Recent Highlights of ATVB

Neoatherosclerosis From a Pathologist’s Point of View

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Percutaneous coronary interventions using drug-eluting stents (DES) or bare metal stents (BMS) are the most commonly performed procedures for the management of acute coronary syndromes and stable angina.1 BMS, introduced >2 decades ago, were limited by complications of in-stent restenosis (ISR) with the need for repeat revascularization in up to one third of patients.2 DES with controlled release of antiproliferative agents developed in the past decade were specifically designed to inhibit neointimal formation and reduce restenosis within the stented coronary segment and have achieved greater success, particularly with the development of newer platforms aimed at improving the safety and efficacy. The commonly used antiproliferative drugs are highly lipophilic molecules that exert immunosuppressive effects in the case of sirolimus group of drugs by inhibiting the mammalian target of rapamycin, which prevents the degradation of p27kip1, a cyclin-dependent kinase inhibitor that plays an important role in regulating vascular smooth muscle cell (VSMC) migration and proliferation,3 whereas paclitaxel is a cytotoxic drug known to suppress VSMC and endothelial cell proliferation and migration by disrupting microtubule dynamics, thus affecting cells in the mitotic phase of the cell cycle.4 The first generation of DES (namely Cypher [Cordis Corp, Miami Lakes, FL] and Taxus [Boston Scientific, Natick, MA]) had unique disadvantages of thick metallic struts, and uneven and thick coatings of poorly biocompatible permanent polymers were used to load drugs. However, despite therapeutic efficacy and recommendations to extend dual antiplatelet therapy for at least 12 months, complications of late stent thrombosis (LST, >1 month to ≤1 year) and very late stent thrombosis (VLST, >1 year) have emerged as a significant limitation, particularly with approved first-generation DES.5 More recently, however, complications of in-stent neoatherosclerosis with associated plaque rupture have seemed to be a major contributing factor for late DES failure and as another cause of very late in-stent thrombosis.6

Neatherosclerosis has been observed for BMS (Figure 1) and first and second-generation DES (Figure 2), the latter with thinner struts and more biocompatible permanent polymer and reduced or rapid release drug dose (CoCr everolimus-eluting stent, EES [XIENCE V, Abbott Vascular, Santa Clara, CA; or PROMUS, Boston Scientific] and zotarolimus-eluting stent [Resolute, Medtronic, Santa Rosa, CA]). The incidence of neatherosclerosis, however, occurs more often and early in DES (median, 420 days) versus BMS (median, 2160 days) where both increase with time.6,7 Although newer generation DES show a lower prevalence of LST/VLST, less uncovered struts, less inflammatory response, and decreased fibrin deposition compared with first-generation DES, the prevalence of neatherosclerosis is similar.

Neatherosclerosis, by definition, represents the accumulation of lipid-laden macrophage foam cells within the neointima of stented arteries, with or without necrotic core formation, or calcification or complications of thrombosis6 (Figures 1 and 2). Its rapid onset further distinguishes neatherosclerosis from native coronary disease, as it generally develops within months to years after stent placement rather than occurring over decades.1

The early neointima after DES implantation mainly consists of peristrut fibrin with minimal VSMCs within proteoglycan-rich extracellular matrix8 with poor strut coverage by endothelial cells. On the contrary, the preceding neointima for BMS is relatively thick and composed primarily of VSMCs in a proteoglycan/collagenous matrix with complete endothelial coverage within 3 to 4 months. This developing neointima in both BMS and DES, although fundamentally distinct considering there is delayed healing in the latter, eventually could become a more viable substrate for neatherosclerosis with a similar end result but a different pathophysiologic process bearing in mind that the intimal soil between DES and BMS is clearly different.9 The infiltration of macrophage foam cells represents the earliest stage in the progression of neatherosclerosis mainly localized to the peristrut region or adjacent to the luminal surface (Figures 2 and 3). Although less clear, this early lesion may progress to a fibroatheromatous plaque, which by definition harbors a necrotic core that may be located near the luminal surface or within the peristrut regions6 and may also show calcification.8 The development of the necrotic core is presumed, which is attributed to apoptotic cell death of foamy macrophages.10 Intraplaque hemorrhage may also be observed, which may come from the lumen or to a lesser extent, plaque vasa vasorum.12 Calcification occurs from cell death or when localized to the peristrut regions involve fibrin, especially in paclitaxel-eluting stent (PES). Unstable plaques are recognized by typical morphological identifiers of necrotic core, intraplaque hemorrhage, and thin fibrous cap with foamy macrophage infiltration, features that are well recognized in clinical or autopsy studies as potential sites of future coronary events.10,11

