INTRODUCTION

Calcific aortic valve disease is a slowly progressive disorder with a disease continuum that ranges from mild valve thickening without obstruction of blood flow, termed aortic sclerosis, to severe calcification with impaired leaflet motion, or aortic stenosis ***(Rosario v. 2005).*** In the past, this process was thought to be "degenerative" because of time-dependent wear-and-tear of the leaflets with passive calcium deposition. Now, there is compelling histopathologic and clinical data suggesting that calcific valve disease is an active disease process akin to atherosclerosis with lipoprotein deposition, chronic inflammation, and active leaflet calcification. The overlap in the clinical factors associated with calcific valve disease and atherosclerosis and the correlation between the severity of peripheral arterial diseases and aortic valve calcification provide further support for a shared disease process ***(Freeman R. otto, 2005).***

Aortic valve diseases are defined by the following features on echocardiography:

If Leaflet thickening, stiffness, and/or increased echogenicity (calcification) are the hallmarks of the condition without commissural fusion, so the diagnosis of aortic valve sclerosis ***(Otto c et al., 1999).*** Also Focal areas of thickening are typically seen on the aortic side of the valve in the center of

the valve cusp, rather than at the leaflet edges, often initially involving the non-coronary cusp ***(Stewart et al., 1997).***

If Leaflet excursion is impaired and the commissures are fused with increase in leaflet calcification and elevation of Doppler flow velocities (>2.5 m/sec) so the diagnosis of aortic valve stenosis ***(White, 2007).***

Peripheral artery disease is a common circulatory problem in which narrowed arteries reduce blood flow to the limbs ***(O'Brien, 2006).*** When someone develops peripheral artery disease (PAD), his extremities — usually his legs — don't receive enough blood flow to keep up with demand. This causes symptoms, most notably leg pain when walking (intermittent claudication) ***(Meijer et al., 2002).*** Peripheral artery disease is also likely to be a sign of a more widespread accumulation of fatty deposits in the arteries (atherosclerosis). This condition may be reducing blood flow to the heart and brain, as well as the legs.Periphral arterial disease can be diagnosed with ankle brachial index) ***(Newman et al., 1993).***

The ankle-brachial index (ABI) is a simple, reliable means for diagnosing PAD. Blood pressure measurements are taken at the arms and ankles using a pencil shaped ultrasound device called a Doppler. The arm and ankle systolic blood pressure measurements are recorded. Then the ankle systolic pressures are divided by the highest arm pressure to establish

an ABI measurement for each leg. The ABI range that is generally considered normal is .95 to 1.2.

The ABI test is simple enough to be performed in any doctor's office or vascular laboratory. Not only is the ABI one of the most reliable tests for PAD, it is also the least expensive ***(McDermott et al., 2000).***

The ABI test is used to document the presence or absence of PAD, and can be performed every year to quickly assess whether PAD is getting worse ***(Allison et al., 2008).***

AIM OF THE WORK

To study the prevalence of Peripheral Arterial Disease in patients with Calcific Aortic Valve Disease, using the ABI.

AORTIC VALVE CALCI-SCLEROSIS

Introduction

Calcific aortic valve disease is identified by thickening and calcification of the aortic valve leaflets in the absence of rheumatic heart disease. It is divided, on a functional basis, into aortic sclerosis, in which the leaflets do not obstruct left ventricular outflow, and aortic stenosis, in which obstruction to left ventricular outflow is present. Aortic sclerosis is present in more than 25% of patients over age 65 ***(Stewart et al., 1997)*** and is associated with a 50% increase risk of cardiovascular events ***(Otto et al., 1999).***

Over the past 10 to 15 years, calcific aortic valve disease, which includes aortic sclerosis and aortic stenosis, has come to be recognized as an active process, based on: (1) epidemiologic studies demonstrating associations of specific risk factors with increased prevalence or rate of progression of aortic valve disease; (2) identification, in valve lesions, of histopathologic features of chronic inflammation, lipoprotein deposition, renin-angiotensin system components, and molecular mediators of calcification; and (3) identification of cell-signaling pathways and genetic factors that may participate in valve disease pathogenesis. These studies will be reviewed and organized into a proposed global hypothesis for the pathogenesis of calcific aortic valve disease.

