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Circulation 2009, 120:391-399: originally published online July 20, 2009
doi: 10.1161/CIRCULATIONAHA.109.854398
Circulation is published by the American Heart Association. 7272 Greenville Avenue, Dallas, TX 72514
 Print ISSN: 0009-7322. Online ISSN: 1524-4539

Correlation of Intravascular Ultrasound Findings With Histopathological Analysis of Thrombus Aspirates in Patients With Very Late Drug-Eluting Stent Thrombosis

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Background—Intravascular ultrasound of drug-eluting stent (DES) thrombosis (ST) reveals a high incidence of incomplete stent apposition (ISA) and vessel remodeling. Autopsy specimens of DES ST show delayed healing and hypersensitivity reactions. The present study sought to correlate histopathology of thrombus aspirates with intravascular ultrasound findings in patients with very late DES ST.

Methods and Results—The study population consisted of 54 patients (28 patients with very late DES ST and 26 controls). Of 28 patients with very late DES ST, 10 patients (1020±283 days after implantation) with 11 ST segments (5 sirolimus-eluting stents, 5 paclitaxel-eluting stents, 1 zotarolimus-eluting stent) underwent both thrombus aspiration and intravascular ultrasound investigation. ISA was present in 73% of cases with an ISA cross-sectional area of 6.2±2.4 mm^2 and evidence of vessel remodeling (index, 1.6±0.3). Histopathological analysis showed pieces of fresh thrombus with inflammatory cell infiltrates (DES, 263±149 white blood cells per high-power field) and eosinophils (DES, 20±24 eosinophils per high-power field; sirolimus-eluting stents, 34±28; paclitaxel-eluting stents, 6±6; *P* for sirolimus-eluting stents versus paclitaxel-eluting stents=0.09). The mean number of eosinophils per high-power field was higher in specimens from very late DES ST (20±24) than in those from spontaneous acute myocardial infarction (7±10), early bare-metal stent ST (1±1), early DES ST (1±2), and late bare-metal stent ST (2±3; *P* from ANOVA=0.038). Eosinophil count correlated with ISA cross-sectional area, with an average increase of 5.4 eosinophils per high-power field per 1-mm^2 increase in ISA cross-sectional area.

Conclusions—Very late DES thrombosis is associated with histopathological signs of inflammation and intravascular ultrasound evidence of vessel remodeling. Compared with other causes of myocardial infarction, eosinophilic infiltrates are more common in thrombi harvested from very late DES thrombosis, particularly in sirolimus-eluting stents, and correlate with the extent of stent malapposition. **(*Circulation*. 2009;120:391-399.)**

Key Words: eosinophils ■ ultrasound ■ thrombus ■ stents

Stent thrombosis (ST) is a rare but devastating adverse event after percutaneous coronary intervention (PCI), which results in abrupt closure of the treated artery with the incumbent risk of sudden death or myocardial infarction (MI).[1,2] Case reports, observational studies, and meta-analyses of randomized trials comparing bare-metal stents (BMS) with drug-eluting stents (DES) suggest that very late (>1 year) ST is more common, with the latter representing a distinct entity complicating the use of DES.[3–9]

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The mechanisms leading to very late ST are complex and incompletely understood. Human autopsy studies of thrombosed DES specimens suggest delayed healing, incomplete endothelialization, and chronic inflammation as prevailing pathological mechanisms.[10,11] Clinical investigations with the use of intravascular ultrasound (IVUS) in patients presenting

Received January 30, 2009; accepted May 26, 2009.

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Circulation **is available at http://circ.ahajournals.org** **DOI: 10.1161/CIRCULATIONAHA.109.854398**

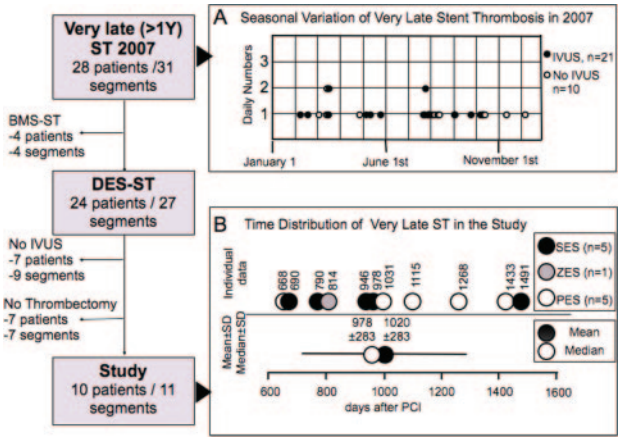

Figure 1. Flow chart of patients presenting with very late definite ST at the Bern University Hospital during 2007. A total of 28 patients presented with very late definite ST in 31 stented segments. From this cohort, 4 events were related to BMS, 7 patients did not undergo IVUS, and 7 additional patients had no thrombectomy attempt. The study population therefore consists of 10 patients with 11 ST segments. A, Admission of patients with very late ST at our institution during 2007. B, Time occurrence of very late ST in days after DES implantation. ZES indicates zotarolimus-eluting stents.

