A winter's tale: Report from the First Annual Canadian Biomarkers and Surrogate Endpoints Symposium

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The International Partnership for Critical Markers of Disease (www.cmod.org), a nonprofit organization, has a mission to accelerate the identification, validation and appropriate application of biomarkers in cardiovascular and related disease. Founded by Drs Therese Heinonen, Peter Libby and Jean-Claude Tardif, the International Partnership for Critical Markers of Disease has sponsored an annual cardiovascular Biomarkers and Surrogate Endpoints Symposium in Bethesda, Maryland (USA), since 2003. These symposia have furnished an unusual forum for ongoing collaboration among academia, pharmaceutical and biotechnology concerns, and regulatory agencies. The United States Food and Drug Administration, Health Canada and the European Agency for the Evaluation of Medicinal Products have all participated in these international conferences.

Despite global involvement, each of the six previous symposia took place in Bethesda. This venue did not reflect the 'international partnership' proclaimed in the organization's name. Health Canada, Canadian researchers and representatives from the Canadian pharmaceutical industry all played important roles in the Bethesda symposia. Thus, Ottawa (Ontario) hosted the First Annual Canadian Biomarkers and Surrogate Endpoints Symposium on January 5, 2009.

The present article summarizes some of the issues and controversies highlighted during the symposium. Over the past four decades, cardiovascular drugs have contributed to a remarkable improvement in the outcomes of patients with cardiovascular disease. The future of cardiovascular drug development now appears uncertain (1). The cost and time required to bring a new drug to market have escalated. Current clinical outcome trials must include more people, which presents a higher hurdle because patients should receive treatment with the standard therapies that considerably reduce events. In principle, surrogate end points provide a way to obtain efficacy data at a fraction of the cost of a large outcomes trial. However, trials that have used surrogate end points have had their own problems. For example, a null carotid intima-media thickness (IMT) trial (2) for an approved and presumably effective drug has recently evoked a firestorm of controversy (3).

ESTABLISHED SURROGATE MARKERS

The morning session began with four presentations related to established surrogate markers: quantitative coronary arteriography (QCA; David D Waters), intravascular ultrasound (IVUS; Jean-Claude Tardif), carotid IMT using ultrasound (Eva Lonn) and carotid plaque using ultrasound (David Spence).

QCA

QCA provided a surrogate end point during the late 1980s and early 1990s in more than a dozen trials testing cholesterol lowering, often with statins. These trials almost unanimously showed that cholesterol lowering slowed the progression of coronary atherosclerosis, slowed the development of new coronary lesions and, in some cases, promoted the regression of coronary lesions (4). Based on either coronary or

carotid end point trials, the United States Food and Drug Administration has given most statins an indication for slowing the progression of atherosclerosis.

QCA as a surrogate end point has both advantages and disadvantages. Three trials have demonstrated that coronary angiographic changes during a trial predict subsequent coronary events (5-7), thus validating the use of this method as a surrogate end point. Coronary angiography, a common tool in clinical practice, presents a familiar option to cardiologists. Progression of coronary disease, although usually asymptomatic, can cause coronary symptoms and events, rendering it clinically relevant.

The disadvantages of QCA as a surrogate end point include its invasiveness, which causes 10% to 25% of patients to not return for follow-up angiography, and its inability to detect coronary atherosclerosis until the later stages, when it encroaches on the lumen. The small changes in QCA measurements reported in clinical trials seem trivial because most segments do not change, and those that do, are diluted when the averages of measurements are considered.

IVUS

Clinical trials have used IVUS to assess the effects of a variety of drugs on the advancement of coronary atherosclerosis. The technique has an extremely low incidence of complications in properly selected patients (8). IVUS images atherosclerosis within the vessel wall before it impinges on the coronary lumen, where angiography would detect it (Figure 1). As shown by Glagov et al (9), the coronary artery adapts to early atherosclerosis by positive remodelling, whereby the vessel expands and the lumen does not narrow until atherosclerosis reaches quite an advanced stage.

