into the base of the plaque, and the plaque tends to grow outwards (expansive remodelling). Top right panel illustrates the thin and locally weakened fibrous cap. Macrophages are abundantly present whereas smooth muscle cells are scarce. Bottom left panel illustrates the corresponding ruptured plaque with consequent thrombus covering the rupture site. (Reproduced with permission from Thim et al. *J Intern Med.* 2008;263:506-16.)

It has been suggested that the pathologies of IRD and atherosclerosis might share some inflammatory and immunologic pathways (4;29;68). In fact, the inflammatory/immunologic

response in atherosclerosis is surprisingly similar to that in RA (69;70); for example, TNF alpha and IL-6 play important roles in both conditions. In addition, a clonal expansion of CD4+CD28- cells has been observed in the peripheral blood of patients with RA and those with acute coronary syndrome, but not healthy individuals. CD4+CD28- cells have been also found in rheumatoid synovitis and unstable atherosclerotic lesions. These senescent T cells lack the co-stimulatory CD28 molecule, and exhibit features of natural killer cells with highly proinflammatory properties (29;70;71). Similarities in the pathogenesis of RA and atherosclerosis are highlighted by some common risk factors, including smoking.

Because cardiovascular morbidity is increased in various disorders associated with chronic inflammation, including autoimmune diseases, chronic infections, diabetes mellitus, depression and obesity, a causal relationship between chronic inflammation and cardiovascular disease has been suggested (4). Indeed, the features of inflammatory processes differ among the respective diseases. For example, in contrast to many other inflammatory conditions, CRP levels usually remain low during active SLE. Nevertheless, chronic inflammatory conditions are likely to share some important common pathways that might play a crucial role in premature atherothrombosis; e.g., defects in immunocompetent cells, metabolic effects of chronic inflammation, and/or endothelial injury/vascular inflammation (4;21).

Although insights into the inflammatory response in atherosclerosis are rapidly being revealed, and several candidate antigens have been identified, the precise mechanism underlying atherosclerosis is still unclear. Several studies have proposed roles for infections (e.g. cytomegalovirus, Chlamydia pneumonia, or pathogen burden) and autoimmunity (72-75). Atherosclerosis is associated with the occurrence of several autoantibodies, including antibodies to oxidized low density lipoprotein, antibodies to beta 2 glycoprotein I, antibodies to heat shock proteins, and antibodies to endothelial cells. According to the “Response-to-Injury” hypothesis, atherosclerosis may be caused by vascular injury induced by various factors, such as pressure, chemical substances, infections, etc. (69).

## **1.4 Resistance to atherosclerosis in different vessels**

Blood vessels have different susceptibilities to atherosclerosis. Veins, for example, are usually less vulnerable than arteries. However, differences exist also within the arterial tree (76;77). For example, the internal mammary artery (IMA), though similar in dimension to the coronary arteries, is highly protected from atherosclerosis (78). The risk for vascular stenosis remains low even when the IMA is used for a coronary graft; IMA conduits have longer patency than other donor vessels, including the saphenous vein (79-81).

In theory, differences in the prevalence of atherosclerosis in different vessels may be due to regional vascular properties, pressure, and/or intraluminal rheologic properties that determine susceptibility to injury and inflammation (82).

## **1.5 The driving force behind the study**

The journey of this project started at a course of autoimmune diseases for rheumatologists. A cardiologist stressed the points that vasculitis was a potential cause of CAD in rheumatic diseases, and that a different therapeutic approach was needed for cardiac vasculitis versus atherosclerosis. However, he was not able to answer the question of how these two conditions can be distinguished. Therefore, we started to look for diagnostic tools that could demonstrate cardiac vasculitis by tissue examination. We focused on the potential of taking biopsies during coronary artery bypass grafting (CABG), because it is a relatively common form of cardiac surgery. We were not able to perform coronary artery biopsies due to the high risk it posed for patients, but we found a safe way to collect aortic and cardiac tissue (containing small cardiac vessels). At that point, we became aware of the challenges involved in the



interpretation of histological criteria for vasculitis (see General discussion). Therefore, we decided to evaluate vascular inflammation in a descriptive manner rather than distinguish between vasculitis and non-vasculitis.

In addition to vasculitis, we were interested in the mechanisms that promote atherosclerosis in IRD. While most research concerns the importance of traditional cardiovascular risk factors and circulating inflammatory mediators, we focused on finding clues directly in the vascular tissue. We hypothesized that patients with IRD might be prone to vascular inflammation (including low-grade forms) that might promote plaque formation and/or destabilization. The susceptibilities of different types of vessels to inflammation might explain differences in their vulnerabilities to atherosclerosis.

We were frequently asked by our colleagues whether patients with different IRDs underwent CABG earlier than patients without IRD, because it might indicate an earlier debut of severe CAD in the respective IRDs. Because relatively little research has addressed the role of CAD in spondyloarthritides, we were particularly interested in the age of patients with spondyloarthritis.

To identify factors that might play a role in the pathogenesis of cardiovascular disease and assess their importance, we wanted to establish a comprehensive long term data- and biobank containing biological material and information about patients with and without CAD.

These grounds gave rise to the aims for our study.

## **2. Main hypotheses and aims**

### **2.1 Hypotheses**

- Aortic inflammation might be detectable in tissue specimens removed during CABG.
- Local vascular inflammation in any vascular layer might, in some cases, lead to formation of atherosclerotic lesions and/or to their destabilization.
- Vascular inflammation, including that localized outside atherosclerotic lesions, might be more common and pronounced in patients with IRD compared to those without IRD, and contribute to the high cardiovascular risk in IRD.
- The susceptibilities of different types of vessels to inflammation might underlie differences in their vulnerabilities to atherosclerosis.
- Patients with spondyloarthritis have premature CAD, and therefore undergo a first CABG earlier than those without spondyloarthritis.

### **2.2 Purpose**

We wanted to extend the knowledge regarding the existence and features of vascular inflammation. In addition, we aimed to identify a diagnostic tool for aortic inflammation and indicators of premature CAD in spondyloarthritis.

### **2.3 Aims**

- To determine whether aortic specimens obtained during CABG would be suitable for detecting aortic inflammation.
- To compare the occurrence and extent of inflammatory cell infiltrates in the aorta of CAD patients with and without IRD.
- To compare vessels with different susceptibilities to atherosclerosis (aorta, saphenous vein, IMA) for the occurrence of mononuclear cell infiltrates outside of atherosclerotic lesions.
- To look for predictors of vascular inflammatory infiltrates in non-atherosclerotic areas.
- To examine whether patients with spondyloarthritis underwent a first CABG at a younger age than those without spondyloarthritis.
- To establish a research data- and biobank for future studies.

### 3. Methods

In this chapter, I describe only the cross-sectional part of the study discussed in Papers I-III. However, we have planned a follow up of the CABG groups to assess the outcomes.

#### 3.1 Patients

##### 3.1.1 Patient groups described in Papers I and II

###### CAD patients with IRD (CAD-IRD group, n=70)

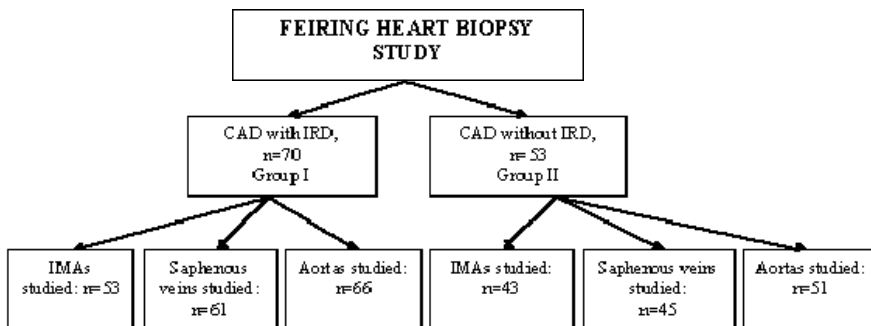
The inclusion into the Feiring Heart Biopsy Study began in 2001. We planned to include all patients with IRD that had been referred to CABG at the Feiring Heart Clinic and the University Hospital of North Norway, Tromsø. However, only one patient (with RA) was enrolled in Tromsø. All the other subjects were consecutively enrolled at the Feiring Heart Clinic, where all subjects referred for CABG between May 2001 and January 2005 were actively screened for rheumatic diseases (due to I. Hollan's illness, no patients underwent biopsies between July 2004 and January 2005). The rheumatologic diagnosis was based on clinical examination performed by one physician (I. Hollan), a review of medical records, and previous X-ray images.

