2018

Plaque Burden Influences Accurate Classification of Fibrous Cap Atheroma by In-Vivo Optical Coherence Tomography in a Porcine Model of Advanced Coronary Atherosclerosis.

Christian B. Poulsen
Aarhus University Hospital

Ryan M. Pedrigi
University of Nebraska-Lincoln, rpedrigi@unl.edu

Nilesh Pareek
Royal Brompton and Harefield NHS Foundation Trust

Ismail D. Kilic
Royal Brompton and Harefield NHS Foundation Trust

Niels R. Holm

Aarhus University Hospital

Follow this and additional works at: <http://digitalcommons.unl.edu/mechengfacpub>

[next page for additional authors](#)
Part of the [Mechanics of Materials Commons](#), [Nanoscience and Nanotechnology Commons](#),
[Other Engineering Science and Materials Commons](#), and the [Other Mechanical Engineering Commons](#)

Poulsen, Christian B.; Pedrigi, Ryan M.; Pareek, Nilesh; Kilic, Ismail D.; Holm, Niels R.; Bentzon, Jacob F.; Botker, Hans Erik; Falk, Erling; Krams, Rob; and de Silva, Ranil, "Plaque Burden Influences Accurate Classification of Fibrous Cap Atheroma by In-Vivo Optical Coherence Tomography in a Porcine Model of Advanced Coronary Atherosclerosis." (2018). *Mechanical & Materials Engineering Faculty Publications*. 302.
<http://digitalcommons.unl.edu/mechengfacpub/302>

This Article is brought to you for free and open access by the Mechanical & Materials Engineering, Department of at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Mechanical & Materials Engineering Faculty Publications by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

Authors

Christian B. Poulsen, Ryan M. Pedrigi, Nilesch Pareek, Ismail D. Kilic, Niels R. Holm, Jacob F. Bentzon, Hans Erik Botker, Erling Falk, Rob Krams, and Ranil de Silva

Title: Plaque Burden Influences Accurate Classification of Fibrous Cap Atheroma by In-Vivo Optical Coherence Tomography in a Porcine Model of Advanced Coronary Atherosclerosis.

Authors: Christian B. Poulsen, M.D, PhD; Ryan M. Pedrigi, PhD; Nilesh Pareek, MB, BChir, MA; Ismail D. Kilic, M.D; Niels R. Holm, M.D; Jacob F. Bentzon, M.D, PhD; Hans Erik Bøtker, M.D, PhD; Erling Falk, M.D, DMSc; Rob Krams, M.D, PhD; Ranil de Silva, MBBS, PhD

DOI: 10.4244/EIJ-D-17-01028

Citation: Poulsen CB, Pedrigi RM, Pareek N, Kilic ID, Holm NR, Bentzon JF, Bøtker HE, Falk E, Krams R, de Silva R. Plaque Burden Influences Accurate Classification of Fibrous Cap Atheroma by In-Vivo Optical Coherence Tomography in a Porcine Model of Advanced Coronary Atherosclerosis. *EuroIntervention* 2018; Jaa-347 2018, doi: 10.4244/EIJ-D-17-01028

Manuscript submission date: 18 November 2017

Revisions received: 14 March 2018

Accepted date: 28 March 2018

Online publication date: 03 April 2018

Disclaimer: This is a PDF file of a "Just accepted article". This PDF has been published online early without copy editing/typesetting as a service to the Journal's readership (having early access to this data). Copy editing/typesetting will commence shortly. Unforeseen errors may arise during the proofing process and as such Europa Digital & Publishing exercise their legal rights concerning these potential circumstances.

Plaque Burden Influences Accurate Classification of Fibrous Cap Atheroma by In-Vivo Optical Coherence Tomography in a Porcine Model of Advanced Coronary Atherosclerosis

Christian B. Poulsen^{1,2*} MD PhD, Ryan M. Pedrigi^{3,4*} PhD, Nilesh Pareek⁵ MB BChir MA, Ismail D. Kilic^{5,6} MD, Niels R. Holm^{1,2} MD, Jacob F. Bentzon^{1,2} MD PhD, Hans Erik Bøtker² MD PhD, Erling Falk^{1,2} MD DMSc, Rob Krams³ MD PhD, Ranil de Silva^{5,7} MBBS PhD. *Joint first authors who contributed equally to this work.

