THE NATURAL HISTORY INVESTIGATION OF *IN VIVO* HUMAN CORONARY ENDOTHELIAL FUNCTION AND ATHEROMA PLAQUE PROGRESSION: INVASIVE CORONARY IMAGING STUDIES

A thesis submitted by

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THESIS SUMMARY

It has been well documented that coronary vasoconstriction, atheroma plaque burden, and 'vulnerable' plaque plays a critical role in the pathogenesis of acute coronary syndrome (ACS); yet, the dynamic interaction among these factors *in vivo* to date have not been evaluated, particularly in the longitudinal study. In this thesis, we described a series of *in vivo* experiments conducted within the intact epicardial coronary tree from patients who presented with unselected clinical presentation, with the aim to explore the underlying relationship between coronary atheroma burden, plaque composition, and coronary endothelial vasoreactive function. We particularly highlight the potential role of a novel intravascular ultrasound (IVUS) platform which co-register with near infrared spectroscopy (NIRS) in delineating plaque composition. Utilizing this technology allows us to examine the evolution of both grey scale measurement and coronary plaque composition over time. Contrary to currently available imaging platforms for plaque composition, such as virtual histology IVUS or optical coherence tomography (OCT) which is predominantly qualitative, NIRS measurements are automated and quantitative, allowing ready interpretation in the catheterization lab.

Of the 2 introductory chapters, chapter 1 explores the developmental history, clinical indication, and research application of IVUS in the past three decades. Chapter 2 reviews the different resistance compartments of the coronary tree and the role of various pharmacological agent in the catheterization lab. This chapter also discusses the role of salbutamol in the evaluation of coronary endothelial function.

Chapter 3-5 comprise the result section of this thesis. Chapter 3 describes the effectiveness of OCT in the evaluation of epicardial coronary endothelial function. OCT

is known to have superior lateral resolution when compared to other intravascular imaging modalities currently available. In this chapter, we underscore the limitation of OCT, which relies on bolus contrast injection for blood clearance from the field of view, in the invasive coronary endothelial function assessment. A vast number of coronary vasoreactive agents, including salbutamol, are largely dependent on shear stress generated by the microvascular compartment to mediate conduit vessel vasodilation. Injection of contrast bolus at high rate hence may increase the shear stress generated and impact on the reliability of vasomotor response.

Chapter 4 outlines the relationship between segmental coronary endothelial dependent and independent function with atheroma plaque volume and associated lipid rich necrotic composition in unselected patients who present with either stable chest pain syndrome or ACS. Utilizing NIRS, we demonstrated that coronary endothelial independent function in response to glyceryl trinitrate (GTN) was strongly associated with atheroma plaque volume irrespective whether the patient presented with stable chest pain or ACS. To a lesser extent, we also found a weak association between plaque containing lipid rich necrotic core with coronary endothelium independent vasodilator function. It was also surprising to note the lack of association between coronary endothelial dependent function with either atheroma volume or composition. Having reviewed previous data in the literature and scrutinised our findings, we concluded that adequate dosing of any vasoreactive agent is crucial to generate the desirable and appropriate vasomotor response.

Chapter 5 describes our prospective and serial imaging analysis which aimed to identify the relationship between epicardial coronary vasomotor function and change in

atheroma plaque volume and necrotic lipid laden core plaque, over time. Several key insights emerge from this experiment. We identified that epicardial coronary endothelial function seems to influence the progression of plaque volume and its composition in a unique manner. Whilst coronary endothelial dependent function was found to mediate plaque composition progression, the segmental coronary endothelial independent function was more associated with atheroma volume progression. Similar to previous observations, we also found baseline plaque burden and positive remodelling to influence formation of future fibroatheroma. Furthermore, baseline NIRS derived LCBI was independently predictive of both lipid core progression and plaque volume progression. Taken together, these findings underline the role of atheroma structure-vessel function in mediating the mechanism of atheroma plaque progression in intact human epicardial coronary tree and may provide an important prognostic tool in identifying patients who are at risk of developing future coronary event above and beyond the established cardiovascular risk predictive tools.

DECLARATION

Name: Samuel L. Sidharta Program: Doctor of Philosophy

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THESIS RELATED PUBLICATIONS

1. **Sidharta S**, Puri R. Coronary Vasoactive Agents: Periprocedural pharmacology, Global Textbook of Interventional Cardiology, 2017, S. Kapadia et al (in press).

2. **Sidharta S**, Worthley MI, Worthley SG. Use of Intravascular Ultrasound in Interventional Cardiology. Imaging Coronary Atherosclerosis. Eds: Nicholls SJ and Crowe T. Springer Publishers, New York. pp. 51-66. 2013.

3. **Sidharta S**, Puri R, Frost L, Kataoka Y, Carbone A, Willoughby S, Nelson A, Nicholls S, Worthley S, Worthley M. The Impact of Lumen Size and Microvascular Resistance on Fourier-Domain Optical Coherence Tomography (FD-OCT) Coronary Measurements, Int J Cardiol 2014 Jun 1;174(1):210-1

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1. **Sidharta S**, Nicholls S, Howell S, Baillie T, Montarello N, Montarello N, Honda S, Shishikura D, Nelson A, Delacroix S, Chokka R, Beltrame J, Worthley S, Worthley M. Association between coronary endothelium independent vasoreactivity and lipid rich plaque burden. Heart 2017 (submitted).

2. **Sidharta S**, Baillie T, Howell S, Nicholls S, Montarello N, Honda S, Shishikura D, Delacroix S, Beltrame J, Psaltis P, Worthley S, Worthley M. *In vivo* evaluation of human coronary structure-function predicts subsequent progression of coronary atherosclerotic plaques: A Near Infrared Spectroscopy Study. European Heart Journal 2017 (submitted).

THESIS RELATED ABSTRACTS

1. **S. Sidharta**, T. Baillie, N. Montarello, N. Montarello, J. Beltrame, S. Worthley, S. Nicholls, M. Worthley. Incidence of Lipid Rich Plaque (LRP) by Near Infrared Spectroscopy (NIRS) in Near Normal Coronary Artery of Patients with Stable Presentation Versus Acute Coronary Syndrome. Heart, Lung and Circulation 08/2016; 25:S48

2. **S. Sidharta**, T. Baillie, N. Montarello, N. Montarello, J. Beltrame, S. Worthley, S. Nicholls, M. Worthley. Relationship Between Human Coronary Endothelial Function and Lipid Rich Plaque (LRP) Burden. A Near-Infrared Spectroscopy (NIRS)/Grey Scale Intravascular Ultrasound (IVUS) Study. Heart, Lung and Circulation 08/2016; 25:S61

3. **S. Sidharta**, T. Baillie, N. Montarello, N. Montarello, J. Beltrame, S. Worthley, S. Nicholls, M. Worthley. Relationship Between Coronary Arterial Plaque Burden and Endothelium Independent Vasoreactivity: An Intravascular Ultrasound Study. Heart, Lung and Circulation 08/2016; 25: S60-1

4. **S Sidharta**, R Puri, S Willoughby, L Frost, Y Kataoka, A Nelson, A Carbone, S Nicholls, S Worthley, M Worthley, The impact of luminal size and microvascular resistance on OCT measurements**,** Global Heart 2014 Mar: 9(1) (Suppl): e16, DOI: 10.1016/j.gheart.2014.03.1275

LIST OF ABBREVIATIONS

ACE-i	Angiotensin Converting Enzyme Inhibitor
ACS	Acute Coronary Syndrome
AR	Adrenergic receptor
ARB	Angiotensin Receptor Blocker
ATP	5'-triphosphate
cAMP	Cyclic Adenosine MonoPhosphate
cGMP	Cyclic Guanosine MonoPhosphate
CBF	Coronary Blood Flow
CT	Computed tomography
CTFC	Corrected Thrombolysis in myocardial infarction Frame Count
FD-OCT	Fourier Domain Optical Coherence Tomography
FFR	Fractional Flow Reserve
GTN	Glyceryl TriNitrate
HDL	High Density Lipoprotein
IC	Intracoronary
IQR	Interquartile range
IV	Intravenous
IVUS	Intravascular Ultrasound
LAD	Left Anterior Descending
LCx	Left Circumflex
LCBI	Lipid Core Burden Index
LDL	Low Density Lipoprotein
L-NMMA	*N* -monomethyl-L-arginine

LRP	Lipid Rich Plaque
LVEF	Left Ventricular Ejection Fraction
MACE	Major Adverse Cardiovascular Event
mcg	Microgram
mg	milligram
min	minute
MLA	Minimum Lumen Area
NIRS	Near Infrared Spectroscopy
NO	Nitric Oxide
NOS	Nitric Oxide Synthase
OCT	Optical Coherence Tomography
PAV	Percent Atheroma Volume
PCI	Percutaneous Coronary Intervention
QCA	Quantitative Coronary Angiogram
RCA	Right Coronary Angiogram
SD	Standard Deviation
SEM	Standard Error of the Mean
SLV	Segmental Lumen Volume
SMV	Salbutamol Mediated Vasoreactivity
VSMC	Vascular Smooth Muscle Cell
VH-IVUS	Virtual Histology - IVUS

CHAPTER 1: USE OF INTRAVASCULAR ULTRASOUND IN INTERVENTIONAL CARDIOLOGY

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ABSTRACT

For the last 60 years, coronary angiography remains the investigation of choice to evaluate symptomatic coronary artery disease. Coronary angiography however only provides a two dimensional "luminogram" of a three dimensional arterial structure. As a result, its interpretation is subjected to interobserver variability and may be compounded by vessel overlapping and tortuosity. Intravascular ultrasound (IVUS), on the other hand is a catheter-based technique which provides a comprehensive assessment of the entire vessel wall, including the extent and distribution of the atherosclerotic plaque. Owing to its high resolution, IVUS has been used extensively in various clinical and research settings. Some of the IVUS diagnostic clinical applications include assessment of angiographically intermediate lesions, assessment of plaque morphology, and evaluating appropriate expansion of an inserted coronary stent. IVUS is also extremely valuable in guiding percutaneous revascularisation strategies, especially in the case of left main coronary artery disease, allowing for detailed pre-intervention evaluation of the target artery as well as post intervention assessment of procedural outcome. This information is ultimately critical for the clinician to devise an appropriate procedural strategy to optimize clinical outcome. In atherosclerosis research, IVUS has provided a significant insight into the understanding of the natural history of atherosclerosis. The serial IVUS-measured atheroma change has been widely used as a surrogate end point in various pharmacological clinical trials. The development of new IVUS technology, such as virtual histology IVUS or combined IVUS with near infrared spectroscopy has allowed further characterization of atherosclerotic plaque to potentially help identify vulnerable plaque, which may provide important risk predictive information regarding focal coronary artery disease.

I. INTRODUCTION

Since Mason Sones performed the world's first diagnostic coronary angiography in 1958, coronary angiography has been widely used as the standard clinical imaging to identify the significance of coronary arterial narrowings and to guide catheter based and surgical revascularisation strategies (Mueller and Sanborn, 1995, Topol and Nissen, 1995). However, despite its excellent ability to visualize the contour of the vascular lumen, coronary angiography has some inherent inadequacies and limitations. Among others, coronary angiography provides little insight regarding tissue composition, atheroma plaque characteristics, as well as its extent and distribution (Nissen, 2001, Nicholls and Nissen, 2009). This is not entirely surprising given angiography merely produces a two dimensional silhouette of the contrast filled lumen and does not evaluate vessel wall, the site in which plaque accumulates. Moreover, coronary angiography is subject to significant intraobserver and interobserver variability, raising the issue regarding its accuracy and reproducibility in assessing the extent of coronary artery disease (Topol and Nissen, 1995, Zir et al., 1976, Murphy et al., 1979, Galbraith et al., 1978). In fact, major discrepancies between angiographic visual estimation of lesion severity and post mortem examination had been reported previously (Roberts and Jones, 1979, Arnett et al., 1979, Topol and Nissen, 1995). The use of intravascular imaging techniques, specifically intravascular ultrasound (IVUS), provides a meticulous characterization of vessel wall as well as information regarding the extent and distribution of atherosclerotic plaque (Puri et al., 2011). IVUS has also demonstrated the ubiquitous presence of plaque in the region which appears normal when assessed with coronary angiography (Nissen, 2001). Thus, from a research perspective, IVUS provides a unique opportunity to examine the natural history of atherosclerosis *in vivo* along with the impact of specific medical therapies on this process, in addition to its

known clinical benefit to guide revascularisation strategy with percutaneous coronary intervention (PCI).

History of IVUS

The first real time catheter ultrasound system designed for intracardiac imaging was introduced by Bom et al in the late 1960s (Bom et al., 1972, Yock et al., 1989). The transducer for this system was a 32 barium titanate piezoelectric (pressure-electric) element circular array mounted at the tip of a 3mm (9F) catheter. Clear images were obtainable with this system however limitation with image quality and ring down artefact led a number of groups to develop mechanical catheter imaging systems (Roelandt et al., 1989, Pandian et al., 1988, Yock et al., 1989). Hodgson et al (Hodgson et al., 1989b) then performed the first *in vivo* IVUS study on 20 patients during cardiac catheterization by inserting a 20 MHz transducer through a 0.014" floppy guidewire. Since these early times, technological advances in IVUS have included a reduction in ultrasound transducer size (0.87-1.17mm) and increasing ultrasound frequency (30-45 MHz). The result is a high resolution, cross sectional, tomographic image of the entire vessel wall resembling histology cross sectional specimen.

Principles of IVUS

Medical ultrasound images are produced by passing an electrical current through a miniaturised crystal that expands and contracts to produce sound waves. The generated sound waves then propagate through the different tissue and subsequently reflected according to the acoustic properties of the tissue it travels through (Mintz et al., 2001, Garcia-Garcia et al., 2011). The final image created is a three-layer appearance of the coronary artery in alternating bright and dark echoes: a bright echo from the intima, a

dark zone from the media, and a bright echo from the adventitia (Gussenhoven et al., 1989, Nishimura et al., 1990, Pandian et al., 1988) (Fig 1). For the usual IVUS transducers (20-40 MHz), the axial resolution is approximately 150-200μm and the lateral resolution is 200-250μm with >5mm depth penetration (Nissen and Yock, 2001).

There are 2 different types of IVUS transducer: mechanical single element rotating transducer and the solid state electronic phased array transducer (Mintz et al., 2001, Garcia-Garcia et al., 2011, Nissen and Yock, 2001, McDaniel et al., 2011). The mechanical system utilizes a single rotating transducer, which is driven by a flexible drive cable at 1800 rotations per minute to sweep a beam almost perpendicular to the catheter. It offers a greater resolution owing to its higher frequency system and also a more uniform pullback. This system is available commercially as the 40 MHz iCross or Atlantis SR Pro catheters (Boston Scientific, Santa Clara, California), the Revolution 45 MHz catheter (Volcano Corp, Rancho Cordova, California), and the 40 MHz Lipiscan IVUS (InfraReDx, Burlington, Massachusetts). The electronic phased array system on the other hand uses multiple transducer elements which are arranged in an annular array and sequentially activated to generate an image. Commercially, it is available as the 5F 20MHz Eagle Eye catheter (Volcano Corp). Benefits of this system include enhanced trackability and lack of non-uniform rotational distortion artefacts. The latter is unique to mechanical system and is due to mechanical binding of the drive cable (ten Hoff et al., 1989).

II. CLINICAL APPLICATION

Evaluation of ambiguous lesions in non-left main coronary artery

Assessment and management of angiographic intermediate lesions (50-70% stenosis) are a regular clinical dilemma faced by an interventional cardiologist. This issue is further compounded by various angiographic limiting aspects, such as lesion eccentricity, vessel overlapping and tortuosity, degree of calcification, and diffuse reference vessel disease (Sipahi et al., 2006). In this context, IVUS has the ability to provide complementary information to coronary angiogram with its excellent spatial orientation. In fact, the use of pre-intervention IVUS in coronary artery disease management has been reported to result in redirection of therapy in up to 40% of patients (Mintz et al., 1994).

Although fractional flow reserve (FFR) is currently being preferred as the investigational tool to assess the functional significance of an intermediate lesion (Tobis et al., 2007, Kern and Samady, 2010, Kern et al., 2006), IVUS has an advantage in permitting precise quantification of anatomical distribution and morphology of a lesion; an important consideration in devising a procedural strategy and device selection prior to stenting. Several studies have also reported reasonable correlation between structural data derived from IVUS with physiological parameters from FFR (Abizaid et al., 1998, Nishioka et al., 1999, Abizaid et al., 1999, Koo et al., 2011, Kang et al., 2012, Ben-Dor et al., 2012). For example, earlier studies have identified IVUS derived minimum lumen area (MLA)$<4mm^2$ as being haemodynamically significant when compared with FFR and SPECT imaging (Nishioka et al., 1999, Abizaid et al., 1998). Furthermore, using this MLA cut off, a long term follow up study reported a low event rate among patients who had their intervention deferred for an MLA$\geq 4mm^2$ (Abizaid et al., 1999). The latter studies however suggest a lower MLA cut off value of 2.4-3.6mm^2 as being haemodynamically significant as compared with FFR (Koo et al., 2011, Kang et al.,

2012, Ben-Dor et al., 2012). This apparent discrepancy is not entirely surprising given a single MLA value is significantly influenced by multiple factors including size of a vessel, lesion location and length, and the presence of plaque rupture (Kang et al., 2012). Therefore, a combination of IVUS derived parameters, such as plaque burden, area stenosis, and lesion length also needs to be taken into consideration when assessing an intermediate lesion (McDaniel et al., 2011, Sipahi et al., 2006, Lee, 2012). Illustration of basic IVUS measurement can be seen in figure 2.

Evaluation and management of left main coronary artery disease

Evaluation of left main disease severity: It has been well documented that quantifying angiographic left main disease severity especially that which involves the proximal segment is particularly challenging, in part, due to measurements that have a significant interobserver variability (Fisher et al., 1982, Isner et al., 1981). This interobserver variability relates to three major anatomical factors which impair left main evaluation, which includes aortic cusp opacification, short length of vessel trunk, and the presence of bifurcation or trifurcation at the distal segment (Nissen and Yock, 2001). Furthermore, streaming in the aortic cusp can obscure the ostium of the left main, leaving the angiographers relying on contrast reflux and disengagement of the catheter to visualize the ostium. On the other hand, the short segment of the left main shaft leaves little reference site for comparison. Also, the bifurcation into sub-branches at the distal end potentially conceal the distal left main. IVUS, in contrast, suffers not from the aforementioned limitation making it an investigation of choice when assessing left main lesion (Fig 3).

In the case of evaluating an angiographic intermediate left main stenosis, an IVUS minimum lumen diameter (MLD) of <2.8mm or an MLA<6mm^2 suggest a physiologically significant stenosis and thus merits revascularisation (Sipahi et al., 2006, Tobis et al., 2007). A study of 55 patients with moderate left main disease demonstrated that these cut off values correlate well with physiologically significant lesion as assessed by FFR with a sensitivity of 93% and a specificity of 98% (Jasti et al., 2004). Additionally, a recent multicentre prospective study showed that deferral of revascularisation among patients with a left main MLA≥6mm^2 carried a similar outcome with the revascularised group who had a left main MLA<6mm^2, during long term follow up (de la Torre Hernandez et al., 2011). Another comparative study used an MLA cut off value of 7.5mm^2 to determine whether a patient should undergo revascularisation or to have a deferral strategy (Fassa et al., 2005). It concluded that during the mean follow up of 3.3 +/- 2 years, there was no difference in major adverse cardiac events (MACE) between the two cohorts. Altogether, in conjunction with clinical information, it is reasonably safe to defer revascularisation in patients with MLA≥7.5mm^2 and to consider revascularisation in patients with MLA<6mm^2. Patients with intermediate MLA value (6-7.5mm^2) would require further physiological assessment, for example with an FFR (Kern and Samady, 2010). Based on the available evidence, IVUS was given a class IIA indication for the assessment of angiographically indeterminant left main coronary artery disease by the recent joint American College of Cardiology/American Heart Association/Society for Cardiovascular Angiography and Interventions (ACC/AHA/SCAI) guideline (Levine et al., 2011).

IVUS guided PCI for unprotected left main disease: Aside from its ability to provide further stratification of left main disease severity, IVUS also made a major contribution

in the area of unprotected left main PCI. Patients with significant left main disease (≥50% stenosis) carry a high-risk mortality and morbidity from cardiovascular events considering the area of myocardium at risk should the flow in this vessel becomes compromised. To date, coronary artery bypass surgery (CABG) remains the "gold standard" treatment for majority of significant left main disease especially in those with multivessel involvement. Nevertheless, the introduction of drug eluting stent (DES) has ushered a new era of complex PCI, such as unprotected left main coronary artery disease and bifurcation lesion PCI due to its low rate of restenosis (Garg and Serruys, 2010). The use of pre-intervention IVUS in left main PCI permits a detailed assessment of the vessel anatomy, the size of the vessel, and to determine the extent of ostial involvement at the daughter sub-branches (Tobis et al., 2007, Kang et al., 2011c). This information helps the operator to optimize stent selection and provide complete disease coverage. IVUS also assists in ensuring adequate stent expansion and apposition; important risk factors for restenosis and stent thrombosis (Sonoda et al., 2004, Sakurai et al., 2005, Palmerini et al., 2011). Finally, IVUS also reveals the extent of calcification. This information will be useful in deciding whether to employ a debulking strategy with rotational atherectomy to optimise stent placement (Park et al., 2001, Takagi et al., 2002, Anderson et al., 2002).

The role of IVUS guided strategy for unprotected left main PCI however remains a controversial issue given the conflicting data in the literature (Palmerini et al., 2011). Nonetheless, several recent reports provide clinical data favouring its routine use. In a large Korean registry study (Park et al., 2009), the use of IVUS guided left main PCI was associated with a 3 year reduction in mortality when compared with angiographic guided PCI. This benefit is particularly observed among the 145 matched pairs of

patients who receive drug-eluting stents (4.7% vs. 16%, p= 0.048). Similar findings were demonstrated in Premier of Randomized Comparison of Bypass Surgery versus Angioplasty Using Sirolimus-Eluting Stent in Patients with Left Main Coronary Artery Disease (PRECOMBAT) trial, which reported a low event rate in the PCI group and comparable outcome with the CABG group (8.7% vs. 6.7%, p=0.02 for non-inferiority) (Park et al., 2011). In this multicenter trial, IVUS was extensively used. Some of this clinical benefit may be derived from the effectiveness of IVUS in determining the extent of disease burden and lesion complexity thus assisting the operator with the appropriate procedural strategy. For instance, the MLA of the confluent zone between the distal segment of the left main coronary artery and its daughter branches has been shown to be an accurate indicator of bifurcation left main disease severity and a predictor of post stent underexpansion and clinical outcomes (Kang et al., 2011c, Kang et al., 2011b). Another study then demonstrated that among patients who undergo bifurcation left main PCI, those with post stenting underexpansion have significantly lower MACE free survival rates at 2 years when compared with the comparator group (Kang et al., 2011a). Acknowledging the above data, it would be reasonable to consider IVUS to guide all left main PCI.

IVUS and PCI

Refinement of PCI technique and practice: IVUS has been instrumental in helping us to understand the arterial responses to coronary intervention (Laskey et al., 1993, Mintz et al., 1992, Potkin et al., 1992, Suarez de Lezo et al., 1993) and has assisted in the improvement of technical details of the devices application and their manufacturing. For instance, IVUS has given a significant insight into the different mechanism of restenosis

post intervention. It has shown that whilst a combination of elastic recoil, concentric remodelling, and to a lesser role, local intimal hyperplasia, account for the mechanism of restenosis in post balloon angioplasty (Costa et al., 2001, Fuessl et al., 1999, Costa and Simon, 2005), neointimal hyperplasia is the sole mechanism of late lumen loss in the stented artery following bare metal stenting (Hoffmann et al., 1996, Mintz et al., 1996). These observations were extremely valuable in guiding the development of management strategies for in stent restenosis. Furthermore, IVUS has also revolutionized the technique of stent deployment and guided us regarding post stent medication recommendations. Following the enthusiasm with the introduction of stents, clinicians began to notice issue with stent implantation in the form of subacute thrombosis (Orford et al., 2004). This issue was further compounded by major bleeding issues due to intensive use of oral anticoagulation. IVUS then began to identify the association between subacute stent thrombosis and stent deployment, such as inadequate stent expansion and incomplete stent apposition (ISA) (Nakamura et al., 1994). Inadequate stent expansion occurs when part of the stent is insufficiently expanded when compared to the proximal and distal reference site. ISA, in contrast, refers to a lack of contact between the stent struts and the underlying arterial wall. Based on these observations, Colombo et al. recommended post stent deployment with high pressure balloon inflation to maximise stent apposition and expansion (Colombo et al., 1995). This strategy not only led to a reduction of stent thrombosis but also allowed oral anticoagulation to be replaced with dual antiplatelet therapy thus reducing the length of hospital stay and vascular complication rates.

IVUS and bare metal stent (BMS) PCI: The benefit of IVUS in coronary intervention was initially demonstrated in the era of balloon angioplasty. Randomised and non-

randomised IVUS trials demonstrated that an IVUS guided strategy resulted in improvement of final lumen diameter and caused significant reduction in clinically driven reintervention rates when compared with the angiography guided group (Stone et al., 1997, Frey et al., 2000). Yet, in Balloon Equivalent to STent (BEST) study which randomised 254 patients, the investigators reported a similar clinical outcome between IVUS guided balloon angioplasty (with provisional stenting) vs. routine angiography guided stenting (Schiele et al., 2003). It is worth noting that in this trial, there is a high crossover rate (44%) in the balloon angioplasty group to stenting. Taken together, the use of IVUS is effective in optimizing final balloon angiographic and clinical outcomes. This approach however was viewed as time consuming and cost ineffective especially as the stent profile continued to improve and its price continued to decline, leading to routine stenting becoming the preferred strategy for PCI (Orford et al., 2004).

The benefit of IVUS guided strategy in BMS PCI has been examined in both observational (de Jaegere et al., 1998, Fitzgerald et al., 2000, Kasaoka et al., 1998) and randomised trials (Russo et al., 2009, Mudra et al., 2001, Oemrawsingh et al., 2003, Schiele et al., 1998). IVUS can be used to perform pre-interventional evaluation of the extent of lumen obstruction and the mechanism of obstruction (eg. thrombus or calcification). This in turn allows for each management strategy to be individualized and tailored according to patient's clinical baseline status. Post intervention, IVUS may assist with optimizing procedural outcome, such as assessment of stent expansion and stent strut apposition, detection of PCI complication (eg. dissection, plaque shift), and identification of residual stenosis and stent fracture. Of these, inadequate stent expansion has been associated consistently with target vessel failure resulting from either in stent restenosis (Moussa et al., 1999, Hoffmann et al., 1998, de Feyter et al.,

1999, Kasaoka et al., 1998) or stent thrombosis (Uren et al., 2002, Moussa et al., 1997). Despite its practical use and demonstrable benefit by case control studies, the data regarding IVUS clinical benefit from randomised trials are conflicting. The Thrombocyte activity evaluation and effects of Ultrasound guidance in Long Intracoronary stent Placement (TULIP) study, a randomised controlled trial involving 144 patients with long disease segments (>20mm), demonstrated a significant improvement in both angiographic and clinical end points in the IVUS guided group when compared to the angiographic directed group (Oemrawsingh et al., 2003). Another trial, Angiography Versus Intravascular ultrasound-Directed stent placement (AVID), however reported a slight different outcome. AVID randomised 800 patients into IVUS vs. angiography guided intervention with total follow up of 12 months. Indeed, AVID is the largest randomised trial evaluating the benefit of IVUS in PCI using BMS. In this trial, an IVUS guided strategy resulted in the improvement of post procedural minimum stent area (a marker of adequate stent expansion) but did not result in the reduction of clinical end points (death/myocardial infarction/target lesion revascularisation) except in the subgroup with moderate size vessel (2.5-3.5mm) and in saphenous vein graft PCI (Russo et al., 2009). In this subgroup, the reduction in clinical end points were mainly driven by target lesion revascularisation. These findings were further replicated in a meta analysis from 7 randomised trials involving 2,193 patients (Parise et al., 2011). It concludes that IVUS guidance was associated with a significantly larger postprocedure angiographic minimum lumen diameter, lower rate of 6-month angiographic restenosis, a reduction in the revascularization rate, and overall MACE. However, no significant effect was seen for myocardial infarction or mortality. Overall, the efficacy of IVUS in optimizing procedural outcomes and reducing restenosis rates in BMS PCI is unquestionable, nonetheless its routine use in clinical practice still needs to be weighed

against a number of factors, including cost, time, availability, and the operators' skill to accurately acquire and interpret the images.

IVUS and DES PCI: The arrival of DES opened a new chapter in PCI. DES had been shown to significantly reduce the risk of restenosis to less than 10% (Abizaid et al., 2004, Colombo et al., 2003, Serruys et al., 2002, Silber et al., 2009, Sonoda et al., 2004) when compared to BMS. It does this by inhibiting neointimal proliferation through its drug coating (Burke et al., 1999, Poon et al., 1996). As a result, operators began to put less attention on achieving optimal final procedural outcome as they would when inserting a BMS (Colombo et al., 1995). However, the discovery of increasing incidence of stent thrombosis, especially very late stent thrombosis in the patients receiving DES has resulted in renewed interest in the use of IVUS to direct therapy.

