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Diagnosis of cardiac allograft vasculopathy: Challenges and opportunities

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1. ABSTRACT

Cardiac allograft vasculopathy (CAV) is one of the most common long-term complications in patients following heart transplantation. Because of its irreversible nature, early detection is essential to impact progression. Thus, imaging techniques play a crucial role in the diagnosis and subsequent treatment. Major advancements in imaging and analysis are required to overcome the limitations of current techniques. Coronary angiography which is the standard method, presents low sensitivity in detection, especially at an early stage. Intravascular ultrasonography is a more reliable alternative but is limited to the epicardial vessels. Novel non-invasive techniques, such as stress echocardiography and nuclear imaging, have been introduced but not without limitations. Here, we review various imaging methods and associated analyses to improve diagnostic predictions. We discuss recent advances in the diagnosis of coronary artery disease and their potential translation in the diagnosis of CAV. Additionally, we present potential biomarkers that have been identified for CAV. Finally, we provide a discussion on microvessels with novel anticoagulant properties that are mostly identified in patients with severe CAV.

2. INTRODUCTION

Currently, over 4,500 transplants are performed in the World each year, with more than 2,500 being conducted in the U.S. alone (1,2). The heart transplant procedure is the most durable therapy for patients suffering from end-stage heart failure (3). Unfortunately, the number of transplants is limited by the number of organs available. As a result, on average there are more than 4,000 patients waiting for a heart transplant in the U.S. at any given time (4). Overall, 112,521 heart transplants were reported to the International Society for Heart and Lung Transplantation (ISHLT) between 1982 and June 2013. Over that period, 1-year and 5-year survival rates were 82% and 69%, respectively. For patients who had received a transplant over 2009-2013, the 1-year survival rate was over 86%, which suggested an improvement in heart transplant outcome.

Post-operative complications are still observed in a large number of patients (1). Cardiac allograft vasculopathy (CAV) is one of such complications. It is characterized by thickening of the intimal layer of coronary blood vessels in the transplanted heart. It can target coronary arteries, capillaries, and occasionally veins. CAV is found in 7.8% of the patients by the end of the 1st year, 30% by the end of the 5th year, and 50% of patients by the end of the 10th year post-transplant (5). During the development of CAV, it appears that smooth muscle cell proliferation occurs from the media to the intima layer. This is followed by the development of lipid-laden foam cells and perivascular fibrosis (6). It results

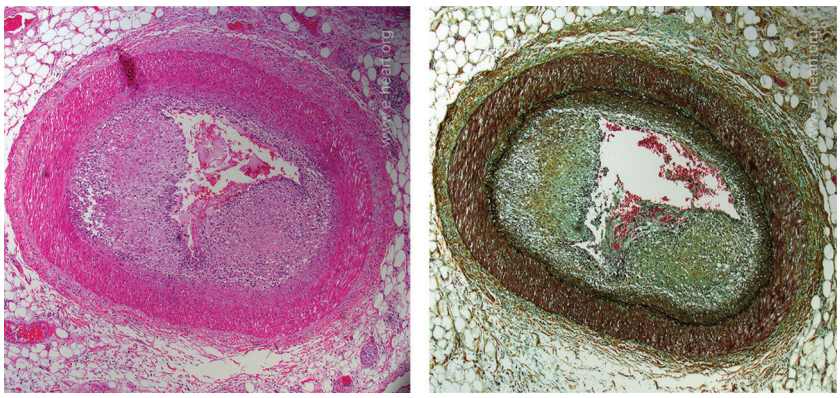

Figure 1. Cardiac allograft vasculopathy. LEFT. Eccentric plaque showing the proliferative intima in the lower half of an epicardial coronary artery. (H&E, x10). RIGHT. The proliferative intima in this case is formed of mature extracellular matrix rich in glycosaminoglycans (green). The internal elastic lamina (black) is intact, and the media of the vessel is also intact (dark red). (Movat pentachrome, x10). Illustration reproduced with permission from www.e-heart.org.

in progressive concentric fibrous intimal hyperplasia, meaning that the vessel wall is thickened homogenously around its entire circumference (Figure 1). The disease is diffused as it progressively spreads along the length of the vessels. A functional decrease of blood flow due to decreased caliber of the affected vessels leads to ischemia which can be followed by ventricular arrhythmias, congestive heart failure, or sudden cardiac death (7-9). CAV accounts for 3% of deaths within 1 year after transplantation and 10% of deaths between 1 to 5 years (10). Risk factors for the disease are numerous as any phenomenon causing endothelial injury can potentially lead to CAV. There is extensive evidence that immunological factors, such as histocompatibility mismatch, acute rejection episodes and chronic inflammation, play a major role in the development of CAV (11,12). Additionally, non-immunological factors, including donor and recipient sex and age, as well as their history of diabetes and hypertension, have also been identified as risk factors (13,14).

Since CAV is irreversible, the ultimate recourse for a patient is often re-transplantation. The lack of organ donors and lower survival rate after a second transplant, however, are obvious limitations for this option (15,16). Thus, the focus of treatments remains mainly on prevention, and early detection of CAV. Before transplantation, significant effort is required during storage and transportation of the donor organ to reduce the cold ischemic time and subsequently reduce tissue damage (3). Immunosuppressive drugs, such as cyclosporine, are then prescribed to the patients following heart transplantation to prevent rejection and subsequent risk of CAV. Unfortunately, such immunosuppressive drugs commonly lead to hyperlipidemia that is also known to be a risk factor for CAV. Thus, lipid-lowering drugs, such as statins, are also prescribed (17). After CAV is diagnosed in a patient, pharmacological treatment options become limited. Various statins, specifically 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, have been shown to slow the progression of the disease and are generally prescribed (18,19). Procedures such as percutaneous coronary interventions, coronary artery bypass grafting, transmyocardial laser revascularization, and heparin-induced/mediated extracorporeal low density lipoprotein plasmapheresis are considered in severe cases when re-transplantation is not possible. There are conflicting reports, however, on their outcome and long-term efficacy (20-23). Drugs such as oral L-arginine, antioxidants such as vitamins C and E, and flavonoids have been shown to have the potential to restore endothelial function in allografts, although a large trial is still required to validate their efficacy in reducing the development and progression of CAV (24-26).