1. Institute of Clinical Medicine, Aarhus University Hospital, Denmark
2. Department of Cardiology, Aarhus University Hospital, Denmark
3. Department of Bioengineering, Imperial College London, United Kingdom
4. Department of Mechanical and Materials Engineering, University of Nebraska-Lincoln, NE, USA
5. Cardiovascular Research Unit, Royal Brompton and Harefield NHS Foundation Trust, London, United Kingdom
6. Department of Cardiology, Pamukkale University, Denizli, Turkey
7. National Heart and Lung Institute, Imperial College London, United Kingdom

Short running title: Comparison of in-vivo OCT derived plaque classification with histology

Address correspondence.

Ranil de Silva, MBBS, PhD
National Heart and Lung Institute (Brompton Campus)
Imperial College London and NIHR Cardiovascular Biomedical Research Unit Royal Brompton and Harefield NHS Foundation Trust
Level 2 Chelsea Wing, Sydney Street
London SW3 6NP, United Kingdom
Tel: +442073518626
Fax: +442073518629
E-mail: r.desilva@imperial.ac.uk

Conflict of interest: NRH has received institutional research grants and speaker fees from St. Jude Medical and Terumo. The other authors report no conflicts

Total word count: 4055

Abstract

Aims: In-vivo validation of coronary optical coherence tomography (OCT) against histology and the effects of plaque burden (PB) on plaque classification remain unreported. We investigated this in a porcine model with human-like coronary atherosclerosis.

Methods and Results: Five female Yucatan *D374Y*-PCSK9 transgenic hypercholesterolemic minipigs were implanted with a coronary shear-modifying stent to induce advanced atherosclerosis.

OCT frames ($n=201$) were obtained 34 weeks after implantation. Coronary arteries were perfusion-fixed, serially sectioned and co-registered with OCT using a validated algorithm. Lesions were adjudicated using the Virmani classification and PB assessed from histology. OCT had a high sensitivity, but modest specificity (92.9% and 74.6%), for identifying fibrous cap atheroma (FCA). The reduced specificity for OCT was due to misclassification of plaques with histologically defined pathological intimal thickening (PIT) as FCA (46.1% of the frames with histological PIT were misclassified). PIT lesions misclassified as FCA by OCT had a statistically higher PB than in other OCT frames (median 32.0% versus 13.4%; $p<0.0001$). Misclassification of PIT lesions by OCT occurred when PB exceeded approximately 20%. **Conclusions:** Compared with histology, in-vivo OCT classification of FCA had high sensitivity but reduced specificity due to misclassification of PITs with high PB.

Condensed abstract

Advanced coronary atherosclerosis assessed by in-vivo optical coherence tomography (OCT) has not been directly compared with histology. In a porcine model with human-like coronary atherosclerosis we compared in-vivo OCT images with histology in five animals. We found a high sensitivity but modest specificity of OCT in classifying fibrous cap atheroma (FCA). The modest specificity was caused by misclassification of FCA lesions as pathological intimal thickening. The misclassification most frequently occurred when plaque burden exceeded ~20%. Compared with

histology, OCT has high sensitivity but modest specificity for identifying FCA. The study suggests that plaque burden should be considered when interpreting OCT images.

Impact on Daily Practice

Optical coherence tomography (OCT) is increasingly used for the evaluation of coronary atherosclerosis. This study suggests that OCT misclassifies advanced fibrous cap atheroma, particularly when plaque burden is high, and suggests that plaque burden should be considered when interpreting OCT images.

