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Osteocardiology: Defining the Go/No-Go Time Point for Therapy

Nalini M. Rajamannan

Division of Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN, and Most Sacred Heart of Jesus Cardiology and Valvular Institute, Sheboygan, WI, USA

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Valvular heart disease · Lipids · Pathophysiology · Atherosclerosis · Experimental models

Abstract
Recent epidemiological studies have revealed that the risk factors associated with coronary artery calcification (CAC), including male gender, smoking, hypertension, and elevated serum cholesterol, are similar to the risk factors associated with the development of calcific aortic valve disease (CAVD). The results of the experimental and clinical studies demonstrate that traditional risk factors initiate early atherosclerosis which over time differentiates to form bone in the heart causing clinical CAC and CAVD. Understanding the cellular mechanisms of cardiovascular calcification, the end-stage process of the atherosclerosis will help define the specific time point to modify this cellular process of bone formation in the heart termed osteocardiology. This time point between subclinical atherosclerosis and clinical calcification is the go/no-go time point, or the point of no return with severe clinical calcification in the heart. This review will summarize the development of bone formation in the heart termed osteocardiology, to define the go/no-go time point for therapy initiation to slow the progression of cardiovascular calcification.

Introduction

As the global population ages, due to advances in medical therapies, calcific atherosclerotic disease is emerging as a common clinical diagnosis. For years cardiovascular calcification was thought to be due to a degenerative phenomenon by which calcium attaches to the surface of the aortic valve leaflet and the lumen of the vasculature. In 2011, the National Heart, Lung, and Blood Institute recognized that calcific aortic valve disease (CAVD) is an active biological osteogenic process [1]. Numerous epidemiological studies were first identified by the Framingham study [2]. The traditional atherosclerotic risk factors include: smoking, male gender, body mass index, hypertension, elevated lipid and inflammatory markers, metabolic syndrome, and renal failure [3–16].

For decades, diagnosing calcification in the heart has been elusive. The advent of computed tomography has opened the window to diagnosing calcification and calculating the amount of calcification using the Agatston Score [16–18]. Understanding why calcification develops secondary to atherosclerosis in specific locations in the heart, which include the coronary artery and left-sided cardiac valves, has not been well defined until recently [19]. Understanding the hemodynamic and molecular mechanisms of calcification is critical towards under-

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E-Mail karger@karger.com
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Nalini M. Rajamannan, MD
Most Sacred Heart of Jesus Cardiology and Valvular Institute
703 N 8th St, Suite 303
Sheboygan, WI 53081 (USA)
E-Mail nrajamannan@gmail.com

standing the end-stage calcified phenotype, or osteocardiology provides the foundation for defining the timing and phenotypic expression of bone formation in the heart. This review will correlate experimental evidence with hemodynamic calculations to define the cellular mechanisms of calcification to turn basic science into future clinical success.

Osteocardiology Risk Factors

For decades, scientific investigations such as the Framingham Heart Study, the Cardiovascular Health Study, and the Multi-Ethnic Study of Atherosclerosis (MESA) have studied risk factors, which contribute to the pathogenesis of atherosclerosis in the development of cardiovascular disease. Atherosclerosis is a disease in which plaque builds up inside the artery over time. Investigators have determined the risk factors for atherosclerosis utilizing large databases of patients and analyzing the risks associated with the specific diagnosis of cardiovascular disease. Over the past 50 years, these large databases have helped to answer several questions as they relate to CAVD [20] and coronary artery calcification (CAC) [21].

The MESA has been instrumental in defining the amount of calcium in the heart, and the associated subclinical risk factors associated with calcification in the heart [22], including the understanding of mitral annular calcification as defined in the MESA and other databases [19, 23], as independently associated with cardiovascular risk factors including age, gender, diabetes mellitus, body mass index, status of current smoking, and use of lipid-lowering therapy, similar to CAC and CAVD.