Native Coronary Atherosclerosis

The natural history of native human coronary atherosclerosis evolves through the progression of well-defined morphological identifiers recognized from early pathological intimal thickening to advanced lesions of thin cap fibroatheroma, which is the primary substrate for plaque rupture.14 These processes arise from
the early insudation of lipid colocalized to areas rich in proteoglycans with subsequent infiltration of inflammatory cells, particularly macrophage foam cells and T lymphocytes accompanied by necrosis and thinning of the fibrous cap with the final progression to potentially fatal thrombosis, taking decades to achieve. Our modified American Heart Association classification based on morphological descriptions divides coronary lesions into 2 categories: the nonprogressive intimal lesions and progressive atherosclerotic plaques. Early nonatherosclerotic intimal lesions consist of adaptive intimal thickening and intimal xanthoma (fatty streak), which are uniformly present in all populations and they typically do not evolve further and are even known to regress. On the other hand, the progressive atherosclerotic lesions emerging as pathological intimal thickening, and fibroatheromas have the potential to develop into lethal plaques of rupture or erosion, which are the major substrates for acute coronary syndromes and sudden death. Phases post rupture considered episodic healed plaque ruptures are often further complicated by calcification (fibrocalcific plaques), which may show severe calcification characterized by sheets of calcification that may evolve into nodular calcification.

Although the precise mechanism of how pathological intimal thickening converts to a fibroatheroma is less clear, the infiltration of macrophages into existing proteoglycan-rich lipid pools is believed to be the defining feature. Immune responses along with lipid deposition have also been implicated both in the initiation and propagation of atherosclerotic plaque that results in the chronic inflammation of the vessel wall. Fibroatheromas, characterized by a thick fibrous cap overlying a necrotic core, can be divided into 2 different stages recognized as early or late, based on the discrete collection of cellular debris within the necrotic core, increased cholesterol crystals, and complete degradation of extracellular matrix observed only in late stages. In contrast, the early fibroatheromas are characterized by macrophage infiltration into the lipid pool with fewer cholesterol crystals and gradual loss of proteoglycan matrix likely through active proteases. Studies conducted in animal models reported apoptosis of macrophages as a fundamental step toward plaque progression accompanied by decreased uptake of apoptotic bodies by macrophages, so-called defective efferocytosis suggesting a deficient mechanism of clearance that contributes to necrotic core. The continued accumulation of lipid-laden macrophages through active proteases promotes thinning of the fibrous cap, which ultimately ruptures from hemodynamic forces likely supported by microcalcifications within the cap causing stress fracture.

In-Stent Neoatherosclerosis
The preceding atherosclerotic lesion that forms on the surface of existing neointima within a stent is observed to progresses at an accelerated rate, within months to years that eventually may lead to coronary syndromes either from plaque rupture or ISR. Unlike native disease, necrotic core formation in neoatherosclerosis is thought to occur from macrophage apoptosis in the absence of a lipid pool, as the necrotic core is typically found within clusters of macrophage foam cells in the peristrut regions or near the luminal surface, as more common for DES. Focal calcification(s) also occurs at sites of macrophage apoptosis in neoatherosclerosis similar to native disease (Figure 3). The temporal onset of neoatherosclerosis is even further accelerated for DES where the earliest atherosclerotic change of foamy macrophage infiltration is recognized at 4 months after sirolimus-eluting stent (SES) implantation, whereas the same change in BMS is typically seen beyond 2 years and rarely occurs until 4 years. Moreover, the study of long-term BMS implants at autopsy demonstrates a dynamic luminal response occurring within BMS characterized by early neointimal thickening and restenosis within 9 months followed by reduction of neointima