Keywords: optical coherence tomography; coronary artery disease; pre-clinical research

Abbreviations:

FCA: Fibrous Cap Atheroma

IVUS: Intravascular Ultrasound

LAD: Left Anterior Descending artery

LCx: Left Circumflex artery

OCT: Optical Coherence Tomography

PIT: Pathological Intimal Thickening

STEMI: ST segment elevation myocardial infarction

SMS: Shear Modifying Stent

TCFA: Thin Cap Fibroatheroma

ThFCA: Thick Fibrous Cap Atheroma

Introduction

Intracoronary optical coherence tomography (OCT) is increasingly used to classify coronary atherosclerotic lesions and guide interventional procedures, due to its higher spatial resolution compared to intravascular ultrasound (IVUS).^{1,2} A challenge with using OCT is the limited penetration of light into the vessel wall³ and the heterogeneity of plaque constituents that have high signal, which can affect image interpretation and lesion classification.¹ Previous studies have compared ex-vivo OCT imaging of cadaveric human coronary arteries with histology.⁴⁻¹¹ To our knowledge, studies comparing in-vivo OCT classification of coronary plaques to histology have not been reported. In the present study, we sought to compare identification of advanced coronary plaque morphologies by in-vivo OCT with co-registered histology in hypercholesterolemic minipigs with human-like advanced coronary atherosclerosis.

Methods

All animal experiments were performed in accordance with the ethical and welfare regulations of the University of Aarhus and approved by the Danish Animal Experiments Inspectorate. Detailed descriptions of the animals and experimental protocols have been described previously by Pedrigi et al.¹² Briefly, five female *D374Y* PCSK9 transgenic minipigs on a Yucatan background were fed a cholate-free high fat high cholesterol (HFHC) diet for 1 to 4 months prior to instrumentation with a shear-modifying stent (SMS). SMS were made by custom modification of 3.0-mm stainless steel coronary stents covered with expandable polytetrafluoro-ethylene with a circular ring placed in the middle of the stent to restrict expansion of the central region during deployment, thus creating a focal stenosis after implantation that caused flow disturbances primarily downstream of the stent.¹³ The SMS was implanted into either the left anterior descending (LAD; N=1) or left circumflex artery (LCx; N=4). Coronary angiograms and intravascular frequency domain optical coherence tomography (FD-OCT) images were acquired immediately before and after SMS implantation, as well as at 19 and 34 weeks follow up (one pig died after follow up at 19 weeks, so only four pigs were evaluated at the additional follow up time of 34 weeks). Coronary angiograms were acquired in orthogonal views during injection of iodinated contrast (iohexol 140mgI/mL, GE healthcare, Norway) with ventilation briefly paused using a clinical grade digital fluoroscopy suite (Allura Xper FD10, Philips, The Netherlands). Intracoronary OCT was performed in both the LAD and LCx of all pigs at a speed of 20 mm/s during pump injection of iohexol (4-8 mL/s) using the Illumien system and Dragonfly imaging catheters (St Jude, USA). Prior to each pullback, the OCT catheter was imaged in orthogonal views during injection of iodinated contrast to enable reconstruction of the catheter path, which could be combined with the OCT frames to obtain a 3-D reconstruction of the instrumented artery. After the final catheterization procedure, the pigs were sacrificed and the coronary arteries were perfusion-fixed at 100 mmHg for 1 hour followed by

immersion fixation for 24 hours using 4% formaldehyde in phosphate buffered saline (VWR, Prolabo, Belgium) to allow histological evaluation of the instrumented artery.

Evaluating plaque type in OCT frames and histology sections

All frames of the OCT pullback (acquired at 200 μm intervals) within the instrumented vessel of each pig were segmented and evaluated for the presence of fibrous cap atheroma (FCA) by three investigators (NP, IDK, and RDS) who were blinded to the histological data. These analyses were performed offline using standard software (St Jude Medical, USA). FCA within OCT frames was characterized using standard criteria.⁴ Specifically, FCA was defined as a plaque containing lipid (signal-poor region, with poorly delineated borders, signal drop-off, and little or no signal backscattering) that was covered by a fibrous cap. The lipid needed to subtend an arc greater than 90° and the fibrous cap was classified as the thinnest portion of tissue layer overlying a signal-poor region.⁴ For each FCA, the fibrous cap thickness was measured to allow delineation of thin cap fibroatheroma (TCFA; classified as having a cap thickness $\leq 85 \mu\text{m}$) from thick fibrous cap atheroma (ThFCA).¹⁰ In histology, the cap needed to be $\leq 65 \mu\text{m}$.¹⁴ Normal vessel was classified as having a tri-laminar architecture without intimal thickening.

