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Evaluation of Four-Year Coronary Artery Response After Sirolimus-Eluting Stent Implantation Using Serial Quantitative Intravascular Ultrasound and Computer-Assisted Grayscale Value Analysis for Plaque Composition in Event-Free Patients

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OBJECTIVES This study sought to evaluate the long-term arterial response after sirolimus-eluting stent implantation.

BACKGROUND Sirolimus-eluting stents are effective in inhibiting neointimal hyperplasia without affecting plaque volume behind the stent struts at six months.

METHODS Serial quantitative intravascular ultrasound and computer-assisted grayscale value analysis over four years were performed in 23 event-free patients treated with sirolimus-eluting stents.

RESULTS In the first two years, the mean plaque volume (155.5 ± 42.8 mm^3 post-procedure and 156.8 ± 57.7 mm^3 at two years, $p = 0.86$) and plaque compositional change expressed as mean percent hypoechogenic tissue of the plaque behind the stent struts (78.9 ± 8.6% post-procedure and 78.2 ± 8.9% at two years, $p = 0.67$) did not significantly change. However, significant plaque shrinking (change in plaque volume = −18.4 mm^3, $p = 0.02$) with an increase in plaque echogenicity (change in percent hypoechogenic tissue = −7.8%, $p < 0.0001$) was observed between two and four years. The mean neointimal volume increased over four years from 0 to 8.4 ± 5.8 mm^3 ($p < 0.0001$). However, no further statistically significant change occurred between two and four years (7.0 ± 6.7 mm^3 vs. 8.4 ± 5.8 mm^3, $p = 0.25$).

CONCLUSIONS Between two and four years after sirolimus-eluting stent implantation, peri-stent tissue shrank with a concomitant increase in echogenicity. These intravascular ultrasound findings suggest that late chronic artery responses may evolve for up to four years after sirolimus-eluting stent implantation. In addition, the fact that the neointima does not significantly change from two to four years may suggest that the biological phenomenon of a delayed healing response has begun to subside. (J Am Coll Cardiol 2005;46:1670–6) © 2005 by the American College of Cardiology Foundation

Polymer-based drug-eluting stents reduce in-stent neointimal hyperplasia in randomized trials and registries (1–3). However, concern exists that the non-erodable polymer, as well as the presence of the drug within the polymer, may exert long-term detrimental biological effects (4,5). Little data are available on long-term arterial responses after either sirolimus-eluting stent (SES) or paclitaxel-eluting stent (PES) implantation. A recent report showed that PES implantation was associated with an increase in plaque volume behind the stent struts at six months (6). Although SES do not affect the plaque volume behind the stent struts at six months, no data at later time points have been reported (7,8). The goal of this study was to evaluate the late progression of the intra-stent neointima as well as the long-term arterial remodeling process and changes in plaque composition inside and outside the stent after SES implantation. To investigate these changes, serial quantitative intravascular ultrasound (IVUS) and computer-assisted grayscale value analyses for plaque compositional imaging over a four-year follow-up period were performed in event-free patients who were treated with SES.

METHODS

Study population. Thirty patients with de novo coronary artery lesions were treated with a single 18-mm sirolimus-eluting Bx-Velocity stent (Cordis, Miami Lakes, Florida) in São Paulo, Brazil, in the first human study of SES as described elsewhere (9). After the procedure, proper risk factor management was mandated for all patients. Of the 30 patients, 26 patients underwent IVUS examination at four years by protocol, three patients had target vessel revascularization before scheduled four-year follow-up angiography, and one asymptomatic patient refused repeat angiog-

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Abbreviations and Acronyms

ANOVA = analysis of variance
ECG = electrocardiogram/electrocardiographic
IVUS = intravascular ultrasound
PES = paclitaxel-eluting stent
SES = sirolimus-eluting stent

raphy. Of these 26 patients, three were excluded: two patients did not undergo IVUS at two-year follow-up, and plaque behind the stent on the immediate post-procedure IVUS could not reliably be assessed in one patient. Thus, serial coronary angiography and intravascular ultrasound analysis were performed post-procedure, at four-month, one-year, two-year, and four-year follow-up in 23 patients who were event-free throughout the four-year follow-up period.

