

**Pathogenesis of aortic valve stenosis:
bench to bedside approach**

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THESIS SUMMARY

Experiments described in this thesis address the pathogenesis of aortic valve sclerosis/stenosis using a bench to bedside approach. In particular, the thesis begins with development of a technique using ultrasonic backscatter analyses to quantitate the early stages of aortic stenosis. Subsequent chapters utilized this methodology to quantitate aortic valve structural changes in a model and intervention study of aortic stenosis in rabbits. The last chapters are human studies designed to identify factors associated with presence of aortic sclerosis/stenosis; with particular interest in potential association of endothelial dysfunction/inflammation/platelet aggregation with abnormal aortic valve structure quantitated by ultrasonic backscatter. In Chapter 1 (Introduction) the relevant literature is reviewed.

Development of ultrasonic backscatter to quantitate aortic sclerosis (Chapter 2)

Aortic valve sclerosis (ASc) is detected when there is visual assessment of focal increases in echogenicity of the aortic valve most commonly assessed by echocardiography. However, there is no previously described method to quantitate degree of aortic valve structural abnormality as ASc is not associated with marked hemodynamic obstruction quantifiable by Doppler echocardiography. The current study used ultrasonic backscatter to quantitate aortic valve structural abnormality in patients assessed as having ASc based on valve appearances, compared to young healthy volunteers with normal aortic valves.

The results of the study indicate: 1) that the mean levels of aortic valve backscatter in ASc patients are approximately 60% greater than in young healthy volunteers (ie aortic valve backscatter scores ≥ 16 dB are not consistent with normal aortic valve structure), 2) ultrasonic backscatter scores in ASc patients are directly correlated with subjective scoring of sclerosis and with a positive trend with transvalvular pressure gradients in patients with mild-moderate aortic stenosis, and most importantly, 3) ultrasonic backscatter is a reproducible technique, with mean differences between estimates based on repeat echocardiograms of 2.3 ± 1.7 (9.1%). These results indicate that ultrasonic backscatter could be used as a quantitative measure of aortic valve structural abnormality in epidemiology and for examination of interventions.

In vivo studies

Development of an animal model of aortic stenosis with vitamin D₂ (Chapter 3)

The aim of the study was to develop an appropriate animal model for AS. The study used vitamin D₂ alone at 25,000IU/4 days weekly (vit-D₂) for 8 weeks to induce AS in rabbits. Results showed that: 1) rabbits in the vit-D₂ group had significantly increased in transvalvular velocity and pressure gradients compared to rabbits in the control group (normal chow + drinking water); this was consistent for aortic valve ultrasonic backscatter scores; 2) aortic valve immunohistochemistry/histology showed marked calcification, neutral lipids, macrophage, and leukocyte infiltrations for rabbits in the vit-D₂ group (ie consistent with histology of human AS); 3) significant elevation of asymmetric dimethylarginine (ADMA) concentrations in the vit-D₂ group occurred

compared to controls over the 8 weeks treatment period; the change in ADMA concentrations correlated significantly with the change in transvalvular pressure gradients for rabbits in the vit-D₂ group; 4) rabbits in the vit-D₂ group had significantly impaired endothelium-dependent acetylcholine-induced aortic relaxation, and this effect was completely abolished by the nitric oxide synthase inhibitor (L-NAME); 5) the addition of 0.5% cholesterol-supplemented diet to the vitamin D₂ regimen did not accentuate the development of AS. Thus, treatment with vitamin D₂ at 25,000IU/4 days weekly for 8 weeks significantly induced AS with similar aortic valve pathology to that of human AS; therefore, the model is suitable for use in examining potential therapeutic interventions in AS.