Cardiac denervation at the time of heart transplantation usually prevents transplant patients from experiencing angina, which is an important warning sign for coronary heart disease. Hence, imaging techniques play a crucial role to detect the disease as early as possible in order to prevent fatal outcomes. Reviews on commonly used imaging techniques, and non-invasive techniques with high potentials are available in the literature (27, 28). Here, we provide a review of the imaging techniques (established as well as emerging) and speculate on the future of CAV diagnosis. In Section 2, we introduce angiography and intravascular ultrasonography (IVUS), the two most commonly used techniques in heart transplant centers for CAV diagnosis. The invasive nature of these methods, as well as the less than optimal sensitivity, have encouraged the development of alternative imaging techniques. These emerging techniques are presented in Section 3. We discuss dobutamine stress echocardiography (DSE) and single-photon emission computed tomography (SPECT) that have been greatly evaluated, and also include more recent methods such as computed tomography angiography (CTA),

cardiovascular magnetic resonance (CMR) and optical coherence tomography (OCT). The combined use of angiography and intravascular ultrasonography, as well as the combined use of angiography and optical coherence tomography, have also been evaluated for CAV diagnosis and are briefly included in the discussion. These emerging methods present limitations which leave room for innovative techniques that can be established in the future. In Section 4, we propose the translation of the innovations in the diagnosis of coronary artery disease (CAD) to CAV diagnosis. For instance, hybrid methods based on imaging and computational analysis, or computed tomography and scaling power laws, have recently shown potential for CAD diagnosis, and could be adopted for CAV as well. Finally, we speculate in Section 5 that there might be possibilities for CAV diagnosis based on immunological factors. We review the biomarkers that have been identified for CAV, and provide an overview of a specific type of microvessel (referred to as capiole) that presents interesting anticoagulant properties and phenotypic characteristics of capillaries and small arterioles, mainly identified in patients with severe CAV. These structures may represent new targets for diagnosis and treatment of microvascular CAV.

3. COMMON IMAGING TECHNIQUES FOR THE DIAGNOSIS OF CAV

Coronary angiography is the standard technique used in all transplant centers for CAV diagnosis. Intravascular ultrasonography is slowly gaining popularity and is now used in combination with angiography in many centers. The International Society for Heart and Lung Transplantation guidelines for the care of heart transplant recipients classify angiography alone for the assessment of CAV as class I with level of evidence C, and the use of IVUS in conjunction with coronary angiography as class II with level of evidence B (29).

3.1. Coronary angiography

Coronary angiography is the method of choice at most cardiac transplant centers to detect CAV (30). The sensitivity of the method is estimated to be 10-20% during year 1, and 35-50% between years 1 and 5 post-transplant (27).

To perform a coronary angiography, a small catheter is inserted into an artery of the leg, arm, or neck. The catheter is then guided until it reaches the heart. A contrast iodine dye is injected using the inserted catheter and X-ray pictures of the heart, called angiograms, are taken. The injected dye, which is based on a chemical modification of a 2,4,6-tri-iodinated benzene ring, enhances the visibility of the coronary blood vessels in the X-ray images (31).

A baseline angiography is commonly performed several weeks after transplantation, while follow-up is performed annually or biannually depending on the protocol of the transplant center. The lumens of the targeted vessels, filled with the contrast dye, appear as distinct dark branches on the X-Ray (Figure 2). It is then possible to observe narrowing in a blood vessel by comparison with the baseline.

Coronary angiography has several limitations. First, it is difficult to assess the “true” diameter from a single x-ray projection if the vessel lumen is not circular. Second, it permits assessment of vessel lumen but not the wall. Third, since reduction in the lumen diameter of the affected blood vessel does not occur initially, detection by angiography is only possible at a more advanced stage (32). Finally, since the disease is diffused and affects the entire length of the vessel, CAV can be missed if the thickening is evenly distributed along the length; i.e., a lack of reference normal vessel (33). As a result, concerns have been expressed regarding the sensitivity of the method for CAV (34). Many studies, mainly based on post-mortem correlation, have also questioned the accuracy and repeatability of the technique (35-39). This invasive technique is also prone to complications. For instance, the injection of contrast iodinated dye has been shown to increase the risk of kidney injury in patients (40). Although CO_2 can be used as a nontoxic, non-allergic, injectable, rapidly absorbable gas that provides a cost-effective alternative to iodinated contrast agents in peripheral vessels (41), the utilization of CO_2 for coronary arteries is contraindicated due to generation of gas embolisms. Studies are currently underway to make CO_2 viable for imaging of coronary arteries which would positively impact the diagnosis of CAV.

3.2. Intravascular ultrasonography

Intravascular ultrasonography is an imaging technique that has been introduced recently for CAV diagnosis. While coronary angiograms provide a 2D profile of the lumen of blood vessels, IVUS imaging allows visualization of the lumen cross-sectional area for observing the morphology of CAV. This is particularly useful, especially during earlier stages of CAV, for tracking the changes occurring on the intima layer. Due to better prognosis of CAV using IVUS, it is now becoming a popular method at many centers. A sensitivity of about 50% is reported in assessing CAV during the 1st year after transplantation (3).