Out of 116 patients with verified IRD, 70 were enrolled into the biopsy study (Figure 6): three patients refused to undergo biopsies, and 43 did not undergo biopsies due to I. Hollan's absence or preoperative time constraints. The inclusion criteria were age >18 years; referred to CABG surgery due to CAD; confirmed IRD according to accepted diagnostic criteria (83-88) and absence of any clinically significant infection or malignancy. In Paper I, we described patients in whom aortic specimens were taken (66 IRD patients and 51 controls), and in Paper II, patients in whom saphenous vein or IMA specimens had been taken (65 IRD and 51 control patients) (Figure 6).

###### CAD patients without IRD (CAD-nonIRD group, n=53)

The CAD-nonIRD group was consecutively included at the Feiring Heart Clinic during the same period of time as the CAD-IRD group, and was matched to the CAD-IRD group for age and sex at a group level. The inclusion criteria were age >18 years; referred to CABG surgery due to CAD; absence of IRD, psoriasis, clinically significant infection, and malignancy.

Figure 6. Patients included for examination by biopsies in the Feiring Heart Study



### **3.1.2 Patient groups described in Paper III**

#### **Spondyloarthritis patients undergoing a first CABG (SpA group, n=30)**

Of the patients with IRD that had been referred to CABG at the Feiring Heart Clinic (screened as described for the CAD-IRD group), we selected those with spondyloarthritis undergoing a first CABG between May 2001 and January 2005. This group included 15 with ankylosing spondylitis, 14 with psoriatic arthritis, and 1 with Reiter's disease.

The AS was diagnosed in accordance with the 1984 modified New York criteria (15), and psoriatic arthritis with the Moll and Wright criteria (16). The patient with reactive arthritis disease had had oligoarthritis, conjunctivitis, and aseptic urethritis 20 years prior to the CABG.

#### **Patients without spondyloarthritis undergoing a first CABG (non-SpA group, n=3822)**

In this group, we selected patients not included in the SpA group that underwent a first CABG at the same clinic during the same period of time as the SpA group.

### **3.1.3 Other patient groups (biobank)**

#### **Healthy controls (n=30)**

Healthy volunteers were recruited among the staff of Lillehammer Hospital for Rheumatic Diseases and their acquaintances in 2004. The inclusion criteria were absence of clinically significant disease, trauma, or surgery during the preceding 6 weeks; absence of pregnancy, alcohol, and drug abuse; age in the same range as patients in the CAD-IRD group. The group was matched to CAD-IRD for sex at a group level.

#### **RA patients without cardiovascular complications (n=30)**

This group was consecutively included at the Lillehammer Hospital for Rheumatic diseases between 2007 and 2008. The inclusion criteria were RA confirmed according to the American College of Rheumatology 1987 criteria (83); absence of cardiovascular disease (judged by medical history, clinical findings, and electrocardiographic findings); absence of clinically significant chronic infection or malignancy; not pregnant; absence of clinically significant acute infection, trauma, or surgery during the preceding 6 weeks; within the same age range as the RA patients from the CAD-IRD group. The group was matched to the subgroup of RA patients in the CAD-IRD group for sex at a group level.

## **3.2 Data collection**

### **3.2.1 CAD-IRD and CAD-nonIRD groups**

These groups were preoperatively examined by interview, self-reported questionnaires, physical examination, ankle brachial index, and blood tests. During the CABG, several tissue specimens were collected, and the surgeons recorded the macroscopic findings.

#### **Medical history and physical findings**

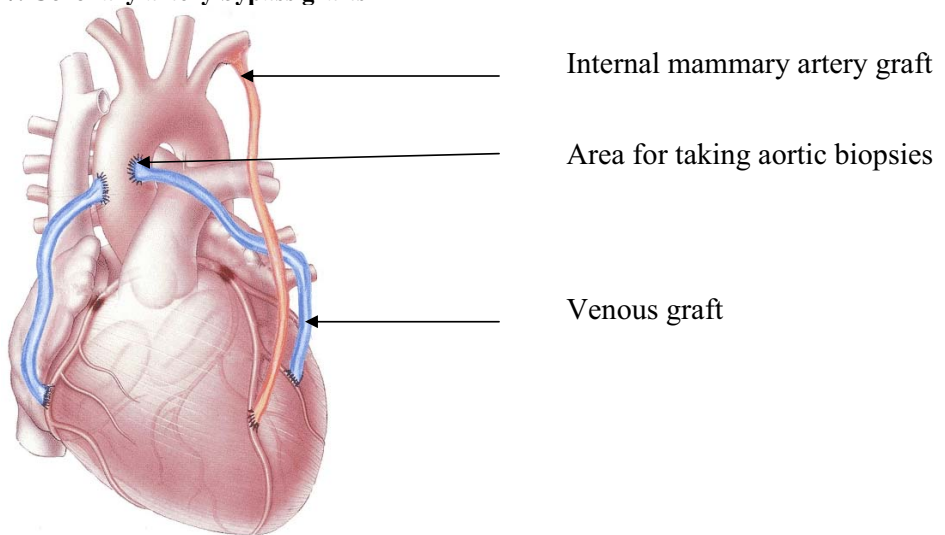
Dr. Hollan performed all physical examinations (including assessments of the disease severity and activity of IRD), and recorded all demographic data, detailed medical history (including angiographic findings), and information on treatments. The data were recorded in a predefined form, using questions requiring "yes" or "no" or other predefined answers whenever applicable. The onset of IRD was defined as the date of diagnosis by a physician. A positive family history of CAD was defined as CAD in first-degree relatives at ages < 65 years

old. Hypercholesterolemia was defined as a total s-cholesterol >5.5 mmol/l recorded in the patient's medical records or current use of lipid-lowering drugs. Significant coronary stenosis was defined as a diameter reduction >50% by visual estimate. Left ventricular hypertrophy was diagnosed by electrocardiography and/or echocardiography. Left ventricle ejection fraction was determined by ventriculography.

### Biopsies

We collected tissue from the aorta and saphenous vein that was routinely removed during a CABG. In addition, we collected several other specimens in a manner that did not increase the complication risk.

**Figure 7. Coronary artery bypass grafts**



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### Biopsy procedures

- **Aorta** (Figure 7): We collected tissue that was removed for the establishment of an aortocoronary anastomoses. Subsequently, part of the adventitia (up to 10×25 mm) covered by the epicardium was removed from the ventral part of the ascending aorta, and one to three punch holes (each 4.8 mm in diameter) were made through the vessel wall for proximal aortocoronary anastomoses in the same area. The punch specimens consisted of the intima and media, and sometimes a thin layer of the adventitia. To avoid thromboembolic complications, the aortic specimens were taken from areas with less pronounced gross signs of atherosclerosis. In two patients that underwent repair of an aortic aneurysm during CABG, specimens were collected from the edge of the aortic operational incision.
- **Saphenous vein**: We collected the excess portion of the prepared saphenous vein (up to several centimetres) that remained after venous grafting was performed.
- **IMA**: We removed a 5-10 mm-long specimen from the IMA, distal to the surgical cut.

- *Right atrium:* Biopsies from the right atrium (up to 3×2 mm) were obtained from a 10-20 mm long incision that was routinely performed during CABG for insertion of a cannula for extracorporeal circulation.
- *Rectus abdominis muscle:* Biopsies from the rectus abdominis muscle (ca. 5×5×3 mm) were taken from the incision in the thorax.
- *Skin:* Two 4×2 mm skin specimens were collected from surgical cuts: one from the thorax, and one from the leg.
- *Cardiac valves:* In patients that had cardiac valve replacement surgery in addition to CABG, the removed valvular tissue was collected.

All specimens were collected and treated according to a detailed protocol. The specimens were divided into small portions: some were fixed in formalin and embedded in paraffin; others were snap-frozen in liquid nitrogen, and stored at -75°C. To simplify the biopsy assessment procedure, we stored only frozen aortic specimens (not paraffin fixed specimens) in eight patients. However, because the frozen sections were lower quality for histological examination, we used paraffin sections in the remaining patients. Nevertheless, both the frozen and paraffin fixed specimens were suitable for demonstrating inflammatory cell infiltrates. For histological analyses, we selected one 3- $\mu$ m thick section from the aortic adventitial tissue (specimen A), one from an aortic punch specimen (specimen B), one from the IMA, and one from the saphenous vein.

The sections were stained with hematoxylin, eosin, and saffron. Two experts, blinded to the clinical data, examined the sections by light microscopy in random order for the presence and location of atherosclerotic lesions, inflammatory cell infiltrates, and other signs of vasculitis, including giant cells, granuloma, leucocytoclasia, and necrosis of the vessel wall. When the two interpretations were discordant, an additional reading was performed by a third expert to adjudicate the findings.