with very late DES thrombosis observed an exceedingly high incidence of incomplete stent apposition (ISA) with evidence of vessel remodeling, suggesting a causal link between the vascular response to DES implantation and very late ST in affected patients.[12–15]

Although autopsy studies are limited to postmortem specimens and therefore lack generalizability, coronary angiography and IVUS do not delineate morphological changes of the vessel wall in response to DES implantation in sufficient detail. To overcome these limitations, we first harvested thrombus for detailed histopathological analysis and then performed IVUS in patients presenting with DES thrombosis ≥1 year after stent implantation. The purpose of the study was to correlate the histopathological findings from aspirated thrombus specimens with structural changes of the arterial wall to elucidate pathophysiological mechanisms underlying the phenomenon of very late DES thrombosis. Moreover, thrombus aspirates from patients with very late DES thrombosis were compared with specimens of patients with spontaneous acute MI, early DES thrombosis, and early and late BMS thrombosis.

Methods

Patient Population

The study population consisted of 54 patients: 28 patients with very late DES thrombosis and 26 controls. During 2007, 28 patients with 31 arterial segments presented with very late definite ST (1329±664 days after the index procedure) at our institution. IVUS was performed in 17 patients with 18 DES thrombosis segments, but thrombus aspiration was possible in only 10 patients with 11 segments. Of note, none of these patients have been part of our previous report but constitute new patients presenting with this adverse event to our institution.[14] Figure 1 summarizes the selection of patients with very late definite ST included in the present study. The histopathological findings of patients with very late DES thrombosis were compared with thrombus aspirates harvested from 26 controls: spontaneous acute MI (n=7), early BMS thrombosis (n=4), late BMS thrombosis (n=5), and early DES thrombosis (n=10). The study complied with the Declaration of Helsinki with regard to investigation in humans and was approved by the ethics committee at the University Hospital of Bern, Switzerland. All patients provided written, informed consent.

Drug-Eluting Stents

DES availability at our institution was as follows: sirolimus-eluting stents (SES) (Cypher, Cordis, Johnson & Johnson) became available in April 2002, followed by paclitaxel-eluting stents (PES) (Taxus, Boston Scientific) in March 2003. From April 2003 to May 2004, both DES were used in a randomized clinical trial.[16] This was followed by use of both DES on an alternating basis until April 2005, at which time the use of PES was discontinued. Zotarolimus-eluting stents (Endeavor Sprint, Medtronic) became available in August 2005.

Definition of ST

Definite ST was defined as the association of clinical symptoms with angiographic confirmation of thrombotic stent occlusion.[14,17] Clinical signs and symptoms were considered to be present if at least 1 of the following criteria was met:

1. Sudden onset of typical chest pain with duration >20 minutes
2. Ischemic ECG changes, such as:
   a. ST-segment elevation in the territory of the implanted stent
   b. ST-segment depression or T-wave inversion in the territory of the implanted stent
3. Typical rise and fall in cardiac biomarkers

Angiographic criteria for ST[14] were fulfilled when the Thrombolysis in Myocardial Infarction flow was as follows:

1. Grade 0 with occlusion originating in the peri-stent region
2. Grade 1, 2, or 3 in the presence of a thrombus originating in the peri-stent region

Timing of ST was categorized as early when occurring during the first 30 days, late when occurring between 1 month and 1 year, and very late when occurring beyond 1 year after DES implantation.

Quantitative Coronary Angiographic Analysis

Coronary angiograms were digitally recorded at baseline, immediately after the procedure, and at the time of very late definite ST and assessed at the angiographic core laboratory of the Bern University Hospital, Bern, Switzerland. Digital angiograms were analyzed with the use of an automated edge-detection system with the use of the CMS-GFT algorithm (MEDIS, Leiden, the Netherlands). Quantitative measurements included the diameter of the reference vessel, minimal luminal diameter, and percent diameter of stenosis (defined as the diameter of the reference vessel minus the minimal luminal diameter, divided by the reference diameter and multiplied by 100).

Thrombus Harvesting

Before emergency PCI, patients underwent thrombus aspiration followed immediately by IVUS acquisition of the affected stent segment. Control patients underwent thrombus aspiration without IVUS. In brief, sublingual or intracoronary nitroglycerin was administered (0.2 to 0.3 mg), and an angiogram with the use of a 6F guiding catheter was performed to identify the site of thrombotic stent occlusion. Then, a 0.014-inch coronary guidewire was passed through the affected stent, followed by aspiration of thrombus with the use of specifically designed aspiration catheters (Export Aspiration System, Medtronic Vascular Incorporation, Santa Rosa, Calif; Proxis Embolic Protection System, St Jude Medical, St Paul, Minn; Diver Aspiration System, Invatec, Italy). Harvested thrombus was passed through a 40-μm Becton Dickinson Cell Strainer filter and fixed in 4% neutral buffer

formalin. Ten milliliters of fresh blood from the occluded coronary artery segment was then sampled and divided into 2 Monovettes containing either EDTA or heparin. After centrifugation (5000 rpm, 10 minutes, 4°C), plasma was stored at −80°C.