Although changes in IVUS end points have not yet been proven to predict subsequent coronary events, evidence from several sources validate IVUS end points as credible surrogates. IVUS trials and trials with clinical end points have yielded analogous results; for example, both Reversal of Atherosclerosis with Aggressive Lipid Lowering Therapy (REVERSAL) (10), an IVUS trial, and Pravastatin or Atorvastatin Evaluation and Infection Therapy (PROVE-IT) (11), a trial with clinical end points, demonstrated the superiority of atorvastatin 80 mg over pravastatin 40 mg. IVUS outcomes also correlate with QCA outcomes, as shown with rosuvastatin in A Study to Evaluate the Effect of Rosuvastatin on Intravascular Ultrasound-Derived Coronary Atheroma Burden (ASTEROID) (12). Despite the Glagov effect, research has demonstrated a fairly strong correlation between selected QCA and IVUS measurements (13).

IVUS trials of torcetrapib and rimonabant (14,15), two drugs that favourably affect lipid levels, showed no beneficial effect on the primary end point (although in each case, a favourable effect occurred for a secondary IVUS end point). The development of both drugs has ceased due to an increase in total mortality in a large outcomes study of torcetrapib, and concern regarding an increased incidence of depression and suicide in the case of rimonabant. However, two IVUS

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Figure 1 Qualitative assessment of intravascular ultrasound by grey-scale imaging. The soft plaque (A) is dark (10 o'clock to 2 o'clock) compared with the fibrous cap (B). The superficial (C) and deep (D) calcifications are radiolucent. Images courtesy of Dr Philippe L'Allier

trials (16,17) have suggested benefit with short-term intravenous infusions of high-density lipoprotein cholesterol-raising compounds, and further studies of drugs that increase high-density lipoprotein cholesterol are underway.

Carotid ultrasound: IMT versus plaque

Compared with QCA and IVUS as surrogate end points, carotid IMT has the obvious advantages of noninvasiveness and lack of radiation, which allow for its repeated use at frequent intervals. In addition to its application in trials, recently published standards direct its use for risk assessment in clinical practice (18). Prospective studies have demonstrated the ability of carotid IMT to predict both stroke and myocardial infarction across a broad range of populations (19-21), thus presenting a well-established surrogate marker in clinical trials.

With improvements in technology and imaging protocols, the reproducibility of carotid IMT measurements has improved remarkably over time. Controversy still exists regarding whether the average of measurements from multiple sites for a patient or a single common carotid measurement provides the preferable option. Combining carotid IMT with an independent measure of risk (eg, C-reactive protein levels) enhances the value of each marker. In the Cardiovascular Health Study (22), C-reactive protein did not predict stroke risk in subjects with a lower carotid IMT, but did become a strong predictor with higher carotid IMT.

Changes in carotid IMT in imaging trials have correlated very well with the outcomes of larger clinical end point trials. For example, in

the pooled Rating Atherosclerotic Disease Change by Imaging with a New CETP Inhibitor (RADIANCE) trials (23), torcetrapib was associated with a significant increase in common carotid IMT, but the large end point event trial was stopped because the drug was associated with an increase in mortality.

However, improving medical treatment has dramatically slowed the progression of carotid IMT in potential study populations – a possible problem for future trials using carotid IMT as the end point measure that necessitates increases in both sample size and overall trial expense. One proposed method, measuring changes in plaque volume, would require much smaller sample sizes. In one study (24) of only 38 patients treated with placebo or atorvastatin 80 mg/day for just three months, large differences ($P < 0.0001$) occurred in plaque volume changes between the treatment groups. Despite these results, plaque volume has not supplanted IMT as the preferred end point in carotid imaging trials. Recently published guidelines (25,26) have the potential to improve the standardization of methods used in clinical trials, and to optimize the use of this technology in clinical practice.

NEWER IMAGING TECHNIQUES

The next session consisted of four presentations related to newer imaging techniques: magnetic resonance imaging (MRI; Dr Alan Moody), computed tomography and positron emission tomography (PET; Dr Zahi A Fayad), PET as a tool for molecular imaging (Dr Rob Beanlands) and newer atherosclerosis imaging technologies (Dr Philippe L'Allier). In the afternoon session, Dr Peter Libby

discussed the related topic of molecular imaging innovations in cardiovascular disease.

