Morphological Predictors of Restenosis After Coronary Stenting in Humans

Andrew Farb, MD; Deena K. Weber, MS; Frank D. Kolodgie, PhD; Allen P. Burke, MD; Renu Virmani, MD

Background—Experimental studies suggest that arterial injury and inflammation lead to increased neointimal growth after stenting. Despite the increased use of coronary stents in humans, there are only limited pathological data on the morphological features of in-stent restenosis.

Methods and Results—Detailed histology was performed on 116 stents, implanted ≥90 days in 87 coronary arteries, from 56 patients (mean age, 59 ± 13 years). The mean duration of stent implant was 10 months. In-stent restenosis was defined as a stent area stenosis of >75%. Lumen area increased as stent area increased \( (r^2 = 0.27, P = 0.0001) \), but there was a much stronger correlation between stent area and neointimal area \( (r^2 = 0.70, P < 0.0001) \). Arterial medial fracture was associated with a 29% increase \( (P < 0.01) \) in neointimal thickness compared with arteries with an intact media. Neointimal thickness \( (P = 0.0001) \), inflammatory cell density \( (P < 0.0001) \), and neointimal vascular channel density \( (P < 0.0001) \) were greater when struts were in contact with a ruptured arterial media compared with fibrous plaque or an intact fibrous cap. Stent strut penetration into a lipid core was associated with increased neointimal thickness \( (P = 0.04) \) and inflammatory cell density \( (P = 0.03) \). Neointimal inflammatory cell content was 2.4-fold greater in stents with restenosis versus no restenosis, and inflammation was associated with increased neoangiogenesis.

Conclusions—Coronary stenting that is accompanied by medial damage or penetration of the stent into a lipid core induces increased arterial inflammation, which is associated with increased neointimal growth. These data suggest the use of stenting strategies that reduce inflammation and neoangiogenesis to reduce the incidence of restenosis. (Circulation. 2002;105:2974-2980.)

Key Words: stents  ■  restenosis  ■  atherosclerosis  ■  pathology

Coronary stents reduce restenosis rates compared with balloon angioplasty and have become the standard of care in catheter-based coronary artery revascularization for atherosclerotic disease. However, in-stent restenosis remains a recognized clinical problem and can be expected to increase in incidence as coronary stenting becomes more frequent and is used in less ideal (non-Benestent) lesions. Clinical and angiographic predictors of in-stent restenosis include long lesions, stenting of small coronary arteries, use of multiple stents, proximal left anterior descending coronary stenting, smaller final minimal lumen diameter, and stenting in diabetic patients. Studies of stenting in experimental animals suggest associations among medial injury, inflammation, and neointimal growth.

Previous studies from our laboratory have demonstrated a significant linear correlation between increased neointimal growth and increased stent size relative to the proximal reference coronary artery lumen. The objectives of the present study were to examine morphological predictors of in-stent restenosis in human coronary arteries focusing on arterial medial injury, neointimal inflammation, and underlying plaque morphology.

Methods

Stented human coronary arteries were accessioned at the Armed Forces Institute of Pathology for diagnostic consultation. Arteries were fixed in 10% neutral buffered formalin, radiographed, and processed for light microscopy. The length of the stented arterial segment was measured from postmortem radiographs. The stented arterial segment was placed intact into processing vials and dehydrated in a graded series of alcohols. Samples were then infiltrated with methylmethacrylate plastic and placed in airtight vials in a 38°C waterbath for polymerization. After polymerization, blocks were cut at 2-mm intervals along the entire length of the stents. Plastic sections (4 to 5 mm thick) were then cut, adhered to glass slides, and allowed to dry. The plastic was then removed, and the sections were rehydrated and stained. In 28 cases, a 2-mm-long segment was cut from the midportion of the stented artery using fine scissors. The
stent wires were carefully removed under a dissecting microscope before paraffin embedding. Plastic and paraffin sections were stained with hematoxylin and eosin and Movat pentachrome.

**Selection of Cases**

Coronary stents that had been implanted for ≥3 months were selected for analysis. In-stent restenosis was defined as a stent lumen cross-sectional area stenosis of >75% (equivalent to a stent lumen diameter stenosis of >50%). No restenosis was defined as a stent lumen area stenosis of ≤75% (equivalent to a stent lumen diameter stenosis of ≤50%).