To validate the characterization of FCA within the OCT frames, histological analysis was performed on $\sim 2.5\text{mm}$ vessel segments immediately upstream and downstream of the implanted stent, where flow disturbances were greatest and plaque development most advanced. Those vessel segments downstream of the stent exhibited the most extensive advanced plaque development. Tissue blocks containing the coronary artery and underlying myocardium were sectioned at a thickness of 3 μm and serially collected to allow assessment of up to ten stains (the sections for each stain were evaluated at 60 μm intervals) over a distance of $\sim 2.5 \text{ mm}$ in the tissue blocks

adjacent to the stent. Three stains were used to evaluate plaque type: haematoxylin and eosin (H&E), muramidase (macrophages), and picrosirius red (collagen). Digitized images of the stained histology sections were acquired using an Olympus BX50 light microscope with a Colorview 2 camera. Plaque type and morphology were classified according to the Virmani classification by expert histopathologists (CBP and EF) who were blinded to the OCT data. Plaques were classified as TCFA, FCA, PIT, early lesions (xanthoma and intimal thickening), or normal vessel wall. In addition, TCFA, FCA, and PIT were considered advanced plaques. Accurate co-registration of the histology sections to the OCT frames was performed using custom Matlab software, which used the attached myocardium, vessel bifurcations, and the implanted stent as circumferential and longitudinal fiducial markers, thus accounting for rotational artefacts and shrinkage. A full description of the methodology for 3D spatial co-registration of in-vivo OCT and histology, including an error propagation analysis have been reported previously.¹² Because plaque type was evaluated in histology sections with a higher longitudinal frequency (60 μm interval between histological sections, which equated to ~ 105 μm in-vivo) than OCT frames (200 μm interval), the closest histology section to a given OCT frame was used to validate the plaque type classification within that frame.

To examine differences in plaque size between OCT frames correctly identified not to contain a FCA (based on examination of co-registered histology) versus those falsely identified to have a FCA, we computed PB within the co-registered histological sections as the percentage of plaque area to external elastic membrane area.¹⁵ PB could not be computed from the OCT frames due to the limited penetration depth of this imaging modality.

Statistics

The ability to accurately identify FCA within OCT frames was evaluated by comparison to co-registered histology sections, which is considered the gold-standard. Performance of this classification was quantified by sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, and diagnostic odds ratio. Statistical comparisons were performed with a one-way ANOVA and $p < 0.05$ was considered statistically significant. All analyses were done in Matlab.

Results

The implanted SMS induced the development of advanced coronary plaques in all pigs. A total of 201 OCT frames from within the diseased vessel segments proximal to the inlet and outlet of the stent over all pigs were segmented and evaluated for the presence of FCA. Histological analysis was performed on these vessel segments, wherein histological sections were evaluated at 60 μm intervals for plaque type using the Virmani classification system¹⁴ and accurately co-registered to the OCT frames to assess the accuracy of OCT-based FCA identification (Figure 1, 2, 3 and 4). OCT had a high sensitivity but modest specificity of 92.9% and 74.6%, respectively, for classifying FCA. The sensitivity and specificity associated with each type of FCA demonstrated 28.6% and 96.4% for TCFA compared to 66.7% and 73.9% for ThFCA (Table 1). The low sensitivity associated with TCFA and ThFCA resulted from an inability to accurately determine the fibrous cap thickness, whereas the lower specificity in all FCA (composed primarily of ThFCA) resulted from misclassification of lesions with histologically defined PIT; 46.1% of OCT frames with histologically defined PIT were misclassified as FCA. Histologically defined PIT lesions misclassified as FCA by OCT had a statistically higher percentage PB of 32.0% [20.3%, 58.2%] (median and [25th, 75th] percentiles) compared to 13.4% [10.5%, 14.9%] within OCT frames in

which lesions were not misclassified as FCA ($p<0.0001$). This misclassification of PIT lesions by OCT occurred more frequently when PB exceeded approximately 20% (Figure 5).