MRI

MRI, a noninvasive procedure, does not involve radiation; consequently, serial imaging studies pose no health risk. Limitations of MRI include the claustrophobic environment of the scanner, difficulty in studying very large patients and an inability to study patients with metal implants. Spatial resolution, temporal resolution and signal generation correlate with one another so that, for example, an improvement in spatial resolution would require a longer scanning time or a decrease in signal generation. Movement, whether arterial pulsatility, respiration or cardiac motion, presents a serious problem for MRI. The limit of resolution of MRI typically falls in the 500 micron range.

MRI quantifies carotid plaque size and composition with high accuracy and reproducibility, and provides an opportunity to correlate plaque features with subsequent cerebrovascular events. Among patients with 50% to 79% asymptomatic carotid stenosis detected by ultrasound in one study (27), cerebrovascular events during follow-up more commonly occurred in those with thin or ruptured fibrous caps, intraplaque hemorrhage, larger mean intraplaque hemorrhage area, larger lipid-rich necrotic core and larger maximum wall thickness. Both low- and high-dose rosuvastatin favourably altered high-risk plaque features, as shown by MRI in a recent randomized trial (28).

Computed tomography and PET

The future of imaging progresses toward combining multiple modalities and using molecular markers to provide insight into the underlying pathophysiological processes. For example, ultra-small superparamagnetic iron oxide (USPIO) particles have been effective for the noninvasive MRI assessment of atherosclerotic plaque inflammation in humans (29). Macrophages in inflamed plaques absorb these particles, where they can be imaged 36 h after injection. Carotid plaque inflammation appears unrelated to the severity of stenosis (30). Both degree of inflammation and stenosis severity likely denote additive prognostic markers, and inflammation within the plaque presents a target for therapy (29), with follow-up imaging to confirm suppression of inflammation.

Fluorine-18 (^{18}F)-fluorodeoxyglucose (FDG) PET imaging can also track glucose uptake within plaques in carotid, iliac and femoral distributions with high-quality reproducibility (31). ^{18}F -FDG PET imaging of uptake in carotid lesions correlates well with macrophage staining of histological sections after carotid endarterectomy (32). FDG uptake did not correlate with plaque area or thickness, and thus, may offer an independent marker of risk.

Trials assessing the effects of new drugs on inflammation use USPIO particles imaged using both MRI and ^{18}F -FDG PET imaging, with sample sizes of only 20 to 30 patients per treatment group required to detect a 10% to 15% change. These measurements of inflammation have not yet been shown to be associated with an increased risk of future events because the studies needed to demonstrate this correlation require longer follow-up times and more subjects.

PET as a tool for molecular imaging

As with ^{18}F -FDG, PET imaging can evaluate broad, nonspecific biological processes such as blood flow or metabolism. PET can also evaluate more specific biological mechanisms, such as neurohumoral function, angiogenesis and apoptosis, and track therapy by labelling the drug or the cells the drug targets.

In the Endothelial Modulation in Angiogenic Therapy (EMAT) trial (33), PET imaging distinguished a treatment effect with very few patients studied. Coronary bypass patients with diffuse left anterior descending artery disease received treatment with arginine and vascular endothelial growth factor injections into the left anterior descending artery territory at the time of surgery. At three months, nitrogen-13-ammonia PET quantified anterior wall myocardial flow

reserve. The combination of these therapies improved not only anterior wall blood flow but also wall motion, compared with a control group in this small study (33).

New developments in atherosclerosis imaging technologies

A variety of new intracoronary imaging approaches can provide detailed images of the tissue characteristics of coronary plaques. Perhaps the simplest approach, grey-scale IVUS imaging, allows classification of plaque tissues based on their different echodensities. Calcified regions of plaques appear bright, while lipid pools within a plaque are dark, with gradations in between. Different grey-scale categories provide the basis for a scoring system, and treatment has led to changes in scores in serial studies. Changes in plaque composition may have more prognostic importance than changes in plaque size.