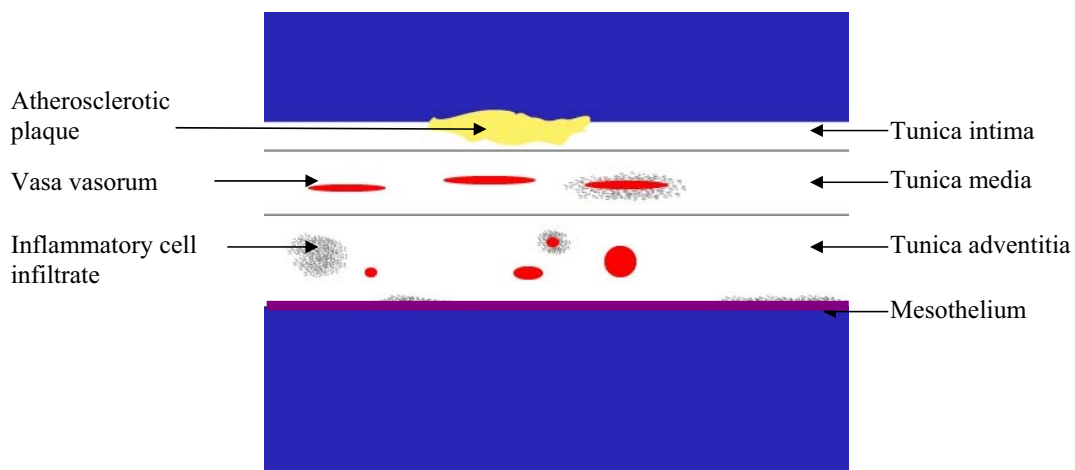
Atherosclerotic lesions were defined according to “A Definition of Advanced Types of Atherosclerotic Lesions and a Histological Classification of Atherosclerosis” (American Heart Association) (89), and calcifications in the intima of the saphenous vein were considered atherosclerotic plaques.

To our knowledge, no similar studies had previously been performed; thus, we established a new scoring system for the inflammatory cell infiltrates. In the aortic adventitia, we distinguished between the inflammatory infiltrates adjacent to the epicardium and those in the inner adventitia (in case they had different causes and different clinical significance) (Figure 8).

There is no clear anatomic border between the epicardial and adventitial loose connective tissues; thus, the differentiation was based on the pathologists’ judgment. Inflammatory infiltrates adjacent to the epicardial mesothelium were classified as epicardial (submesothelial), unless there was diffuse spreading into the inner adventitia. Infiltrates freely localized in the adventitia and large epicardial infiltrates that diffusely spread into the adventitia were considered adventitial. Submesothelial mononuclear cell infiltrates (MCIs) were scored as follows: 0= no infiltration, 1= mild infiltration, and 2= pronounced infiltration. MCIs in the inner adventitia were semi-quantified as follows: 0= no infiltrates, 1= 1–3 infiltrates, and 2=  $\geq 3$  infiltrates or diffuse infiltration. The size of the largest infiltrate in a single section was classified according to the number of mononuclear cells, where 0= none, 1= 3–49 cells, 2= 50–99 cells, 3= 100–199 cells, and 4=  $\geq 200$  cells. IMA specimens were also examined for the presence of inflammatory cell infiltrates in the perivascular tissue. In addition, a single evaluation was performed to determine the intima/media ratio ( $\leq 1$  or  $>1$ ) and intimal thickening (yes/no) in the IMA and saphenous vein. The sizes of MCIs in the saphenous vein were semi-quantified ( $>50$  inflammatory cells per infiltrate: yes/no).

The surgeons recorded gross signs of inflammation, defined as redness, induration, or edema of the vessel wall (yes/no), and the presence of atherosclerotic plaques (yes/no) in the aorta. They also evaluated gross signs of inflammation in the saphenous vein and the IMA and the quality of the venous conduit (good, medium, or poor).

**Figure 8. Assessment of the aortic specimens**



*We investigated the presence and location of atherosclerotic lesions and inflammatory cell infiltrates. We semi-quantified the number of inflammatory cell infiltrates and the size of the largest infiltrate per section.*

### **Blood samples**

Blood samples were collected preoperatively for biochemical, hematological, immunological, and microbiological tests. Routine biochemical and hematological tests were performed consecutively. For most analyses, multiple small portions of serum, EDTA, and citrate from plasma and whole blood were stored in a freezer at  $-75^{\circ}\text{C}$  to be analyzed in batches.

### **3.2.2 Healthy controls and RA patients without cardiovascular complications**

These groups were examined by blood tests. In addition, the RA patients were given a rheumatologic examination and self-reported questionnaires, similar to those given to the CAD-IRD group.

### **3.2.3 Non-SpA group**

In this group, demographic and clinical data from medical records were recorded. The use of non-steroidal anti-inflammatory drugs (NSAIDs) and coxibs was recorded only in a random sample of 84 control patients.

### 3.3 Ethics

The Regional Ethics Committee for Medical Research approved the study protocol. The data- and biobank has approval until 2018. The Norwegian Directorate for Health and Social Affairs approved specimen analysis in specified laboratories in foreign countries.

### 3.4 Statistics

We used the chi-square and Fisher's exact tests to assess differences in proportions in independent samples, and McNemar's test to assess differences in proportions in two related samples (for example, the occurrence of pathologies in two different arteries from the same individual).

The independent sample t-test and Mann-Whitney U test were used to examine differences in continuous variables in two independent samples.

Logistic regression analyses were used to identify predictors of vascular mononuclear cell infiltrates (MCIs) and of vascular MCIs $\geq$ 50 cells. Due to the small sample size of our study, we built the logistic regression models using clinical judgment. For simple analyses, we selected independent variables that might have a logical relationship to the chosen dependent variables (MCIs and MCIs $\geq$ 50), including variables reflecting disease severity, activity, and duration of rheumatic disease and CAD; cardiovascular risk factors; treatment; coexisting atherosclerosis; CRP; ESR; and demographic data. Crucial variables (age and gender) and variables that had  $p < 0.10$  in the simple analyses were used further in the multiple logistic regression models (Papers I and II).

To identify predictors of the age at CABG, we used linear regression analyses. The selection of independent variables for these analyses was again based on clinical judgment. Because we wanted to compare the effects of spondyloarthritis and traditional cardiovascular risk factors on the age at first CABG, we used the spondyloarthritis diagnosis and the traditional cardiovascular risk factors as independent variables in multiple linear regression, model I. In a second linear regression, model II, we examined whether the effect of these factors were independent of current systemic glucocorticosteroid treatment. Preceding the multiple regression modeling, we performed simple regression analyses for all the mentioned independent variables.

The analyses were performed with SPSS for Windows, version 14-15 (SPSS Inc, Chicago, IL). The level of statistical significance was set at 0.05, and all statistical tests were two-sided.



## 4. Summary of results

### 4.1 Paper I

Aortic specimens were obtained in 66 IRD and 51 non-IRD patients; aortic punch specimens were obtained in 64 IRD and all the non-IRD patients; and aortic adventitia specimens were obtained in 63 IRD and 44 non-IRD patients. The patient characteristics are described in Paper I, Table 1. The IRD group comprised patients with RA (n=23), psoriatic arthritis (n=10), undifferentiated seronegative arthritis (n=1), SLE (n=3), ankylosing spondylitis (n=6), Reiter's disease (n=1), GCA (n=6), polymyalgia rheumatica (n=14), primary Sjögren's syndrome (n=1), and undifferentiated collagenosis (n=1).

Acute inflammatory infiltrates in the aorta occurred in one control patient (in the adventitia). Atherosclerotic lesions were present in 21% of IRD and 14% of non-IRD patients ( $p=0.35$ ). MCIs within atherosclerotic lesions occurred in 5% of IRD and 6% of non-IRD patients ( $p=0.69$ ). Epicardial MCIs occurred in 48% of IRD and 51% of non-IRD patients ( $p=0.78$ ). Medial MCIs were present in 10% of IRD and no non-IRD patients ( $p=0.035$ ); they were found only in patients that also had adventitial MCIs. Adventitial MCIs occurred in 30 of 64 (47%) IRD and 10 of 51 (20%) non-IRD patients (odds ratio=3.6,  $p=0.002$ , 95%CI: 1.6-8.5). The adventitial MCIs in IRD patients were likely to be larger and more numerous than those in non-IRD patients. Adventitial MCIs were present in most of the examined rheumatic diagnoses, and they were especially pronounced in patients with GCA/polymyalgia rheumatica or spondyloarthritis.

Of the examined clinical parameters, only IRD and current cigarette smoking independently predicted the occurrence and size of adventitial MCIs in adjusted analyses.