The first step in performing IVUS is similar to coronary angiography, and involves inserting a catheter in the artery of the leg, arm, or neck. A catheter with a miniature ultrasound probe mounted on its tip is inserted and pushed until it reaches the heart, and then guided to the vessel of interest. The IVUS probe emits high ultrasound frequencies, typically centered around 20–50 MHz. The ultrasound signal reflected back to the probe from the arterial wall is processed through a dedicated console. Using a 30 MHz probe, axial and lateral resolutions of approximately 150 and 250 μm,

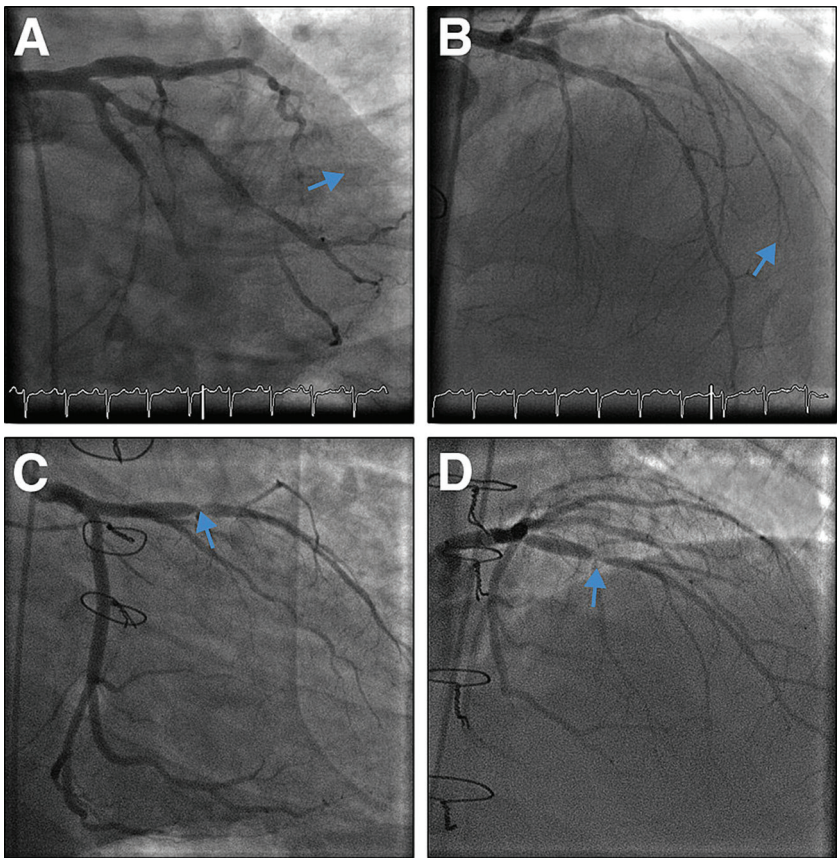

Figure 2. Coronary angiography of a proximal left anterior descending artery demonstrating distinct manifestations of cardiac allograft vasculopathy (blue arrows). Panels A and B show the classical angiographic appearance of CAV with multiple sequential lesions, diffuse narrowing of the coronary arteries, and prominent pruning of the distal vasculature (arrows). Panels C and D expose that CAV can also appear similar to typical atherosclerotic coronary artery disease in a native heart (arrows). Illustration reproduced with permission from (27).

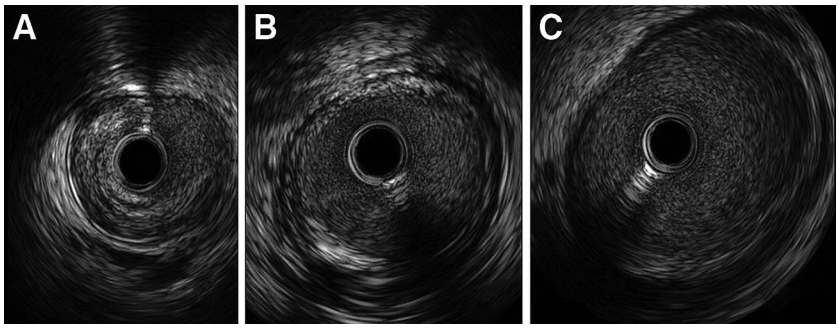

Figure 3. Intravascular ultrasound frames corresponding to (A) middle left anterior descending artery, (B) proximal left anterior descending artery, and (C) distal left main of the same patient after 1 year of heart transplantation. Concentric thickening of the intima layer, suggesting presence of CAV, is identified. Illustration reproduced with permission from (27).

respectively, are obtained. A detailed description of the procedure is available in (42).

During check-up, the probe is placed beyond the target lesion site and the ultrasound catheter is then withdrawn with continuous imaging, resulting in a series of tomographic images of the vessel wall (Figure 3). It is known that in a normal coronary artery, intimal thicknesses can be up to 0.3 mm. Thus, it is accepted that CAV is potentially present in a patient when the intimal thickness exceeds this value.

Although IVUS provides a clear advantage over angiography, it is currently not recommended as

a stand-alone diagnosis method, but is conducted in combination with angiography due to several limitations. First, the size of currently available IVUS catheters (smallest diameter about 1 mm) limits imaging only from proximal to mid-epicardial vessels (42). Moreover, only one vessel is usually targeted at a time due to difficulty in accessing multiple vessels with the current technology, which limits the sensitivity of the method (43). Finally, the invasive nature of the technique and the cost are additional shortcomings.

4. EMERGING TECHNIQUES

Due to limitations and the invasive nature of angiography and IVUS, numerous (mainly non-invasive) techniques have been developed for CAV diagnosis. Although these techniques are currently used mainly for research, they are slowly emerging as clinical alternatives. Dobutamine stress echocardiography (DSE) and single-photon emission computed tomography (SPECT) are the most promising candidates. Both are classified by the International Society for Heart and Lung Transplantation guidelines as class IIa with level of evidence B for the assessment of CAV in patients who are not able to undergo an invasive procedure (29). The use of a clearly defined methodology and lack of a large study are restricting these methods from being widely accepted for CAV diagnosis. Additional novel techniques, such as computed tomographic angiography (CTA), cardiac magnetic resonance (CMR) and optical coherence tomography (OCT) along with co-registration using angiography and IVUS, as well as co-registration using angiography and optical coherence tomography have also been proposed. More studies are required to confirm their validity.