Discussion

In the present study, we present the first report of direct comparison of in-vivo OCT classified coronary plaque morphology with co-registered histology. We found that OCT was able to classify FCA with a high sensitivity. However, plaques with histopathologic features of PIT and histologic PB exceeding 20% were often misclassified as FCA by OCT, resulting in a modest specificity of in-vivo OCT for classifying FCA. In this pilot study, the sensitivity for discriminating TCFA was low.

Using standard criteria definition⁴, the sensitivity and specificity for detecting TCFA by ex-vivo OCT has been reported to range between 72-100% from imaging studies of cadaveric human coronary arteries with paired histology.^{10,11,16} Though the specificity for identification of TCFA in the current study falls within the reported range, the sensitivity was low at 28.6%. This may be attributable to the small number of TCFA lesions in the current study; the error associated with co-registration of the in-vivo OCT frames and corresponding histological sections, particularly given the heterogeneity of plaque type that was observed when it was delineated on a section-to-section basis at 60 μm intervals. Furthermore changes in the dimensions of the plaque resulting from histological processing, such as perfusion fixation and shrinkage of the tissue, affected the appearance of the lesions and increased the error in measurements of cap thickness causing discrepancies between plaque classification by in-vivo imaging and histology particularly when a common set of diagnostic criteria was applied to both modalities.^{10,12}

A pooled analysis combining both TCFAs and ThFCAs was performed to evaluate the ability of in-vivo OCT to classify FCA plaque morphologies, in general. The sensitivity of in-vivo OCT was

comparable to previous ex-vivo OCT imaging studies¹⁷, though the specificity was modest.¹⁷ The latter was due to misclassification of lesions within the OCT frames as FCA, which actually had histological features of PIT. This was particularly problematic in lesions with higher PB. The light sources used in the various OCT systems have reported tissue depth penetration between 1-3.5 mm.^{3,17} Our findings accord with recent publications from other groups. Phipps et al.⁹ showed that irrespective of the atherosclerotic lesion composition, an important cause of FCA misclassification by ex-vivo OCT was foam cell accumulation close to the luminal surface and consequent reduced backscatter of light due to their lipid content, which is consistent with our findings (Figures 1, 2, 3 and 4). In line with these observations, Lutter et al.¹⁸ reported that plaque constituents classified by OCT did not correlate well with histological features. Our study extends these findings by showing that PB is an important determinant of misclassification of FCA plaque morphologies by in-vivo OCT. This may be improved by using multi-modality intracoronary imaging systems which combine OCT and intravascular ultrasound, with the latter enabling evaluation of PB by virtue of better depth penetration.²⁰

When comparing animal and human studies, important differences should be noted. The coronary lesions encountered in porcine models have developed over months, not decades as in humans.²¹ Though the lesions assessed in the present study showed features of advanced atherosclerosis, including necrotic core formation, calcium depositions and neovascularisation, they appeared more cellular than human lesions^{21,22} with foam cells frequently appearing close to the luminal surface in both PIT and FCA lesions (Figures 1,2, 3 and 4), which may have influenced the results.⁹

Study Limitations

Two limitations of this study should be considered. First, a small cohort of 5 pigs was studied and human-like FCA developed in only 3 of 5 animals, including TCFA in 2 of 5 animals. Secondly, the animals were only followed for 34 weeks; a longer follow-up period may have resulted in the

development of more advanced lesions. However, even with this sample size, the high-resolution analysis provided sufficient power to allow identification of statistically significant associations between OCT-derived vessel wall/plaque features and histology.¹²

Conclusions

We present, to the best of our knowledge, the first comparison between in-vivo OCT classification of coronary FCA compared with co-registered histology. We found that in-vivo OCT has a high sensitivity but only a modest specificity for classification of FCA. While these data require confirmation in a larger sample, they may provide important considerations for the clinical interpretation of in-vivo OCT images of coronary atherosclerotic plaques.