In 2013, Thanassoulis et al. [24] studied the role of common genetic variation in valvular calcification. The investigation was initiated within the Cohorts for Heart and Aging Research in Genome Epidemiology consortium. They then performed a 2-stage analysis to discover the associations of genetic loci with the presence of mitral annular calcification and aortic-valve calcification, and to confirm the findings in the first cohort during the replication phase of the study the investigators used several databases including the Framingham Heart Study and the MESA database. The findings discovered that the role of genetic variation in the *LPA* locus, mediated by Lp(a) levels, is associated with aortic-valve calcification across multiple ethnic groups and with incident clinical aortic stenosis [24]. Lp(a) has also been associated with coronary artery disease [25].

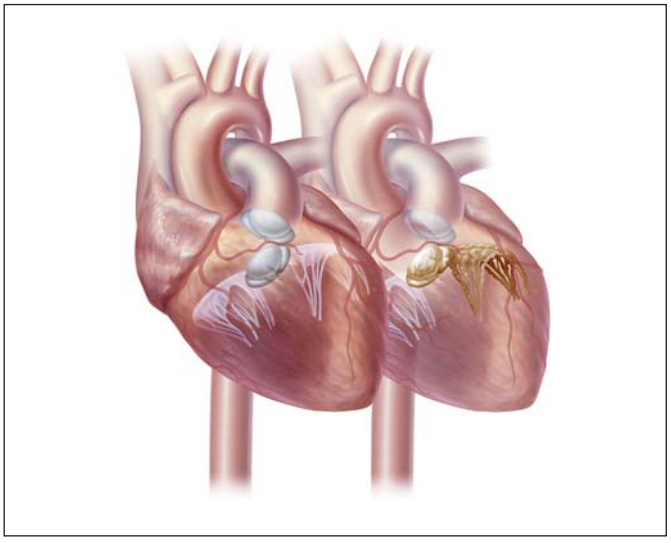

Fig. 1. The osteocardiology phenotype. The figure demonstrates the areas in the heart where calcification develops including coronary artery calcification, calcific aortic valve disease, calcific aortic disease, and mitral annular calcification.

All of these studies have demonstrated that atherosclerotic risk factors are in part responsible for the development of CAVD, mitral annular calcification, and CAC, associated with variable calcification expression depending on the anatomic location. Defining the osteocardiology phenotype recognizes that in the presence of these traditional risk factors, calcification can develop in specific locations in the heart which include the left-sided heart valves and the coronary artery. Figure 1 demonstrates the locations in the heart where atherosclerotic calcification develops; these include the aortic valve, coronary artery, and mitral valve annulus.

In addition, large epidemiological databases have further developed the concept that atherosclerosis and osteoporosis develop simultaneously secondary to traditional cardiovascular risk factors [26]. Calcification in the heart and osteoporosis in the bone are a common diagnosis in the aging population. The paradox of bone formation in the heart and thinning bone in the femur secondary to atherosclerosis has been confirmed in an LDLR (low-density lipoprotein receptor) null mouse model [27]. Understanding of the parallel role of bone in the heart is becoming increasingly important since the phenotype of calcification in the valve is similar to an osteogenic process [28]. This paradox provides a foundation for the theory correlating risk factors, epidemiology, disease mechanisms, and possibility for medical therapy.