Stent thrombosis, despite its rare occurrence (annual risk ~0.5%), carries significant morbidity and mortality rates (Serruys and Daemen, 2007). The overall prognosis is poor with 10-30% mortality rate. Several precipitating factors have been implicated to cause stent thrombosis and these essentially can be categorized as patient factors, lesion characteristics, device factors, and procedural factors. IVUS has provided significant insights into the morphologic pattern and possible causes of stent thrombosis following DES implantation, specifically stent underexpansion and ISA. Fuji et al conducted a retrospective analysis on 15 patients who developed stent thrombosis following successful DES implantation (Fujii et al., 2005). They reported that lesions leading to stent thrombosis had more stent underexpansion, smaller minimum stent area, and residual edge stenosis. The strong association between IVUS detected stent underexpansion and stent thrombosis was also observed and well established in various

other DES trials (Liu et al., 2009, Okabe et al., 2007, Alfonso et al., 2007, Takebayashi et al., 2005, Vautrin et al., 2012, Cheneau et al., 2003). The role of ISA in causing stent thrombosis, on the other hand, is still controversial. Firstly, ISA is common, occurring in 10-20% of DES cases and can be acute or late (Mintz, 2007a). Whilst acute ISA is largely procedural related, late ISA may be due to a combination of positive vessel remodelling (ie. vessel expansion), intimal hyperplasia, or dissolved thrombus which can lead to a gap between vessel wall and stent (Mintz, 2007b). Secondly, there remains uncertainty regarding the link between ISA and stent thrombosis. In a case control study by Cook et al, a significantly high rates of ISA were noted in the very late stent thrombosis patients as compared to the DES control group (Cook et al., 2007). This was also seen in another observational study (Siqueira et al., 2007) but not identified in follow up studies of large randomised DES trials (Jimenez-Quevedo et al., 2006, Ako et al., 2005, Kimura et al., 2006, Degertekin et al., 2003, Colombo et al., 2003). In a recent sub-analysis of a randomised DES trials, Cook et al reported that very late stent thrombosis and MACE occur more frequently in patients with ISA than without ISA (Cook et al., 2012). In this study, there was no difference in mortality.

Based on these IVUS observations, several trials have been conducted to assess the clinical benefit of IVUS directed therapy in DES PCI. A study by Roy et al. compared 1-year clinical outcomes in 884 patients who underwent IVUS-guided PCI with a propensity-matched cohort of angiographically guided patients (Roy et al., 2008). IVUS directed therapy was found to be associated with lower incidence of stent thrombosis at 30 days (0.5% vs. 1.4%, p=0.05) and 1 year (0.7% vs. 2.0%, p=0.01) with no difference in the rates of myocardial infarction or death. Claessen et al. subsequently reported that an IVUS guided strategy resulted in a significant reduction of early, medium, and long

term clinical outcomes (Claessen et al., 2011), with this benefit largely driven by a reduction in myocardial infarction. In another large observational study involving 8,371 patients, IVUS guided DES PCI was also found to be associated with a reduction in mortality rates at 3 years (HR 0.46; 95% CI 0.33– 0.66, P < 0.001) (Hur et al., 2011). Despite these observed benefits, to date there is still a lack of evidence regarding clinical benefits in randomised controlled IVUS studies. Indeed, there is only one randomised trial assessing the efficacy of IVUS use in DES implantation. Angiography Vs. IVUS Optimization (AVIO) trial randomised 284 patients with complex lesion (eg. small and diffuse vessel disease, bifurcation lesion, chronic total occlusion) into IVUS guided or angiography guided arms (Colombo, 2010). Optimal stent expansion was defined as achieving ≥70% of the cross sectional area of the post dilation balloon. The results at 9 months follow up indicated that an IVUS guided strategy resulted in a larger post procedural minimum lumen diameter but no difference was observed in the rate of MACE. On balance, there is no current strong recommendation for routine use of IVUS to guide PCI. However, IVUS use should be considered in patients with high risk of stent thrombosis or in patients whereby the consequence of stent thrombosis is fatal, such as left main coronary artery disease. IVUS should also be considered for evaluation of in stent restenosis or stent thrombosis, especially when it is a recurring event.

Safety

Despite its clinical advantages over coronary angiography, the widespread use of IVUS has been somewhat limited by its invasive nature. The safety of IVUS has been investigated extensively and has a low rate of complications (Hausmann et al., 1995, Batkoff and Linker, 1996, Bose et al., 2007). The most common complication recorded

is transient vessel spasm, which occurs in the order of 2% of procedures. Major IVUS complications, such as dissection and abrupt vessel closure are rare, occurring in <0.5% of procedures (Bose et al., 2007). Importantly, IVUS has not been shown to result in accelerating disease progression (Ramasubbu et al., 2003, Guedes et al., 2005).

III. RESEARCH APPLICATION

Evaluation of atherosclerotic plaque progression/regression

Atheroma burden and its rate of progression, as measured by serial IVUS has been associated with increased risk of future cardiac events (von Birgelen et al., 2004, Nicholls et al., 2010). As a result, serial change in IVUS-measured atheroma burden has been used extensively in randomised clinical trials as a surrogate clinical end point to assess various novel (Tardif et al., 2004, Serruys et al., 2008, Nissen et al., 2008b, Nissen et al., 2007, Nissen et al., 2003, Nissen et al., 2006b) or existing cardiovascular medications (Nissen et al., 2006a, Nissen et al., 2008a, Nissen et al., 2004b, Nicholls et al., 2011, Nissen et al., 2004a, Nasu et al., 2009, Hirohata et al., 2010). The use of IVUS as a surrogate end point allows trials to be conducted in a shorter time frame with smaller number of participants; thus, it helps in expediting the process of drug development rather than progressing early to a costly Phase 3 trial, which may yield a negative result. Amongst these trials, IVUS has provided especially a significant insight into the impact of statin therapy on the natural history of atherosclerosis. The use of intensive statin therapy is not only shown to halt disease progression but may also results in disease regression (Nissen et al., 2006a, Nicholls et al., 2011). Furthermore, virtual histology-IVUS (VH-IVUS) has extended this observation by demonstrating that statin therapy also results in a change of plaque composition (Nasu et al., 2009, Nozue et al., 2012). Altogether, these trials demonstrate that pharmacological therapy has the

potentials to halt, reverse, and alter the course of the natural history of atheroma progression.

The quest of accurately defining vulnerable plaque

In the last decade, IVUS technology has been constantly used in the research arena in an attempt to identify vulnerable plaque, the major substrate for acute coronary syndrome (ACS). Vulnerable plaque or thin-capped fibroatheroma (TCFA) is defined histologically as a plaque with thin fibrous cap (<65μm), which is associated with a large necrotic core (often containing haemorrhage or calcification), reduced smooth muscle content, and a large number of infiltrative inflammatory cells, such as macrophages and activated T cells (Friedewald et al., 2008). Several other pathological features have also been observed to be present in TCFA, such as positive remodelling (expansion of the external elastic membrane in response to plaque accumulation) (Varnava et al., 2002) and abundant intraplaque vasa vasorum, indicating neoangiogenesis and active inflammation (Kolodgie et al., 2003, Galis and Lessner, 2009). Some of these TCFA features like positive remodelling are easily identified by standard grayscale IVUS, however it falls short in its capability to characterize plaque composition. Grayscale IVUS is also limited due to its considerable postprocessing to produce an image and reliance on visual inspection of acoustic reflections to determine plaque component.

In an effort to improve the detection of plaque composition, an IVUS capability called VH-IVUS was developed. VH-IVUS uses mathematical autoregressive spectral analysis of the radiofrequency backscatter data to generate a tissue map of 4 different plaque components: fibrous (green), fibrofatty (yellow green), necrotic core (red), and dense

calcium (white) (Nair et al., 2002) (Fig 4). An alternative algorithm to characterize plaque composition called integrated backscatter IVUS (IB-IVUS) has also been developed. This algorithm uses fast-Fourier transform analysis of the backscatter signal of a tissue volume to generate a tissue colour map (Kawasaki et al., 2002, Kawasaki et al., 2001). VH-IVUS' accuracy in detecting these plaque types has been validated *in vivo* with a predictive accuracy of 87.1%, 87.1%, 88.3%, and 96.5% for fibrous, fibrofatty, necrotic core, and dense calcium respectively (Nasu et al., 2006). VH-IVUS however is limited in its ability to visualize thrombus and may misclassify it as fibrous or fibrofatty plaque. VH-IVUS derived TCFA (VH-TCFA) has been defined as confluent necrotic core rich plaque (>10%) without evidence of overlying fibrous tissue on 3 consecutive frames with the arc of the necrotic core in contact with the lumen for 36 degrees along the lumen circumference (Garcia-Garcia et al., 2009). It must also have a percent atheroma of ≥40% (Rodriguez-Granillo et al., 2005). Based on these criteria, investigators have found that VH-TCFA is more prevalent in the patients with ACS than in patients with chronic stable angina, inferring it as a reasonable surrogate for vulnerable plaque (Hong et al., 2007, Nakamura et al., 2009).

Information derived from both IVUS and VH-IVUS has been evaluated as potential tools to individually risk predict focal atherosclerotic narrowings, as a possible better tool than traditional angiographic assessment. Yamagishi et al analysed 106 patients with angiographic minimal coronary artery disease with baseline conventional IVUS (Yamagishi et al., 2000). At follow up, plaques that resulted in ACS were found to exhibit higher baseline plaque volume, eccentric disease, and echolucent characteristics. Another landmark study which analysed the impact of baseline plaque composition on future coronary event is Providing Regional Observations to Study Predictors of Events

in the Coronary Tree (PROSPECT) trial (Stone et al., 2011). PROSPECT is the first prospective study utilising 3 separate imaging modalities: coronary angiogram, conventional greyscale IVUS, and VH-IVUS to assess the natural history of vulnerable plaque. In this study, 697 patients presenting with an ACS underwent 3 vessel IVUS and VH-IVUS imaging post PCI with the primary end point of MACE (death/cardiac arrest/myocardial infarction/rehospitalisation). After a median follow up of 3.4 years, 20.4% of patients experienced a MACE event. Events were adjudicated to be related to culprit lesions in 12.9% of patients and to non-culprit lesions in 11.6%. Predictors for MACE in non-culprit lesion were identified to be diabetes, IVUS plaque burden of ≥70%, MLA ≤4mm^2, and presence of VH-TCFA. In the absence of VH-TCFA, the event rate in the non-culprit lesions were reduced to 1.3-1.9% in 3 years depending on the presence of other risk factors. In another serial VH-IVUS study by Kubo et al, VH-TCFA may stabilise, remain unchanged, or even evolve in a new territory suggesting that the natural history of vulnerable plaques is a dynamic process (Kubo et al., 2010). In summary, these new intravascular imaging modalities may provide incremental risk predictive information to standard measures of lesion specific vulnerability.

Besides VH-IVUS, there are several other IVUS based technologies that have been developed as potential newer risk predictive tools. Some examples include IVUS elastography/palpography (Choi et al., 2007, Carlier et al., 2002, Suh et al., 2011) and contrast enhanced IVUS (CE-IVUS) (Vavuranakis et al., 2008, Vavuranakis et al., 2007, Carlier et al., 2005). The former attempts to identify the plaque vulnerability by analysing the mechanical strain property of the arterial wall (Doyley et al., 2001). The differences in tissue deformity may allow differentiation of various plaque phenotypes. On the other hand, CE-IVUS uses a microbubble contrast agent in order to quantify

vasa vasorum density and plaque perfusion, which is a feature of inflammation, one of the major findings associated with vulnerable plaque (Carlier et al., 2005, Staub et al., 2010). These technologies however remain unstudied concerning their ability to identify a high risk lesion subset that will lead to an adverse clinical event.

IVUS has made a major leap in atherosclerosis imaging with the continual expansion of its new capabilities. In the near future, we will see a more IVUS synergism with other imaging modality to improve tissue characterization and arterial imaging. A co-registration with optical coherence tomography (OCT), for example is already on the horizon (Li et al., 2010). The principle of OCT image acquisition is analogous to ultrasound imaging; however, a near infrared light source is used instead of sound to create an image. Its spatial resolution is 10 to 20 μm, which is approximately 10 times greater than that of IVUS and hence it provides an excellent contrast between lumen and vessel wall (Maehara et al., 2009). Owing to this, OCT is excellent in visualising the thin cap component of TCFA or stent strut coverage. It also has several other tissue characterization capabilities, such as red blood cell rich thrombus, platelet rich thrombus, and macrophages infiltration (Bezerra et al., 2009). With further evaluation, OCT, may lead future serial intravascular imaging studies looking at changes in these adverse findings located at the lumen-vessel wall interface when evaluating effects of novel new therapies.

Use of IVUS in coronary endothelium vasodilator assessment

Another exciting area from IVUS research is the use of IVUS to assess the relationship between segmental coronary endothelial function and regional plaque burden. It has been well established that endothelial dysfunction, as assessed by the vasodilator

responses to different endothelial dependent stimuli is associated with increased risk of future cardiovascular event (Halcox et al., 2002, Schachinger et al., 2000, Al Suwaidi et al., 2001). Recently, we published data from our sets of experiment that looked at the relationship between plaque burden and endothelial function as a plausible explanation for the association of this phenomenon with adverse clinical events (Puri et al., 2012a). Having considered the inherent limitation and image resolution of the traditional quantitative coronary angiogram (QCA), we utilized IVUS to evaluate the vasodilator properties of the study artery to the endothelial dependent stimulus, intracoronary salbutamol. The study showed a strong relationship between *in vivo* segmental human coronary endothelial dependent macrovascular and microvascular function with associated underlying atheroma burden. In addition, this coronary structure-function relationship seems to hold true irrespective of the nature of patient's clinical presentation, indicating that the greater endothelial dysfunction observed in patients with ACS compared to stable angina was due to the underlying greater volume of atherosclerosis in ACS (Puri et al., 2013).

The observations from these seminal works suggest that intravascular imaging modality with enhanced resolution and topographic capabilities may be of benefit when evaluating small, subtle changes in lumen size when assessing coronary vasodilator function. This concept leads to the first sets of experiments discussed in a separate chapter below which aims to assess the effectiveness of OCT in measuring changes in luminal area in response to vasoreactive stimulus. It was felt that the combination of OCT and IVUS would offer both superior luminal visualisation along with adequate depth penetration; therefore, the subtle relationship between segmental conduit vasoreactivity and regional atheroma volume with its composition may be readily

ascertained. This study, and our aforementioned works however, did not evaluate the impact of this dynamic process on the natural history of atherosclerosis progression over time. Furthermore, it did not investigate the impact of an endothelial independent stimulus, such as glyceryl trinitrate (GTN) and its association with regional plaque burden. These issues are a key focus of this thesis.

Since the completion of these studies, further technological advances have been made in IVUS technology, particularly in relation to evaluating plaque composition. Recently, a combined catheter of IVUS and near infrared spectroscopy (NIRS) has been developed and performed *in vivo* (Schultz et al., 2010, Garg et al., 2010, Gardner et al., 2008, Waxman et al., 2009). NIRS provides information of atheroma composition by analysing the pattern of near infrared light absorbance by different tissue (Figure 5). It is superior to IVUS in terms of its ability to detect cholesterol lipid crystals, a major component of fibroatheroma's necrotic core (Moreno et al., 2002); however, it does not provide any structural information. Several investigators have shown the association between NIRS positive lesions and acute coronary events (Madder et al., 2013, Madder et al., 2012); indeed, the finding of positive NIRS signal in a vessel may yield significant prognostic implication (Goldstein et al., 2011, Oemrawsingh et al., 2014). It will be the first time that NIRS imaging technology has been used to assess coronary endothelial function in a longitudinal imaging study, and we hope the result of these experiments will provide important new insight regarding the significance of human coronary structure function and its impact upon the natural history progression of coronary atherosclerosis.

IV. SUMMARY

The role of IVUS in the world of interventional cardiology continue to evolve in parallel with the refinement of PCI technique, devices, and approach. IVUS has played an important role in helping us to understand the different arterial response to various interventional approach as well as guiding the clinician with the device selection and strategy. Its role is even more paramount in the area of complex PCI, such as left main or bifurcation disease whereby the consequences of complication could be severe. Advances in IVUS, such as VH-IVUS and IVUS with NIRS capability also permit a more optimal characterization of atherosclerotic plaque. This provides not only a significant insight into the natural history of atherosclerosis but also an opportunity to evaluate the impact of different pharmacological therapy on modifying the disease progression. Furthermore, the utilisation of these imaging tools may be extended not only to evaluate fixed plaque composition, but also to evaluate dynamic function of the coronary arterial system. Finally, large prospective clinical trials are needed to assess the clinical benefit of emerging novel IVUS technology.

FIGURE 1: The three-layered appearance of a cross sectional coronary artery as assessed by IVUS.

Abbreviations: I (intima); M (media); A (adventitia); L (lumen); C (IVUS catheter).

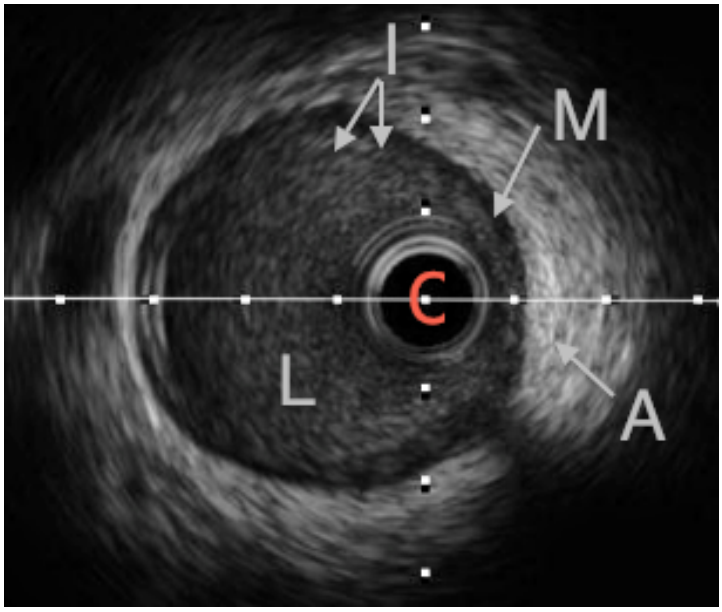


FIGURE 2: Basic IVUS measurement.

A depicts the proximal reference segment. B is a cross sectional image taken from the most stenotic segment. C illustrates the calculation of the area stenosis and D demonstrates the IVUS quantification of plaque burden. Area stenosis is a measure of luminal stenosis relative to the normal reference segment. In contrast, plaque burden refers to the area within the EEM (external elastic membrane) which is occupied by atheroma. (Reproduced with permission from McDaniel et al (McDaniel et al., 2011)).

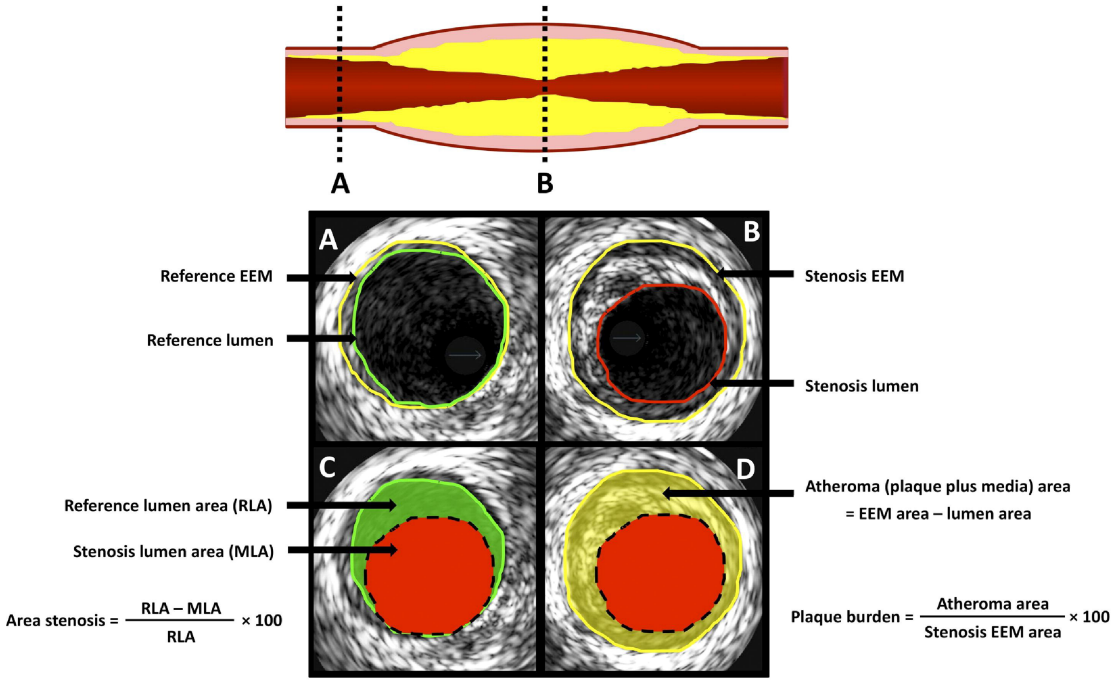


FIGURE 3: Indeterminant ostial left main stenosis.

This is the coronary angiogram picture taken from an 82 year old man who presents with chest pain and strongly positive stress test (left). Black arrow demonstrates area of haziness involving the ostium of the left main coronary artery. This segment correlates with the IVUS image on the right which shows an eccentric, calcified left main lesion, with minimum lumen area (MLA) of 3.9mm^2. The patient was revascularised percutaneously and had a good outcome.

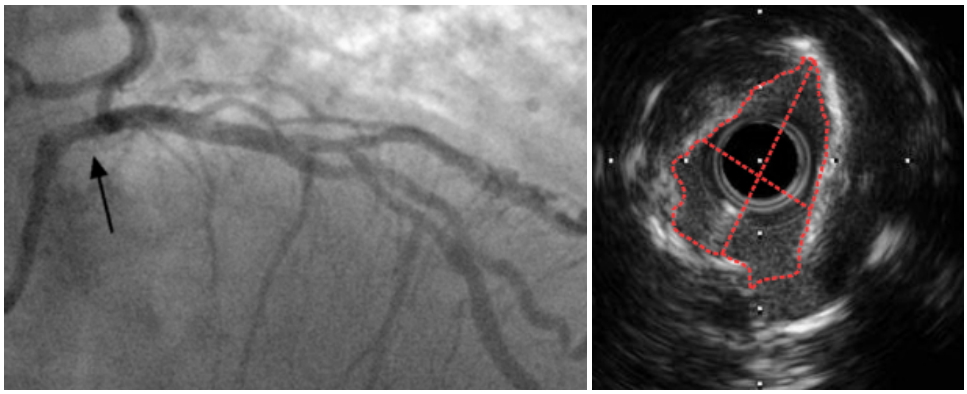


FIGURE 4: Lesion type classification by VH-IVUS.

Based on the VH-IVUS algorithm, coronary lesion or plaque can be characterized according to the combination of different tissue colour map (fibrous=green; fibrofatty=yellow green; necrotic core=red; dense calcium=white). (A) Pathological intimal thickening. (B) VH-IVUS derived thin capped fibroatheroma. (C) Thick capped fibroatheroma. (D) Fibrotic plaque. (E) Fibrocalcific plaque. Reproduced with permission from Kubo et al (Kubo et al., 2010).

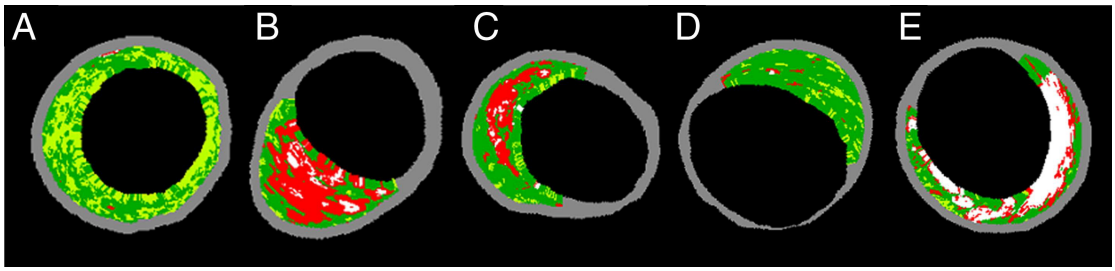
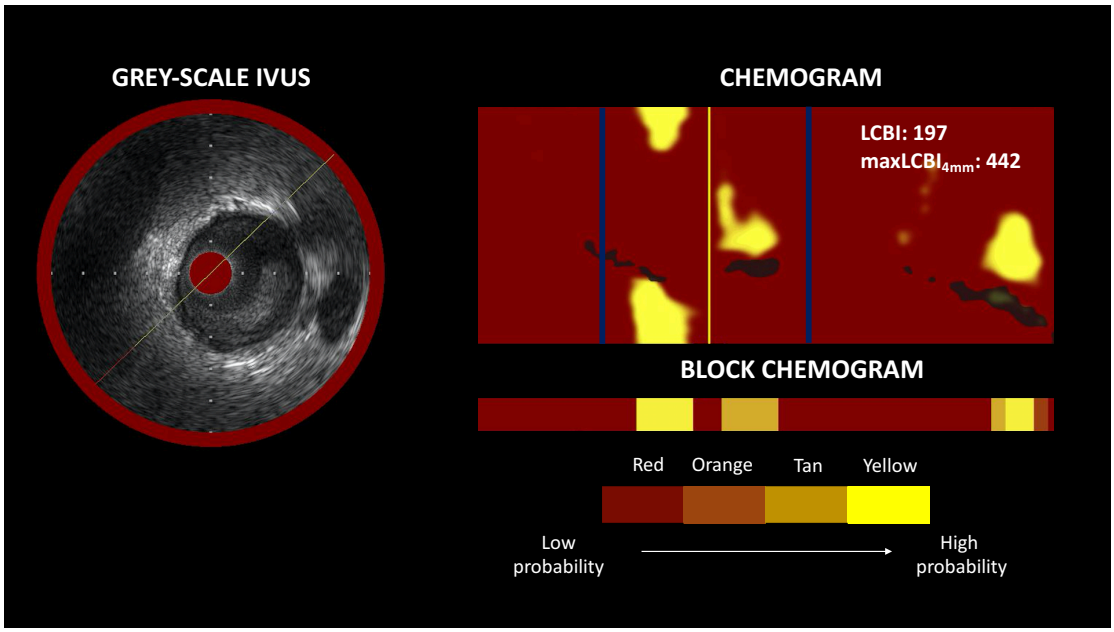


FIGURE 5: Near infrared spectroscopy output with the corresponding greyscale image (left). (Top right) Chemogram map of the artery wall indicating the location and intensity of lipid core plaque. (Middle right) Block chemogram displaying the presence of lipid core at 2mm segments in four probability categories (red<orange<tan<yellow). The lipid core burden index (LCBI) of the region of interest (subtended by the blue lines) is 197, with max(LCBI)$_{4mm}$ of 442.



CHAPTER 2: CORONARY VASOACTIVE AGENT: PERIPROCEDURAL PHARMACOLOGY AND INVASIVE ASSESSMENT OF CORONARY ENDOTHELIAL FUNCTION

Adapted from: Sidharta, S. and Puri, R. Coronary Vasoactive Agents: Periprocedural pharmacology, Global Textbook of Interventional Cardiology, 2016, S. Kapadia et al (in press).

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Contribution to the Paper	Preparation of manuscript		
Overall percentage (%)	95%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	20/1/2017

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

iv. the candidate's stated contribution to the publication is accurate (as detailed above);

v. permission is granted for the candidate in include the publication in the thesis; and

vi. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Contribution to the Paper	Correction and critical review of manuscript		
Signature		Date	20/1/2017

I. INTRODUCTION

Coronary vasoactive pharmacotherapeutic agents form an essential component of the interventional cardiologists armamentarium during diagnostic coronary angiography and percutaneous coronary intervention (PCI). Complications such as slow coronary flow or the 'no-reflow' phenomenon may arise during PCI as a result of several compounding mechanisms, including distal macro and micro-embolization, neutrophil plugging, vasoconstriction, myocyte contracture, local intracellular and interstitial oedema, intramural haemorrhage, and endothelial blistering (Niccoli et al., 2009, Kunadian et al., 2008). No-reflow is thought predominantly a microvascular disorder and therefore does not respond to mechanical intervention such as aspiration thrombectomy or coronary stenting, necessitating the administration of intracoronary (IC) vasoactive agents. IC vasoactive agents have also been utilized in a variety of clinical and research settings to evaluate the hemodynamic significance of angiographic indeterminate lesions, or during IC provocation for the evaluation of coronary vasospasm, microvascular dysfunction, and coronary endothelial function.

This chapter aims to provide an overview of various IC vasoactive pharmacotherapeutic agents, including their clinical and research indications, mechanisms of action, and side effect profile. For the purpose of this discussion, we divide their site of action as either at the macrovascular level (epicardial), microvascular compartment, or both.

Coronary Blood Flow Regulation. As in any vascular bed, myocardial blood flow depends upon the upstream coronary artery driving pressure and the resistance produced

by the downstream serial vascular compartments (Poiseuille's Law). Owing to the fact that myocardial oxygen extraction from the coronary circulation is near maximal under basal conditions (up to 75% of arterial oxygen content), myocardial blood flow control is critical in safeguarding the homeostatic myocardial oxygen supply and demand relationship in preventing ischaemia or infarction (van de Hoef et al., 2012). Central to this role is the intricate network of coronary resistance vessels (Kern et al., 2006). Coronary arterial resistance is determined by the summed resistances of the entire epicardial coronary conductance (R1), pre-capillary arterioles (R2), and intramyocardial capillary resistance circuits (R3) (Kern and Lim, 2006).