Effects of ramipril on development of AS in rabbits (Chapter 4)

Using this animal model, this study aimed to examine the effects of the angiotensin-converting enzyme inhibitor (ACEi) ramipril on development of AS. Rabbits (n=28) treated for 8 weeks were divided into 2 groups: (a) vitamin D₂ alone (n=10) (normal chow + 25,000IU vitamin D₂ in drinking water); (b) vitamin D₂/Ramipril (n=12) (normal chow+25,000IU vitamin D₂/Ramipril (0.5mg/kg) in drinking water). Six further rabbits constituted a normal reference group (no treatment was given). The results for comparisons between vitamin D₂/ramipril vs vitamin D₂ alone were as follows: 1) ramipril-treated rabbits had significantly less severe hemodynamic obstructions (p<0.05, for both) as assessed by transvalvular velocity, and aortic valve area; with borderline reduction in aortic valve backscatter (p=0.08); 2) ramipril significantly reduced plasma

ADMA concentrations; 3) there was improvement in acetylcholine-induced aortic relaxation ($p=0.056$), with significant improvement in sodium nitroprusside-induced relaxation ($p<0.05$); 4) there was a strong inverse correlation between acetylcholine-induced aortic relaxation and aortic valve backscatter score ($p<0.001$), thus providing further evidence of the potential role of nitric oxide in retarding the development of AS in this model.

These data provide a strong rationale for the inception of a randomized trial of ACE inhibition as a strategy for limitation of AS progression in humans.

Human studies

Aortic stenosis is associated with elevated plasma levels of asymmetric dimethylarginine (ADMA) concentrations in humans (Chapter 5).

Given the findings that aortic stenosis induced by vitamin D₂ in rabbits also caused elevation of plasma ADMA concentrations, a physiological inhibitor of nitric oxide synthase, a mediator and marker of endothelial dysfunction and an indicator of incremental cardiovascular risk. The study sought to determine whether plasma ADMA concentrations are elevated independently of pre-existing coronary risk factors in subjects with at least moderate aortic stenosis (n=42) compared to age-matched patients with normal aortic valves (n=42): as determined both by visual assessment and with aortic valve backscatter scores < 16dB. Results for this study were as follows: 1) plasma ADMA concentrations were not statistically different between the AS and non-AS group

(median 0.59 vs 0.54 $\mu\text{mol/L}$, $p=0.13$, Mann-Whitney test) on univariate analysis; 2) backward stepwise multiple linear regression showed the presence of AS was a significant predictor of elevated ADMA concentrations ($p=0.04$, 95% CI =0.001, 0.072). 3) in addition, elevated plasma ADMA concentrations were also associated with history of atrial fibrillation ($p=0.009$, 95% CI=0.015, 0.100), and negatively associated with creatinine clearance ($p=0.01$, 95% CI=-0.002, 0.000), and the use of statin therapy ($p=0.01$, 95% CI=-0.081, -0.011). Therefore, in conclusion, this study found that AS is independently associated with elevation of ADMA concentrations, beyond that implied by “conventional” risk factors for endothelial dysfunction. The clinical status of AS as an incremental marker of cardiovascular risk may reflect ADMA-mediated endothelial dysfunction.

Assessment of factors associated with ASc in a random ageing population study
(Chapter 6).

There have been few clinical studies of factors associated with ASc. Previous population studies have established that ASc is an independent correlate of incremental risk of coronary events. Having established that patients with AS have increased plasma ADMA concentrations (Chapter 5), it was now aimed to determine whether subjects with increased aortic valve backscatter scores (ASc) also have other markers of endothelial dysfunction/NO effects, independent of preexisting coronary risk factors. The study was designed to identify such anomalies, if they existed, on an incremental basis to other

putative correlates of ASc, including coronary risk factors, renal dysfunction and vitamin D levels.

Random selected subjects (n=253) aged between 51 to 77 years were evaluated. All patients underwent transthoracic echocardiography examination; aortic valve ultrasonic backscatter score (AV_{BS}), was used to quantitate echogenicity of the aortic valve. Conventional coronary risk factors were identified on history. Integrity of NO generation/response was assessed via (i) plasma ADMA concentrations; (ii) inhibition of platelet aggregation by the NO donor sodium nitroprusside (SNP); (iii) aortic augmentation index (AIx), a measure of arterial stiffness/wave reflection. All putative correlations with AV_{BS} were examined by univariate and stepwise multiple linear regression analyses.