IRD and non-IRD patients with adventitial MCIs had higher CRP levels than those without adventitial MCIs. Adventitial MCIs were observed in a higher proportion of patients with unstable, compared to those with stable, atherosclerotic lesions (67% vs. 17%). However, none of these differences were statistically significant.

Patients with RA, spondyloarthritis, or GCA/polymyalgia rheumatica that used systemic glucocorticosteroids showed a trend towards a lower prevalence and size of adventitial MCIs (statistically non significant). The difference in the prevalence of adventitial MCIs in glucocorticosteroid users and non-users almost reached statistical significance in the RA subgroup ( $p=0.051$ ). Among patients with RA, psoriatic arthritis, or undifferentiated arthritis, adventitial MCIs were observed in 2 of 8 (25%) patients treated with methotrexate, neither of 2 (0%) patients treated with TNF alpha inhibitors, 6 of 9 (67%) patients treated with other disease modifying antirheumatic drugs, and 6 of 14 (43%) patients not currently receiving disease modifying antirheumatic drugs ( $p=0.20$ ).

One non-IRD and 3 IRD patients had undergone abdominal aneurysm repair and 3 IRD patients had a thoracic aneurysm. All patients with thoracic aneurysms had MCIs in the media and adventitia, and 3 of 4 patients with a history of abdominal aneurysm had adventitial MCIs. Medial and adventitial MCIs in the 7 patients with current or former aortic aneurysms were significantly more frequent than in patients without aortic aneurysms ( $p=0.003$  and  $p=0.007$ , respectively).

The MCIs in non-atherosclerotic areas were localized around the vasa vasorum and/or throughout the connective tissue. They consisted mainly of lymphocytes, with a marked involvement of plasma cells in some patients (Figure 10). Giant cells were observed in adventitial MCIs in one patient with psoriatic arthritis. No leucocytoclasia, granuloma, or vessel wall necrosis was observed in any specimen.

Aortic inflammation was clinically silent in all patients. The surgeon's gross assessment of aortic inflammation had a low positive predictive value (0.30), but a high negative predictive value (0.90) for adventitial MCIs.

## 4.2 Paper II

We examined 65 IRD and 51 non-IRD patients from the Feiring Heart Biopsy Study that had specimens taken from the saphenous vein and/or the IMA (Figure 6). Patient characteristics are described in Paper II, Table 1. All but one patient, in whom an aortic specimen was not obtained, were part of the patient population from Paper I. Saphenous vein specimens were available in 106 patients, IMA specimens in 103 patients (however, the IMA was observed in only 96 of these specimens), and aortic specimens in 115 patients.

MCIs in the vascular media and/or adventitia were present in the saphenous vein in 18 of 106 subjects (17%), in the IMA in 2 of 96 subjects (2%), and in the aorta in 40 of 115 subjects (35%). In this chapter, we focus mainly on findings in the saphenous vein and the IMA because findings in the aorta were thoroughly described in Paper I. The medial and adventitial MCIs in the saphenous vein and IMA consisted mainly of lymphocytes localized around the vasa vasorum and/or diffusely in connective tissue, often at the junction between the media and adventitia (Figure 10). No acute inflammatory infiltrates, giant cells, granulomas, leucocytoclasia, or necroses of the vessel walls were found in any venous or IMA specimens.

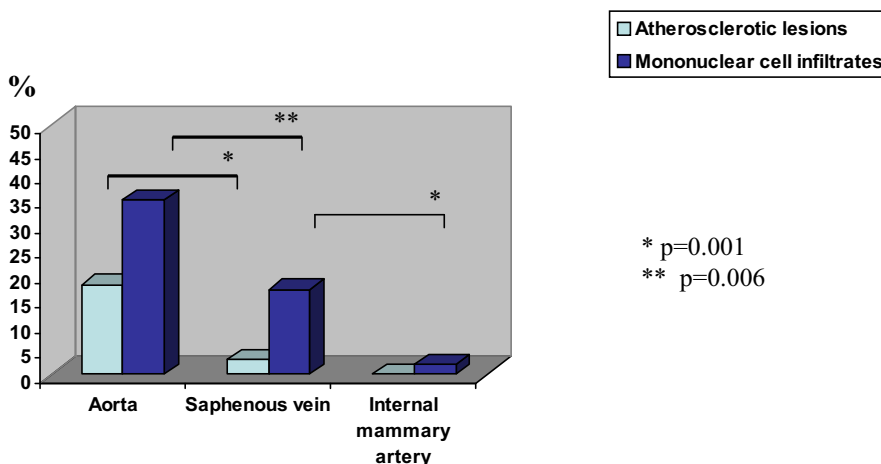
We did not observe any significant relationships between the occurrences of MCIs in the aorta, saphenous vein, and IMA when they occurred in a single individual. Current cigarette smoking and IRD were independent predictors of the occurrences of medial or adventitial MCIs in the aorta, but not in the saphenous vein or IMA.

Atherosclerotic lesions were observed in 3 of 106 (3%) saphenous vein specimens (from IRD patients) and no IMA specimens (Figure 9). Thickening and fibrosis of the saphenous vein vessel wall was common, and intimal thickening in the saphenous vein was more pronounced than in the IMA.

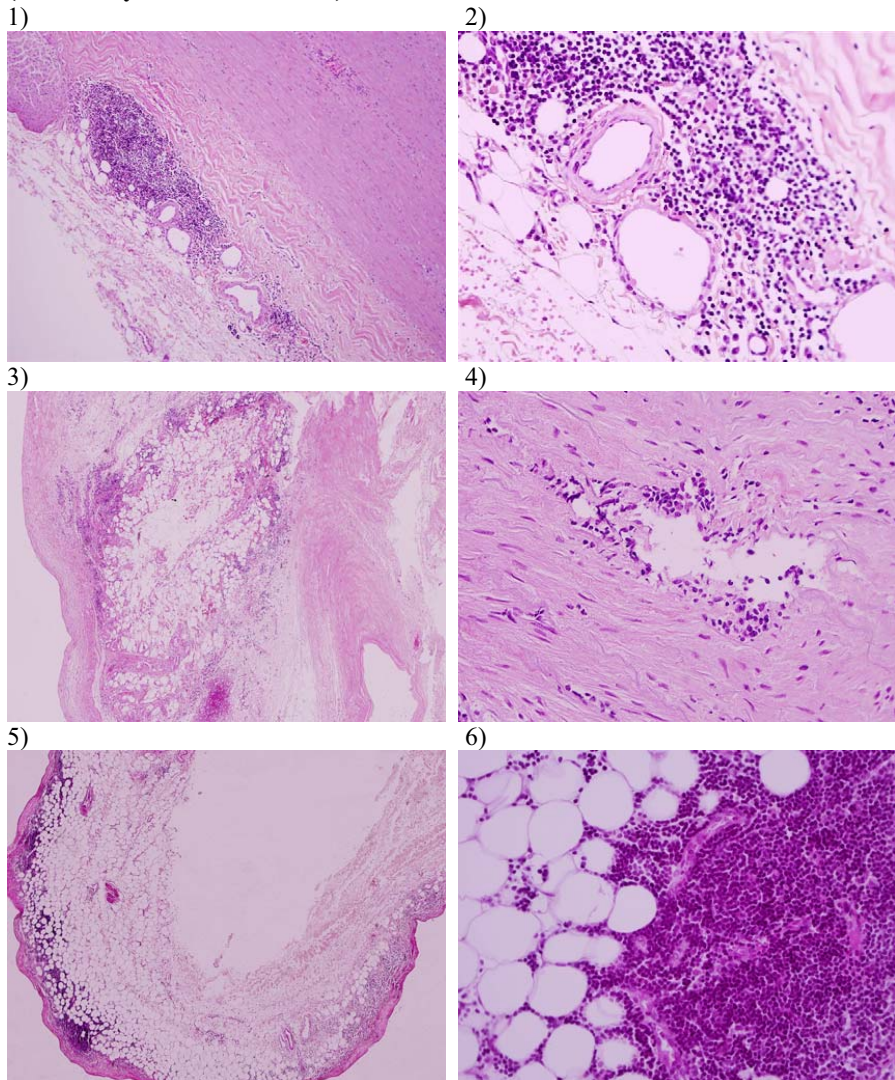
Seven of 96 patients had inflammation in fatty and/or muscular tissue around the IMA.

Gross examination by surgeons did not reveal inflammation in any saphenous vein or IMA specimens. The gross quality of venous conduits was similar in the IRD and non-IRD groups, and was not related to the occurrence of MCI in the saphenous vein.