**Morphological and Morphometry Measurements**

The arterial segment from each stent demonstrating the greatest severity of in-stent stenosis was selected for analysis. Histological sections were magnified (>×20) and digitized, and computer-guided morphometry measurements were performed using IPLab Spectrum software (Scanalytics Inc). The following arterial measurements were made on each section: areas of internal elastic lamina, stent, underlying plaque, lipid core, calcific foci, lumen, and in-stent neointima. Stent area stenosis was calculated. Neointimal thickness was measured at each stent strut, and mean intimal thickness was calculated. The presence or absence of medial dissection was noted, and if present, the length of medial discontinuity (medial fracture length) was measured. Sections were additionally magnified (>×200), and 4 fields containing stent struts (12, 3, 6, and 9 o'clock) were selected for analysis of neointimal thickness, neointimal inflammatory cell density (cells/mm²) around stent struts, and neointimal neoangiogenesis (area of endothelialized vascular channels [confirmed by von Willebrand factor staining] adjacent to stent struts). The stent strut was categorized as being associated with fibrous plaque (or an intact fibrous cap or intact media), medial injury, or lipid core penetration. Neointimal and plaque macrophages, T cells, B cells, and fibrin associated with stent struts were identified by immunohistochemistry (KP-1, UCHL, L26, and fibrin II stains, respectively) in paraffin sections.

**Statistical Analysis**

Numerical data are presented as mean±SD. Continuous variables were compared using an ANOVA and simple linear regression. *P*≤0.05 was considered significant.

**Results**

**Patient Characteristics**

The study group consisted of 56 individuals (36 men, 20 women, mean age 59±13 years) with coronary artery stents in place 10±7.1 months (range, 3 to 36 months) antemortem (25 patients ≥6 months duration of stenting; 31 patients, >6 months). The indications for stenting were unstable or post-myocardial infarction angina (n=23), stable angina (n=15), acute myocardial infarction (n=2), and unknown (n=16). Two patients underwent rotational atherectomy; no patients received brachytherapy. A total of 116 stents were placed in 87 coronary arteries, as follows: MULTI-LINK, n=53; AVE, n=28; Palmaz-Schatz, n=18; NIR, n=11; Gianturco-Roubin II, n=3; BardXT, n=2; and Gianturco-Roubin, n=1. Stent diameters ranged from 1.95 to 4.20 mm with 47 stents <3.0 mm in diameter, 23 stents 3.0 to 3.4 mm in diameter, and 17 stents ≥3.5 mm in diameter. The mean length of deployed stents was 22±14 mm (range, 8 to 73 mm). The arteries stented were as follows: left anterior descending, n=36; right coronary, n=26; left circumflex, n=17; left obtuse marginal, n=3; left diagonal, n=2; ramus intermedius, n=2; and left main, n=1. Patient mortality was attributable to sudden cardiac death (n=18), postcoronary revascularization (n=9), acute myocardial infarction (n=6), heart failure (n=4), and noncoronary death (n=19). Of the 87 stented arteries, in-stent restenosis was present in 42, and no restenosis was present in 45.

**Morphometry**

Final lumen area correlated with stent area (*r*=0.27, *P*<0.0001, Figure 1A), but there was a much stronger correlation (*r*=0.70, *P*<0.0001) between stent area and neointimal area (Figure 1B). The lumen area was significantly smaller and the neointimal area was significantly greater in in-stent restenosis versus nonrestenosis cases (Table). The area within the internal elastic lamina (IEL) was slightly smaller in restenosis cases, but differences were not statistically significant. Plaque area as a function of IEL area,

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**Basic Morphometry: Long-Term Stent Patency Versus In-Stent Restenosis**