Acknowledgements

We thank Zahra Nasr, Lisa Maria Røge, and Dorte Qualmann for technical assistance.

Conflicts of interest:

CBP: Non, RMP: Non, NRH: Received institutional research grants and speaker fees from St. Jude Medical and Terumo, NP: Non, IDK: Non, ALP: Non, CDM: Non, JFB: Non, HEB: Non, EF: Non, RK: Non. RDS: Non.

Funding Sources

This work was supported by the Cardiovascular Research Unit, Royal Brompton and Harefield NHS Foundation Trust (RdS); BHF Program Grant (RG11/13/29055); Helga and Peter Kornings Fund and Aarhus University.

References

1. Otsuka F, Joner M, Prati F, Virmani R, Narula J. Clinical classification of plaque morphology in coronary disease. *Nat Rev Cardiol*. 2014;11:379–389.
2. Tian J, Ren X, Vergallo R, Xing L, Yu H, Jia H, Soeda T, McNulty I, Hu S, Lee H, Yu B, Jang I-K. Distinct morphological features of ruptured culprit plaque for acute coronary events compared to those with silent rupture and thin-cap fibroatheroma: a combined optical coherence tomography and intravascular ultrasound study. *Journal of the American College of Cardiology*. 2014;63:2209–2216.
3. Jang I-K. Optical coherence tomography or intravascular ultrasound? *JACC Cardiovasc Interv*. 2011;4:492–494.
4. Tearney GJ, Regar E, Akasaka T, Adriaenssens T, Barlis P, Bezerra HG, Bouma B, Bruining N, Cho J-M, Chowdhary S, Costa MA, de Silva R, Dijkstra J, Di Mario C, Dudek D, Dudeck D, Falk E, Falk E, Feldman MD, Fitzgerald P, Garcia-Garcia HM, Garcia H, Gonzalo N, Granada JF, Guagliumi G, Holm NR, Honda Y, Ikeno F, Kawasaki M, Kochman J, Koltowski L, Kubo T, Kume T, Kyono H, Lam CCS, Lamouche G, Lee DP, Leon MB, Machara A, Manfrini O, Mintz GS, Mizuno K, Morel M-A, Nadkarni S, Okura H, Otake H, Pietrasik A, Prati F, Räber L, Radu MD, Rieber J, Riga M, Rollins A, Rosenberg M, Sirbu V, Serruys PWJC, Shimada K, Shinke T, Shite J, Siegel E, Sonoda S, Sonada S, Suter M, Takarada S, Tanaka A, Terashima M, Thim T, Troels T, Uemura S, Ughi GJ, van Beusekom HMM, van der Steen AFW, van Es G-A, van Es G-A, van Soest G, Virmani R, Waxman S, Weissman NJ, Weisz G, International Working Group for Intravascular Optical Coherence Tomography (IWG-IVOCT). Consensus standards for acquisition, measurement, and reporting of intravascular optical coherence tomography studies: a report from the International Working Group for Intravascular Optical Coherence Tomography Standardization and Validation. 2012. p. 1058–1072.
5. Kume T, Akasaka T, Kawamoto T, Watanabe N, Toyota E, Neishi Y, Sukmawan R, Sadahira Y, Yoshida K. Assessment of coronary arterial plaque by optical coherence tomography. *Am J Cardiol*. 2006;97:1172–1175.
6. Yabushita H, Bouma BE, Houser SL, Aretz HT, Jang I-K, Schlendorf KH, Kauffman CR, Shishkov M, Kang D-H, Halpern EF, Tearney GJ. Characterization of human atherosclerosis by optical coherence tomography. *Circulation*. 2002;106:1640–1645.
7. Manfrini O, Mont E, Leone O, Arbustini E, Eusebi V, Virmani R, Bugiardini R. Sources of error and interpretation of plaque morphology by optical coherence tomography. *Am J Cardiol*. 2006;98:156–159.
8. Zimarino M, Prati F, Stabile E, Pizzicannella J, Fouad T, Filippini A, Rabozzi R, Trubiani O, Pizzicannella G, De Caterina R. Optical coherence tomography accurately identifies intermediate atherosclerotic lesions--an in vivo evaluation in the rabbit carotid artery. *Atherosclerosis*. 2007;193:94–101.
9. Phipps JE, Hoyt T, Vela D, Wang T, Michalek JE, Buja LM, Jang I-K, Milner TE, Feldman MD. Diagnosis of Thin-Capped Fibroatheromas in Intravascular Optical Coherence Tomography Images: Effects of Light Scattering. *Circulation: Cardiovascular Interventions*.