The epicardial coronary tree measures approximately 0.5–5mm in diameter and offers little resistance to coronary blood flow until atherosclerotic obstructions occurs (van de Hoef et al., 2012). These arteries are lined with vascular endothelium and smooth muscle cells which contract and relax in response to various circulating factors including vasoactive substances, neurohormonal stimuli, and flow mediated endothelium-dependent vasodilators or vasoconstrictors (Deussen et al., 2012). The dynamic nature of these interactions will be discussed in greater detail below. The principal control and regulation of coronary blood flow occurs at the level of coronary arterioles (100-500μm in diameter) and arterial microvessels (<100μm), collectively known as coronary microvessels (Marzilli et al., 2006). At this level, vascular tissue possesses intrinsic control mechanisms to maintain the homeostasis of the local microenvironment in the form of myogenic response to changes in intravascular pressure and flow induced dilation response to changes in shear longitudinal stress. Myogenic control is largely endothelium-independent and critical in modulating basal tone for maintenance of physiological intraluminal pressure, whilst flow mediated

dilation is largely endothelium-dependent (Marzilli et al., 2006). Altogether, coronary vascular resistance is a product of several inter-related mechanisms, including myocardial metabolism, autoregulation, myogenic control, neural control, and endothelial function.

II. PREDOMINANTLY CORONARY MACROVASODILATORS

Nitrates

Nitric oxide (NO) is an endothelium-derived vasoactive substance which has critical function in mediating coronary vasodilation. This substance counters the effects of other endothelium-derived vasoconstrictors, such as thromboxane and endothelin (Davignon and Ganz, 2004). It also inhibits platelets activation (Andrews et al., 2001), leucocyte adhesion (Kubes et al., 1991), vascular smooth muscle cell proliferation (Garg and Hassid, 1989), and preventing oxidative modification of low-density lipoprotein cholesterol (LDL-C) (Rubbo et al., 2002). Nitric oxide is synthesized in a pulsatile manner within the endothelium from its precursor, L-arginine by the enzymatic action of nitric oxide synthase (NOS). Central to the regulation of NOS activity is the Ca^{2+}-activated calmodulin complex (Forstermann and Sessa, 2012). The rise in intracellular Ca^{2+} induces the binding of calmodulin to NOS which in turn activates NO synthesis. Cofactors such as flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), nicotinamide adenine dinucleotide phosphate (NADPH) and tetrahydrobiopterin (BH_4) are also involved in the regulation of NO production (Forstermann and Sessa, 2012). Following synthesis, NO diffuses out into the adjacent vascular smooth muscle cells resulting in vasorelaxation by activating guanylate cyclase and subsequent increase in

the level of intracellular cyclic guanosine monophosphate (cGMP) (Davignon and Ganz, 2004) (Fig 1).

Since the initial description of its clinical use by William Murrel in 1879 (Murrell, 1879), nitrate remains a cornerstone therapy for stable angina pectoris and plays a significant role during invasive cardiovascular practice. Nitrate is an organic nitrovasodilator producing vascular relaxation by liberating NO within the vasculature. It is distinguishable from the inorganic nitrovasodilators, such as sodium nitroprusside, by its possession of a nitrate ester bond (R-O-nitric oxide$_2$) (Anderson et al., 1994). Important similarities and differences exist in the biotransformation of these organic and inorganic nitrates, but it is generally accepted that they share a final common pathway via nitric oxide production. (For the purpose of discussion in this section, organic nitrate will be referred as nitrate. Inorganic nitrate such as sodium nitroprusside will be discussed in the separate section below).

A number of studies have shown that nitrates dose-dependently dilate both normal and atherosclerotic epicardial coronary arteries, while producing only a submaximal, brief effect on coronary flow. Feldman and colleague (Feldman et al., 1979) demonstrated that intracoronary nitrates can increase epicardial coronary artery diameter by 10-30%. Brown et al. (Brown et al., 1984) furthermore reported that about 70% of coronary lesions, from mild to severe, dilate measurably in response to nitroglycerin largely as a result of dilation of the normal portion of the artery within the eccentric stenosis. Nitrates, however has minimal or no effect on coronary microvessels. In an experiment using porcine coronary arteries, Sellke and colleagues (Sellke et al., 1990) demonstrated

that vessels >200μm in diameter were very responsive to nitrates, whereas those <100μm in diameter dilated only minimally. This finding was supported by Kurz et al. (Kurz et al., 1991) in their *in vivo* coronary microvessel model. One plausible explanation for this phenomenon is due to the inability of the smaller coronary microvessels to convert nitroglycerin to its active vasodilator intermediate (Harrison and Bates, 1993). Taken together, nitrates predominantly dilate large epicardial coronary arteries and arterioles >100μm in diameter with little or no effect at the coronary microvascular level. Moreover, given its site of action on the vascular smooth muscle cells, nitrates likewise has the capacity to dilate the venous system (Rosen et al., 1987) and collateral vessels (Feldman et al., 1981). Indeed, several studies demonstrated a greater sensitivity of nitrates for veins than arteries (Rosen et al., 1987, Stiefel and Kreye, 1984, Toyoda et al., 1986). The outcome of this increased venous capacitance is a decrease in preload which then leads to reduction in ventricular volume and ultimately oxygen consumption.

Due to its effects, nitrates are widely used in the catheterization lab to reduce or reverse coronary vasospasm as a primary observation, or secondary to catheter placement, ballooning, or stenting a vessel. Furthermore, nitrates are frequently used during invasive endothelial function research protocols when assessing endothelium-independent vasodilator function (Flammer et al., 2012). A dose ranging from 50-400 microgram (mcg) is typically used depending on the clinical circumstances and patient's tolerance. Its main side effect is hypotension and headache secondary to vasodilation of the venous system and the cerebral vasculature (Bagdy et al., 2010).

III. PREDOMINANTLY CORONARY MICROVASODILATORS

Adenosine

Adenosine is an endogenous purine nucleoside. It is produced from dephosporylation of extracellular adenosine 5'-triphosphate (ATP) released from the parenchymal tissue (including endothelium) by the enzymatic action of ecto-5′-nucleotidase (Mustafa et al., 2009). It functions as a cytoprotective modulator under both physiological and pathological conditions. This protective response might take the form of increased blood supply (vasodilation or angiogenesis), ischaemic preconditioning (in the heart, brain, or skeletal muscle), and/or suppression of inflammation (activation and infiltration of inflammatory cells, production of cytokines and free radicals) (Jacobson, 2009). Although originally identified within the myocardium in 1929 (Drury and Szent-Gyorgyi, 1929), it took another 60 years to uncover its effect within the human coronary circulation (Wilson et al., 1990). Adenosine is a potent arteriolar vasodilator and mediates vasodilation largely via an endothelium-independent and partly, endothelium-dependent manner (Dinckal et al., 2003, Duffy et al., 1999, Smits et al., 1995, Jones et al., 1995). Currently, there are four known adenosine receptor (AR) subtypes, namely A_1, A_{2A}, A_{2B}, and A_3 receptors (Mustafa et al., 2009). Although all four AR subtypes are found within coronary smooth muscle cells, only the A_{2A}AR and A_{2B}AR have been identified on coronary endothelial cells (Olanrewaju et al., 2000, Olanrewaju et al., 2002). Among these receptors, A_{2A}AR plays a significant role in controlling coronary vasodilation, while other receptors play a lesser role (Frobert et al., 2006, Hodgson et al., 2007, Morrison et al., 2002, Talukder et al., 2003). The binding of adenosine on A_{2A}AR activates adenylate cyclase and results in accumulation of cyclic adenosine monophosphate (cAMP) and subsequent opening of intermediate calcium-activated

potassium channels (Mustafa et al., 2009). The end result is hyperpolarization of smooth muscle and vasorelaxation. Adenosine has a differential effect on resistance coronary vascular beds, chiefly dilating vessels with diameter <100μm. Larger resistance vessels or epicardial conduit coronary arteries dilate via a NO-dependent mechanism from the concomitant increase in local shear stress (flow-mediated dilation). Adenosine has an extremely short half-life (<10 seconds) due to both its high affinity red blood cell uptake and rapid inactivation by adenosine deaminase. Several medications, such as ticagrelor and dipyridamole have been shown to increase the level of adenosine plasma concentration by inhibiting the red blood cells reuptake of adenosine in both healthy volunteers (Biaggioni et al., 1986, Conradson et al., 1987, Wittfeldt et al., 2013) and acute coronary syndrome (ACS) patients (Bonello et al., 2014). Therefore, in patients receiving ticagrelor or dipyridamole, a reduction of adenosine dose is suggested to prevent supernormal response to adenosine used for both therapeutic and diagnostic applications (Voci and Pizzuto, 2014).

Indication: *(1) Hemodynamic evaluation of coronary stenosis*. The use of fractional flow reserve (FFR), the ratio of maximal blood flow in a stenotic artery to normal maximal flow (Pijls et al., 1996), allows physicians to determine whether an angiographic lesion is functionally significant. FFR-guided PCI has been demonstrated to result in superior clinical outcomes compared with angiographic-guided PCI (Bech et al., 2001, Pijls et al., 2007, Tonino et al., 2009, Pijls et al., 2010). Indeed, in a large-scale randomized controlled trial, FFR-guided PCI coupled with optimal medical therapy was associated with significant reductions in the composite endpoint of death, myocardial infarction, and urgent revascularization when compared with optimal

medical therapy alone (De Bruyne et al., 2012). This benefit seems to be preserved in the ACS population as well (Sels et al., 2011). An FFR value of ≤ 0.8 is considered to be a functionally significant value (Tonino et al., 2009, De Bruyne et al., 2012).

Valid FFR measurements are obtained following achievement of maximal coronary hyperaemia. Adenosine has been used extensively in various FFR studies due to its ability to achieve maximal reduction in coronary microvascular resistance, its brief duration of action, and minimal toxicity profile. Intravenous adenosine with doses ranging between 140-170 mcg/kg/min are considered "gold standard" and have been shown to be safe and effective in achieving steady-state coronary hyperaemia (Kern et al., 2006). Consequently, intravenous adenosine permits clinicians to analyse the functional significance of segmental epicardial disease, including the evaluation of tandem stenosis during pressure wire pullback (Kim et al., 2012a, Pijls et al., 2000). Limitations associated with the use of intravenous adenosine include longer preparation time, higher cost, the need for central vein cannulation (or at minimum a large bore antecubital vein access), and higher occurrence of systemic side effects (eg. flushing, angina-like chest pain, transient bradyarrhythmia (sinus bradycardia, 2:1 AV block, asystole, and hypotension). An IC bolus dosing of adenosine, on the other hand, is simple, inexpensive, and relatively free of systemic side effects, thus making it an attractive option, especially when transradial coronary angiography approach was used and femoral access has not been prepared. The use of IC adenosine also presents different challenges, notably, in obtaining steady response of maximal hyperaemia. Indeed, a failure to produce maximal hyperaemia has been reported to occur in 10-15% cases following IC adenosine (Jeremias et al., 2000). To circumvent this issue, it is

recommended that serial incremental IC adenosine doses should be given until a plateau of response is achieved. Recent data have demonstrated that higher dose of adenosine (300-720 mcg) increased the sensitivity of FFR for the detection of haemodynamically significant coronary stenosis (De Luca et al., 2011, Lopez-Palop et al., 2013). Taken together, adenosine is generally safe and effective in achieving maximal hyperaemia in the recommended intracoronary and intravenous dosages.

*(2) Managing No reflow***.** The ultimate goal of reperfusion therapy, either via a thrombolytic agent or PCI, is to achieve optimal vessel patency and re-establishment of coronary flow. A number of reports however have shown that up to 40% of patients fail to achieve adequate myocardial tissue perfusion (defined as Thrombolysis in Myocardial Infarction (TIMI) flow grade >2) despite patent coronary artery and absence of coronary dissection or vasospasm; a phenomenon known as 'no reflow' (Piana et al., 1994, Kondo et al., 1998, Iwakura et al., 1996). No-reflow is associated with adverse clinical outcomes and is caused by microvascular obstruction, arising from multiple pathophysiological mechanisms such as distal embolization of plaque and/or thrombus, intracellular oedema and endothelial damage, *in situ* thrombosis, microvascular spasm, activation of the inflammatory cascade with leucocyte stasis and extravasation, and finally reperfusion injury (Niccoli et al., 2009, Jaffe et al., 2010).

The data from various animal models of reperfusion injury have consistently demonstrated the efficacy of adenosine in reducing infarct size, improving left ventricular function, and improving coronary blood flow (Olafsson et al., 1987, Velasco et al., 1991, Babbitt et al., 1989). Likewise, in a number of prospective observational

studies involving acute myocardial infarction patients who undergo primary angioplasty, the use of both IC and IV adenosine were found to be well tolerated and associated with smaller infarct size and reductions of the no reflow phenomenon (Garratt et al., 1998, Marzilli et al., 2000, Assali et al., 2000, Claeys et al., 2004). However, randomized controlled trials have yielded somewhat conflicting results. In the Acute Myocardial Infarction Study of Adenosine (AMISTAD) trial (Mahaffey et al., 1999), a total of 236 ST elevation myocardial infarction (STEMI) patients receiving thrombolysis were randomized into 3-hour IV adenosine infusions (70 mcg/kg/min) or placebo. A significant 33% relative reduction in infarct size was noted in the adenosine group compared with placebo. This trial nonetheless was not powered for clinical endpoints. AMISTAD-II was subsequently designed to address this clinical question in much larger patient population (n = 2118) (Ross et al., 2005). However, the trial failed to show the benefit of IV adenosine in reducing adverse clinical events (death, congestive heart failure, rehospitalization), although the study medication was well tolerated and resulted in a dose-related reduction in infarct size. Lastly, in a meta-analysis of trials investigating the impact of adenosine on angiographic and clinical outcomes of ACS patients undergoing PCI or thrombolysis for STEMI, Navarese and colleague found that the use of adenosine was associated with a significant reduction of post-procedural no-reflow (OR [95% CI] = 0.25 [0.08–0.73], p = 0.01), even though this failed to translate into clinical benefits in the long term (Navarese et al., 2012). It is possible that the lack of clinical benefit noted in these studies was due to small sample size or suboptimal study design. Taken together, adenosine appears to reduce the incidence of no reflow periprocedurally in the setting of primary PCI. Nevertheless, the clinical significance of these findings remains unclear. For the management of no reflow, we recommend IC adenosine, initially at low dose e.g. 30 mcg bolus, with

escalating doses of 60, 90, 120 injected every 20–30 seconds, as AV conduction allows is considered by many as an effective first option (Wong et al., 2013).

Papaverine

Originally discovered by Georg Merck in 1848 (Merck, 1848), papaverine is an opiate derivative with smooth muscle relaxing properties. It exerts its smooth muscle relaxing action by inhibiting cyclic nucleotide phosphodiesterase, resulting in an increase of cAMP and smooth muscle relaxation (Boswell-Smith et al., 2006). Papaverine is a potent, direct coronary microvascular dilator with nominal effects in epicardial conduits. Historically, it has been the agent of choice for evaluating coronary flow reserve (Carlson et al., 1988, Christensen et al., 1991, Wilson and White, 1986). IC papaverine administration has been shown to increase coronary blood flow velocity up to four to five times above baseline in patients with normal epicardial coronary arteries (Wilson and White, 1986). Its half-life is approximately 2 minutes with peak effect after administration at 10–30 sec and the duration of plateau is around 45–60 sec, four times longer than that of IC adenosine (Zijlstra et al., 1986). Understandably, this makes papaverine an attractive vasoactive agent for FFR evaluation especially in the setting of diffuse disease due to its ability to achieve maximal and steady hyperaemia. IC papaverine of 10-20mg produces a similar complete, true steady-state hyperaemic response compared with 140mcg/kg/min of IV adenosine (De Bruyne et al., 2003), or 0.56-0.84mg/kg of IV dipyridamole (Wilson and White, 1986). Its use during FFR evaluation however has been superseded by adenosine due to its side effect profile and thus akin to nitroprusside, its use should be reserved for patients with contraindications to adenosine. Papaverine may induce significant electrocardiographic changes such as

ST-segment depression or QT interval prolongation, and polymorphic ventricular tachycardia and fibrillation (Talman et al., 1990, Wilson and White, 1988, Kern et al., 1990). Also, it has been shown to generate myocardial lactate production irrespective of the degree of coronary flow reserve and electrocardiographic changes in patients with normal coronary arteries (Takeuchi et al., 1996). Lastly, as a potent coronary microvascular dilator, papaverine may improve TIMI flow in some cases of no reflow (Ishihara et al., 1996, Wilson et al., 1989). However due to lack of prospective, randomized data and its side effect profile, it is currently not recommended for use outside of a research setting.

IV. MIXED CORONARY VASODILATORS

Sodium Nitroprusside

Sodium nitroprusside is a direct NO donor with fast release kinetics and potent vasorelaxant effects on conduit vessel, arterial resistance, and venous capacitance vessels. It has a preferential vasodilatory action on coronary arterioles without affecting myocardial contractility or other types of smooth muscle. Its vascular smooth muscle relaxing effects are analogous to the actions of nitrates (via NO-cyclic GMP pathway). Nitroprusside was first described by Hermann (Hermann, 1886) in 1886, however its potential clinical utility was not unravelled until 1928 when Johnson (Johnson, 1928) described its therapeutic effect in reducing hypertension by peripheral vascular relaxation independent of the autonomic innervation (Tuzel, 1974). Since then, nitroprusside has been widely used, particularly in the intensive care setting, for treating hypertensive crises or severe left ventricular failure.

Indication: Like adenosine, nitroprusside has been evaluated as an adjunctive pharmacotherapeutic option to PCI for improving coronary flow in the setting of acute myocardial infarction or other high risk PCI situations, such as saphenous vein graft intervention. Whilst observational studies demonstrated improvement in the degree of microvascular dysfunction following nitroprusside when assessed with both angiographically (TIMI flow or corrected TIMI frame count) (Hillegass et al., 2001, Airoldi et al., 2007, Pasceri et al., 2005, Wang et al., 2004) and via microvascular resistance indices (Morimoto et al., 2012), randomized trial data have been inconsistent. Hendler and colleagues (Hendler et al., 2006) randomized 40 patients undergoing primary PCI into 3 investigational groups of IC nitroprusside, IC adenosine, and IC verapamil (with IC nitroglycerin as the control). Nitroprusside was associated with greater myocardial blush scores, correlating with a significant improvement in LVEF when compared with adenosine and verapamil. Subsequent randomized studies by Amit and colleagues (Amit et al., 2006), as well as the large multicenter Randomized Evaluation Of Intracoronary Nitroprusside vs Adenosine After Thrombus-aspiration During Primary PErcutaneous Coronary Intervention for the Prevention of No Reflow in Acute Myocardial Infarction (REOPEN-AMI) trial (Niccoli et al., 2013) on the other hand failed to demonstrate improvement of microvascular obstruction and no reflow with IC nitroprusside. It seems likely that the discordance between these findings is possibly due to underpowering differential dosing regimens and potential elements of selection and measurement bias. In terms of the dosage for managing no reflow, a 100 mcg to a total of 1mg bolus is recommended and can be administered via the coronary guide catheter. Common side effects include hypotension, headache, and nausea.

With increasing clinical use of FFR, there is also growing interest in finding an alternative agent to adenosine which is inexpensive yet as effective as adenosine in achieving maximal hyperaemia. Parham et al (Parham et al., 2004) compared the hyperaemic and haemodynamic responses of IC nitroprusside with IC adenosine (30-50 mcg bolus) in 21 patients undergoing cardiac catheterization with angiographically normal left anterior descending arteries. Using a Doppler wire, time to peak and average peak velocity were similar between nitroprusside and adenosine with the 3 doses of IC nitroprusside that were administered (0.3, 0.6, and 0.9 mcg/kg). The duration of coronary hyperaemia was ~25% greater with IC nitroprusside in comparison with IC adenosine. FFR measurements with IC nitroprusside were identical to those obtained with IC adenosine ($r = 0.97$). As an extension to this work, Leone and colleagues (Leone et al., 2012) evaluated correlations of FFR readings using IC nitroprusside (0.6 mcg/kg), incremental doses of IC adenosine (60, 300, and 600 mcg), and IV adenosine (140 mcg/kg/min). The IC adenosine dose used in this study was higher than the dose used by Parham et al. (Parham et al., 2004). Both IC adenosine doses and nitroprusside induced significant decreases of FFR compared with baseline levels ($p < 0.001$), however the mean FFR with nitroprusside was significantly higher compared with IV adenosine. Nevertheless, higher doses of IC nitroprusside (100 mcg) have been reported to correlate well with that of IV adenosine (Rudzinski et al., 2013). This suggests that IC nitroprusside is safe and effective for the induction of maximal coronary hyperaemia. However, given its limited body of evidence, it is currently difficult to advocate its use during routine clinical practice except perhaps in the setting whereby adenosine is absolutely contraindicated such as drug hypersensitivity or severe chronic obstructive airways disease.

Verapamil

Verapamil is a non-dihydropyridine calcium channel blocker which acts on the voltage operating "slow Ca^{++} channels" located on the vascular smooth muscle cell and myocardium during depolarization, thereby producing relaxation of coronary vascular smooth muscle and subsequent coronary vasodilation (Durand et al., 1991, Nayler and Krikler, 1974). Since its introduction in 1962 as an antiarrhythmic and coronary vasodilator (Haas, 1964), verapamil has been used widely as an effective anti-angina and antihypertensive agent. In contrast to nitrates which dilate epicardial conduits in a flow-independent manner, verapamil mediates epicardial vasodilation in flow-independent as well as a flow-dependent manner, making this vasoactive agent both a macro- and microvascular dilator, although its action upon resistance coronary arterioles seems to predominate (Oldenburg et al., 2000, Adachi et al., 1987, Messing et al., 1991). Using a coronary Doppler guide wire in the left anterior descending artery, Ishihara et al. (Ishihara et al., 1997) demonstrated that verapamil caused a dose-dependent increase in coronary blood flow velocity and decrease in coronary vascular resistance index, confirming its vasodilating effects on coronary resistance vessels.

Indication: Early animal experiments demonstrated that timely administration of verapamil was associated with reduction in the index of myocardial ischaemic injury and increased contractile function of the post-ischaemic stunned myocardium following myocardial infarction (Campbell et al., 1986, Reimer and Jennings, 1984, Przyklenk and Kloner, 1988). Plausible mechanisms to account for these beneficial verapamil effects include reduction in global oxygen demand (Taniyama et al., 1997), inhibition of platelet aggregation and thus thrombus formation (Ikeda et al., 1981), and coronary

microvascular vasodilatation (Messing et al., 1991). Intuitively, verapamil would be a useful vasoactive agent in the setting of no reflow and indeed its efficacy in improving myocardial perfusion or decreasing the incidence of no reflow has been documented in a number of observational trials of infarct PCI (Piana et al., 1994, Pomerantz et al., 1991, Werner et al., 2002, Rezkalla et al., 2010), degenerated vein graft PCI (Kaplan et al., 1996), and rotational atherectomy (Nunez et al., 1996). Similar observations were noted in various randomized trials. In a trial involving 40 patients with acute myocardial infarction who underwent primary PCI, Taniyama and colleague (Taniyama et al., 1997) observed that IC administration of 0.5mg verapamil followed by oral administration significantly reduced the myocardial contrast echocardiographic derived low reflow zone, a measure of improved microvascular function, by 25% ($p < 0.05$), compared with placebo. Other angiographic or contrast echo-derived indices of coronary microvascular function also improved with verapamil compared with placebo. Furthermore, a recent meta analysis (Su et al., 2013) reported that in the setting of PCI for ACS, verapamil was associated with decreased incidence of no-reflow, a decreased corrected thrombolysis in myocardial infarction (TIMI) frame count (CTFC), improvement in the TIMI myocardial perfusion grade, and a reduced incidence of major adverse cardiac events (MACE) during hospitalization and 2 months following PCI. Although the benefit of verapamil on no reflow seems compelling, its impact on clinical outcome appears modest due to small number of patients and short-term follow-up available in these randomized trials. Finally, IC verapamil should also be considered in the setting of acute episodes of vasospastic angina refractory to nitrate therapy (Babbitt et al., 1988). A dose of 100 μg up to 1 mg administered via IC route is recommended for the management of no reflow with careful attention to be paid on verapamil's side effects which include hypotension, bradycardia, and transient heart block.

V. NOVEL AGENT

Nicorandil

Nicorandil is a vasoactive agent with two modes of function. It activates ATP-sensitive K^+ channels and also possess a nitrate group in its chemical structure. Whilst its potassium channel opening effect induces hyperpolarization of the vascular smooth muscle cell membrane resulting in vasodilation of peripheral and coronary resistance arterioles, its nitrate-like activity also produces vasodilation of the systemic veins and epicardial conduit vessels (Suryapranata, 1993). The precise mechanism of nicorandil-mediated vasodilation has not been fully elucidated but does not appear to involve cholinergic, adrenergic, histaminergic, or adenosine-potentiating mechanisms (Suryapranata, 1993). IC nicorandil produces a dose-dependent increase in coronary blood flow velocity and associated coronary hyperaemic response, comparable to that of IC papaverine (Hongo et al., 1995) and IV adenosine (Jang et al., 2013). Its peak effect occurs at 17-20 seconds following administration with plateau duration of 25-27 seconds (Hongo et al., 1995). Furthermore, when administered via IC route, nicorandil also produced fewer changes in blood pressure, heart rate and PR interval, less chest pain, and no atrioventricular block when compared with other vasoactive agent such as papaverine or adenosine (Jang et al., 2013).

Indication: Nicorandil has been shown to improve a number of surrogate endpoints in the setting of acute myocardial infarction (Miyazawa et al., 2006, Ota et al., 2006, Ito et al., 1999), vasospastic angina (Lablanche et al., 1993), and complex PCI with rotational

atheretectomy (Tsubokawa et al., 2002). In a recent meta analysis involving 1680 infarct PCI patients, Wu et al. found nicorandil to significantly reduce the incidence of TIMI flow grade scores ≤2, reduce the TFC, increase left ventricular ejection fraction (LVEF), along with reducing the incidence of ventricular arrhythmia and congestive heart failure (Wu et al., 2013). It is worth noting however that the largest randomized trial included in this meta-analysis, the Japan-Working Groups of Acute Myocardial Infarction for the Reduction of Necrotic Damage by Atrial Natriuretic Peptide or Nicorandil (J-WIND) study reported no difference in the primary end point of total infarct size (estimated by creatine kinase) and LVEF between nicorandil and control (Kitakaze et al., 2007). Several mechanisms have been postulated to explain the unique, cardioprotective effects of nicorandil, including a reduction in preload and afterload, anti-free radical and neutrophil modulating properties (Pieper and Gross, 1992, Yasu et al., 2002), vasodilatation of small coronary arteries, and beneficial effects on ischaemic preconditioning (Matsubara et al., 2000). Many of these effects, particularly the latter are likely mediated via its K_{ATP} channel opening effects (Genda et al., 2002). The efficacy of nicorandil in the setting of elective PCI nonetheless is modest (Hwang et al., 2013, Kim et al., 2012b) except in cases when rotational atherectomy is considered. Matsuo and colleagues (Matsuo et al., 2007) randomized 200 patients undergoing rotational atherectomy to an IC nicorandil versus verapamil infusions. Compared with verapamil, nicorandil was associated with a significantly lower incidence of no reflow/slow flow, persistent ST-segment elevation and myocardial infarction with no differences in rates of restenosis and MACE at 6 month follow up. Taken together, nicorandil provides an additional and interesting therapeutic option in the catheterization lab and its use could complement the aforementioned vasoactive agents.

VI. SPASM TESTING AGENT

Ergonovine

Vasospastic (variant or Prinzmetal) angina is a clinical syndrome characterized by rest angina, typically with onset between midnight and early morning, and associated with transient ST segment changes on electrocardiogram (Maseri and Chierchia, 1982). A positive diagnostic ECG changes encompass an ST elevation of 0.1 mV or more, an ST depression of 0.1mV or more, or new appearance of negative U waves recorded in at least two contiguous leads on the 12-lead ECG during an episode (Group, 2010). Episodes are caused by focal or diffuse epicardial coronary spasm and typically occur in patients with minor coronary artery stenosis. Multiple factors have been postulated that contribute to the pathogenesis of coronary spasm including endothelial dysfunction, vascular smooth muscle hypersensitivity and increased autonomic tone, oxidative stress and genetic disorders (Shimokawa, 2000). Prognosis of patients with variant angina is relatively benign with more than 80% of patients experiencing myocardial infarction free survival at 10 years follow up (Yasue et al., 1988, Ong et al., 2011). Nonetheless, coronary vasospasms can lead to ACS and arrhythmias, including ventricular arrhythmias and sudden cardiac arrest (Togashi et al., 2013, Ong et al., 2008). Diagnosis of variant angina can be extremely challenging due to the elusive nature of the disease. The gold standard investigation is a catheterisation lab based coronary provocation test. Although numerous agents have been described, in clinical practice, ergonovine and acetylcholine are the 2 agent most commonly used.

