Subsequent Development of Fibroatheromas With Inflamed Fibrous Caps Can Be Predicted by Intracoronary Near Infrared Spectroscopy

Dhavalkumar Patel, Damir Hamamdzic, Raul Llano, Daivesh Patel, Lan Cheng, Robert S. Fenning, Khalid Bannan, Robert L. Wilensky

Objective—To prospectively evaluate whether the development of fibroatheromas exhibiting features of potential instability can be detected and predicted by serial invasive imaging.

Methods and Results—Multivessel intracoronary ultrasound and near infrared spectroscopy (NIRS) were performed in diabetic/hypercholesterolemic pigs 3, 6, and 9 months after induction. Animals were euthanized at 9 months and histological/immunohistochemical evaluation of the arteries was performed (n=304 arterial segments). Intravascular ultrasound demonstrated, over time, a progressive increase in plaque + media and necrotic core areas and positive vascular remodeling. By histology, NIRS+ lesions were significantly more likely to be a high-risk fibroatheroma (P=0.0001) containing larger plaque (P<0.0001) and necrotic core areas (P<0.0019) and thinner fibrous caps (P=0.04). NIRS + fibroatheromas possessed a greater concentration of inflammatory cells demonstrating protease activity (P=0.006), and proliferating (P=0.016), and apoptotic cells (P=0.04) within the fibrous cap. Eighty-eight percent of NIRS+ lesions at 3 and 6 months subsequently developed into a fibroatheroma at 9 months (P<0.01). By multivariate analysis NIRS positivity at 6 months predicted the subsequent presence of a fibroatheroma at 9 months (P=0.005; odds ratio, 2.71).

Conclusion—The future development of inflamed fibroatheromas with thinner fibrous caps, greater plaque, and necrotic core areas, and possessing characteristics of increased plaque instability were detected by intravascular ultrasound/NIRS imaging. (Arterioscler Thromb Vasc Biol. 2013;33:347-353.)

Key Words: acute coronary syndromes ■ fibroatheromas ■ macrophages ■ myocardial infarction ■ vulnerable plaque

Coronary artery disease is an inflammatory disease with focal clinical manifestations. Even with optimal medical therapy, the risk of death, myocardial infarction, and unstable angina occurring in stable coronary artery disease patients is ~19% during a 4.6-year follow-up.1 To understand the process leading to increased risk, investigators have attempted to image those lesions with an increased likelihood of subsequent instability. However, prediction of future development of high-risk lesions is limited by the paucity of animal models exhibiting fibroatheromas, including both thin-cap fibroatheromas (TCFAs), the pathological substrate of the majority of unstable lesions, and thick-cap fibroatheromas (ThCFAs), which can progress into TCFAs. Hence, studies assessing the ability of invasive imaging systems to detect high-risk plaques generally study ex vivo human preparations or in vivo lesions after an acute event. Such studies demonstrate the ability of imaging systems to detect putative high-risk lesions but not predict future development.

Near infrared spectroscopy (NIRS) measures the wavelength-dependent interaction of electromagnetic radiation with matter (see online-only Data Supplement for detailed description of NIRS). NIRS provides simultaneous, multicomponent, and nondestructive chemical analysis of arterial tissue with a rapid acquisition time based on the absorbance of light by organic molecules in the spectral range between 800 and 2500 nm. NIRS is effective in biological media because it offers much of the same specificity of spectral features as infrared, but the light penetrates deeply through water and water-containing tissue. Different chemical species can be identified by proportion of diffusely reflected returned light as a function of wavelength. The scattering and absorption properties of different chemical structures within the arterial wall vary thereby providing a unique spectroscopic signature.2 The intracoronary NIRS catheter (Infraredx Corp, Burlington, MA) has been shown to accurately determine the presence of lipid core plaques in necropsy samples,3 an important defining characteristic of TCFAs.5 However, NIRS does not provide...
Materials and Methods

Three-vessel coronary angiography, IVUS, and NIRS were performed 3, 6, and 9 months after DM/HC induction with the results correlated to 9-month histology and immunohistochemistry. DM, defined as a consistent blood glucose level >150 mg/dL, was successfully induced in 21 male Yorkshire pigs (25–30 kg), with 125 mg/kg streptozotocin (Sigma-Aldrich, St. Louis, MO). Animals were then chronically fed a diet containing 0.5% cholesterol, 5% lard, and 1.5% sodium cholate (Animal Specialties Inc, Quakertown, PA). Serum glucose and cholesterol levels were monitored, and insulin administered to keep glucose levels <350 mg/dL to prevent ketoacidosis. The study was approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania.