Serial quantitative IVUS analysis. All follow-up IVUS were performed using an automated pull-back system at 0.5 mm/s and recorded on VHS videotapes. Images were digitized for quantitative analysis. The lumen, stent, and external elastic membrane contours were detected with CURAD QCU analysis software (Curad B.V., Wijk bij Duurstede, the Netherlands) (7). The stented segment and the 5-mm segments proximal and distal to the stent were analyzed. Over the four-year follow-up period, different IVUS catheters and ultrasound consoles were used. Post-procedure, four-month, and one-year follow-up, 20- or 30-MHz non–electrocardiogram (ECG)-gated IVUS were performed. At two-year follow-up, 30- or 40-MHz ECG- or non–ECG-gated IVUS were performed. At four-year follow-up, 30- or 40-MHz non–ECG-gated IVUS were performed. Three IVUS consoles were used (In-Vision [Volcano Therapeutics, Rancho Cordova, California], Clearview, and Galaxy [both Boston Scientific Corp., Natick, Massachusetts]). To analyze and compare the IVUS data consistently, all IVUS examinations were retrospectively ECG-gated using the Intelligate method, which automatically selects the end-diastolic frames from pre-recorded non–ECG-gated IVUS data (10). In addition, an automatic adjustment for 30-MHz Boston Scientific catheters that were connected to a Clearview console was applied as previously described (11).

Image-based plaque characterization (echogenicity). We used a computer-aided, in-house–developed grayscale value analysis program for plaque characterization (12). Based on the mean gray level (brightness) of the adventitia, plaque was classified as more (hyperechogenic) or less bright (hypoechogenic) in relation to the adventitia (Fig. 1A). The volume bounded externally by the surface that lies 0.5 mm outside the media and internally by the surface that lies 0.2 mm outside the media was defined as the adventitia. In cross-sectional images, it appears as a 0.3-mm thick band just outside the media. Upper tissue was defined as tissue that has a mean gray value higher than the mean adventitial intensity plus two times its standard deviation (12). Calcified plaque and stent struts were in this upper tissue range. The percentage of hypoechogenic plaque was calculated for the entire region of interest, excluding upper tissue (Fig. 1B) (12).

Statistical analysis. Continuous variables were expressed as mean values ± standard deviations and compared by means of the paired *t* test. Overall IVUS parameters (volume and echogenicity) across all time points were compared using repeated-measure analysis of variance (ANOVA). Two post-hoc tests (between post-stent and two years, and between two years and four years) were performed with Bonferroni corrections (p value for significance <0.025) for p values <0.05 on ANOVA.

RESULTS

Patient characteristics. Mean age of patients was 57 years, and two-thirds were male (Table 1). The left anterior descending artery was treated in 14 patients, right coronary artery in 6 patients, and left circumflex artery in 3 patients. After stent implantation, risk factor management was instituted in all patients. Aspirin and statins were prescribed in all patients during the four years.

Plaque volume behind the stent struts. Table 2 shows the serial quantitative IVUS data. Plaque volume behind the stent struts did not change significantly in the first two years ($p = 0.86$), but showed a significant change between two and four years because of a decrease in plaque volume behind the stent struts at four years (156.8 ± 57.7 mm^3 vs. 138.4 ± 42.2 mm^3, $p = 0.02$). Although plaque volume at the proximal edge of the stents increased significantly at four-month follow-up compared with post-procedure (32.5 ± 12.2 mm^3 vs. 39.8 ± 15.8 mm^3, $p = 0.005$), it did not differ significantly between post-procedure and four-year follow-up (32.5 ± 12.2 mm^3 vs. 33.0 ± 11.7 mm^3, $p = 0.81$). At the distal edge, no significant changes in plaque volume were observed at any time point ($p = 0.18$).