Ergonovine is a naturally occurring argot alkaloid which produces direct vasoconstriction by activation of serotonergic (5-HT2) receptors located on the vascular

smooth muscle (Suyama and Kuriyama, 1984). Ergonovine is predominantly metabolized by the liver and serves as a major substrate of CYP3A4 hepatic enzymes. The use of ergonovine as a provocative agent in the setting of coronary disease was first described by Stein to diagnose evidence of "coronary insufficiency" (Stein, 1949, Stein and Weinstein, 1950). Since then, a growing body of literature has demonstrated the utility and safety of ergonovine as a diagnostic tool for variant angina (Curry et al., 1979, Curry et al., 1977, Heupler et al., 1978, Schroeder et al., 1977). A positive ergonovine test is defined as transient occlusion (>90% stenosis) of a coronary artery accompanied by signs and symptoms of ischaemia, such as angina and ST-segment changes on coronary angiography, at 1-2 minutes following its IV or IC administration (Group, 2010). Ergonovine commonly causes a generalized or diffuse reductions of epicardial lumen diameters of the order of 15% to 30% on angiography and therefore in isolation, this is not representative of variant angina (Conti et al., 1979, Chahine, 1980). The key value of the administration of ergonovine is that, in selective patients, namely those with true variant angina, focal spasm can be elicited with the full spectrum of its signs and symptoms. If the patient develops mild, diffuse coronary artery constriction, the test is considered negative; if severe, focal spasm develops, with associated diagnostic ECG changes, the test is interpreted as positive. Prior to performing the test, a washout period of 1-2 days or longer is required for any calcium channel blockers and nitrate therapy.

Both IC and IV ergonovine have been shown to be sensitive and specific in diagnosing coronary vasospasm (Sueda et al., 2003, Sueda et al., 2004, Waters et al., 1983, Hackett et al., 1987). Nevertheless, IV ergonovine may present some drawback such as induction of perpetuating multivessel spasm and haemodynamic instability, making

angiographic acquisition challenging (Hackett et al., 1987). Also, it does not permit individual provocation of left and right coronary artery as with IC administration. For this reason, the Japanese Cardiac Society Guidelines recommend an IC administration of ergonovine with an incremental dose of 20-60 mcg (slow injection over 2-5 minutes) into the left coronary artery then right coronary artery with 5 minutes apart between each administration (Group, 2010). The safety of IC ergonovine was recently evaluated in a multicenter trial involving 1244 patients with vasospastic angina (Takagi et al., 2013). Takagi and colleague reported that the use of IC ergonovine has an acceptable safety profile with low incidence of arrhythmia (1.6% ventricular ectopic beats, 0.8% VT/VF, 0.6% AV block, and 0.4% bradycardia/sinus pause) (Takagi et al., 2013). Other adverse reactions to ergonovine include ischaemia, myocardial infarction, nausea, and abdominal cramps (Chahine, 1980).

Acetylcholine

Acetylcholine is a muscarinic cholinergic agonist which mediate coronary vasodilation via an endothelium-dependent mechanism. Early animal experiments using canine (Feigl, 1969, Vanhoutte and Cohen, 1984) and simian (Toda, 1983) models demonstrated the IC administration of acetylcholine to produce both epicardial and resistance coronary artery vasodilation. The pioneering work by Furchgott and Zawadzki (Furchgott and Zawadzki, 1980) showed that acetylcholine causes vasodilation by inducing the vascular endothelium to release endothelium-derived relaxing factor, subsequently identified as NO, which then diffuses out into the vascular smooth muscle to produce relaxation via a cGMP dependent pathways (Furchgott and Vanhoutte, 1989). This observation was further extended in human by Ludmer and

colleagues (Ludmer et al., 1986) using quantitative coronary angiography (QCA) to evaluate the change in conduit artery diameter in response to graded infusion of acetylcholine. The use of acetylcholine in the catheterization laboratory at this stage is mainly limited as a provocative agent for the evaluation of variant angina or research protocol-driven evaluation of coronary endothelial function.

The administration of IC acetylcholine as a provocative agent for variant angina testing was first described by Yasue and colleagues (Yasue et al., 1986). They conducted a study involving 28 patients with a clinical diagnosis of variant angina on the basis of typical angina pain associated with transient electrocardiographic ST elevation. These investigators reported that IC acetylcholine at doses of 10-50 mcg in the right coronary artery and 10-80 mcg in the left coronary artery produced focal spasm in 94% of the coronary arteries suspected to be the culprits for the patients' symptoms. Similar findings of acetylcholine as a reliable and effective provocative agent for evaluating coronary vasospasm were also reported by subsequent investigators (Miwa et al., 1991, Okumura et al., 1988a, Sueda et al., 2002). IC acetylcholine is known to have high sensitivity (90%) and specificity (99%) for the diagnosis of variant angina (Okumura et al., 1988b). It has a shorter half-life yet similar potency when compared with ergonovine (Sueda et al., 2003, Sueda et al., 2004). Data from Sueda and coworkers showed that residual effects of acetylcholine are not observed within 10 minutes following injection (Sueda et al., 2003). Compared with ergonovine, IC acetylcholine also caused more diffuse and distal spasms, whereas IC ergonovine is thought to have a more 'focal' effect; indicating that ergonovine may provoke more vasoreactivity (Sueda et al., 2003). A careful and judicious approach is crucial when employing IC acetylcholine as some major side effects, such as sustained ventricular tachycardia /

fibrillation, shock, bradyarrhythmia (atrioventricular block, bradycardia, or sinus pause) – especially with right coronary injection have been reported (Sueda et al., 2000). The event rate for these arrhythmic complications however is low (<5%) (Takagi et al., 2013, Ong et al., 2014). A dose of 20, 50, and 100mcg for left coronary artery and 20 and 50 mcg for the right coronary artery injected over 20 seconds is recommended with at least a 5-minute interval between boluses (Group, 2010). An insertion of a temporary wire is also suggested for an additional haemodynamic support.

VII. CORONARY ENDOTHELIAL FUNCTION ASSESSMENT

The endothelium plays a critical role in maintaining the vascular homeostasis by secreting a wide range of factors which regulate vascular tone, cellular adhesion, thrombus formation, smooth muscle cell proliferation, and vessel wall inflammation (Farouque and Meredith, 2001). Altered endothelial function (endothelial dysfunction), largely evaluated by vasodilator responses to endothelium- dependent stimuli occurs in the setting of established atherosclerosis, hypertension, smoking, dyslipidaemia, systemic inflammation, and diabetes mellitus (Davignon and Ganz, 2004). Importantly, endothelial dysfunction, measured in the peripheral (Gokce et al., 2003) or coronary circulations (Halcox et al., 2002, Schachinger et al., 2000, Suwaidi et al., 2000) has been shown to be associated with a greater risk of future MACE, independent of the traditional cardiovascular risk factors. Altogether, this leads to a well-regarded hypothesis that coronary endothelial dysfunction plays a significant role in triggering the event of atherogenesis and may contribute to the atherosclerosis associated ischaemic sequelae.

Coronary endothelial function assessment involves pharmacological or physiological stimulation of endothelial release of NO and other vasoactive compounds. These vasodilator responses are then compared to the corresponding responses to endothelium-independent stimuli (eg. nitroglycerin) to demonstrate the integrity of vascular smooth muscle. Some of the pharmacological agents which have been used to assess endothelium dependent coronary vasomotor effects include acetylcholine (Ludmer et al., 1986), salbutamol (Barbato et al., 2005), serotonin (McFadden et al., 1991), substance P (Quyyumi et al., 1997), and bradykinin (Aptecar et al., 2000). To date, acetylcholine remains the "gold standard" vasoactive agent for the invasive evaluation of coronary endothelial function (Flammer et al., 2012).

The basic methodology for coronary endothelial function assessment is now well established in coronary research laboratories (Flammer et al., 2012). Typically, during standard invasive coronary endothelial function assessment, QCA or intravascular ultrasound (IVUS) is used, and changes in conduit vessel diameters and cross-sectional areas in response to graded concentration of endothelium-dependent stimuli are documented. In addition, the integrity of coronary microvascular function is evaluated simultaneously by measuring the changes in coronary blood flow using a flexible and steerable 0.014-inch diameter Doppler guidewire. Coronary blood flow is measured as a product of Doppler-derived average peak velocity and the cross sectional area of the vessel obtained by angiography or IVUS (Doucette et al., 1992). In coronary vessels or segments with preserved endothelial function, the normal response is vasodilation and increase coronary blood flow. In the setting of endothelial dysfunction however, NO production is insufficient and/or outweighed by the production of endothelium-derived vasoconstrictors, such as endothelin, and hence endothelium-dependent vasoactive

agent administration results in paradoxical vasoconstriction due to its direct effect on the vascular smooth muscle (Ludmer et al., 1986).

Endothelial adrenergic system

Adrenergic stimulation plays a key role in the regulation of coronary vasomotor tone and requires a complex interaction between receptors acting as vasoconstrictors (α-adrenergic) and receptors acting as vasodilators (β-adrenergic) (Barbato, 2009). Presently, three β-adrenergic receptors subtypes have been identified within the human coronary circulation: β1, β2, and β3-subtypes (Hodgson et al., 1989a, Sun et al., 2002, Dessy et al., 2004). Data from ex vivo and *in vivo* human studies demonstrated greater (up to 5-fold) densities of β-adrenergic receptors within arteriolar resistance coronary vessels when compared with small to medium sized arteries and significantly more when compared with proximal epicardial vessels (up to 34-fold) (Ferro et al., 1995, Amenta et al., 1991, Barbato, 2009, Barbato et al., 2003). Among these receptor subtypes, β1 receptors are predominantly located within the epicardial coronary arteries whereas β2 and β3 receptors are present at the level of coronary microvessels. Interestingly, in a morphology characterization study of adrenergic receptors within human epicardial arteries, Jensen and coworkers found that two thirds of all adrenergic receptors are of the β adrenoreceptor type, with 99% of these being the β2-subtype (Jensen et al., 2009). Whether these receptors are fully functional nonetheless remains to be elucidated. Both β1 and β2 are characteristically expressed on endothelial and vascular smooth muscle cells, whereas β3 adrenoreceptors are mainly expressed on the vascular endothelium. A number of studies have demonstrated that β2 adrenergic receptors mediated coronary vasodilation via endothelium-dependent pathways, either

by inducing NO release or activation of K+ channel induced hyperpolarization (Ferro et al., 2004, Grossini et al., 2009, Jayachandran et al., 2001, Barbato et al., 2005, Puri et al., 2012a, Wilkinson et al., 2002). A number of investigators have shown that this process can be altered in the setting of atherosclerosis, such that vasodilation can be typically blunted or even paradoxical vasoconstriction can occur (Barbato et al., 2005, Puri et al., 2012a, Wilkinson et al., 2002, Puri et al., 2013).

Salbutamol is a short-acting, direct β2 agonist which has been used to treat asthma since 1969 (Choo-Kang et al., 1969, Palmer and Diament, 1969). Its administration into the human coronary circulation with incremental doses of 0.125 mcg up to 5 mcg/min produces increase in left ventricular contractility yet without significant changes in the systemic blood pressure, left ventricular end-diastolic pressure, and heart rate (Newton et al., 1999). In their seminal work, Wilkinson and colleagues demonstrated the potential use of salbutamol as a vasoreactive agent for endothelial function assessment (Wilkinson et al., 2002). Using pulse wave analysis for non-invasively assessing peripheral endothelial function, they showed that inhaled salbutamol induced increase in forearm blood flow and this response was blunted with the NO inhibitor, *N* -monomethyl-L-arginine (L-NMMA), indicative of its NO-dependent properties. This observation was validated in the human coronary microcirculation by Barbato et al. (Barbato et al., 2005) and within both the micro-and epicardial circulations by Puri et al. (Puri et al., 2012a). In unobstructed coronary arteries, even in the setting of non-ST-segment elevation myocardial infarction, IC salbutamol appears safe with low rate of coronary spasm (0.06%) and no arrhythmia documented (Barbato et al., 2005, Puri et al., 2012a). These findings are important for a number of reasons. Firstly, although

endothelial dysfunction has been demonstrated to be an independent predictor of cardiovascular events, its use is impractical and limited due to its invasive nature. Secondly, the “gold standard” provocative agent, acetylcholine, potentially causes a number of significant adverse effects, such as major coronary vasospasm and bradyarrhythmia thus limiting its use for non-invasive evaluation. Salbutamol on the other hand is an effective but gentler vasomotor provocative agent with no arrhythmic complications. Its inhaled delivery also permits for non-invasive endothelial function evaluation and therefore possible for use in clinical and larger-scale settings. Its wider application however still awaits further observational and interventional studies in a much larger population spanning longer duration of follow up.

VIII. CONCLUSIONS

Coronary blood flow regulation is complex and is a function of the dynamic interplay between perfusion pressure, conduit obstruction, and resistance vessels. These interactions can be modulated for diagnostic and therapeutic purposes with various coronary vasoactive agents. Their use has been pivotal in the cardiac catheterization lab as an aid to diagnostic coronary angiography for diagnosing haemodynamically significant stenosis or coronary vasospasm, as well as an important therapeutic adjunct to PCI for improving myocardial perfusion and ultimately the clinical outcome. Understanding the role, mechanisms, and side effects of these medications are crucial to ensure safe and appropriate administration to optimize the clinical evaluation and treatment of the wide-spectrum of coronary arterial disease.

FIGURE 1. Mechanism of nitric oxide (NO) synthesis by endothelial cells. Stimulation of endothelial NO synthase in response to various stimuli leads to increased nitric oxide (NO) production in endothelial cells by receptor and non-receptor and calcium-dependent and non-calcium-dependent pathways. NO then diffuses to vascular smooth muscle and causes relaxation by cGMP dependent pathways. (Reproduced with permission from Herrmann J et al. (Herrmann et al., 2010))

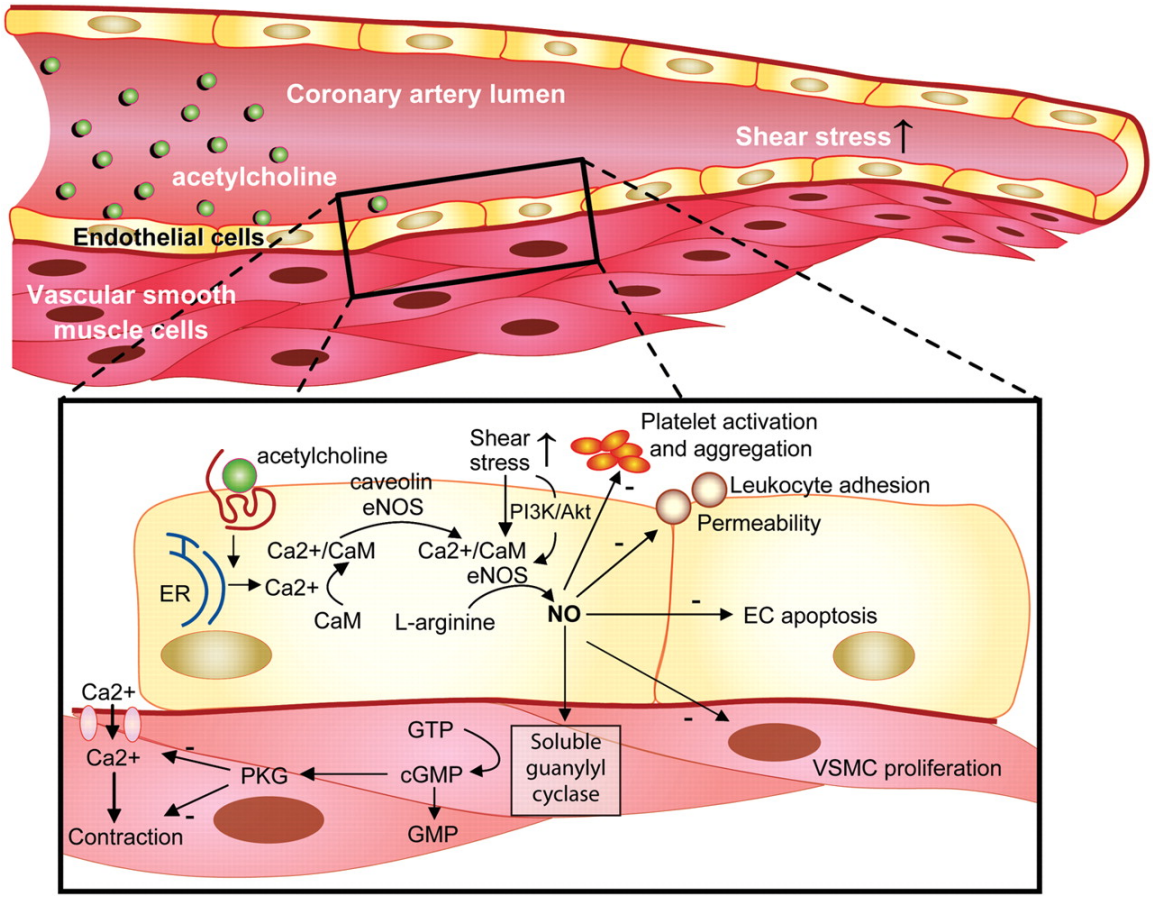

TABLE 1. Summary of coronary vasoactive agents

Medication	Mechanism of action	Site of action	Dosage	Side effects
Nitrate	Nitric oxide donor	Epicardial	50-400mcg (IC)	Hypotension, headache,
Adenosine	Purine nucleoside	Resistance	FFR: 60 mcg LCA; 40 mcg RCA (IC); ↑ 30-60mcg up to 150mcg if FFR remains suboptimal 140mcg/kg/min to 170mcg/kg/min (IV) If antecubital vein is used, then start with 170mcg/kg/min No reflow: Start with 30mcg (IC) then ↑every 30mcg every 20 sec until favorable response	Flushing, angina, transient bradyarrhythmia, hypotension
Nitroprusside	Nitric oxide donor	Mixed	No reflow: 100 mcg then titrate up to max 1 mg FFR: 0.6mcg/kg (IC) – not routinely recommended at present	Hypotension, headache, nausea
Papaverine	Phosphodiesterase inhibitor	Resistance	FFR: 15-20 mg LCA; 10-12mg RCA (IC)	ST depression, QT prolongation, VT, VF,

				↑myocardial lactate
Verapamil	Calcium channel blocker	Mixed	No reflow: 100 mcg up to 1 mg (IC)	Hypotension,
Nicorandil	K^+ channel opener and NO donor	Mixed	No reflow: 500 mcg to a total of 5 mg (IC)	Arrhythmia

CHAPTER 3: THE IMPACT OF LUMEN SIZE AND MICROVASCULAR RESISTANCE ON FOURIER-DOMAIN OPTICAL COHERENCE TOMOGRAPHY (FD-OCT) CORONARY MEASUREMENTS

Adapted from: Sidharta S, Puri R, Frost L, Kataoka Y, Carbone A, Willoughby S, Nelson A, Nicholls S, Worthley S, Worthley M, The Impact of Lumen Size and Microvascular Resistance on Fourier-Domain Optical Coherence Tomography (FD-OCT) Coronary Measurements, Int J Cardiol 2014 Jun 1;174(1):210-1

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Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	20/1/2017

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

vii. the candidate's stated contribution to the publication is accurate (as detailed above);

viii. permission is granted for the candidate in include the publication in the thesis; and

ix. the sum of all co-author contributions is equal to 100% less the candidate's stated

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ABSTRACT

Backgrounds: Optical Coherence Tomography (OCT) is a catheter based intravascular imaging modality which provides a very high resolution image of vessel wall. In acquiring OCT images, angiographic contrast media is injected via an injection pump (total of 14mls, over 4 seconds, 300psi) to achieve effective intracoronary clearance of blood for optimal image acquisition. The influence of dye load and subsequent luminal measurements may vary at differing macro and microvascular conditions.

Aims: To investigate the impact of luminal dimensions and differing microvascular resistance on human coronary artery luminal measurements acquired by OCT.

Method: A total of 10 patients underwent serial imaging with intravascular ultrasound (IVUS) at baseline (used as a 'gold standard' comparator), followed by OCT. The OCT measurements were acquired during incremental intracoronary infusions of salbutamol as a proven microvascular vasodilator. Each IVUS and OCT runs were matched and analysed as 4 mm segments. To evaluate the impact of differing microvascular resistance and lumen dimension on OCT lumen measurement, we dichotomised the groups into small and large lumen groups divided around the IVUS segmental lumen volume (SLV) median.

Results: A total of 49 matched segments were available for analysis. The mean SLV of all segments with IVUS was 79.29 ± 4.78mm^3 compared with corresponding mean SLV of all segments with OCT, 73.52 ± 4.56mm^3. OCT measurements in the small lumen group increased in response to incremental salbutamol dose (baseline 49.22 [30.76-69.15] mm^3, 0.3mcg salbutamol 62.47 [38.40-72.35] mm^3, 0.6 mcg salbutamol 64.35 [43.85-68.56] mm^3, p = 0.0077) however this response was not observed in the large lumen group (baseline 102.6 [78.83-115.1] mm^3, 0.3mcg salbutamol 89.28 [83.39-103.3] mm^3, 0.6mcg salbutamol 90.88 [81.36-107.2] mm^3, p = 0.205).

Conclusion: Volumetric lumen assessment with OCT is increased by 25% in small vessels in the setting of a reduction in microvascular resistance. In the presence of significant epicardial coronary stenosis, this finding may have implications on the effectiveness of OCT to guide stent sizing.

INTRODUCTION

Fourier-Domain Optical Coherence Tomography (FD-OCT) is a catheter based intracoronary imaging modality that provides high-resolution images using near-infrared interferometry principle (Muller et al., 2010). The spatial resolution of OCT is 10 to 20 μm, which is approximately 10 times greater than that of intravascular ultrasound (IVUS) and hence it provides an excellent contrast between the lumen and vessel wall. FD-OCT also provides a high frame rate and rapid pullback speed, thus enabling fast scanning of long coronary segments. Some of its applications include the identification of thin cap fibroatheroma (TCFA) (Kubo et al., 2007), thrombus imaging (Kume et al., 2006), and increasingly used to guide percutaneous coronary intervention (PCI) (Gomez-Lara et al., 2011).

Coronary endothelial/vasodilator dysfunction is thought to play a mechanistic role in mediating coronary atheroma plaque disruption (Bogaty et al., 1994) which may eventually lead to adverse coronary events (Halcox et al., 2002, Schachinger et al., 2000). Our group has recently shown, using IVUS imaging, that this vasodilator function is associated with plaque burden (Puri et al., 2012a). In light of a greater spatial resolution associated with OCT, future research in this area may gain benefit using this newer imaging modality.

Despite emerging as a potentially attractive imaging modality for the assessment of epicardial coronary endothelial/vasodilator function, several technical aspects from OCT image acquisition need to be considered. Growing reports have documented significant discrepancy in the lumen evaluation between OCT and IVUS whereby IVUS derived lumen measurement tends to be larger than OCT, particularly in the smaller

epicardial coronary vessels (Okamura et al., 2011, Bezerra et al., 2013). Furthermore, during OCT image acquisition, angiographic contrast media is injected via an infusion pump to achieve effective intracoronary (IC) clearance of blood for optimal image acquisition. It could be speculated that this bolus injection may increase the arterial wall shear stress and hence impact upon the coronary vasodynamic assessment. FD-OCT lumen measurement analysis also has a potential to vary at differing macro and micro vascular conditions (Guagliumi et al., 2013). We aim to investigate the impact of lumen dimensions and differing microvascular resistance (in response to IC salbutamol provocation) on human coronary artery luminal measurements acquired by OCT, comparing this with baseline IVUS and OCT acquired measurements. Particularly, we are interested in observing these effects in both small and large epicardial vessels.

METHODS

Study Subjects

Following informed consent, 10 consecutive patients who underwent elective coronary angiogram at the Royal Adelaide Hospital catheterization laboratories were enrolled. The target artery of interest must be "smooth" or have minimal disease, defined as <30% visual angiographic stenosis and not undergone previous PCI. All vasoactive medications were held 24 hours prior to the study. Patients with recent acute coronary syndrome, renal failure, cardiac failure, severe valvular heart disease, and prior coronary revascularization were excluded. This study was approved by the Royal Adelaide Hospital Research Ethics Committee.

Intravascular imaging protocols.

Coronary angiography was performed using standard 6 French technique. Intravenous

heparin (70 IU/kg) was administered for the research protocol. Each study participant underwent serial intravascular imaging with initial IVUS (40 MHz Atlantis® SR Pro catheter, Boston Scientific, Natick, MA, USA) run with automated pullback speed of 0.5mm/s (used as a 'gold standard' comparator) and followed by serial FD-OCT (2.7-F C7 Dragonfly Imaging Catheter, LightLab Inc., Westford, Massachusetts) interrogation. Prior to FD-OCT catheter pullback, angiographic contrast media was injected through the guiding catheter via an injection pump (settings: 14 mL volume contrast, 4mL/s, 300 psi per OCT run) to achieve effective IC clearance of blood for optimal image acquisition. All IVUS and FD-OCT acquisition were performed without glyceryl trinitrate (GTN) pre-treatment of the target artery. All the images were saved and recorded on a DVD for off-line analysis.

During FD-OCT acquisition, serial 5-minute IC infusions via the guiding catheter at 2 mL/min was administered: 5% dextrose, followed by 0.3 mcg/min and then 0.6 mcg/min of salbutamol as the vasoactive agent. These doses are known to increase coronary blood flow by approximately 60% (Puri et al., 2012a, Barbato et al., 2005). At 3 minute into each IC infusion, we recorded the patient's haemodynamics (blood pressure and heart rate) followed by FD-OCT pullback at an automated pullback speed of 20 mm/s. The infusion was then continued for the remainder 2 minutes whilst FD-OCT acquisition was undertaken. Each pullback recorded 54 mm of coronary artery over a 2-3 second period. Prior to the commencement of imaging, the Z-offset was adjusted for appropriate image calibration for accurate image measurements offline.

Image analysis

All IVUS and FD-OCT data was analysed using echoPlaque 3.0.60 (Indec Systems,

Santa Clara, CA, USA). For each run, the common most distal and proximal fiduciary markers (anatomical side-branches) were identified from corresponding IVUS and OCT pullbacks in order to define the region of vessel to be analysed. For IVUS imaging, cross-sectional images were selected every 30 frames (0.5 mm) apart. Coronary lumen and the external elastic membrane were traced by manual planimetry upon each selected IVUS frame, to enable the calculation of lumen area and plaque burden within each segment. Plaque burden was calculated as percent atheroma volume (PAV) within each segment (Nissen et al., 2004b). Briefly, the leading edges of the lumen and EEM were traced. Plaque area was defined as the area occupied between these leading edges. The PAV within each coronary segment was calculated as the proportion of the entire vessel cross-sectional area (of the respective coronary segment) occupied by atherosclerotic plaque. For OCT imaging, cross-sectional images were selected every 2nd frame (0.4 mm) apart. Each IVUS and OCT run was precisely divided into pre-defined consecutive 4 mm segments, comprising of 9 cross-sectional IVUS frames (with 8 x 0.5 mm intervals) and 11 cross-sectional OCT frames (with 10 x 0.4 mm intervals). Each segment was therefore analysed separately. The segmental lumen volume (SLV) was calculated as the average of lumen area within that particular segment and normalized as previously reported (Puri et al., 2012a). Using MIB software (Indec Medical Systems, Santa Clara, CA, USA), the corresponding IVUS and OCT pullbacks (with numbered frames) were simultaneously played in order to accurately frame match anatomical fiduciary markers between each run (Fig 1). This technique ensured that precisely the same arterial segments were consistently analysed between each IVUS and OCT run per patient. Only cross sectional images deemed acceptable for complete lumen tracing (frames that contained a complete lumen circumference) were included.

To evaluate the effects of different microvascular resistance on FD-OCT measurements, coronary segments were dichotomized as larger and smaller segments divided around the median IVUS-derived SLV. Furthermore, we dichotomized the coronary segments surrounding the mean IVUS-derived PAV to determine the impact of plaque burden on FD-OCT measurements.

Statistical analysis

Sample characteristics are summarized as counts and percentages, means with standard deviation, or median with interquartile range as appropriate. Comparisons between SLV measured by IVUS versus OCT was conducted using paired Student t test. Group comparisons between serial OCT were performed with repeated measures one-way ANOVA. Log transformations were applied where appropriate. Statistical analysis was performed with GraphPad Prism (version 6.0, GraphPad Software, La Jolla, California).

RESULTS

Clinical characteristics

Baseline demographics are shown in table 1. The average age of our cohort was 60 years and 70% were male. A total of 49 epicardial segments were analysed out of 10 coronary arteries. The mean IVUS-derived SLV of all segments at baseline was 79.29 ± 4.78 mm^3, compared with a corresponding mean FD-OCT-derived SLV of 73.52 ± 4.56 mm^3 ($p<0.0001$), representing a relative difference of 7.3%.

Microvascular resistance and FD-OCT measurement: impact of lumen size

The mean FD-OCT derived SLV of all coronary segments at baseline, 0.3 mcg, and 0.6 mcg salbutamol were 74.15 ± 31.92 mm^3, 75.68 ± 26.79 mm^3, and 74.35 ± 25.74 mm^3

respectively (p = 0.55). FD-OCT measurements in the smaller IVUS-derived SLV group increased in response to IC salbutamol infusion at both the 0.3 and 0.6 mcg/min (baseline 49.22 mm^3 [30.76-69.15]; 0.3mcg/min salbutamol 62.47 mm^3 [38.40-72.35]; 0.6 mcg/min salbutamol 64.35 mm^3 [43.85-68.56], p = 0.0077) however this vasodilatory response was not observed in the larger IVUS-derived SLV group (baseline 102.6 mm^3 [78.83-115.1]; 0.3mcg/min salbutamol 89.28 mm^3 [83.39-103.3]; 0.6mcg/min salbutamol 90.88 mm^3 [81.36-107.2], p = 0.205) (Fig 2).

