THE PATHOBIOLOGY OF HUMAN CORONARY ATHEROMA: CONTRIBUTIONS OF INTERVENTIONAL CARDIOLOGY

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DE PATHOBIOLOGIE VAN HUMANE CORONAIR ATHEROSCLEROSE: BIJDRAGEN VAN DE INTERVENTIE CARDIOLOGIE

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"Looking into the heart of light, the silence".
(T.S. Eliot, The Waste Land)

a María
a mis padres

CONTENTS

General overview

The development of coronary angiography facilitated a complete new assessment of coronary circulation in humans, opening a new age in the study and treatment of coronary artery disease. A second revolution came from further developments of cardiac catheterisation that made possible the performance of percutaneous therapeutic procedures in the coronary arteries. During the last 10 years balloon angioplasty has become not only a useful therapeutic tool for clinicians, but also as a model of myocardial ischaemia and vessel wall damage for researchers. More recently, the development of new percutaneous intracoronary devices has provided new opportunities in the study of the pathophysiology of coronary artery disease.

The central topic of this thesis is the use of three of these new technologies for the investigation of different aspects of the pathobiology of coronary atheroma: directional coronary atherectomy, a recanalisation technique based on debulking the obstructing atheromatous plaque; coronary angioscopy, which can be used percutaneously and allows direct visualizacion of luminal changes in the coronary arteries; and intravascular ultrasound imaging, which provide information on the structure of vascular wall and atheromatous plaque.

The use of directional coronary atherectomy as a research tool in the investigation of coronary atherosclerosis constitutes the kernel of the first part of this thesis (Chapters 1-6). Atherectomy specimens represent a form of biopsy from the target atheromatous lesion. With the generalisation in the use of this technique, atherectomy samples can be obtained from a variety of coronary syndromes, including stable and unstable angina, restenosis post-intervention, and cardiac allograph vasculopathy, providing a unique opportunity for the study of the pathobiology of the atheromatous plaque in each of these conditions. In the work presented in Chapter I, directional coronary atherectomy was used as a sampling device to compare the characteristics of the atheromatous plaques in stable and unstable angina pectoris. The histological information collected in-vivo was analysed taking into account a number of clinical and angiographic variables. A unique characteristic of coronary atheroma obtained with directional atherectomy is that it is biologically viable, and the cellular components present can be dedicated to cell culture. Chapters 2-5 focus in the use of this technique to study a number of aspects on the biology of smooth muscle cells present in the retrieved specimens. The topics of research included the relationship between the ability of smooth muscle cells to outgrow or colonize the culture environment and the clinical and histological substrate of the specimen (Chapter 2), the determination of growth characteristics and extracellular matrix production by smooth muscle cells from primary and restenotic atheromatous lesions (Chapter 3), and the reactivity

towards blood platelets of the extracellular matrix synthetised by the cells (Chapter 4). The information obtained from atherectomy specimens can be combined with data obtained during patient follow-up, offering the unique opportunity of studying prospectively the relationship between the anatomopathological substrate of the lesion and the subsequent restenosis process. This constitutes the topic of Chapter 5, where information derived from cell culture and histology was analysed jointly with clinical and angiographic data collected prospectively. To conclude with the first part of this thesis, Chapter 6 investigates the effect of directional atherectomy in patients with cardiac allograft atherosclerosis from the twofold angle of the retrieved atheromatous specimens and the treated vessel, studied at the time of re-trasplantation or during necropsy.

In the second part of the thesis, coronary angioscopy and intravascular ultrasound imaging were used as investigative tools in studying the substrate of acute coronary syndromes (Chapters 7 to 10). The advantages of angioscopy over coronary angiography in assessing the nature of coronary stenoses, the problems derived from the lack of adequate validation of angioscopic observations, and the limitations dictated by the state-of-the-art angioscopic equipment are discussed briefly in Chapter 7. Chapter 9 reports on the angioscopic findings in patients with unstable angina refractory to medical treatment, and on the comparison of visual findings with histological information obtained in a number of atherectomy specimens retrieved in the same study population. Chapters 8 and 10 explores the feasibility of studying simultaneously the characteristics of the lumen and the structure of the arterial wall of unstable patients using combined angioscopy and intravascular ultrasound imaging. Finally, in Chapter 11 the relationship between the computerised detection and analysis of coronary stenoses by quantitative angiography and the underlying degree of atherosclerotic disease, as judged from intravascular ultrasound imaging, is investigated.

Part I

Directional Atherectomy as a Research Tool in the Study of the Pathobiology of Human Coronary Atheroma

Chapter I

Histological Characteristics of Tissue Excised During Directional Coronary Atherectomy in Stable and Unstable Angina Pectoris

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Abstract

Background

The collection and analysis of tissue removed during coronary atherectomy has the considerable advantage of allowing the pathologic assessment of coronary artery disease "in-vivo", thus avoiding the selection bias inherent to post-mortem studies.

Aim of the study

To compare the histopathologic characteristics of atherectomy specimens retrieved in patients with stable (SAP) and unstable (UAP) angina pectoris undergoing percutaneous coronary recanalisation.

Methods

Tissue samples were obtained from 93 atherectomy procedures performed in 48 SAP (52%) and 45 UAP (48%) patients. Primary and restenotic lesions were included. Clinical variables considered were age, sex, previous myocardial infarction, previous coronary intervention, and coronary risk factors. The presence of neointimal hyperplasia (NH), fibrous tissue, cholesterol crystal clefts, necrotic debris, calcium deposits, macrophages, thrombus, media or adventitia was recorded.

Results

Several differences were noted in both syndromes: First, thrombus showing variable degrees of organisation was found more frequently in patients with UAP than with SAP (10/45, 22%, versus 1/48, 2%, respectively, p = 0.007). The presence of thrombus in UAP bore no relationship to the angiographic stenosis morphology. Second, calcium deposits were more frequent in UAP than in SAP (18/45, 48% versus 9/48, 33% respectively, p = 0.042). The presence of NH correlated strongly with previous coronary interventions (17/24 restenotic vs 14/69 primary lesions, p=0.0001), and showed similar characteristics irrespective of the technique (balloon or laser angioplasty, directional atherectomy and stenting). In primary lesions, the presence of NH bore no relation with SAP or UAP but was observed in younger patients (51±12 versus 59±10 years respectively, p=0.017).

Conclusions

These observations provide further insight into the histopathological substrate of SAP and UAP and reemphasise the potential of directional atherectomy in the study of coronary syndromes.

Introduction

Unstable angina is an acute coronary syndrome associated with substantial short and medium term morbidity and mortality.[1] The understanding of the pathogenesis of this syndrome has been based largely on post-mortem studies of coronary arteries[2] and supported by indirect evidence of coronary thrombosis in relation to the syndrome.[3-5] Since directional coronary atherectomy is unique in extracting intact atheromatous tissue during coronary recanalisation, it may facilitate the study of the processes taking place in the vessel in different coronary syndromes. In the present study the histopathological characteristics of atherectomy samples retrieved in 93 patients with stable or unstable angina pectoris were compared and related to different clinical variables.

Methods

We studied 93 patients who underwent directional coronary atherectomy providing histological material at the Thoraxcenter during the period 1989-1992. Following the coronary atherectomy protocol approved by the Thoraxcenter Institutional Review Board, informed consent was obtained in all patients prior to intervention. Directional coronary atherectomy was performed using the femoral approach. An average of 6±3 passes in multiple directions were performed across the stenosis.

Clinical variables

Clinical variables recorded included age, sex, previous myocardial infarction, current stable or unstable angina pectoris, previous coronary intervention and risk factors for coronary artery disease (history of hypercholesterolaemia, non-insulin dependent diabetes mellitus, cigarette smoking, hypertension and family coronary artery disease). Primary unstable angina was defined as continuous or intermittent chest pain at rest requiring hospitalisation, associated with electrocardiographic evidence of myocardial ischaemia but without associated increase in the cardiac enzymes. The time interval between the onset of chest pain and the atherectomy procedure was 7±5 days.

Histological analysis of the specimens

The obtained specimens were fixed in 10% formalin. Routine processing for light microscopy and haematoxylin-azophloxin and Verhoeff-van Giesson staining was performed. All specimens were reviewed by two independent observers who were blinded to the clinical data. The recommendations layed out in the American Heart Association Medical/Scientific Statement on the Definition of the Intima of Human Arteries and of its Atherosclerosis-Prone Regions[6] were followed in collecting information regarding intimal constituents. Medial tissue was identified on the basis of parallel arrangement of smooth muscle cells, embeded in collagen and frequently associated with a fragment of the internal or external elastic lamina.

Adventitia was recognised by the the presence of coarse bundles of dense collagen intermingled with elastin fibers, sometimes in association with fragments of the external elastic lamina and media. Fibrous tissue was classified as dense when composed of acellular or poorly cellular connective tissue formed predominantly by dense collagen, and classified as loose when the tissue fragments showed a moderate cellularity and collagen bundles separated by accumulations of extracellular matrix. Neointimal hyperplasia was defined as fibromuscular connective tissue showing a random orientation of spindle shaped and stellate cells embedded in abundant extracellular matrix. Cholesterol crystal clefts, necrotic debris and calcium deposits were recorded independently. No special staining was used to identified calcium. The presence of macrophages was recorded only when these formed clusters or when they were present in unusually high number. Thrombus and/or intraplaque hemorrhage were identified as amorphous material, in close apposition with atheromatous material, frequently showing collections of leucocytes between layers of fibrin. Areas consisting mainly of fibrin and not clearly related to the plaque that could have formed during the procedure were not recorded. The Verhoef-van Giesson staining was used to discriminate between fibrin and dense collagen. Organisation was judged when infiltration by cellular elements, e.g. smooth muscle cells, fibroblasts, capillary sprouts, was observed, and graded from I to III on the basis of the number and characteristics of infiltrating cellular elements.

Angiographic morphology

In the 43 patients with unstable angina lesion morphology was classified according to the criteria proposed by Ambrose et al.[7] by two independent cardiologists blinded to the result of the histopathological studies. Complex lesion morphology was recorded when eccentric lesions with overhanging or ragged edges, or lesions with multiple irregularies were noted. In case of disagreement, the opinion of a third cardiologist was taken into account.

Statistical analysis

Mean values and standard deviations are presented for continuous variables. Comparison of mean values was performed using two-tailed unpaired Student's t-tests. Discrete variables were compared using chi-square tests, and Yates' continuity correction applied when indicated. Statistical significance was accepted at the 5% level.

Results

No significant differences were found between clinical characteristics of both groups, with the exception of a higher prevalence of previous myocardial infarction in the unstable group (13/48, 27%, versus 21/45, 47%, in stable and unstable patients respectively, $p = 0.05$). Several associations between clinical variables were observed in the patient population. The mean age of male patients was signi-

Table I. Characteristics of the study population

	Stable	Unstable	p
Clinical variables			
Age (years, mean±SD)	57.89±10.38	56.84±10.85	NS
Previous myocardial infarction	13/48 (27%)	21/45 (47%)	0.05
Male sex	39/48 (81%)	37/45 (82%)	NS
Serum cholesterol ≥ 8 mmol/L	3/48 (6%)	3/45 (7%)	NS
Diabetes mellitus	1/48 (2%)	-	
Systemic hypertension	12/48 (25%)	10/45 (22%)	NS
Cigarrete smoking	18/48 (37%)	16/45 (36%)	NS
Family history coronary disease	6/48 (12%)	9/45 (20%)	NS
Previous coronary intervention	13/48 (27%)	11/45 (24%)	NS
Angina class (NYHA)	II :22, III : 26	III: 3 , IV : 42	
Histological variables			
Dense fibrous tissue	40/48 (83%)	39/45 (86%)	NS
Loose fibrous tissue	12/48 (25%)	5/45 (11%)	NS
Neointimal hyperplasia	14/48 (29%)	17/45 (38%)	NS
Cholesterol clefts	4/48 (8%)	4/45 (9%)	NS
Necrotic debris	3/48 (6%)	6/45 (13%)	NS
Calcium deposits	9/48 (19%)	18/45 (40%)	0.042
Thrombus	1/48 (2%)	10/45 (22%)	0.007
Macrophages	6/48 (12%)	9/45 (20%)	NS

NYHA = New York Heart Association

ficantly lower than that of females (56±10 vs 64±11 years respectively, p = 0.004). Patients with hypercholesterolemia frequently belonged to families with a history of coronary artery disease (67% vs 13% in other patients, p = 0.003). Twenty four patients had a previous history of coronary intervention, including 14 balloon angioplasties, 6 stent implantations, 3 atherectomy procedures and 1 excimer laser angioplasty. The mean time interval between previous intervention and atherectomy was 147±108 days.

Prevalence and characteristics of thrombus in the retrived specimens

The most striking difference between the syndromes was the presence of foci of thrombus or intraplaque hemorrhage in 10 of 45 (22%) unstable and only in 1 of 48 (2%) stable patients (p = 0.007). Only one of these patients had had a previous coronary intervention. All the samples showed some degree of cellular organisation, including the presence of endothelial cells covering newly formed channels

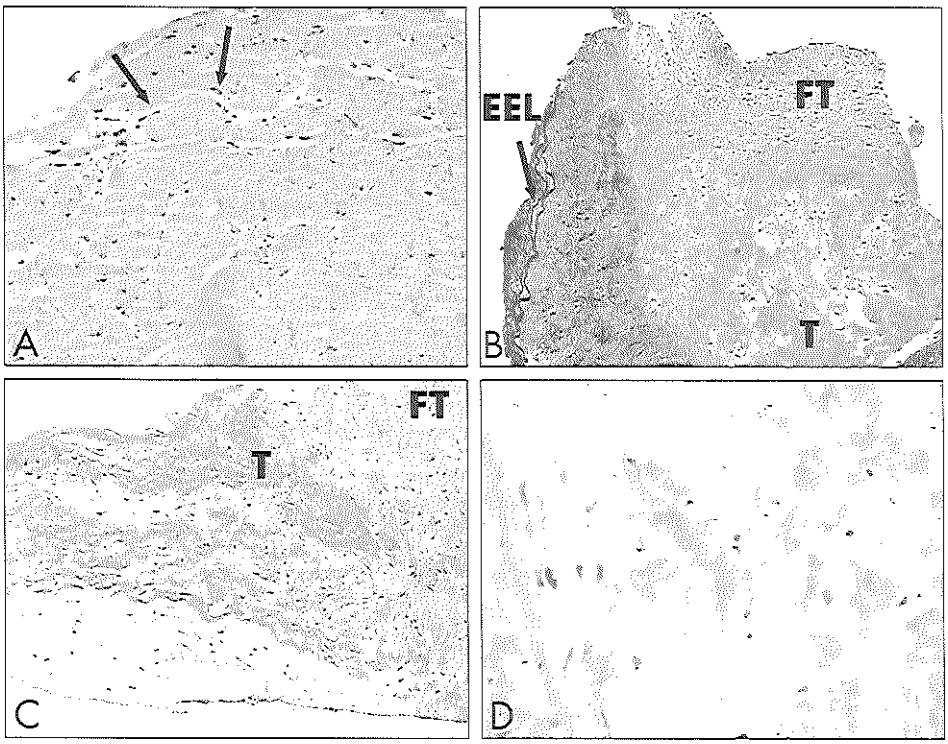

Figure 1

Thrombotic material in atherectomy specimens from patients with unstable angina showing different stages of organisation. A: Early organisation 5 days after the onset of angina at rest, showing lacunar spaces (arrows) in the thrombotic bulk that are partially covered by endothelial cells (confirmed by positive staining with lectin immunochemistry using Ulex europeus). B: Large area of thrombus (T) 7 days after the onset of angina at rest in close association to newly formed fibromuscular tissue (FT) and showing partial infiltration by myofibroblasts. Fragments of external elastic lamina (EEL)(arrow) and media are evident, indicating that deep vessel resection ocurred during atherectomy. C and D: Advanced thrombus organisation by fibromuscular tissue 2 days after the onset of angina at rest. Although virtual incorporation to the vessel wall has taken place it is possible to identify strands of thrombotic material surrounded by connective tissue (D). A. C and D : Hematoxylin-azophloxin; B: Verhoeff-van Giesson (original magnification: A, x60; B and C, x30; and D, x125).

or capillary vessels present in thrombotic mass originating from the surrounding tissue, the appearance of smooth muscle cells or myofibroblasts, and the presence of thrombotic material embeded in fibrocellular tissue, the latter characteristic suggesting that the areas of fibrin and platelets derived from an episode of thrombosis or plaque hemorrhage was being integrated in the atheromatous plaque (Fig. 1). Thrombus was apposed to fibrous tissue in all cases, without endothelial cells in the interface between both. A lack of relation between the interval between the onset of angina at rest and thrombotic organisation was evident. Likewise, the relation between angiographic morphology and the presence of thrombus in the

retrieved tissue did not reach statistical significance. Complex angiographic morphology was noted in 17 of 45 (37%) unstable patients (Fig. 2). Thrombus or plaque hemorrhage was present in 6 (35%) of these cases, and in 4 (14%) of those with non-complex angiographic morphology (p=NS).