Neointimal volume. Neointimal volume increased at each measured time point, and was 8.4 ± 5.8 mm^3 at four-year follow-up ($p < 0.0001$). Between post-procedure and two-year follow-up, a significant change in neointimal volume was noted (0 mm^3 vs. 7.0 ± 6.7 mm^3, $p < 0.0001$). However, between two-year and four-year follow-up, the increase in neointimal volume was not statistically different (7.0 ± 6.7 mm^3 vs. 8.4 ± 5.8 mm^3, $p = 0.25$). Figure 2A shows the correlation between neointimal volume and plaque volume behind the stent struts over four years.

Plaque composition. Serial computer-assisted grayscale value analyses for plaque composition are shown in Table 3. With respect to the plaque behind the stent struts, there was a significant decrease in percent hypoechogenic tissue, that is, an increase in percent hyperechogenicity up to four years ($p < 0.0001$). Between post-procedure and two years, there were no significant changes in plaque echogenicity ($78.9 \pm 8.6\%$ vs. $78.2 \pm 8.9\%$, $p = 0.67$). However, between two

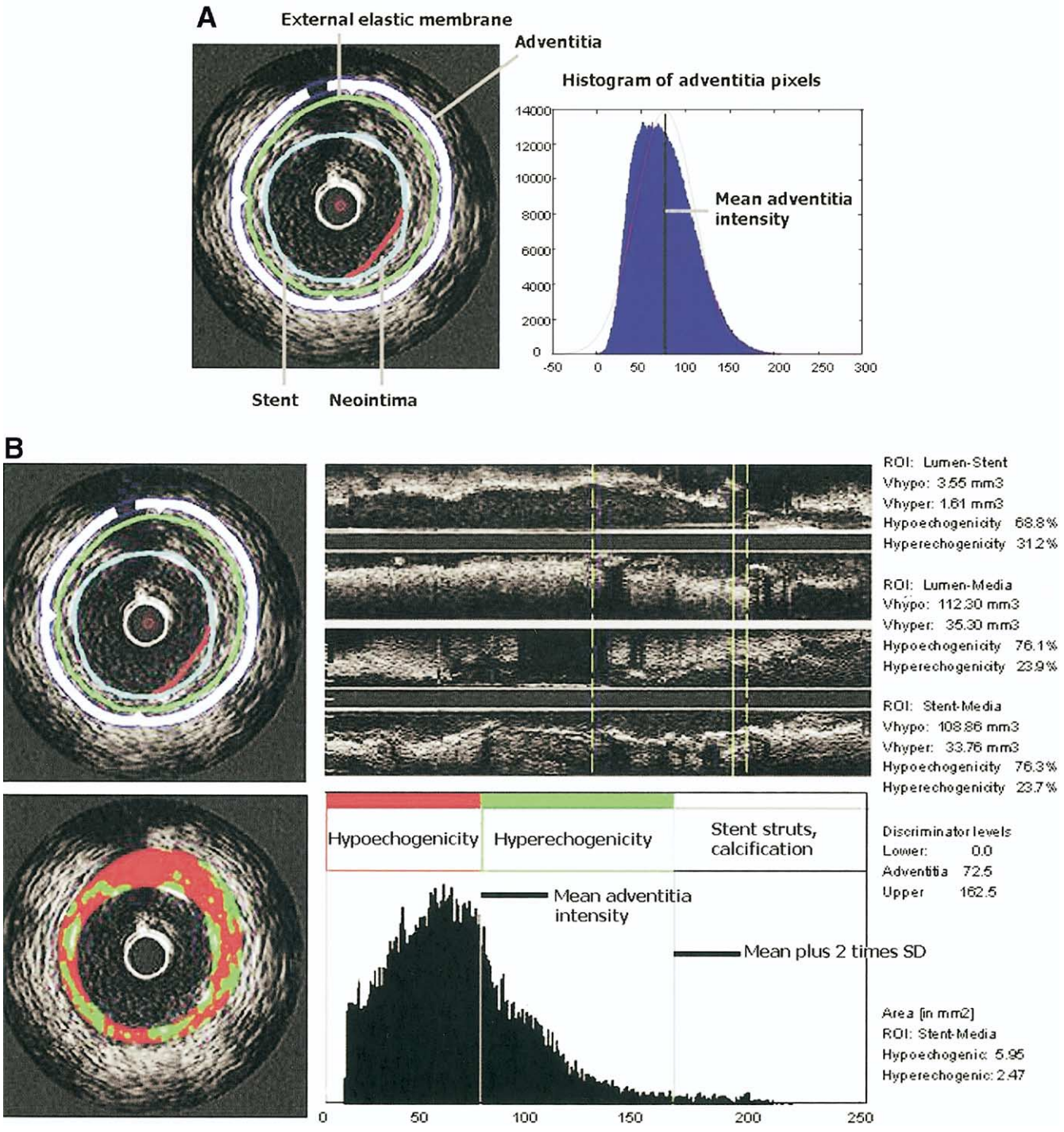

Figure 1. **(A)** The adventitia is defined as tissue outside the external elastic membrane contour. For all non-shadowed adventitia pixels, the mean value and standard deviation are calculated. To observe the suitability, a normal distribution curve based on the same mean and standard deviation histogram is created. **(B)** Cross-sectional image of echogenicity and distribution graph of plaque echogenicity behind the stent struts. Hyperechogenic areas are colored **green**. Hypoechogenic areas are colored **red**. ROI = region of interest.

and four years, significant changes were noted (78.2 ± 8.9% vs. 70.4 ± 10.6%, $p < 0.0001$). A similar pattern was observed for plaque at the proximal stent edge with a significant reduction in percent hypoechogenic tissue at four-year follow-up ($p = 0.02$). At the distal stent edge, no significant changes in plaque echogenicity were observed across time points ($p = 0.78$). Figure 3 is a representative example of a patient with plaque shrinkage and reduction of hypoechogenic plaque composition from two-year to four-year follow-up.