**Figure 1.** Regression plots of the correlations of stent areas with lumen area (A) and neointimal area (B). The weaker correlation between stent size and lumen area indicates that stent size alone does not determine long-term lumen area.
Inflammation and Neointimal Growth
Medial fracture, present in 40 stented arteries and absent in 47 arteries, was associated with a 29% greater mean neointimal thickness \((P<0.01)\) compared with stents without medial fracture (Figure 2, A through C). The mean stent area stenosis was 77 ± 15% when medial fracture was present versus 68 ± 20% when medial fracture was absent \((P<0.04)\). Medical fracture length as a percent of the circumference of the internal elastic lamina was greater in restenotic stents (15.0 ± 18.8%) compared with nonrestenotic cases (7.3 ± 12.6%, \(P<0.03\), Figure 2D). In an analysis by stent struts, the neointima was thinner when stent struts were in contact with fibrous plaque, an intact fibrous cap, or normal media (0.68 ± 0.36 mm) versus struts associated with arterial medial disruption (0.91 ± 0.32 mm, \(P=0.0001\)).

Neointimal vascular channel density was associated with increased inflammatory cell accumulation around struts (intact fibrous plaque, medial disruption, or lipid core penetration). Peri-strut inflammatory cell density was increased in regions of medial disruption (2259 ± 2619 inflammatory cells/mm²) compared with struts in contact with fibrous plaque or an intact fibrous cap (819 ± 1094 inflammatory cells/mm², \(P<0.0001\), Figures 3B through 3D). The percent of the neointima occupied by macrophages was 3-fold higher in restenosis versus no-restenosis cases (Figure 4).

Neangiogenesis, Intimal Growth, and Inflammation
Peri-strut neangiogenesia area density correlated significantly but weakly with mean in-stent neointimal thickness \((r^2=0.10, P<0.0001)\), and increased neangiogenesia area density was associated with increased inflammatory cell density (Figure 5). Struts associated with medial disruption had a greater vascular channels area density (0.010 ± 0.011 mm²/×200 field) compared with struts in contact with fibrous plaque or an intact fibrous cap (0.004 ± 0.006 mm²/×200 field, \(P<0.0001\), Figure 6).

Lipid Core Penetration by Stent Struts
Core penetration by stent struts was present in 16 cases. Lipid core penetration was associated with an increased neointimal thickness (0.88 ± 0.50 mm, \(P=0.04\); Figure 7, A and B) and increased numbers of peri-strut inflammatory cells (1493 ± 2105 inflammatory cells/mm²; \(P=0.03\)) compared with struts in contact with fibrous plaque (0.68 ± 0.36 mm neointimal thickness and 819 ± 1094 inflammatory cells/mm²). Neointimal vascular channel density was similar in cases of lipid core penetration versus struts in contact with fibrous plaque. Plaques with large lipid core areas and a greater percentage of the plaque occupied by a lipid core were more likely to be penetrated by stent struts; core area and the ratio of core area to plaque area in arteries with core penetration was 1.72 ± 1.71 mm² and 37 ± 44%, respectively.
compared with 0.84±1.28 mm² and 14±18%, respectively, in arteries without core penetration (P<0.03, Figure 7E).

Discussion

Angiographic factors associated with in-stent restenosis include small diameter reference coronary arteries, longer lesion length, and longer stent length. The present study adds important morphological data (extent of medial injury, inflammation, and neoangiogenesis) to the list of clinical and angiographic variables that are associated with outcome after coronary stenting. Notably, these local morphological parameters are not fully appreciated with present coronary artery imaging techniques.

Arterial Injury and Restenosis

Not unexpectedly, in-stent neointimal area correlated strongly (r²=0.70) with increased stent size, because a larger-diameter deployed stent can accommodate a greater accumulation of neointimal tissue than a smaller stent. However, the much weaker correlation between stent size and lumen area (r²=0.27) confirms that other factors besides stent size must be operative in determining long-term stent patency. In the present study, the potential benefit of “bigger is better” to reduce restenosis when applied to stenting is limited by increased arterial injury and inflammation. Clearly, medial injury and increased medial fracture length cause increased neointimal growth.