![Figure 1. Progression of neoatherosclerosis occurring within bare metal stent (BMS). A and B, Low- and high-power images of pathological intimal thickening showing the presence of lipid pool (LP) with focal areas of microcalcification (arrow heads). C and D, Low- and high-power images of fibroatheroma showing the presence of necrotic core (NC) and an overlying thick cap. E and F, Low- and high-power views of a thin cap fibroatheroma; high-power image shows the area of thin cap and adjacent NC. G and H, Low- and high-power images of plaque rupture note luminal thrombus (Th) and a thin-ruptured cap (arrow). Images G and H are reproduced from Nakazawa et al with permission of the publisher. Copyright © 2011, Elsevier.](http://atvb.ahajournals.org/Download)
beyond 18 months with late luminal renarrowing occurring sec-
ondary to neoatherosclerosis beyond 4 years.23

The introduction of BMS in the late 1980s reduced the
rate of restenosis attributed to balloon angioplasty because
of the elimination of elastic recoil and negative remodeling.24
However, the cellular response(s) to stent implantation with
today’s BMS and current generation DES represents a driving
substrate for late and very late thrombotic events.25 In an autopsy
study from our laboratory, the extracellular matrix within the
neointima of BMS essentially consists of proteoglycan (mainly
versican), hyaluronic acid, and collagen type III, whereas, there-
after, type I collagen increases with a corresponding decrease
in cellularity17 and extracellular matrix, indicating the sta-
bility of the neointima beyond 18 months.8 Endothelial cells
typically cover the luminal surfaces of BMS by 3 to 4 months
after implantation. In arteries stented for >4 years, VSMCs are
sparse although there is abundant proteoglycan/collagen, which
can serve as a potent nidus for foamy macrophage infiltration
(neoatherosclerosis) near the luminal surface. In a recent study,
we found that the development of neoatherosclerosis is delayed
in BMS relative to first-generation DES.7

It is becoming clear that the incidence of neoatheroscle-
rosis increases with the duration of the stent implantation,
especially after 3 years, with thrombotic events observed
only beyond 4 years for BMS. Furthermore, because neoath-
erosclerosis is rarely observed in BMS <2 years, late throm-
botic event, that is, >30 days but <2 year, is likely triggered
by causes other than neoatherosclerosis.25 Other possibilities
include endothelial senescence via a p57–p21–dependent
pathway, which has been reported in areas of disturbed flow
where atherosclerotic lesions occur.16

**Evolution of DES**

Since the initial approval of first-generation DES in 2003,
worldwide benefits from this technology.25 Although clearly there has
been an improvement in efficacy over BMS, persistent compli-
cations of hypersensitivity27 and thrombogenicity,28 a rare but
serious complication of treatment prompted further enhance-
ments in stent platforms and selection of drugs and polymer
coatings. Second-generation DES offer improved biocompat-
ible with a thinner durable polymer coating but with less drug
particularly for EES when compared with zotarolimus-eluting
stents. Otsuka et al6 highlighted that although the effective-
ness of the first- and second-generation DES was secondary to
delayed arterial healing, the amount of neointimal growth and
late catch-up were similar.6 Nonetheless, the incidence of LST
and VLST is significantly less for second-generation DES when
compared with first-generation DES. The extent of fibrin and
inflammatory response is significantly less in the second-gen-
eration DES, together with greater neointimal coverage and re-
endothelialization over struts, particularly for EES. Surprisingly,
however, we have reported a similar incidence of neoatheroscle-
rosis between first and second-generation CoCr-EES.29

The overall prevalence of neoatherosclerosis after coro-
mary CoCr-EES implantation was 29%, which did not differ
significantly from SES (35%) and PES (19%).29 Similarly,
no significant differences were observed in the frequency of
foamy macrophage infiltration or fibroatheromas between
early and new generation stents. However, there was no evi-
dence of unstable plaque features consistent with thin cap
fibroatheromas or plaque ruptures in second-generation DES,
which is likely related to the fact that all cases were within 3
years of implantation.