**Figure 9. Occurrence of atherosclerotic lesions and mononuclear cell infiltrates in non-atherosclerotic areas of the aorta, saphenous vein and internal mammary artery (%)**

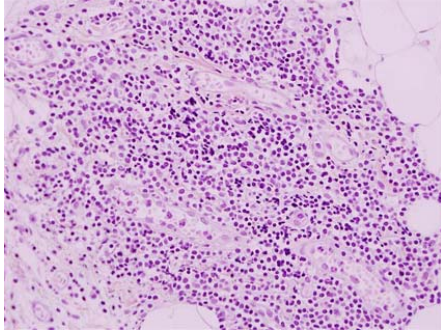


**Figure 10. Light microscopy of the aorta, saphenous vein, and internal mammary artery (haematoxylin and eosin stain)**

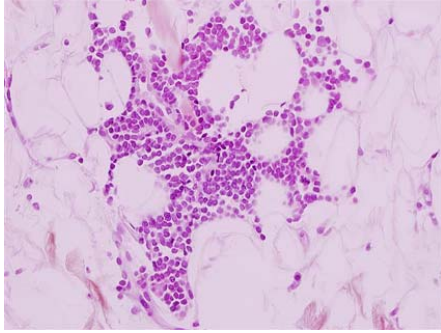


1) A large MCI in the inner aortic adventitia of a patient with giant cell arteritis (GCA) that underwent repair of an ascending aortic aneurysm during the CABG operation. The patient also had perivascular MCIs in the media and an atherosclerotic plaque in the intima. The inflammatory changes in the deep vascular layers may have been secondary, but also primary, to atherosclerosis and/or aneurysm (100×). 2) High-power view of the adventitia from the specimen shown in 1 (400×). 3) Pronounced MCI in the aortic adventitia with fibrosis in a patient with GCA (40×). 4) MCI in the aortic media of the patient shown in 3 (400×). 5) Pronounced diffuse submesothelial MCIs in the aortic specimen of a patient with RA and thoracic aneurysm. MCIs spread diffusely into the inner adventitia and were also present in the media (100×). 6) High-power view of the adventitia from the specimen shown in 5 (400×).

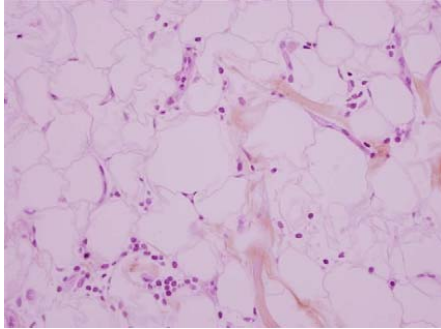
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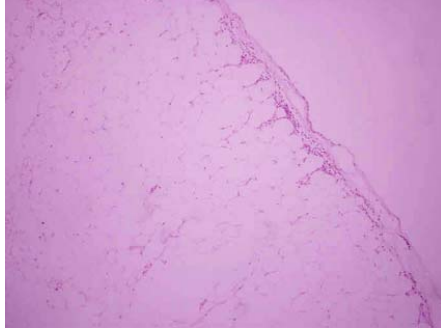
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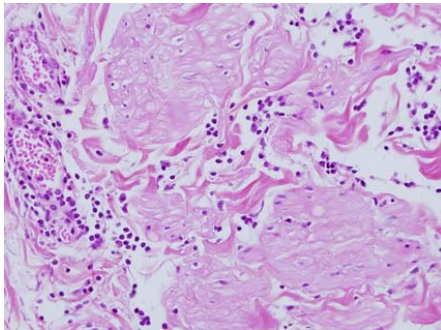
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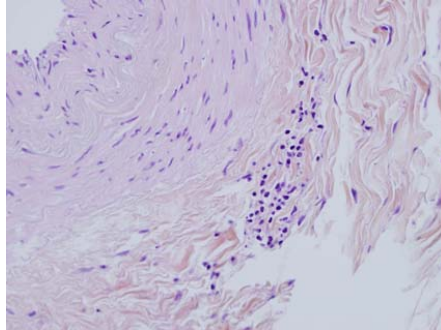
10)



11)



12)



7) MCI in the inner part of the aortic adventitia of a patient with ankylosing spondylitis that also had a MCI in the media (400×). 8) MCI in the inner aortic adventitia of a patient with psoriatic arthritis (400×). 9) Small MCI in the inner aortic adventitia of a control patient (400×). 10) Spread aortic submesothelial MCI, not considered to affect the inner adventitia, in a patient with polymyalgia rheumatica (100×). 11) MCIs in the saphenous vein of a patient with psoriatic arthritis (400×). 12) MCI in the internal mammary artery of a control patient (400×).

### 4.3 Paper III

Of the patients undergoing a first CABG, 30 (0.78%; 95% CI: 0.50-1.06) had CAD with spondyloarthritis (SpA group) and 3822 had CAD alone (non-SpA group). We did not find any statistically significant differences in the frequency of a concomitant aortic or cardiac valve surgery or in the distribution of traditional cardiovascular risk factors between the SpA and non-SpA groups. The mean ages (SD) of patients with and without spondyloarthritis were 60±9 and 67±10 years, respectively ( $p < 0.001$ ). 53% of patients with spondyloarthritis and 25% of patients without spondyloarthritis were <60 years old. Patients with ankylosing spondylitis had a mean age of 58±10 years, and patients with psoriatic arthritis had a mean age of 62±7 years ( $p = 0.15$ ).

Patients with spondyloarthritis had fewer coronary arteries with significant stenoses compared to the non-SpA group (2.5±0.6 vs. 2.7±0.5, respectively;  $p = 0.025$ ). However, 60% of patients with spondyloarthritis versus 52% of patients without spondyloarthritis had previously sustained a myocardial infarction ( $p = 0.4$ ). Most of the other cardiovascular prognostic factors, including the left ventricle ejection fraction, class of the New York Heart Association Functional Classification (NYHA), left main coronary artery stenosis, left ventricle hypertrophy, occurrence and the mean number of previous myocardial infarctions, were more altered in the SpA than in the non-SpA patients, but the differences were not statistically significant. Use of lipid lowering drugs, ACE inhibitors, and betablockers was similar in both groups, but patients with spondyloarthritis were more likely to use acetylsalicylic acid ( $p = 0.046$ ) and systemic glucocorticosteroids ( $p = 0.012$ ) than the control patients. Most control patients used systemic glucocorticosteroids due to a chronic obstructive pulmonary disease/asthma, or due to autoimmune diseases.

We used two multiple linear regression models to look for predictors of age at the first CABG (Table 1). In the first model, we adjusted for traditional cardiovascular risk factors and a spondyloarthritis diagnosis. In the second model, we also adjusted for use of systemic glucocorticosteroids. The results from both models were consistent. Spondyloarthritis was a stronger predictor of early CABG than most of the examined cardiovascular risk factors; only current cigarette smoking had a similar effect. Also male sex, hypercholesterolemia, positive family history of CAD, higher body mass index, absence of hypertension, and absence of glucocorticosteroid treatment correlated with a lower mean age at the first CABG. Non-insulin-dependent diabetes and former smoking had no effect on the mean age at the first CABG. Separate analyses of the ankylosing spondylitis and psoriatic arthritis subgroups showed similar results to those found for the whole SpA group.

Use of NSAIDs or coxibs had no significant effect on the age at the first CABG. The age at spondyloarthritis onset correlated positively with the age at the first CABG (beta = 0.33,  $p = 0.005$ , 95%CI: 0.11-0.55,  $r^2 = 0.239$ ).

Table 1. Predictors of age at the first CABG – results from 2 multiple linear regression models (n=3852)\*

	Age at the first CABG			
	Model I†		Model II‡	
	Beta (95% CI)	p	Beta (95% CI)	P
Spondyloarthritis	-6.2 (-9.5, -2.8)	<0.001	-6.6 (-9.9, -3.3)	<0.001
Female sex	4.8 (4.1, 5.5)	<0.001	4.7 (4.0, 5.4)	<0.001
BMI	-0.05 (-0.07, -0.03)	<0.001	-0.05 (-0.07, -0.03)	<0.001
Hypercholesterolemia	-4.2 (-5.0, -3.4)	<0.001	-4.2 (-5.0, -3.4)	<0.001
Hypertension	0.9 (0.3, 1.5)	0.005	0.9 (0.3, 1.5)	0.004
DM-insulin	-1.4 (-2.7, -0.1)	0.033	-1.5 (-2.7,-0.2)	0.022
DM-oral antidiabetics	0.5 (-0.6, 1.6)	0.368	0.5 (-0.6, 1.6)	0.391
DM-diet	-0.3 (-2.0, 1.2)	0.674	-0.4 (-2.0, 1.2)	0.608
Current smoking	-5.7 (-6.5, -5.0)	<0.001	-5.7 (-6.5,-5.0)	<0.001
Former smoking	-0.5 (-1.2, 0.2)	0.149	-0.5 (-1.2, 0.1)	0.122
Family history of CAD	-2.6 (-3.3, -2.0)	<0.001	-2.6 (-3.2,-2.0)	<0.001
Systemic glucocorticosteroids	–	–	4.8 (3.1, 6.5)	<0.001

\*CABG = coronary artery bypass graft surgery. BMI = body mass index. CAD= coronary artery disease. DM insulin = diabetes mellitus treated with insulin. DM-oral antidiabetics = DM treated with oral antidiabetics. DM-diet = DM treated with diet.