IVUS Image Acquisition and Analysis

The technique of IVUS acquisition was performed as described previously but with different IVUS hardware and software.[14] Briefly, a commercially available IVUS catheter (Atlantis SR 40-MHz scanner, Boston Scientific Corporation, Santa Clara, Calif) was advanced >10 mm beyond the distal edge of the thrombosed stent. The IVUS catheter was withdrawn with the use of a motorized pullback (0.5 mm/s), and images were continuously recorded throughout the stent and at least 10 mm distal and proximal to the stent. After IVUS images were obtained, the imaging catheter was withdrawn, and PCI with the use of standard techniques was begun. IVUS imaging was repeated at the end of the procedure. All IVUS procedures were digitally recorded on CD-ROM.

Quantitative IVUS analyses were performed offline according to the criteria of the clinical expert consensus document on IVUS with the use of computerized planimetry (CAAS-QIVA, Pie Medical Imaging, the Netherlands). Quantitative measures included the external elastic membrane (EEM), the lumen cross-sectional area (CSA), and stent CSA at stented and reference segments. The image slice with the smallest stent and lumen CSA and the image slice with the greatest EEM were also analyzed. The proximal and distal reference segments selected for analysis were the most normal-looking cross sections within 10 mm proximal or distal to the lesion but before any side branch. ISA was defined as lack of contact between at least 1 strut and the underlying arterial wall intima that did not overlap a side branch with evidence of blood flow behind the strut. Stent expansion was defined as the ratio of minimal stent CSA divided by the reference lumen CSA. Neointima was calculated as stent minus lumen CSA measures. Remodeling index was calculated as the ratio of maximal in-stent EEM CSA divided by the reference EEM CSA. Positive remodeling was defined as a remodeling index ≥1.05.[18]

Histopathological Sampling and Analysis

The thrombectomy specimens were fixed in 4% neutral buffered formalin and processed for paraffin sectioning. Paraffin blocks of the thrombotic material were sectioned on a rotary microtome and stained with hematoxylin-eosin, Movat pentachrome, and Luna stains. All sections were examined by light microscopy for platelets, fibrin, red blood cells, plaque constituents, and inflammation. In addition, 5 high-power fields (×40) demonstrating the greatest severity of inflammation were selected for quantitative analysis. Total white blood cell (WBC) and eosinophil counts were performed and summed for the selected 5 high-power fields. Data were recorded as total WBCs and total eosinophils. Additionally, neutrophil (cathepsin-G) and macrophage (KP-1) stains were performed to further characterize the nature of the inflammatory cell infiltrate. The severity of the inflammation was graded on the basis of a 3-tiered scale defined as (1) mild (<100 WBCs per high-power field), (2) moderate (101 to 300 WBCs per high-power field), and (3) severe (>300 WBCs per high-power field).

Inflammatory Markers

IgG, IgE (Immundiagnostik, Bernsheim, Germany), interleukin-6, tumor necrosis factor-α, C-reactive protein (Quantikine HS, R&D Systems, Abingdon, United Kingdom), and myeloid-related protein complex 8/14 (MRP8/14) (Bühlmann Laboratory, Schönenbuch, Switzerland) were measured in samples harvested from descending aorta and coronary artery by enzyme-linked immunosorbent assay in patients with very late DES thrombosis but not control patients.

Statistical Analysis

Continuous data were compared with least-squares linear regression models and 1-way ANOVA, with the use of robust SEs that account for the correlation of characteristics of thrombus specimens within patients. Continuous characteristics of patients at baseline were compared with an independent *t* test. Comparisons of IVUS measures within a segment were performed with a linear regression model with robust SEs, which respected the paired nature of the data. Then we used a univariable least-squares linear regression model with robust SEs to determine the association between ISA CSA and WBC and total eosinophil count, eosinophilic fraction in thrombus, IgE, high-sensitivity C-reactive protein, tumor necrosis factor-α, interlelukin-6, and MRP8/14. Categorical data were compared with the Fisher exact test. Averages of continuous data are reported as mean±SD, and categorical data are reported as counts and percentages. All *P* values are 2-sided. Analyses were performed in SPSS 12.0.1 (SPSS Institute, Chicago, Ill) and Stata 10 (Stata Corporation, College Station, Tex).

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

The results of the present study pertain to 10 patients with 11 DES ST segments, in whom both IVUS and thrombus aspiration were successfully performed at the time of very late ST (1020±283 days after the index procedure) (Figure 1). Baseline clinical characteristics of patients undergoing IVUS (n=10) and patients not undergoing IVUS (n=14) at the time of emergency PCI for very late ST were similar (Table 1). Baseline angiographic characteristics of the 11 very late ST segments analyzed at the time of the index procedure are summarized in Table 2. No residual dissections were observed immediately after the index procedure. All patients with very late definite ST presented clinically as having acute ST-segment elevation MI with angiographic evidence of thrombus at the target lesion. At the time of very late definite ST, patients had been treated with acetylsalicylic acid (100 mg/d) without concomitant thienopyridine, and 1 patient was under oral anticoagulation.