2016;9.

10. Brown AJ, Obaid DR, Costopoulos C, Parker RA, Calvert PA, Teng Z, Hoole SP, West NEJ, Goddard M, Bennett MR. Direct Comparison of Virtual-Histology Intravascular Ultrasound and Optical Coherence Tomography Imaging for Identification of Thin-Cap Fibroatheroma. *Circulation: Cardiovascular Imaging*. 2015;8:e003487.
11. Fujii K, Hao H, Shibuya M, Imanaka T, Fukunaga M, Miki K, Tamaru H, Sawada H, Naito Y, Ohyanagi M, Hirota S, Masuyama T. Accuracy of OCT, grayscale IVUS, and their combination for the diagnosis of coronary TCFA: an ex vivo validation study. *JACC Cardiovasc Imaging*. 2015;8:451–460.
12. Pedrigi RM, Poulsen CB, Mehta VV, Holm NR, Pareek N, Post AL, Kilic ID, Banya WAS, Dall'Ara G, Mattesini A, Bjørklund MM, Andersen NP, Grøndal AK, Petretto E, Foin N, Davies JE, Di Mario C, Bentzon JF, Bøtker HE, Falk E, Krams R, de Silva R. Inducing Persistent Flow Disturbances Accelerates Atherogenesis and Promotes Thin Cap Fibroatheroma Development in D374Y-PCSK9 Hypercholesterolemic Minipigs. *Circulation*. 2015;
13. Foin N, Sen S, Petraco R, Nijjer S, Torii R, Kouser C, Broyd C, Mehta V, Xu Y, Mayet J, Hughes A, Mario C, Krams R, Francis D, Davies J. Method for Percutaneously Introducing, and Removing, Anatomical Stenosis of Predetermined Severity In Vivo: The “Stenotic Stent.” *J of Cardiovasc Trans Res*. 2013;6:640–648.
14. Virmani R, Kolodgie FD, Burke AP, Farb A, Schwartz SM. Lessons From Sudden Coronary Death : A Comprehensive Morphological Classification Scheme for Atherosclerotic Lesions. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2000;20:1262–1275.
15. Antonacci G, Pedrigi RM, Kondiboyina A, Mehta VV, de Silva R, Paterson C, Krams R, Török P. Quantification of plaque stiffness by Brillouin microscopy in experimental thin cap fibroatheroma. *J R Soc Interface*. 2015;12.
16. Nakano M, Yahagi K, Yamamoto H, Taniwaki M, Otsuka F, Ladich ER, Joner M, Virmani R. Additive Value of Integrated Backscatter IVUS for Detection of Vulnerable Plaque by Optical Frequency Domain Imaging: An Ex Vivo Autopsy Study of Human Coronary Arteries. *JACC Cardiovasc Imaging*. 2016;9:163–172.
17. Lowe HC, Narula J, Fujimoto JG, Jang I-K. Intracoronary optical diagnostics current status, limitations, and potential. *JACC Cardiovasc Interv*. 2011;4:1257–1270.
18. Lutter C, Mori H, Yahagi K, Ladich E, Joner M, Kutys R, Fowler D, Romero M, Narula J, Virmani R, Finn AV. Histopathological Differential Diagnosis of Optical Coherence Tomographic Image Interpretation After Stenting. *JACC Cardiovasc Interv*. 2016;9:2511–2523.
19. Komukai K, Kubo T, Kitabata H, Matsuo Y, Ozaki Y, Takarada S, Okumoto Y, Shiono Y, Orii M, Shimamura K, Ueno S, Yamano T, Tanimoto T, Ino Y, Yamaguchi T, Kumiko H, Tanaka A, Imanishi T, Akagi H, Akasaka T. Effect of atorvastatin therapy on fibrous cap thickness in coronary atherosclerotic plaque as assessed by optical coherence tomography:

the EASY-FIT study. *Journal of the American College of Cardiology*. 2014;64:2207–2217.