†Model I: adjusted for spondyloarthritis, sex, BMI, hypercholesterolemia, hypertension, DM-diet, DM-oral antidiabetics, DM-insulin, current smoking, former smoking, and family history of CAD ( $r^2 = 0.16$ ).

‡Model II: adjusted for the same factors as Model I and for current systemic glucocorticosteroid treatment ( $r^2 = 0.17$ ).

## **5. General discussion**

### **5.1 Methodological aspects**

It is important to interpret the statistically significant as well as non-significant results with caution. As in any study, our findings may be accidental or influenced by biases and confounding factors; thus, further research is needed for validation.

#### **5.1.1 Study design**

The part of the study described in Papers I-III has a cross-sectional design. Cross-sectional studies do not allow the determination of causal relationships. On the other hand, they tend to be easier and more rapidly completed than other forms of observational studies, and may therefore generate hypotheses in a feasible way.

To minimize the role of potential confounding factors, we chose a controlled matched design, and adjusted statistical analyses for possible confounders. Indeed, a complete exclusion of any confounder is impossible because there may be some unknown factors and some may not be detectable in a study. In addition, statistical analyses may not allow control of all potential confounders, e.g., due to a small sample size. All these sources of uncertainty may play a role in our study.

#### **5.1.2 Patients**

We wished to examine as large a population as feasible of patients that required a CABG. Therefore, we originally planned to include patients at two or several centers of cardiothoracic surgery. However, there were many practical challenges in running the project: the screening, examination, and treatment of the blood and tissue samples were time consuming, and some parts of this process were difficult to integrate into the tight program of preoperative procedures. In addition, it was necessary to maintain close, flexible cooperation between the cardiologist and rheumatologist. As the only physician involved in the rheumatologic examination, I was required to travel to the clinic to examine the patients. Therefore, despite a positive attitude at other centers, we enrolled all but one patient at the Feiring Heart Clinic, which was closest to my place of residence. We interrupted the inclusion of patients into the CAD groups after approximately 4 years, when, due to illness, I was not able to visit the Feiring Heart Clinic (it is over 100 kilometers from my residence).

At the Feiring Heart Clinic, we collected biological specimens in 70 out of 107 IRD patients that underwent CABG between May 2001 and July 2004. We were not able to include all 107 patients mainly due to a short hospitalization time before the CABG. Often, there was little time (frequently outside of ordinary working hours) to examine the patient and obtain the necessary medical information to confirm an IRD diagnosis. Furthermore, the study tasks were given a lower priority than the necessary preoperative procedures. In addition, in spite of our active approach, we might have overlooked some few IRD cases because the screening might be underprioritized in some circumstances, e.g. in critically diseased patients.

Due to the logistic problems, the number of patients was relatively small. Thus, the lack of statistical significance in some results might be caused by type 2 errors that may have masked true differences. However, the study remains useful because it extends the limited available cellular and molecular data that describe vascular processes underlying accelerated atherosclerosis in IRD. To our knowledge, this is the only study of its kind, and our findings may provide important information for guiding further research. Compared to other studies on surgical vascular specimens in IRD, our sample size is still relatively large (90). In addition, the study population is well characterized, and a variety of biological specimens from each patient was gathered. Thus, it is possible to analyze the relationships between various tissue

and circulatory biomarkers in individual patients. Another strength of the study was the large size of the non-SpA control group.

A potential drawback of this study is that we lumped together patients with different IRDs that have different disease mechanisms. However, chronic inflammatory conditions may share some common mechanisms that contribute to premature atherosclerosis, and vascular inflammation might be one such mechanism. Furthermore, our approach enabled the demonstration of trends in different diagnostic subsets. Because the subgroups were too small to draw definitive conclusions, larger studies of homogenous diagnostic subgroups are needed. However, obtaining aortic surgical specimens from large numbers of CAD patients with the particular rheumatic diseases will probably be extremely difficult as the IRDs are rare, low numbers of these patients undergo CABG, and the logistics in collecting specimens during cardiovascular surgery are challenging. By our approach, we were able to provide information about the trends in the whole IRD group as well as in the respective diagnostic subgroups. However, we acknowledge that similar vascular changes could be caused by different inflammatory mechanisms, and this could not be demonstrated in this study.

We based most of the IRD diagnoses on relatively strict criteria, which enabled a lucid definition of the IRD population. However, we therefore could not provide information about vascular pathologies in patients with earlier and milder forms of the diseases involved. Also, the non-SpA group might have contained patients with spondyloarthritis that did not meet the criteria for spondyloarthritis. This might cause an underestimation of the differences between the rheumatic and control patients. The non-SpA group also comprised patients with other inflammatory conditions, e.g. autoimmune diseases, infections, obesity, or diabetes. Thus, we examined the effect of defined spondyloarthritis diagnosis per se, not of chronic inflammation, on the age at CABG.

The study results may not be generalized to patients with advanced renal failure or severe multi-organ complications, because those patients were not typically accepted for CABG at the Feiring Heart Clinic.

### **5.1.3 Data collecting and processing**

To assure the quality of the collected data, we used questionnaires with questions requiring “yes” or “no” or other predefined answers whenever applicable. For most self-reported measures, we used internationally validated instruments. The rheumatologic examination was performed by one investigator. Further, most laboratory tests were performed in batches in a standard, randomized, blinded manner.

An important advantage of our method was the ability to collect surgical vascular and cardiac biopsies without increasing risk for the patients.

The interpretation of a histological assessment is somewhat subjective, particularly in the distinction between submesothelial and adventitial infiltrates, and sometimes the distinction between large and multiple infiltrates. To minimize bias, the specimens were evaluated independently by at least 2 pathologists that were blinded to the patients’ medical histories. We documented the findings with photographs to facilitate interpretations. We plan to conduct immunohistochemical analyses that may contribute to the validation of our histochemical findings. These analyses may also delineate fine differences in the content and nature of lymphocytic and mononuclear cell infiltrates in different diagnostic categories e.g. SLE, spondyloarthritis, and RA; and thus reflect different mechanisms of inflammation in these disorders. Histochemical and immunohistochemical techniques have different strengths and weaknesses; therefore, a combination of these approaches is useful.

The area of the examined specimens was not measured, and we cannot exclude slight variations in specimen size between patients; larger specimens offer a greater chance of finding pathologies. However, systematic errors are fairly unlikely, as the biopsies were



performed in the same way in both groups. In further analyses, we intend to use computerized immunohistochemical methods that allow area measurements.

Although paraffin sections were used for histological analyses in most patients, frozen sections were used in eight patients. This probably did not substantially influence the results, because the important structures and MCIs were apparent in both types of sections.

As mentioned in Chapter 1.5, we became aware that diagnosing vasculitis was quite subjective when based on the presence of inflammatory cells in the vessel walls. The interpretation is often influenced by clinical symptoms, and there is substantial variation among experts. Therefore, for the time being, we avoided classifications of the findings with regard to vasculitis, and instead, provided more objective, semi-quantitative descriptions of the findings.

The occurrence of atherosclerotic lesions in the aorta is probably underestimated because the aortic specimens were taken from areas with less pronounced atherosclerosis for the patients' safety.

Calcifications in the intima of saphenous vein were considered atherosclerotic lesions, although they might represent a different pathology. Our judgment was in accordance with current criteria (89).

We compared arteries of different sizes and structures. In future studies, it would be interesting to investigate whether the occurrence of atherosclerosis parallels the occurrence of inflammatory changes in vessels of similar caliber.

It is impossible to collect all potentially important information in a study, due to e.g., feasibility and the limited general level of knowledge in the planning stages. In our study, many, but not all, cardiovascular risk and prognostic factors were recorded. The non-SpA group was less well characterized than groups where biological material was collected. This control group lacked information on CRP levels and former medical treatments. The current use of coxibs and NSAIDs was recorded in only one subgroup of these patients. In addition, the cardiovascular risk factors lacked detailed characterization; for example, we did not investigate proinflammatory properties of mediators of lipoprotein metabolism. These and other unrecognized factors might have influenced the premature need for CABG.