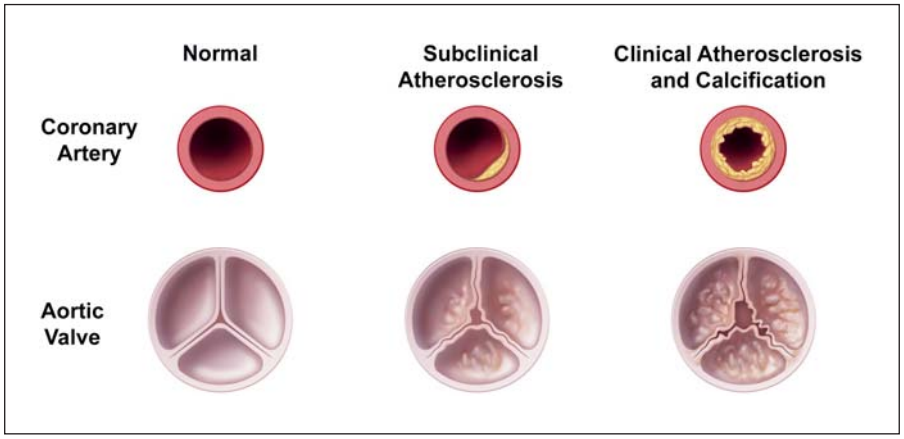

Fig. 2. Progression of atherosclerosis to calcification in the coronary artery and the aortic valve. Demonstrates the disease development in the left-sided cardiac aortic valve and coronary artery over time in 2 phases: from subclinical atherosclerosis to clinical calcification.

Osteocardiology Phenotype: LDL-Density-Pressure Theory

The osteocardiology phenotype is specific to areas of high-pressure differential in the heart. Left-sided heart valves and coronary artery develop calcification, as compared to no evidence of calcification in the right-sided heart valves and pulmonary veins in patients with traditional risk factors for atherosclerosis. The LDL-density-pressure theory demonstrates that in the presence of oxidative stress secondary to traditional cardiovascular risk factors, calcification develops in the aortic valve, coronary artery, and the mitral annulus [1, 29]. Figure 2 demonstrates that the disease develops in the left-sided cardiac aortic valve and coronary artery over time in 2 phases: from subclinical atherosclerosis to clinical calcification. CAC associated with atherosclerosis has also been well delineated in patients with familial hypercholesterolemia [30], as well as CAVD [28, 31] and mitral valve disease [32]. Mitral annulus calcification is a process characterized by fibrosis and calcification of the mitral valve annulus [33].

Phenotypic Expression of Calcification in the Heart: The LDL-Density-Radius Theory

Figure 3 defines the role of fluid hemodynamics in the heart as it affects the calcification phenotype. Fluid flow in the heart is dependent on multiple factors as derived by the Bernoulli equation for fluid flow [34]. Bernoulli described flow through a column is directly proportional to the change in pressure across the column and indirectly proportional to the resistance. The formula for flow through the heart is similar to the Ohm law for electricity as shown in equation 1, with Q = flow, P = pressure, and R = resistance [35]:

$$Q = \frac{\Delta P}{R}. \quad (1)$$

The entire formula for resistance for steady state flow through a circular tube is shown in equation 2, where η = viscosity and r = radius of the tube:

$$R = \frac{8\eta L}{\pi r^4}. \quad (2)$$

Equations 1 and 2 can be combined to give the flow rate through a circular tube in terms of P = pressure and ΔP = pressure drop, which is described as Poiseuille's law:

$$Q = \frac{\pi r^4}{8\eta L}\Delta P. \quad (3)$$

The differences in the rate of fluid flow are dependent on the radius of the anatomic structure, which is inversely proportional to the resistance. In addition, it is important to note the inverse r^4 dependence of the resistance to fluid flow. If the radius of the tube is halved, the pressure drop for a given flow rate and viscosity is increased by a factor of 16. Since the flow rate is then proportional to the fourth power of the radius, the size of the radius becomes important as blood flows through the heart [35].