Pathology and Pathogenesis

Macroscopicall:

Degenerative aortic valve disease, characterized macroscopically as increased leaflet thickness, stiffening and calcification, without commissural fusion, is common among the elderly (Fig. 1) ***(Lindroos et al., 1993).***

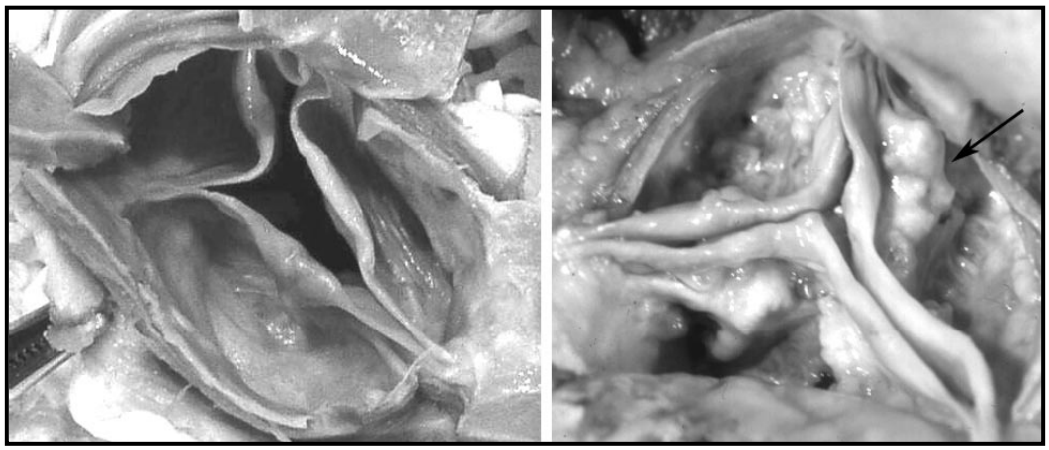

Figure (1): Gross specimen of minimally diseased aortic valve (left) and severely stenotic aortic valve (right). In the severely stenotic valve, there are prominent lipocalcific changes on aortic side of valve cusps (arrow), with sparing of commissures ***(Freeman, 2005).***

Histopathology:

Histopathologic studies have shown striking similarities between aortic valve sclerosis and atherosclerosis ***(Otto et al., 1994; Olsson et al., 1994; Edep et al., 2000)*** including:

1- Chronic Inflammation:

In 1994, a series of 3 studies reported that aortic valve lesions contained the cell types characteristic of chronic inflammation: macrophages ***(Otto et al., 1994; Olsson et al., 1994)*** and T lymphocytes ***(Olsson et al., 1994; Olsson et al., 1994)*.** One also found expression of important chronic inflammation effector molecules, including interleukin (IL)-2 and the Class II human leukocyte antigen, HLA-DR ***(Olsson et al., 1994)*.** More recently, mast cells ***(Helske et al., 2004)*** and the proinflammatory cytokines, IL-1β ***(Kaden et al., 2003)*** and tumor necrosis factor (TNF)-α ***(Kaden et al., 2005)***, also have been identified in stenotic aortic valves.

In addition, aortic valve lesions contain a number of matrix-metalloproteinases (MMPs) ***(Soini et al., 2001; Kaden et al., 2003)***, which degrade various components of the extracellular matrix. Results differ as to whether levels of the natural inhibitors of MMPs, tissue inhibitors of matrix metalloproteinases (TIMPs), are increased ***(Soini et al., 2001)*** or unchanged ***(Kaden et al., 2005)*** in valve lesions. These molecules typically are expressed in inflammatory and fibrosing illnesses.

2- Endothelial Dysfunction:

At the tissue level, aortic sclerosis is characterized by focal areas of subendothelial thickening on the aortic side of the

valve leaflet. The normal aortic valve leaflet consists of three well-defined layers: the fibrosa is the central dense collagen layer that provides tensile strength to the leaflet; the ventricularis is an elastin-rich layer on the ventricular side of the leaflet; and the spongiosa is a layer of loose connective tissue typically confined to the basal one-third of the leaflet.