## **5.2. Main results**

### **5.2.1 Inflammation and atherosclerosis in the aorta, saphenous vein, and IMA**

In surgical specimens obtained during a CABG from patients with CAD, we detected inflammation in terms of inflammatory cell infiltrates in the thoracic aorta, saphenous vein, and IMA (Figure 10). Because acute inflammatory infiltrates occurred only in one specimen (aortic adventitia), we primarily focused on chronic, mononuclear cell infiltrates (MCIs), which occurred surprisingly frequently. MCIs were found within and outside atherosclerotic lesions. The latter, which were more common, were localised in the media and adventitia. The occurrences of MCIs in the vascular media and/or adventitia followed the same pattern as the occurrences of atherosclerotic lesions: most occurred in the aorta, fewer in the saphenous vein, and the least in the IMA. The pattern of atherosclerotic lesion occurrence in the respective vessels was in accordance with common knowledge and trends observed in other studies (77;79;80;91-94). The true occurrence of aortic atherosclerotic lesions is likely to be higher than that reported here, because the aortic specimens were specifically taken from areas with less pronounced macroscopic signs of atherosclerosis (see Chapter 3.2).

In the aorta and saphenous vein, atherosclerotic lesions were slightly more frequent in patients with IRD than in those without IRD (21% vs. 14% in the aorta and 5% vs. 0% in the saphenous vein), but the differences were not statistically significant. No atherosclerotic

lesions were found in the IMA in any group. Inflamed atherosclerotic lesions, found only in the aorta, occurred at similar frequencies in both groups. However, we cannot rule out an underestimation of differences between the groups in frequency and characteristics of atherosclerotic lesions, because the total number of observed atherosclerotic lesions was small. Moreover, the lack of significant differences may be due, in part, to the similar severities of CAD; all patients were selected for CABG according to the same clinical criteria.

MCIs outside atherosclerotic lesions occurred both in IRD and non-IRD patients. These MCIs were subclinical in all cases and consisted predominantly of lymphocytes. Except for the presence of giant cells in one adventitial specimen, we did not find any other characteristic signs of vasculitis in any patient (Papers I and II).

In the aorta, we distinguished between MCIs localized diffusely in the vessel wall and those adjacent to the epicardial mesothelium. The latter might represent a normal phenomenon or might be related to an epicardial disease. Submesothelial MCIs occurred in half the patients, with similar frequencies in IRD and non-IRD groups (Figure 11). The submesothelial MCIs often colocalized with fibrosis (Paper I). Gross signs of epicardial fibrosis are commonly observed during a CABG and might contribute to restricted epicardial circulation. However, the clinical importance of epicardial fibrosis is still unclear.

Adventitial MCIs in the aorta were more common in the IRD than the non-IRD group (47% vs. 20%, odds ratio=3.6,  $p=0.002$ ). IRD patients also had greater sizes and numbers of MCIs. Aortic adventitial MCIs were found in almost all the rheumatic diseases; they were especially large and frequent in GCA/polymyalgia rheumatica and spondyloarthritis subgroups, diseases that are typically complicated with aortic involvement (31;95). MCIs in the aortic media only occurred in the IRD group (in 10%), and were especially frequent in patients with aortic aneurysms. They were present only in patients that also had aortic adventitial MCIs. In theory, MCIs spreading from the adventitia into the media may represent a more advanced stage of vascular injury than MCIs localized in only the adventitia.

The extent and size of aortic adventitial MCIs were independently related to current smoking. Smoking is linked to inflammation in the lungs and joints, trombangiitis obliterans, and inflammatory aortic aneurysms (96). In theory, the harmful cardiovascular effects of smoking might also be related to enhanced vascular inflammation (Paper I).

The frequencies of medial and/or adventitial MCIs in the saphenous vein or the IMA were not related to either IRD comorbidity or smoking status (Paper II). This could stem from a type 2 error due to the low frequency of MCIs or from the inherent resistance of these vessels to systemic cardiovascular risk factors. In a recent study, the IMA seemed to be resistant to atherosclerosis in patients with renal failure (97).

Patients treated with systemic glucocorticosteroids, methotrexate, or TNF alpha inhibitors had a tendency (though statistically insignificant) to less pronounced adventitial inflammation in the aorta. This may reflect the effects of these drugs on vascular inflammation and should be investigated in further studies (Paper I).

The clinical significance of the observed chronic inflammation in the vascular media and adventitia is not known, and may be different in the different vessels. It may be independent of or secondary to atherosclerosis, but it may also reflect an inflammatory process that induces plaque formation or instability (98-101). The fact that we did not find atherosclerotic lesions in all patients with subintimal MCIs, and vice versa, could not rule out a possible causal role of chronic vascular inflammation in atherosclerosis. The atherosclerotic lesions may evolve from vascular inflammation over time, and only in certain circumstances. Atherosclerotic lesions may represent a late stage of a process that is triggered or enhanced by subintimal inflammation (similarly to calcifications that evolve only in some cases of pulmonary tuberculosis). Nevertheless, the inflammation may be resolved at the end-stage. In addition, we could not rule out the possibility that atherosclerotic lesions or subintimal

inflammation existed in an area near the place the specimens were obtained (especially since the specimens were taken from less diseased areas). The low power of this study prevented determinations of clear associations between the co-existence of subintimal MCIs and inflamed and stable atherosclerotic plaques.

Chronic vascular inflammation may contribute to the pathogenesis of atherosclerosis and confer high cardiovascular risk in smokers and individuals with IRD. The inherent resistance of some vessels to inflammation may influence the susceptibility to stenosis, and therefore their suitability as grafts. In addition, vascular inflammation may lower vessel wall resistance, and therefore contribute to the higher risk of aortic aneurysms in IRD (54;95;102-104) (Paper I).

The cause of chronic vascular inflammation is unknown. Inflammation might be triggered by systemic inflammatory mediators, pressure, chemical substances, infections, or autoantigens (e.g., oxidized low density lipoproteins, heat shock proteins, or citrullinated proteins) (105;106). The development of atherosclerosis may depend both on systemic factors (e.g., traditional cardiovascular risk factors, dysregulation of the immune system, infection, or decreased number or function of endothelial progenitor cells) and on local vascular properties, including the biochemical composition of the vessel wall (e.g., the content of different proteoglycans), the presence and characteristics of the immunoreactive cells, neurohumoral regulation, or microcirculation (107-115). Both the intima and the deeper vascular layers may play a substantial role in plaque formation (100;116-119). We note that adventitial MCIs were often localized around the vasa vasorum (Paper I). Microvascular injury with increased endothelial permeability plays an important role in various rheumatic manifestations, including synovitis, and may be involved in the pathogenesis of atherosclerosis (116;120-125).

The adventitia consists predominantly of fatty tissue. The proinflammatory effects of adipose tissue are well recognized, as are the contributions of lipid metabolism and obesity to cardiovascular risk. Hypothetically, the observed high occurrence of MCIs in the perivascular and adventitial fat may be a sign of generalized adipose tissue dysfunction and inflammation. The fat related to blood vessels may play a particular role in atherogenesis (109;113;126;127).

Vascular inflammation, e.g., in primary vasculitides, may occur in patchy patterns. We examined very small aortic specimens that were not selected by gross signs of inflammation. On the contrary, they were removed from less diseased areas for safety. Thus, aortic inflammation may be more common than shown by our results.

Higuchi et al. demonstrated that the number of lymphocytes in the adventitia of coronary arteries was related to the presence of ruptured atherosclerotic plaques in the same part of the vessel. That suggested that adventitial inflammation may contribute to plaque instability (98). In our study, inflamed atherosclerotic lesions were associated with the size of adventitial MCIs. However, the association lost statistical significance after adjustment for possible confounders. Moreover, the presence of MCIs in the inner adventitia was not associated with the presence of acute coronary syndrome.

The tools for detecting aortic inflammation are limited. Our sampling method allowed diagnosis in patients undergoing CABG, a relatively common surgery, without increasing perioperative risk. Perioperative macroscopic examination was useful for predicting aortic adventitial inflammation, but not for predicting MCIs in the saphenous vein and the IMA.