Neointimal echogenicity. The echogenicity of the neointima increased over the four-year follow-up period ($p < 0.0001$) (Fig. 2B). On post-hoc testing with Bonferroni corrections, the decrease in hypoechogenicity between four

Table 1. Patient Characteristics

	n = 23
Age, yrs (mean ± SD)	57 ± 9
Male, %	65.2
Hypertension, %	69.6
Hyperlipidemia, %	47.8
Diabetes mellitus, %	26.1
Family history, %	30.4
Current smoking, %	65.2
Treated vessel	
LAD, %	60.9
LCX, %	13.0
RCA, %	26.1

LAD = left anterior descending artery; LCX = left circumflex artery; RCA = right coronary artery.

months and two years was not significant (74.9 ± 12.7% vs. 66.7 ± 19.1%, p = 0.03), but was significant between two-year and four-year follow-up (66.7 ± 19.1% vs. 57.0 ± 22.6%, p = 0.01).

DISCUSSION

Previous reports have shown that SESs are effective in inhibiting neointimal hyperplasia without affecting total vessel volume or plaque volume behind the stent struts at six months (7,8). In the present study, this pattern of tissue growth inhibition inside and outside the SES was maintained at two years. However, at four-year follow-up, a significant negative remodeling (shrinking) of the plaque behind the stent struts with an increase in hyperechogenicity was observed.

Several studies have shown that plaque echogenicity in the carotid artery is related to the histologic components of plaques and that echolucency (low echogenicity) can predict clinical events (13,14). For coronary plaque studies, plaque echogenicity has also been related to the histologic components of plaque (15–17). Hyperechogenic tissue has been shown to be associated with a predominance of dense fibrous or elastic tissue, whereas hypoechogenic plaque was correlated with predominance of loose fibrous, lipid, or necrotic tissue. Recently, other analysis systems such as IVUS elastography, IVUS palpography, IVUS radiofrequency analysis (so-called virtual histology), and optical coherence tomography have been used to evaluate plaque vulnerability and plaque composition (18–20). However, these methodologic approaches require prospective acquisition of data, for which their respective devices were not available for human use at the time of initial assessment. Plaque echogenicity analysis by computer-assisted grayscale value is currently the only method that has the potential to analyze plaque composition in a retrospective manner.