For example, the average diameter of a left main coronary artery is 4.5 ± 0.5 mm [36], and the average diameter of the left ventricular outflow tract is 2.0 ± 0.2 cm [37]. From a circulatory perspective, these differences in the radius lengths become relevant as the effect of the calcification is correlated with the hemodynamic flow properties. These differences in radii will have different effects

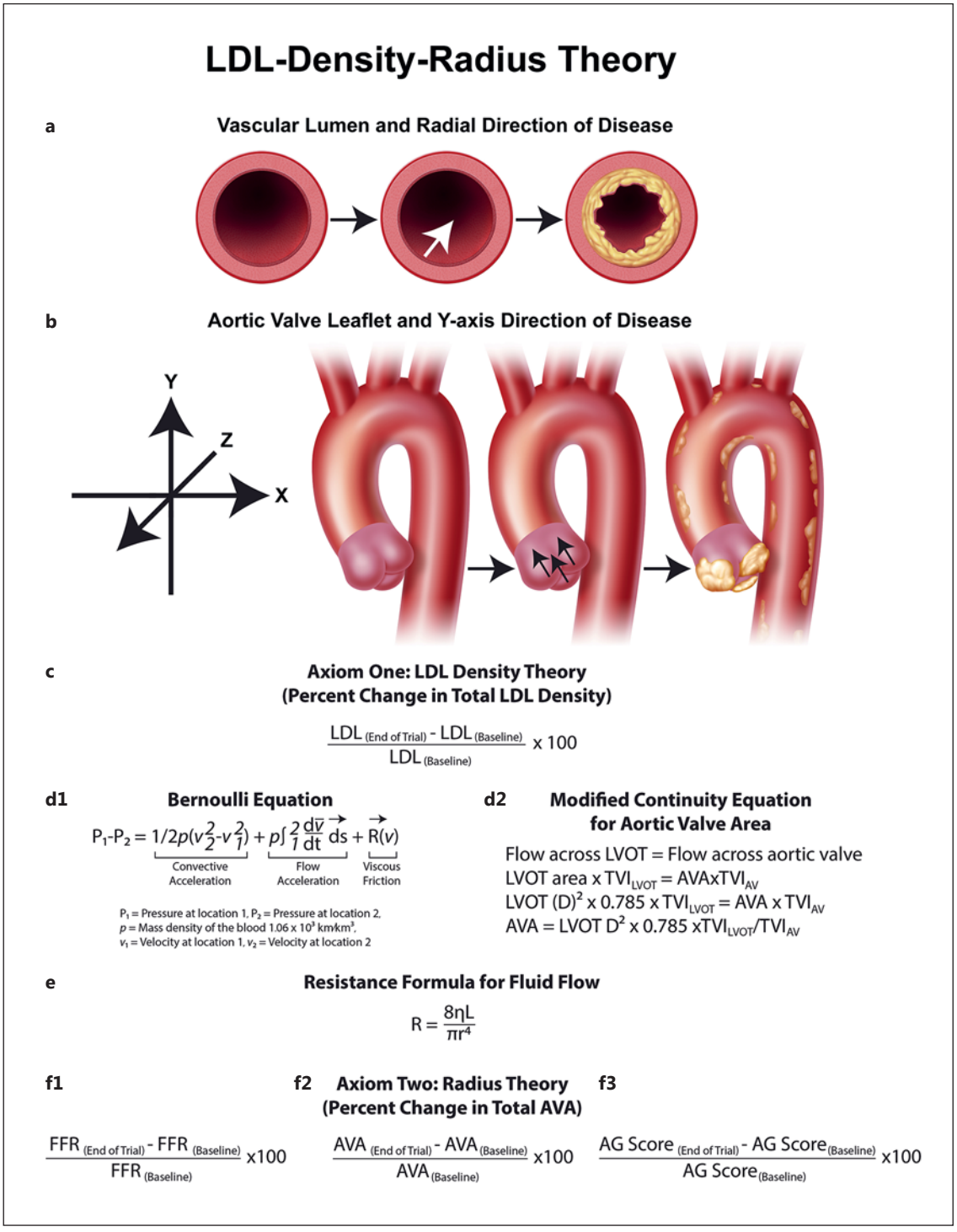

Fig. 3. The LDL-density-radius theory [35]. **a** Vascular lumen and radial direction of disease. **b** Aortic valve leaflet and y-axis direction of disease. **c** LDL-density theory. **d1** Bernoulli equation. **d2** Modified continuity equation for aortic valve area. **e** Resistance for fluid flow. **f** Radius theory. **f1** FFR in CAC. **f2** Aortic valve area in CAVD. **f3** AG score in CAD. LVOT, left ventricular outflow tract; TVI, trans velocity index; AG, Agaston score.