IVUS Findings

Results from quantitative IVUS analysis are summarized in Table 3. The maximal vessel CSA was significantly larger for the in-stent than for the reference segment (EEM CSA, 20.2±3.7 versus 12.8±2.5 mm^2; $P=0.001$), resulting in a remodeling index of 1.6±0.3. ISA was present in 8 very late definite ST segments (73%), and maximal ISA area and length measured 6.2±2.4 mm^2 and 9.4±9.5 mm, respectively. ISA area exceeded 5.0 mm^2 in 5 of 8 segments (63%) and was most likely related to vessel remodeling. ISA foci were located at the proximal stent segment in 13% and within the stent body in 87% of cases. Only 1 segment had a minimal stent CSA <4.0 mm^2, and the stent expansion index amounted to 0.86±0.26.

Histopathological Findings

A total of 11 thrombectomy specimens were submitted for histopathological analysis. One specimen showed aspirated fragments of necrotic core devoid of thrombus, and 2 specimens, despite serial sectioning, contained only minute fragments of thrombus insufficient for histopatho-

Table 1. Baseline Clinical Characteristics of Patients With Very Late ST

	Study Population, IVUS/Thrombus Available (n=10)	Not Included Into Study, IVUS/Thrombus Not Available (n=14)	P
Age, mean±SD, y	63±13	58±12	0.40
Male sex, n (%)	9 (90)	13 (93)	0.67
Diabetes mellitus, n (%)	1 (10)	0 (0)	0.42
Hypertension, n (%)	7 (70)	9 (64)	0.56
Dyslipidemia, n (%)	7 (70)	10 (71)	0.64
Smoking, past and current, n (%)	4 (40)	10 (71)	0.13
BMI >30, n (%)	3 (30)	4 (29)	0.98
Previous CABG, n (%)	0 (0)	1 (7)	0.46
Previous MI, n (%)	4 (40)	3 (21)	0.30
Stable angina pectoris/silent ischemia,* n (%)	2 (20)	3 (21)	0.87
Acute coronary syndromes,* n (%)	8 (80)	11 (79)	0.87
STEMI, n (%)	2 (20)	6 (43)	0.36
NSTEMI, n (%)	3 (30)	3 (21)	0.69
Unstable angina, n (%)	3 (30)	2 (14)	0.52
Multivessel disease, n (%)	7 (70)	10 (71)	0.64
LVEF, mean±SD, %	56±10	58±8	0.63

BMI indicates body mass index; CABG, coronary artery bypass grafting; STEMI, ST-segment elevation MI; NSTEMI, non–ST-segment elevation MI; and LVEF, left ventricular ejection fraction.

*Clinical indication of PCI at time of index procedure.

logical analysis. The thrombus fragments measured 4.6±3.5×2.0±1.5 mm.

Qualitative histopathological analysis of the remaining 8 thrombi showed fragments of fibrin and platelet-rich thrombus with variable numbers of trapped red blood cells. All 8 thrombi demonstrated a preponderance of acute inflammatory cells consisting of neutrophils highlighted by immunostaining with cathepsin G. In addition, all thrombi showed some degree of chronic inflammation consisting of macrophages and lymphocytes. Macrophages were identified by immunostaining with KP-1 and ranged from a few scattered cells to diffuse infiltrates, which in 2 cases formed focal aggregates accompanied by proteoglycan deposition consistent with early organization of the thrombus. One thrombus demonstrated grade 1 inflammation (mild), 4 thrombi demonstrated

Table 2. Lesion Characteristics at Time of Index Procedure

Target Lesion Segments (n=11)	
Before procedure	
Lesion length, mm	14.3±5.6
Reference vessel diameter, mm	2.76±0.37
Minimal luminal diameter, mm	0.57±0.49
Stenosis, % of luminal diameter	78.1±19.9
During procedure	
No. of stents per lesion	1.4±0.7
Total stent length, mm	28.6±19.3
Stent diameter, mm	2.94±0.40
DES stent type	
SES, n (%)	5 (45)
PES, n (%)	5 (45)
ZES, n (%)	1 (9)
Stent overlap, n (%)	2 (18)
Maximal pressure, atm	14.4±3.5
Immediately after procedure	
Final minimal luminal diameter	
In stent, mm	2.51±0.36
In segment, mm	2.10±0.49
Final stenosis	
In stent, % of luminal diameter	6.8±11.0
In segment, % of luminal diameter	24.4±5.2

ZES indicates zotarolimus-eluting stent. Values are mean±SD unless otherwise specified.

Table 3. IVUS Measurements

	Very Late ST
No. of segments	n=11
Reference segment	
EEM CSA, mm^2	12.8±2.5*
Lumen CSA, mm^2	7.5±1.5**
Stent segment	
Maximal EEM CSA, mm^2	20.2±3.7*
Remodeling index	1.6±0.3
Stent CSA, mm^2	7.8±3.3
Minimal stent CSA, mm^2	6.6±2.8
Minimal stent CSA <4 mm^2, n (%)	1 (9)
Stent expansion	0.86±0.26
In-stent lumen CSA, mm^2	7.6±2.9**
Neointimal hyperplasia, mm^2	0.7±1.1
ISA, n (%)	8 (73)
Maximal ISA CSA, mm^2	6.2±2.4
Maximal ISA length, mm	9.4±9.5

Values are mean±SD. P values are from a least-squares linear regression model with robust standard errors, which respects the paired nature of the data. *p=0.001, **p=0.87.