Imaging Protocols

Animals were medicated with ketamine (2 mg/kg), medetomidine (0.1 mg/kg), atropine (0.04 mg/kg), and isoflurane to induce and maintain anesthesia. Clopidogrel (75 mg) and aspirin (325 mg) were administered 3 days before and continued for 2 days after the procedures, to reduce the risk of thrombosis during intravascular imaging. Percutaneous vascular access was obtained and a 6 Fr hockey stick guide was used for angiography and imaging. Heparin 100 IU/kg was administered as well as additional heparin, if needed, to achieve and maintain an activated clotting time of >300 seconds.

IVUS and NIRS evaluations were performed in duplicate with the pullback length recorded. Angiographic images were acquired before and after IVUS and NIRS pullbacks to determine the location of the start and end points. IVUS imaging used a 30 MHz transducer system (Boston Scientific Corporation, Natick, MA). Both catheters were advanced to the same distal side-branch and a motorized pullback at a constant speed of 0.5 mm/s from the branch point to the ostium was performed. The NIRS coronary imaging system consists of a scanning near infrared laser, a fiberoptic coronary catheter (3.2 Fr), and an automated pullback and rotation device.

Quantitative and qualitative analyses of IVUS were performed according to the criteria of the American College of Cardiology clinical expert consensus document on IVUS. A lesion with >40% atherosclerotic lesions was used. Lesions with >40% atherosclerotic lesions were used.6–8 For NIRS, we compared the block chemogram with histology. The block chemogram display provides a summary for each 2-mm section of artery by mapping the 90th percentile algorithm probability for specific colors to aid in visual interpretation (red, orange, tan, yellow) based on increasing algorithm probability that a lipid core plaque is present in that 2-mm block. For this study, a NIRS+ arterial section was defined as a segment containing a yellow (high probability), orange, or tan (both intermediate probability) block. Black represents the presence of the guide wire or calcifications.

Tissue Retrieval and Image/Histology Coregistration

After the 9-month in vivo evaluation, the animals were euthanized, heart removed, and the coronary arteries perfused with 10% neutral buffered formalin for at least 2 hours at physiological pressure. The extent of longitudinal vascular recoil after removal from the heart was determined by measuring the length of each artery, in situ, before fixation and after fixation and removal from the myocardium. The ratio of the fixed, removed artery to the in vivo interrogated segment was calculated and applied to the in vivo IVUS and NIRS pullback lengths. The exact location of the IVUS- and NIRS-interrogated sections was then determined based on angiographic images, arterial landmarks, and pullback lengths to accurately coregister the IVUS and NIRS images with the histological sections.

The arteries were cut into 5-mm blocks and sectioned from the proximal end of the block. IVUS and NIRS data were then subdivided into corresponding segments which were compared with the histological sections, controlling for the in vivo/ex vivo arterial length ratio. Only those IVUS and NIRS images corresponding to the proximal histological arterial sections were then analyzed. Proper alignment of segments visualized in vivo by IVUS and ex vivo by histology was demonstrated by a highly significant correlation in plaque + media areas (r=0.87; P<0.001; Figure I in the online-only Data Supplement). Chemograms containing black sections were excluded from further analysis.

Statistical Analysis

See online-only Data Supplement.

Results

Only animals that underwent 3 invasive evaluations were included in the analysis. At baseline, the mean blood glucose level was 62±14 mg/dL, and mean serum cholesterol 97±9 mg/dL. After DM/HC induction, the glucose levels averaged 304±49 mg/dL and cholesterol levels 511±64 mg/dL during the course of the study. Of the 21 induced pigs, 5 died unexpectedly because of ischemic sudden death with 4 demonstrating multivessel disease with superimposed thrombus, whereas 1 had a single TCFA without thrombus. Two additional animals had multivessel disease and died of procedurally related complications and 1 of a noncardiac cause. The remaining 13 animals underwent diagnostic evaluation at 3, 6, and 9 months after DM/HC induction and were included in the final analysis.
IVUS demonstrated progressive growth of plaque + media, necrotic core, and external elastic laminal areas over time (Table 1). Necrotic core area increased from 0 at 3 months to 0.71±0.80 mm² at 9 months. The growth of plaque + media exceeded the increase in external elastic lamina area so, despite positive remodeling, the mean lumen area decreased between 6 months and 9 months. IVUS lacks the resolution to precisely determine cap thickness so no attempt was made to differentiate TCFA (fibrous caps <100 μm) from ThCFA (fibrous caps >100 μm).