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on resistance. This concept becomes important as the rates of occlusion for vascular occlusion versus valvular stenosis are considered in the treatment of these 2 disease processes. Theoretical understanding of the effect of fluid hemodynamics on the calcification phenotype, and the signaling mechanisms involved in the development of calcification, provides the foundation for why randomized vascular trials demonstrate positive results more rapidly than randomized valvular trials, if the trials are designed in the same manner.

The direction of the LDL affects the vascular lumen in an inward direction causing occlusion overtime, as shown in Figure 3a. The direction of this LDL which affects the valve is upward along the y-axis along the aortic surface of the valvular fibrosa. Over time, the leaflets stiffen and can fuse in some valves. The overall effect on the radius is a reduction in the aortic valve opening and obstruction which leads to progressive stenosis of the valve as shown in Figure 3b. Figure 3c demonstrates a formula to calculate the percent reduction of the LDL density before and after therapy similar to the calculation derived in the Reversal Trial measuring reductions in atheroma volume in coronary artery disease [38]. Calculation of the percent lowering of LDL density in a valve trial allows for the potential to calculate the improvement of the biological effect of LDL on this disease.

The second part to this theory is the effect of the anatomical radius. This hemodynamic radius principle is based on the biological direction of this disease. The second part of this formula calculates the biological effect of the changes in the radius on the specific anatomical location in the heart. Figure 3d1 shows the formula for Bernoulli flow through a pipe, as modified [39] for echocardiography. Figure 3d2 presents the formula to calculate aortic valve areas by echocardiography using the Doppler technique [40]. The derivation of the Bernoulli principle for this equation includes the drop of the calculation for the flow acceleration and the viscous friction because the velocity profile in the center of the lumen is usually so low that the effect of viscous friction becomes insignificant and not necessary to calculate. Clinically, the viscous friction factor has been ignored as part of the continuity equation in aortic valve disease as defined by the echocardiography physiologists [41].

However, the concept of viscous friction becomes important when comparing vascular to valvular trials. The size of the radius plays a very important role in the time to see treatment effects, which are defined by vascular clinical end points such as ischemia and acute myocardial infarction. Clinical results from the trial entitled FAME [42] revealed the most stringent results for the role of fractional flow reserve (FFR) in the diagnosis of physiological critical stenosis in coronary artery disease in lesions: FFR as the continuity equation measure flow via pressure differential versus velocity differential as derived from the Bernoulli equation. In 20 centers in Europe and the USA, 1,005 patients undergoing percutaneous coronary intervention with stent implantation, were randomized based on angiography or based on FFR in addition to angiography. Results demonstrated improved end points in the FFR group with less stent use in patients with angiographically significant lesions and an FFR less than 0.80.

To date SEAS and SALTIRE, randomized clinical trials in valvular heart disease, were designed using the vascular trialists' approach, which resulted in negative results in slowing progression of CAVD [43]. However, because the flow in the lumen of the vasculature is not flat due to a smaller radius [34], the viscous friction factor must be taken into account when evaluating the treatment effects within the vasculature as derived by Bernoulli's original equation [34]. Therefore, LDL lowering will have a more rapid effect on the vasculature as compared to the heart valve.

The importance of the smaller radius is shown in Figure 3e, which is the calculation of resistance of fluid through a pipe. If the size of the radius (r) is significant in the calculation of flow, then the inverse r^4 dependence of the resistance becomes important in the treatment of a smaller radius versus a larger radius in the aortic valve area as viscosity increases by a factor of 16. Therefore, comparing the rates of improvement in a vascular versus a valvular trial will be different due to the differences in the size of the radius and the derivation of the modified Bernoulli equation for the echocardiographic formula for valve areas. The continuity equation drops the calculation of viscous friction due to the large size of the radius of the outflow tract of the left ventricle.