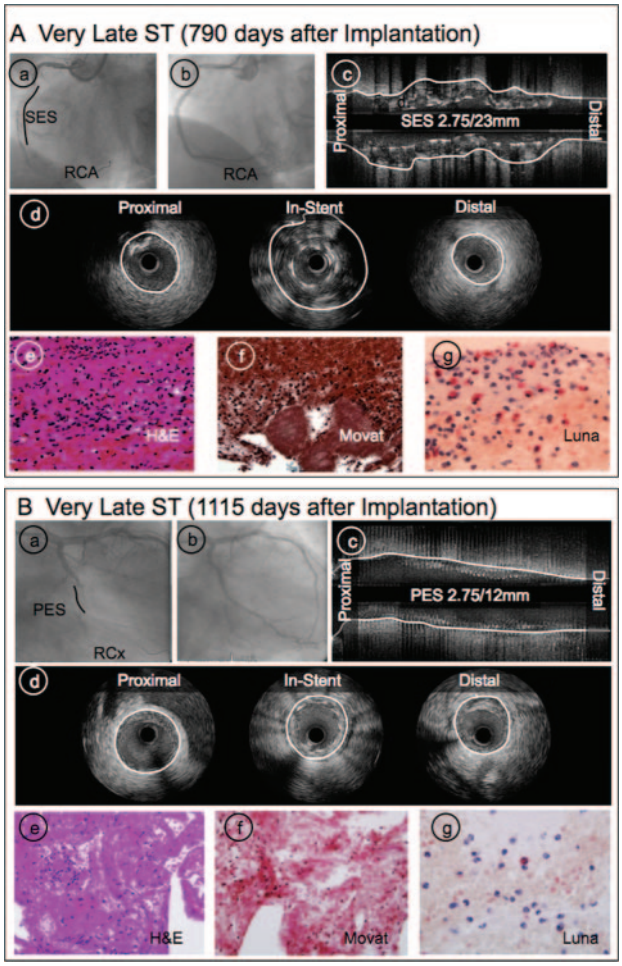

Figure 2. Correlation of angiographic and IVUS findings with histopathological analysis of thrombus aspirates harvested from very late ST segments with (A) and without (B) ISA. A, a and b, Angiographic findings; c and d, IVUS findings; e through g, histopathological analysis. A, Very late ST occurred 790 days after implantation of SES in the right coronary artery (RCA) of a 76-year-old woman treated for a non–ST-segment elevation MI. IVUS demonstrates ISA with evidence of positive arterial remodeling. Histopathology shows fresh thrombus with an intense inflammatory infiltrate of WBCs and numerous eosinophils. H&E indicates hematoxylin-eosin. B, Very late ST was observed 1115 days after implantation of PES into the left circumflex artery (RCx) in a 54-year-old man treated for non-ST segment elevation MI. IVUS shows no ISA and no evidence of vessel remodeling. Histopathological analysis shows thrombus with an intense inflammatory infiltrate of neutrophils but only sparse eosinophils.

grade 2 (moderate), and 3 demonstrated grade 3 inflammatory cell infiltrates (severe).

Quantitative histopathological analysis revealed 263±149 WBCs per high-power field and a total mean eosinophil count of 20±24 cells per high-power field. Segments treated with SES (34±28) tended to show a higher eosinophil count than those treated with PES (6±6; $P_{\text{SES versus PES}}=0.09$) or zotarolimus-eluting stents (0; Figure 2). The mean fraction of eosinophils amounted to 9.6±12.5% of WBCs and was highest in segments treated with SES (17.8±13.8%) followed by PES (1.5±1.0%; $P_{\text{SES versus PES}}=0.051$; Figures 2 and 3). Detailed information of individual patients relative to time of ST, DES type, and IVUS and histopathological findings is provided in Table 4.

Histopathological data of control specimens are summarized in Table 5. The mean number of WBCs was similar among specimens from very late DES thrombosis (263±149) and thrombi collected from spontaneous acute MI (291±94) but lower in specimens from early BMS ST (146±117), early DES ST (73±117), and late BMS ST (84±50; $P=0.0001$, ANOVA). The mean number of eosinophils per high-power field was higher in specimens from very late DES thrombosis (20±24) than in those with all other settings: spontaneous acute MI (7±10), early DES ST (1±2), early BMS ST (1±1), and late BMS ST (2±3; $P=0.038$, ANOVA).

Serum levels of high-sensitivity C-reactive protein showed a difference between the systemic arterial (13±8 ng/mL) and coronary circulation (1559±247 ng/mL; $P<0.001$). The coronary concentration of MRP8/14 was markedly increased (26±16 g/mL, with an upper limit of normal 4.7 g/mL), but levels of IgE (61±57 kU/L), tumor necrosis factor-α (1.1±2.2 ng/mL), and interleukin-6 (20±36 ng/mL) were not.