Of the 304 arterial segments with matching IVUS/NIRS and histological sections, the presence of a fibroatheroma was identified in 130 histological sections of which 63 were TCFA and 67 ThCFA (examples in Figure II in the online-only Data Supplement). Other arterial sections were pathological intimal thickening (PIT) (n=66), intimal hyperplasia (IH) (n=67), or were without a lesion (n=41). NIRS positivity (yellow, tan, or orange coregistered chromogroms) significantly predicted the presence of a fibroatheroma by histology with significant correlations for both TCFA and ThCFA (both \( P<0.0001 \); Table 2). Sensitivity of NIRS positivity for identifying a fibroatheroma was 45%, specificity 95%, positive predictive value 88% negative predictive value 70%, and accuracy of 74%. For TCFAs alone the sensitivity was 46%, specificity 85%, positive predictive value 49% negative predictive value 86%, with an accuracy of 77%.

NIRS+ arterial sections were associated with greater plaque area, necrotic core area, percent necrotic core area, and thinner fibrous caps than NIRS– arterial sections (Table 3). When examining only fibroatheromas by IVUS, NIRS+ lesions had greater plaque and necrotic core areas and thinner fibrous caps than NIRS– fibroatheromas (Table 3). When assessing only histological TCFAs, NIRS+ was associated with larger plaque area than NIRS– TCFA (7.55±4.95 mm² versus 5.44±1.91 mm²; \( P=0.04 \)) and larger necrotic core area (4.58±3.95 mm² versus 2.92±1.57 mm²; \( P=0.04 \)). Lesions which were consistently NIRS+ for 9 months or were NIRS+ at 6 and 9 months had the largest necrotic core areas, by IVUS, at 9 months and more likely to become fibroatheromas and TCFA (Table 4). Lesions NIRS+ at 6 and 9 months (\( P<0.0001 \)) or converted to NIRS+ at 9 months (\( P=0.0003 \)) had a significant increase in necrotic core area between 6 and 9 months compared with those that converted to or remained NIRS– (Table 1 in the online-only Data Supplement). The results were confirmed by histology (Table II in the online-only Data Supplement).

NIRS+ lesions demonstrated a significantly greater concentration of inflammatory cells exhibiting augmented protease activity in the entire plaque and importantly within the fibrous cap than NIRS– sections (Figure 1). There was a greater percentage of apoptotic cells in the fibrous caps of NIRS+ sections and significantly greater number of proliferating cells in both the entire plaque and the fibrous cap (Figure 2). Compared with fibroatheromas which were NIRS–, NIRS+ fibroatheromas had a greater concentration of inflammatory cells (\( P=0.004 \)) and apoptotic cells within the fibrous cap (\( P=0.04 \); Figure 2).

Arterial sections which demonstrated NIRS positivity at both the 3- and 6-month timepoints or only at 6 months were more likely to be a fibroatheroma at 9 months (Figure 3; \( P=0.0004 \)). Regardless of the subsequent NIRS status, 54% of 3 month NIRS+ arterial sections developed into a fibroatheroma at 9 months, whereas 78% of sections NIRS+ at 6 months were a fibroatheroma at 9 months. Of the 16 arterial sections which were NIRS+ at both the 3- and 6-month timepoints 5 were NIRS– at 9 months and only 1 of the 5 was a TCFA. Of the 11 sections which remained positive at 9 months 7 were a TCFA. At 6 months 54 sections were NIRS+ with 28 sections becoming NIRS– at 9 months. Only 9 were a TCFA. Prediction of TCFA and fibroatheroma at 9 months in relation to IVUS and NIRS results at 3, 6, 9 months by univariate and multivariate logistic analysis are as shown in Table III in the online-only Data Supplement. By multivariate logistic regression analysis NIRS positivity at 9 months (\( P<0.0001 \); odds ratio, 13.37; confidence interval, 5.18–34.45) and cross-sectional narrowing >40% by IVUS (\( P=0.001 \); odds ratio, 5.14; confidence interval, 1.98–13.35) successfully detected the presence of TCFA, whereas NIRS positivity at 6 months significantly predicted the presence of fibroatheromas at 9 months (\( P=0.005 \); odds ratio, 2.71; confidence interval, 1.34–5.49). Glucose and cholesterol levels did not significantly predict development of TCFAs or fibroatheromas.