20. Bourantas CV, Jaffer FA, Gijsen FJ, van Soest G, Madden SP, Courtney BK, Fard AM, Tenekecioglu E, Zeng Y, van der Steen AFW, Emelianov S, Muller J, Stone PH, Marcu L, Tearney GJ, Serruys PW. Hybrid intravascular imaging: recent advances, technical considerations, and current applications in the study of plaque pathophysiology. *European Heart Journal*. 2016;
21. Shim J, Al-Mashhadi RH, Sørensen CB, Bentzon JF. Large animal models of atherosclerosis - new tools for persistent problems in cardiovascular medicine. *J Pathol*. 2016;238:257–266.
22. Falk E, Nakano M, Bentzon JF, Finn AV, Virmani R. Update on acute coronary syndromes: the pathologists' view. *European Heart Journal*. 2013;34:719–728.

Figure Legends

Figure 1. Representative images of a histologically-defined TCFA with a PB of 65.6%. The TCFA is shown by OCT at low and high magnification with corresponding histology including H&E (basic; 2x and 20x), muramidase (macrophages; 2x and 20x) and picrosirius red (collagen; 2x and 20x). Yellow arrows indicate the fibrous cap.

Figure 2. Representative images of histologically-defined ThFCA with a PB of 51.7% in OCT and histology, which includes H&E (basic; 2x and 20x), muramidase (macrophages; 2x and 20x), and picrosirius red (collagen; 2x and 20x) stainings. Yellow arrows indicate the fibrous cap..

Figure 3. Representative images of histologically-defined PIT with a large PB of 26.5% that was misclassified as FCA by OCT. Histology included the following stains: H&E (basic; 2x and 20x), muramidase (macrophages; 2x and 20x), and picrosirius red (collagen; 2x and 20x). Yellow arrows indicate part of the plaque that from OCT could be assessed as a fibrous cap.

Figure 4. Representative images of histologically-defined PIT with a small PB of 16.2% in OCT and histology that was correctly ignored in OCT (i.e., not classified FCA). Histology included the following stains: H&E (basic; 2x and 20x), muramidase (macrophages; 2x and 20x), and picrosirius red (collagen; 2x and 20x). Yellow arrows indicate the center of the plaque.

Figure 5. Boxplot showing median, interquartile range, and whiskers, which indicate the minimum and maximum data points not considered outliers. Outliers (indicated by red dots) are 1.5 times the interquartile range added to the third quartile or subtracted from the first quartile, which is approximately 2.7 standard deviations from the mean for normally distributed data. Plaque burden (%) of histologically-defined PIT either correctly ignored in OCT (true negative) or classified as FCA (false positive) are shown. **** indicates $p < 0.0001$.

Table 1. Performance of blinded investigators in identifying FCA within OCT compared to the gold-standard of histology ($n=201$ co-registered OCT frame-histology section pairs).

	OCT Frames	Histology Sections	Sensitivity (%)	Specificity (%)	LR+	LR-	DOR
All FCA	70	28	92.9	74.6	3.7	0.1	38.1
TCFA	9	7	28.6	96.4	7.9	0.7	10.7
ThFCA	61	21	66.7	73.9	2.6	0.5	5.7
PIT	-	89	-	-	-	-	-
Early	-	51	-	-	-	-	-
Normal	-	33	-	-	-	-	-

LR+: positive likelihood ratio; LR-: negative likelihood ratio; DOR: diagnostic odds ratio.

Low Magnification

High Magnification

Low Magnification

High Magnification

A

B

C

D

Low Magnification

High Magnification

A

B

C

D

Low Magnification

High Magnification