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Neoatherosclerosis: Another Consequence of Endothelial Dysfunction?

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The long-term vascular response to coronary stenting can involve transformation of an otherwise benign neointima to one that resembles *de novo* atherosclerosis in an unstented vessel. The neointima may be infiltrated by foamy macrophages, undergo neovascularization, and develop unstable thin-cap fibroatheromas (TCFA) that contribute to neointimal disruption and thrombus formation *in situ*, similar to what is observed in the presence of a vulnerable plaque.^{1, 2} This process is known as neoatherosclerosis and is independently associated with mean in-stent neointimal thickness (OR-2.53; 95% CI 1.96, 3.27, $p < 0.001$). When present, neoatherosclerosis leads to high rates of target lesion revascularization and stent thrombosis.^{1, 2}

There has been growing interest in the use of intravascular optical coherence tomography (OCT) imaging to examine neointimal tissue characteristics and detect neoatherosclerosis in bare metal stents (BMS) or drug-eluting stents (DES). A number of small clinical studies using this approach found that early (< 6 months) after BMS implantation, the neointima was thin and homogeneous; however, after 5 years, the neointima contained lipid pools and microvessels with areas of neointimal disruption consistent with neoatherosclerosis.³ Similar neoatherosclerotic changes have been observed in DES neointima although the overall lipid content is reportedly higher than what was seen in BMS.⁴ In one study of 50 patients with DES in-stent restenosis, OCT revealed that 52% had at least one in-stent neointimal TCFA and 58% had at least one area of in-stent neointimal disruption suggesting that neoatherosclerosis was highly prevalent in restenosis.⁵ DES neoatherosclerosis exhibits spatial heterogeneity with a predilection for the proximal and distal segments of the stent and occurs more frequently when there is an adjacent lipid-rich plaque.⁶ OCT studies also indicate that neoatherosclerosis occurs earlier in DES than BMS. While neoatherosclerosis was observed in BMS after 5 years, it was detected in DES after a median of only 32.2 months.⁵ While the aforementioned studies enrolled relatively few patients, their collective

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Disclosures

None.

findings add to an accumulating body of evidence that identifies neoatherosclerosis as a significant contributor to late stent failure.⁵

The OCT findings from clinical studies are supported by detailed histopathological examinations of in-stent neoatherosclerosis. Early pathological studies of BMS implanted for 2–7 years revealed chronic inflammation, neointimal microvessels, and extracellular matrix collagen deposition and matrix metalloproteinase activity.⁷ A contemporary autopsy study that analyzed 197 BMS and 209 DES found similar evidence of neointimal remodeling and confirmed that neoatherosclerosis occurred earlier in DES compared to BMS (70–120 vs. 900 days). Although TCF As and plaque rupture were detectable earlier in DES than BMS (2 vs. 5 years), neointimal TCFA were found more frequently in BMS (7.4%) than in DES (3.1%).⁸ In this series, the incidence of neoatherosclerosis was higher in DES than BMS (31% vs. 16%) despite the fact that at the time the analyses were performed, BMS had been implanted for ~2,160 days compared to only 420 days for DES. These findings are not unique to first-generation DES and a similar histopathological profile has been reported for second-generation DES.^{8,9} Clinical characteristics associated with in-stent neoatherosclerosis included advanced age, DES, stent implantation > 4 years, tobacco use and chronic kidney disease.^{8,10} Another study that performed histopathological analysis of aspirated thrombectomy material from 42 patients with BMS implanted > 1 year identified fragments of atherosclerotic plaques that included TCF As, foamy macrophages, and cholesterol crystals in the aspirates.¹¹ While it is not known whether or not the thrombus was retrieved from within the stent or an adjacent area, these findings add strength to the argument that in-stent neoatherosclerosis behaves the same as unstable atherosclerotic plaques.¹²