Discussion

This is the first study comparing in vivo NIRS and IVUS imaging, obtained at multiple timepoints, with histological and immunohistochemical end points in an animal model which demonstrates complex, human-like coronary artery lesions. The results show that although lipid core plaque development is dynamic, the early and persistent accumulation of lipid within the arterial wall leads to the subsequent development of fibroatheromas. NIRS and IVUS imaging detected but more importantly predicted the future development of fibroatheromas possessing such characteristics of instability as increased plaque and necrotic core areas, and thinned fibrous cap as well as an increased concentration of activated inflammatory cells.
as well as proliferating and apoptotic cells within the fibrous cap; all characteristics of high-risk coronary lesions.

At necropsy the majority of ruptured coronary lesions causing death possess a thin fibrous cap overlying a large necrotic core in the setting of an active inflammatory infiltrate. Such high-risk lesions contain an increased concentration of activated macrophages expressing cathepsins, serine proteases, and matrix metalloproteinases which in turn disrupt macrophage function and survival and promote enzymatic degradation of the fibrous cap. Mechanical stress on the fibrous cap is the primary determinant of subsequent plaque rupture and hence plaque instability and results from a dynamic combination of

| Table 4. Association of NIRS Status and Mean Necrotic Core Area by IVUS. By Histology NIRS Positivity at 9 Months was Associated with a Higher Percentage of Fibroatheromas (Thin-Cap and Thick-Cap Fibroatheromas) or TCFA |
|-----------------|-----------------|-----------------|-----------------|
| NIRS+ 0.0 mm² (65) | NIRS+ 0.0 mm² (22), P=0.039 | NIRS+ 0.11 mm² (22), P=0.039 | NIRS+ 0.11 mm² (22), P=0.039 |
| NIRS+ 0.11 mm² (65) | NIRS+ 1.02 mm² (6), P=0.024 | NIRS+ 1.02 mm² (6), P=0.024 | NIRS+ 1.02 mm² (6), P=0.024 |
| NIRS+ 0.44 mm² (43) | NIRS+ 0.37 mm² (37) | NIRS+ 0.37 mm² (37) | NIRS+ 0.37 mm² (37) |
| NIRS+ 0.79 mm² (58), P<0.0001 | NIRS+ 1.81 mm² (19), P=0.0083 | NIRS+ 1.81 mm² (19), P=0.0083 | NIRS+ 1.81 mm² (19), P=0.0083 |
| NIRS– 0.0 mm² (278) | NIRS– 0.91 mm² (39) | NIRS– 0.91 mm² (39) | NIRS– 0.91 mm² (39) |
| NIRS– 0.17 mm² (218) | NIRS+ 1.00 mm² (42), P=0.0002 | NIRS+ 1.00 mm² (42), P=0.0002 | NIRS+ 1.00 mm² (42), P=0.0002 |
| NIRS– 0.0 mm² (28) | NIRS– 0.26 mm² (176) | NIRS– 0.26 mm² (176) | NIRS– 0.26 mm² (176) |

P values pertain to the differences in necrotic core area between the 2 groups, based on NIRS status at the specific time point. Number of samples noted in parentheses. IVUS indicates intravascular ultrasound; NIRS, near infrared spectroscopy; and TCFA, thin-cap fibroatheromas.

Figure 1. Near infrared spectroscopy (NIRS) positivity is associated with markers of increased plaque instability. Adjacent sections obtained from a NIRS+ thin-cap fibroatheroma (ThCFA; A–F) and a NIRS+ thick-cap fibroatheroma (ThCFA; G–L). A and G, Movat’s stain. B and H, picro-sirius red. C, D, I, J, cathepsin S. E and K, terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL). F and L, Ki67. Arrows in A and B point to the thin fibrous cap. Note the absence of collagen in the fibrous cap of the ThCFA (B) compared with the ThCFA (H) as well as the increased concentrations of macrophages (C, D vs I, J) apoptotic (E vs K) and proliferating cells (F vs L) in and near the fibrous cap (dark brown) in the NIRS+ ThCFA sections. Collagen-poor areas deep within the artery, are either foam cell rich (B) or calcifications (H) are noted by the asterisk. The scale bars for A–C and G–I, 200 μm and for D–F and J–L, 50 μm.
biomechanical forces dependent not only on cap thickness but also on relative necrotic core thickness and inversely with the extent of positive arterial remodeling.¹²