To measure the treatment effect for coronary artery disease, Figure 3f1 shows the calculation for the percent improvement for the FFR. To measure the treatment effect for aortic valve disease, Figure 3f2 shows the calculation for percent improvement for the aortic valve area. To measure the treatment effect for aortic disease, Figure 3f3 shows the calculation for the percent improvement for aortic disease. Mathematically and biologically, clinical trials for aortic valve disease may consider the following 2 concepts for targeting the disease biology in terms of the radial direction of disease and the magnitude of the LDL density to activate the atherosclerotic process ac-

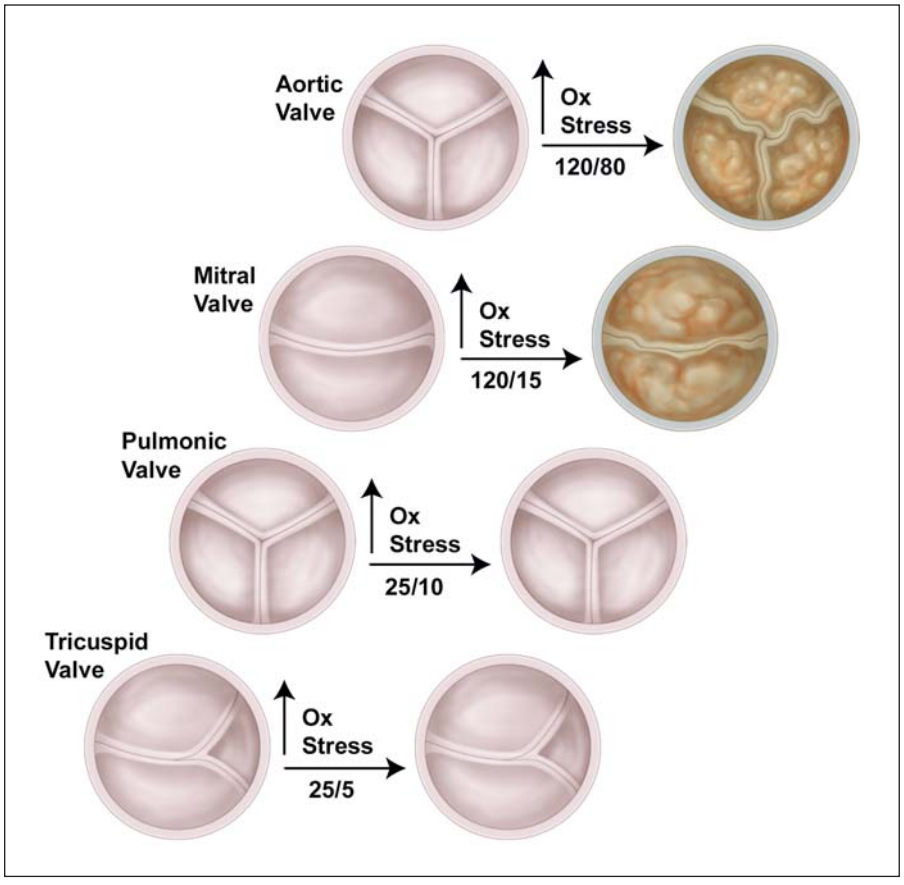

Fig. 4. The LDL-density-pressure theory. The LDL-density-pressure theory implicates the role of pressure in the heart as a mechanism to signal the Lrp5 receptor via the identified mechanostat effect on the protein to signal the Wnt pathway in the various anatomical locations of the heart based on the pressure and in the presence of hyperlipidemia [50] and oxidative stress [29]. When the pressure is high across the aortic valve, calcification develops but on the right side of the heart where the pressures are low, there is no calcification. Ox, oxidative.

cording to Bernoulli's original formula and the effect on resistance and fluid flow. The effect as described in the LDL-density-radius theory to date has only been described in coronary arteries and not in valves [35]. Hopefully, future clinical trials in this field will address the effect of radius, genetics, and lipid profiles in the future design to slow the progression of valvular heart disease with medications.