Calcium deposits were also observed more frequently in patients with unstable (18 of 45 samples, 67%) than stable angina (9 of 39 samples, 33%)(p = 0.024). No significant differences were found with regard to the presence of fibrous tissue, cholesterol clefts, necrotic core or clusters of macrophages. In the overall population complex atheromatous samples (containing dense fibrous tissue, calcium deposits and necrotic debris) were obtained in older patients (58±10 vs 51±12 years, p < 0.031). Necrotic debris was observed in 7 of 34 cigarette smokers (21%) versus 2 of 59 (4%) non-smokers (p=0.019). Macrophages were identified in 4 of 15 (27%) and 5 of 78 (6%) samples with and without necrotic debris, respectively (p = 0.05).

Prevalence neointimal hyperplasia in the retrived specimens
Neointimal hyperplasia was observed in 17 of 24 (71%) patients with previous coronary intervention and in 14 of 69 (20%) patients with primary lesions (p = 0.0001). Neointimal hyperplasia had identical characteristics in patients with previous balloon angioplasty, stenting, atherectomy or laser angioplasty. Particular attention was paid to the 14 cases with primary lesions showing typical neointimal hyperplasia. When compared to other primary lesions, no relationship with the type of coronary syndrome was observed: 6 patients had stable and 8 unstable angina pectoris (p=NS). Likewise, no association with sex, coronary artery disease risk factors or previous myocardial infarction was found. However, the mean age of patients with primary lesions showing neointimal hyperplasia was significantly lower than that of patients with primary lesions and other histological characteristics (51 ±13 versus 59±10 years respectively, p = 0.017).

Discussion

The retrieval of atheromatous material during directional coronary atherectomy has created new possibilities in the study of coronary syndromes. Although limited by lesion selection and sampling characteristics,[8] the collection and analysis of the removed tissue has the considerable advantage of allowing the pathologic assessment of coronary artery disease "in-vivo", thus avoiding the selection bias inherent to post-mortem studies. To our knowledge, the present work represents the first comparative study of the histopathological subtrate of two different coronary syndromes using atherectomy retrieved material.

Primary unstable angina is considered to be an acute thrombotic syndrome[2-5] occurring predominantly in patients with widespread coronary artery disease.[9] Coronary thrombosis does not result initially in transmural myocardial necrosis

because of incomplete, episodic vessel obstruction, intermittent spontaneous vessel recanalisation, or the presence of well developed collateral anastomoses.[3,4] Different observations suggest that the associated mural thrombus is very rich in platelet aggregates and shows a layered appearence.[2]

In the present study a higher prevalence of mural thrombus and/or plaque hemorrhage in unstable angina was also observed: thrombus was identified in atherectomy samples obtained from the ischaemia-related coronary lesion of 22% of unstable and in only 2% of stable patients. This figure is lower than the prevalence of thrombus suspected in angiographic studies[3] but similar to that reported in a necropsy study of patients with unstable angina.[9] It is remarkable that no stadistical relation between complex angiographic morphology and presence of thrombus in the tissue retrieved could be found, although several explanations can be given for this. The persistence of complex angiograpic morphology in the long-term has been reported in 57% of cases by Haft et al.[10] A complex angiographic morphology may also result from multiluminal channels that are frequent in atheromatous plaques of unstable patients[11] (Fig. 3D). Unstable angina may also result

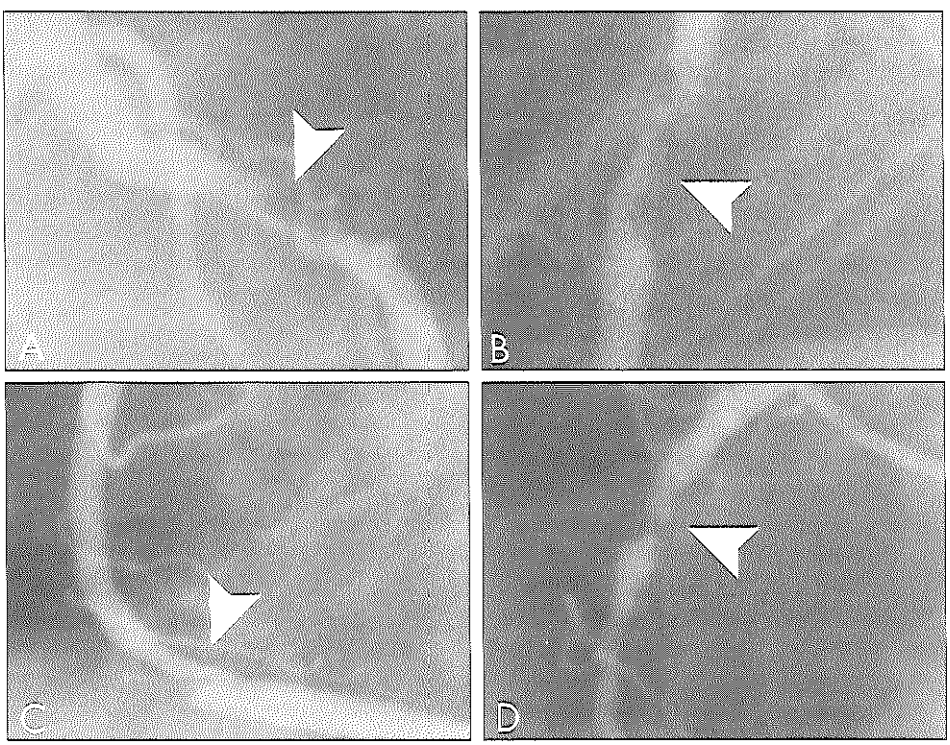

Figure 2

Complex angiographic morphology in 4 patients with unstable angina and histological evidence of coronary thrombosis. Figures A-D show complex eccentric lesions with overhanging edges (arrows).

from changes in plaque geometry secondary to intraplaque hemorrhage, which may be difficult to diferentiate from mural thrombus during the study of isolated fragments of the arterial wall.

An interesting finding is that all samples containing thrombus or intraplaque hemorrhage material showed different degrees of cellular organisation which, on the grounds of the time scale of thrombus organisation observed in experimental models,[12] bore no relationship to the time interval between the onset of chest pain and atherectomy. This may suggest that the onset of coronary thrombosis or plaque hemorrhage had preceeded by several days or weeks the development of angina at rest. The retrieved organising thrombus might thus correspond to either an episode of plaque hemorrhage or to a first episode of subocclusive thrombosis that after episodic growth or rethrombosis led to the development of symptoms[2] (Fig. 3A). The absence of fresh thrombus in these samples could be due to spontaneous lysis and inhibition of further thrombosis by continued systemic heparinisation (Fig. 3B) or embolisation of that labile fraction of thrombus during catheter manipulation (Fig. 3C).

These observations may have implications for therapeutic and diagnostic approaches in unstable patients. The low prevalence of thrombus observed and the degree of organisation and/or embedment of thrombus in the atheromatous plaque may explain the therapeutic failure of thrombolytic agents in primary unstable angina.[13,14] It might be also important to elucidate whether some of the angioscopic characteristics of coronary thrombus observed in unstable patients, such as the characteristic greyish appearance reported by Mizuno et al.,[5] could be related no only to platelet-rich but also to organising characteristics of thrombus, since it is well known that the macroscopic appearance and colour of thrombus shifts progressively towards a pale, whitish color as organisation increases.[15]

The cause of the initial event in the development of mural thrombosis, plaque rupture or fissuring, remains controversial. In this study macrophages, which have been identified in areas of the fibrous cap that area prone to rupture,[16] were not observed preferentially in unstable plaques but preferentially in plaques with necrotic core. Only fibrous tissue was found in close association with thrombus, an observation that may be relevant to the kind of initiating thrombogenic stimuli. Although no endothelium could be identified in the area covered by thrombus, no firm conclusions can be drawn from this as experimental studies have shown that endothelial cells are rarely observed 3 days after being engulfed by mural thrombosis.[12]

The higher prevalence of calcium deposits in unstable plaques may be related to the frequent existence of severe and widespread coronary artery disease in unstable patients.[11] A proportional relation between complex atheroma and age was also evident in the overall study population, in agreement with the current knowledge on the sequence of events leading to the progression of coronary artery disease.[17]

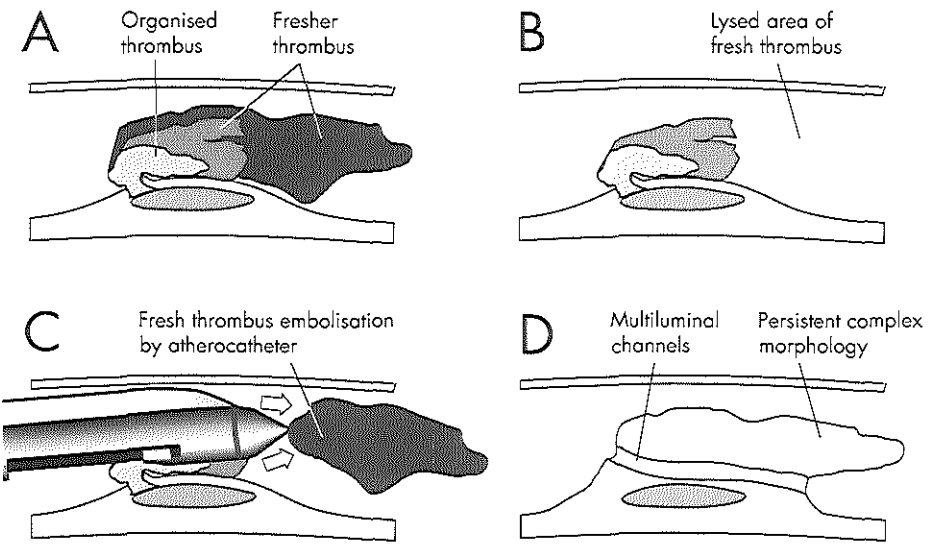

Figure 3

Mural thrombosis in unstable angina and histological findings in atherectomy specimens. A: Episodic thrombus growth has been proposed as a characteristic feature of unstable angina, yielding areas of different degree of organisation. Fresher areas of thrombus may have been missed in atherectomy specimens due to spontaneous lysis and concommitant treatment with intravenous heparin (B) or by dislodgement and embolization of the more labile fraction of thrombus by the atherocatheter (C). In some cases, complex angiographic morphology may have resulted from persisting irregularities or multiluminal channels in relation to the recanalisation of prior epiodes of plaque ulceration (D)

Our results also support previous observations in coronary atherectomy specimens showing that neointimal hyperplasia constitutes the pathological subtrate of restenosis after coronary intervention,[18] irrespectively of the revascularisation technique used previously. Typical atherosclerotic tissue was also retrieved in a substantial number of restenotic lesions, although this is probably due to the sampling characteristics of the device or in circumstances where restenosis was due to other mechanisms than neointimal hyperplasia.[8] In accordance with a previous study,[19] neointimal hyperplasia was also found in a substantial number of primary lesions. We noted that these patients were significantly younger than others with typical primary atherosclerotic lesions, a fact that may have particular relevance since fibromuscular neointimal proliferation has been reported as the pathological substrate for coronary artery disease in the young resulting in sudden death.[20] The ultimate meaning of this observation as to the natural history of atherosclerosis remains unclear. Neointimal hyperplasia represents an unspecific vessel wall response to different kinds of injury that lead to accelerated forms of atherosclerosis. Whether the presence of this type of tissue in primary lesions of young, symptomatic patients is a reflection of less-known factors initiating the atherosclerotic process (e.g. viral endothelial injury, genetical predisposition) remains hypothetical.

Histological studies based on atherectomy specimens are biassed by selective plaque sampling,[8] although in the present study this limitation was partially overcome by routinely performing multiple cuts in different sectors of the vessel. Case selection may have occurred since only vessels judged suitable for the technique were intervened (e.g., coronary atherectomy was performed in only 1 patient with total occlusion). The differenciation between mural thrombosis and foci of plaque hemorrhage is strongly limited by the analysis of isolated fragments of atheroma. Atherectomy was performed in stenoses that were identified on the grounds of clinical, angiographic and electrocardiographic data as ischaemia related stenoses. However, this "culprit lesion" approach may have not been free from a number of confounding factors, including the persistence of complex angiographic morphology from a previous event[10] (Fig. 3D), and the development of myocardial ischaemia "at a distance" by a different coronary narrowing.

In spite of these limitations, the results of the present study emphasise the use of directional coronary atherectomy as a means of investigation during its therapeutic use. The identification of such features as an increased prevalence of organised thrombus in patients presenting with unstable angina, and of neointimal hyperplasia in primary coronary lesions of younger patients contributes further to our knowledge of the processes that take place in the coronary arteries during the natural history of coronary syndromes.