Most vulnerable eccentric plaques possess a low positive remodeling index and a large necrotic core in the setting of a thinned fibrous cap. These plaques often are dynamic: expanding or regressing over time. As such, determination of future vulnerability is difficult to predict. Clinical observational studies using VH-IVUS have shown, >12 months, that 10% of lesions characterized as PIT and 6% of ThCFAs developed into TCFAs, whereas 75% of TCFAs regressed to either a ThCFAs or a fibrotic lesion.¹³ The Providing Regional Observations to Study Predictors of Events in Coronary Tree (PROSPECT) study also demonstrated evolution of fibroatheromas into TCFAs during a 3.4-year follow-up. Of the recurrent events from the nonculprit lesion 51% occurred at the site of a documented TCFA, whereas the remaining events were caused by lesions most commonly characterized as ThCFAs at the baseline assessment.¹⁴ Such ThCFAs were associated with an intermediate risk of future cardiac events. The current study supports the dynamic nature of coronary lesions, demonstrating that those lesions exhibiting early and persistent NIRS positivity were more likely to develop into TCFAs, whereas some lesions, originally NIRS+ became NIRS-. Indeed, the change from NIRS+ to NIRS− was associated with a smaller necrotic core area at 9 months than those which remained NIRS+, whereas those lesions which were NIRS+ at both 6 and 9 months had the largest necrotic core areas.

A fibrous cap thickness of <65 μm is a strong determinant of plaque rupture, however, ruptures also occur in ThCFAs. Tanaka et al¹⁵ showed by optical coherence tomography, that 67% of ruptured plaques in acute coronary syndrome patients possessed a fibrous cap thickness of <65 μm, whereas the remaining 33% had a rupture in a fibrous cap up to 140 μm in thickness. Toutouzas et al¹⁶ demonstrated, also by optical coherence tomography, that fibrous cap thickness ranged from 30 to 140 μm in patients presenting with a ST elevation myocardial infarction treated with thrombolytic therapy. In only 50.9% of patients did an optical coherence tomography-defined TCFA cause the infarct.

Although NIRS successfully differentiated fibroatheromas from other lesion subsets, it did not differentiate TCFAs from ThCFAs. This finding is not surprising as NIRS is most sensitive to cholesterol-rich, necrotic lesions with connective tissue degradation, the pathophysiologic substrate of both TCFAs and ThCFAs.²,³ This inability to differentiate TCFAs from ThCFAs may take on less importance given the aforementioned clinical data suggesting that a plaque rupture causing an acute coronary syndrome¹⁵,¹⁶ can occur in ThCFAs with fibrous caps up to 140 μm in thickness and evidence of a dynamic progression of ThCFAs to TCFA.¹³ The current data showing increased markers of plaque instability in NIRS+ fibroatheromas is supportive and demonstrates that vascular inflammation is dynamic (ie, changes in NIRS positivity over time; Figure 3). Of interest is that our results with NIRS showed similar sensitivity, specificity, positive and negative predictive values to detect the presence of a TCFA in DM/HC coronary arteries in vivo to results demonstrated by Gardner et al³ in ex vivo human samples. Nonetheless, the data suggest that the clinical contribution of a combined NIRS and IVUS approach would be to exclude the presence of inflamed fibroatheromas whether TCFAs or ThCFAs rather than detect only TCFAs.

Figure 2. Compared with near infrared spectroscopy (NIRS)– fibroatheromas NIRS+ fibroatheromas are associated with increased inflammatory cells in the plaque and the cap, increased apoptotic cells in the fibrous cap and increased proliferation in the plaque and cap.

Figure 3. Early and persistent near infrared spectroscopy (NIRS) positivity predicts the subsequent development of fibroatheromas. NIRS+ at either 3 or 6 months, predicted development of a thin-cap fibroatheromas (TCFA; P=0.0001) and a fibroatheroma at 9 months (P=0.0001) at 9 months. For both 3 and 6 months NIRS+ P=0.03 for TCFA and P=0.0004 for fibroatheroma. For NIRS+ at 6 months only P=0.004 for diagnosis of TCFA and P=.00001 for fibroatheroma. The number of histological sections evaluated is noted. IH indicates intimal hyperplasia; PIT, pathological intimal thickening; and ThCFA, thick-cap fibroatheromas. Fibroatheromas include both TCFAs and ThCFAs.
The variable presentation of advanced atherosclerotic lesions, produced over a predictable time period, makes the DM/HC porcine model an attractive model for diagnosing and assessing the presence of high-risk lesions. The percentage of IVUS documented advanced atheromas at 6 months was 47.7% increasing to 68.5% at 9 months. The relatively high percentage of animals which suffered acute ischemic sudden death (5/21, 24%) illustrates the model’s relevance by demonstrating the potential of rapidly progressive lesions becoming unstable resulting in sudden death. In this model, high-risk lesion development is often focal and progression is dependent in large part on persistent vascular inflammation. Increased expression of numerous proinflammatory genes, many important for macrophage and T lymphocyte recruitment and functioning as well as upregulation of inflammatory mediators has previously been demonstrated.17 The synergistic action of DM and HC is associated with increased nuclear factor kappa-B levels and hypoactivation of the Akt pathway, a central signaling node, is involved in both the development of atherosclerotic lesions and inflammation. However, no induced animal model perfectly reflects the human condition which develops insidiously over decades. DM/HC pigs have persistently elevated glucose and cholesterol levels and it can be anticipated that the dynamic changes in NIRS status occur more rapidly than in humans.