**Clinical Prevalence of Neoatherosclerosis**

The clinical prevalence of neoatherosclerosis can only be
detected by invasive intravascular imaging, which means its
diagnostic accuracy is highly dependent on type of imag-
ing modality. Intravascular ultrasound, which has a spatial

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**Figure 2.** Progression of neoatherosclerosis occurring within drug-eluting stent (DES). **A** and **B**, Low- and high-power images of early ath-
erosclerotic change characterized by luminal foam cells (arrows) with underlying fibrointimal thickening. **C** and **D**, Low- and high-power
images of foamy macrophage clusters (arrows) in the peristrut region adjacent to luminal surface. **E** and **F**, Low- and high-power images
of fibroatheroma with necrotic core (NC) within neointima. **G** and **H**, Low- and high-power images of thin cap fibroatheroma (arrows) with
NC. **I** and **J**, Low- and high-power images of plaque rupture resulting in continuity between the overlying thrombus (Th) and the NC. *^ rep-
resents stent strut. Images **E**, **F**, and **I** are reprinted from Yahagi et al1 with permission of the publisher.
Figure 3. A and B, In-stent neoatherosclerosis involving drug-eluting stent (DES) showing its progression within the neointima from infiltration of macrophage-derived foam cells, to more advanced lesions of fibroatheroma, thin cap fibroatheroma (TCFA), and complications of luminal thrombosis attributed to plaque rupture. Thrombi from plaque rupture involving DES are typically nonocclusive. Calcification is recognized as microcalcifications intermixed with apoptotic foam cells or calcification of persistent fibrin in peristrut regions because of delayed healing caused by drug occurring in the absence of macrophages. The temporal onset of neoatherosclerosis is accelerated for DES where the earliest atherosclerotic change of foamy macrophage infiltration is recognized at 4 months after sirolimus-eluting stent implantation, whereas the same change in bare metal stent (BMS) is typically seen beyond 2 years and rarely occurs until 4 years of implantation. C and D, Illustrations describing neoatherosclerosis for BMS where proteoglycan-rich lipid pools are recognized as the earliest lesion where subsequent macrophage infiltration is thought responsible for conversion to fibroatheromatous plaque with the potential to form more complicated lesions, such as TCFA, and rupture. Unlike DES, the character of neointima for BMS is generally extensive and hypocellular, as there is a focal loss of smooth muscle cells, which likely contributes to lipid pool formation.
resolution of 150 to 250 μm, is only capable of detecting necrotic core. In comparison, optical coherence tomography with an axial resolution of 10 to 20 μm improves the likelihood of identifying foamy macrophages, and therefore, it is additionally capable of distinguishing neoatherosclerosis. Although intravascular ultrasound studies demonstrate a good correlation of necrotic core with stent duration, the prevalence of neoatherosclerosis is ultimately underestimated with this imaging technique. On the other hand, Kang et al reported a 90% prevalence of neoatherosclerosis in patients presenting with stable and unstable angina with 52% showing thin cap fibroatheromas and 58% demonstrating at least 1 site of in-stent plaque rupture with a 32.2-month implant duration. Yonetsu et al conducted a systematic assessment of in-stent neoatherosclerosis in lesions >100 μm of thickness and showed that 47% have optical coherence tomography–detected neoatherosclerosis confirmed by accumulated lipid or calcification. Vergallo et al further showed that implant duration (≥48 months), DES usage, current smoking, chronic kidney disease, and an absence of angiotensin-converting enzyme inhibitors or angiotensin II receptor blockade usage were independent determinants of optical coherence tomography–detected neoatherosclerosis. Although the study by Vergallo et al reported high rates of neoatherosclerosis, we show a much lower prevalence in our autopsy registry (31% with a mean duration of 361 days); however, the incidence will ultimately vary with the duration of implant. The clinical prevalence of neoatherosclerosis and the percentage of cases presenting with late and VLST remain unknown. Taniwaki et al proposed a biologically relevant association between the presence of in-stent neoatherosclerosis and the progression of native atherosclerosis in a prospective study (SIRTAX LATE OCT [Sirolimus-Eluting Stent Compared With Paclitaxel-Eluting Stent for Coronary Revascularisation]) 5 years after DES implantation, giving a different perspective about the progression of atherosclerosis. Their findings suggest that patients presenting with in-stent neoatherosclerosis are more likely to have nontarget lesion revascularization in 79% of cases than 45% without neoatherosclerosis despite having a similar incidence of plaque progression in native coronary arteries (6% versus 4.3%, respectively). Hence, pathogenic factors contributing to the progression of native atherosclerosis seem to be similar to those involved in neoatherosclerosis formation, although the underlying mechanism(s) and presentation are clearly different. On the basis of our histology studies, younger age, longer duration of the implant, and SES and PES usage were identified as an independent predictor for the formation of neoatherosclerosis. The clinical study by Taniwaki et al highlights that secondary prevention by high-dose statins along with newer pharmacological agents will be a requirement and we need to remain vigilant in patients receiving DES to prevent accelerated neoatherosclerosis.