Although damage to the arterial media is readily apparent by histology, arterial injury is difficult to quantify clinically. In present interventional cardiology practice, the operator cannot tightly control the degree of arterial injury (plaque disruption, medial stretch, medial dissection, or medial rupture) produced during catheter-based procedures. Intravascular ultrasound can recognize disruption in plaques (in the

Figure 3. Restenotic stents had a greater accumulation of neointimal inflammatory cells (A), and inflammatory cell density was increased at stents strut sites associated with medial injury (B). Photomicrographs demonstrate increased neointimal chronic inflammation adjacent to stents struts (*) overlying focal medial (m) injury (arrows in C). Fewer inflammatory cells were observed near struts in contact with fibrous plaque (fp in D). Inflam indicates inflammatory. C and D, Movat pentachrome stain. Scale bars=0.16 mm in C and 0.19 in D.

Figure 4. Neointimal macrophages and neointimal growth. A, In-stent restenosis of a left circumflex artery stent placed 6 months antemortem. A hypercellular neointima (B) surrounds stents struts (*), and KP-1 immunohistochemistry (C) identifies numerous brown-staining macrophages. Fewer intimal macrophages are associated with the widely patent left anterior coronary artery stent deployed 4 months antemortem (D through F). F, Peri-strut calcium (Ca²⁺) deposits are indicated. Increased neointimal macrophage content was associated with in-stent restenosis (G). Neo indicates neointima. A, B, D, and E, Movat pentachrome stain; C and F, anti–KP-1 immunostain. Scale bars=0.85 mm in A and D, 0.13 mm in B and C, and 0.25 mm in E and F.
absence of calcification) but cannot precisely visualize the arterial media. The present study also underscores an important difference in human stenting compared with most experimental animal studies. In a normal animal artery, a properly sized (ie, nonoversized) stent is placed in apposition to the internal elastic lamina, causing mild arterial trauma. In humans, stents are expanded in a stenotic arterial segment to a diameter that matches a presumed normal reference segment; plaque disruption and arterial injury of varying degrees are unavoidable if lumen dilatation is to be achieved. The present study suggests that proper stent sizing and avoidance of excessive medial injury in the restoration of normal physiological blood flow are desirable goals in coronary stent placement rather than only maximizing lumen area.

**Lipid Core Penetration and Restenosis**

Fibrous cap rupture and stent strut penetration into the plaque lipid core were associated with increased neointimal growth. Plaques with large absolute lipid core size (\( \geq 2.0 \text{ mm}^2 \)) and the percent of plaque occupied by the lipid core (\( \geq 30\% \) of total plaque area) identify lesions at increased risk for lipid core penetration by stent struts. These lipid-rich plaques may be predisposed to increased neointimal growth based on the presence of numerous resident chronic inflammatory cells associated with lipid cores. Stenting highly lipid-rich vulnerable lesions and disrupted plaques might be improved via the use of devices to aspirate large cores or through intensive lipid lowering or photodynamic therapy to reduce plaque lipid and inflammatory cell content before stent deployment. Like medial injury, lipid core penetration by stents is associated with increased neointimal inflammation.

In contrast to intravascular ultrasound (IVUS), we were unable to demonstrate a correlation between underlying plaque burden (plaque area/IEL area) and in-stent restenosis. We postulate that this difference reflects the more detailed analysis of arterial injury, inflammation, and neoangiogenesis afforded by histology that is beyond the resolution of IVUS. The present study does show that stent penetration into a plaque core, rather than just plaque size itself, is a determinant of increased neointimal growth.

**Figure 5.** Progressive increase in neointimal inflammatory cell density with increased density of neointimal angiogenesis.

**Figure 6.** Increased neointimal neoangiogenesis was associated with medial injury (A). Photomicrographs show multiple vascular channels (arrows in B) adjacent to stent struts (*) in areas of medial injury (arrowheads in B) with positive von Willebrand factor staining of neovessels in C. Few vascular channels are present near struts in contact with fibrous plaque (fp in D). VC indicates vascular channels. B and D, Movat pentachrome stain. Scale bars=0.20 mm in B and D and 0.11 mm in C.

