PATHOGENESIS OF AORTIC STENOSIS: IMPLICATIONS REGARDING IMPAIRMENT OF NITRIC OXIDE SIGNALLING

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for the degree of Doctor of Philosophy

Dedicated to my beautiful

wife Doan and son Joshua

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Abstract

Aortic valve stenosis (AS) is now the most common valve disease in Western world and its prevalence and incidence are rising. The earliest clinically detectable stage of this process, aortic valve sclerosis (ASc), reflects abnormal aortic valve morphology in the absence of haemodynamic obstruction, but may progress to AS. The prevalence of ASc is as high as 25% in populations over 65 years of age: - thus it carries important epidemiological, clinical and pathophysiological implications. Despite the increased interest into studies of ASc/AS, the pathogenesis of this condition remains largely elusive, except to say that rather than the notion of being just a "wear and tear" inevitable process, it is now accepted to be an active pathophysiological process. The relevant literature is reviewed in Chapter 1.

Studies described in this thesis address the determinants of occurrence and progression of ASc in a cohort of aging subjects followed for 4 years. Novel methodology of aortic valve ultrasonic backscatter was utilized to quantitate ASc severity and progression. In the subsequent studies the effects of ASc on left ventricular hypertrophy (LVH) were evaluated in a separate cohort of healthy aging individuals, with no significant cardiovascular risk factors or hypertensive therapy. Finally, effects of aging on integrity of the nitric oxide (NO) signalling cascade were examined in the population cohort recruited for evaluation of progression of ASc.

The key findings from this thesis are:

(1) Platelet NO responsiveness is a determinant of both the occurrence and progression of ASc, while age and BMI are determinant of occurrence only. Calcium levels and arterial stiffness correlate only with progression. Categorical assessment of progression reveals that use of inhibitors of the renin-angiotensin system is associated with lack of ASc progression.

(2) Whilst ASc is not correlated with the development of LVH in the absence of treated hypertension, markers of NO generation and of the NO/ cyclic GMP signalling cascade in the peripheral circulation predict both LV mass index and LV diastolic function in a normal, untreated, aging population, irrespective of ASc status.

(3) Aging is associated with both increases in ADP-induced platelet aggregation and plasma asymmetric dimethyarginine (ADMA) concentrations, and with reductions in platelet NO responsiveness. Female gender is associated with more severely impaired platelet NO responsiveness, greater arterial stiffness and a more pronounced fall in platelet NO responsiveness with time, which in turn was also observed in subjects with lower plasma vitamin D concentrations. There is a significant relationship between deterioration in platelet NO responsiveness and increases in ADMA concentrations. Finally, use of angiotensin convertin enzyme inhibitors/angiotensin receptor blockers is associated with preserved platelet NO responsiveness and lower arterial stiffness.

In summary, the aging process is associated with a remarkable degree of attenuation of NO generation and signalling, which constitutes both a correlate of ASc development/progression and of the development of LVH (although the latter is not closely associated with ASc in "normal" populations). Furthermore, the rate of deterioration of NO signalling is greatest in females, in the presence of low vitamin D levels and correlates with rises in ADMA concentrations.

Declaration

I, Aaron Leonid Sverdlov, certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Aaron Leonid Sverdlov

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Published works in whole or in part contained within this thesis

1. **Sverdlov AL**, Ngo DT, Horowitz JD. Pathogenesis of aortic sclerosis: association with low BMI, tissue nitric oxide resistance, but not systemic inflammatory activation. *Am J Cardiovasc Dis* 2012; 2(1):43-9.
2. **Sverdlov AL**, Ngo DT, Horowitz JD. Redefining the natural history of calcific aortic stenosis: lessons from Laennec. *J Intern Med* 2012. In press. Doi: 10.1111/j.1365-2796.2012.02520.x.
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4. **Sverdlov AL**, Ngo DT, Nightingale AK, Rajendran S, Mishra K, Heresztyn T, Ritchie RH, Marwick TH, Frenneaux MP, Horowitz JD. The endogenous NOS inhibitor asymmetric dimethylarginine (ADMA) predicts LV mass independent of afterload. *Nitric Oxide* 2011; 25(1): 41-46.
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1. **Sverdlov AL**, Ngo DT, Horowitz JD. Redefining the natural history of calcific aortic stenosis: lessons from Laennec. *J Intern Med* 2012. In press. Doi: 10.1111/j.1365-2796.2012.02520.x.