4.1. Dobutamine stress echocardiography

Stress echocardiography allows evaluation of myocardial function, and subsequent assessment of functional integrity of both the macro and micro vessels. Dobutamine stress echocardiography is the preferred stress modality in transplant patients as it has been shown to provide better sensitivity than exercise-based stress echocardiography. Sensitivity values ranging between 53% to 96%, and specificity values ranging from 53% to 100% have been reported (44-53).

Dobutamine increases heart rate to mimic the effects of exercise to stress the heart. It is infused continuously into a vein, with a step-wise increase in concentration every 3 to 5 min. Generally, an initial concentration of 10 mcg/kg/min is introduced which is progressively increased to 20, 30, and 40 mcg/kg/min, until the patient attains the desired sub-maximum heart rate (defined as 85% of the maximum heart rate established for his age). Transducers that send ultrasonic sound waves are placed at several locations on the chest. The waves reflect back from the heart wall and are captured by the transducers that transmit them to a computer. Images of the heart structures are thus continuously recorded. A detailed protocol to perform this technique can be found in reference (54).

Generally, 2D echocardiogram and M-mode echocardiogram are used for the diagnosis of CAV (51). The post-systolic strain index (PSI) is used to assess CAV in the imaged heart. This index is calculated based on various myocardial strain measurements obtained from the echo images using a dedicated software (55). End-diastole is chosen as the reference time point for strain calculations. The end-systolic strain ε_{sys} is defined as the magnitude of deformation between the reference time point and the end of systole at aortic valve closure. The peak strain ε_{peak} is defined to be the highest strain value obtained for the radial direction (and subsequently the lowest strain value for the longitudinal direction). The post-systolic strain index is then defined as the ratio between $[\varepsilon_{peak} - \varepsilon_{sys}]$ and ε_{peak}. A post-systolic strain index greater than 34% is prescribed by a study to identify patients suffering from CAV with a high sensitivity ratio of 88% (53). The use of Doppler echography to measure coronary blood velocity and assess CAV has also been reported (56). Such echography allows measurement of blood flow velocity in the targeted arteries. The coronary flow reserve (CFR) can be calculated from the echo images as the maximum increase in blood flow between rest and stress conditions. A coronary flow reserve value lower than 2.9 has been found to indicate CAV with high sensitivity (56). A limitation of coronary flow reserve is that it cannot differentiate between large coronary artery disease and microcirculation.

Dobutamine stress echocardiography has been shown to be non-suitable for early detection of CAV (46). A recent study concluded that the technique had an inadequate sensitivity to detect CAV in the first five years after transplant (57). Generally, it is accepted that dobutamine stress echocardiography provides preliminary assessments that could assess the need for an invasive follow-up (58).

4.2. Single-photon emission computed tomography

Single-photon emission computed tomography is a nuclear imaging technique that uses gamma rays. Sensitivity values ranging from 21% to 92% and specificity values ranging from 55% to 100% have been reported by various studies for diagnosis of CAV, with better values in more recent studies (59-66). It can be noted that the use of positron emission tomography (PET), another nuclear imaging technique has also been assessed for the diagnosis of CAV by few studies with positive outcomes (67-69).

The procedure for single-photon emission computed tomography resembles that of the dobutamine

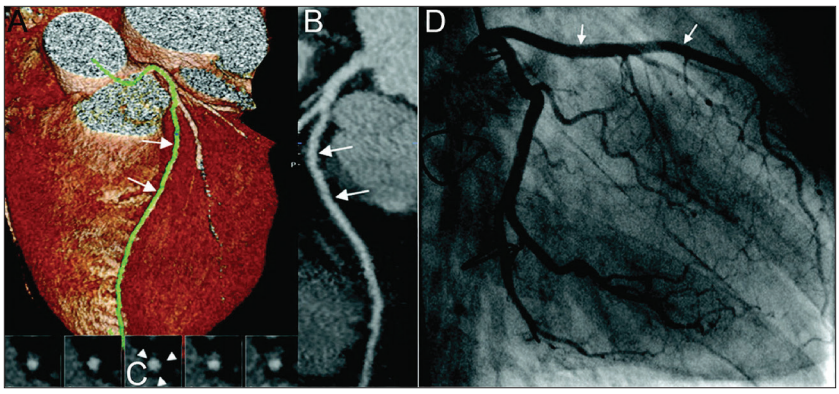

Figure 4. Computed tomographic angiography of a patient 5 years after transplant showing diffuse concentric thickening of the wall of the mid left anterior descending artery (A, arrows), best seen in curved multi-planar reformatted (B, arrows) and short-axis images (C, arrowheads), but difficult to appreciate on invasive angiography (D, arrows). Illustration reproduced with permission from (28).

stress echocardiography. A radioactive tracer is first injected into the blood. This tracer emits gamma rays that are picked up by a single-photon emission computed tomography camera and converted into 2D pictures for visualizing coronary blood flow. The position and angle of the camera are varied to visualize various vessels. On the pictures, areas with good blood flow will appear lighter in color whereas areas with poor blood flow will have darker color. The American Society for Nuclear Cardiology provides a comprehensive guideline for the use of this technique (70).

It has been shown that there is a correlation between CAV and abnormal blood flow (67). This correlation is used in single-photon emission computed tomography (SPECT) to diagnose CAV based on the observation of the blood flow in various vessels. The myocardial perfusion reserve (MPR) is the index used to assess CAV, which is calculated at rest and during stress test. The myocardial perfusion reserve is defined as the ratio between the myocardial blood flow velocity during stress and the resting myocardial blood flow velocity (69). It has been shown that the myocardial perfusion reserve is inversely proportional to intima thickness. Hence, a decrease of myocardial perfusion reserve over time compared to a baseline is viewed as an indicator of CAV development and/or progression. The use of single-photon emission computed tomography (SPECT) for CAV diagnosis is limited primarily due to the risks associated with frequent exposure to nuclear dose, and the cost of the test.