Increased cathepsin S staining within the fibrous cap of NIRS+ lesions (Figure 1), reflecting augmented protease activity, may signal those lesions with an increased likelihood of progression to a TCFA and plaque rupture. Within the arterial wall cathepsin S is a major elastolytic and collagenolytic enzyme and plays a role in inflammation as well as turnover of cells, cellular matrix, and cholesterol.20 This cysteine protease is involved in both the development of atherosclerotic lesions as well as a reduction in fibrous cap thickness by reducing lesion smooth muscle and collagen content.21 In murine knock-out models cathepsin S deficiency was associated with a reduced number of plaque ruptures.22 Mice deficient in leukocyte cathepsin S also developed less-advanced lesions with a reduced number of plaque ruptures.22 Mice deficient in hypoxia knock-out models cathepsin S deficiency was associated with low shear stress predisposing to increased lipid accumulation and inflammation. However, no induced animal model perfectly reflects the human condition which develops insidiously over decades. DM/HC pigs have persistently elevated glucose and cholesterol levels and it can be anticipated that the dynamic changes in NIRS status occur more rapidly than in humans.

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Furthermore, these animals demonstrated impaired elastolysis which generally lacked, or possessed a smaller necrotic core. In murine knock-out models cathepsin S deficiency was associated with low shear stress predisposing to increased lipid accumulation and inflammation. However, no induced animal model perfectly reflects the human condition which develops insidiously over decades. DM/HC pigs have persistently elevated glucose and cholesterol levels and it can be anticipated that the dynamic changes in NIRS status occur more rapidly than in humans.

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In practice, there are potential difficulties in comparing in vivo imaging results obtained at multiple timepoints and comparing those images with histological results. Growth of the animal, albeit reduced in DM pigs, results in increased arterial length over time. Coronary artery length shortens after removal from the heart due to a loss of arterial branch anchoring and the effect of fixation. We addressed these issues by comparing the pullback lengths of the NIRS and IVUS in vivo with the length of this segment immediately after removal of the fixed artery from the heart as well as using fiduciary points obtained from the angiogram and IVUS images. We also used the macroscopic assessment of the entire fixed artery, taking into account the location of each side-branch and its distance from the arterial ostium to calculate the ratio of the in vivo to ex vivo postfixation lengths. As such, the location of each IVUS and NIRS interrogation could be pin-pointed and compared with each other for the 3 arterial interrogations and then to histological samples. The excellent correlation of plaque + media areas derived from IVUS and morphometry supports this approach (Figure I in the online-only Data Supplement). An additional potential source of error is that analysis of IVUS-derived images were performed every 1 mm of the coronary artery, whereas histological analysis was performed on a 5-μm slice. With regard to the coregistration of NIRS and histology, the NIRS block chemogram analyzes a segment 2 mm in length and so images an arterial segment longer than the IVUS or histological slice. Hence, some unavoidable mismatch may have been present. Given the potentially small size of individual fibroatheromas it is possible that some, visualized by IVUS and/or NIRS, were missed by histology or vice versa. Finally, we did not measure the necrotic core angle and it is possible that some smaller necrotic cores seen by histology but possessing a <60° angular extent may have been missed thereby reducing NIRS sensitivity.

The development of an approach to determine the presence of those lesions with an increased risk of rupture over the subsequent 1 to 2 years would be of considerable importance in the prevention of sudden death and myocardial infarction. Noninvasive CT imaging or positive emission tomography (PET) scanning could identify patients with potential high-risk plaques with subsequent lesion assessment performed by directed invasive imaging with IVUS/NIRS as described in the present study. The recently developed IVUS/ NIRS combination catheter will eliminate problems with coregistration. The current data suggest that such a directed diagnostic evaluation could detect, and even predict the development of coronary lesions demonstrating the salient features of unstable or vulnerable plaques.

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Disclosures
None.