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Peer-reviewed full text publications arising from and/or related to the work conducted towards this thesis - in submission.

1. **Sverdlov AL***, Ngo DT*, Chan, WPA, Chirkov YY, Gersh BJ, McNeil JJ, Horowitz JD. Determinants of aortic sclerosis progression: implications regarding impairment of nitric oxide signalling and potential therapeutics. *Eur Heart J* - under review.

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Publications/presentations in abstract form related to this thesis

1. **Sverdlov AL**, Ngo DTM, Chan WP, Chapman MJ, Chirkov YY, Gersh BJ, McNeil JJ, Horowitz JD. Progression of Early Aortic Valve Disease: Are ACE Inhibitors Protective? Presented at AHA Scientific Sessions 2011, Orlando, USA.

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List of abbreviations

5-HT	Serotonin
ACE	Angiotensin converting enzyme
ACEI	ACE inhibitor
ADMA	Asymmetric dimethylarginine
ADP	Adenosine diphosphate
ANCOVA	Analysis of co-variance
Ang II	Angiotensin II
apo	Apolipoproteins
ARB	Angiotensin receptor blockers
AS	Aortic stenosis
ASc	Aortic sclerosis
AVA	Aortic valve area
AVBS	Aortic valve ultrasonic backscatter score
AVp	Aortic valve pressure gradient (transvalvular pressure gradient)
AVR	Aortic valve replacement
AVv	Aortic valve velocity (transvalvular velocity)
BAV	Bicuspid aortic valve
BMI	Body mass index
BMP	Bone morphogenic protein
BNP	Brain natriuretic peptide
BSA	Body surface area
CAD	Coronary artery disease
CFR	Coronary flow reserve
CMs	Cardiomyocytes

CrCl	Cretinine clearance
CRP	C-reactive protein
CT	Computed tomography
CTx	C-terminal telopeptide of collagen type 1
DDAH	Dimethylarginine dimethylaminohydrolase
DNA	Deoxyribonucleic Acid
EF	Ejection fraction
eNOS	Endothelial nitric oxide synthase
EPC	Endothelial progenitor cells
FMD	Flow mediated dilatation
GMP	Guanosine monophosphate
GP	Glycoprotein
GTN	Glyceryl trinitrate
HF/HC	High fat/high carbohydrate
hs-CRP	High sensitivity CRP
HT	Hypertension
IL	Interleukin
INOS	Inducible nitric oxide synthase
LDL	Low density lipoprotein
LDLr -/-	LDL receptor deficient
L-NAME	L-Nitro-Arginine Methyl Ester
LV	Left ventricle
LVH	Left ventricular hypertrophy
MC	Mast cell
MGP	Matrix Gla-protein

MMP	Matrix metalloproteinases
MRI	Magnetic resonance imaging
NAD(P)H	Nicotinamide Adenine Dinucleotide (Phosphate) Hydrogen
nNOS	Neuronal nitric oxide synthase
NO	Nitric oxide
NOS	Nitric oxide synthase
P1NP	N-terminal peptide of procollagen I
PAI-I	Plasminogen activator inhibitor-1
PRMT	Protein arginine methyltransferase
RAAS	Renin-angiotensin-aldosterone system
RANK	Receptor Activator of Nuclear Factor κ B
RANKL	Receptor Activator of Nuclear Factor κ B ligand
ROS	Reactive oxygen species
sGC	Soluble guanylate cyclase
SNP	Sodium nitroprusside
TAVI	Percutaneous transcatheter aortic valve implantation
TGF-β	Transforming growth factor beta
TNF-α	Tumor necrosis factor-alpha
TXNIP	Thioredoxin-interacting protein
VICs	Valvular interstitial cells
vWf	von Willebrand factor