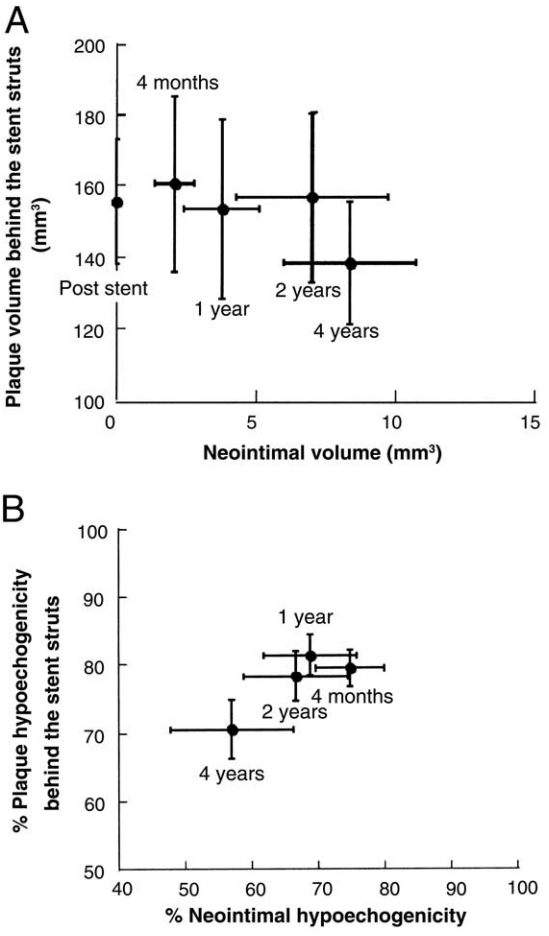

Figure 2. **(A)** Correlation between neointimal volume and plaque volume behind the stent struts over four years. **(B)** Correlation between neointimal percent hypoechogenicity and plaque percent hypoechogenicity behind the stent struts over four years. **Error bars** indicate 95% confidence interval.

Table 2. Serial Three-Dimensional Intravascular Ultrasound Analysis of Neointima and Plaque Volume

	After Procedure	4 Mo	1 Yr	2 Yrs	4 Yrs	ANOVA* p Value	Post Hoc After Procedure to 2 Yrs	Post Hoc 2 to 4 Yrs
Neointima, mm^3	0	2.1 ± 1.7	3.8 ± 3.3	7.0 ± 6.7	8.4 ± 5.8	<0.0001	<0.0001	0.25
Plaque (stented segment), mm^3	155.5 ± 42.8	160.4 ± 60.4	153.5 ± 61.6	156.8 ± 57.7	138.4 ± 42.2	0.04	0.86	0.02
Vessel (stented segment), mm^3	305.1 ± 53.7	310.8 ± 99.4	305.4 ± 83.8	309.8 ± 84.1	290.5 ± 65.3	0.09	—	—
Plaque (proximal edge), mm^3	32.5 ± 12.2	39.8 ± 15.8	36.5 ± 11.9	37.4 ± 13.0	33.0 ± 11.7	0.006	0.03	0.05
Vessel (proximal edge), mm^3	82.6 ± 21.9	88.6 ± 23.8	80.1 ± 17.2	82.6 ± 22.1	78.3 ± 21.6	0.001	0.98	0.10
Plaque (distal edge), mm^3	26.2 ± 15.4	28.6 ± 11.1	28.6 ± 14.0	31.1 ± 13.4	25.8 ± 11.3	0.18	—	—
Vessel (distal edge), mm^3	63.3 ± 22.0	68.5 ± 20.8	66.5 ± 26.3	67.3 ± 21.8	61.4 ± 22.7	0.07	—	—

*Repeated-measures analysis of variance (ANOVA) was performed among five periods. Post hoc analysis between after the procedure and two years, and between two and four years were performed with Bonferroni corrections (significant level of p value is 0.025).