References

1. *Betriu A, Heras M, Cohen M, Fuster V. Unstable angina: Outcome according to clinical presentation. J Am Coll Cardiol 1992; 19:1659-63.*

2. *Falk E. Unstable angina with fatal outcome: dynamic coronary thrombosis leading to infarction and/or sudden death. Circulation 1985; 71:699-708*

3. *Ambrose JA. Plaque disruption and the acute coronary syndromes of unstable angina and myocardial infarction: If the substrate is similar, why is the clinical presentation different? J Am Coll Cardiol 1992; 19:1653-8.*

4. *Fuster V, Badimon L, Cohen M, Ambrose JA, Badimon JJ, Chesebro J. Insights into the pathogenesis of acute ischemic syndromes. Circulation 1989; 77:1213-20.*

5. *Mizuno K; Miyamoto A; Satomura K; Kurita A; Arai T; Sakurada M; Yanagida S;Nakamura H : Angioscopic coronary macromorphology in patients with acute coronary disorders. Lancet, 1991; 337:809-12.*

6. *Stary HC, Blackenhorn DH, Chandler B, Glagov S, Insull W, Richardson M, Rosenfeld ME, Schaffer SA, Schwartz CJ, Wagner WD, Wissler RW. A definition of the intima of human arteries and of its atherosclerosis-prone regions. Circulation 1992; 85:391-405.*

7. *Ambrose JA, Winters SL, Tern A. Angiographic morphology and the pathogenesis of unstable angina pectoris. J Am Coll Cardiol 1985; 5:609-16.*

8. *Waller BF, Pinkerton CA: "Cutters, scoopers, shavers and scrappers": The importance of atherectomy devices and clinical relevance of tissue removed. J Am Coll Cardiol 1990; 15:426-8.*

9. *Kragel AH, Gertz SD, Roberts WC: Morphologic comparison of frequency and types of acute lesions in the major epicardial coronary arteries in unstable angina pectoris, sudden coronary death and acute myocardial infarction. J Am Coll Cardiol 1991; 18:801-8.*

10. *Haft JI, Al-Zarka AM: The origin and fate of complex coronary lesions. Am Heart J 1991; 121:1050-61.*

11. *Kragel AH, Reddy SG, Wittes JT, Roberts WC: Morphometric analysis of the composition of coronary arterial plaques in isolated unstable angina pectoris with pain at rest. Am J Cardiol 1990; 66:562-7.*

12. *Hand RA, Chandler AB: Atherosclerotic metamorphosis of autologous pulmonary thromoemboli in the rabbit. Am J Pathol 1962; 40:469-86.*

13. *Davies MJ. Sucessful and unsuccessful coronary thrombolysis. Br Heart J 1989; 61:381-4.*

14. *Vetrovec GW, Leinbach RC, Gold HK, Cowley MJ. Intracoronary thrombolysis in syndromes of unstable ischaemia: Angiographic and clinical results. Am Heart J 1982; 104:946-52.*

15. *Pearson TA, Dillman J, Solez K, Heptinstall RH: Monoclonal characteristics of organising arterial thrombi: Significance in the origin and growth of human atherosclerotic plaques. Lancet, 1979; 1:7-11.*

16. *Lendon CL, Davies MJ, Born GVR, Richardson PD. Atherosclerotic caps are locally weakened when mancrophage density is increased. Atherosclerosis 1991; 87:87-90.*

17. *Stary HC. The sequence of cell and matrix changes in atherosclerotic lesions of coronary arteries in the first forty years of life. Eur Heart J 1990; 11 (Supp E):3-19.*

18. *Garrat KN, Edwards WD, Kaufmann UP, Vlietstra RE, Holmes DR Jr Differencial histopathology of primary atherosclerotic and restenotic lesions in coronary arteries and saphenous vein bypass grafts: analysis of tissue obtained from 73 patients by directional atherectomy. J Am Coll Cardiol 1991; 17: 442-8.*

19. *Safian RD, Gelbfish JS, Erny RE, Schnitt SJ, Schmidt DA, Baim DS. Coronary atherectomy. Clinical, angiographic, and histological findings and observations regarding potential mechanisms. Circulation 1990; 82: 69-79*

20. *Corrado D, Thiene G, Pennelli N. Sudden death as the first manifestation of coronary artery disease in young people (≤35 years). Eur Heart J 1988; 9:139-44.*

Chapter II

Clinical and Histological Determinants of Smooth Muscle Cell Outgrowth in Cultured Atherectomy Specimens: Importance of Thrombus Organization

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Abstract

Background

Coronary atherectomy provides a unique opportunity to obtain plaque tissue from a wide variety of clinical syndromes. We investigated the relationship between the clinical and histopathological substrate of tissue retrieved during directional coronary atherectomy and the proliferative and migratory potential of smooth muscle cells as judged from successful outgrowth during cell culture.

Methods

Following directional coronary atherectomy tissue samples were examined macroscopically, divided in two equal pieces and separately subjected to cell culture and histopathological study. Cell culture was performed using an explant technique. In-vitro smooth muscle cell outgrowth was related to clinical (age, sex, coronary syndrome, previous MI, previous intervention, risk factors) and histological variables (presence of neointimal hyperplasia, connective tissue, cholesterol clefts, necrotic debris, calcium deposits, macrophages, thrombus, internal elastic lamina, media or adventitia).

Results

Atherosclerotic tissue was obtained from 98 consecutive atherectomy procedures. Histological examination revealed a broad spectrum of appearances ranging from complex plaque containing dense fibrous tissue, calcium deposits, macrophages and necrotic debris to neointimal proliferation and organised thrombus. Smooth muscle cell outgrowth was observed in 43 of 98 samples (44%). Cell outgrowth was not influenced by any of the clinical variables. It was influenced however by histological variables and in particular the presence of organizing thrombus. Smooth muscle cell outgrowth was successful in 8 of 10 samples with (80%) and only 35 of 88 (40%) without thrombus ($p = 0.03$).

Conclusion

The presence of organizing thrombus in the retrieved tissue facilitates smooth muscle cell outgrowth and suggests an enhanced proliferative and migratory potential. These findings may be relevant to the understanding of neointimal proliferation in coronary syndromes where mural thrombosis is likely to occur.

Introduction

Smooth muscle cell proliferation plays a key role in the development of typical and accelerated forms of atherosclerosis.[1] Cell culture of those present in atheromatous tissue retrieved during directional coronary atherectomy allows us to study smooth muscle cells from the pathological substrate of a variety of human coronary syndromes. In studying the pathobiology of coronary atheroma, the use of coronary atherectomy specimens for cell culture offers several distinct methodological advantages over other sources of atheromatous material, such as that obtained during peripheral atherectomy, carotid endarterectomy or animal models of atherosclerosis. Vascular smooth muscle cells are embryologically derived from local mesoderm,[2,3] and those found in the coronary arteries are likely to have biological differences with those located in other vessels. Likewise, the development of the atheromatous plaque is strongly influenced by local factors[4] that may influence cell populations, extracellular matrix composition and plaque architecture. The use of human coronary material makes also possible to avoid some of the pitfalls associated with the use of animal models of atherosclerosis.[5,6]

Even cell culture studies using human coronary smooth muscle cells however suffer with some technical limitations. Once in culture these cells undergo a progressive phenotypic modulation that is time dependent and enhanced by successive cell passages.[7-9] This process may facilitate the selection of cell clones with a higher proliferating capacity. Furthermore, although isolated cell studies provide information regarding cellular function and pathopysiological conditions, their extrapolation to the clinical situation is limited because they ignore the complex cell-cell and cell-extracellular matrix interactions which modulate smooth muscle cell growth in vivo.[10]

To minimise these limitations in the present study we have used an explant culture technique[11,12] to maintain a representative section of the atherosclerotic plaque in culture medium, thus retaining the intrinsic distribution and anatomical relationships of the participating cells, cell-cell interactions, the correct chemical configuration of the extracellular matrix, and the general in vivo millieu of the atherosclerotic plaque. Only coronary material was used. The initial outgrowth of smooth muscle cells was then used as a surrogate index for their in-vivo migratory and proliferative potential while still under the influence of other histological and humoral variables that were present in the atheromatous plaque at the time of atherectomy.

Methods

Procedural

Percutaneous directional coronary atherectomy was performed on 98 lesions in 98 patients. Informed consent was obtained from all patients prior to the procedure

according the protocol approved by the Thoraxcenter Institutional Review Board. Atherectomy was performed using the Simpson's Atherocath and a conventional technique.[13] Multiple cuts in different sectors of the vessel were routinely performed. Under sterile conditions, the specimens were removed from the housing of the atherocatheter, washed with 0.9% saline and placed in M199 Hepes buffered culture medium (GIBCO Laboratories) with antibiotics (penicillin 100 IU/ml and streptomycin 0.1 mg/ml). They were immediately transferred to the laboratory where they were flushed with fresh culture medium and examined with the help of a dissecting microscope. A representative section was then fixed in 3.6% buffered formalin for histopathological examination and the remainder placed in culture.

Clinical variables

For each patient a number of clinical variables were recorded. These included age, sex, previous myocardial infarction, stable or unstable angina pectoris, previous coronary intervention and risk factors for coronary artery disease (hypercholesterolaemia, diabetes mellitus, cigarette smoking, hypertension and family history of coronary artery disease). Unstable angina was defined as continuous or intermittent chest pain at rest requiring hospitalisation, associated with electrocardiographic evidence of myocardial ischaemia and no increase in cardiac enzymes.

Tissue analysis

Specimens for histopathological study were routinely processed for light microscopy and stained with Haematoxylin-azophloxin and Verhoeff-van Giesson. All specimens were reviewed independently by two observers, blinded to the clinical data. In case of disagreement the opinion of a third pathologist was sought and consensus agreement reached. For the analysis of intimal constituents the recommendations of the AHA Medical/Scientific statement on the definitions of the intima of human arteries and of its atherosclerosis-prone regions were followed.[14] Fibrous tissue was classified as dense when composed of acellular or poorly cellular connective tissue formed predominantly by dense collagen, and loose when the tissue fragments showed a moderate cellularity and collagen bundles separated by accumulations of extracellular matrix. Fibromuscular hyperplasia was defined as fibrous connective tissue showing a random orientation of spindle shaped and stellate cells embedded in abundant extracellular matrix. Thrombus was identified as amorphous material, often in close apposition with atheromatous material, frequently showing collections of leucocytes between layers of fibrin. Discrimination between fibrin and dense collagen was performed using Verhoeff-van Giesson staining. The thrombus was regarded as organizing when infiltration by cellular elements such as smooth muscle cells or fibroblasts was observed. Cholesterol crystal clefts, necrotic debris and calcium deposits were recorded independently. The presence of macrophages was recorded only when these formed clusters or when they were present in unusually high number. Medial tissue was identified on the basis of parallel arrangement of smooth muscle cells, embedded in collagen and frequently associated with a fragment of the internal or external elastic lamina.

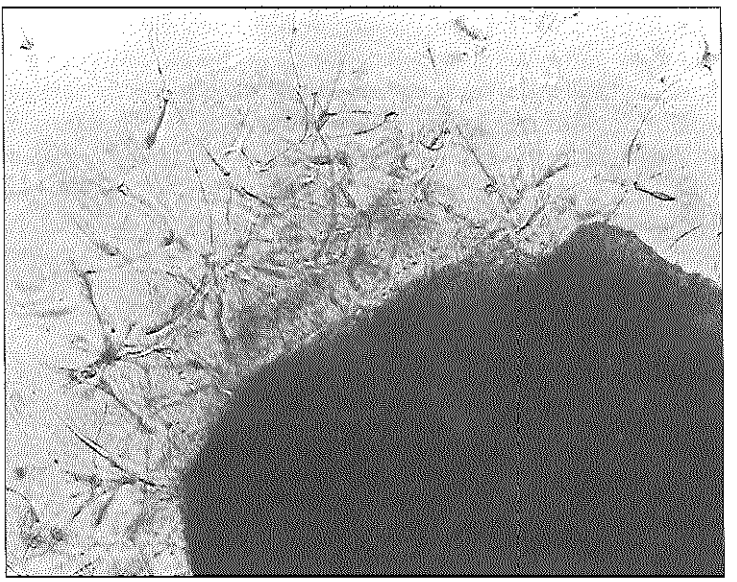

Figure 1
Fragment of retrieved tissue after 12 days in culture examined with phase-contrast microscopy. A dense population of cells migrating out of the atherectomy explant are evident. These cells fulfill the morphological criteria of myofibroblasts discussed in detail in the text. (Original magnification: x60).

Adventitia was recognised by the presence of coarse bundles of dense collagen intermingled with elastin fibers, sometimes in association with fragments of the external elastic lamina and media.

Cell culture

The atheromatous tissue was cultured by a cell biologist (MdJ) blinded to clinical data. An explant technique was used. Tissue explants were placed on human fibronectin coated (10 μg/cm^2) glass cover slips in 2 cm^2 wells (Four well plates, Nunc) and cultured in 300μl culture medium (M199 with $NaHCO_3$ (GIBCO Laboratories) supplemented with glutamine, 10% human serum, 10% fetal calf serum, penicillin 100 IU/ml, streptomycin 0.1 mg/ml and mixed in a ratio of 1:1 with conditioned medium from established smooth muscle cell lines actively growing in our laboratory). Cultures were maintained in a CO_2 incubator at 37°C in a humidified atmosphere equilibrated with 5% (v/v) CO_2 in air. The culture medium was changed every 3-4 days. Smooth muscle cell outgrowth was identified using inverted light microscopy and morphological criteria. These included a characteristic growth pattern of multiple layers of spindle or stellar shaped cells showing stress fibers and lamellipodia (Fig. 1). These morphological criteria were reinforced by positive immunostaining against smooth muscle cell α-actin (DAKO, Denmark) with human skin fibroblasts as negative controls.

Statistical analysis

Mean values and standard deviations were calculated for all continuous variables. Comparison of mean values was performed using two-tailed unpaired t-test. Discrete variables were compared using chi-square test, and continuity correction applied when indicated. A p value of < 0.05 was considered significant.

Results

Clinical

Of the 98 patients in the study 49 presented with stable and 47 with unstable angina pectoris. The remaining 2 were post-cardiac transplantation patients with cardiac allograph vasculopathy (Table 1). Twenty four of the patients had a previous history of coronary intervention (14 balloon angioplasty, 6 stent implantation, 3 atherectomy procedures and 1 excimer laser angioplasty) and had restenosis at the site of previous intervention. The mean time interval between the previous revascularisation procedure and the atherectomy was 147±108 days. The target lesion was located in the left anterior descending coronary artery in 62 cases, in the circumflex in 12, in the right coronary in 20 and in saphenous vein grafts in 4 cases. An average of 6 ± 3 passes in multiple directions were made across each lesion.

In the study population there was a history of hypercholesterolaemia (serum cholesterol ≥ 8 mmol/dl) in 7 patients, systemic hypertension in 27, smoking in 36 and a family history of coronary artery disease in 18. There were no patients with a history diabetes mellitus. None of these risk factors appeared to influence smooth muscle cell outgrowth (Table 1). Likewise, none of the other clinical variables discussed above could be related with enhanced cell outgrowth.

Histological

Thrombus was present in 10 of the 97 sections examined, predominantly in unstable angina patients (9/49 (19%), versus 1/49 (2%) stable patients, p = 0.019). Some degree of organization was present in all thrombotic specimens examined (Fig. 2). This ranged from the presence of endothelial-like cells in lacunar spaces or capillaries and the presence of scarce myofibroblasts infiltrating the thrombotic mass from the adjacent fibrous tissue to infiltration by high numbers of myofibroblasts with the production of extracellular matrix. The thrombotic material appeared to be embedded in the fibrocellular tissue suggesting that areas of fibrin and platelets derived from an episode of thrombosis or plaque haemorrhage was being integrated into the atheromatous plaque. In 4 cases this process was seen in conjunction with extensive fibromuscular proliferation.

Neointimal hyperplasia was observed in 31 samples, predominantly in restenotic (17/24, 71%) rather than de novo (14/69, 20%) lesions (p=0.0001). In 7 cases (22%) a neovascularisation network was found in the interphase between neointimal hyperplasia and surrounding fibrous dense or loose fibrous tissue (4 in primary and 3 in restenotic lesions). In secondary lesions neointimal proliferation had identical characteristics irrespective of the nature of the previous intervention. Dense and loose fibrous tissue was found in 79 and 17 samples respectively. Calcium deposits were observed in 27 samples. Macrophages were identified in 15 samples, predominantly in those with necrotic debris. Media and adventitia was found in 23 (23%) and 7 (7%) specimens respectively.