Microvascular resistance and FD-OCT measurement: impact of plaque burden

Coronary segments were also divided around the mean IVUS-derived segmental plaque burden (PAV) into low PAV group (mean: 22.39 ± 4.29 mm^3) and high PAV group (mean: 45.43 ± 9.71 mm^3) to determine the impact of plaque burden on epicardial vasomotor response as measured with FD-OCT. There was no significant difference in the vasodilatory response observed either in the low plaque volume group (dextrose: 91.41 ± 28.05 mm^3, 0.3 mcg salbutamol: 89.04 ± 23.82 mm^3, and 0.6 mcg salbutamol 85.88 ± 24.59 mm^3; p = 0.088) or in the high plaque volume group (dextrose: 61.18 ± 28.76 mm^3, 0.3 mcg salbutamol: 66.47 ± 25.10 mm^3, and 0.6 mcg salbutamol: 66.4 ± 23.77 mm^3; p = 0.16).

DISCUSSION

This is the first human *in vivo* study evaluating the impact of lumen size, plaque burden, and differing microvascular resistance state on FD-OCT coronary lumen measurement. We demonstrated that volumetric lumen assessment with FD-OCT is significantly increased in response to the microvascular vasodilator salbutamol in small vessels, while no change is observed in larger vessels. This finding was not seen under different

plaque burden conditions. Consistent with the previous reports (Okamura et al., 2011, Puri et al., 2012b, Kubo et al., 2013), at baseline, we found that coronary lumen dimension measured with IVUS was 7.3% larger when compared with FD-OCT. A few possible explanations may be provided to explain this measurement disparity, including: (1) sharper lumen-intima interface delineation by FD-OCT, resulting in more precise lumen visualization than IVUS; (2) faster FD-OCT pullback creating smoother longitudinal view; and (3) discrepancy in image gating between the two imaging modality (Bezerra et al., 2013). In spite of these suggestions, whether this discrepancy is due to FD-OCT underestimation or IVUS overestimation remains uncertain.

We then explored the impact of various microvascular resistance condition on FD-OCT luminal measurements. Although there was no significant difference in the vasomotor response in the entire coronary segments following salbutamol injection, on further exploratory analysis, we noted an incremental epicardial vasodilatory response by 25% to increasing doses of IC salbutamol in small lumen segments which was not observed in the large lumen segments. Salbutamol, a beta 2 adrenoreceptor (AR) agonist, is a novel vasoreactive agent which is known to increase coronary blood flow by 50-60% via nitric oxide dependent pathway (Barbato et al., 2005, Puri et al., 2012a). It is generally thought that salbutamol exerts its conduit vasodilating property by reducing the coronary microvascular resistance, which in turn produces a shear stress dependent vasodilation (Barbato, 2009). Of note, vascular wall shear stress is indirectly proportional to the arterial radius (Davies, 2009). The impact of contrast injection during FD-OCT derived vasoreactive assessment needs to be mentioned as well. Even though at standard conditions the impact of this contrast injection may be minimal, it has been reported that contrast media may induce conduit vasodilation through various

mechanisms (Limbruno et al., 2000, Baile et al., 1999). We also speculate, with such an injection rate, it would be possible to generate a higher shear rate. Mechanistically therefore, this difference in luminal response likely relates to the greater increase in shear stress observed in smaller vessel, particularly in the setting of an increase coronary blood flow associated with a reduction in microvascular resistance. Given that reduction in lumen size and microvascular resistance occurs with significant epicardial coronary stenosis (Chamuleau et al., 2003), it is possible that these findings could have implications on stent sizing.

The discrepancy in FD-OCT derived vasodilatory response in small versus large lumen was not seen in segments with different IVUS derived plaque volume. In our previous coronary endothelial function studies using IVUS, we were able to characterize systematically the segmental coronary structure-function relationship between atheroma plaque burden and epicardial coronary vasoreactivity (Puri et al., 2012a). We therefore hypothesized that the combined use of FD-OCT and IVUS, which leverage on both near field and far field resolution would provide an excellent imaging platform to conduct a 'gold standard' coronary structure-function *in vivo* evaluation. The negative result of the current study however is important in highlighting the limitation of FD-OCT in coronary vasoreactive assessment. The injection of bolus contrast medium prior to image acquisition and its aforementioned impact on lumen measurement would make any dose response experiment challenging. Furthermore, characterization of the relative expression of alpha and beta AR subtypes within the human epicardial coronary arteries has uncovered that two-thirds of all such AR's are of the beta-AR type, of which 99% are of the beta 2-AR sub-type (Jensen et al., 2009). Hence, the rapid contrast flush with FD-OCT acquisition may provide inadequate time for salbutamol to interact with its

corresponding epicardial receptor to generate adequate vasoreactive response, which in turn restrict the capacity to evaluate the underlying relationship between regional plaque burden and segmental coronary vasomotion. Lastly, the trade-off of the superior lateral resolution of FD-OCT is poor depth penetration, which limit its ability to assess atheroma volume. Hence, it may not be feasible to use FD-OCT as a standalone imaging modality for systematic evaluation of coronary atheroma structure and vasoreactive function in the same manner as IVUS.

Limitations in our study should be acknowledged. First, the sample population is small and limited to patients with stable chest pain presentation. Second, we did not evaluate the impact of normal saline as an alternative agent to achieve blood clearance for optimal FD-OCT image acquisition. Next, our data is limited to the *in vivo* evaluation of coronary segments that do not contain angiographically significant stenosis. Consequently, our results may not be applied to segments with critical disease. Finally, a Doppler wire was not used during our vasodilatory assessment which would have provided incremental understanding between the changes in luminal volumes and its associated coronary blood flow/microvascular vasodilation.

CONCLUSION

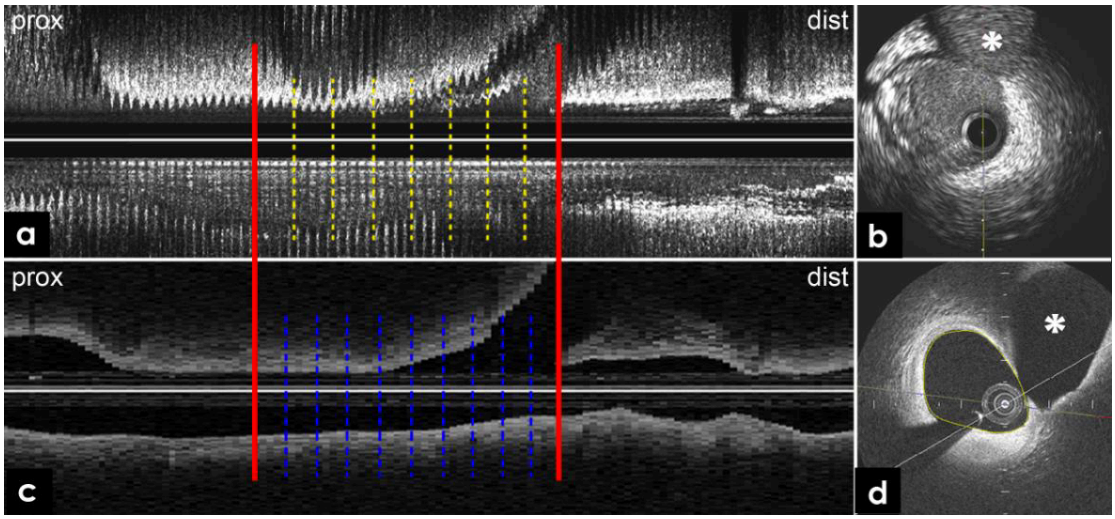
Volumetric lumen assessment with OCT results in a smaller measurement compared to IVUS for a given epicardial segment analysed. Furthermore, in smaller vessels, a large measurement is seen when microvascular resistance is reduced. This finding is not related to underlying coronary plaque burden. As lumen size reduction and microvascular resistance occurs with significant epicardial coronary stenosis, this finding may have implications on stent sizing.

TABLE 1: Clinical characteristics

	Entire cohort (n=10)
Age, years	61 ± 10
Male	7
Hypertension	2
Smoker	4
Medications:	
Aspirin	6
Statin	3
ACE-i/ARB	3
Calcium channel blocker	1
Lipids, mmol/L	
Total cholesterol	4.73 ± 1.11
TG	0.89 ± 0.45
HDL	1.32 ± 0.34
LDL	3.0 ± 0.91
hsCRP, mg/L	5.1 ± 8.4
Artery	
LAD	8
LCx	1
RCA	1

ACE-I = angiotensin converting enzyme inhibitor; ARB = angiotensin receptor blocker; TG = triglyceride; HDL = high density lipoprotein; LDL = low density lipoprotein; LAD = left anterior descending artery; LCx = left circumflex artery; RCA = right coronary artery

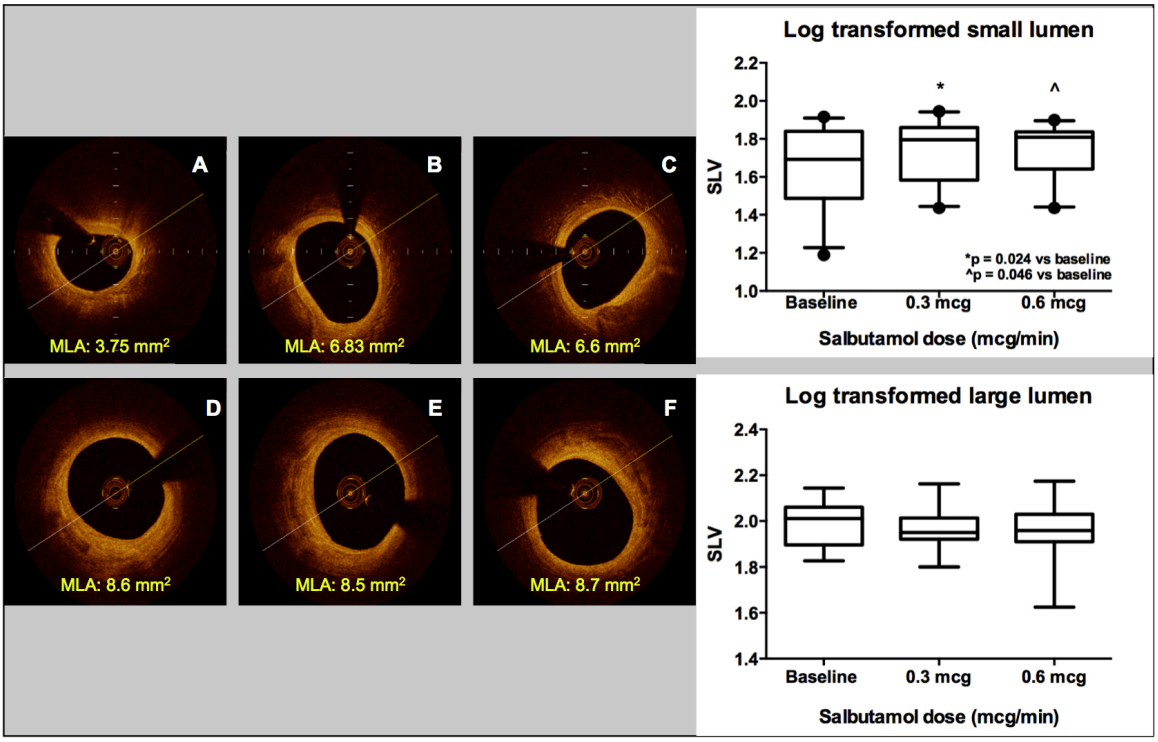
FIGURE 1: Methodology for grey scale intravascular ultrasound and FD-OCT analysis



Corresponding horizontal views of IVUS (a) and OCT (c) imaging of the same coronary segment. Start frames (cross-sectional views) of IVUS and OCT shown in (b) and (d) respectively illustrating distal fiducial markers (white asterix). For IVUS, 9 consecutive cross sectional frames (yellow dotted lines, spaced at 0.5 mm intervals), and for OCT 11 consecutive cross sectional frames (blue dotted lines, spaced at 0.4 mm intervals) were traced to depict each 4 mm coronary segment (Reproduced with permission from Puri et al. (Puri et al., 2012b)

FIGURE 2: OCT-derived minimum lumen area (MLA) dichotomized into small lumen (top panel) and large lumen (below panel) with their corresponding graph.

A & D represent the baseline MLA whereas B & E and C & F represent MLA in response to salbutamol infusion of 0.3 mcg and 0.6 mcg respectively.



CHAPTER 4: RELATIONSHIP BETWEEN SEGMENTAL CORONARY ENDOTHELIAL FUNCTION WITH LIPID RICH PLAQUE BURDEN: A NEAR INFRARED SPECTROSCOPY STUDY

Adapted from: Sidharta SL, Nicholls SJ, Howell S, Baillie TJ, Montarello N, Montarello N, Honda S, Shishikura D, Nelson AJ, Delacroix S, Chokka RG, Beltrame JF, Worthley SG, Worthley MI. Association between coronary endothelium independent vasoreactivity and lipid rich plaque burden (submitted to Heart).

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Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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By signing the Statement of Authorship, each author certifies that:

x. the candidate's stated contribution to the publication is accurate (as detailed above);

xi. permission is granted for the candidate in include the publication in the thesis; and

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ABSTRACT

Objectives: Plaque burden and endothelial dysfunction are both key pathological mechanisms associated with adverse coronary events. In this study, we aim to investigate the relationship between atherosclerotic lipid rich plaque and segmental coronary endothelial function utilizing near infrared spectroscopy (NIRS) in patients with stable chest pain presentation and acute coronary syndrome (ACS).

Methods: 53 patients were enrolled. Coronary vessels with <30% visual angiographic disease were studied. If significant angiographic disease was identified, the 'study' artery was a non-culprit vessel. Macrovascular response [% change segmental lumen volume (SLV)], plaque burden [per cent atheroma volume (PAV)], lipid rich plaque (LRP), and lipid core burden index (LCBI) were studied in 2-mm coronary segments.

Results: Segmental PAV (r = -0.41, p = 0.006), LRP (r = -0.165, p< 0.0001), and LCBI (r = -0.202, p< 0.0001) were significantly associated with endothelium independent vasoreactivity. Greater LRP (31.3% vs 17.8%, p = 0.0008) and LCBI (32 (90-214) vs 0 (0-82), p = 0.0002) were observed in segments with impaired endothelium independent function whereas greater PAV was noted in the groups with both impaired endothelium dependent (32 [24.4-39.7]% vs 29.9 [12.4-38.3]%, p = 0.0013) and independent (35.7 [28.2-46.5]% vs 30 [21.4-37.6]%, p = <0.0001) response when compared with normal segmental vasoreactivity. In a multivariate analysis, PAV was found to be the sole independent predictor for endothelium independent function (OR: 1.07; 95% CI: 1.04-1.10, p < 0.0001). No association was observed between endothelial dependent function and any of the atheroma plaque indices.

Conclusion: Our findings show that atheroma volume, and to a lesser extent, lipid rich necrotic core is linked with impaired coronary endothelial independent function. Further studies are required to determine if these segmental indices are associated with specific

risk of plaque progression and clinical events.

INTRODUCTION

Coronary endothelial dysfunction has been associated with adverse cardiac events, independent of traditional risk factors (Lerman and Zeiher, 2005). Similarly, in various large prospective trials, intravascular ultrasound (IVUS) derived plaque burden and its rate of progression are also known to be predictors of adverse cardiac outcomes (Stone et al., 2011, Stone et al., 2012, Nicholls et al., 2010). Previously, we described a novel methodology to evaluate segmental epicardial coronary endothelial function utilizing IVUS and intracoronary (IC) salbutamol infusion as the coronary endothelial dependent vasoreactive stimulus (Puri et al., 2012a). This study demonstrated a strong association between *in vivo* segmental epicardial endothelial dependent function and its underlying atheroma burden. Indeed, irrespective of clinical presentation whether patients presenting with stable chest pain syndrome or acute coronary syndrome (ACS), coronary atheroma plaque burden has been shown to be independently associated with coronary endothelial function (Puri et al., 2013). However, these studies did not explore the relationship between coronary endothelial function and plaque composition, notably qualitative and quantitative measurements of lipid within the plaque, thought a key factor of plaque vulnerability.

Pathological studies have demonstrated thin cap fibroatheroma with lipid rich necrotic core as the substrate of plaque responsible for ACS (Virmani et al., 2006). Therefore, identifying and delineating such plaque characteristic *in vivo* may be important in improving patient's risk assessment and management. A novel intracoronary catheter-based near infrared spectroscopy (NIRS) imaging system has been developed to enable the detection of lipid rich containing plaque *in vivo*. This system, which now also co-registers with IVUS, has undergone rigorous ex vivo (Gardner et al., 2008, Kang et al.,

2015) and *in vivo* (Waxman et al., 2009) validation experiments and identifies lipid in coronary atheroma with a high degree of accuracy. The use of NIRS in the invasive coronary endothelial function assessment with quantitative coronary angiography (QCA) has been reported previously however it only included the left anterior descending (LAD) artery of patients with stable disease (Choi et al., 2013). We therefore sought to evaluate whether segmental coronary endothelial function, as assessed with IVUS, is associated with NIRS derived lipid rich plaque (LRP) and segmental plaque burden in patients presenting with both ACS and stable chest pain syndrome.

METHODS

Subjects

We consecutively enrolled 53 patients (age ≥ 18 years) who were referred to the Royal Adelaide Hospital Cardiac Catheterization Laboratories for diagnostic coronary angiography or percutaneous coronary intervention (PCI) for ACS or stable chest pain presentation. The target artery of interest must be "smooth" or have minimal disease, defined as <30% visual angiographic stenosis and not undergone previous PCI. If significant angiographic, culprit disease was identified, the study artery was the non-culprit vessel. Informed consent was obtained >48 hours prior to index coronary angiography and all vasoactive medications were withheld 24 hours prior to the study. Exclusion criteria include previous surgical coronary revascularization, significant valvular heart disease or left ventricular systolic dysfunction (ejection fraction ≤ 35%), ST elevation myocardial infarction (STEMI), known predilection to coronary vasospasm, uncontrolled hypertension, significant renal or pulmonary disease, and chronic beta blocker therapy or use of long or short acting β2 agonist within the

previous 24 hours. This study was approved by the Royal Adelaide Hospital Research Ethics Committee (ACTRN12612000594820).

Endothelial function assessment

Coronary angiography was performed via a standard 6-French technique. Following this, coronary endothelial function was evaluated similar to our previously described methodology (Puri et al., 2012a). Should PCI be indicated, this was performed immediately after the completion of the endothelial function assessment protocol. Intravenous heparin (100 IU/kg) was administered for the research protocol. Briefly, a 0.014-inch Doppler flow wire (FloWire; Volcano therapeutics Inc. Rancho Cordova, CA, USA) was placed in the proximal segment of the target artery and the average peak velocity (APV) of the coronary blood flow (CBF) was recorded. This wire was also used to monorail the 3.6F TVC Insight Catheter (InfraReDx, Burlington, Massachusetts, USA), a co-registration between IVUS and NIRS system, into the study artery. IVUS was performed according to standard protocol, without glyceryl trinitrate (GTN) pre-treatment of the target artery. Serial 5-minute IC infusions via the guiding catheter at 2 mL/min were administered in the following sequence: (a) 5% dextrose, (b) salbutamol (0.3 μg/min) to assess endothelium dependent vasodilation, and (c) bolus injection (not IC infusion) of GTN (100 μg) to assess endothelium-independent vasodilation. At 3 minute into each IC infusion, we recorded the APV and the patient's haemodynamics (blood pressure and heart rate) followed by repositioning of the wire into the distal vessel and commencement of IVUS pullback at 0.5mm/s. The infusion was then continued for the remainder 2 minutes whilst IVUS acquisition was undertaken.

IVUS analyses

The method of IVUS grey scale evaluation was described previously (Puri et al., 2012a, Mintz et al., 2001). All grey scale data were analysed using echoPlaque 3.0.53 (Indec Systems, Santa Clara, CA, USA). Common distal and proximal fiduciary points (anatomical side branches) were chosen to match each IVUS run using ImageJ software. Cross sectional images were analysed every 16 frames (0.5mm) and each IVUS pullback was divided into a pre-defined 2mm segments comprising 5 cross sectional images at 0.5mm apart (Figure 1). Frames that precluded complete lumen or vessel wall planimetry were excluded from analysis, as were segments that involved branch points. The leading edge of the luminal borders and external elastic membrane (EEM) were contoured for each designated frame. Segmental percent atheroma volume (PAV) and percent change in segmental lumen volume (SLV) were the primary determinant of segmental plaque burden (Mintz et al., 2011) and vasomotor response respectively. Segmental PAV was calculated according to this equation:

$$PAV = \frac{\Sigma\,(EEM_{area} - lumen_{area})_{segment}}{\Sigma\,(EEM_{area})_{segment}} \times 100$$

SLV was calculated as the average of lumen area within that particular segment and normalized as previously reported (Puri et al., 2012a). Impaired endothelial function is defined as % change SLV < 0 (vasoconstriction) whilst % change SLV > 0 denotes normal endothelial function (vasodilation) (Schachinger et al., 2000). The coronary blood flow (CBF) was calculated as: Average IVUS $MLA_{entire\ vessel}$ X (APV/2). Also at the vessel level, we collected the PAV for the entire vessel (PAV_{vessel}), calculated according to the equation above except that it is the summation of plaque area and EEM area involving all the measured images in that vessel. The percent change CBF and

PAV_{vessel} were the determinants of endothelial dependent microvascular function and the entire vessel plaque burden; these were explored in our secondary analysis.

NIRS analyses

During the NIRS automated pullback at 0.5mm/s, raw spectra were acquired at 40Hz, producing an image with data points spaced every 0.1 mm and every 1^0 apart. A NIRS algorithm defines a lipid containing plaque as fibroatheroma with lipid core $>60^0$ circumferential extent, >200μm thick, with a fibrous cap having a mean thickness <450μm (Gardner et al., 2008). Using this algorithm, the position of lipid core in each scanned artery segment is plotted on a digital map or 'chemogram', whereby the x-axis denotes the pullback position (mm) and the y-axis represents the circumferential position (degree). The probability of lipid core presence is displayed in chemogram as a colour scale from red (low probability) to yellow (high probability). A summary metric (block chemogram) of lipid core presence in 2mm chemogram interval is also generated in 4 probability categories using the top 10^{th} percentiles pixel information (yellow: $P > 0.98$, tan: $0.84 \leq P \leq 0.98$, orange: $0.57 \leq P \leq 0.84$, red: $P < 0.57$) to enhance interpretation. A lipid core is considered to be present if at least one 2mm segment in the block chemogram contains a strong signal, i.e. bright yellow (>95% specificity). For our primary analysis, we defined segmental LRP as yellow or tan on the block chemogram (Kang et al., 2015). The final NIRS output is the lipid core burden index (LCBI), which is the quantitative measurement of lipid core and calculated by dividing the number of yellow pixels with the rest of viable NIRS pixel within the region of interest and scaled from 0-1000. We collected LCBI data at the segmental level (2mm) and also the total LCBI and $maxLCBI_{4mm}$, which reflect LCBI information for the entire vessel. The NIRS images and the block chemogram were analysed off line using

LipiScan analyser software (LipiScan, InfraReDx, Burlington, MA, USA) (Figure 2).

Statistical analysis

Sample characteristics are summarized as counts and percentages; means with standard deviation or median with interquartile range as appropriate. Pearson correlation was performed to evaluate the linear association between coronary endothelial function and plaque burden. Spearman correlation was used to assess the association between coronary endothelial function and LRP and LCBI as well as between coronary microvascular function and total vessel LCBI and plaque burden. Differences between impaired versus normal coronary endothelial dependent and independent function or between stable and ACS patients were compared using Mann-Whitney rank sum test and intergroup data were analysed with the Kruskal-Wallis test.

Univariate associations with the outcome of interest are reported as odds ratios. The estimates were obtained using a linear mixed effects model with a binomial distribution and a logit link function. Subjects were treated as a random factor in all analyses. Multivariate modelling started with a saturated model which included all variables with a univariate p-value < 0.2. Variables were systematically removed until only significant variables remained. All tests were two-tailed and assessed at the 5% alpha level. The analyses were completed using SAS v9.3 (SAS Institute Inc., Cary, NC, USA) and GraphPad Prism v6 (La Jolla, CA, USA).

RESULTS

Patient Characteristics. Baseline demographics are shown in table 1. A total of 53 patients were enrolled into the study. Two patients were excluded due to inability to acquire IVUS and NIRS data. One patient who presented with stable chest pain syndrome experienced complication attributable to IVUS catheter related clot at the end of the study protocol. This complication resulted in very small myocardial infarction. 28 patients (53%) presented with stable clinical presentation and were found to have smooth coronary arteries. In our 51 patients, a total of 849 IVUS segments were available for greyscale IVUS analysis, and 789 segments were available for co-registered greyscale and NIRS analysis. The mean length of the total vessel evaluated per patient was 37.54 ± 13.46mm (LAD 39.84 ± 14.73mm; LCx 32.51 ± 10.36mm; RCA 35.92 ± 9.29mm). The mean and median of segmental PAV was 31.79 ± 11.82% and 31.05 [12.28-77.35] % respectively. In the chemogram analysis, the median segmental LCBI was 0 [0-100] and 20% of the segments were LRP positive (Table 2).

Relationship between segmental plaque burden, LRP, and conduit endothelial function. The median % change SLV was 2.61 [-4.16-10.36] % following IC salbutamol infusion and 15.81 [4.59-29.38] % following IC GTN administration. A linear association was not observed between salbutamol-mediated % change SLV and segmental PAV (r = -0.19, p = 0.221), LRP (r = -0.044, p = 0.213), or segmental LCBI (r = -0.056, p = 0.110). In contrast, significant correlation was observed between vasoreactive response to GTN with segmental PAV (r = -0.41, p = 0.006), LRP (r = -0.165, p< 0.0001), and segmental LCBI (r = -0.202, p< 0.0001). Table 2 summarizes the IVUS greycale and NIRS data between different vasoreactive responses. Greater

PAV was found in the coronary segments with impaired vasoreactivity in response to both salbutamol (32 [24.4-39.7] % vs 29.9 [12.4-38.3] %, p = 0.0013) and GTN (35.7 [28.2-46.5] % vs 30 [21.4-37.6] %, p = <0.0001). Similarly, segments with endothelial independent dysfunction were found to have higher percentage of LRP (31.3% vs 17.8%, p = 0.0008), greater LCBI (32 [90-214] vs 0 [0-82], p = 0.0002), and smaller EEM area (12.1 [9.4-17.3] mm^2 vs 15.6 [12.5-19.4] mm^2, p <0.0001) when compared with segments with normal function. This observation was not noted following IC salbutamol infusion.

We then conducted further analysis to see if this relationship held true at different amounts of plaque burden and LCBI measurements. Figure 3 and 4 describe the differences in segmental endothelium dependent and independent response in different tertiles of plaque burden and LCBI that were derived from the total coronary segments in the entire cohort. The consistent finding was that there was an inverse relationship in a dose dependent fashion between the burden of segmental conduit atherosclerosis and its lipid core with segmental coronary endothelium independent function whereby the segments in the lowest tertile of plaque exhibit the greatest vasodilation compared with the intermediate and highest tertile plaque and lipid group (Figure 5). This segmental plaque burden and lumen response relationship was also noted in the salbutamol group, albeit less, with no relationship demonstrated in different LCBI tertiles.

Observer variability. The observer variability for grey scale IVUS measurement was high. The intraclass correlation of coefficient was 0.937 (95% CI: 0.863-0.971) and 0.959 (0.885-0.985) for inter- and intra-observer variability respectively.

Coronary microvascular function and lipid rich plaque. The median % change CBF to salbutamol was 33.24 [10.77-54.17] %. There was no correlation observed between PAV_{vessel} (r = 0.0446, p = 0.766), total LCBI (r = -0.0913, p = 0.556), and $maxLCBI_{4mm}$ (r = -0.1272, p = 0.411) with % change CBF in response to salbutamol. A significant correlation was noted between % change CBF and salbutamol mediated % change SLV (r = 0.36, p = 0.015).

Multivariable predictors of segmental coronary endothelium dependent and independent function. Figure 6A and Table 3 summarizes the univariate and multivariate predictors for segmental coronary endothelial function in response to salbutamol. In the multivariate analysis, ACS presentation (OR: 0.43; 95% CI: 0.20-0.95; p = 0.038), smoker (OR: 0.48; 95% CI: 0.23-1.00; p = 0.05), and reduced % change CBF (OR 0.99; 95% CI: 0.97-1.00; p = 0.034) were significantly predictive of impaired coronary vasoreactivity. Figure 6B summarizes the univariate predictors for GTN mediated segmental coronary vasoreactivity. The predictors for greater degree of endothelial independent function include negative LRP (OR: 0.54; 95% CI: 0.31-0.93; p = 0.026), lower LCBI (OR: 1.00; 95% CI: 1.00-1.00, p = 0.048), and lower segmental PAV (OR: 1.07; 95% CI: 1.04-1.09, p = 0.000). In the multivariate model, greater PAV was found to be the sole independent predictor for reduced luminal response to GTN (OR: 1.07; 95% CI: 1.04-1.10, p < 0.0001).