Table 3. Percent Hypoechogenic Tissue Component in the Plaque and Neointima

	After Procedure	4 Mo	1 Yr	2 Yrs	4 Yrs	ANOVA* p Value	Post Hoc After Procedure to 2 Yrs	Post Hoc 2 to 4 Yrs
Neointima, %	—	74.9 ± 12.7	68.9 ± 17.1	66.7 ± 19.1	57.0 ± 22.6	0.0001†	0.03‡	0.01
Plaque (stented segment), %	78.9 ± 8.6	79.4 ± 6.7	81.3 ± 7.4	78.2 ± 8.9	70.4 ± 10.6	<0.0001	0.67	<0.0001
Plaque (proximal edge), %	88.7 ± 6.9	87.2 ± 6.5	87.1 ± 6.0	87.7 ± 7.9	81.7 ± 9.3	0.02	0.67	0.008
Plaque (distal edge), %	86.1 ± 9.8	86.8 ± 6.0	87.0 ± 10.4	88.5 ± 9.5	85.9 ± 9.4	0.78	—	—

*Repeated-measures analysis of variance (ANOVA) was performed among five periods. Post-hoc analysis between after the procedure and two years, and between two and four years were performed with Bonferroni corrections (significant level of p value is 0.025). †Repeated-measures ANOVA was performed among four periods, except after the procedure. ‡Post-hoc analysis was performed between four months and two years.

For SES, 80% of the drug load is eluted within 30 days of stent implantation (1). However, concern has arisen about long-term biological reactions to the non-biodegradable polymer (5). Long-term exposure to these polymers may cause chronic inflammation. Complete natural healing (i.e., re-endothelialization and a reduction in proteoglycan content) may take three to six months after bare metal stent implantation (21). In this present study, plaque volume behind the stent struts decreased between two and four years, and this shrinkage was accompanied by a change in plaque echogenicity, suggestive of a change in plaque composition. This may indicate that the peri-stent area has undergone a slower healing process after SES implantation that results in a more fibrous content at four-year follow-up.

In animal experiments, it has been documented that the exuberant proliferative process observed after stenting is largely inhibited in the early stage with SES, but the normal healing process is also delayed with persistence of fibrin deposition and inflammatory cells (22). In humans, one study reported the histologic findings of atherectomy specimens of restenotic lesions after implantation of a paclitaxel derivative-eluting polymer stent in which persistent fibrin accumulation with smooth muscle cells and proteoglycan and collagen type III-rich matrix with inflammation was observed at 12 months (4,5). An autopsy performed on a patient four years after SES implantation showed complete healing, indicating that the delayed healing response had abated, concurring with our findings (23).

In this study, neointimal volume continued to increase over the four-year period. However, the increase between two and four years failed to reach statistical significance (7.0 vs. 8.4 mm^3, $p = 0.25$), and the change in neointimal volume between two years and four years was significantly less than between the post-procedure and two-year measurements (1.4 vs. 7.0 mm^3, $p = 0.04$), which possibly indicates that the delayed tissue proliferation has subsided. Carter et al. (24) reported long-term effects of SES in a porcine coronary model. They found that inhibition of neointimal hyperplasia was not sustained at 90 days compared with the bare metal stent, and the mean neointimal area was similar between 90 days and 180 days. These results differ from observations seen in clinical trials, including this study. Several trials have reported low target vessel revascularization rates up to three years (25). This discrepancy is likely explained by different monocyte and lymphocyte responses to similar concentrations of sirolimus in human and porcine vessels, and the different anatomical