References


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NIRS system
The NIRS system performs 8000 chemical measurements per 100 mm of scanned artery. By predictive algorithm (constructed on the basis of histologic controls) it calculates the probability that a lipid core plaque is present within the interrogated site. In spectroscopic terms, the algorithm classifier distinguishes a near infrared spectra that contains signatures of high amounts of pooled cholesterol versus spectra that exhibit characteristics of normal tissue; cholesterol has prominent features within the spectrum which distinguishes from collagen and other arterial components. Immediately following the pullback the data is displayed in a 2-dimensional arterial map, the “chemogram,” while the block chemogram, is a summary of the results for each 2-mm section of artery is mapped to the same color scale as the chemogram. The block chemogram display provides a summary for each 2-mm section of artery by mapping the 90th percentile algorithm probability for spectra with the 2-mm block to the same color scale, binned to four discrete colors to aid in visual interpretation (red: $P<0.57$, orange: $0.57 \leq P < 0.84$, tan: $0.84 \leq P < 0.98$, yellow: $P > 0.98$) algorithm probability that a lipid core plaque is present in that 2-mm block. Black areas within the chemogram indicate regions where spectra were statistical outliers compared to the necropsy validation set and are thus indeterminate for lipid core presence.

Early feasibility studies have shown that NIRS can identify plaque composition in post-mortem human aortic specimens (1). The NIRS catheter was prospectively validated for detection of LCP in human coronary arteries obtained at necropsy, with the LCP defined as containing over >60° of angular extent and a length which exceeded 200µm. This validation study demonstrated an area under the receiver operating characteristic (ROC) curve (AUC) for detection of lipid core plaque of 0.80. The depth of penetration is approximately 1mm (2). Goldstein et al clinically demonstrated that extensive lipid core plaques determined by NIRS were associated with a high risk of periprocedural myocardial infarction, due to embolization of plaque contents, presumably lipid (3).

Tissue processing.
Adjacent histologic sections were analysed using Movat’s pentachrome staining, Picor-sirius red staining and immunohistochemistry. Picro-sirius red red was used to more clearly delineate the fibrous cap by taking advantage of collagen’s birefringence under a polarizing light source. All histology sections were quantitatively analyzed using Image-Pro Plus (MediaCybernetics) to determine the plaque + media, intimal and necrotic core areas. Necrotic core area was defined as those areas in which extracellular matrix was absent and replaced by necrotic debris by Movat’s (loss of collagen and reticular fibers) and Picro-sirius Red staining (loss of collagen) (4). Assessment of cap thickness was determined by measuring the thinnest cap width between the necrotic core and the lumen. Sections were then classified using modified AHA criteria by 2 independent observers, blinded to both the IVUS and NIRS data as either TCFA, ThCFA, pathological intimal thickening (PIT), intimal hyperplasia (IH) and normal tissue (5,6). We differentiated TCFAs from ThCFAs but subsumed both under the term fibroatheroma. NIRS, IVUS and histologic data is presented as results per 5 mm arterial sections.
Since high-risk lesions have been shown to demonstrate increased concentration of activated macrophages, increased cellular proliferation and apoptosis (9) fibroatheromas (n=117) underwent further immunohistochemical evaluation to determine the activated inflammatory cell content (generally macrophages), using Cathepsin S and the presence of proliferating (Ki-67) and apoptotic cells (TUNEL) both in the entire plaque and an 100 μm luminal slice of the fibrous cap. Assessment of the Cathepsin S, Ki67 and TUNEL staining was performed using bright field microscopy. Quantitative analysis of these sections was performed using Image-Pro Plus software under 400 x magnification by investigator blinded to the results of NIRS/IVUS.

**Statistical analysis.**
Categorical variables were expressed as frequencies and continuous variables were presented as Mean ± SD. Categorical variables were compared by chi-square test or Fisher Exact Test. Comparison of continuous variable for ≥2 unmatched groups was performed using student’s independent t-test and one-way ANOVA test, respectively. To determine significance in change in continuous variables measured over time (3,6,9-m) mixed effect ANOVA with animal and artery a random effect was used. Correlation between plaque + media area on histology and IVUS was examined by Pearson Correlation. To identify 3, 6 and 9-m IVUS/ NIRS predictors of TCFA and fibroatheromas at 9-m univariate analysis was performed with the following variables evaluated: mean glucose and cholesterol levels, NIRS positivity at 3,6,9-m, plaque + media area, remodeling index, lumen area, cross sectional narrowing >40% and the presence of a necrotic core on IVUS at 3,6,9-m. All variables showing an association with TCFA and fibroatheromas on univariate analysis were then entered into multivariable logistic regression model. SPSS version 12 was used for all statistical analysis. P<0.05 was considered significant.