4.3. Additional promising techniques

Additional diagnostic methods have emerged although they currently lack full assessment for CAV. Coronary computed tomography angiography is a major example (71). It is the only one classified in the International Society for Heart and Lung Transplantation guidelines (as class IIb with level of evidence C) among all the methods listed in this section. Sensitivity values ranging from 70% to 100% and specificity values varying between 67% and 97% have been reported by various studies (72–78). It combines the injection of iodine-rich contrast and computed tomography scanning to provide a high resolution 3D view of the coronary vessels. Variation of position and angle allow visualization of cross sectional areas along the length of the vessels, which is very useful for CAV diagnosis (Figure 4). The major limitations that have been identified so far are the risks due to exposure to radiation, the risks associated with the use of iodine-rich contrast in patients with renal complications, and the resolution limits to vessels with diameters greater than 1.5 mm (74,79).

Cardiovascular magnetic resonance has been proposed by several studies to assess CAV (80–83). Sensitivity values ranging between 67% and 100%, with specificity values of 63% to 85% have been reported in the aforementioned studies. The method consists of injecting gadolinium contrast, and conducting magnetic resonance imaging on the patient (Figure 5). Various parameters have been suggested to access CAV from these images, such as the myocardial perfusion reserve (MPR), peak systolic strain, and the mean infarct mass. Similar to computed tomography angiography, vessels with diameters smaller than 1.5 mm cannot be analyzed. Another limitation of this technique is the risk of developing nephrogenic systemic fibrosis that has been associated with gadolinium administration (84).

Optical coherence tomography (OCT) is another promising method that was first evaluated for measurement of coronary intima-media thickness in 2005 (85), and has been successfully applied, especially in the last 4 years, to assess CAV (86–89). The procedure for OCT is similar to intravascular ultrasonography, with the only difference being that light near the infrared region is used instead of ultrasound (Figure 6). Although the depth of penetration is reduced with using light rays, the pixel resolution is significantly higher as it ranges

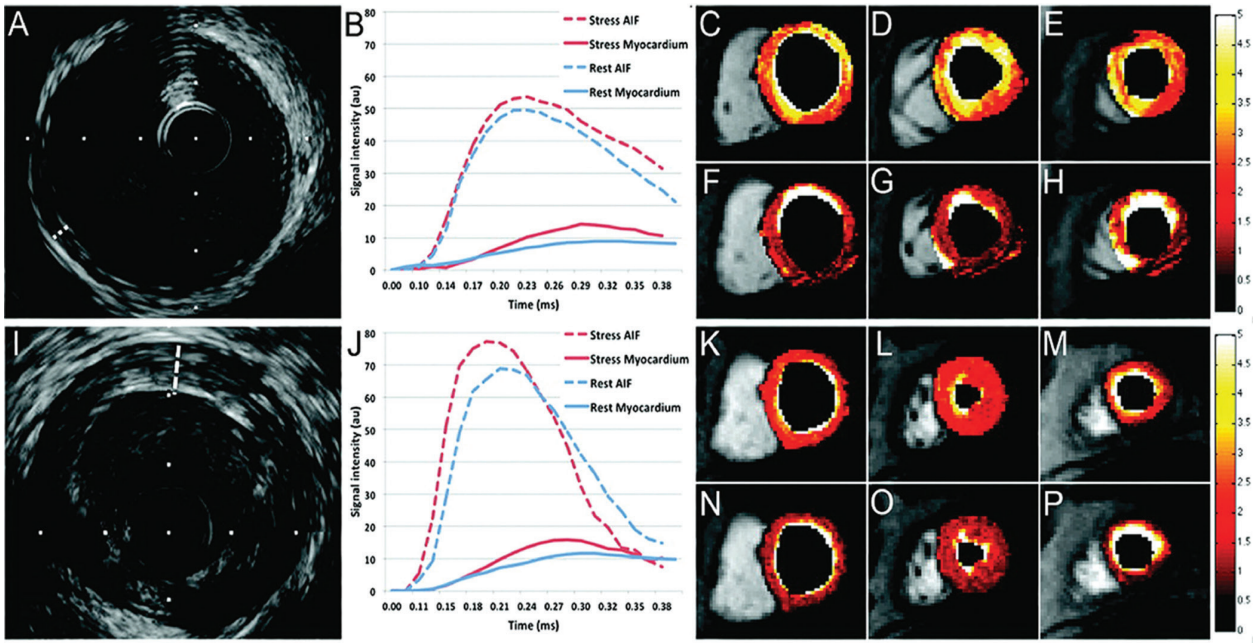

Figure 5. Quantitative adenosine-stress perfusion cardiac magnetic resonance in 2 transplant recipients; one with minimal (A through H) and one with severe cardiac allograft vasculopathy (I through P). Intravascular ultrasonography images (A and I) are also provided. Illustration reproduced with permission from (28).

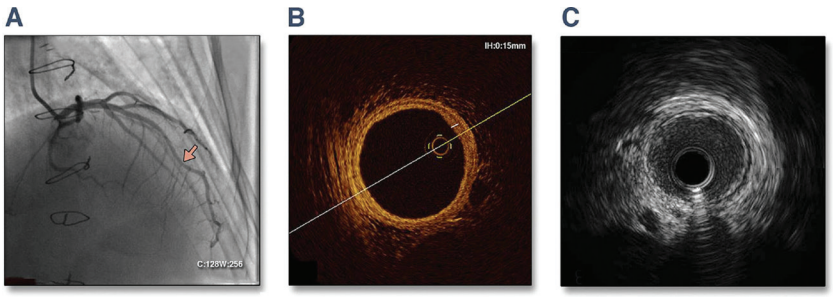

Figure 6. Coronary angiography (A), optical coherence tomography (B), and intravascular ultrasonography (C) imaging in a patient 8 years after heart transplantation. (A) Quantitative angiogram analysis showed 14% diameter stenosis in the mid–left anterior descending artery (arrow). (B) Optical coherence tomography revealed intimal hyperplasia with thickness of 150 μm. (C) Accurate measurement of intimal hyperplasia was difficult with intravascular ultrasound. Illustration reproduced with permission from (87).

between 12 μm to 18 μm (compared to 150 to 250 μm with IVUS) (90). The invasive nature and cost of the technology are currently the major drawbacks.