Understanding the cellular mechanisms involved in neoatherosclerosis has led to a focus on the endothelium. A functional endothelium is key to preventing neoatherosclerosis and this point was ably demonstrated by an experiment performed in normocholesterolemic rabbits implanted with a ³²P β-emitting stent. At 6 and 12 months, there was evidence of neoatherosclerosis with neointimal infiltration of inflammatory cells, foam cells, and calcification that occurred in the setting of poor reendothelialization. Since this model does not develop atherosclerosis in the absence of a high cholesterol diet, the in-stent lesions were determined to be neoatherosclerosis that was attributable to the lack of a functional endothelium.¹³ This is not surprising as early studies in the era of balloon angioplasty demonstrated that the regenerated endothelial cells are structurally and functionally abnormal. These cells were irregularly sized, did not align in the direction of blood flow, and were functionally impaired with abnormal endothelium-dependent vascular reactivity.¹⁴ Based on these observations, it should be expected that after stent placement the regenerated endothelium would be functionally incompetent. These regenerated cells are likely to have lost many of their typical atheroprotective features and have incomplete or poorly formed cell-cell junctions that are permissive for lipid or inflammatory cell migration into the vessel wall.^{8,15}

Several strategies have been investigated as mechanisms to restore the endothelium and, thereby, prevent neoatherosclerosis. The combination DES-endothelial progenitor cell capture stent has been evaluated as a mechanism to improve reendothelialization. While a

randomized trial indicates that these stents are safe and non-inferior to paclitaxel-eluting stents, there are no long-term intravascular studies to determine whether or not they prevent neoatherosclerosis. This seems unlikely as current preclinical evidence confirms that regenerated endothelial cells originate from adjacent cells and not from endothelial progenitor cells suggesting that the main benefit of the combination stent may extend beyond restoring the endothelial monolayer.^{16, 17} Drug-coated balloons, which avoid the pro-inflammatory effects of stent struts and polymers, may also be insufficient to prevent neoatherosclerosis. In fact, OCT performed 9 months after treatment with a drug-coated balloon for DES in-stent restenosis revealed a distinct heterogeneous pattern in the neointima with bright speckled structures consistent with macrophage infiltration and neoatherosclerosis.¹⁸

To address the issue of neoatherosclerosis, efforts need to focus on methodologies to promote reendothelialization with functional endothelial cells. The first step towards accomplishing this goal is to identify the phenotypic differences between normal and regenerated endothelium at a molecular, metabolic, and genetic level. Investigators have already started to perform gene-profiling studies comparing native and regenerated endothelial cells in preclinical models. In one study, normal and regenerated endothelial cells were isolated from hypercholesterolemic pigs 28 days after balloon angioplasty of the LAD and examined using gene expression microarrays. This experiment identified 5 genes with atheroprotective functions that were downregulated in the regenerated cells while another 9 pro-atherogenic genes were upregulated.¹⁹ Information derived from this and other deep phenotyping studies could be used further to identify genes or proteins that could be targeted for genetic manipulation to restore normal endothelial function. In addition, profiling studies should take into account the effects of antiproliferative drugs eluted from stents on the endothelium. This becomes an important consideration as recent work has shown that normal endothelial cells exposed to sirolimus become dysfunctional and have impaired barrier function leading to increased vascular permeability.²⁰ The second part of the equation is to determine the best mechanism to repopulate the stent luminal surface with competent endothelial cells and prevent neoatherosclerosis. This could be accomplished by delivery of fully functional cells either by coating a stent with an endothelial cell-specific antibody (i.e., von Willebrand factor or vascular endothelial-cadherin) coupled with stop-flow infusion of genetically engineered endothelial cells (based on gene profiling studies) that are expanded ex vivo and infused immediately after stent placement. Another plausible method is to generate a stent, such as a bio absorbable self-expanding platform (to avoid crush injury to the cells) that is prepopulated with a layer of endothelial cells. Similarly, a modified balloon delivery system could also be used to adhere functional endothelial cells to the luminal surface. Clinical realization of these strategies will require that we continue to apply advances made in basic endothelial biology to vascular scaffold design to limit neoatherosclerosis and prevent this mechanism of stent failure.

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