DISCUSSION

Our study demonstrated that in coronary vessels with normal or minimal angiographic disease, segments which vasocontrict in response to salbutamol were not strongly associated with high-risk vulnerable plaque features. Similarly, % change of CBF in response to salbutamol, a determinant of microvascular endothelial function, was not related to total lipid or plaque burden. On the other hand, we found a strong inverse relationship between plaque burden, and to a lesser extent, LRP and LCBI, with respect to segmental vascular response to our endothelial independent vasodilator, GTN.

Atherogenesis is a complex process which includes a dynamic interplay between multiple pathological factors including, but not restricted to inflammation, endothelial dysfunction, vascular smooth muscle cell (SMC) proliferation and apoptosis, lipid rich necrotic core formation, intraplaque haemorrhage, remodelling, and plaque rupture. It has been postulated that functional changes in the endothelium predates plaque and/or lipid formation (Ludmer et al., 1986). It is known that SMC also play a critical role in propagating the atherosclerotic process. For instance, in response to various local environmental cues, such as growth factors, inflammatory mediators, cell to cell or cell to matrix signaling, SMCs are able to undergo phenotypic modulation from its contractile to proliferative state in early stage atheroma formation (Shi and Chen, 2015). In addition, increased superoxide production by SMCs in response to oxidative stress impacts on the soluble guanylyl cyclase and cGMP-dependent protein kinase activity which in turn leads to eNOS uncoupling and endothelial dysfunction (Munzel et al., 2005). Finally, SMCs and foam cells apoptosis and necrosis leads to lipid pool deposition and necrotic core formation (Virmani et al., 2000). Atherosclerosis, in essence, profoundly affects the vascular wall architecture and this structural and

chemical alteration may explain the variability in vascular response to exogenous stimuli.

The present study did not find association between either lipid rich containing plaque or plaque burden with segmental vascular response to salbutamol, an endothelium-dependent vasoreactive agent. This finding is indeed intriguing in light of previous findings (Puri et al., 2012a, Puri et al., 2015). We feel the cause of these discrepant findings is multifactorial. First, a single dose of 0.3 μg/min of salbutamol was used in this study compared with the incremental dosing of 0.15, 0.3, and then 0.6 μg/min used previously. Salbutamol possesses a half-life of 2-4 hours, therefore the lack of relationship between coronary salbutamol mediated vasoreactivity (SMV) and various plaque indices in our cohort might be due to salbutamol under dosing. We opted for the 0.3 μg/min dose in the present study because this dose elicited the greatest response of SMV both at the conduit and microvascular level in our previous experiments (Puri et al., 2012a); it could be suggested that the reason for this maximal response may not necessarily be due to the 0.3 μg/min dose but rather as a result of cumulative dosing inclusive of the prior salbutamol (0.15 μg/min) infusion. This 'under dosing' theory is supported by the dynamic changes seen with our single dose strategy. The overall increase of % change SLV (2.61% vs 5.5%) and coronary blood flow (33.24% vs 54%), in response to salbutamol in the present study was lower when compared with our previous data (Puri et al., 2012a). Furthermore, the size of baseline SLV in the segments with impaired endothelium dependent function was larger when compared with segments with normal endothelium dependent luminal response (93.5 vs 80 mm^3). It is thought that salbutamol mediates vasoreactivity by reducing coronary microvascular resistance and in part, through a shear stress mechanism, leading to conduit vessel

dilation (Barbato, 2009, Barbato et al., 2005). Since the relationship between shear stress and lumen is indirectly proportional (Davies, 2009), the lack of epicardial SMV demonstrated in our population may be due to greater baseline lumen, an observation we have noted previously (Sidharta et al., 2014, Puri et al., 2013). Interestingly, despite this apparent disagreement, we were able to demonstrate a strong linear relationship between coronary macro and microvascular endothelial dependent function. Also, patients who present with ACS or smoking history demonstrated greater degree of impaired coronary luminal response when compared with those who present with stable chest pain syndrome or non-smokers, an observation that was noted in previous coronary endothelial function studies (Bogaty et al., 1994, Zeiher et al., 1995). Taken together, this suggests that SMV reflects the systemic or generalized nature of atherosclerotic disease burden. Its conduit function however is dependent on shear stress generated by the coronary microvascular driving force and further influenced by epicardial lumen size.

The inverse relationship between high-risk plaque features, such as plaque burden and to a certain extent, lipid rich necrotic core, with segmental endothelium independent function were not entirely unforeseen given the observation from previous studies (Adams et al., 1998, Schachinger and Zeiher, 1995). We are the first group to demonstrate the direct mechanistic link between these factors; especially the GTN vascular response's association with specific plaque phenotype, NIRS derived LRP. Indeed, we showed that adjacent segments with various degree of plaque burden and/or lipid rich necrotic plaque exhibit abnormal endothelium independent function in a dose dependent manner irrespective of clinical presentation and traditional risk factors. In the clinical endothelial function assessment, GTN is normally administered to evaluate the

integrity of vascular SMC function given its direct, nitric oxide (NO) action at the epicardial level. However, results from previous *in vivo* observational studies regarding the role of endothelium independent function in the setting of coronary atherosclerosis have been conflicting, contrary to the general consensus regarding acetylcholine mediated luminal vasoreactivity. Whilst some groups noted impairment of nitroglycerin mediated dilation in patients with atherosclerotic risk factors (Adams et al., 1998) or minimal atherosclerotic disease (Schachinger and Zeiher, 1995), many others did not show this association (Ludmer et al., 1986, Lavi et al., 2009, Choi et al., 2013); leading to suggestion that vascular SMC function remains intact in spite of progressive endothelial dysfunction in various stages of atherosclerotic disease (Zeiher et al., 1991). In a sense, this is surprising given that vascular SMC has been shown to play a critical role in the pathogenesis of atherosclerosis even at the early stage. We believe the main difference observed above relates to the variability in GTN dosing. Indeed, to date there is no consensus as to what constitute the appropriate GTN dose in the evaluation of coronary endothelial function although majority of researchers seem to use an IC bolus dose of 200-300 microgram. In their IC GTN dose response experiment, Feldman et al. demonstrated that most epicardial coronary vasodilation usually occurred after relatively small doses of IC GTN, with two-thirds of the total dilation detected after 5 μg and three-fourths after the cumulative dose of 55 μg (Feldman et al., 1982). Similar vasodilatory dose response was also shown in a non-invasive experiment, whereby sublingual GTN doses in the aforementioned range produces change in brachial artery diameter and other non-invasive NO mediated endothelial vasomotor parameters, such as radial artery or aorta augmentation index, comparable to that of the endothelium dependent stimuli at healthy subjects (Oliver et al., 2005). Taken together, only relatively small doses of IC GTN are required to produce significant coronary artery

dilatation; hence removing issues such as hypotension, which is not uncommonly seen with the higher doses. Furthermore, higher dose of GTN would theoretically assess the maximum vasodilator capacity (E_{max}), not a more appropriate EC_{50} effect required to correctly assess vascular smooth muscle function.

Several other explanations may also be provided to explain the segmental impairment of GTN mediated vasodilation. In this study, segments with impaired endothelium independent vasoreactivity do not only have greater plaque burden and lipid core but also smaller EEM area compared to the segments which exhibit normal vascular response (12.1 vs 15.6 mm^2). Although this is likely due to plaque-related negative remodelling, it is also plausible that the smaller EEM area may represent reduced baseline vasomotor tone as a reflection of segmental functional remodelling process (Schachinger and Zeiher, 1995). Second, the impairment in GTN vasodilator capacity may also due to vascular NO resistance. Reduced or non-responsiveness to GTN, distinguishable from nitrate tolerance, has been reported in patients with congestive heart failure (Varriale et al., 1991), stable angina, or ACS (Chirkov et al., 2001) and may extend beyond vascular smooth muscle and include platelets. Predominant mechanisms of NO resistance include reduction of soluble guanylate cyclase activity or inactivation of NO by superoxide anion production (Chirkov et al., 2001). Lastly, smooth muscle cell dysfunction secondary to SMC atrophy and/or apoptosis in conjunction with extracellular matrix deposition at the intima occurs in the atherosclerotic process and these architectural changes alone may impact on the GTN vasomotor response (Adams et al., 1998). Taken together, our findings suggest: (1) early in the process of atherogenesis, structural and functional changes are not limited at the endothelium level alone but also involves the vascular SMC; (2) the extent of plaque

burden and to a lesser extent, lipid rich necrotic core, predicts GTN vasodilator capacity irrespective of traditional risk factors; (3) concerning evaluation of the NO mediated effects on the epicardial vascular SMC, irrespective of being endogenous driven (via shear stress or direct conduit effects) or exogenous directed (NO donor), appropriate dosing of vasoactive agent may be critical to achieve the desirable response.

Limitations. Given the invasive nature of our study protocol and the potential for inducing coronary vasospasm, we only performed the endothelial function assessment in the non-culprit artery with normal appearance or mild angiographic disease; hence these findings should be repeated in culprit segments with greater plaque burden. Secondly, due to the constraint and difficulties with conducting true dose response effects in human coronary arteries, only a single dose of salbutamol and GTN were used in this study. Finally, in contrast to relatively well-accepted algorithm for necrotic core identification in VH-IVUS, to date, there is no consensus of what constitute NIRS derived lipid rich plaque. For instance, in their endothelial function analysis, Choi and colleague (Choi et al., 2013) defined lipid core plaque as orange, tan, and yellow on block chemogram, contrary to our LRP definition, which only included tan and yellow colour on block chemogram (Kang et al., 2015).

CONCLUSION

This study further adds to the growing body of evidence linking plaque burden and certain aggressive plaque phenotype, such as lipid rich necrotic core with impaired segmental vascular function. The strong inverse relationship between segmental

endothelium independent function with high-risk plaque feature was a novel mechanistic observation and may offer a proof of concept of a simpler methodology to evaluate coronary endothelial function. Further studies will need to be undertaken to evaluate if a simple readily available drug (IC GTN) can provide incremental risk predictive information regarding focal coronary atheroma identified in the invasive catheterization laboratory.

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Conflict of interest: none declared.

FIGURE LEGENDS

Figure 1. Methodology for grey scale intravascular ultrasound and NIRS analysis. The IVUS pullback was divided into 2-mm segments (denoted by red lines on longitudinal view). Within each segment, plaque, and lumen volumes were calculated upon sequential numbered frames spaced 0.5 mm apart, numbered 1–5 (cross-sectional views).

Figure 2. Near infrared spectroscopy output with the corresponding greyscale image (left). (Top right) Chemogram map of the artery wall indicating the location and intensity of lipid core plaque. (Middle right) Block chemogram displaying the presence of lipid core at 2mm segments in four probability categories (red<orange<tan<yellow). The lipid core burden index (LCBI) of the region of interest (subtended by the blue lines) is 197, with max$(LCBI)_{4mm}$ of 442.

Figure 3. Segmental endothelial dependent and independent vasoreactivity stratified according to different plaque burden tertile. Data are expressed as median.

Figure 4. Segmental endothelial dependent and independent vasoreactivity stratified according to different LCBI tertile. Data are expressed as median.

Figure 5. Representative images and changes to minimal luminal area (MLA) following intracoronary glyceryl trinitrate (GTN) administration. Panel A represents a segment with

high plaque burden/LRP positive with corresponding lack of vasodilator response seen in the same segment to GTN seen in Panel B. Panel C shows a segment with low plaque burden/LRP negative with a strong vasodilator response seen in the same segment to GTN seen in Panel D.

Figure 6. Forest plot summarizing the univariate predictors of (A) segmental coronary endothelium dependent function and (B) endothelium independent function

TABLE 1. Clinical, biochemical, and angiographic characteristics

Clinical Characteristics n =53	
Age	60.1 ± 8.97
Female	23 (43%)
Clinical Presentation:	
Stable (smooth coronaries)	28 (53%)
Stable (with culprit)	9 (17%)
ACS	16 (30%)
Artery:	
Left anterior descending	33 (62%)
Left circumflex	15 (28%)
Right coronary artery	5 (10%)
Current smoking	30 (57%)
Diabetes	10 (19%)
Hypertension	37 (70%)
Hyperlipidaemia	38 (72%)
Medications:	
Aspirin	46 (87%)
ACE inhibitors / ARB's	34 (64%)
Statins	37 (70%)
Calcium channel blocker	9 (17%)
Nitrate	1 (1.9%)
Total cholesterol (mmol/L)	4.54 ± 1.5
Low density lipoprotein cholesterol (mmol/L)	2.89 ± 1.1
High density lipoprotein cholesterol (mmol/L)	1.0 [0.9-1.3]
Triglyceride (mmol/L)	1.3 [0.6-2]
C-reactive protein (mg/L)	3.5 [0.85-9.05]
Brain natriuretic peptide (ng/L)	98 [50-283]

Data are expressed as mean ± SD or median and interquartile range when appropriate

ACS, Acute Coronary Syndrome - defined as troponin positive presentation; ARB Angiotension Receptor Blocker

Table 2. Segmental grey-scale and NIRS data according to the pattern of coronary vasomotor response.

	All segments (N = 849)	% change SLV (SALBUTAMOL)			% change SLV (GTN)		
		Impaired	Normal	P value	Impaired	Normal	P value
Baseline SLV (mm^3)	85.1 [61.1-114]	93.5 [63.7-122.9]	80 [60-106]	0.0005	79.1 [53.2-118.6]	85.6 [63.3-113.4]	0.15
PAV (%)	31.1 [22-38.8]	32 [24.4-39.7]	29.9 [12.4-38.3]	0.0013	35.7 [28.2-46.5]	30 [21.4-37.6]	<0.0001
EEM (mm^2)	15 [11.6-19.1]	15.8 [11.9-19.6]	14.7 [11.3-18.5]	0.13	12.1 [9.4-17.3]	15.6 [12.5-19.4]	<0.0001
LRP (%)	20	23.1	18.1	0.09	31.3	17.8	0.0008
LCBI	0 [0-100]	0 [0-130]	0 [0-83]	0.33	32 [0-214]	0 [0-82]	0.0002

Data are expressed as median [interquartile range].

SLV, Segmental Lumen Volume; PAV, Percent Atheroma Volume; EEM, External Elastic Media; LRP, Lipid Rich Plaque; LCBI, Lipid Core Burden Index

TABLE 3. Multivariate analysis for % change in SLV

Effect	Odds ratio	Lower CL	Upper CL	P value
Stable presentation	0.43	0.2	0.95	0.038
Non smoker	0.48	0.23	1.0	0.05
% change CBF	0.99	0.97	1.0	0.034
PAV*	1.07	1.04	1.10	<0.0001

*Independent predictor for endothelium independent response

CBF Coronary Blood Flow; PAV Percent atheroma volume

FIGURE 1

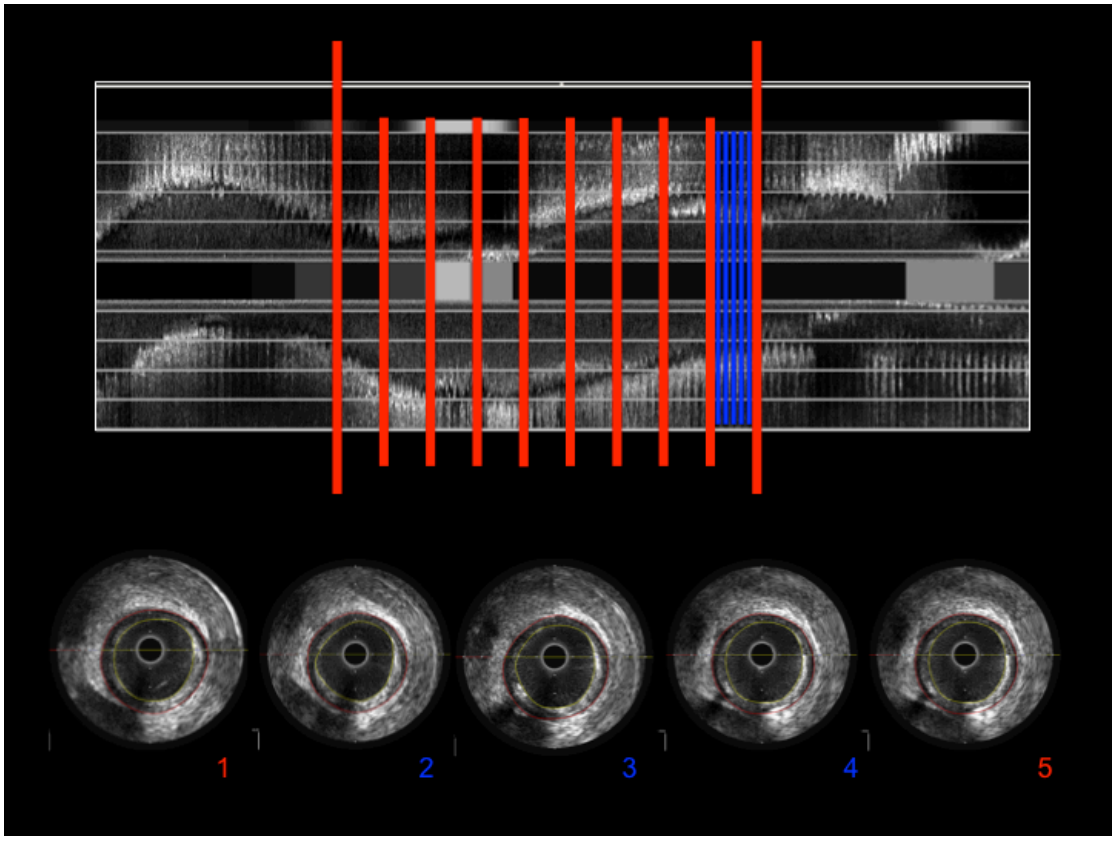


FIGURE 2

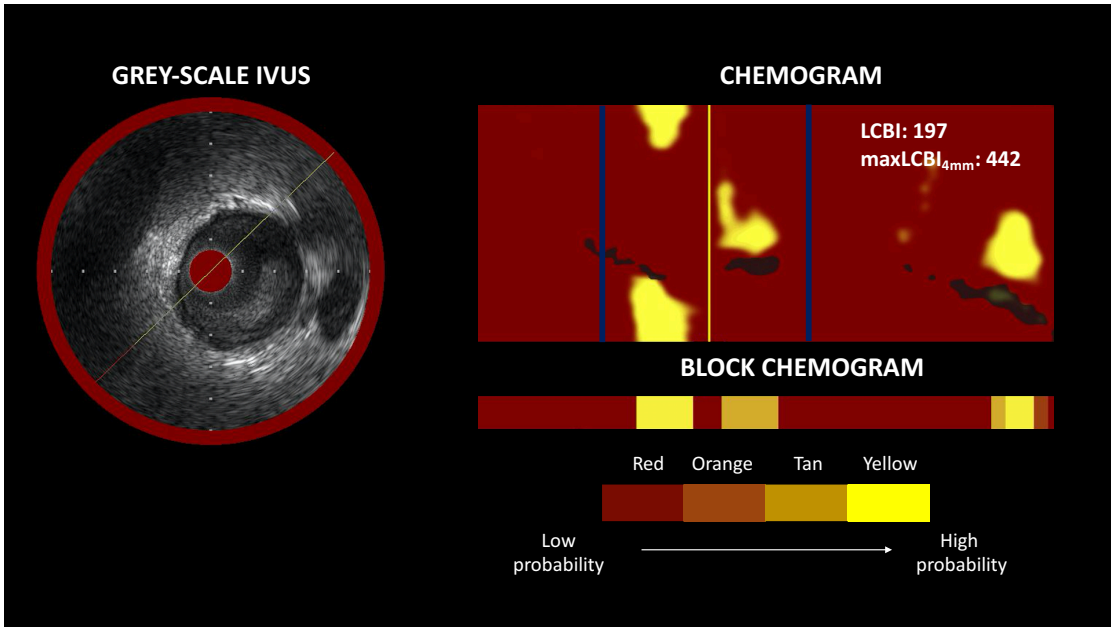

GREY-SCALE IVUS
CHEMOGRAM
LCBI: 197
maxLCBI4mm: 442
BLOCK CHEMOGRAM
Red
Orange
Tan
Yellow
Low probability
High probability

FIGURE 3.

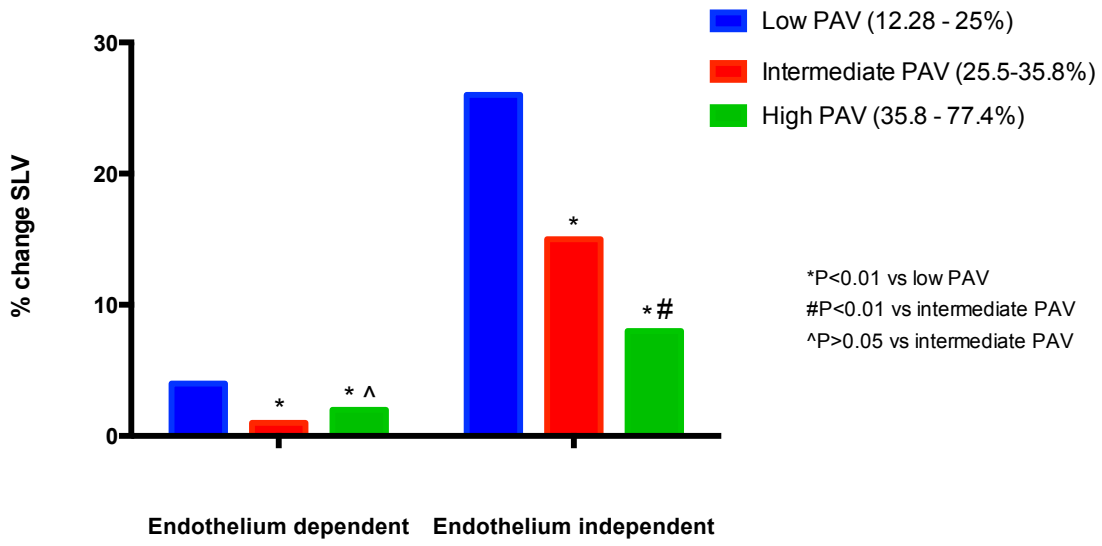
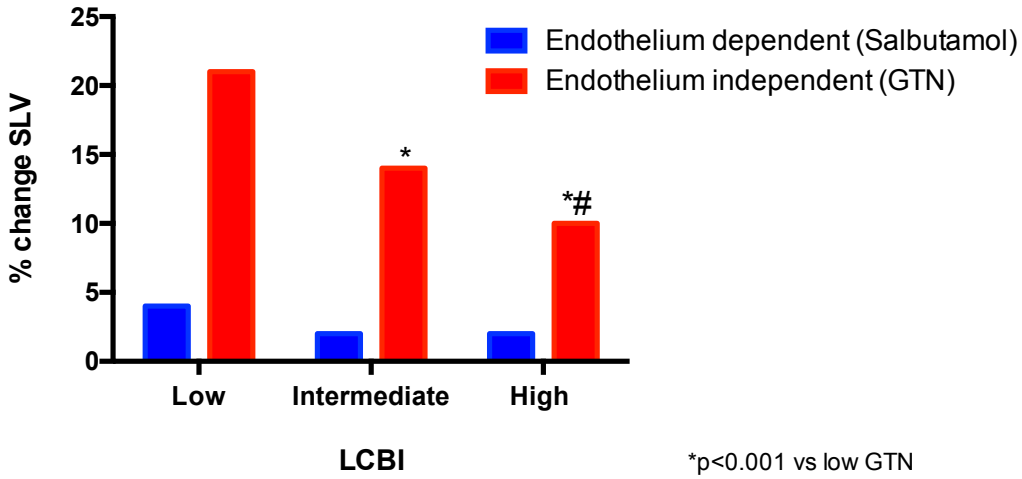

Low PAV (12.28 - 25%)
Intermediate PAV (25.5-35.8%)
High PAV (35.8 - 77.4%)
% change SLV
30
20
10
0
*
* ^
*
*#
*P<0.01 vs low PAV
#P<0.01 vs intermediate PAV
^P>0.05 vs intermediate PAV
Endothelium dependent
Endothelium independent

FIGURE 4.



*p<0.001 vs low GTN
#p<0.05 vs intermediate GTN

Low LCBI: 0-0	Intermediate LCBI: 0-45	High LCBI: 45-717

FIGURE 5.

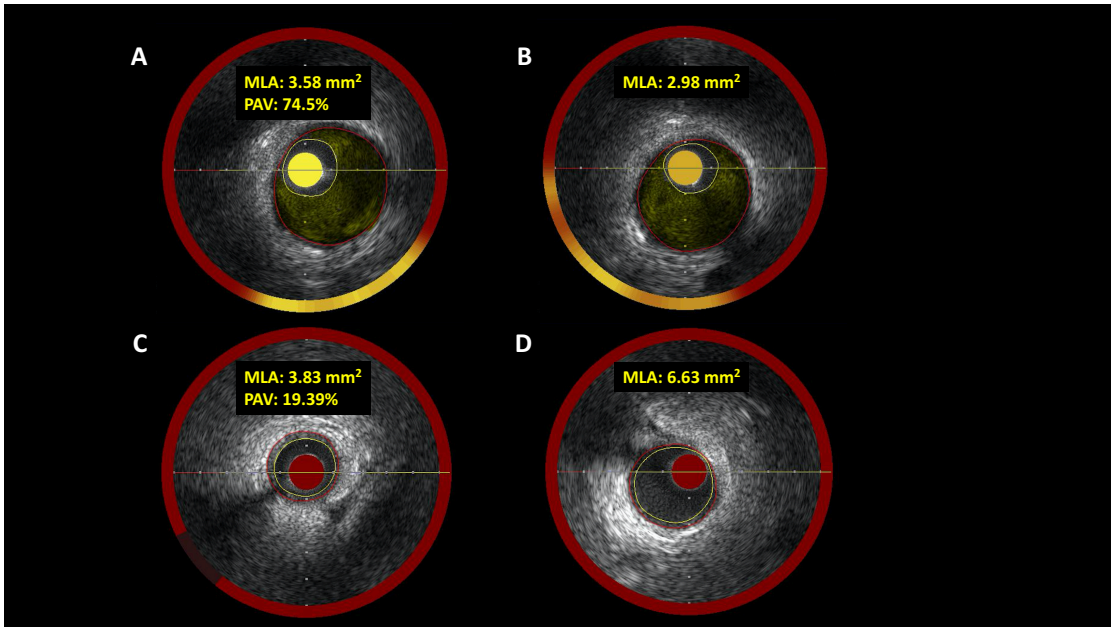
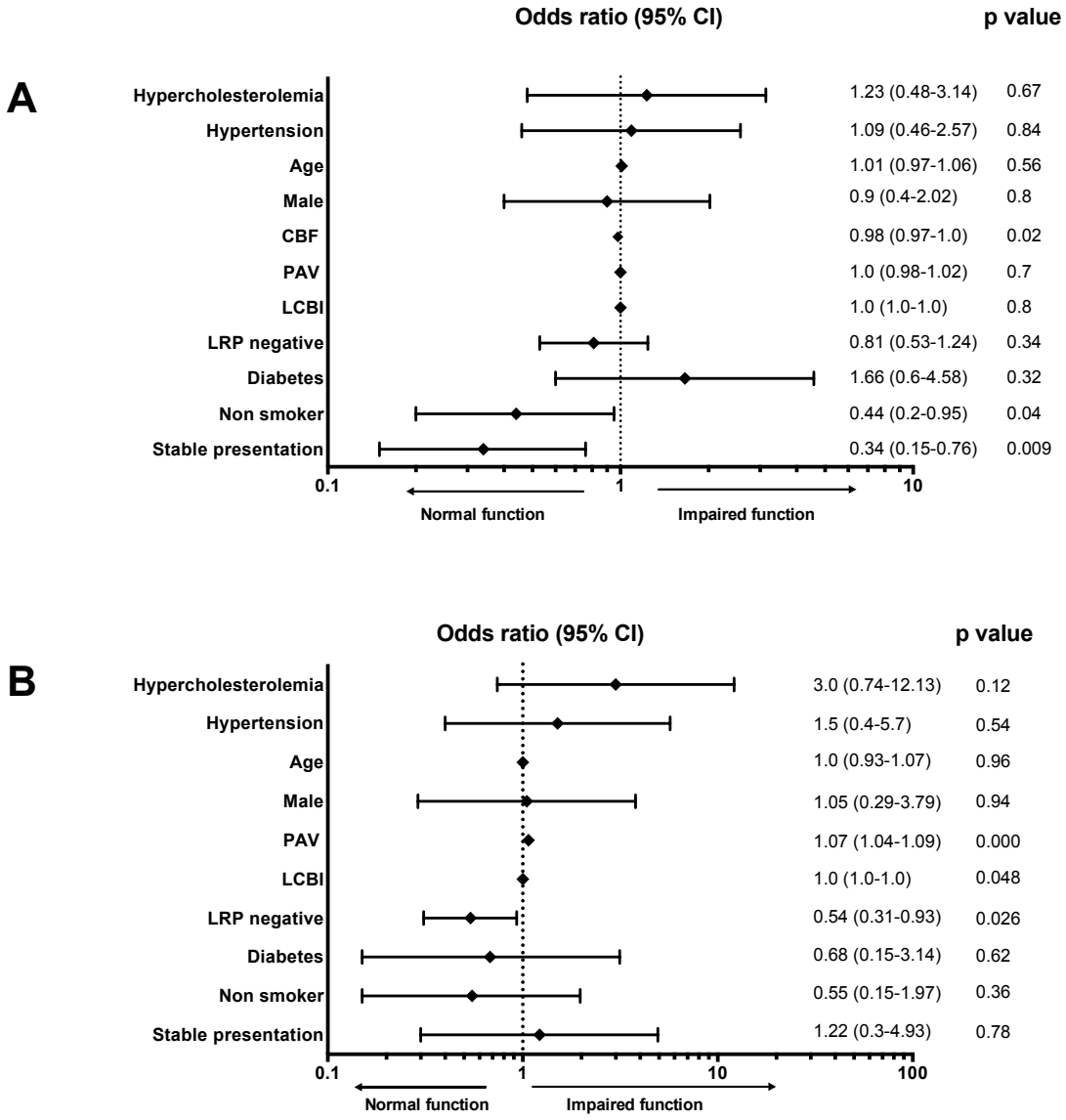

A
MLA: 3.58 mm²
PAV: 74.5%
B
MLA: 2.98 mm²
C
MLA: 3.83 mm²
PAV: 19.39%
D
MLA: 6.63 mm²

FIGURE 6


A
Odds ratio (95% CI)
p value
Hypercholesterolemia
Hypertension
Age
Male
CBF
PAV
LCBI
LRP negative
Diabetes
Non smoker
Stable presentation
1.23 (0.48-3.14)
1.09 (0.46-2.57)
1.01 (0.97-1.06)
0.9 (0.4-2.02)
0.98 (0.97-1.0)
1.0 (0.98-1.02)
1.0 (1.0-1.0)
0.81 (0.53-1.24)
1.66 (0.6-4.58)
0.44 (0.2-0.95)
0.34 (0.15-0.76)
0.67
0.84
0.56
0.8
0.02
0.7
0.8
0.34
0.32
0.04
0.009
0.1
1
10
Normal function
Impaired function
B
Odds ratio (95% CI)
p value
Hypercholesterolemia
Hypertension
Age
Male
PAV
LCBI
LRP negative
Diabetes
Non smoker
Stable presentation
3.0 (0.74-12.13)
1.5 (0.4-5.7)
1.0 (0.93-1.07)
1.05 (0.29-3.79)
1.07 (1.04-1.09)
1.0 (1.0-1.0)
0.54 (0.31-0.93)
0.68 (0.15-3.14)
0.55 (0.15-1.97)
1.22 (0.3-4.93)
0.12
0.54
0.96
0.94
0.000
0.048
0.026
0.62
0.36
0.78
0.1
1
10
100
Normal function
Impaired function

CHAPTER 5: THE INVESTIGATION OF THE RELATIONSHIP BETWEEN SEGMENTAL CORONARY ENDOTHELIAL FUNCTION AND ATHEROSCLEROTIC PLAQUE PROGRESSION/REGRESSION

Adapted from: Sidharta SL, Baillie TJ, Howell S, Nicholls SJ, Montarello N, Honda S, Shishikura D, Delacroix S, Beltrame JF, Psaltis PJ, Worthley SG, Worthley MI. *In vivo* evaluation of human coronary structure-function predicts subsequent progression of coronary atherosclerotic plaques: A Near Infrared Spectroscopy Study (submitted to European Heart Journal).