Finally, the feasibility of co-registration using angiography and IVUS for surveillance of CAV has been demonstrated (91). A contrasted angiogram of coronary artery is acquired at an end diastolic cardiac phase followed by pullback of the catheter tip that captures IVUS images at around 30 frames per second. The results provide more accurate cross-sectional imaging of the left anterior descending artery while exposing the heart to minimal radiation. Another co-registration technique to obtain images using angiography and optical coherence tomography (OCT) (92) has been utilized to provide procedure planning information to the cardiologists prior to intervention. The invasive nature of these approaches is again the major drawback.

5. TRANSLATION OF CAD DIAGNOSIS TO CAV

Coronary artery disease (CAD) encompasses various conditions that arise typically from atherosclerosis, which is characterized by the thickening of the intimal layer due to deposition of lipids and various cells (93). Although there are discrepancies between their patho-physiology, CAV is often viewed as an accelerated form of CAD in the transplanted heart (3,94). This parallel encouraged the adoption of angiography for the diagnosis of CAV while it was originally developed

for atherosclerotic CAD diagnosis. Since CAD affects a larger population, more effort and resources have been allocated to diagnosis of this disease. Thus, we hope that novel techniques for CAV diagnosis (that overcome the limitations of the methods presented in the previous sections) can be adopted from techniques developed for CAD. The following sections present some of those techniques for detection of CAD.

5.1. Integration of imaging and computational analysis

Many studies have linked blood flow-induced shear stresses on the arterial wall to the development of atherosclerosis (95–97). Since stress cannot be measured, but only computed, the idea to use vessel images in conjunction with computational fluid dynamics (CFD) to compute fluid stresses has been widely adopted (98). The initial computational fluid dynamics (CFD) study on coronary artery stenosis can be dated back to work published in the year 2000 by Banerjee and colleagues who determined the effects of blood flow using computational fluid dynamics on coronary artery stenosis post-angioplasty (99). With advances in imaging techniques and greater computing power, computational fluid dynamics (CFD) has been able to predict the role of hemodynamic forces in developing CAD (100).

Although the role of hemodynamics in progression of CAV is poorly understood, recent research shows a strong hemodynamic basis for occlusive atherosclerosis in patients with CAV; *i.e.* low and oscillatory wall shear stresses due to regional fluid dynamics, which can be determined using computational fluid dynamics (CFD) (101). Timmins *et al*. (102) calculated the wall shear stresses by generating 3D coronary reconstructed geometry from a patient's 7-year post-transplant angiograms (when the patient did not have significant CAV) and performed computational fluid dynamics (CFD) simulation on the 3D model. The simulations were able to identify regions of low velocity of blood flow and hence low wall shear stresses. Moreover, the regions experiencing oscillatory wall shear stresses matched the sites of subsequent lesion formation in the patient at 15-years post-transplant follow-up. The positive results using simulations suggested an association between low and oscillatory wall shear stresses which may lead to subsequent development of a clinically manifested CAV. Based on these findings, it appears that combining computational fluid dynamics (CFD) with coronary imaging can be used to assess future risk and predict potential regions of CAV lesions in patients as well. This may be extremely useful to identify patients at risk, and adjust diagnosis frequency with invasive follow-up accordingly. More studies are required to confirm the potential of this computational analysis approach.

An extension of this hybrid imaging-computational method for the diagnosis and prediction of CAD, is the computational fluid dynamics-derived fractional flow reserve (CFD derived FFR) method. The fractional flow reserve (FFR) method provides a physiologic assessment of lesion severity and is regarded as the gold standard for detecting stenosis. Conventionally, the pressure wire-based fractional flow reserve (FFR) method has been used by advancing a guidewire to the distal vessel. This invasive procedure adds time (especially in side branches and coronaries with complex anatomy (103)) and cost to the procedure and hence limits utility in everyday clinical practice. Recent improvements in the fractional flow reserve (FFR) method rely on the use of computational fluid dynamics (CFD) techniques in 3D models obtained from noninvasive computed tomography angiography or invasive quantitative coronary angiography (104,105). These studies report improvement in the diagnostic accuracy of the fractional flow reserve (FFR) method in predicting lesion-specific ischemia, with accuracy values of 86-88%. The accuracy was superior to anatomical assessment by coronary computed tomography angiography alone (diagnostic accuracy of 65%) (106).

The use of the fractional flow reserve (FFR) method with invasive coronary sensor pressure and flow wires has also been reported for CAV. Chih *et al*. (107) reviewed that normal fractional flow reserve (FFR) with reduced coronary flow reserve across macro- and micro-vascular compartments represents diffuse epicardial or microvascular CAV. For a given epicardial plaque burden, increased fractional flow reserve (FFR) was found to associate with deteriorated index of microcirculatory resistance. They concluded that, "both scenarios reflect the reduced physiological impact of epicardial disease in the presence of microvascular dysfunction and increased microvascular resistance as the maximal achievable coronary flow is diminished". The graft microvasculature is affected early after transplantation and microvascular dysfunction (reduced coronary flow reserve, increased index of microcirculatory resistance, abnormal vasoconstrictor response to acethylcholine) predicts development of CAV (107–109). Again, the invasive nature of these methods is a limitation. Following the promising results for CAD, the computational fluid dynamics-derived fractional flow reserve (CFD derived FFR) method should be attempted for CAV to overcome this limitation.