Smooth muscle cell outgrowth

Depending on the volume of the retrieved tissue an average of 4.5 fragments (range 2-8), each measuring approximately 1 mm^3, were placed in culture. Cells started to grow out from explants by 4-14 days (Fig. 3) reaching a steady state by 4-6 weeks. If no outgrowth was observed by 3-4 weeks the samples were discarded. Despite the use of antibiotics in the culture medium six of the cultured specimens developed infections and were discarded. The infections tended to develop at 3 to 4 weeks by which time cell outgrowth should have occurred. In none of these specimens had it done so however.

Primary cell outgrowth was observed in 43 of 98 samples (44%). Under light microscopy when primary outgrowth occurred the majority of cells tended to form multiple layers and had a spindle or polygonal shape with multiple stress

Table I. Clinical variables and outcome of smooth muscle cell culture

	Successful	Failed	p value
Clinical			
Age (years, mean±SD)	57±10	57±11	NS
Previous MI	14/43	21/55	NS
Syndrome			
-Stable angina	20/49 (41%)	29/49 (59%)	NS
-Unstable angina	23/47 (49%)	24/47 (51%)	NS
-Transplant vasculopathy	0/2	2/2	-
Risk factors CAD:			
-Male sex	34/43 (79%)	46/55 (83%)	NS
-Hypercholesterolemia	3/39 (8%)	4/59 (8%)	NS
-Hypertension	10/40 (25%)	17/58 (29%)	NS
-Smoking	17/42 (40%)	19/56 (34%)	NS
-Family history CAD	8/43 (19%)	10/55 (18%)	NS
Previous intervention	11/43 (26%)	13/55 (24%)	NS
Histological			
Neointimal hyperplasia	14/30 (46%)	29/68 (43%)	NS
Thrombus (organizing)	8/10 (80%)	35/88 (40%)	0.03
Dense fibrous tissue	32/79 (40%)	11/19 (57%)	NS
Loose fibrous tissue	10/16 (62%)	33/82 (40%)	NS
Cholesterol clefts	4/8 (50%)	39/90 (43%)	NS
Calcium deposits	13/27 (48%)	30/71 (42%)	NS
Nechrotic debris	6/9 (67%)	37/89 (46%)	NS
Macrophage clusters	7/15 (47%)	36/83 (43%)	NS
Media	11/23 (48%)	32/75 (43%)	NS

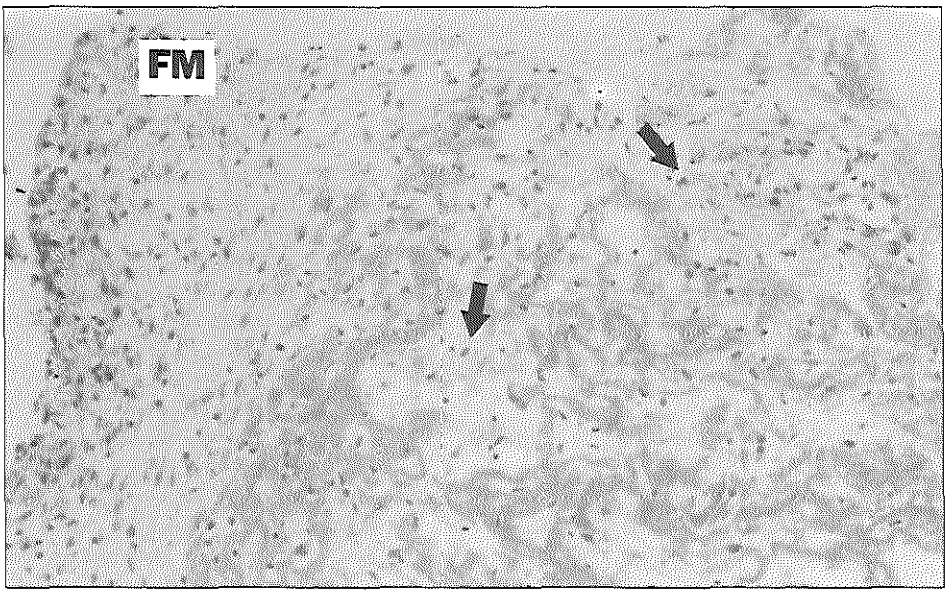

Figure 2

Histological cross section showing thrombus partially infiltrated by myofibroblasts (arrows) in close association with newly formed fibromuscular tissue (FM). (Original magnification: x30).

fibers extending to lamellipodia. These light microscopic appearances are characteristic of smooth muscle cells. The cells were confirmed to be smooth muscle cells by positive staining on immunocytochemistry with monoclonal antibody to α-actin. In addition to smooth muscle cells a second cell type oval in shape, with eccentrically placed small, indented nuclei was identified in 6 cultures. Immunoperoxidase staining with macrophage specific HAM 56 confirmed these cells to be macrophages. Macrophages typically disappeared by the 10-14th day of culture.

Cell outgrowth was not significantly influenced by any of the clinical variables recorded including patient sex or age, coronary syndrome (stable or unstable angina), type of coronary lesion (de novo or secondary) or risk factors for coronary artery disease (hypercholesterolaemia, hypertension, smoking, family history) (Table I). Although cell culture failed in samples obtained from both cardiac transplant patients, the small number involved precludes any conclusions as to the statistical significance of this finding. Smooth muscle cell outgrowth was significantly influenced however by the presence of organizing thrombus on histological examination. Smooth muscle cell outgrowth was documented in 8 of 10 (80%) samples with and only 35 of 88 (40%) without thrombus ($p = 0.03$, Table I). None of the other histological variables analysed including the presence of neointimal hyperplasia, fibrous tissue (dense or loose), lipid deposits, necrotic debris, macrophages, media or adventitia influenced cell outgrowth. Finally

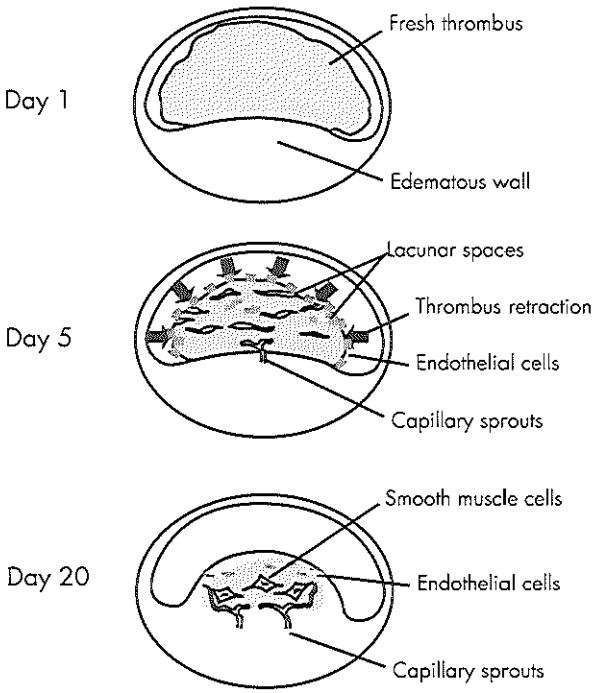

Figure 3

A graphic update on the sequence of events leading to thrombus organization, based on observations in experimental animal models.[37,38,41] Following the initial episode of thrombosis, a significant retraction of the thrombotic mass occurs, with the formation of lacunar spaces. These, as the thrombotic surface, become quickly endothelialised. A granulation reaction with capillary ingrowth from the vessel wall occurs simultaneously. By day 20 smooth muscle cells that may have migrated from the vascular wall during the granulation reaction or that are derived from circulating cells that have infiltrated the thrombus are seen along newly formed capillaries. Some of the endothelialised lacunar spaces may give origin to multiluminal channels that constitute a classical histopathological landmark of prior thrombotic recanalisation.

there was no correlation between the number of explants used in each case and the outgrowth of smooth muscle cells (4.5±1.6 and 4.5±1.9 explants in those cases with successful or failed culture respectively, p=NS).

Discussion

Atherectomy has facilitated not only the study of the histological constitution of the atheromatous plaque[13,15-17] but also the culture of smooth muscle cells present in human atheroma.[11,12,18-22] Several groups,[19,21] including ours,[18] have reported on improved cell outgrowth rates when an explant cell culture technique is used. The advantage of this method is that it minimizes the modifications of cell phenotype associated with enzymatic dispersion, prolonged culture, cell division and successive cell passages[7-9] and therefore allows a better appreciation of the in-vivo proliferative and migratory potential of smooth muscle cells. Since cell outgrowth occurs in the presence of the normal constituents of the atheromatous plaque present in in the culture, it makes possible to examine the influence of the existing plaque milieu at the time of intervention on the process of cell proliferation.

We found a broad range of histological appearances in both de novo and restenotic lesions ranging from predominant neointimal hyperplasia to typical atheroma containing dense fibrous tissue, calcium deposits, macrophages and necrotic debris. In agreement with previous communications based on the study of atherectomy specimens, neointimal proliferation was seen not only in the classical scenario of restenosis[1,23] but also in a substantial number of primary lesions.[15,17,24] Although the prevalent view on the development of fibromuscular proliferation is that it constitutes a non-specific response to various types of vessel wall injury,[1] it remains unclear the type of vascular insult responsible for its development in de novo lesions. Its occurrence in younger patients[17,24,25] suggests that it may differ from the classical sequence of events observed in the formation of atheroma,[26] leading to a more aggressive form of atherosclerotic disease. Finally, we have found that organization is common in thrombotic atherectomy samples from unstable patients,[17] a fact that is in agreement with a recent work by Isner et al.[27] using atherectomy specimens but which was not highlighted in post-mortem studies, underlining the distinct advantages derived from the use of atherectomy in the in-vivo study of coronary syndromes.

Our explant culture success rate (44%) is low in comparison with other studies[12, 21] although this must be due to the sole use of coronary material. Cell culture from coronary atherectomy specimens is difficult however because of the small amounts of tissue involved and yields significantly lower outgrowth success rates than peripheral tissue. When culture of both coronary and peripheral tissue has been attempted, a lower success rate and a longer time span until outgrowth has been observed in the coronary samples.[12] This was explained on the grounds of a smaller number of specimens and their lower wet weight.[12] However, it must be remembered that there are major differences in the histopathological characteristics of coronary and peripheral artery samples. The prevalence of thrombus in peripheral artery specimens obtained during directional atherectomy is as high as 61%,[13] which is significantly higher than that found in the present and previous studies in the coronaries.[16,17] Although the relevance of this fact for cell culture is highlighted by the conclusions of the present study, it is unfortunate that none of the previous studies reported on the histologic characteristics of the material used for culture, a limitation recently acknowledged in a recent report by Pickering et al.[21]

Our study is the first to consider the influence of a broad spectrum of histopathological features of retrieved tissue in addition to clinical features on cell outgrowth. Like Bauriedel and colleagues[12] we found that no clinical variables, including unstable angina and drug therapy influence the outcome of plaque cultivation. This is reflected in the clinical situation where there is little evidence that clinical factors influence the restenosis rate and all therapeutic strategies have been singularly unsuccessful. Common sense dictates that if clinical factors are operative it would be through the histological milieu of the atherosclerotic plaque. In spite that human smooth muscle cells cultured from restenotic lesions appear to migrate

more rapidly than those from primary atheroma[12] and show accelerated growth curves,[22] we did not find significant differences between the primary outgrowth of smooth muscle cells from explants of restenotic and primary lesions. A possible reason for this discrepancy is that some of these studies used isolated smooth muscle cells, obtained after several passages, and free of the complex cell-cell and cell-extracellular matrix interactions which modulate smooth muscle cell proliferation and migration in the atherosclerotic plaque in vivo.[10]

Evidence suggesting an enhanced proliferative potential of smooth muscle cells present in restenotic lesions can be found in the literature. An improved smooth muscle cell outgrowth from the injured vascular wall has been demonstrated by Grunwald[18] in a rat model. Smooth muscle cell outgrowth has been found to occur more rapidly in restenotic than de novo atherectomy specimens obtained in peripheral vessels,[12,21] although the initial outgrowth was similar. In our study cell outgrowth was not significantly different in explants from de novo or restenotic lesions. There are a number of possible reasons for this. Our experiments were performed only using coronary atherectomy specimens which, as discussed before, may have substantial differences in their histological substrate with those obtained in peripheral vessels. The time interval from the former percutaneous intervention may also be of importance as there is evidence suggesting that smooth muscle cells experience a process of senescence during its migration to the neointima[29] and decrease their proliferation rate after a period of time.[30]

We found an enhanced proliferating potential of smooth muscle cells present in coronary atheroma where thrombotic organization is taking place. This may be related to three major factors. First, mural thrombus constitutes a milieu rich in circulating elements, such as platelets, monocytes and lymphocytes, which can secrete a number of vascular growth factors,[1] promoting smooth muscle proliferation. Thrombin and fibrin[31] have both been shown to have chemotactic and mitogenic activity on vascular smooth muscle cells, an effect which may be prolonged after thrombus has been incorporated in the plaque. Thrombin may also act as a competence factor, stimulating the expression of growth factors including PDGF and their receptors[32,33] and thus helping to perpetuate the activation of smooth muscle cells. Any or all of these mechanisms may have been operative in our study, resulting in the increased migratory and proliferative activity of the smooth muscle cells when surrounded by thrombus.

Second, the process of thrombus organization may have played a key role in the observed outgrowth of cells. In fact, since organization was taking place in all thrombotic specimens we believe that our conclusions should be restricted to the presence of organizing thrombus. There is growing evidence suggesting that thrombus organization plays a key role in the development of neointimal hyperplasia after vascular injury.[34-37] It has been suggested that the smooth muscle cell involved in this process are derived from circulating mononuclear cells rather than being of intimal or medial origin[37,38] (Fig. 4). Thrombus would serve as a biode-

gradable fibrin matrix, colonized by circulating mononuclear cells which heal the injury site from the lumen side inwards, towards deeper vascular layers.[37] In this scenario the mononuclear cells which have colonized and started organizing the thrombus are self selected for their migratory and proliferative ability, a fact that would provide an unexpected explanation to a previous report showing that smooth muscle cells in organizing thrombus have a monoclonal origin.[39] Although the smooth muscle cell cultured from thrombotic and non thrombotic origin were morphologically indistinguishable the above tentative scenario remains plausible. Finally, a third explanation for our findings is that organizing thrombus may have facilitated cell outgrowth by optimizing cell transfer to the culture medium.

In our study we tried to reach a compromise between obtaining combined information from histopathology and cell culture. As discussed above, meticulous inspection of the samples in the dissecting microscopy was performed to ensure that the tissue fragment dedicated to histology and cell culture sample were representative of the whole specimen. However, the possibility that the two pieces were significantly different cannot be ruled out. Atherosclerosis is a segmental disease process, and in this regard our study shares the limitations of all histopathological studies using atherectomy specimens, where conclusions are reached using fragmented samples of the arterial wall.[23] A second limitation is that pretreatment of the culture wells with fibronectin may have facilitated the transformation of smooth muscle cells from the contractile to the synthetic phenotype[10] affecting explant outgrowth. We believe that this mechanism is unlikely to be operative in this case however as fibronectin is already present in serum and any additional effect that fibronectin coating of the wells may have had is likely to have been constant in all specimens. Furthermore, in the absence of fibronectin poor adherence of atherosclerotic tissue to the culture medium occurs resulting in decreased explant success. The final potential source of error is variability in the area of contact between the explant tissue and the fibronectin. This is likely to have been randomly distributed amongst all the specimens studied however making it unlikely to account for the observed differences. We believe that these differences in cell outgrowth are a true reflection of the growing potential of the cells in the explant.