**Neoatherosclerosis as a Cause of In-Stent Thrombosis**

In the current and ongoing CVPath stent registry comprised of 266 BMS and 348 cases of first- and second-generation of DES, there are 10 coronary stents with plaque rupture and thrombosis developing from in-stent neoatherosclerosis. Considering the evolution of stent technology, the overall implant duration in our registry is clearly longer for BMS (median, 832 days) when compared with DES (median, first-generation DES, 383 days; second-generation DES, 210 days). Nonetheless, however, plaque rupture was observed in equal numbers of BMS and DES (4 SES and 1 PES), whereas 4 ruptures occurred in the presence of ISR, (3 BMS and 1 SES), ranging in duration from 5 to 8 years. The remaining 6 stents showed plaque rupture in the absence of ISR with 4 cases of DES, ranging in duration from 2 to 5 years, and 2 cases of BMS, with durations of 7 and 8 years. Taken together, these data suggest that ISR is not a prerequisite for plaque rupture and occlusive thrombosis.

In contrast, in-stent erosion is even a more rare underlying cause of late and very late thrombosis where in our experience, underlying neoatherosclerosis has been documented in only 1 of 3 erosion cases involving a 5-year SES implant overlying a contributing fibroatheroma with microcalcification. The 2 remaining erosion cases involved a BMS of 4-month duration with underlying severe ISR, whereas another case of SES at 2 years without underlying ISR or neoatherosclerosis showed focal neointimal tissue with an adherent thrombus extending from the 2 strut surfaces causing complete occlusion of the lumen.

**Mechanisms of Neoatherosclerosis**

The precise mechanism(s) of accelerated in-stent neoatherosclerosis remains unknown although nonspecific antiproliferative effects of the drugs eluted from the stent are considered the most likely mechanism. One issue is the selected drugs used for DES that not only target VSMCs but also endothelial cells, which results in incomplete regenerating, dysfunctional endothelial coverage of the stented segment with poor cell-to-cell junctions, reduced expression of antithrombotic molecules, platelet deposition, and decreased nitric oxide production, all favoring a greater permeability of low-density lipoproteins into subendothelial spaces, especially the small dense low-density lipoproteins that exhibit greater binding to arterial wall proteoglycans. Also, we have reported markedly greater accumulated proteoglycan in DES when compared with BMS, which may serve as a possible nidus for greater lipid retention within the subendothelial spaces with secondary macrophage infiltration. In the well-established rabbit model of iliofemoral artery stenting, Joner et al demonstrated varying degrees of re-endothelialization in different polymeric DES at 14 days post implantation. There was greater expression of platelet endothelial cell adhesion molecule-1 in EES compared with SES, PES, and EES supporting the mechanism of delayed re-endothelialization. Moreover, relative to BMS, all DES showed a lower expression of platelet endothelial cell adhesion molecule and antithrombotic cofactor thrombomodulin, a measure of endothelial function. Low wall shear stress contributes to activation of endothelial cells to promote the expression of adhesion molecules, such as intercellular adhesion molecule-1 and vascular cell adhesion protein-1 especially in DES, promoting monocyte binding and transmigration into subendothelial spaces with subsequent transformation into macrophage-derived foam
cells. Also, persistent endothelial injury caused by nonlaminar, disturb blood flow in bifurcation lesions, curvature of large arteries and nonstreamlined stent struts in both DES and BMS promotes atherosclerosis. Proinflammatory mediators (e.g., monocyte chemotactic protein-1, interleukin-8, tumor necrosis factor, reactive oxygen species, and interleukin-1) along with the activation of endothelial cells by selectins arrange the capture, rolling, and activation of monocytes to undergo firm adhesion to endothelial cells, likely as reported in native disease. It is also conceivable that macrophage proliferation may be involved, similar to native disease. Continued inhibition of VSMC growth and persistent disruption of lamina