Keywords: Near infrared spectroscopy, Lipid rich plaque, Endothelial function, Plaque Progression

STATEMENT OF AUTHORSHIP

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Publication Details	Submitted to European Heart Journal

Principal Author

Name of Principal Author (Candidate)	Samuel Sidharta		
Contribution to the Paper	Study design, patient recruitment, analysis, preparation of manuscript		
Overall percentage (%)	85%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	20/1/2017

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

xiii. the candidate's stated contribution to the publication is accurate (as detailed above);

xiv. permission is granted for the candidate in include the publication in the thesis; and

xv. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Signature		Date	20/1/2017

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Contribution to the Paper	Correction and critical review of manuscript		
Signature		Date	20/1/2017

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Contribution to the Paper	Correction and critical review of manuscript		
Signature		Date	20/1/2017

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Signature		Date	20/1/2017

ABSTRACT

Background: Coronary vasodilator function and atherosclerotic plaque progression have both been shown to be associated with adverse cardiovascular events. However, the relationship between these factors and the lipid burden of coronary plaque remains unknown. These experiments focus on investigating the relationship between impaired coronary vasodilator function [endothelium dependent (salbutamol) and endothelium independent (glyceryl trinitrate, GTN)] and the natural history of lipid rich plaque (LRP).

Methods and results: 33 patients with stable chest pain syndrome or acute coronary syndrome underwent serial assessment of coronary vasodilator function and plaque imaging by dual modality intravascular ultrasound (IVUS) and near infrared spectroscopy (NIRS). Coronary segmental macrovascular response [% change segmental lumen volume (ΔSLV)], plaque burden [per cent atheroma volume (PAV)], and lipid core [LRP and lipid core burden index (LCBI)] were measured at baseline and after an interval of 12-18 months (N=520 segments). Coronary segments which develop LRP over the study time period demonstrated greater reduction in vasodilator response to salbutamol (-0.24 ± 2.96 vs 5.60 ± 1.47%, p = 0.04) and GTN (13.91 ± 4.45 vs 21.19 ± 3.19%, p = 0.036), at baseline. By multivariate analysis, reduced baseline coronary endothelium dependent vasodilator function independently predicted increase in lipid core whereas baseline coronary endothelium independent predicted increase on atheroma volume. In the secondary analysis, serial changes in coronary endothelial independent function was associated with changes in plaque volume.

Conclusions: Epicardial coronary vasodilator function is a major determinant of LRP progression irrespective of the nature of clinical presentation.

Keywords: Endothelial function, lipid rich plaque, near infrared spectroscopy, plaque progression.

INTRODUCTION

Coronary heart disease is the leading cause of death in the western world, accounting for 1 in 7 deaths in the United States (Writing Group et al., 2016). This underscores the importance of identifying 'at risk' or vulnerable patients who are likely to develop future adverse cardiac events. The pathogenesis of acute coronary syndrome (ACS) nevertheless is complex and remains to be fully elucidated. Altered vasodilator function or vascular nitric oxide resistance measured in both peripheral (Neunteufl et al., 2000, Yeboah et al., 2009) and coronary circulation (Halcox et al., 2002, Schachinger et al., 2000, Suwaidi et al., 2000) has been shown to be associated with future adverse cardiac events, independent of traditional cardiovascular risk factors. However, the exact mechanism behind how this impacts on clinical events remains uncertain.

In the past few years, data from several prospective trials have identified plaque burden and certain plaque characteristics, such as virtual histology (VH) derived thin cap fibroatheroma and minimum lumen area, as independent predictors of adverse events; which applies to culprit and non-culprit vessels (Stone et al., 2011, Cheng et al., 2014, Calvert et al., 2011, Stone et al., 2012, Nicholls et al., 2010). Utilizing intravascular ultrasound (IVUS), we demonstrated a strong relationship between in-vivo segmental human coronary endothelial dependent conduit function and underlying atheroma burden (Puri et al., 2012a). This coronary structure-function relationship holds true regardless the nature of patient's clinical presentation (Puri et al., 2013).

However, these studies have not evaluated the impact of this dynamic process on the natural history of plaque burden or composition over time. One characteristic of plaque that has gained increasing recent attention as a marker of vulnerability is its lipid

content. Near infrared spectroscopy (NIRS) technology has greatly improved the ability to detect and quantify lipid rich necrotic core in real time and can be acquired at the same time as gray scale IVUS images, using dual modality catheter systems. Studies have shown that lipid rich plaque (LRP) is a key pathological substrate of ACS (Gardner et al., 2008, Waxman et al., 2009). We have undertaken this study, using serial NIRS-IVUS imaging to assess the impact of coronary vasodilator function on the natural history of coronary plaques and their associated lipid content over time.

METHODS

Study design

This study was approved by the Royal Adelaide Hospital Research Ethics Committee and registered with Australian New Zealand Clinical Trials Registry (ACTRN12612000594820). We consecutively enrolled patients (age ≥ 18 years) who were referred to the Royal Adelaide Hospital Cardiac Catheterization Laboratories for diagnostic coronary angiography and/or percutaneous coronary intervention (PCI) with ACS or stable chest pain presentation. Each patient underwent a clinical evaluation and IVUS/NIRS study protocol at baseline and at 12-18 month follow-up. The evaluated artery had to be angiographically "smooth" or containing only minimal disease, defined as <30% visual angiographic stenosis, with no previous percutaneous coronary intervention (PCI) undertaking to it. If a significant angiographic, culprit artery was found, the study artery was a non-culprit vessel. Informed consent was obtained >48 hours prior to index coronary angiography and all vasoactive medications were withheld 24 hours prior to the study. Exclusion criteria included previous surgical coronary revascularization, significant valvular heart disease or left ventricular systolic dysfunction (ejection fraction ≤ 35%), ST elevation myocardial infarction (STEMI),

known predilection to coronary vasospasm, uncontrolled hypertension, significant renal or pulmonary disease, and chronic beta blocker therapy or use of long or short acting β2 agonist within the previous 24 hours.

Coronary vasodilator function testing

Coronary angiography was performed according to standard clinical indication. Upon arterial sheath insertion, 25 mL of blood was collected for measurement of lipid profile, brain natriuretic peptide (BNP) and C-reactive protein (CRP). Lipid profile was also measured at follow-up. Following completion of angiography, coronary endothelial function was evaluated similar to our previously described methodology (Puri et al., 2012a). If PCI to culprit vessel was indicated, this was performed immediately after the endothelial function testing. Intravenous heparin (100 IU/kg) was administered for the research protocol. Briefly, the 3.6F TVC Insight Catheter (InfraReDx, Burlington, Massachusetts, USA) was inserted into the study artery. IVUS was performed according to standard protocol, without glyceryl trinitrate (GTN) pre-treatment of the target artery. Serial 5-minute intracoronary (IC) infusions via the guiding catheter at 2 mL/min were administered in the following sequence: (a) 5% dextrose, (b) salbutamol (0.3 μg/min) to assess the endothelium dependent vasodilation, and (c) bolus injection of GTN (100 mcg) to assess endothelium independent vasodilation. At 3 minute into each IC infusion, we recorded the patient's hemodynamics (blood pressure and heart rate) followed by commencement of IVUS-NIRS pullback at a rate of 0.5mm/s. The infusion was then continued throughout the remaining 2 minutes that it took for IVUS-NIRS acquisition to be completed.

IVUS analyses

All gray scale IVUS data were analysed using echoPlaque 3.0.53 (Indec Systems, Santa Clara, CA, USA) using previous methodology (Puri et al., 2012a). Common distal and proximal fiduciary points (anatomical side branches) were chosen to match baseline and follow-up IVUS runs using ImageJ software (Mintz et al., 2011). Cross sectional images were analysed every 16 frames (0.5mm) and each IVUS pullback was divided into a pre-defined 2-mm segments comprising 5 cross sectional images at 0.5mm apart; the 2-mm segment was selected to allow for co-registration analysis with the NIRS output both at baseline and during follow-up. Frames that precluded complete lumen or vessel wall planimetry were excluded from analysis, as were segments that involved branch points. Manual planimetry of the leading edge of the luminal borders and external elastic membrane (EEM) were performed for each designated frame. Segmental percent atheroma volume (PAV) and percent change in segmental lumen volume (ΔSLV) were used to determine segmental plaque burden and vasomotor response respectively. Segmental PAV was calculated according to the following equation:

$$PAV = \frac{\Sigma\,(EEM_{area} - lumen_{area})_{segment}}{\Sigma\,(EEM_{area})_{segment}} \times 100$$

SLV was calculated as the average of lumen area within that particular segment and normalized as previously reported (Puri et al., 2012a). Remodelling index (RI) was calculated as EEM at follow-up minus EEM at baseline (Mintz et al., 2011).

NIRS analyses

A NIRS algorithm defines a lipid containing plaque as fibroatheroma when the lipid core is $>60^0$ circumferential extent, >200μm thick, and a fibrous cap mean thickness of

<450μm (Gardner et al., 2008). Using this algorithm, the position of lipid core in each scanned artery segment is plotted on a digital map or 'chemogram', whereby the x-axis indicates the pullback position (mm) and the y-axis represents the circumferential position (degree). The probability of lipid core presence is displayed in a chemogram as a colour scale from red (low probability) to yellow (high probability). To enhance interpretation, another NIRS output called block chemogram is generated, which is a summary metric of lipid core presence in 2-mm chemogram intervals; it is classified in 4 probability categories using the top 10^{th} percentiles pixel information (yellow: $P > 0.98$, tan: $0.84 \leq P \leq 0.98$, orange: $0.57 \leq P \leq 0.84$, red: $P < 0.57$). For our primary analysis, we defined segmental LRP as yellow or tan on the block chemogram (Kang et al., 2015). The final NIRS output is lipid core burden index (LCBI), which is the 'quantitative measurement' of lipid output and derived from a fraction of valid yellow pixels within a longitudinal range of the chemogram and scaled from 0-1000. The NIRS images and the block chemogram were analysed off line using LipiScan analyser software (LipiScan, InfraReDx, Burlington, MA, USA).

Statistical analysis

Categorical measures were summarized as counts and percentages; continuous data were summarized as means with standard deviation and medians with interquartile range as appropriate. Differences between baseline and follow-up data were made using Student *t* test for the patients' biochemical and clinical parameters, and mixed models for segmental variables (LCBI, PAV, SLV, EEM). Segments were also stratified according to their baseline LRP (derived from block chemogram) and 'progressor' status as measured by changes in LCBI (△LCBI). Adjusted means were calculated for

each group for ΔSLV, SLV, PAV, EEM, and RI using mixed models, accounting for multiple segments measurements within a patient.

In constructing multivariate predictor model, LRP was treated as a dichotomous measure and analysed using a linear mixed effects model with a binomial distribution and a logit link function. The remaining outcomes were assessed as continuous measures using linear mixed effects models with a Gaussian distribution and an identity link function. Subject was treated as random factor in all models. Multivariable modelling started with a fully saturated model which included all variables with a p-value less than 0.20. Variables were then systematically removed from the model until only significantly variables remained. All tests were two-tailed and assessed at the 5% alpha level. The analyses were completed using SAS v9.3 (SAS Institute Inc., Cary, NC, USA) and GraphPad Prism v6 (La Jolla, CA, USA).

RESULTS

Patient characteristics. Baseline demographics are summarized in table 1. From 4th March 2013 – 12th January 2015, 51 patients were enrolled into the study. Complete follow-up IVUS/NIRS data were obtained from 33 patients, with mean follow-up duration of 15 ± 3.8 months. During baseline imaging, one patient developed procedural complication due to IVUS catheter related clot at the end of the study protocol. The complication resulted in small myocardial infarction with no regional wall motion abnormality detected. This patient was excluded from the analysis. Five patients who were studied at baseline developed adverse events during follow-up, unrelated to study

protocol, with 1 death and 4 target vessel intervention (to the study vessel) due to stable angina (2 patients) and unstable angina (2 patients).

61% patients had smooth coronary arteries and presented with stable chest pain syndrome at baseline and 30% presented with ACS. The left anterior descending (LAD) artery was studied in 58%. The mean length of the coronary vessel evaluated per patient was 37.85 ± 13.10mm (LAD 40.43 ± 14.22mm; LCx 32.42 ± 11.34mm; RCA 41.40 ± 6.32mm). The mean total cholesterol, HDL-C, and triglyceride at follow-up were 3.97 ± 0.24 mmol/L ($p < 0.05$ vs baseline), 1.04 ± 0.04 mmol/L ($p < 0.05$ vs baseline), and 1.06 ± 0.13 mmol/L ($p < 0.01$ vs baseline) respectively, representing an absolute differences of -0.62 ± 1.42, -0.09 ± 0.21, and -0.55 ± 1.17 from baseline. There was no difference in the level of LDL-C (2.74 ± 0.2 vs 2.45 ± 0.16, $p = 0.13$), systolic (129.6 ± 3.71 vs 133.1 ± 3.69, $p = 0.41$), and diastolic blood pressure (77.66 ± 1.76 vs 77.94 ± 1.67, $p = 0.89$) between baseline and follow-up.

Baseline coronary vasodilator function and follow-up LRP. Table 2 summarizes all coronary segments according to its LRP status at baseline. A total of 520 paired 2-mm segments were available for complete baseline and follow-up IVUS and NIRS analysis. Of these, 412 (79%) did not have LRP at baseline. During follow-up, 35 segments (8%) progressed to develop LRP whilst 377 segments remained LRP negative (Figure 1). The coronary segments which eventually progressed demonstrated impaired vasodilator function in response to salbutamol
(-0.24 ± 2.96 vs 5.6 ± 1.47%, $p = 0.04$) and GTN (13.91 ± 4.45 vs 21.19 ± 3.19%, $p = 0.036$) (Figure 2 & 3) as well as greater PAV (34.35 ± 2.01 vs 29.57 ± 1.52%, $p = 0.001$) and LCBI (76.33 [67.48-83.05] vs 19.32 [12.09-26.43], $p = 0.0001$) at baseline.

On the other hand, amongst the segments which contained lipid core at baseline, 40% "healed" during follow-up. When compared with the segments which remained LRP positive at follow-up imaging, these segments were noted to have smaller LCBI (235 [219-291] vs 326 [288-386], p = 0.002) and a statistical trend to smaller PAV (38.6 ± 2.67 vs 42.59 ± 2.51%, p = 0.097) at baseline. There was no statistical difference in the baseline vasomotor response between LRP segments which healed compared to those that did not (salbutamol: 5.57 ± 2.77 vs 4.95 ± 2.63%, p = 0.79; GTN: 14.05 ± 3.39 vs 9.88 ± 3.03%, p = 0.235) (Figure 2).

The univariate predictors of follow-up LRP are summarized in figure 4. Every 5-unit reduction in baseline salbutamol-mediated vasomotor (SMV) response was associated with 11% increase in the odds of developing positive LRP during follow-up. On multivariate analysis, only the baseline LCBI (OR: 1.24, 95% CI (1.17 to 1.31), p < 0.0001) and changes in LCBI (OR: 1.29, 95% CI (1.22 to 1.37), p < 0.0001) were independent predictors of follow-up LRP.

Baseline coronary vasodilator function, changes in LCBI, and changes in PAV. Table 3 summarizes the mean and median of segmental LCBI and PAV at baseline and follow-up. When the groups were dichotomized into 'non-progressor' (Δ LCBI ≤ 0) vs 'progressor' (Δ LCBI > 0) on the basis of changes in LCBI, the 'progressor' group was noted to have smaller baseline lumen and EEM, and greater PAV and remodelling index (Fig 5). Furthermore, there was no difference in the baseline vasoreactive response between the two groups. In the multivariable prediction analysis however, reduced baseline SMV response (β coefficient: -3.03, 95% CI (-5.81 to -0.25), p = 0.033) was

associated with greater changes in LCBI as were segmental LCBI (β coefficient: -5.10, 95% CI (-5.75 to -4.46), $p < 0.001$), PAV (β coefficient: 12.65, 95% CI (7.80 to 17.51), $p < 0.001$) and remodelling index (β coefficient: 6.36, 95% CI (0.77 to 11.95), $p = 0.026$) (Table 4).

No relationship between changes in PAV and changes in LCBI was seen (β coefficient -0.35, 95% CI (-2.24 to 1.55), $p = 0.721$). In the multivariate analysis, changes in PAV was significantly associated with baseline GTN-mediated vasoreactive function whereby every 5-unit increase in vasodilation was associated with an 0.21-unit increase in mean changes in PAV. Similarly, changes in PAV was associated with baseline LCBI (β coefficient: 0.07, 95% CI (0.04 to 0.10), $p < 0.0001$) (Table 5).

Changes in coronary vasodilator function, changes in LCBI, and changes in PAV. Due to the disparity in the manner baseline coronary endothelial dependent and independent function affecting either plaque composition or atheroma volume progression, we performed secondary analysis to determine whether these relationships were indeed related to the predictive nature of coronary endothelial function or merely an associated phenomenon. Complete serial coronary endothelial function at baseline and follow-up were available in 25 out of 33 patients (454 segments). Following adjustment for baseline coronary vasoreactive response, lumen volume, plaque burden, and segmental LCBI; changes in LCBI was not associated with either changes in serial vasomotion in response to salbutamol (β coefficient: -0.65, 95% CI (-1.36 to 0.05), $p = 0.07$) or GTN (β coefficient: -0.2, 95% CI (-0.75 to 0.35), $p = 0.477$). Changes in PAV, on the other hand, was significantly associated with changes in serial vasoreactivity in

response to GTN (β coefficient: -0.06, 95% CI (-0.09 to -0.02), p = 0.001) but not salbutamol (β coefficient: 0.01, 95% CI (-0.03 to 0.05), p = 0.671).

DISCUSION

The salient findings of this serial IVUS-NIRS study were: (i) epicardial coronary endothelium dependent vasodilator function is a major determinant and predictor of LRP progression; (ii) serial changes in coronary GTN vasomotor response is associated with atherosclerotic burden progression but not changes in plaque composition; (iii) the natural history of coronary atherosclerosis *in vivo* is dynamic with potential to progress or heal.

Coronary vasodilator function and atherosclerotic plaque progression. Coronary endothelium vasodilator dysfunction has been proposed as the key component in the pathophysiology of ACS given its association with various traditional risk factors of atherosclerosis (Zeiher et al., 1995, Drexler and Zeiher, 1991), unstable clinical presentation (Bogaty et al., 1994), and future adverse cardiac events (Halcox et al., 2002, Schachinger et al., 2000, Suwaidi et al., 2000). In spite of this, the mechanistic links which provide plausible explanation between altered vascular function and clinical events have not been fully elucidated. Several investigators, including us have demonstrated that coronary vasoreactivity in response to either acetylcholine, salbutamol, or GTN is significantly impaired in coronary segments which exhibit 'vulnerable plaque' features, such as high plaque burden (Puri et al., 2012a, Schachinger and Zeiher, 1995), necrotic core (Lavi et al., 2009), and inflammation (Choi et al., 2014). Others have shown that nitric oxide resistance is also associated with increased platelet aggregation (Chirkov et al., 2001) and reduction in the acute

fibrinolytic activity (Newby et al., 1999). Collectively, these findings provide a rationale linking altered nitric oxide mediated vascular function with adverse cardiac events. However, these studies lacked serial evaluation of these surrogate markers to validate the effects of vasomotor function on plaque biology over time. In this present study, we demonstrated for the first time that in the 'near normal' non-culprit artery of the patients who present with either stable chest pain syndrome or ACS, impaired endothelium dependent vasodilator function is an independent predictor of atherosclerotic LRP progression, irrespective of the nature of their clinical presentation. Importantly, coronary endothelial function remained an independent predictor of disease progression even after controlling for other 'vulnerable plaque' features, such as plaque burden, LCBI, and remodelling index. These findings therefore would support the notion that coronary endothelium dependent vasodilator dysfunction occurs early in the process of atherogenesis and play a crucial role in the acceleration of atheroma necrotic core formation and progression.

Coronary endothelium independent vasoreactivity, on the other hand, is not associated with plaque composition evolution but with plaque volume progression in a direct, linear fashion. Of note, we did not observe any association between coronary SMV and atheroma burden progression. Previous reports have documented that the greatest degree of plaque progression tend to occur in subjects with the least amount of plaque at baseline (Nicholls et al., 2010, Nicholls et al., 2006). Second, PAV, which is commonly accepted as the primary determinant of plaque burden in various randomized trials (Mintz et al., 2011), expresses atheroma volume in proportion to the EEM volume (Nicholls et al., 2007). Consequently, the association between endothelium independent response and changes in plaque volume in this study may in fact reflect the degree of

underlying plaque burden and arterial remodelling, leading to increased vessel wall stiffness. To explore this interaction further and to establish whether this coronary structure-function relationship is of predictive nature or merely an associated phenomenon, we conducted a secondary analysis on a subset of patients who had complete baseline and follow-up endothelial function assessment. We discovered that serial changes in GTN-mediated vasoreactive response were inversely correlated with plaque burden progression, not with plaque composition evolution, suggesting that coronary endothelium dependent vasodilator function mediates the progressive activity of atherosclerosis; the relationship between plaque growth and coronary endothelium independent vasomotor response however seems to be an associated phenomenon.

Dynamic nature of coronary atherosclerosis. Data from serial VH-IVUS studies have shed some light on the dynamic nature of coronary lesions (Kubo et al., 2010). Consistent with this observation, we found that coronary segments with certain environmental atheroma milieu demonstrate propensity to progress or heal. Yet, it is worth commenting that the trajectory of the plaque compositional changes in this present study does not seem to correlate with the changes in atheroma thickness. This finding is indeed intriguing in light of the previous statin trials which showed that regression of plaque volume tends to be accompanied by the change in plaque composition (Hong et al., 2009). Several possible conjectures may be provided to explain these findings. First, this study is a non-interventional, natural history observational study. Not surprisingly, the change in plaque volume is minimal and may not be substantial enough to detect the association signal with the corresponding plaque characteristics. Furthermore, plaque compositional change tends to precede the change in atheroma burden (Dohi et al., 2015). Indeed, pathology data indicated that plaque

growth occurs as a result of recurrent events of plaque disruption followed by healing (Mann and Davies, 1999); a process downstream to necrotic core formation, which is characterized by non-resolving inflammation, macrophage infiltration, and foam and smooth muscle cells (SMC) apoptosis (Virmani et al., 2000). Incidentally, NIRS LRP positive lesions are not only known to contain rich free cholesterol monohydrate and cholesterol ester but also heightened inflammatory and apoptotic cellular activity within the plaque and fibrous cap (Virmani, 2011, Patel et al., 2013). Lastly, the disparity in the plaque growth-characteristic relationship may reflect the dynamic interplay between the coronary endothelium-SMC complex in mediating atherosclerotic progression as demonstrated by the varying coronary endothelium dependent and independent response shown in this study.

Clinical implications. NIRS has emerged as an attractive intravascular imaging option in clinical setting due to its ability in detecting lipid component of fibroatheroma, which may yield important prognostic information (Oemrawsingh et al., 2014). Hence, serial NIRS imaging may be useful in generating novel clinical surrogate marker in the same manner as serial IVUS has done in the past. Although our study is not designed or powered to detect hard clinical endpoint, its results have provided some rationale explaining the mechanistic relationship between coronary vasoreactive response and the natural history of atherosclerosis. We postulate that ‘near-normal’ coronary segments with impaired endothelial function and LRP are at higher risk to progress to flow limiting stenosis, plaque rupture, and increased clinical events. All in all, the use of coronary structure-function assessment may serve as an important prognostic tool to identify segments with early accelerated and progressive atherosclerosis, and as a means to monitor disease activity (Kitta et al., 2009) above and beyond traditional

cardiovascular risk factor score. However, its widespread clinical use would solely depend on the validation of the findings of this invasive study with its non-invasive imaging modalities equivalent.

Limitation. Some limitations of this study need to be acknowledged. First, the relatively small number of patients and adverse cardiac events did not allow us to evaluate whether coronary vasodilator function or changes in LCBI relate to clinical outcomes. Second, given the invasive nature of our study protocol and the potential for inducing coronary vasospasm, we only performed the endothelial function assessment in the non-culprit artery with normal appearance or mild angiographic disease and hence these findings should not be extrapolated to segments with greater plaque burden or culprit lesions. Thirdly, due to time limitations with an invasive protocol, only a single dose of salbutamol and GTN were used in this study, where a formal dose assessment would have been more appropriate. Fourth, we only included patients who are clinically stable given that all vasoactive agents, including anti anginals such as beta-blocker and nitrate therapy needed to be withheld prior to the procedure; in this way, allowing for a potential selection bias to a more stable cohort.

CONCLUSION

This study adds to the growing body of evidence linking coronary vasodilator function with adverse cardiac events by demonstrating the association between baseline coronary vasoreactive response with lipid content and plaque volume progression. Furthermore, in the context of dynamic nature of coronary atherosclerosis, LCBI may provide an interesting prognostic and diagnostic tool to identify patients who are at increased risk of future adverse cardiovascular events. Larger clinical studies are needed to explore

whether changes in LCBI over time may be used as surrogate for hard clinical endpoints.

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Conflict of interest: none declared

FIGURE LEGENDS

Figure 1. Changes in plaque characteristics evaluated by NIRS between baseline and follow-up. (Red = LRP negative; Yellow = LRP positive)

Figure 2. Relationship between baseline coronary vasodilator function and changes in lipid rich plaque (LRP) between baseline and follow-up. (Top) Segments which progress to contain LRP exhibit greater vasodilator dysfunction when compared to those which remains LRP negative at follow-up. (Bottom) 40% of segments with LRP at baseline "healed" during follow-up and demonstrated no significant difference in the baseline vasodilator function. SLV, segmental lumen volume; *$p < 0.05$; #$p > 0.20$

Figure 3. Representative Images of Serial IVUS/NIRS at Baseline and Follow-Up. At baseline, this negative LRP segment had blunted coronary endothelium-dependent vasoreactive response. At follow-up imaging, it "progressed" to become LRP positive. MLA, minimum lumen area; PAV, percent atheroma volume.