5.2. Computed tomography and scaling power laws based approach

Another method, combining computed tomography and scaling power laws, has been shown to provide a simple, accurate and non-invasive diagnosis tool for Glagov's positive remodeling of coronary arteries, a characteristic of atherosclerosis at early stages (110). This approach consists of obtaining computed tomography scans of the blood vessels of interests, and using them to reconstruct their 3D structures based on validated

algorithm (111,112). The reconstructed structures are then used to fit coefficients in power laws-type relations defined between various parameters of the vessels such as length, volume, and blood flow. Significant variation in the coefficients values of these power laws compared to healthy patients has been shown to provide a reliable diagnosis of Glagov's positive remodeling. The method was introduced by Huo *et al*. (113) and applied to swine coronary arteries. It was found that the coefficients from the length–volume scaling power law varied significantly between healthy and diseased swine, and provided a quantitative rationale for the diagnosis of Glagov's positive remodeling and subsequent atherosclerosis. The authors applied a similar method on a patient cohort with metabolic syndrome and concluded that the coefficients from the length-volume power law also provide a quantitative rationale for diagnosis of diffuse CAD (114). The scaling law approach for diagnosis of diffuse disease holds promise for early diagnosis of CAV.

6. IMMUNOLOGICAL APPROACHES TO CAV DIAGNOSIS

6.1. Biomarkers

In the last few years, strong focus has centered on biomarkers for diagnosis of CAV. In 2002, Labarrere *et al.* (115) found a correlation between increased concentration of C-reactive protein and risk of developing CAV. A decade later, the same group showed that early absence of atherothrombotic risk (measured using predictive markers such as myocardial fibrin deposition, and loss of vascular antithrombin and tissue plasminogen activator) identifies a patient subgroup that rarely develops CAV or graft failure, implying that this low-risk subgroup could possibly be followed with less frequent invasive diagnosis procedures (116).

A study published in 2008 concluded that higher levels of von Willebrand factor were found in patients who later developed CAV, and proposed the use of von Willebrand factor as a biomarker to identify patients with higher risk of developing CAV (117). A study published in 2013 found that higher levels of vascular endothelial growth factors (VEGF)-A, (VEGF)-C and platelet factor-4 are strongly associated with CAV (118). Finally, a recent study published in 2015 demonstrated that the median plasma levels of endothelium-enriched microRNAs are higher in patients with CAV as compared to patients without CAV, and represent biomarkers with significant potential for use in CAV diagnosis (119).

Although these studies are promising, a comparative study to identify the biomarkers with highest potential is clearly required. Subsequently, the development of a non-invasive technique to measure these biomarkers that are currently evaluated through invasive biopsies is also needed. We have seen that hybrid methods provide improved detection, so methods combining imaging, computational analysis and multiple biomarkers may provide a predictive matrix for optimal CAV diagnosis.

6.2. Capioles: size matters, little things in the large panorama!

CAV is a disease that affects all vessels from epicardial arteries to veins compromising the entire graft vasculature. Since it is a panvascular disease affecting all vessels of the transplanted heart, imaging tools that evaluate large arteries, small arteries and arterioles and even smaller microvessels are needed.

Following transplantation, Labarrere *et al.* were the first to identify antithrombin-reactive microvessels that confer a favorable prognosis in patients with CAV (120). The antithrombin-reactive microvessels have particular phenotypic characteristics that differ from normal arteries, capillaries and veins. Capillaries binding antithrombin (Figure 7) are larger than normal capillaries (estimated range: 10-30 μm), and unlike normal capillaries (< 10 μm), they react with antibodies to smooth-muscle-specific alpha actin and the Pathologische Anatomie Leiden-Endothelium (PAL-E) antigen (121,122): an antigen normally found only in venules, small to medium-size veins, and capillaries with the altered vascular permeability observed in angiogenesis (123,124). It may be that this particular capillary antithrombin-binding is associated with neo-vessel formation or vascular remodeling involving pericytes or smooth muscle cells and capillary endothelial phenotypic changes (121,122). These capillary-like vessels may be involved in capillary arterialization secondary to ischemic injury, undergoing diameter increase and recruitment of smooth muscle actin-reactive cells to become new arterioles (125–127). An increased capillary arterialization in the presence of thrombin supports this hypothesis (128).

It is possible that capioles are capillaries involved in arteriogenesis (128), since arteriogenesis has been attributed to enlargement of a pre-existing collateral network or de novo formation of new arterial vessels by means of capillary arterialization (129,130). Angiographic studies performed in patients with capioles showed the presence of small vessel disease with a blush pattern (131). Although the artery-to-artery or arteriole to arteriole connection cannot be completely demonstrated by histology or immunopathology, the extraordinary phenotypic similarity between capioles and the collateral capillary arterialization following arteriolar ligation in mice (127); and the presence of small vessel disease with a blush pattern in transplanted hearts with capioles strongly suggest these particular vessels are involved in collateral arterial/arteriolar formation.

The generation of new (collateral) arteries from capioles may occur in response to occlusion of arterial trunks in areas of microinfarction with extensive

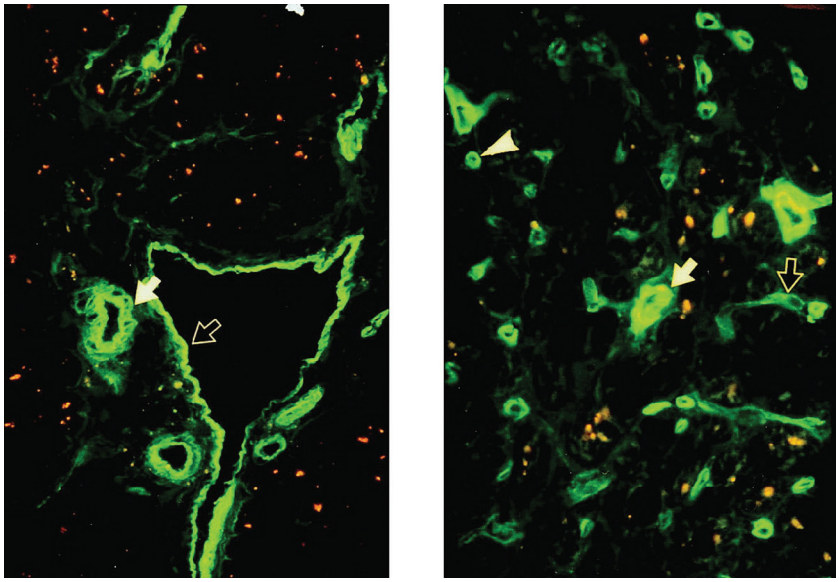

Figure 7. Reactivity of heart biopsy specimens to antithrombin antibody. Note arterial (arrow) and venous (open arrow) antithrombin in normal donor heart (Left). Note intense antithrombin-reactivity in arteries (arrow) and capioles (arrowhead) in a patient with CAV that managed to survive (Right). Illustration modified with permission from (120).