The lesions of aortic sclerosis displace the subendothelial elastic lamina on the aortic side of the leaflet and extend into the adjacent fibrosa. It is presumed that endothelial disruption related to altered shear stress on the aortic side of the leaflet may initiate the disease process, although there is no direct evidence for this hypothesis (Fig.2).

3- Lipoprotein Deposition:

Another hallmark of athero**sclerosis** is deposition of plasma lipoproteins in plaques. Similarly, the "atherogenic" lipoproteins, LDL ***(Walton et al., 1970; Olsson et al., 1999)*** and Lp(a) ***(O'Brien et al., 1996)***, are deposited in human aortic valve lesions, and aortic valve cholesterol content is increased in a hyper-cholesterolemic rabbit model of aortic valve disease ***(Rajamannan et al., 2002*)**.

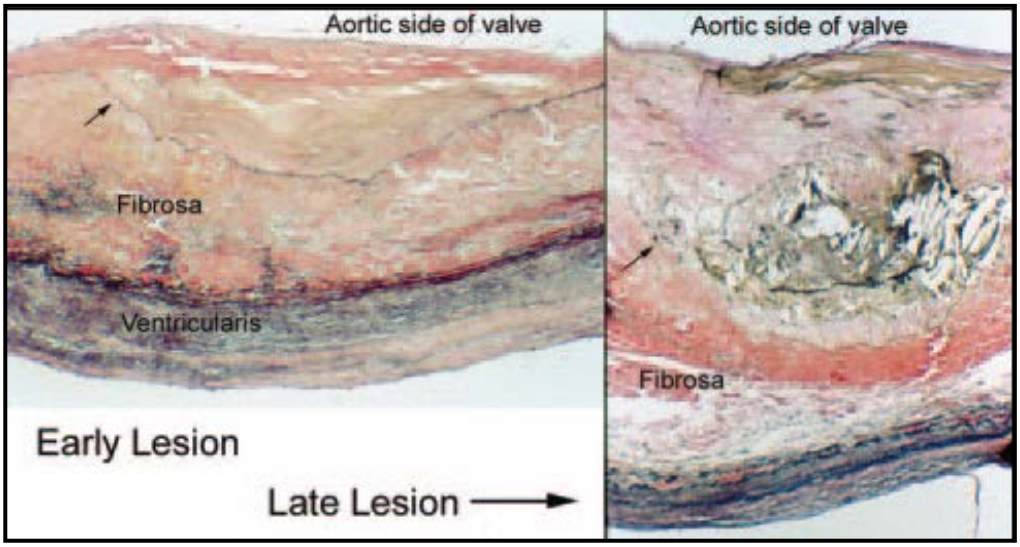

Figure (2): Examples of histological findings in early and late lesions of calcific aortic valve disease ***(Freeman, 2005).***

Similar to atherosclerosis ***O'Brien et al. (1998)***, aortic lesion lipoprotein deposition likely is mediated, at least in part, by accumulated extracellular matrix proteoglycans, including biglycan and decorin ***(Wight, 1989).*** In general, lipoproteins bind to proteoglycans via charge-charge interactions between positively-charged basic amino acids on apolipoproteins and negatively-charged glycosaminoglycan side chains of proteoglycans ***(Olsson et al., 1997).*** Interestingly, a mutation of the single basic amino acid in apoB that mediates LDL binding to proteolgycans markedly decreases atherosclerosis in a murine model ***(Skalen et al., 2002).*** Therefore, lipoprotein-proteoglycan interactions may not only link elevated plasma

LDL and Lp(a) levels with increased aortic valve disease risk ***(Stewart et al., 1997)***, but also may represent a therapeutic target.

Oxidized lipids also have been detected in human aortic valve lesions ***(Olsson et al., 1999)***, particularly in areas of developing calcification. In vitro studies have shown that oxidized cholesterol stimulates calcified nodule formation by valve fibroblasts ***(Mohler et al., 1997)***, and that calcified nodule formation by these cells is inhibited by simvastatin ***(Wu et al., 2005).*** Together, these observations provide a potential link between accumulated lesion lipoproteins and calcification and also suggest that statins might have therapeutic benefit.