Correlation of IVUS Findings With Inflammatory Markers

In linear regression models, we explored the association between ISA area in patients with very late DES thrombosis and inflammatory markers as assessed by histopathology and serology. ISA area was associated with total eosinophil count (and eosinophil fraction) with an average increase of 5.4 eosinophils (2.6%) per 1-mm^2 increase in ISA CSA. Conversely, we found no association with other inflammatory markers, including levels of IgE, high-sensitivity C-reactive protein, tumor necrosis factor-α, interleukin-6, and MRP8/14 (Table 6).

Discussion

The present study demonstrates in vivo that very late ST after DES implantation is associated with histopathological and serological signs of inflammation. The amount of eosinophils was 3 times higher in thrombus aspirates from patients with very late ST compared with other causes of MI. Eosinophilic infiltrates were associated with evidence of vessel remodeling leading to presumably secondary stent malapposition. These findings suggest eosinophilic coronary arteritis due to delayed-type hypersensitivity reaction as 1 of the causes of very late DES thrombosis.

Very late ST after DES implantation is a multifactorial process, which is understood only incompletely. Delayed healing manifested by persistent fibrin deposition and incomplete reendothelialization emerged as the prevailing mechanism of thrombosis in a recent necropsy study.[11,19] In addition, chronic inflammation and hypersensitivity reactions, stenting over major side branches or bifurcation stenting with the crush technique, malapposition related to positive arterial remodeling or incomplete stent expansion, in-stent restenosis with superimposed thrombus, and pen-

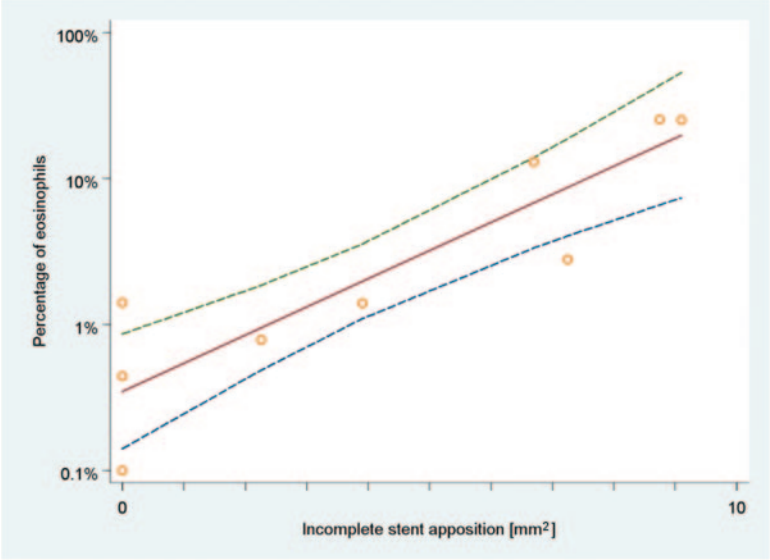

Figure 3. Association between area of ISA and eosinophil count fraction. The dotted lines indicate the predicted logarithm of the percentage of eosinophils with 95% confidence interval from linear regression with ISA used as the explanatory variable.

etration of necrotic core through stent struts were observed as pathological mechanisms leading to very late ST.

Virmani and colleagues[10] first described a case of local hypersensitivity reaction with extensive vasculitis of intima, media, and adventitia consisting predominantly of lymphocytes and eosinophils in a patient suffering from very late DES thrombosis. Histopathological analysis revealed aneurysmal dilatation of the vessel wall within the stented segment with evidence of stent malapposition and thick fibrin thrombus between the stent and the arterial wall. Further support of hypersensitivity reactions after DES implantation comes from the Research on Adverse Drug Events and Reports (RADAR) registry, with 17 of 5783 cases reporting hypersensitivity symptoms probably or certainly related to DES.[20] Although these previous reports were limited to either autopsy series or clinical case descriptions, the present study corroborates these findings in vivo in patients presenting with very late ST undergoing emergency PCI. Thrombus aspirates harvested from very late ST segments showed signs of infiltration with neutrophils, lymphocytes, macrophages, and eosinophils, which was accompanied by IVUS evidence of vessel remodeling. Moreover, serological data revealed markedly increased concentrations of high-sensitivity C-reactive protein and MRP8/14, reflecting an inflammatory reaction. Previously, Altwegg and colleagues[21] reported that MRP8/14 is expressed at the site of coronary occlusion by phagocytes in patients with acute coronary syndromes. It was suggested that the appearance of elevated MRP8/14 in the circulation before markers of myocardial necrosis makes MRP8/14 a prime candidate for the detection of unstable plaques.