fibrin deposits in and around damaged cardiomyocytes (Figure 8). Capioles are predominantly identified next to areas of microinfarction in transplanted hearts with severe lesions of CAV (121–123). Quiescent capillaries found in normal hearts and hearts without microvascular fibrin deposits never express antithrombin compared to the antithrombin-reactive capioles. Capioles are most probably capillaries becoming arterialized with outstanding anticoagulant properties.

The relevance of the microvasculature in patients with severe CAV is highlighted by the demonstration that myocardial perfusion in transplanted hearts increases significantly after reduction of low-density lipoprotein-cholesterol, lipoprotein (a), C-reactive protein and fibrinogen plasma levels following apheresis treatment in transplanted patients with severe CAV (132). This further suggests that improving the status of the microvessels in transplanted hearts with severe CAV may be considered as a novel therapy to increase survival. Understanding the physiopathology of endothelial and microvascular dysfunction in CAV will certainly play a crucial role in the development of novel diagnosis techniques and therapies (133).

7. CONCLUSIONS

Cardiac allograft vasculopathy (CAV) is one of the most common long-term complications of heart transplantation. Because of its irreversible nature, early detection is crucial to implement a strategy to slow down the progression of the disease. The most common diagnosis techniques, namely coronary angiography and IVUS, are invasive and have low sensitivity especially at early stages of the disease. There is clearly a need for better alternative for the diagnosis of CAV. Many techniques, mainly non-invasive, have been developed. Among these, the non-invasive dobutamine stress echocardiography (DSE) and single-photon emission computed tomography (SPECT) have been studied largely with mostly positive outcomes. However, low sensitivity at early stages and risk from exposure to frequent nuclear dose, respectively, have been reported as major drawbacks. Additional techniques such as coronary computed tomography angiography (CTA), cardiovascular magnetic resonance (CMR) and optical coherence tomography (OCT) have also been evaluated with promising results. Large patient studies are still required in order to establish definitive efficacy and accuracy of these various approaches.

To date, none of the methods developed have provided an optimal predictive power of CAV without limitations. Hence, there is opportunity for novel techniques yet to come. We propose the translation of recent developments in CAD to CAV diagnosis. Use of non-invasive imaging techniques in conjunction with computational analysis method, such as computational fluid dynamics, have shown promising results for CAD diagnosis. The combined use of computed tomography images and scaling power laws is another potential tool for CAV diagnosis.

Various studies have reported different potential biomarkers for CAV. Agreement on a common biomarker and method of procurement (blood, tissue, etc.) is still to be determined. It is unlikely that a single biomarker

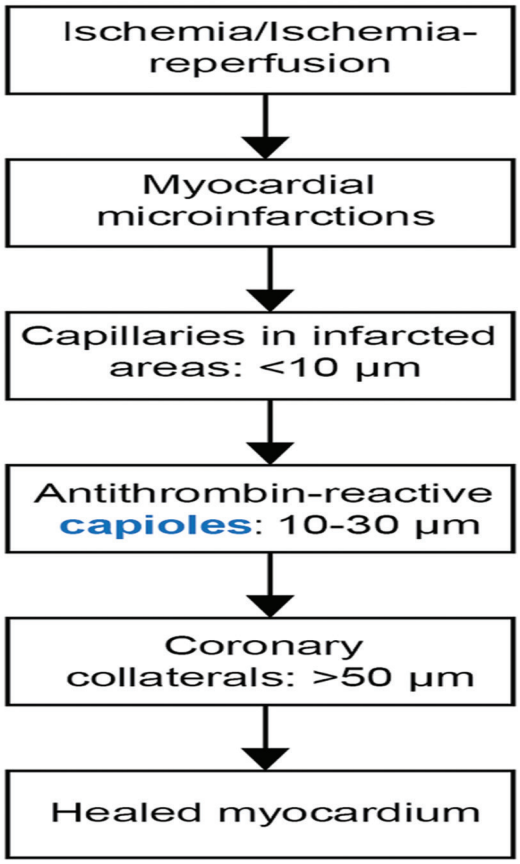

Figure 8. Antithrombin-reactive microvessels (capioles) are involved in development of collaterals in areas of microinfarction.

can be completely predictive of CAV but rather a set of biomarkers may be necessary. Hence, an integration of biological, imaging and computational indices may provide a reliable biomarkers matrix to identify patients at high risk and adjust assessment frequency accordingly. Finally, we identified microvessels with particular anticoagulant properties and phenotypic characteristics of capillaries and small arterioles, named capioles, which are mostly seen in patients with severe CAV. We argue that these microvessels may be new targets for the diagnosis of CAV and can be added to the biomarker matrix.

Although current methods for CAV diagnosis have limitations, it appears that non-invasive, highly reliable alternatives will emerge in the near future. Methods combining multiple techniques and fields, such as imaging, computational analysis, and biomarker detection, would likely provide a biomarker matrix that demonstrates higher accuracy and sensitivity.

8. ACKNOWLEDGEMENT

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Abbreviations: CAV: cardiac allograft vasculopathy; IVUS: intravascular ultrasonography; SPECT: single-photon emission computed tomography; OCT: Optical coherence tomography; CFD: computational fluid dynamics; FFR: fractional flow reserve; CAD: coronary artery disease

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