Our study emphasizes the research utility of clinically indicated directional coronary atherectomy and suggests an enhanced migratory and proliferating potential for smooth muscle cells present in atheromatous plaque where thrombotic organization is taking place, supporting the concept that plaque composition may influence progression of atherosclerosis. Furthermore it also suggests that monitoring in vitro cell outgrowth may provide a means of assessing important biological features of the pathobiology of the atheromatous plaque.

References

1. *Ip JH, Fuster V, Badimon L, Badimon J, Taubman MB, Chesebro JH. Syndromes of accelerated atherosclerosis: Role of vascular injury and smooth muscle cell proliferation. J Am Coll Cardiol 1990; 15:1667-87.*

2. *Schwartz SM, Heimark RL, Majesky MW. Developmental mechanisms underlying pathology of arteries. Physiol Rev 1990; 70: 1177-1209.*

3. *Robertson AL. Pathobiology of vascular cells in vitro in relation to human atherogenesis. Organ and species differences. Ann N Y Acad Sci 1990; 598:200-16.*

4. *Friedman MH. How haemodynamic forces in the human affect the topography and development of atherosclerosis? In Glagov S, Newmann WOP, Schaffer SA, eds. Pathobiology of the human atherosclerotic plaque. Springer-Verlag, 1990, pp 303-16.*

5. *Muller DWM, Ellis SG, Topol EJ. Experimental models of coronary artery restenosis. J Am Coll Cardiol 1992; 19: 418-32.*

6. *Ferrel M , Fuster V, Gold HK, Chesebro JH. A dilema for the 90's: Choosing the appropriate experimental animal model for the prevention of restenosis. Circulation 1992; 85: 1630-1.*

7. *Stadler E, Campbell JH, Campbell GR. Do cultured smooth muscle cells resemble those of the arterial wall? If not, why not? J Cardiovasc Pharmacol 1989; 14 (Suppl. 6): S1-S8.*

8. *Campbell G, Campbell J, Manderson J, Horrigan S, Rennick R. Arterial smooth muscle cell. A multifunctional mesenchimal cell. Arch Pathol Lab Med 1988; 112:977-86.*

9. *Campbell GR, Campbell JH. Phenotypic modulation of smooth musce cells in primary culture. In: Campbell JH, Campbell GR, eds. Vascular smooth muscle cell in culture. Boca Ratón, FL: CRC Press, 1987:39-56.*

10. *Madri JA, Kocher O, Merwin JR, Bell L, Yannariello-Brown J. The interactions of vascular cells with solid-phase (matrix) and soluble factors. J Cardiovasc Pharmacol 14(Suppl. 6): S70-S75.*

11. *Bauriedel G, Dartsch PC, Voisard R, Roth D, Simpson JB, Hofling B, Betz E. Selective percutaneous "biopsy" of atheromatous plaque tissue for cell culture. Basic Res Cardiol 1989; 84: 326-31.*

12. *Bauriedel G, Windstetter U, DeMaio SJ, Kandolf R, Hofling B. Migratory activity of human smooth muscle cells cultivated from coronary and peripheral primary and restenotic lesions removed by percutaneous atherectomy. Circulation 1992; 85: 554-564.*

13. *Johnson DE, Hinohara T, Selmon MR, Braden LJ, Simpson JB. Primary peripheral arterial stenoses and restenoses excised by transluminal atherectomy: a histopathologic study. J Am Coll Cardiol 1990; 15: 419-25*

14. *Stary HC, Blackenhorn DH, Chandler B, Glagov S, Insull W, Richardson M, Rosenfeld ME, Schaffer SA, Schwartz CJ, Wagner WD, Wissler RW. A definition of the intima of human arteries and of its atherosclerosis-prone regions. Circulation 1992; 85:391-405.*

15. *Safian RD, Gelbfish JS, Erny RE, Schnitt SJ, Schmidt DA, Baim DS. Coronary atherectomy. Clinical, angiographic, and histological findings and observations regarding potential mechanisms. Circulation 1990; 82: 69-79.*

16. *Garrat KN, Edwards WD, Kaufmann UP, Vlietstra RE, Holmes DR Jr Differencial histopathology of primary atherosclerotic and restenotic lesions in coronary arteries and saphenous vein bypass grafts: analysis of tissue obtained from 73 patients by directional atherectomy. J Am Coll Cardiol 1991; 17: 442-8.*

17. *Escaned J, van Suylen RJ, MacLeod DC, Umans VAWM, de Jong M, Bosman FT, de Feyter PJ, Serruys PW. Histological characteristics of tissue excised during directional coronary atherectomy in stable and unstable angina pectoris. Am J Cardiol 1993 (In press).*

18. *Strauss BH, de Jong M, Verkerk A, van Suylen RJ, Umans VAWM, de Feyter PJ, van Hooije CMC, MacLeod DC, Visser WJ, Jongkind JF, Verdouw PD, Serruys PW. Human coronary smooth muscle cells in culture: Phenotypic features, proliferation and extracelular matrix production. Circulation 1991; 84: II-295.*

19. *Dartsch PC, Voisard R, Bauriedel G, Hofling B, Betz E. Growth characteristics and cytoskeletal organization of cultured smooth muscle cells from human primary stenosing and restenosing lesions. Arteriosclerosis 1990; 10: 62-75.*

20. *Chao K, Ko YL, Cheng JJ, Lien WP. Cell cultures of coronary atherectomized target lesions. Eu-Heart-J 1991; 12 (Abstr. Suppl.): 291.*

21. *Pickering GJ, Weir L, Rosenfield K, Stetz J, Jekanowski J, Isner JM. Smooth muscle cell outgrowth from human atherosclerotic plaque: Implications from the assessment of lesion biology. J Am Coll Cardiol 1992; 20:1430-9.*

22. *MacLeod DC, Strauss BH, de Jong M, Escaned J, Umans VA, van Suylen RJ, Verkerk A, de Feyter PJ, Serruys PW. Proliferation and extracellular matrix synthesis of smooth muscle cells cultured from human coronary therosclerotic and restenotic lesions. J Am Coll Cardiol 1993 (In press).*

23. *Waller BF, Pinkerton CA. "Cutters, scoopers, shavers and scrapers": The importance of atherectomy devices and clinical relevance of tissue removed. J-Am Coll Cardiol 1990; 15: 426-8.*

24. *Miller MJ, Kuntz RE, Friedrich SP, Leidig GA, Fishman RF, Schnitt SJ, Baim DS, Safian RD. Frequency and consequences of intimal hyperplasia in specimens retrieved by directional atherectomy of native primary coronary artery stenoses and subsequent restenoses. Am J Cardiol 1993; 71:652-58.*

25. *Corrado D, Thiene G, Pennelli N. Sudden death as the first manifestation of coronary artery disease in young people (≤35 years). Eur Heart J 1988; 9:139-44.*

26. *Stary HC. The sequence of cell and matrix changes in atherosclerotic lesions of coronary arteries in the first forty years of life. Eur Heart J 1990; 11 (Supp E):3-19.*

27. *Isner JM, Brinker JA, Gottlieb RS, Leya F, Masden RR, Shani J, Kearney M, Topol EJ ,for CAVE-AT. Coronary thrombus: Clinical features and angiographic diagnosis in 370 patients studied by directional coronary atherectomy. Circulation 1992 (Suppl. I); 86:I-648.*

28. *Grunwald J, Chobamiam A, Haudenschild C. Smooth muscle cell migration and proliferation. Atherosclerosis 1987; 67:215-21.*

29. *Ross R et al. Human atherosclerosis: I. Cell constitution and characteristics of advanced lesions of the superficial femoral artery. Am J Pathol 1984; 114: 79-93.*

30. *Strauss BH, Umans VA, van Suylen RJ, de Feyter PJ, Marco J, Robertson GC, Renkin J, Heyndrickx G, Vuzedvski VD, Bosman FT, Serruys PW. Directional coronary atherectomy for treatment of restenosis within coronary stents: Clinical, angiographic and histological caharacteristics. J Am Coll Cardiol 1992; 20:1465-73.*

31. *Naito M, Hayashi T, Kuzuya M, Funaki C, Asai K, Kuzuya F. Effects of fibrinogen and fibrin on the migration of smooth muscle cells in vitro. Atherosclerosis 1990; 83:9-14.*

32. *Thyberg J, Hedin U, Sjolund M, Palmberg L, Bottger BA. Regulation of differentiated properties and proliferation of arterial smooth muscle cells. Arteriosclerosis 1990; 10:966-90.*

33. *Casscells W. Smooth muscle cell growth factors. Prog Growth Factor Res 1991; 3:177-206.*

34. *Poole JCF, Cromwell BS, Benditt EP. Behaviour of smooth muscle cells and formation of extracellular structures in the reaction of arterial walls to injury. AmJ Pathol 1971; 62:391-404.*

35. *Sumiyoshi A, More RH, Weigensberg BI. Aortic fibrofatty type atherosclerosis from thrombus in normolipidemic rabbits. Atherosclerosis 1973; 18:43-57.*

36. *Jorgensen L, Rowsell HC, Hovig T, Mustard JF. Resolution and organisation of platelet-rich mural thrombi in carotid arteries of swine. Am J Pathol 1967; 51:681-719.*

37. *Schwartz RS, Holmes DR, Topol EJ. The restenosis paradigm revisited: An alternative proposal for cellular mechanisms. J Am Coll Cardiol 1992; 20:1284-93.*

38. *Feigl W, Susani M, Ulrich W., Matejka M, Losert U, Sinzinger H. Organisation of experimental thrombosis by blood cells. Evidence of the transformation of mononuclear cells into myofibroblasts and endothelial cells. Virchows Arch (Pathol Anat) 1985; 406:133-48.*

39. *Pearson TA , Dillman J, Solez K, Heptinstall RH. Monoclonal characteristics of organising arterial thrombi: Significance in the origin and growth of human atherosclerotic plaques. The Lancet 1979; I: 7-11.*

40. *Hedin U, Johan T. Plasma fibronectin promotes modulation of arterial smooth-muscle cells from contractile to synthetic phenotype. Diferentiation 1987; 33:239-46.*

41. *Hand RA, Chandler AB: Atherosclerotic metamorphosis of autologous pulmonary thromoemboli in the rabbit. Am J Pathol 1962; 40:469-86.*

Chapter III

Proliferation and Extracellular Matrix Synthesis of Smooth Muscle Cell Cultures from Human Coronary Atherosclerotic and Restenotic Lesions

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Abstract

Background

Common to both primary atherosclerosis and restenosis are vascular smooth muscle cell proliferation and production of extracellular matrix proteins. The applicability to man of experimental animal models of these processes has been questioned.

Aim of the study

To examine the proliferative capacity and extracellular matrix synthesis of human coronary plaque cells in vitro.

Methods

Primary atherosclerotic and restenotic lesions were excised by percutaneous directional coronary atherectomy in 93 patients. Smooth muscle cells were cultivated by an explant technique and identified by their morphology in culture, ultrastructural features under electron microscopy and immunostaining using monoclonal antibodies to smooth muscle cell α-actin. Proliferation in secondary culture was assessed with growth curves, and the synthesis of collagen and sulfated glycosaminoglycans by the incorporation of ^{3}H-proline and ^{35}S-sulfate respectively. These studies were also performed in cells derived from human umbilical artery media.

Results

Success rates for primary (45%) and secondary (12%) culture of coronary cells were not influenced by clinical variables or lesion category. Primary culture success was improved by the presence of organised thrombus in the plaque and in relation to increased maximum cell density of the atherectomy specimen. Restenotic cells displayed more rapid growth than cells of primary atherosclerotic origin which grew similarly to umbilical artery cells; calculated population doubling times (hours, means and 95% CI) for the three cell groups were 52 (48-58), 71 (62-83) and 74 (65-84) respectively. Restenotic and primary atherosclerotic cells did not differ in the synthesis of collagen (0.034±0.004 and 5.43±1.00 respectively, both $p<0.05$).

Conclusions

The data suggest that an increase in smooth muscle cell proliferative capacity contributes to coronary restenosis in man, and support the concept that the extracellular matrix synthesis of adult smooth muscle cells is important to lesion formation.

Introduction

Migration, proliferation and extracellular matrix synthesis are properties of the vascular smooth muscle cell central to both primary atherosclerosis and restenosis, the vascular response to injury which may be provoked by balloon dilatation.[1-8] In cell culture, vascular smooth muscle cells retain many of their in vivo characteristics thus allowing the study of specific cellular properties under controlled conditions in vitro. Early experimental work with smooth muscle cells derived from human atherosclerotic lesions depended upon tissue removed from peripheral arteries at surgery or post-mortem[3,9-11] but fresh human atherosclerotic tissue can now be retrieved from both peripheral and coronary arteries by the procedure of directional atherectomy.[12,13] The feasibility of culture, characterisation and investigation of smooth muscle cells from tissue obtained by directional atherectomy has been demonstrated.[14-17]

Recent reviews of mechanisms in atherosclerosis[6] and in the arterial response to mechanical injury[7,8] have focused more on the migration and proliferation of vascular smooth muscle cells than on extracellular matrix synthesis. However, the contribution of the extracellular matrix to primary atherosclerotic and restenotic lesions is well defined histologically[3,18,19] and there is experimental evidence that following balloon injury, extracellular matrix deposition outlasts cellular proliferation and accounts for the bulk of the resulting intimal lesion.[20] It has been proposed, too, that the extracellular matrix may exert important effects on the migration and proliferation of the vascular smooth muscle cell.[21,22]

We cultivated smooth muscle cells from primary atherosclerotic and restenotic coronary lesions in man, obtained by directional atherectomy, in order to study both the proliferative behaviour and the synthesis of collagen and sulfated glycosaminoglycans, principal components of the extracellular matrix, of the cultured cells. In establishing this in vitro model of processes central to the formation of vascular intimal lesions, we also examined whether adult coronary plaque smooth muscle cells displayed properties in culture different to those of smooth muscle cells form an alternative immature arterial source; the normal media of human umbilical arteries.

Methods

Patient population and procedure

A series of 93 patients underwent percutaneous directional coronary atherectomy. Details of the population are summarised in Table 1. Lesions were designated restenotic when associated with a ≥50% diameter stenosis at the site of a previous intervention of which 18 (75%) were conventional (balloon) percutaneous transluminal coronary angioplasty, 4 (17%) were stents and 2 (8%) directional atherectomy. The target vessels included 4 long-standing saphenous vein grafts, all with

Table I. Baseline characteristics of the study population

Age (years)		58±1
Gender	Male	77 (83%)
	Female	16 (17%)
Angina	Stable	48 (52%)
	Unstable	45 (48%)
Lesion	Primary*	69 (74%)
	Restenotic	24 (26%)
Vessel	LAD	59 (63%)
	CX	11 (12%)
	RCA	19 (21%)
	Graft	4 (4%)

Unstable angina was defined as typical pain occurring at rest, with or without accompanying electrocardiographic changes, despite maximal medical therapy. *Primary = primary atherosclerotic . LAD: left anterior descending coronary artery; CX: circumflex coronary artery; RCA: right coronary artery; Graft: saphenous vein coronary bypass graft.

restenotic lesion. Informed consent was obtained from all patients prior to the procedure which was performed using the Simpson Coronary AtheroCath (Devices for Vascular Intervention, Redwood City, California, USA) according to a protocol approved by our hospital ethics committee and described elsewhere.[23] Under sterile conditions, specimens were flushed from the dived with 0.9% saline and placed immediately in serum-free M199 culture medium for transfer to the culture laboratory.