**Figure 7.** Lipid core penetration by stent struts (low power in A and high power in B) was associated with increased neointimal thickness (arrows) compared with stent struts in contact with fibrous plaque (C and D). Cholesterol clefts are indicated (arrowheads in B). Plaques with large lipid cores, occupying more than one third of the total plaque area, were at increased risk of lipid core penetration by the stent (E). A though D, Movat pentachrome stain. Scale bars=0.37 mm in A, 0.22 mm in B, 0.47 mm in C, and 0.20 in D.
Importance of Inflammation
Preclinical studies suggest that intimal inflammation is a determinant of in-stent neointimal growth. In stented rabbit iliac arteries, monocyte adherence correlated with neointimal size ($r^2=0.96$). A subsequent study showed peak monocyte adherence to the lumen surface 3 days after stenting, which correlated with intimal cellular proliferation ($r^2=0.916$). In porcine coronary stents, lymphohistocytic cell infiltration around stent struts was associated with increased intimal thickness and percent lumen area stenosis. Furthermore, multiple studies demonstrate that treatments that inhibit inflammation and neointimal cell adhesion molecules reduce intimal growth. In rabbit iliac artery stents, M1/70, a monoclonal antibody to the adhesion molecule Mac-1, reduced leukocyte recruitment >2-fold and reduced intimal area. P-selectin knockout mice treated with carotid ligation had a 76% reduction in neointima/media area and reduced vessel wall leukocytes. A monoclonal antibody against intercellular adhesion molecule-1 reduced the intima/media ratio by $\approx 50\%$ in the rat carotid balloon injury model.

Inflammatory cells associated with coronary stent placement in humans have been previously described. Neutrophils surrounding stent struts, typically observed early after stent placement, are absent beyond 30 days after deployment. In contrast, chronic inflammatory cells (macrophages and lymphocytes) are seen both early (3 to 7 days) and late (≥6 months) after stenting. Furthermore, early after stenting (≥3 days), the severity of inflammation in stented human coronary arteries is related to the underlying arterial wall morphology; stents in apposition to damaged arterial media or that penetrate the necrotic core of lipid-rich plaques are associated with greater numbers of inflammatory cells compared with struts in contact with fibrous plaque. The present study extends these observations to intermediate-term to long-term stent implants. Arterial medial disruption and lipid core penetration by stent struts are both associated with chronic inflammation. These factors are in turn associated with greater neointimal growth. Because some degree of arterial injury is unavoidable during stenting, therapies directed against local inflammation are a logical target to reduce neointimal growth. Recent experimental and human studies have shown low in-stent restenosis rates with stents coated with rapamycin, an agent with both antiproliferative and antiinflammatory effects.

Significance of Neangiogenesis
Neovessels, originating from the vasa vasorum, are commonly found in atherosclerotic plaques, and their growth is proportional to underlying plaque burden. Intimal smooth muscle cells and macrophages express vascular endothelial growth factor (VEGF) in human atherosclerotic lesions. Experimental studies have shown that arterial injury is associated with increased intimal and medial expression of VEGF and smooth muscle cell and endothelial cell proliferation in response to VEGF. Neointimal vascularization within stents, noted previously by Brasen et al, colocalizes with oxidative epitopes, iron deposits, VEGF, platelet-derived growth factor-BB, and microhemorrhages. We postulate that microvessel growth is stimulated by the release of VEGF by inflammatory cells. Chronic inflammation is augmented by the presence of a foreign body (stent) and arterial injury. It is possible that the constant motion of the semirigid stent damages peri-strut neovessels resulting in local hemorrhage and fibrin deposition. The presence of neovessels within the neointima offers a potential target for antirestenosis therapies.

Limitations
As an autopsy study, the results presented may not be representative of persons who receive stents and survive without stent-associated morbidity. In the present study, however, a large number of stents were analyzed, most of which did not have histological in-stent restenosis.

Conclusion
Morphological studies of human coronary stent implants confirm the links among arterial injury, inflammation, and neointimal growth. Neangiogenesis is associated with inflammation and may play a secondary role in restenosis. These data underscore the limitation of the “bigger is better” hypothesis when applied to stenting. Improved arterial imaging techniques may ultimately offer the interventional cardiologist the ability to optimally deploy stents with minimal arterial injury and avoidance of lipid core penetration. Reduction in stent-associated inflammation is a promising therapeutic target to improve late outcome.

References
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