Radiofrequency data analysis goes a step beyond grey-scale IVUS by characterizing tissues according to spectral analysis of radiofrequency backscatter. Studies of this technology (34), termed IVUS virtual histology, have demonstrated its reproducibility and correlation with autopsy coronary lesions. In a recent trial (35) that used multiple imaging techniques and biomarkers to assess the effect of darapladib, a lipoprotein phospholipase A₂ inhibitor, IVUS virtual histology demonstrated that the drug prevented necrotic core expansion compared with placebo. Whether this effect correlates with a reduction in coronary events has yet to be determined.

Optical coherence tomography (OCT), a light-based technology, offers very high resolution but limited depth penetration (Figure 2). OCT measures cap thickness much more precisely than other techniques. Thin caps tend to rupture, and in one study (36), serial OCT showed that statin treatment increased plaque thickness. Coronary angiography, another light-based technology, allows direct visualization of plaques and has shown that plaque stability relates to plaque colour. However, such light-based techniques have a key limitation: the artery typically has to be occluded and the blood washed out before imaging. A very new technique, optical frequency domain imaging, may overcome at least part of this limitation.

Raman spectroscopy allows characterization of plaque tissue using optical fiber technology. Strong correlations have emerged between Raman tissue spectra and pathological findings (37). Palpography assesses the local mechanical properties of tissue by measuring its deformation caused by changes in intraluminal pressure (38). High-strain plaques have a propensity to rupture and palpography measures strain directly.

Molecular imaging innovations in cardiovascular disease

The cardiovascular imaging modalities currently in use remain fundamentally based on anatomy rather than biology. Studies using x-ray, echocardiography and nuclear cardiology focus on anatomical and functional measurements, while molecular imaging remains poorly charted territory. The potential for molecular imaging during the early stages of atherosclerosis can be associated with the mechanisms of endothelial activation, leukocyte accumulation, phagocytic capacity and proteinase activity. We provide an example for each of these four mechanisms below.

In rabbits fed an atherogenic diet, the aortic endothelium can express vascular cell adhesion molecule-1 (VCAM-1) as early as one week (39). MRI using the linear peptide affinity ligand VHPKQHR, homologous to sequences in very late antigen-4, a known VCAM-1 ligand, has visualized VCAM-1 accumulation in apolipoprotein E-deficient mice (40).

Monocytes have a significant role in atherogenesis and molecular imaging can now track their recruitment from the circulation. Studies using labelled monocytes in apolipoprotein E-deficient mice show that monocytes accumulate continuously during atheroma formation, accumulation increases in proportion to lesion size and hypercholesterolemia augments recruitment (41). Once monocytes accumulate in the vessel wall, they fulfill multiple functions, including phagocytosis.

Figure 2 Optical coherence tomography (OCT) images from a cadaver study. **A** OCT image of a lipid-rich carotid plaque showing a signal-poor lipid pool (L) with poorly delineated borders, beneath a thin fibrous cap (arrows). **B** Corresponding histological section of a lipid-rich carotid plaque. **C** OCT image of a fibrocalcific aortic plaque showing a sharply delineated region with a signal-poor interior. **D** Corresponding histological section of a fibrocalcific aortic plaque. H&E Hematoxylin and eosin stain. Adapted with permission from reference 46

As discussed earlier, macrophages phagocytose iron oxide nanoparticles (USPIO), which can assess lesion size and inflammatory burden by MRI (29,30).

Imaging in the aortas of apolipoprotein E-deficient mice fed an atherogenic diet can detect matrix metalloproteinases (MMPs) (42). The technique involves an activatable near-infrared fluorescence probe for MMP gelatinases. The imaged MMP activity colocalized with macrophage accumulation and MMP-2 and MMP-9, as shown by in situ zymography. The near-infrared fluorescence probe has also visualized cathepsin K activity in mice and endarterectomy specimens (43). Cathepsin K, a potent elastolytic and collagenolytic cysteine protease, likely participates in the evolution and destabilization of atherosclerotic plaques.