Cell Culture

Excised coronary tissue was placed in cell culture after the method described by Bauriedel et al.[13] Tissue explants were placed on round glass cover slips coated with fibronectin ($10\mu g.cm^{-2}$) in 2 cm^2 wells and culture medium (M199 supplemented with glutamine, 10% human serum, 10% fetal calf serum, penicillin 100 $IU.ml^{-1}$ and streptomycin 0.1 $mg.ml^{-1}$) was added. For primary cultures, conditioned medium from actively growing umbilical artery medial smooth muscle cell strains was mixed 1:1 with the standard culture medium. In secondary culture, the culture medium was not supplemented with conditioned medium. For human umbilical artery smooth muscle cell culture, 3-4 mm^3 pieces of arterial wall were

dissected free from fresh human umbilical cord sections and cultured in fibronectin-coated 24 well plates using standard unconditioned culture medium. All cultures were incubated at 37°C in a moist atmosphere under 5% CO_2. At confluency, cells were subcultured by trypsinizing.

Histology.
A small portion of the atherectomy specimen was set aside for histology. This tissue was divided into approximately 1 mm^3 pieces with a sterile scalpel, fixed in 10% buffered formalin and processed for light microscopy with haematoxylin-azofloxin and Verhoeff-van Giesson stains.

Assessment of maximum cell density. Early studies used tissue from the initial 12 patients to assess maximal cell density. Cell nuclei were counted in several microscope fields in haematoxylin-azofloxin stained sections by means of a computerized morphometry system (IBAS, Kontron, Oberkochen, Germany). The maximum value recorded was used for the estimation of maximum cell density, expressed as cells. mm^{-2}.

Identification of smooth muscle cells by immunostaining and electron microscopy. Cells from passages 2-4 were seeded on round glass cover slips at a density of 5000 cells.cm^{-2}. After 48 hours, the cover slips were rinsed in phosphate-buffered saline (PBS, pH 7.4) before fixing in acetone at -20°C. Immunostaining was performed with monoclonal antibodies directed against smooth muscle cell α-actin

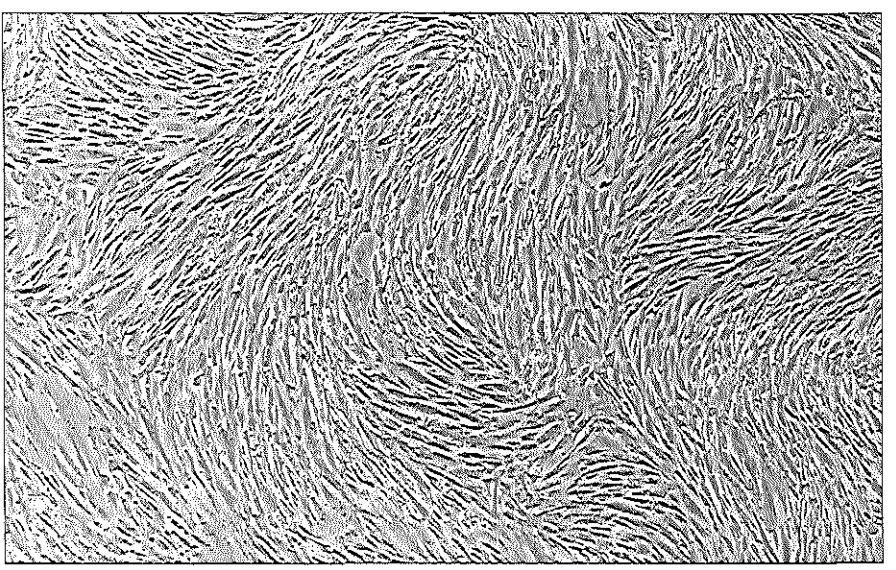

Figure 1
Vascular smooth muscle cells in secondary culture showing the typical "hill-and-valley" growth pattern.

(DAKO, Denmark) using an indirect immuno-peroxidase procedure.[24] For electron microscopy, cells cultured in 10 cm^2 diameter Petri-dishes were fixed in a phosphate-buffered mixture of 4% paraformaldehyde and 1% glutaraldehyde, postfixed in 1% OsO_4 and dehydrated in alcohol. Thereafter, embedding-capsules filled with epon were placed upside down on the cell monolayer and after polymerisation of the epon, the epon blocks were separated from the plastic by immersion in liquid nitrogen. Ultra-thin sections were cut on a Reichert OmL13 Ultramicrotome, stained with uranyl acetate and lead citrate and examined in a Philips E.M. 400 electron microscope.

Proliferation studies. Cells from passages 2-5 were seeded in 10 cm^2 culture plates at a density of 2000 cells.cm^{-2}. One day after seeding and on every third day thereafter, the medium was exchanged. At appropriate times, cells of two to four wells were washed with PBS, trypsinized for 3-5 minutes at 37°C, and counted in a haematocytometer. Cell numbers were used to plot growth curves.

Extracellular matrix synthesis. To determine collagen synthesis, subconfluent cells of passage 2-4 were incubated for 48 hours at 37°C in culture medium containing 2µM ascorbic acid and 10µCi.ml^{-1} ^{3}H-proline (specific activity 231 mCi.mg^{-1}). Incubations were performed in triplicate in 2 cm^2 culture wells. Subsequently, the medium was removed and combined with two cell layer washes (PBS) before precipitating with 20% trichloroacetic acid (TCA): 1 mM proline (24 hours, 4°C). Following centrifugation, the pellet was washed with graded concentrations of TCA:1 mM proline and dissolved in 0.2N NaOH overnight. Thereafter, collagen and non-collagen protein synthesis were quantified by a bacterial collagenase digestion method (clostridium histolyticum Type III, Calbiochem) modified from Peterkofsky et al.[25] Collagen synthesis was expressed as nmol ^{3}H-proline.µg total cell $protein^{-1}$.

Sulfated glycosaminoglycan synthesis was determined as follows. In triplicate 2 cm^2 culture wells, subconfluent cells of passage 3-5 were incubated for 48 hours at 37°C in culture medium containing 0.5 ml ^{35}S-sulfate (specific activity 20µCi.ml^{-1}). Thereafter, the medium was washed with PBS to a final volume of 2.5 ml and 250µl of this solution was applied onto a disposable Sephadex G-25M gel filtration column (PD10, Pharmacia) equilibrated and run in 8M urea:0.5% TritonX100:0.15M NaCI:50 mM sodium acetate (pH 6.0). Column fractions of 300µl were collected and fractions 8-45 counted in Instagel II. The synthesis of sulfated glycosaminoglycans, expressed as nmol ^{35}S-sulfate.µg total cell $protein^{-1}$, was derived from the sum of the counts in the initial peak.

For total cell protein, cells were detached from culture wells by gentle scraping, washed in PBS and precipitated in 20% TCA (24 hours, 4°C). After centrifugation, the pellet was dissolved in 0.2N NaOH overnight and total protein estimated according to the Pierce BCA assay (Pierce Rochford, Illinois, USA) using bovine serum albumin as the standard.[26]

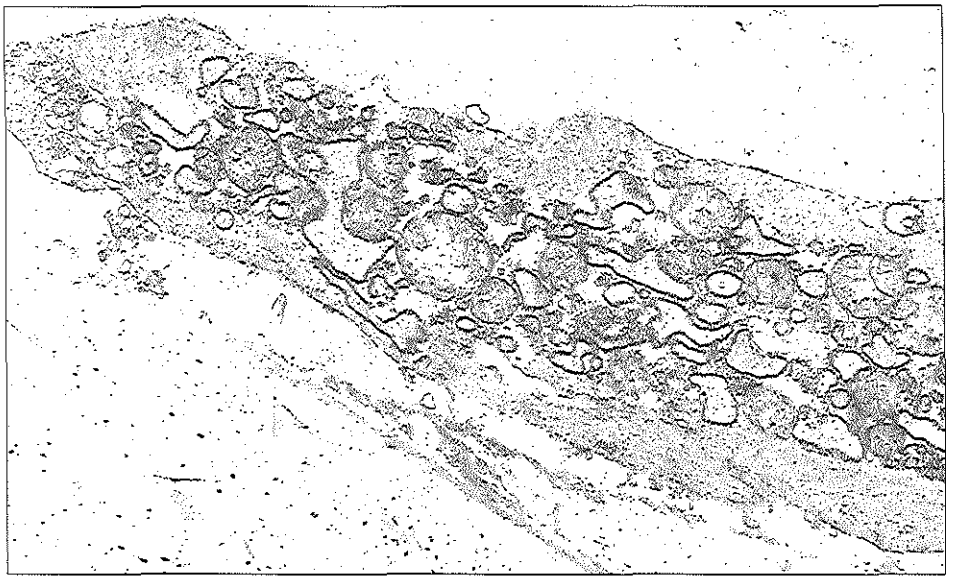

Figure 2

Transmission electron microphotography illustrating the synthetic phenotype of cultured smooth muscle cells, showing dense perinuclear organelles with endoplasmic reticulum, Golgi apparatus and abundant ribosomes (x12,000).

Statistical Analysis

Results are presented as means ± s.e.m..Group differences in initial smooth muscle cell outgrowth (primary culture), success of secondary culture, patient age, gender and anginal class, patient medications and histologic features were assessed by means of chi-squared tests for categorical variables with a Yate's correction where appropriate. Cell population doubling times, expressed as means and 95% confidence intervals, were derived following linear regression of log transformed cell population growth curves. Otherwise, comparisons between primary atherosclerotic and restenotic coronary artery smooth muscle cells and human umbilical artery smooth muscle cells were performed using a two-sample t test. Significance was accepted at the 5% level.

Results

Culture success: relationship to clinical parameters and histologic features.

Initial cell outgrowth (primary culture) was seen in 37 (40%) of 93 attempted cultures and successful secondary culture (up to 7 serial passages) was achieved in 11 (12%). Clinical data for the patients whose tissue provided secondary cultures were the following: 8 male (73%), 3 female (27%); age 56 ± 1 years; stable angina 6 (55%), unstable 5 (45%); primary atherosclerotic lesions 7 (64%), restenotic 4 (36%).

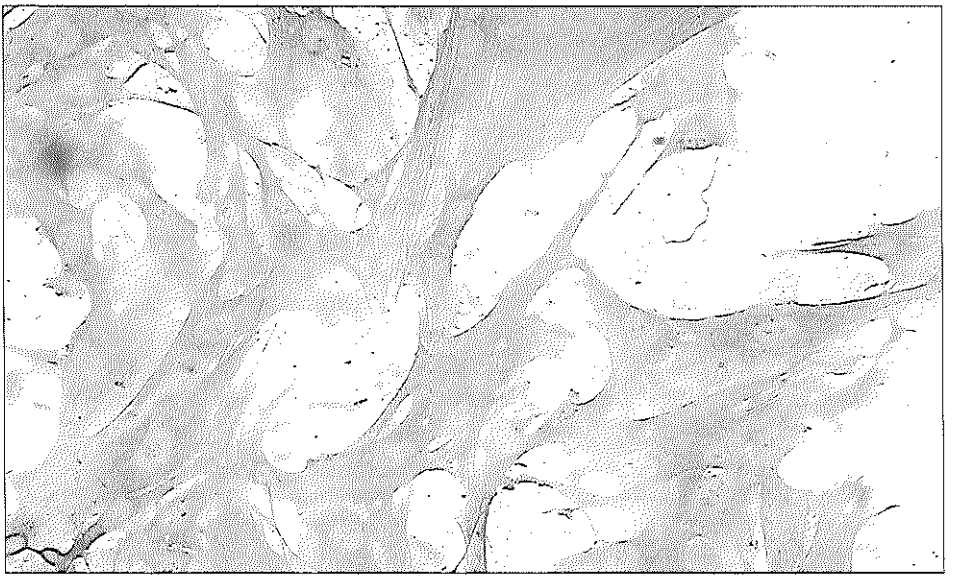

Figure 3
Immunohistochemical staining with monoclonal antibody (1/400 dilution) directed against smooth muscle cell specific α-actin (x500). Stress fibers are clearly seen.

Cells started to grow out from explants after 4-8 days and confluent multilayer primary cultures from the 11 patients were established after 4-6 weeks. The success of initial outgrowth of coronary smooth muscle cells and of serial passage in secondary culture was not related to the nature of the lesion; primary atherosclerotic or restenotic, or to any patient characteristic including current medication. Cell outgrowth was however related to the presence of organized thrombus in the plaque. The rate of outgrowth in the presence of thrombus was 80% (8/10), but was 35% (29/83) in the absence of thrombus ($p<0.025$). In the initial 12 patients, cell outgrowth improved with greater tissue maximum cell densities (outgrowth: 922 ± 43 cells.mm^2 vs. no outgrowth: 213 ± 34, $p< 0.01$). The four successful secondary cultures from restenotic lesions were not associated with unusually aggressive restenosis (interval from previous intervention: 130 ± 36 days).

Morphology and identification of cells cultured from human coronary lesions. Subconfluent cultures took the form of a network of multilayered elongated cells in interlacing bands separated by lacunae. In confluent multilayer cultures, the cells appeared as whorls producing the "hill-and-valley" pattern typical of vascular smooth muscle cells in culture (Fig. 1). This pattern was observed for all serially passaged cells. Electron microscopy of cultured coronary cells revealed features of synthetic phenotype[27] with abundant perinuclear organelles and peripheral myofibrils (Fig. 2). Immunostaining for α-actin was positive for both coronary (Fig. 3) and umbilical cell cultures, identifying the cells as smooth muscle cells. However,

a lesser proportion, approximately 20%, of the coronary cells showed typical staining with stress fibers, consistent with the electron micrographic evidence of synthetic phenotype.

Proliferation studies

When subcultured at the same initial densities, the three groups of cells grew similarly until day 5 (Fig. 4). Thereafter, the cells of restenotic origin demonstrated accelerated proliferation with significantly greater cell numbers compared to the primary atherosclerotic cells whose growth pattern closely resembled that of the umbilical artery cells. Derived population doubling times (hours, means, 95% CI in parentheses) were 52 (48-58) for restenotic cells, 71 (62-83) for primary atherosclerotic cells and 74 (65-84) for umbilical artery cells. The correlation coefficients for the regressions were 0.95 (restenotic cells), 0.87 (primary atherosclerotic cells) and 0.88 (umbilical artery cells).

Extracellular matrix synthesis

The results of the extracellular matrix assays are summarised in Table 2. Collagen synthesis, reflected by the incorporation of ^{3}H-proline, did not differ between restenotic and primary atherosclerotic cells in culture but was, however, significantly greater for cells of coronary than umbilical artery origin. Production of sulfated glycosaminoglycans, as assessed by the incorporation of ^{35}S-sulfate, was less for restenotic than for primary atherosclerotic smooth muscle cells, although not significantly so, and was more than twofold greater for coronary than for umbilical artery cells.