Different mechanisms may underlie the process of neoatherosclerosis in BMS, which is likely more reminiscent of native coronary disease with the formation of lipid pools within a well-developed neointima rich in VSMCs (Figure 3). Although relatively delayed when compared with DES, the process of neoatherosclerosis for BMS accompanied by necrotic core formation likely occurs at a comparative accelerated pace to native coronary artery disease. Moreover, apoptosis of smooth muscle cells and foam cells coinciding with maturation of the neointima within BMS likely contributes toward the transition from lipid pool lesion to necrotic core. Similar to native disease, VSMC apoptosis is thought to be the underlying consequence of lipid pool formation with proteoglycan deposition, which promotes retention of lipoproteins, thus initiating the neoatherosclerotic process. Therefore, BMS complicated by ISR represents a more mature neointima with greater endothelial coverage, which may be the main reason for delayed neoatherosclerosis contrary to DES where clearly macrophage infiltration of lipid pools with subsequent apoptosis would trigger development and expansion of a necrotic core.

The character of the primary lesion, as an indication for DES seems to be a significant risk factor for neoatherosclerosis, and in unstable lesions, stent struts are likely embedded in the avascular necrotic core where the drug likely persists for long periods of time, causing sustained delays in endothelial recovery when compared with those DES implanted in stable plaques, thus accelerating neoatherosclerosis in unstable lesions. Furthermore, histopathology analysis showed that the majority of neoatherosclerotic plaques originate and remain confined to the territory of the stent and are less likely an extension from proximal or distal nonstented arterial segments. Communication of underlying necrotic core into the overlying neointima is also an unlikely possibility, as we excluded cases that show a continuity between native disease and neoatherosclerosis.

Ultimately, the development of neoatherosclerosis after stent implantation is likely multifactorial and may involve the inability to maintain a fully functional-endothelialized luminal surface within the stented segment. Delayed arterial healing in response to DES characterized by poor strut coverage by endothelial cells, disturbed flow, and focal platelet aggregation and adhesion have been identified as the major pathological substrate responsible for LST/VLAST after first-generation DES among several other factors, such as hypersensitivity reactions, strut malapposition with excessive fibrin deposition, and stent fracture. Recently, platelet aggregation has been associated with interaction with oxidized low-density lipoproteins, which promotes phenotypic change in monocyte enhancing foam cell formation. Several clinical related risk factors have also been described, which include younger age, longer implant duration, SES or PES usage, and acute myocardial infarction stenting.

Progressive neoatherosclerosis is a rare and potentially fatal process characterized by a necrotic core consisting of acellular debris with significant amounts of free cholesterol with near-to-complete depletion of extracellular matrix. Further infiltration of foamy macrophages within the neointima in response to proatherogenic stimuli along with VSMC apoptosis continues to weaken the thin fibrous cap that may eventually rupture. Other features of neoatherosclerosis consist of calcification within the neointima of the implanted stent, which presents as microcalcifications or calcified sheets. The mechanism(s) of calcification in DES may be similar to native atherosclerosis; however, unique to neoatherosclerosis is calcification of fibrin deposits, particularly with PES, which is hallmark by extensive and persistent peristrut fibrin deposition when compared with SES and EES.

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**References**

Pathology of Neoatherosclerosis


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