These molecular imaging modalities are increasing our understanding of experimental atherosclerosis. They have the potential to extend to human use and to play a role in a more sophisticated approach to drug development.

Cardiovascular drug development: A Canadian perspective

Dr Peter Liu, Scientific Director of the Institute of Circulatory and Respiratory Health of the Canadian Institutes of Health Research (CIHR), discussed the research funding priorities of CIHR, such as innovations in cardiorespiratory imaging. The types of research supported by CIHR imaging initiatives include the creation of national imaging research resources, the development of consensus on standards, the standardization of quantitative imaging measures, biomarker development and validation, studies leading to the development of new imaging methods, population-based longitudinal studies and research comparing diagnostic imaging strategies. In 2008, the CIHR funded four teams with \$2 million/year for five years, including the Canadian Atherosclerosis Imaging Network.

Health Canada's Dr Agnes Klein discussed surrogate markers from a Canadian regulatory perspective. She noted that surrogate markers differ widely from one disease state to another and that physicians routinely use biomarkers in practice. Examples of accepted biomarkers

outside the cardiovascular area include development of antibodies as evidence of immunity with vaccines, carcinoembryonic antigen as a marker of disease activity in ovarian cancer and hemoglobin A1c reductions for diabetes treatment. Surrogate end points offer much promise from a regulatory perspective to shorten drug development. A new regulatory approach under development in Canada, entitled the 'Progressive Licensing Framework', includes a seamless integration between premarket and postmarket activities, and allows unconventional pathways to drug approval.

Dr Allison Mueller, the Neurology Group Leader for General Electric Healthcare Medical Diagnostics Research and Development, discussed the challenges involved in the commercial development of molecular imaging agents, which, as a result of their drug classification, follow a similar development process to that of drugs. However, they also face imaging and analysis issues such as standardization of image interpretation. One interesting imaging agent under development would detect beta-amyloid within the brain. Detection of amyloid plaques – the pathological hallmark of Alzheimer's disease – would have important implications for early diagnosis and assessment of therapy.

Finally, Dr Catriona McMahon, Vice-President of Medical Affairs at AstraZeneca Canada, provided a pharmaceutical industry perspective on surrogate markers and drug development. Drug development now takes 10 to 15 years and costs anywhere from \$500 million to \$2 billion. For every 5000 to 10,000 compounds that enter the research and development pipeline, 250 survive to preclinical studies, five begin clinical trials in human subjects and one gains approval. Validated biomarkers do not present a viable replacement for well-conducted clinical outcome trials; however, they can facilitate clinical development without sacrificing regulatory standards of safety and efficacy.

DISCUSSION

The discussion periods throughout the symposium revealed diversity of opinion on a variety of issues and consensus on others, with the

streamlining of drug development identified as a priority. Biomarkers and surrogate end points have contributed a great deal to the advancement of cardiovascular science, but have been fallible when used as a regulatory crutch. Newer molecular imaging agents hold great promise in unravelling the pathophysiology of atherosclerosis, but are not yet widely available or validated in patients.

The role of surrogate end points in the drug approval process and the standards of evidence required for cardiovascular drug approval presented the largest unresolved issue – and the most vigorously debated. One popular position was that only a drug that has significantly reduced serious events in large clinical trials that also demonstrate safety should receive approval. Critics of this notion pointed out that such a high standard inhibits new drug development because of the huge expense of large trials. While approval of the first statin occurred in 1988 based on

the surrogate end point of low-density lipoprotein cholesterol reduction in a relatively small number of patients, the first trial showing event reduction with a statin was not published until 1994 (44).

A newer drug, ezetimibe, which was approved based on its ability to lower low-density lipoprotein cholesterol, often takes the place of a statin, despite the absence of proof that it reduces events. Rosiglitazone was approved for use in patients with diabetes because it significantly lowered hemoglobin A1c; however, a recent meta-analysis suggests that it may increase cardiac events (45). Balancing the need for new cardiovascular drugs with the risk of approving an ineffective or dangerous drug poses a difficult problem for regulators. This issue will likely provide a subject for debate again next year at the Second Annual Canadian Biomarker and Surrogate Endpoints Symposium.

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