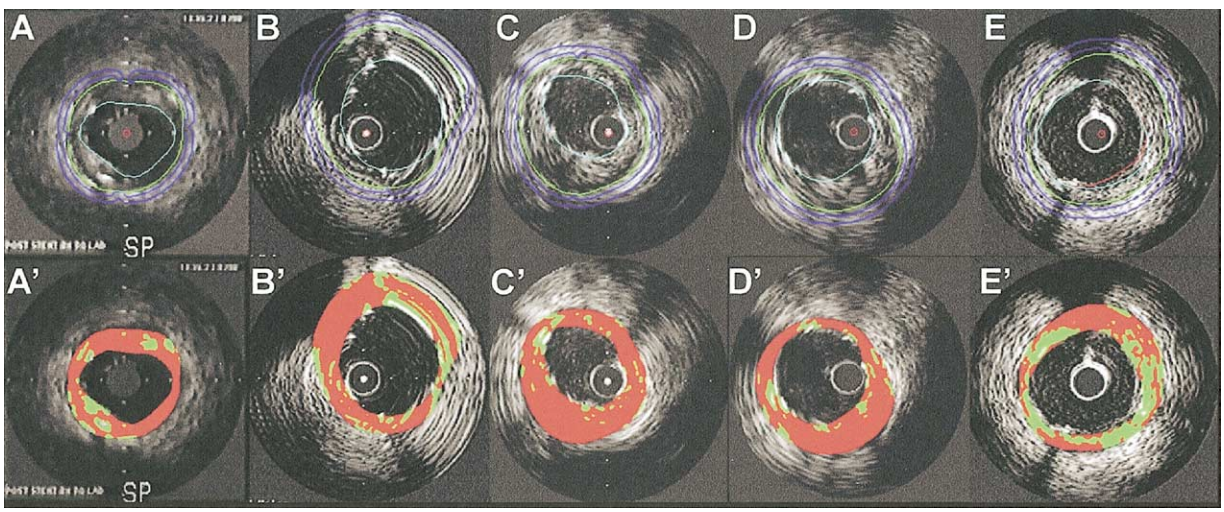

Figure 3. A representative example of a patient with plaque compositional changes between two-year and four-year follow-up. **Top row** shows cross-sectional vessel image at **(A)** post-procedure, **(B)** four months, **(C)** one year, **(D)** two years, and **(E)** four years. **Red line** indicates lumen. **Light blue line** indicates stent. **Green line** indicates media. **Dense blue line** indicates adventitia. **Bottom row** shows cross-sectional echogenicity images. Hyperechogenic areas are colored **green**. Hypoechogenic areas are colored **red**. **(A')** Percent plaque behind stent hypoechogenicity was 84.0% post-procedure, **(B')** 85.3% at four months, **(C')** 88.4% at one year, **(D')** 84.2% at two years, and **(E')** 74.8% at four years.

features of normal porcine coronary arteries versus atherosclerotic human coronary arteries (26).

Furthermore, the increase in neointimal echogenicity as observed in this study would also suggest that a fibrotic process is now operative and predominant. The rate of growth of the neointima has slowed—both features suggest that the completion of the healing process may be expected in the near future.

Study limitations. This study is limited by the sample size of 23 patients. Secondly, patients without events were used for the serial IVUS analysis. Consequently, these results may not apply to all patients treated with SES. Different IVUS catheters and consoles were used over the four-year period, and most data were acquired without ECG gating. These differences may have hampered the consistency of volumetric and echogenic analysis (27). For instance, BSC 30-MHz catheters connected to a Clearview console underestimates true dimensions (11). To correct for these discrepancies, the results from the 30-MHz catheter were adjusted using a previously reported mathematical model (11), and image artifacts resulting from cardiac cycle motion observed in non–ECG-gated images were circumvented by the use of the Intelligate method, which converts non–ECG-gated data to ECG-gated data retrospectively (10,28).

The adventitia was taken as a reference to determine the change in echogenicity occurring in the peri-stent and intrastent tissue. Any alterations to the adventitia (e.g., inflammatory and fibrotic reactions) after stent deployment may alter the echogenicity during the follow-up period. Because any potential increase in echogenicity of the adventitia cannot be totally excluded or measured, the change in echogenicity of the peri-stent and intra-stent tissue may be underestimated and the interference of stent struts with plaque echogenicity may not be completely excluded. Finally, any remaining bias after correction from the use of different IVUS systems cannot be totally excluded in the present study. Despite the inherent limitations, plaque echogenicity analysis by computer-assisted grayscale value is to date the only method with the potential to analyze plaque composition retrospectively.

Conclusions. After SES implantation, negative plaque remodeling and a decrease of hypoechogenic plaque composition were observed at four-year follow-up. In addition, the fact that the neointima does not significantly change from two to four years may suggest that the biological phenomenon of a delayed healing response has begun to subside.

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