On the basis of echocardiographic appearances, ASc was present in 63 subjects (25.4%); mean AVBS scores was 14.9 ± 4.6 dB (SD) vs 11.2 ± 3.9 dB (SD) in the presence vs absence of ASc ($p < 0.001$). Univariate analyses revealed that platelet responsiveness to NO was inversely correlated with AV_{BS} ($\beta = -0.16$, $p = 0.02$); but [ADMA] and AIx were not. On multiple linear regression, significant correlates of increased AV_{BS} were: (i) advanced age ($\beta = 0.21$, $p = 0.003$), (ii) low body mass index ($\beta = -0.23$, $p = 0.001$); and (iii) impaired platelet responsiveness to NO ($\beta = -0.16$, $p = 0.02$).

In Chapter 7, the implications of the overall findings in this thesis are discussed in relation to future perspective.

DECLARATION

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.

I give consent to this copy of my thesis being made available in the University Library.

Doan Thi Minh Ngo

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PUBLICATIONS/PRESENTATIONS

Peer reviewed articles relating to this thesis

- i) **Ngo DT**, Herestyzn T, Mishra K, Marwick TH, Horowitz JD. Aortic stenosis is associated with elevated plasma levels of asymmetric dimethylarginine (ADMA). *Nitric Oxide: Biology and Chemistry* 16: 197-201 (2007).
- ii) **Ngo DT**, Wuttke RD, Turner S, Marwick TH, Horowitz JD. Quantitative assessment of aortic sclerosis using ultrasonic backscatter. *J Am Soc Echocardiogr.* 17: 1123-1130 (2004).

Accepted presentations at national/international meetings:

- i) **Ngo DT**, Sverdlov AL, Willoughby SR, Nightingale AK, Chirkov YY, McNeil JJ, Horowitz JD. Aortic valve sclerosis is associated with impairment of platelet responsiveness to nitric oxide. Accepted oral abstract European Society of Cardiology Congress 2007. Abstract number 83029.
- ii) **Ngo DT**, Stafford I, Wuttke RD, Weedon H, Smith MD, Sverdlov AL, Kennedy JA, Horowitz JD. Ramipril retards development of aortic valve stenosis in an animal model. Accepted oral abstract European Society of Cardiology Congress 2007. Abstract number 83028.
- iii) **Ngo DT**, Wuttke RD, Weedon H, Nightingale AK, Sverdlov AL, Kennedy JA, Horowitz JD. Vitamin D₂ supplementation induces aortic stenosis in rabbits. Accepted oral abstract at World Congress of Cardiology 2006, European Society of Cardiology. Abstract number 83197.
- iv) **Ngo DT**, Wuttke RD, Stafford I, Rosenkranz AR, Sverdlov AL, Chirkov YY, Kennedy JA, Horowitz JD. Effects of vitamin D₂ supplementation on aortic valvular and vascular endothelial functions in a rabbit model of aortic stenosis. Accepted poster abstract at World Congress of Cardiology 2006, European Society of Cardiology. Abstract number 85356.
- v) **Ngo DT**, Wuttke RD, Weedon H, Stafford I, Nightingale AK, Rosenkranz AR, Sverdlov AL, Chirkov YY, Kennedy JA, Horowitz JD. Development of aortic valve stenosis (AS) induced by vitamin D₂ in a rabbit model. Accepted abstract at Cardiac Society of Australia and New Zealand 2006.

- vi) **Ngo DT**, Sverdlov AL, Willoughby SR, Nightingale AK, Chirkov YY, Horowitz JD. Correlates of aortic sclerosis: a population study. Accepted abstract at Cardiac Society of Australia and New Zealand 2006.
- vii) **Ngo DT**, Wuttke RD, Nightingale AK, Rosenkranz AC, Weedon H, Kennedy JA, Horowitz JD. Vitamin D induced aortic stenosis: an animal model. Accepted abstract in the oral prize finalist ASCEPT/APSA 2005.
- viii) Nightingale AK, Rajendran S, Mishra K, Sverdlov AL, **Ngo DT**, Horowitz JD. Vascular responses to GTN but not salbutamol decline with age. Accepted moderated abstract at EuroEcho 2006. Abstract number 644.
- ix) **Ngo DT**, Herestyzn T, Mishra K, Marwick TH, Horowitz JD. Aortic stenosis is associated with elevated plasma levels of asymmetric dimethylarginine (ADMA). Accepted abstract at Scientific Sessions American Heart Meeting 2005. Session number APS.56.1M, presentation number 3227.