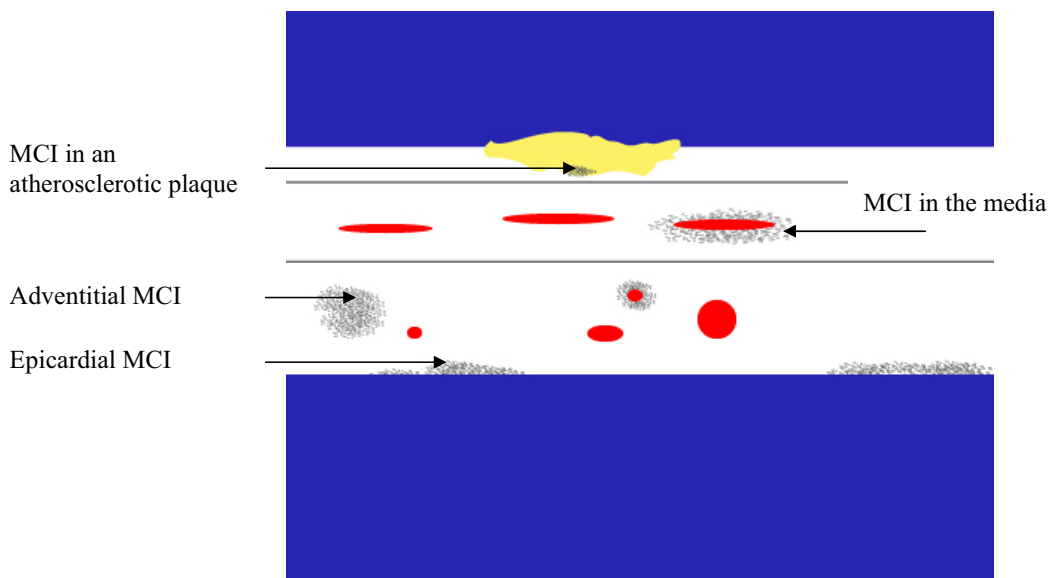
Infiltration of the vessel wall by inflammatory cells is one of the criteria for the histological diagnosis of vasculitis. However, there is no defined cut-off for the size or localization of the infiltrates. For example, inflamed atherosclerotic plaques or rare inflammatory cells in the aortic wall are generally not classified as vasculitis. Therefore, additional criteria are needed for distinguishing between vasculitis and atherosclerosis with inflammation. Another approach is to consider atherosclerosis as an inflammatory process, a low-grade vasculitis.

Due to general uncertainty and disparities in interpretations of the histological criteria of vasculitis, we preferred to present our data in a descriptive form.

Intimal thickening with calcifications and medial hyperplasia with fibrosis are usually considered to be a result of arterial pressure on a venous graft (“arterialization” of the vein). Like Milroy et al., we observed those signs in native saphenous veins (128). The graft patency might be related to preexisting pathologies in the conduit (128;129).

Additional studies are needed to shed light on the role of subclinical vascular inflammation in the development of vascular pathologies, especially the formation and instability of atherosclerotic lesions and aneurysms. Elucidation of the causes of cardiovascular disease, the number one global cause of death, may have implications for its prevention and treatment. Chronic vascular inflammation may hypothetically be reduced with anti-infectious or immunomodulatory treatments, such as antirheumatic drugs, or statins (130).

**Figure 11. Mononuclear cell infiltrates (MCIs) in the aorta**



*We observed a high occurrence of MCIs in the inner adventitia, localized diffusely in the connective tissue, and/or around the vasa vasorum. Adventitial MCIs were more frequent and extensive in IRD than in non-IRD patients. MCIs in the media were observed only in IRD patients. MCIs along the mesothelium (epicardial MCIs) were common in both IRD and non-IRD patients. Atherosclerotic lesions and inflamed atherosclerotic lesions occurred at similar frequencies in IRD and non-IRD patients.*

### **5.2.2 Premature coronary artery bypass surgery in spondyloarthritis**

At the time of a first CABG, patients with spondyloarthritis were, on average, approximately seven years younger than those without spondyloarthritis. The age at spondyloarthritis onset was positively correlated to the age at the first CABG. Half the patients with spondyloarthritis compared to 25% of control patients underwent a first CABG before the age of 60.

Spondyloarthritis was a stronger independent predictor of early CABG than most of the traditional cardiovascular risk factors; only current cigarette smoking had a similar effect. At the time of a first CABG, the mean age of current smokers was approximately six years younger than that of non-smokers. However, former smoking was not associated with premature CABG, which underlines the importance of smoking cessation. Surprisingly, patients with hypertension underwent CABG at a later age than normotensive patients. This might be due to a better cardiovascular prophylaxis in hypertensive patients or to positive effects of antihypertensive therapy. Glucocorticosteroid users underwent a first CABG at a later age than non-users. This might reflect a protective cardiovascular effect of the drug or a late debut of conditions requiring glucocorticosteroid treatment.

The SpA and non-SpA groups were expected to have similar severities of CAD, as the groups were selected for CABG according to the same clinical criteria. Compared to the non-SpA group, the SpA group had a significantly lower mean number of significant coronary artery stenoses. However, other markers of the severity of CAD (NYHA class, left ventricle function, occurrence of left main coronary artery stenosis, and particularly, the occurrence and mean number of former myocardial infarctions) were more altered in the SpA compared to the non-SpA group, but the differences were not statistically significant. Thus, although the spondyloarthritis patients were significantly younger than the controls, they seemed to have similar severities of CAD. The discrepancy between severity of CAD and the occurrence of significant coronary stenoses observed in our study may indicate a different pathogenesis of CAD in patients with spondyloarthritis compared to the general population. For example, patients with spondyloarthritis might be more susceptible to plaque instability (also in non-significant stenoses) and/or thromboembolism and/or to aortic inflammation spreading to coronary arteries and causing left main coronary stenosis. Alternatively, the discrepancy might be due to a type I error.

The early need for a CABG may indicate that patients with spondyloarthritis have an earlier onset of severe CAD than in the general population. This might be caused by vasculitis and/or accelerated atherothrombosis (131). As premature atherosclerosis occurs in various inflammatory diseases, the systemic inflammation might enhance atherothrombosis also in spondyloarthritis. We demonstrated in Paper I that patients with CAD and spondyloarthritis frequently had subclinical aortic inflammation. In theory, vascular inflammation might cause vascular stenosis and/or contribute to the development of atherosclerotic lesions. Thus, antirheumatic treatment could be useful for reducing cardiovascular morbidity in spondyloarthritis.

Although the age difference between the groups could not be explained by differences in the traditional risk factors or current medications, the role of these factors may not be ruled out definitively. The lack of observed relationships might be due to type 2 errors. Moreover, a life time burden of medications and cardiovascular risk factors, including those not recorded in our study, might influence the premature need of CABG in spondyloarthritis.

## **5.2 Prospective for future studies**

### **Main questions to be addressed in further studies with our bio- and databank:**

- What are the features and causes of chronic vascular inflammatory infiltrates?
- Does chronic vascular inflammation influence the risk for perioperative and late complications?
- Why are some vessels more prone to inflammation than others? Are there differences in composition; e.g., in the characteristics of extracellular matrix or in the presence of immunoreactive cells?
- Do patients with and without IRD have different risks for perioperative and late complications?

- Do patients with IRD have more inflammation in small vessels and in the heart than patients without IRD?

Several projects are currently in progress. We have also planned to collect vascular specimens from a larger patient population.

## 6. Conclusions and clinical implications

### Conclusions

- It was possible to detect aortic inflammation in tissue specimens obtained in a CABG.
- IRD patients had similar occurrence of inflamed atherosclerotic lesions and epicardial MCIs as non-IRD patients, but a significantly higher occurrence and extent of medial and adventitial MCIs in aortic specimens. Acute inflammatory cell infiltrate was observed only in 1 patient, in the aortic adventitia.
- MCIs outside of atherosclerotic lesions, in the media or adventitia, occurred most frequently in the thoracic aorta, less frequently in the saphenous vein, and least frequently in the IMA; these frequencies paralleled susceptibilities of the vessels to atherosclerosis.
- IRD and current cigarette smoking were independent predictors of MCIs in non-atherosclerotic regions of the aorta, but not the saphenous vein or the IMA (i.e., vessels with lower frequencies of atherosclerosis and inflammation).
- At the time of a first CABG, patients with spondyloarthritis were, on average, 7 years younger than patients without spondyloarthritis. Spondyloarthritis was a stronger independent predictor of early CABG than most traditional cardiovascular risk factors.
- We have established a unique data- and biobank for future research.

### Clinical implications

Clinicians should realize the opportunity of diagnosing aortitis in patients referred for a CABG. Inflammation in the vascular media and adventitia might play a role in the early pathogenesis of cardiovascular disease, in particular in atherosclerosis and aneurysms. If true, prophylaxis or treatment of vascular inflammation might reduce cardiovascular risk. Spondyloarthritis might be associated with premature CAD; therefore, these patients might require effective cardiovascular prophylaxis, treatment, and follow-up.

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