Table 4. Findings in 10 Patients With 11 Very Late ST Segments

ST Segment No.	Age, y	Sex	Indication at Time of Index Procedure	Time to ST, d	Target Lesion Artery	DES Type	Overlap	Remodeling Index	ISA	Average WBCs in Thrombus	Average Eosinophils in Thrombus	Platelets	Plaque Material	Inflammation Score*
1	48	M	STEMI	1268	RCA	PES	No	1.2	No	170	2	Yes	No	2
2	48	M	Unstable angina pectoris	1031	LAD	PES	No	1.3	No	NA	NA	NA	NA	NA
3	52	M	STEMI	946	RCA	SES	Yes	1.8	Yes	NA	NA	No	Yes	NA
4	73	M	NSTEMI	690	LCX	SES	No	1.4	Yes	230	2	Yes	No	2
5	73	M	NSTEMI	790	RCA	SES	No	1.9	Yes	230	58	Yes	No	3
6	56	M	Unstable angina pectoris	814	RCA	ZES	No	1.5	Yes	NA	NA	NA	NA	NA
7	54	M	NSTEMI	1115	LCX	PES	No	1.3	No	150	1	Yes	No	1
8	81	M	Stable angina pectoris	978	LAD	SES	No	1.9	Yes	175	56	Yes	No	2
9	54	M	Stable angina pectoris	1433	LAD	PES	Yes	1.4	Yes	>500	14	Yes	No	3
10	50	F	Unstable angina pectoris	668	LAD	PES	No	1.9	Yes	>500	7	Yes	No	3
11	80	M	STEMI	1491	LAD	SES	No	1.8	Yes	150	20	Yes	No	2

NA indicates not enough thrombus material available; RCA, right coronary artery; LAD, left anterior descending artery; LCX, left circumflex artery; STEMI, ST-segment elevation MI; NSTEMI, non–ST-segment elevation MI; and ZES, zotarolimus-eluting stent.

*Inflammation score: 1, mild (<100 WBCs per high-power field); 2, moderate (101-300 WBCs per high-power field); and 3, severe (>301 WBCs per high-power field).

Table 5. Quantitative Histopathological Findings in 34 Thrombi

		WBCs		Eosinophils		Eosinophilic Fraction, %	
Setting of Infarction	n	Mean±SD	*P*	Mean±SD	*P*	Mean±SD	*P*
Spontaneous acute MI	7	291±94	0.0001	7±10	0.038	2.4±3.4	0.12
Early ST-BMS	4	146±117		1±1		0.8±0.8	
Early ST-DES	10	73±117		1±2		2.7±3.4	
Very late ST-BMS	5	84±50		2±3		2.8±3.8	
Very late ST-DES	8	263±149		20±24		9.6±12.5	

P values are from ANOVA for differences across groups.

However, the majority of patients with very late ST in the present study showed evidence of vessel remodeling in the vicinity of the stent leading to secondary stent malapposition. This morphological pattern corresponds to a previous report from Feres and colleagues[12] as well as our group.[14] Thrombi extracted from these segments showed ample inflammatory cells, and the number of eosinophils was directly related to the extent of vascular remodeling. This suggests an intense hypersensitivity reaction with inflammation of all 3 layers of the vessel wall. In the remaining patients with very late ST, aspirated thrombi revealed inflammatory cells but with only few or no eosinophils without evidence of vessel remodeling. The latter observation suggests a different inflammatory reaction mainly at the luminal side of the vessel wall.

Eosinophilic infiltrates amounted to ≈10% of WBCs among aspirates from very late DES ST segments. Conversely, only 1% to 3% of WBCs were eosinophils among patients with spontaneous acute MI, early DES thrombosis, and early and late BMS thrombosis, closely resembling the proportion of eosinophils in the peripheral circulation. Of note, eosinophilic infiltrates were predominantly observed in thrombus aspirates from SES-treated segments, whereas the fraction of eosinophils was much lower in thrombus aspirates from PES-treated segments. The inflammatory infiltrates in thrombus aspirates from very late ST segments, especially the elevated total and fractional count of eosinophils, suggest an immunologic reaction in response to the implanted DES. It is therefore tempting to hypothesize that isolated components of DES give rise to a delayed-type hypersensitivity reaction (type IV) in certain patients.[22,23] Several types of delayed hypersensitivity (type IV) may occur according to the respective T-cell reactions, which by releasing cytokines and chemokines preferentially activate and recruit monocytes (type IVa), eosinophils (type IVb), or neutrophils (type IVd). Moreover, cytotoxic functions by either CD4- or CD8-positive T cells (type IVc) seem to participate in all type IV reactions. Because activation of monocytes as indicated by levels of MRP8/14 was similar among SES and PES very late ST specimens, it is attractive to speculate that SES preferentially stimulate eosinophils (type IVb delayed hypersensitivity reaction), whereas PES-related thrombosis is less T cell regulated and reflects a nonspecific inflammatory process (Figure 2). The former may result in eosinophilic coronary arteritis with tissue necrosis and erosion around the stent strut leading to vessel remodeling and aneurysmal dilatation. These structural and inflammatory changes may allow for fibrin and platelet deposition and in conjunction with altered flow dynamics may promote local thrombosis. Moreover, eosinophils can themselves promote local thrombosis by stimulation of the coagulation pathway via inhibition of thrombomodulin cofactor activity by eosinophil granule proteins[24] or direct platelet activation and degranulation mediated by adhesion molecules and lipid mediators.[25] Along this line, thrombotic disease manifestations have been described in the hypereosinophilic syndrome.[26]