4- Renin-Angiotensin System Activation:

A subset of aortic valve lesion macrophages express ACE ***(O'Brien et al., 2002; Helske et al., 2004).*** Surprisingly, a large proportion of valve lesion ACE colocalizes with LDL in the extracellular matrix ***(O'Brien et al., 2002).*** Ang II also is localized to these regions, suggesting that the LDL-associated ACE is enzymatically active ***(O'Brien et al., 2002).*** In addition, AT1 receptor is expressed by fibroblasts only in lesions ***(O'Brien et al., 2002; Helske et al., 2004).*** Degranulated mast cells also are present in lesions ***(Helske et al., 2004).*** This latter observation is important, because mast cell granules contain

chymase, a non-ACE enzyme that also can generate Ang II ***(Helske et al., 2004).***

Thus, aortic valve lesions contain a number of potential sources of Ang II: (1) LDL-associated ACE; (2) macrophage-associated ACE; and (3) mast cell chymase. Moreover, the major pathogenic receptor for Ang II is present in valve lesion fibroblasts. However, whereas smooth muscle cells constitutively express AT-1 receptor, this receptor is only expressed by valve fibroblasts of lesions. Thus, unlike atherosclerosis, where Ang II may affect normal non-plaque smooth muscle cells, valve fibroblasts may be protected from the adverse effects of Ang II until they begin to express AT-1 receptor in early-stage lesions, thereby blunting any potential effects of Ang II on valve lesion pathogenesis.

This also may account for the mixed results of retrospective studies, with one showing a strong association between ACE inhibitor use and decreased rate of valve calcification ***(O'Brien et al., 2005)*** and another finding no effect on progression of AS ***(Rosenhek et al., 2004).*** However, in the latter study, AS was severe in nearly half of all subjects and mean follow-up was only 24 months ***(Rosenhek et al., 2004).*** It therefore may be that, if ACE inhibitors or angiotensin receptor antagonists are to have any benefit, treatment will need to be extended over longer periods of time and/or targeted to either aorticsclerosis ***(O'Brien et al., 2005)*** or earlier-stage AS.

5- Calcification:

Aortic valve calcification now has been shown unequivocally to be an active, rather than a passive, process. Valvular calcium deposits contain both calcium and phosphate ***(Anderson, 1983; O'Brien et al., 1996; Mohler et al., 1999)*** as hydroxyapatite ***(Anderson, 1983; Mohler et al., 1999)***, the form of calcium-phosphate mineral present in both calcified arterial tissue ***(Bostrom et al., 1993)*** and bone. Proteins involved in regulation of tissue calcification have been detected in calcified valvular tissue, including osteopontin ***(O'Brien et al., 1995; Mohler et al., 1997)***, bone morphogenic proteins (BMPs) 2 and 4, ***(Mohler et al., 2001)*** and receptor activator of nuclear factor NF-κB ligand (RANKL) ***(Kaden et al., 2004)***. Osteoprotegrin (OPG), which prevents mineral resorption in bone tissue, is a soluble decoy receptor that resembles RANK and acts as a competitive inhibitor of RANK binding to RANKL. RANK is expressed in normal valve leaflets, but is downregulated in aortic valve lesions ***(Kaden et al., 2004)***. The osteoblast-specific transcription factor, Runx2/Cbfa1, has been detected in rabbit models of experimental aortic valve disease ***(Rajamannan et al., 2002; Rajamannan et al., 2003; Arishiro et al., 2005)***, and osteoblast-like cells have been identified both in a rabbit model ***(Rajamannan et al., 2003)*** and in calcified human valves ***(Mohler et al., 2002)***. Finally, a subset of end-stage human aortic valve lesions contain heterotopic bone ***(Mohler et al., 2002)***,

further confirming the dysregulated nature of aortic valvular calcification.