Table 2. Production of extracellular matrix collagen and sulfated glycosaminoglycans.

Cell source	Collagen		Glycosaminoglycans	
Restenotic	0.034 ± 0.004 (n=6)	*	11.47 ± 1.07 (n=6)	#
Primary **	0.033 ± 0.004 (n=5)	†	15.37 ± 3.10 (n=4)	§
Umbilical	0.019 ± 0.004 (n=3)	*†	5.43 ± 1.00 (n=4)	#§

Values are mean ± s.e.m., expressed as nmol isotope.µg total cell protein-1. **Primary = primary atherosclerotic. Each observation (n) represents the mean result of triplicalte assay. *†# $p<0.05$, § $p<0.005$

Discussion

It is clear that the proliferation and extracellular matrix synthesis of vascular smooth muscle cells are phenomena important in atherosclerosis, and highly relevant to restenosis. We describe the first attempt to investigate both of these processes in smooth muscle cells cultured from human coronary plaque tissue. In particular, we compared primary atherosclerotic lesions with restenotic lesions, and adult coronary cells with cells derived from the media of umbilical arteries.

The difference in proliferation we show for human coronary restenotic and primary atherosclerotic smooth muscle cells in secondary culture is in close agreement with that reported for cells from human peripheral arterial lesions in primary and early sub-culture.[15,17] Our data suggests that the proliferative behaviour of restenotic cells in vitro reflect a prior phenomenon of phenotypic modulation and selection in vivo, rather than some effect of the cell culture process, as the success rates of primary and secondary culture for cells derived from restenotic and primary atherosclerotic lesions were similar. This concept is supported by the previous demonstration of a metabolically active subpopulation of smooth muscle cells in arterial intima and media[28] and human peripheral arterial plaques, where the active subpopulation was more dominant in restenotic lesions and grew more rapidly in secondary culture.[15] Also, experimental studies have shown accelerated smooth muscle cell proliferation following balloon injury,[4,29] and in relation to early but not late atherosclerotic lesions in animals and man.[9,11,30,31] It might be proposed that increased growth of restenotic smooth muscle cells in secondary culture actually reflects senescence and attenuated growth of cells from chronic primary atherosclerotic lesions.[3] However, this seems unlikely, as the growth pattern of primary atherosclerotic cells in the present study was very similar to that of healthy umbilical artery medial cells which have undergone far fewer in vivo population doublings. Interestingly, the population doubling times we report for primary atherosclerotic and umbilical artery cells are identical to those previously described for smooth muscle cells cultivated from adult human atherosclerotic aortic plaques and control aortic media.[9]

For all three cell sources, the combination of culture morphology, electron microscopy and immunostaining for smooth muscle cell α-actin was consistent with vascular smooth muscle cell identity. Vascular smooth muscle cells placed in cell culture alter from contractile to synthetic phenotype within a few days and this change becomes irreversible beyond 5 cumulative population doublings.[32] The precise in vitro age of our cultured coronary cells is not known as a tissue explant culture method was used, but in these early passage cells, the ultrastructural features and the attenuated expression of smooth muscle cell α-actin,[33,34] as evidenced by immunostaining, indicated that the coronary cells in culture were of synthetic phenotype. The morphology and behaviour of the cells might therefore be attributed to the culture process. However, the differences in proliferation between restenotic and primary smooth muscle cells shown in this study and in others[15-17]

suggests that this is not the case; cells from comparable individuals would be expected to react similarly to cultivation. Further, a number of histopathologic studies have revealed that smooth muscle cells in primary atherosclerotic and restenotic lesions are frequently of synthetic phenotype.[3,11,35,36] Also, human coronary smooth muscle cells in atherectomized tissue were shown recently to express mRNA for non-muscle myosin heavy chain (MHC-B), associated with smooth muscle cell synthetic phenotype;[37] expression was greater in restenotic than primary atherosclerotic tissue. In experimental atherosclerosis too, the modulation of smooth muscle cell phenotype from contractile to synthetic has been reported.[38] We believe therefore that the nature of the coronary cells in vitro reflected their nature in vivo.

There is much current interest in the stimulation of smooth muscle cell proliferation and extracellular matrix synthesis in vivo by diverse growth factors, particularly in relation to restenosis. It has been suggested that endogenous mediators, platelet-derived growth factor (PDGF) for example, may influence the initial outgrowth of smooth muscle cells from cultured atherosclerotic plaque tissue.[17] In our study, the success of primary culture was enhanced in specimens containing organized thrombus. Despite the low prevalence of thrombus in the specimens (10/93, 9%), this finding supports the role of thrombus, recently reviewed by Schwartz et al.,[39] as the fertile matrix fostering the growth of smooth muscle cells, perhaps by being a rich source of growth factors and chemoattractants.

Coronary intimal smooth muscle cells from both restenotic and primary lesions synthesised more extracellular matrix protein than human umbilical artery media cells. Two explanations may be offered. Either the difference arose from the obvious discrepancy in in vivo age between the coronary and umbilical artery cells, or the increased extracellular matrix synthesis of the coronary cells was a manifestation of disease. The total arterial content of glycosaminoglycans, particularly sulfated glycosaminoglycans, certainly increases with age,[40] but this increase in total content is heavily influenced by the increase in vascular intimal and medial thickness which occurs with age. The relative tissue concentration of sulfated glycosaminoglycans, a better source of reference for the the measurement made in the present study, varies to a lesser extent. Relevant to our findings is the evidence that glycosaminoglycans are increased in atherosclerosis; in coronary lesions in man[40,41] and in lesions induced by diet or genetic predisposition in animals.[38,42] Arterial collagen appears to alter little with age[43] but is associated with more severe of chronic lesions[19,41] and has also been shown to be elevated in experimental atherosclerosis.[44] Thus, the increased matrix synthesis of the coronary cells is likely to be related to vascular disease as opposed to in vivo age.

Having observed a difference in the proliferative behaviour of restenotic smooth muscle cells compared to primary atherosclerotic cells, reasons for not detecting a difference in matrix synthesis merit consideration. Although collagen synthesis appears to be unaffected by cell proliferation rate,[4] glycosaminoglycan synthesis in

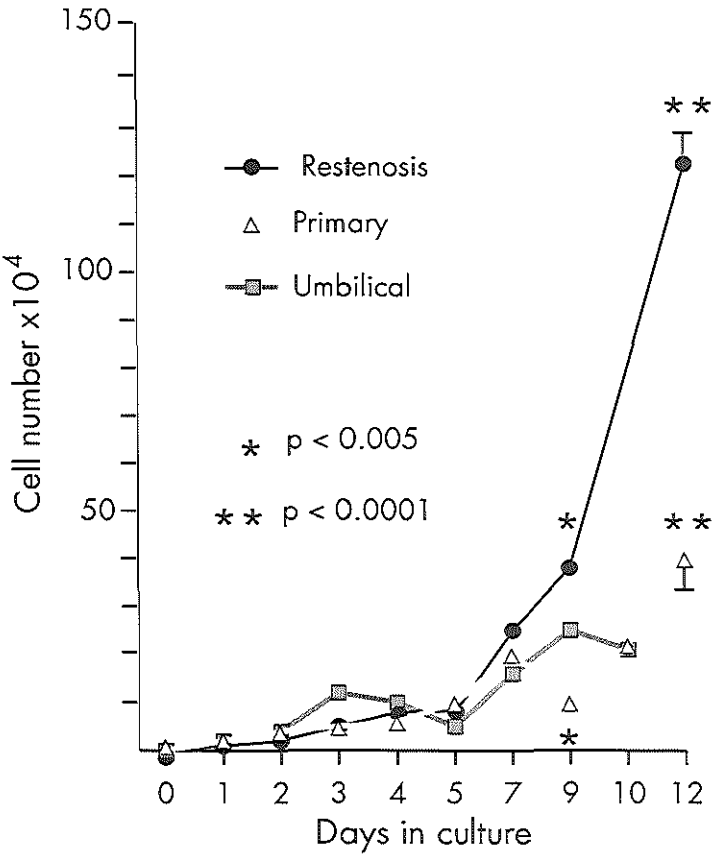

Figure 4

Growth curves in secondary culture of smooth muscle cells derived from restenotic, primary atherosclerotic and umbilical vein media. Each point represents the mean (±S.E.M.) of a minimum of 4 observations from at least two passages of different cell strains. Restenotic cell numbers significantly exceed primary atherosclerotic cell numbers at the times indicated

cell culture has been shown to fall during log phase growth[46] and with increasing cell density.[47] Accordingly, the more rapid proliferation of the restenotic cells may have led to a reduction in ^{35}S-sulfate uptake, obscuring any potential difference from the primary atherosclerotic cells. This is speculative, as we did not estimate the glycosaminoglycan synthesis of restenotic cells under other conditions, such as growth arrest.

There were limitations to the study. First, the small amount of tissue retrieved during directional coronary atherectomy, typically 15-20 mg[16,48] particularly when

of low cellularity, limits the success of secondary culture, and primary culture success rates fall to 60% of those with tissue from peripheral atherectomy.[16] A quarter of our procedures were for restenotic lesions which provided only 4 secondary cultures. Although our proliferation results are wholly consistent with other recent studies, they may only be extrapolated to the general population with caution. Second, in view of the proliferation finding, timing and cell density may have affected the estimation of sulfated glycosaminoglycan synthesis. New studies are under way to address this question. Third, with regard to the influence of vascular disease as opposed to in vivo cell age on extracellular matrix synthesis, it would be advantageous to study adult arterial smooth muscle cells cultivated from ostensibly healthy vessels, such as the internal mammary artery. This is also a focus of current experimental work.

In summary, the more rapid proliferation rate of restenotic lesion smooth muscle cells subcultured in vitro attests to a mechanism of likely importance in the vascular response to injury, and thus highly relevant to the clinical problem of post-angioplasty restenosis. The increased ability of adult coronary smooth muscle cells in secondary culture to synthesise collagen and sulfated glycosaminoglycans supports the putative role of the extracellular matrix in lesion formation and consolidation. Further research into this aspect of vascular smooth muscle cell (dys)function is required to explore the possibility of manipulating extracellular matrix synthesis by pharmacological means. Smooth muscle cells cultivated from primary atherosclerotic and restenotic coronary lesions following directional atherectomy constitute a useful experimental model for the identification of pathophysiological phenomena and thus, potentially, for the selection and testing of novel therapies.

References

1. *Ross R, Glomset JA: Atherosclerosis and the arterial smooth muscle cell. Science 1973;180:1332-1339*

2. *Ross R, Glomset JA: The pathogenesis of atherosclerosis. (First of two parts). New Eng. J. Med. 1976;295:369-377*

3. *Ross R, Wight TN, Strandness E, Thiele B: Human atherosclerosis. I. Cell constitution and characteristics of advanced lesions of the superficial femoral artery. Am J Pathol 1984;114:79-93*

4. *Grunwald J, Haudenschild CC: Intimal injury in vivo activates vascular smooth muscle cell migration and explant outgrowth in vitro. Arteriosclerosis 1984;4:183-188*

5. *Austin GE, Ratliff NB, Hollman J, Tabei S, Phillips DR: Intimal proliferation of smooth muscle cells as an explanation for recurrent coronary artery stenosis after percutaneous coronary angioplasty. J Am Cardio 1985;6:369-375*

6. *Ip JH, Fuster V, Badimon L, Badimon J, Taubman MB, Chesebro JH: Syndromes of accelerated atherosclerosis: role of vascular injury and smooth muscle cell proliferation. J Am Coll Cardiol 1990;15:1667-1687*

7. *Ferns GAA, Stewart-Lee AL, Anggard EE: Arterial response to mechanical injury: balloon catheter de-endothelialization. Atherosclerosis 1992;92:89-104*

8. *Casscells W: Migration of smooth muscle and endothelial cells. Critical events in restenosis. Circulation 1992;86:723-729*

9. *Eskin SG, Sybers HD, Lester JW, Navarro LT, Gotto AM, DeBakey ME: Human smooth muscle cells cultured from atherosclerotic plaques and uninvolved vessel wall. In Vitro 1981;17:713-718*

10. *Bjorkerud S, Ekroth R: The growth of human atherosclerotic and non-atherosclerotic aortic intima and media in vitro. Artery 1980;4:329-335*

11. *Orekhov AN, Kosykh VA, Repin VS, Smirnov VN: Cell proliferation in normal and atherosclerotic human aorta. II. Autoradiographic observation on deoxyribonucleic acid synthesis in primary cell culture. Lab Invest 1983;48:749-754*

12. *Simpson JB, Selmon MR, Robertson GC et al: Transluminal atherectomy for occlusive peripheral vascular disease. Am J Cardiol 1988;62:96G-101G*

13. *Hinohara T, Silmon MR, Robertson GC, Braden L, Simpson JS: Directional atherectomy. Now approaches for treatment of obstructive coronary and peripheral vascular disease. Circulation 1990;81 (Supp.IV):79-91*

14. *Bauriedel G, Dartsch PC, Voisard R et al.: Selective percutaneous "biopsy" of atheromatous plaque tissue for cell culture. Basic Res Cardio 1989;84:326-331*

15. *Dartsch PC, Voisard R, Bauriedel G, Hofling B, Betz E: Growth characteristics and cytoskeletal*

organization of cultured smooth muscle cells from human primary stenosing and restenosing lesions. Arteriosclerosis 1990;10:62-75

16. *Bauriedel G, Windstetter U, DeMaio SJ, Kandolf R, Hofling B: Migratory activity of human smooth muscle cells cultivated from coronary and peripheral primary and restenotic lesions removed by percutaneous atherectomy. Circulation 1992;85:554-564*

17. *Pickering GJ, Weir L, Rosenfield K et al.: Smooth muscle cell outgrowth from human atherosclerotic plaque: implications for the assessment of lesion biology. J Am Coll Cardiol 1992;20:1430-1439*

18. *Stary HC, Blankenhorn DH, Chandler AB et al.: A definition of the intima of human arteries and of its atherosclerosis-prone regions. A report from the committee on vascular lesions of the Council on Arteriosclerosis, American Heart Association. Arteriosclerosis and Thrombosis 1992;12:120-134*

19. *Nobuyoshi M, Kimura T, Ohishi H et al.:Restenosis after percutaneous transluminal coronary angioplasty: pathologic observations in 20 patients. J Am Coll Cardio 1991;17:433-439*

20. *Clowes AW, Reidy MA, Clowes MM: Mechanisms of restenosis after arterial injury. Lab Invest 1983;49:208-215*

21. *Madri JA, Kocher O, Merwin JR, Bell L, Tucker A, Basson CT: Interactions of vascular cells with transforming growth factors-ß. Ann Y Acad Sci 1990;593:243-258*

22. *Madri JA, Bell L, Marx M, Merwin JR, Basson C, Prinz C: Effects of soluble factors and extracellular matrix components on vascular cell behaviour in vitro and in vivo: models of de-endothelialization and repair. J Cell Biochem 1991;45:123-130*

23. *Serruys PW, Umans VAWM, Strauss BH, van Suylen RJ, de Feyter PJ: Percutaneous directional coronary atherectomy: Short-term clinical and angiographic results. Br Heart J 1991;66:122-129*