Lrp5 Signaling in Osteocardiology

The role of lipids in the vasculature is a well-known risk factor of atherosclerosis and bone formation in the heart [27, 44]. Studies have demonstrated the role of lipids and calcification in the vasculature [1] and in the valve [1, 44]. The discovery of the Lrp5 receptor in the gain-of-function [45] and loss-of-function [46] mutations in bone diseases led to several studies showing that activation of the canonical Wnt pathway is important in osteoblastogenesis [1, 47, 48]. The role of lipid signaling via the Lrp5 receptor has been defined in experimental in vitro and in vivo lipid models of vascular atherosclerosis.

Lrp5 receptor biology has also been described in the calcification of the aortic valve [1, 27, 47, 48]. Furthermore, experimental hypercholesterolemia is associated with the upregulation in Lrp5 receptor expression and activation of cell proliferation and extracellular matrix production critical in bone formation in the valve [49]. The mechanism by which Lrp5 signals bone formation in the heart is via lipids and mechanical-force activation. This dual mechanism of signaling via the Lrp5 receptor is known as the pressure theory [50]. Figure 4 demonstrates the role of hemodynamics in the development of calcification in the specific locations in the left-sided heart valves. Calcification develops in the different anatomical locations secondary to the role of oxidative stress or cardiovascular risk factors and the effect of the hemodynamic pressure on the aortic valve, mitral annulus, and the coronary artery [29].

Garg et al. [51] discovered that a loss of function mutation in Notch1 was associated with accelerated aortic

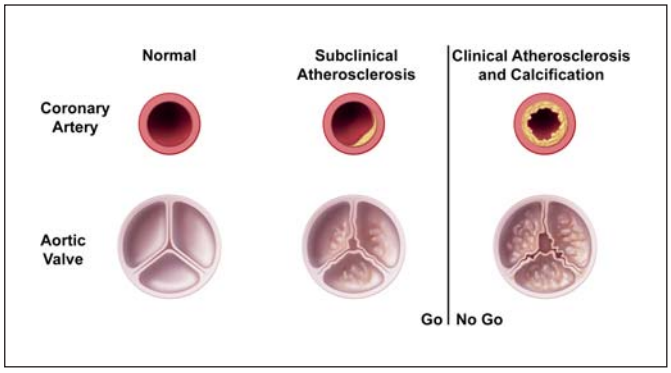

Fig. 5. The go/no-go osteocardiology time point. Timing to treat calcification in the cardiovascular system while the disease process is in the subclinical stage of atherosclerosis prior to the development of severe disease and calcification in the coronary artery and aortic valve, with the black line delineating the time point to treat prior to the black line which demarcates the point of no return in this patient population.

valve calcification and a number of congenital heart abnormalities. The normal Notch1 receptor regulates inhibition of osteoblastogenesis [52, 53]. The Notch1 splicing may be the regulatory switch important for the activation of the Wnt pathway and downstream calcification in these diseased valves [49, 53, 54]. The concept that cell-cell communication within a stem cell niche is necessary for the development of valvular heart disease provides a foundation for the cell architecture, risk factors and the gradient involved during the initiation phase of oxidative stress in the aortic valve. The 2 corollaries necessary for an adult stem cell niche is to first define the physical architecture of the stem cell niche and second the gradient of proliferation to differentiation within the stem cell niche. The endothelial lining cell located along the aortic surface is responsible for the secretion of growth factors including TGF-β, BMP, PDGF, FGF, and interleukin as outlined by the National Heart, Lung, and Blood Institute working group on CAVD [1, 55]. These cells interact with the subendothelial cells that are resident below the endothelial layer of cells. These cells have been characterized as myofibroblast cells [56–58].