In aortic valve lesions, calcified nodules appear to first form in regions of lipid deposition ***(O'Brien et al., 1996; Olsson et al., 1999)***, particularly those with oxidized lipids ***(Olsson et al., 1999).*** They also contain tenascin C ***(Jian et al., 2001)***, an extracellular matrix glycoprotein found in developing bones ***(Jian et al., 2001).*** Recently, groups have isolated a subset of valvular fibroblasts that express osteoblast markers ***(Mohler et al., 1999; Kaden et al., 2004)*** and spontaneously form hydroxyapatite-containing calcified nodules in vitro ***(Mohler et al., 1999; Kaden et al., 2004).*** In response to oxidized cholesterol ***(Mohler et al., 1999)***, transforming growth factor (TGF) β1 ***(Mohler et al., 1999)***, BMP2 ***(Mohler et al., 1999)***, and RANKL ***(Kaden et al., 2004)***, these cells increase their expression of osteoblast markers and increase their rate of calcified nodule formation.

In addition, tenascin C upregulates matrix metalloproteinase (MMP)-2 expression in these cells ***(Jian et al., 2001).*** Importantly, it recently has been shown that statins inhibit calcified nodule formation in these cells, at least in part through inhibition of protein prenylation ***(Wu et al., 2005).*** Finally, hyperphosphatemia has been shown to induce calcified vesicle formation in myofibroblasts ***(Reynolds et al., 2004)***, thereby suggesting a

potential mechanism linking chronic kidney disease to valvular calcification ***(Raggi et al., 2004).*** Osteopontin may be an important inhibitor of valvular calcification.

In one recent study, calcification was dramatically increased in glutaraldehyde-fixed aortic valve leaflets after subcutaneous implantation into osteopontin-deficient as compared with wild-type mice ***(Steitz et al., 2002).*** These authors also demonstrated that, by inducing macrophage carbonic anhydrase expression (thereby creating an acidic extracellular environment), osteopontin actively promoted the dissolution of hydroxyapatite ***(Steitz et al., 2002).*** Carbonic anhydrase has also been demonstrated to play an inhibitory role in a rat model of aortic medial elastocalcinosis ***(Essalihi et al., 2005).*** Interestingly, osteopontin is expressed by infiltrating macrophages in both atherosclerotic ***(Giachelli et al., 1993)*** and aortic valve ***(O'Brien et al., 1995)*** lesions.

However, whereas atherosclerotic plaque smooth muscle cells also express osteopontin ***(Giachelli et al., 1993)***, valve lesion fibroblasts do not ***(O'Brien et al., 1995).*** Thus, as a result of differences in osteopontin expression by the dominant mesenchymal cell type, the relative efficacy of osteopontin as a calcification inhibitor may differ in atherosclerosis and aortic valve disease.

Together, these findings implicate oxidized lipids and macrophage- and T-lymphocyte-produced cytokines in valvular

calcification. They also suggest that specific signaling mechanisms are involved in valvular calcification. For example, the presence of chronic inflammation, inflammatory cytokines, oxidized lipids, and RANKL in lesions suggests that NF-κB activation is a crucial step in the vascular calcification process. NF-κB is upregulated by inflammatory cytokines, oxidant stress, and Ang II, and it signals through the mitogen-activated protein (MAP) kinase pathway.

Atherogenic factors, including oxidized lipids ***(Olsson et al., 1999; Mohler et al., 1999)***, TNF-α, and hyperglycemia ***(Aronow et al., 1987; Aronow et al., 2001; Katz et al., 2006)***, all might mediate valvular calcification, at least in part, through pathways activated by BMP2. BMP2 is present in human aortic valve lesions and stimulates calcified nodule formation by valvular fibroblasts in vitro ***(Mohler et al., 1999).*** BMP2 can upregulate both an "osteogenic" pathway involving the transcription factor Msx2 (which activates Wnt signaling), and a chondro-osteogenic pathway involving the transcription factor Runx2/Cbfa1 ***(Wozney and Rosen, 1998).*** Shao and colleagues have demonstrated that Msx2-overexpressing mice have increased vascular calcification ***(Shao et al., 2005).*** That this effect was mediated through Wnt activation was supported by additional evidence by Msx2 overexpression experiments in TOPGAL+ (Wnt reporter) mice ***(Shao et al., 2005).*** Rajamannan and colleagues have directly implicated the Wnt/LDL receptor-related protein 5 (Lrp5)/β-catenin pathway