Figure 4. Forrest plot summarizing the univariate predictors of LRP at follow-up.

Figure 5. Comparison between lipid progression (ΔLCBI > 0) versus non-progressor (ΔLCBI ≤ 0). SLV, segmental lumen volume; PAV, percent atheroma volume; EEM, external elastic media; RI, remodelling index (calculated as EEM at follow-up - EEM at baseline).

Figure 1.

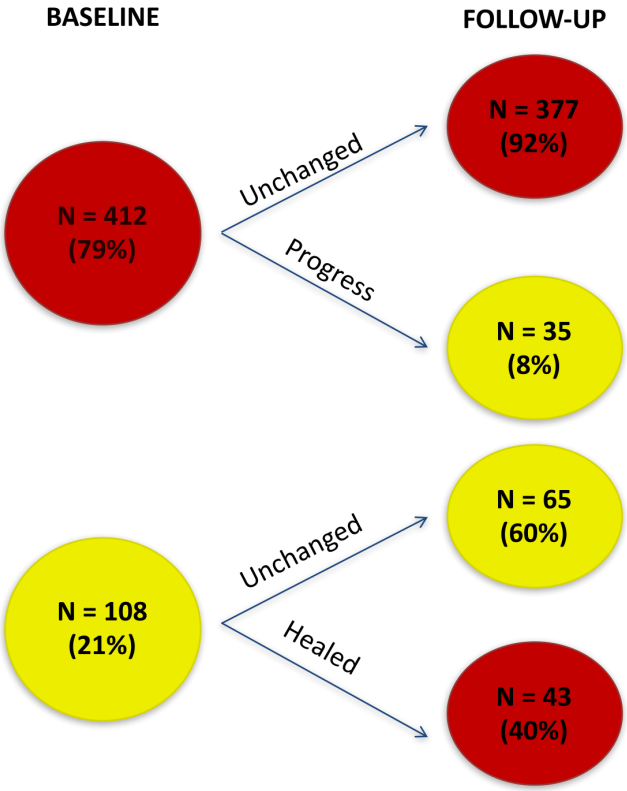
BASELINE
FOLLOW-UP
N = 412
(79%)
Unchanged
N = 377
(92%)
Progress
N = 35
(8%)
N = 108
(21%)
Unchanged
N = 65
(60%)
Healed
N = 43
(40%)

Figure 2.

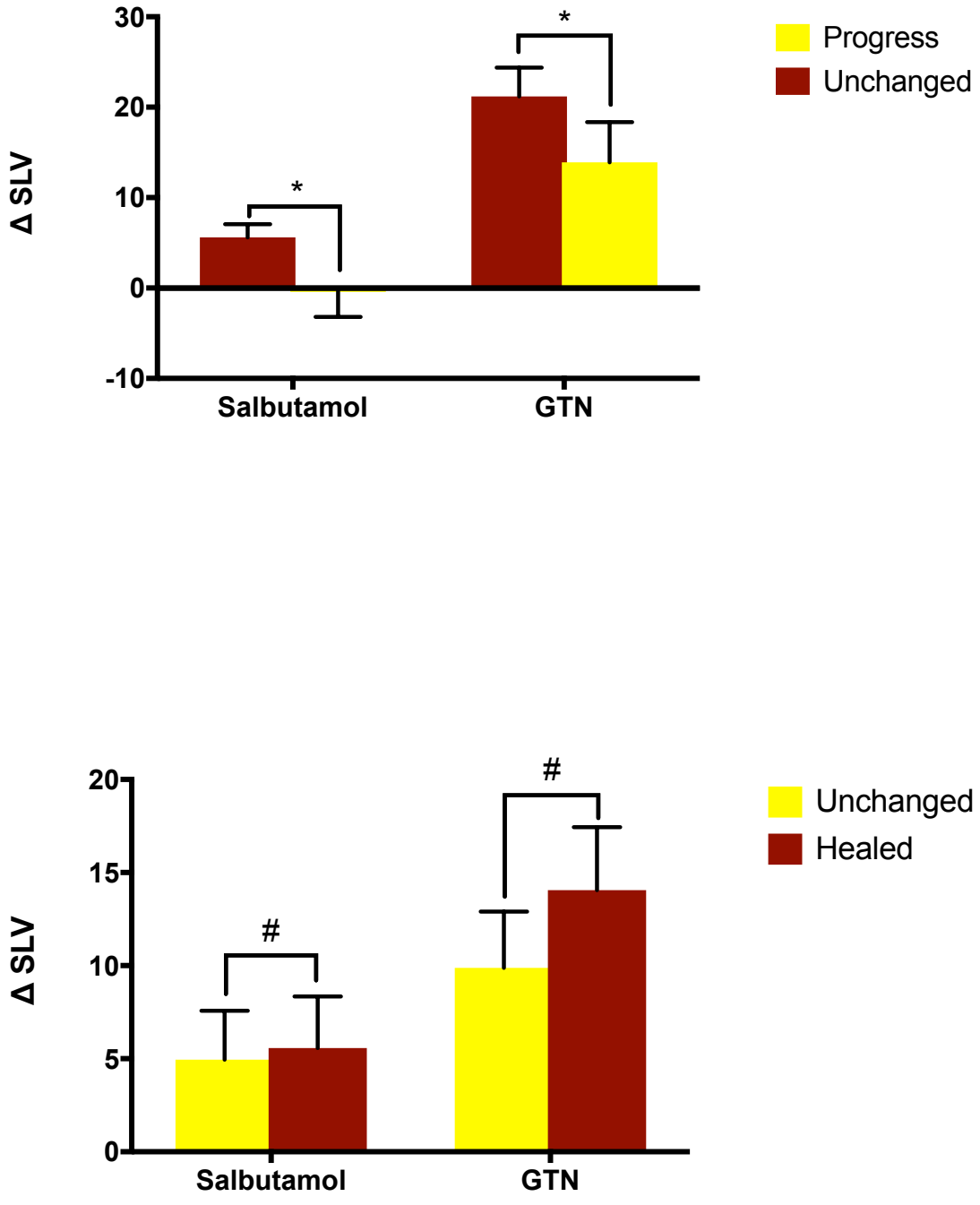
30
20
10
0
-10
Δ SLV
*
*
Salbutamol
GTN
Progress
Unchanged
20
15
10
5
0
Δ SLV
#
#
Salbutamol
GTN
Unchanged
Healed

Figure 3.

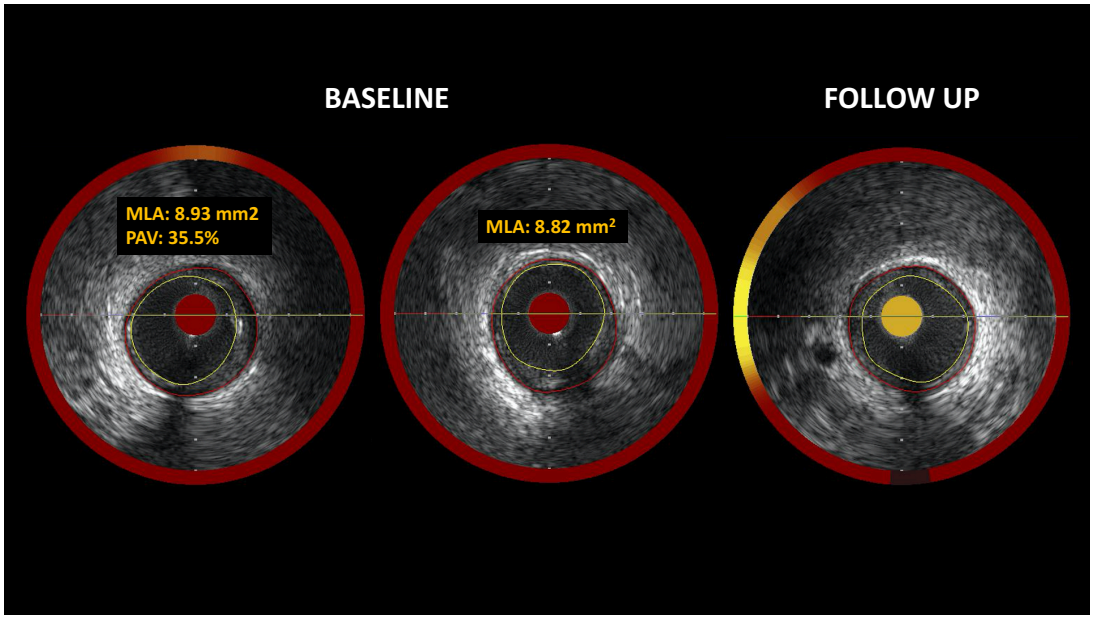

Figure 4.

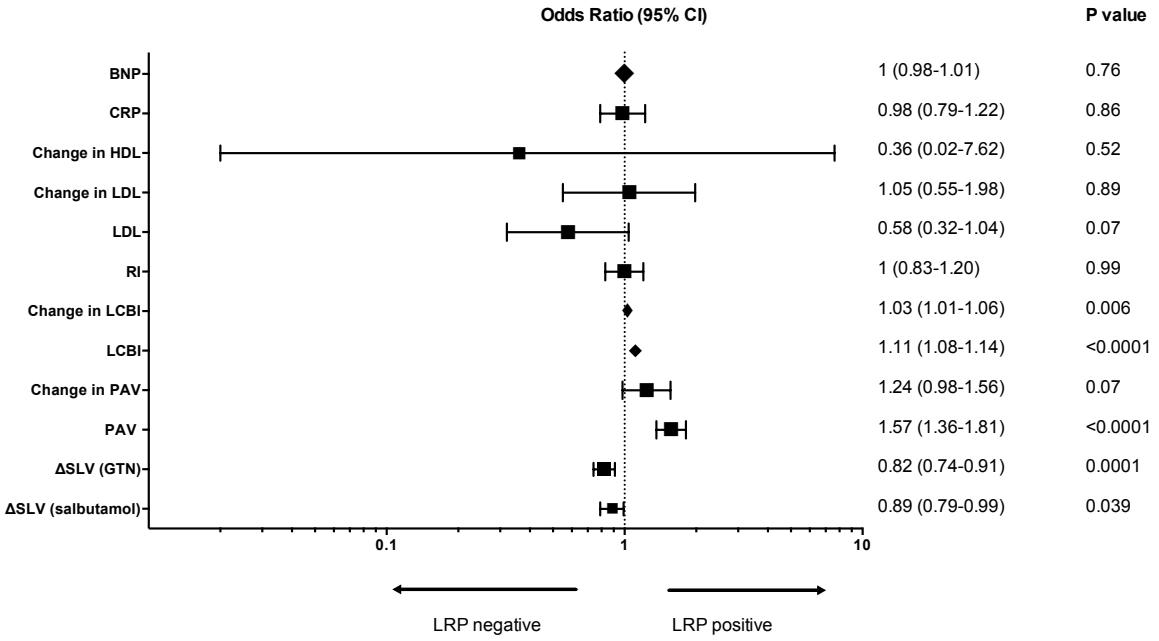
Odds Ratio (95% CI)
P value
BNP
CRP
Change in HDL
Change in LDL
LDL
RI
Change in LCBI
LCBI
Change in PAV
PAV
ΔSLV (GTN)
ΔSLV (salbutamol)
1 (0.98-1.01)
0.98 (0.79-1.22)
0.36 (0.02-7.62)
1.05 (0.55-1.98)
0.58 (0.32-1.04)
1 (0.83-1.20)
1.03 (1.01-1.06)
1.11 (1.08-1.14)
1.24 (0.98-1.56)
1.57 (1.36-1.81)
0.82 (0.74-0.91)
0.89 (0.79-0.99)
0.76
0.86
0.52
0.89
0.07
0.99
0.006
<0.0001
0.07
<0.0001
0.0001
0.039
0.1
1
10
LRP negative
LRP positive

Figure 5.

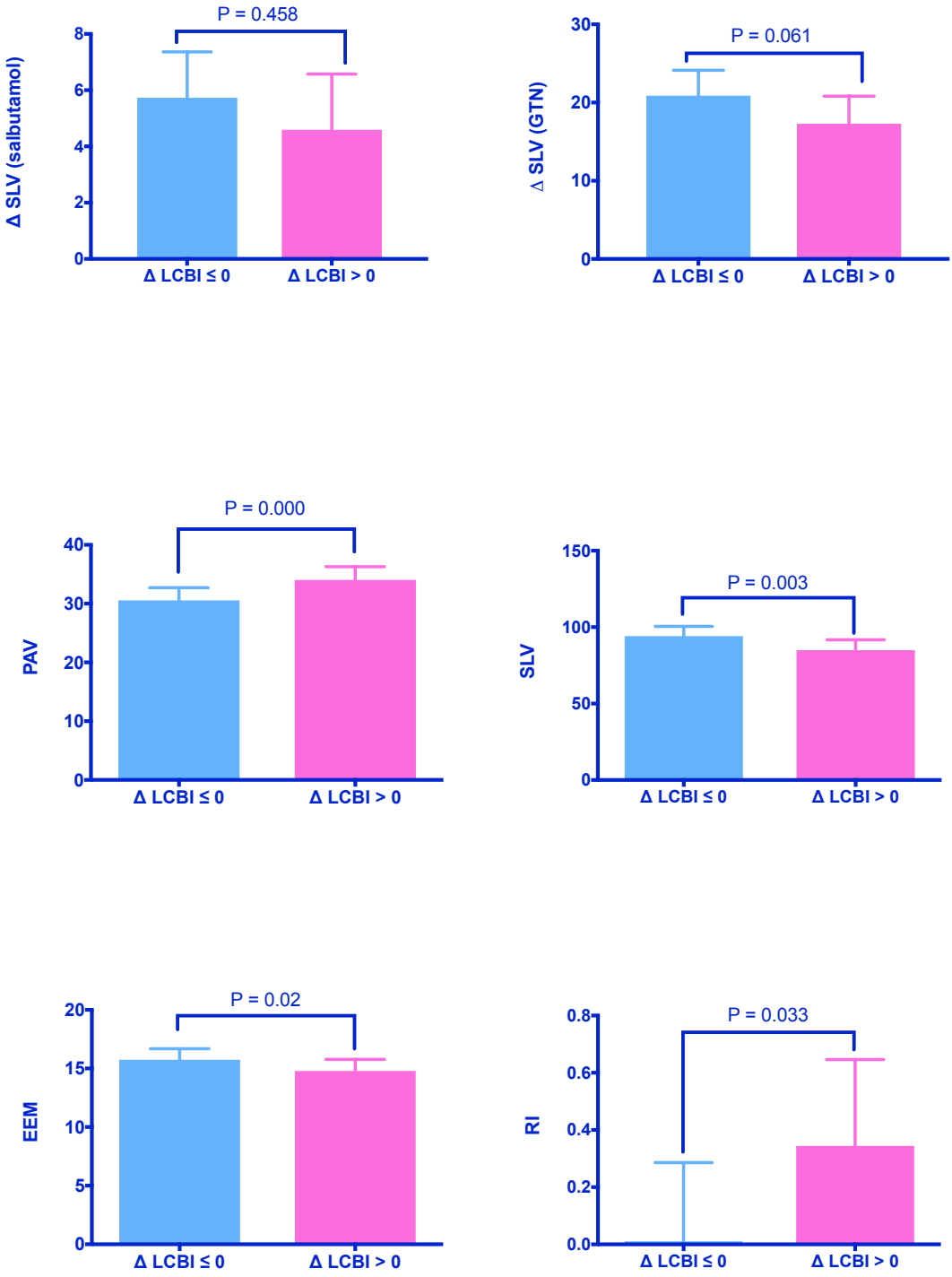
P = 0.458
Δ SLV (salbutamol)
8
6
4
2
0
Δ LCBI ≤ 0
Δ LCBI > 0
P = 0.061
Δ SLV (GTN)
30
20
10
0
Δ LCBI ≤ 0
Δ LCBI > 0
P = 0.000
PAV
40
30
20
10
0
Δ LCBI ≤ 0
Δ LCBI > 0
P = 0.003
SLV
150
100
50
0
Δ LCBI ≤ 0
Δ LCBI > 0
P = 0.02
EEM
20
15
10
5
0
Δ LCBI ≤ 0
Δ LCBI > 0
P = 0.033
RI
0.8
0.6
0.4
0.2
0.0
Δ LCBI ≤ 0
Δ LCBI > 0

Table 1. Clinical, angiographic, and biochemical characteristics.

Clinical Characteristics n = 33 patients	
Age	60.8 ± 9.13
Female	14 (42%)
Clinical Presentation: Stable (smooth coronaries) Stable (with culprit) ACS	 20 (61%) 3 (9%) 10 (30%)
Artery: Left anterior descending Left circumflex Right coronary artery	 19 (58%) 11 (33%) 3 (9%)
Current smoking	20 (60%)
Diabetes	8 (24%)
Hypertension	25 (76%)
Hyperlipidaemia	25 (76%)
Medications: Aspirin ACE inhibitors / ARB's Statins Calcium channel blocker Nitrate	 28 (85%) 23 (70%) 24 (73%) 5 (15%) 1 (3%)
Total cholesterol (mmol/L) Low density lipoprotein cholesterol (mmol/L) High density lipoprotein cholesterol (mmol/L) Triglyceride (mmol/L) C-reactive protein (mg/L) Brain natriuretic peptide (ng/L)	4.6 [3.6-5.0] 2.60 [2.2-3.4] 1.1 ± 0.3 1.6 ± 1.03 4.6 [0.8-9.2] 98 [50-254]

Data are expressed as mean ± SD or median [interquartile range] when appropriate. ACS, Acute Coronary Syndrome - defined as troponin positive presentation; ACE Angiotension Converting Enzyme; ARB Angiotension Receptor Blocker

Table 2. Changes of Near Infrared Spectroscopy (NIRS) findings between baseline and follow-up.

LRP NEGATIVE (N = 412)	**Persist (N = 377)**	**Progress (N = 35)**	**P value**
SLV (mm^3)	93.86 ± 6.45	84.93 ± 8.12	0.104
PAV (%)	29.57 ± 1.52	34.35 ± 2.01	0.001
RI	0.15 ± 0.28	0.41 ± 0.37	0.32
EEM (mm^2)	15.58 ± 0.94	14.24 ± 1.14	0.06
LCBI	19.32 [12.09-26.43]	76.33 [67.48-83.05]	0.0001
LRP POSITIVE N = 108	**Persist (N = 65)**	**Healed (N = 43)**	**P value**
SLV (mm^3)	74.38 ± 7.32	79.01 ± 7.54	0.357
PAV (%)	42.59 ± 2.51	38.6 ± 2.67	0.097
RI	-0.09 ± 0.4	-0.08 ± 0.42	0.99
EEM (mm^2)	14.45 ± 1.21	14.32 ± 1.24	0.87
LCBI	326 [288-386]	235 [219-291]	0.002

Data are expressed as mean ± SEM or median [interquartile range]

LRP, lipid rich plaque; SLV, segmental lumen volume; PAV, percent atheroma volume; RI, remodelling index; EEM, external elastic media; LCBI, lipid core burden index; RI, Remodelling index

Table 3. IVUS and NIRS data at baseline and follow-up

		Baseline	Follow-up	p-value
LCBI	Mean	83 ± 13.47	69 ± 13.47	0.074
	Median	0 [0-113.5]	0 [0-85]	
	Change	-13.31 ± 7.45 (mean)	0 [-25.75-0] (median)	
PAV (%)	Mean	31.11 ± 1.77	32.13 ± 1.77	0.068
	Median	31.5 [22.59-39.68]	31.4 [23.14-40.50]	
	Change	1.02 ± 0.56 (mean)	0.66 [-2.01-3.87] (median)	
EEM (mm^2)	Mean	15.54 ± 0.94	15.61 ± 0.94	0.73
SLV (mm^3)	Mean	92.17 ± 6.48	92.57 ± 6.52	0.82

Value are expressed as mean ± SEM or median [interquartile range]

SLV, segmental lumen volume; PAV, percent atheroma volume; EEM, external elastic media; LCBI, lipid core burden index.

Table 4. Baseline predictors of the change in LCBI in 2-mm coronary segments.

Effect	Univariate				Multivariate			
	Estimate	*Lower 95% CL*	*Upper 95% CL*	*p*				
ΔSLV (GTN)	-0.84	-3.45	1.77	0.529				
LDL-C	-2.12	-22.11	17.87	0.835				
Change LDL-C^	5.53	-17.54	28.59	0.639				
Change HDL-C^	-102.90	-207.5	1.69	0.054				
CRP	0.70	-7.67	9.06	0.870				
BNP	0.18	-0.44	0.80	0.566				
Total cholesterol	3.03	-13.16	19.22	0.714	*Estimate*	*Lower 95% CL*	*Upper 95% CL*	*p*
ΔSLV (salb)	-3.17	-6.57	0.23	0.069	-3.03	-5.81	-0.25	0.033
LCBI	-4.35	-4.95	-3.75	0.000	-5.10	-5.75	-4.46	0.000
PAV	-4.77	-9.95	0.41	0.072	12.65	7.80	17.51	0.000
RI^	6.74	-0.06	13.54	0.053	6.36	0.77	11.95	0.026
Clinical presentation	-26.15	-73.51	21.22	0.289	-36.63	-81.0	7.75	0.106

SLV, segmental lumen volume; PAV, percent atheroma volume; LCBI, lipid core burden index; RI, remodelling index; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; CRP, c-reactive protein; BNP, brain natriuretic peptide; GTN, glyceryl trinitrate. ^variable incorporating baseline and follow-up data

Table 5. Baseline predictors of the change in PAV in 2-mm coronary segments.

Effect	Univariate				Multivariate			
	Est	*Lower CL*	*Upper CL*	*p*				
ΔSLV (salb)	0.04	-0.11	0.20	0.581				
PAV	-0.1	-0.30	0.19	0.657				
RI^	0.08	-0.24	0.39	0.634				
LDL	0.11	-0.85	1.06	0.829				
Change in LDL^	0.48	-0.63	1.58	0.398				
Change in HDL^	-0.8	-5.96	4.38	0.765				
CRP	-0.03	-0.43	0.37	0.875				
BNP	-0.02	-0.05	0.01	0.212				
Total chol	0.06	-0.71	0.84	0.872	*Est*	*Lower CL*	*Upper CL*	*p*
ΔSLV (GTN)	0.18	0.06	0.30	0.004	0.21	0.09	0.33	0.0005
LCBI	0.06	0.03	0.10	0.0002	0.07	0.04	0.10	<0.0001
Clinical presentation	1.12	-1.25	3.49	0.353	1.03	-1.51	3.58	0.426

SLV, segmental lumen volume; PAV, percent atheroma volume; LCBI, lipid core burden index; RI, remodelling index; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; CRP, c-reactive protein; BNP, brain natriuretic peptide; GTN, glyceryl trinitrate. ^variable incorporating baseline and follow-up data

CHAPTER 6: CONCLUSION AND FUTURE DIRECTIONS

We performed a number of experiments utilizing OCT, IVUS, and NIRS in order to: (i) evaluate the fundamental relationship between coronary atheroma volume and composition with segmental coronary vasoreactive function *in vivo* and, (ii) to assess the impact of this dynamic relationship on coronary atherosclerosis progression over time. The key findings of these investigation are summarized below:

- Salbutamol-mediated epicardial coronary vasoreactivity is dependent on shear stress generated by the coronary microvascular driving force and further influenced by the conduit lumen size. Owing to this observation, careful consideration needs to be given when evaluating human coronary endothelial function using OCT.
- In coronary endothelial function assessment, adequate dosing of coronary vasoactive agent of choice is critical to achieve appropriate, accurate, and desirable conduit response, irrespective of whether assessing coronary endothelial dependent or independent vasomotor response.
- In the cross sectional analysis, atheroma plaque volume inversely correlates with segmental coronary epicardial endothelial independent function irrespective of patients' systemic risk factors and their clinical presentation. Furthermore, certain 'vulnerable' plaque phenotype, such as lipid rich necrotic core appears to show an inverse association with coronary endothelial independent function.
- In contrast to the previously validated studies, we found a lack of association between segmental coronary endothelial dependent function (in response to salbutamol) with either atheroma plaque volume or lipid rich plaque burden. We

suggest the reason for this observation is more related to dosing issue rather than innate biological interaction.

- In our serial imaging study, we observed the dynamic nature of the natural history of human coronary atherosclerosis whereby some atheroma plaque appears to progress and evolve into plaque with necrotic, lipid laden core, whilst others 'healed'. This transformation seems to occur at the plaque composition rather than the atheroma volume.
- Irrespective of patients' clinical presentation and baseline demography, coronary endothelial function predicts progression of atheroma plaque in a unique manner. Whereas baseline coronary endothelial dependent function predicts lipid content progression, segmental endothelial independent vasoreactivity predicts atheroma volume progression.
- The presence of NIRS derived lipid rich plaque, measured quantitatively with LCBI within coronary segments at baseline imaging were significantly associated with both plaque volume and lipid content evolution over time.

These findings have several possible clinical implications. Collectively, these data demonstrated that in the prospective analysis using serial intravascular imaging studies, the combination of 'functional' and 'morphological' data from each epicardial coronary segment may add significant prognostic information on how that particular conduit segment behaves over time. Our studies also validate previous observation on the inherent heterogeneity of conduit coronary segments, suggestive that in addition to systemic and traditional risk factors, there are also local environmental milieu, such as impaired vasodilating ability, which are responsible for the segmental arterial vulnerability, and in turn, increases the propensity of that segment to develop necrotic,

lipid laden core plaque, and adverse cardiac event in the future. This thesis adds to the growing body of evidence from prior studies which demonstrated prognostic utility and association between coronary endothelial dysfunction and future adverse coronary event. Secondly, our ability to demonstrate a vasomotor signal with GTN, a readily available drug in the catheterization lab, in the coronary endothelial function assessment both during single time point and serial intravascular imaging study potentially offer an attractive option for a simpler and more straightforward pharmacological agent in the coronary endothelial function evaluation *in vivo*. These observations may also resurrect the old debate concerning the appropriateness of routine use of the term endothelial 'dependent' and 'independent' function versus the suggestion to redesignate the term 'endothelial dysfunction' to 'nitric oxide resistance.' The findings from our cross sectional data appear to support the latter hypothesis. Third, the ability of NIRS derived LCBI, a quantitative measure of lipid core, to predict both coronary atheroma volume progression and future fibroatheroma formation independently of baseline clinical presentation, risk factors, and traditional IVUS grey-scale derived plaque burden present us with a lipid biomarker which may become a novel surrogate measure for coronary disease burden, and cardiovascular mortality and morbidity. Evidently, a larger prospective, serial imaging study would be needed to validate this hypothesis. The combined imaging of IVUS and NIRS offers a compelling option for detailed coronary plaque evaluation given its practicality, ease of use, and real time data generation. In contrast to other imaging modality, such as VH-IVUS or OCT which require some qualitative interpreting skills for lipid core detection, NIRS parameter measurements are fully automated, quantitative, and ready to be incorporated in the catheterization lab work to guide clinical decision making.

Future direction

In spite of advances which have been made in the area of intravascular imaging, current strategies for evaluating coronary artery as a means for primary cardiovascular prevention still suffer from lack of specificity and accuracy. Consequently, we still need to rely on the already established risk factor calculator to determine the patient's likelihood to develop future coronary event although this methodology also possess a number of limitations. Equally important, there remains the dilemma in fully translating and demonstrating prior knowledge from post mortem human studies or animal studies into the *in vivo* natural history of human coronary atherosclerosis. Therefore, future research needs to tackle the following:

- Extending the observation relating to the prognostic utility of NIRS derived LCBI and its changes over time in a larger serial imaging and prospective study. Ideally, the serial imaging would need to be conducted at several time point in order to gain more comprehensive insights concerning the natural history progression of coronary atheroma. This is crucial to validate whether NIRS parameter measurement may yield a reliable surrogate measure for cardiovascular mortality and morbidity over standard plaque volume measurement.
- Improving the diagnostic capability of currently available IVUS and/or OCT platform by refining the far field and near field resolution to create a 'gold standard' intravascular imaging device.
- Designing and conducting large scale simultaneous examination of coronary endothelial function and atheroma plaque volume and/or composition non-invasively either as a cross-sectional or longitudinal study.

- Given the invasive nature of IVUS, NIRS, and OCT, it is essential to validate the findings from these imaging trials with their non-invasive imaging modalities equivalent (such as computed tomography and magnetic resonance imaging) with the view to generate a standardized approach of plaque analysis and practice guidelines for non-invasive evaluation of human coronary atheroma.
- The dynamic nature of human coronary atheroma progression is not limited only to the epicardial coronary tree but also involving the coronary microcirculation. Indeed, this 'compartment' has been thought to play a critical role as a proverbial canary in a coal mine and dysfunction in this part of coronary tree is evident prior to the onset of angiographic coronary disease. Yet, to this date, there is still considerable lack of imaging modality (invasive or non-invasive) with direct visualization capability of *in vivo* human coronary microcirculation. Accordingly, the pivotal task of the cardiology researchers is to develop imaging platform and research design which can accurately analyse the integrity of the entire human coronary tree.

All things considered, the major objective of the series of studies presented in this thesis is to decipher the prospective key participants in the *in vivo* pathogenetic mechanisms of human coronary atherosclerosis, with special emphasis on atheroma architecture and vessel function. Greater translational work ahead is desperately needed to develop a fuller understanding on natural progression of coronary atheroma so better prevention strategy could be devised to arrest or modify the cardiovascular disease progression.

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