![Figure I](image)

**Figure I.** Correlation of segment plaque+media areas obtained by IVUS and histology.
Figure II. NIRS and IVUS findings at 3-m (A), 6-m (B), and 9-m (C) after DM/HC induction and histology using Movat’s pentachrome staining at 9-m (C). Progressive NIRS positivity is observed at locations 1 and 3 over time. While IVUS and histology (bottom panel) demonstrate a similar sized lesion in the two locations, the lesion at 1 is a TCFA and at 3 a ThCFA. Red indicates arterial wall without lipid, yellow indicates the presence of lipid and black the presence of calcifications or the guide wire.
Table I. Persistent NIRS positivity or a change from a NIRS– to NIRS+ status between 6-m and 9-m is associated with a significantly greater increase in necrotic core area by IVUS.

<table>
<thead>
<tr>
<th>NIRS status change from 6 to 9-m</th>
<th>NIRS+ (n=80)</th>
<th>NIRS– (n=261)</th>
<th>P value</th>
<th>NIRS+ (n=30)</th>
<th>NIRS– (n=50)</th>
<th>NIRS+ (n=48)</th>
<th>NIRS– (n=213)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No change: 37.5%</td>
<td>Change + to -: 62.5%</td>
<td>P value</td>
<td>Change – to +: 18.4%</td>
<td>No change: 81.6%</td>
<td>P value</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Status at 9-m</td>
<td>NIRS+ (n=30)</td>
<td>NIRS– (n=50)</td>
<td></td>
<td>NIRS+ (n=48)</td>
<td>NIRS– (n=213)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change in plaque + media area (mm²)</td>
<td>+ 5.26 ± 4.66</td>
<td>+ 4.27 ± 4.51</td>
<td>0.359</td>
<td>+ 5.52 ± 6.57</td>
<td>+ 2.57 ± 5.04</td>
<td>0.005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change in necrotic core area (mm²)</td>
<td>+ 0.98 ± 1.11</td>
<td>- 0.01 ± 0.92</td>
<td>0.0001</td>
<td>+ 0.92 ± 1.46</td>
<td>+ 0.09 ± 0.65</td>
<td>0.0003</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table II. Plaque area, percent of the plaque subtended by necrotic core and fibrous cap thickness by histology. Percent necrotic core area was significantly greater at 9 months, by histology, in lesions which were NIRS+ at 6 and 9 months or was NIRS– at 6 months and converted to NIRS+ at 9 months. Mean cap thickness was non-significantly thinner in lesions which were NIRS+ at both time periods or converted to NIRS+ at 9 months.
<table>
<thead>
<tr>
<th>Months</th>
<th>Predictors of TCFA, by histology at 9 months</th>
<th>Predictors of fibroatheromas by histology at 9 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Univariate logistic regression</td>
<td>Multivariate logistic regression</td>
</tr>
<tr>
<td>9-m</td>
<td>NIRS positive (P&lt;0.001)</td>
<td>NIRS positive (P&lt;0.0001)</td>
</tr>
<tr>
<td></td>
<td>P+M area (P&lt;0.001)</td>
<td>(OR= 3.97, CI=1.88-8.36)</td>
</tr>
<tr>
<td></td>
<td>NC area (P&lt;0.001)</td>
<td>CSN&gt;40% (P=0.046)</td>
</tr>
<tr>
<td></td>
<td>CSN &gt;40% (P&lt;0.001)</td>
<td>Positive RI (P= 0.002)</td>
</tr>
<tr>
<td></td>
<td>Positive RI (P= 0.002)</td>
<td>Lumen area (P=0.01)</td>
</tr>
<tr>
<td></td>
<td>Lumen area (P=0.01)</td>
<td>(OR=3.81, CI= 1.60-7.05)</td>
</tr>
<tr>
<td>6-m</td>
<td>NIRS positive (P=0.001)</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>P+M area (P&lt;0.001)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NC area (P=0.001)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CSN &gt;40% (P&lt;0.00001)</td>
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</tr>
<tr>
<td>3-m</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

Table III. Univariate and multivariate analysis of NIRS and IVUS. CI= confidence interval, CSN- cross-sectional luminal area by IVUS, NC= necrotic core by IVUS, OR-Odds ratio, P+M-Plaque plus media area by (IVUS), RI- remodeling index by IVUS.