Timing of Therapy: Osteocardiology Go/No-Go Theory

The timing of treatment to slow the progression of calcification in the heart has been difficult to achieve with randomized clinical trials. Clinical trials targeting calcification in the coronary artery and aortic valve have been variable, but for the most part negative in part for several reasons. First for years this disease was thought to be due to a degenerative process secondary to passive calcification in the heart.

Further understanding of the biology of atherosclerotic calcification in the heart will help to understand the initiation, early atherosclerosis manifesting in subclinical disease, and late calcification manifesting in severe clinical disease. The concept of identifying and treating early preclinical atherosclerosis versus late calcification defines the principle of go/no-go (subclinical/clinical) binary classification of the disease progression. Figure 5 demonstrates from a biological and clinical perspective the go/no-go timing for the treatment of calcification in the cardiovascular system. It is critical to treat the modifiable disease while it is treatable in a "go" state, meaning the atherosclerotic cellular process is still reversible with lipid-lowering treatments, versus working on treatments before it is too late – severe calcification or the "no-go" state. The trial will be positive only when the go conditions are met, but if the treatment is started too late, then the no-go condition fails such as the randomized control trials in aortic valve disease [59–61]. In the future, the design of clinical trials in atherosclerotic heart disease needs to focus on the early subclinical phase of atherosclerosis – the go phase of disease – to try and reverse atherosclerosis as found in the Reversal Trial [51]. Once calcification starts, then the possibility of medical therapy halting progression may be limited [59–61].

The MESA provides the pivotal evidence from a clinical database which has helped to further define the go/no-go time point for future trials in osteocardiology. The MESA defined the concept that atherosclerosis is a chronic, progressive, inflammatory disease with a long asymptomatic phase. This long asymptomatic phase is the critical time point for identifying risk factors, measuring the initial stages of disease to define early coronary artery calcification to treat, to modify and try to halt, slow, or reverse progression [22]. The MESA also defined, among those with CAVD at baseline, the median rate calcification progression as 2 Agatston units/year [62]. The baseline Agatston Score was a strong, independent predictor of progression, especially among those with high calcium scores at baseline. In conclusion, in this MESA preclinical cohort, the rate of incident CAVD increased significantly with age, risk factors, and association with coronary artery calcification [22].

In 2017, incorporation of risk factor evaluation, diagnosis of subclinical and clinical disease, and the overall

health of the patient in future cardiovascular risk management will be the most important clinical and scientific approach towards treatment of our patients. Understanding the biology of calcification will help to understand the timing, the future clinical trial design, and the duration of therapy to achieve success in treating osteocardiology secondary to atherosclerosis.

Conclusion

For the past 50 years, catheter hemodynamics, echocardiography, angiography, computed tomographic imaging and timing of surgery have evolved as the diagnostic and therapeutic approach for CAC and CAVD. In the past decade, with the advent of experimental models and genetic testing, it has been recognized that the aortic valve has an active cellular biology which incorporates risk factors, osteogenic phenotype and the potential for medical therapy to slow progression. The Lrp5 signaling and other growth factor pathways activate mesenchymal cell differentiation in the valve and vasculature for the development of an osteoblast phenotype. The future management of this disease process will include the understanding of these different mechanisms for future medical therapy of this disease. If the physician can define the traditional risk factors in patients who present with an aortic valve murmur, then targeting these risk factors may slow progression. The stethoscope can become an inexpensive screening tool for this pathological process and possible subclinical atherosclerosis. If there are no identifiable risk factors, then genetic considerations may play a role. Progress in this field will make a difference for the future delay in the timing of surgery for these patients in the future.

Acknowledgements

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Conflict of Interest

The author is the inventor of a patent for methods to slow progression of valvular heart disease. The Mayo Clinic owns this patent, and the author does not receive any royalties from this patent.

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