Table 6. Association of Vascular Remodeling With Inflammatory Markers

	Change (95% CI)	*P*
No. of eosinophils	5.4 (2.0 to 8.8)	0.008
Eosinophilic fraction, %	2.6 (0.6 to 4.7)	0.02
IgE, kU/L	4.4 (−12.9 to 21.8)	0.56
High-sensitivity C-reactive protein, ng/mL	14.7 (−47.3 to 76.7)	0.59
Tumor necrosis factor-α, ng/mL	0.1 (−0.4 to 0.7)	0.62
Interleukin-6, ng/mL	2.1 (−6.8 to 11.1)	0.60
MRP8/14, μg/mL	−0.25 (−4.2 to 3.7)	0.88

Values are change (and confidence interval) per 1-mm^2 increase in ISA.

Limitations

The following shortcomings must be taken into consideration when the results of the present study are interpreted. First, the study population with both IVUS and thrombus aspirates in the setting of very late DES thrombosis is rather small. Moreover, histopathological analysis was possible in only 8 cases, limiting the robustness of the data. Furthermore, a sampling error of the histological analysis can also be an issue. However, we believe that thrombus aspiration was performed properly because a significant amount of thrombus was obtained, as reflected by the mean size of 4.6×2.0 mm, which allowed us to sample the entire thrombus with serial sectioning through the thrombus. Subsequently, the histological sections were assessed by an experienced pathologist by random selections of 5 high-power fields, in which variability of cell counts was minimal between the fields. Furthermore, similar assessment was obtained by a second pathologist, and no significant difference was observed. Therefore, the histological data were obtained with a high degree of reliability.

Moreover, very late DES thrombosis is a rare entity, and the performance of IVUS, thrombus aspiration, and tissue processing for histopathological analysis requires the presence of an experienced team, limiting the acquisition to daytime hours. Second, histopathological analysis of thrombus aspirates rather than tissue samples is limited to the qualitative and quantitative description of aspirated cell types without addressing the degree of endothelialization, fibrin deposition, and assessment of surrounding tissue architecture. Furthermore, sampling by thrombus aspiration allows us to assess only retrievable pieces of thrombus, limiting the analysis to these fragments. Nevertheless, the study methodology employed in the present investigation allowed, to the best of our knowledge for the first time, correlation of IVUS findings in the setting of very late DES thrombosis with thrombus aspirates acquired in vivo rather than examination of autopsy specimens. Third, eosinophilic infiltrates and associated arterial remodeling are not omnipresent in patients with very late DES ST and were observed primarily in SES-treated segments. Accordingly, other mechanisms, including incomplete endothelialization and delayed healing, may be operative either alone or in concert with the aforementioned observations.

Conclusion

Very late DES thrombosis is associated with histopathological signs of inflammation and IVUS evidence of vessel remodeling. Compared with other causes of MI, eosinophilic infiltrates are more common in thrombi harvested from very late DES thrombosis, particularly in SES, and correlate with the extent of stent malapposition.

Disclosures

This study was investigator initiated and investigator driven. Dr Meier has received educational and research support from Cordis, Boston Scientific, Medtronic, and Abbott. Dr Meier also received consulting and lecture fees from Cordis, Boston Scientific, Medtronic, and Abbott. Dr Windecker has received consulting and lecture fees from Abbott, Biosensors, Biotronik, Boston Scientific, Johnson & Johnson, and Medtronic. Dr Virmani has received research support from Medtronic AVE, Atrium Medical Corp, Conor Medsystems, Paracor Medical Inc, Terumo Corp, CardioKinetix, Osiris Therapeutics Inc, Edwards Life Sciences, Nitinol Device and Components, Hancock Jaffee Labx Inc, Biotegra, and CVRx Inc. Dr Virmani also has served as a consultant to or on the advisory board for Medtronic AVE, Abbott Vascular, W.L. Gore, Volcano Therapeutics Inc, Prescient Medical, CardioMind Inc, Direct Flow, and Atrium Medical Corp. The other authors report no conflicts.

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CLINICAL PERSPECTIVE

Very late stent thrombosis (ST) is a distinct entity complicating the use of first-generation drug-eluting stents, resulting in abrupt coronary artery closure, myocardial infarction, or sudden death. Autopsy studies of patients with very late ST showed extensive vasculitis of the intima, media, and adventitia as well as hypersensitivity reactions. With the use of intravascular ultrasound in patients presenting with very late ST, an exceedingly high prevalence of incomplete stent apposition has been reported in concert with positive arterial remodeling. The present report correlates intravascular ultrasound findings with the results of histopathological analysis of thrombi harvested from the occluded coronary artery at the time of very late ST. We observed signs of acute and chronic inflammation. Eosinophilic infiltrates were more common in patients with very late ST compared with control specimens. In addition, the number of eosinophils correlated with the extent of stent malapposition.