24. *Bosman FT, Cramer-Knijnenburg H, van Bergen-Henegouw J: Efficiency and sensitivity of indirect immunoperoxidase methods. Histochemistry 1983;77:185-194*

25. *Peterkofsky B, Diegelmann R: Use of a mixture of protein-free collagenases for the specific assay of radioactive collagen in the presence of other proteins. Biochemistry 1971;10:988-999*

26. *Redinbaugh MG, Turley RB: Adaptation of the bicinchoninic acid protein assay for use with microtiter plates and sucrose gradient fractions. Analytical Biochem 1986;153:267-271*

27. *Thyberg J, Hedin U, Sjolund M, Palmberg L, Bottger BA: Regulation of differentiated properties and proliferation of arterial smooth muscle cells. Arteriosclerosis 1990;10:966-989*

28. *Bjorkerud S: Separation of arterial smooth muscle cell subpopulations with different growth patterns. Acta Path Microbiol Immunol Scand 1984;92:293-301*

29. *Rhee CY, Herz F, Spaet TH: Accelerated culture of aortic smooth muscle cells. Thrombosis Res 1977;11:90-94*

30. *Pietila K, Nikkari T: Enhanced growth of smooth muscle cells from atherosclerotic rabbit aortas in*

culture. Atherosclerosis 1980;36:241-248

31. *Pietila K, Yla-Herttuala S, Rantala I, Nikkari T: Characterisations of cells cultured from advanced atherosclerotic lesions of the rabbit. Med Biol 1982;60:221-225*

32. *Campbell JH, Kocher O, Skalli O, Gabbiani G, Campbell GR: Cytodifferentiation and expression of smooth muscle actin mRNA and protein during primary culture of aortic smooth muscle cells. Correlation with cell density and proliferative state. Arteriosclerosis 1989;9:633-643*

33. *Kocher O, Skalli O, Bloom WS, Gabbiani G: Cytoskeleton of rat aortic smooth muscle cells. Normal conditions and experimental intimal thickening. Lab Invest 1984;50:645-652*

34. *Kocher O, Gabbiani G: Cytoskeletal features of normal and atheromatous human arterial smooth muscle cells. Hum Pathol 1986;17:875-880*

35. *Mosse PRL, Campbell GR, Wang ZL, Campbell JH: Smooth muscle phenotypic expression in human carotid arteries. I. Comparison of cells from diffuse intimal thickenings adjacent to atheromatous plaques with those of the media. Lab Invest 1985;53:556-562*

36. *Strauss BH, Umans VA, van Suylen RJ et al.:Directional atherectomy for treatment of restenosis within coronary stents: clinical, angiographic and histologic results. J Am Coll Cardio 1992;20 (December, in press)*

37. *Leclerc G, Isner JM, Kearney M et al.: Evidence implicating nonmuscle myosin in restenosis. Use of in situ hybridization analyze human vascular lesions obtained by directional atherectomy. Circulation 1992;85:543-553*

38. *Pietila K, Yla-Herttuala S, Jaakkola O, Nikkari T: Metabolism of glycosaminoglycans and lipids in smooth muscle cells from atherosclerotic rabbit aortas in culture. Atherosclerosis 1980;37:449-456*

39. *Schwartz RS, Homas DR, Topol EJ: The restenosis paradigm revisisted: an alternative proposal for cellular mechanisms. J Am Coll Cardiol 1992;20:1284-1293*

40. *Yla-Herttuala S, Sumuvuori H, Karkola K, Mottonen M, Nikkari T: Glycosaminoglycans in normal and atherosclerotic human coronary arteries. Lab Invest 1986;45:402-407*

41. *Tammi M, Seppala PO, Lehtonen A, Mottonen M: Connective tissue components in normal and atherosclerotic human coronary arteries. Atherosclerosis 1978;29:191-194*

42. *Wight TN: Differences in the synthesis and secretion of sulfated glycosaminoglycans by aorta explant monolayers cultured from atherosclerotic-susceptible and -resistant pigeons. Am J Pathol 1980;101:127-142*

43. *Hosoda Y, Kawano K, Yamasawa F, Ishii T, Shibata T, Inayama S: Age dependent changes of collagen and elastin content in human aorta and pulmonary artery. Angiology 1984;35:615-621*

44. *Pietila K, Nikkari T: Enhanced synthesis of collagen and total protein by smooth muscle cells from atherosclerotic rabbit aortas in culture. Atherosclerosis 1980;37:11-19*

45. *Ang AH, Tachas G, Campbell JH, Bateman JF, Campbell GR: Collagen synthesis by cultured rabbit aortic smooth-muscle cells. Alteration with phenotype. Biochem J 1990;265:461-469*

46. *Wight TN, Ross R: Proteoglycans in primate arteries. II. Synthesis and secretion of glycosaminoglycans by arterial smooth muscle cells in culture. J Cell Biol 1975;67:675-686*

47. *Schmidt A, Grunwald J, Buddecke E: [35S] Proteoglycan metabolism of arterial smooth muscle cells cultured from normotensive and hypertensive rats. Atherosclerosis 1982;45:229-310*

48. *Safian RD, Gelbfish JS, Erny RE, Schnitt SJ, Schmidt DA, Baim DS: Coronary atherectomy. Clinical, angiographic, and histologic findings and observations regarding potential mechanisms. Circulation 1990;82:69-79*

Chapter IV

Increased Thrombus Formation of Blood Platelets on the Extracellular Matrix of Smooth Muscle Cells From Atherosclerotic and Restenotic Coronary Artery Lesions

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Abstract

Background
Production of extracellular matrix by vascular smooth musce cells is an important aspect of the development of atherosclerotic lesions, since not only contributes to the overall increase in plaque bulk, but also may promote incorporation of lipids or facilitate thrombotic events. In this study, we focused on the reactivity towards blood platelets of the extracellular matrix produced by vascular smooth muscle cells derived from atheroma obtained in primary and restenotic coronary stenoses and coronary intima and media.

Methods
Coronary specimens were obtained during the performance of percutaneous directional atherectomy or at the time of cardiac transplantation from the heart of the receptor. After successful primary culture, the cells were subcultured on glass coverslips. Later, the extracellular matrix generated from these cells was exposed to flowing blood in an in vitro perfusion system, and the reactivity towards blood platelets quantified using specific immunostaining against glycoprotein 1B. The procoagulant activity of the extracellular matrices was also estimated from the rate of activated factor X generated after adding human coagulation factors VII and X.

Results
A similar platelet coverage was found in coverslips with matrices from intimal (14.55±1.2, n=16) and medial smooth muscle cells (16.57±0.6%, n=28). Platelet coverage was significantly increased in smooth muscle cell cultures from primary atheroma (22.8±1.3%, n=41), and was maximal in cultures from restenotic atheroma (41.9±1.7%, n=12). The high platelet coverage observed in restenotic matrices was due to increased platelet spreading. The procoagulant activity of the various matrices varied considerably, but an increase in tissue factor activity was not observed for matrices from atherosclerotic and restenotic smooth muscle cells.

Conclusions
The results of this study suggest that smooth muscle cells derived from atherosclerotic coronary lesions synthesize extracellular matrix that is more reactive towards blood platelets than that from normal smooth muscle cells.

Introduction

Atherosclerosis is a disease predominantly of the intimal vascular layer in which the vascular smooth muscle cell plays a key role.[1-3] In addition to the typical slow development of atherosclerotic plaques, which evolve over decades,[4,5] several forms of accelerated atherosclerotic disease have been described,[6] including restenosis after percutaneous vascular recanalisation, saphenous vein graft attrition and cardiac allograft vasculopathy. Although smooth muscle cells can be found in the normal vascular intima, it is generally accepted that those found in the atherosclerotic intima have a medial origin.[7] Prior to or during the process of migration, smooth muscle cells undergo a phenotypical shift from "contractile" to "synthetic" phenotype, the latter having an increased capacity for extracellular matrix production.[8,9] The production of large amounts of extracellular matrix by synthetic smooth muscle cells constitutes the main factor leading to a progressive increase of plaque bulk and gradual obstruction of the vascular lumen. Alternatively, thrombotic complications can take place when the extracellular matrix and other plaque components are exposed to the blood after spontaneous fissuring of the plaque,[10,11] or after mechanical disruption of the lesion by mechanical percutaneous recanalisation devices.[12] In earlier studies,[13,14] we observed that the connective tissue of the atherosclerotic plaque was more reactive towards blood platelets than that observed in the healthy vessel wall. At present it is not known whether extracellular matrices synthesized in vitro show similar differences.

Several groups[15-17] have reported on successful smooth muscle cell culture from coronary specimens obtained during directional atherectomy. This technique may be of use in studying the pathology of coronary disease, and may be preferable to the use of atheroma obtained from peripheral arteries (in which smooth muscle cells have a different embryological origin than those from the coronaries[18,19]) or animal models of atherosclerosis, which present a variety of limitations recently reviewed by Muller et al.[20] and Ferrel et al.[21]

The aim of the present study was to investigate whether the extracellular matrix synthetised by smooth muscle cells present in primary atherosclerotic and restenotic coronary lesions is more reactive towards platelets than that synthetised by smooth muscle cells present in the media. For this purpose, smooth muscle cells were cultured on glass coverslips, and the produced extracellular matrices were exposed to flowing blood in a rectangular perfusion chamber.[22,23] We also measured the procoagulant activity of these matrices by adding purified human coagulation factors VII and X and assessing the rate of activated factor X generated.

Materials and methods

Smooth muscle cells were obtained by using an explant culture technique. Fresh coronary arteries were obtained from the explanted hearts of patients undergoing

cardiac transplantation (Department of Cardiology, University Hospital Utrecht). The specimens were rinsed in cold sterile phosphate buffered saline (PBS) and transferred to tubes containing cold culture medium without serum supplement. Within 12 hours, the adventitia was removed, and the media was microscopically separated from the (atherosclerotic) intima. The distinct layers were cut into small pieces of about 1 mm^3, and directly placed on fibronectin-coated tissue culture plates (Costar, Cambridge, MA, USA). Fresh culture medium was supplemented with 20% conditioned medium to facilitate outgrowth of cells. After outgrowth this was immediately replaced by 100% fresh medium (Incubation at 37°C under 5% CO_2). Specimens for histological examination were formalin fixed and stained with Haematoxylin-Eosin.

Table 1. Patient and sample characteristics

Patient/ cell passage	Substrate	Age (Years)	Gender	Vessel	Lesion
U69/P3	*Dilated cardiomyopathy	52	Male	RCA	none
U81/P3	*Ischaemic heart disease	42	Male	LCirc	prim. ath.
U85/P3	*Ischaemic heart disease	52	Male	RCA	prim. ath.
U86/P3	*Ischaemic heart disease	38	Male	RCA	prim. ath.
R193/P4	†Unstable angina	71	Male	LAD	resteno-tic
R84/P4	†Unstable angina	69	Male	LAD	prim. ath.
R109/P3	†Unstable angina	44	Male	LAD	prim. ath.

*Tissue obtained from explanted hearts after transplantation; † tissue obtained during directional coronary atherectomy.

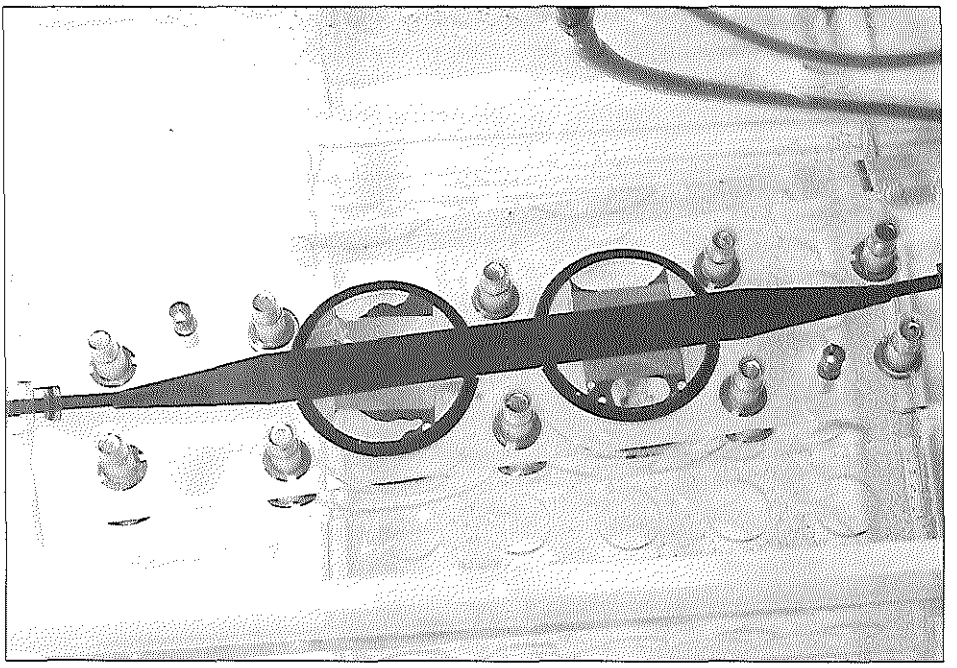

Figure 1

Rectangular perfusion chamber used in the study. Coverslips with extracellular matrix from cultured smooth muscle cells were exposed to non pulsatile heparinised blood flow at a shear rate of 1000 s^{-1}. See text for details.

Atherosclerotic plaque material was also obtained from patients who were undergoing percutaneous coronary atherectomy at theThoraxcenter (Erasmus University, Rotterdam) using the Simpson atherectomy device and following a conventional protocol described elsewhere.[24] The retrieved material was also cut in small pieces of about 1 mm^3 and was handled as described above.

After cell outgrowth, the cells were transferred towards culture bottles (Costar). At confluence, 50% of the cells were frozen and stored in liquid nitrogen (for other experiments), the remaining cells were subcultured on gelatin coated glass coverslips (Menzel, Braunschweig, Germany). Smooth muscle cells were identified by their typical growth pattern and by staining with a monoclonal antibody (mAb) against α-actin (Boehringer, Mannheim, Germany). The cultured smooth muscle cells failed to react with a mAb against desmin (Boehringer), which was in agreement with the studies of Thomas and Kim[25] and Dartsch et al.[26] After the smooth muscle cells had grown to confluence, matrices were isolated by exposing the cells to 0.1 M NH_4OH (5 min. at room temperature; Houdijk et al.[27]) This step was followed by tree washes with PBS (pH 7.35). Coverslips with matrices were stored in PBS at -20°C and were used within 2 weeks for perfusion experiments.

Culture medium
Cells were cultured in M199 Modified Earle's Salts (Gibco Europe Ltd., Paisley, UK) supplemented with 10% pooled human serum, 10% fetal calf serum (Gibco), Penicillin (100 U/ml), Streptomycin (100µg/ml) and Fungizone (2.5 µg/ml). Conditioned medium was prepared by mixing 20% medium from proliferating umbilical smooth muscle cells line and 80% fresh medium.

Perfusions
Fresh blood from normal donors who denied to have taken aspiring in the preceding ten days was anticoagulated with 1/10th volume of 50 U/ml unfractionated heparin (Organon, Oss, The Netherlands). Heparin did not cause increased platelet clumping under the flow conditions of our experiments. Platelet deposition under non pulsatile flow conditions was performed using a rectangular perfusion chamber as described.[22,23] Whole blood (15 ml) was prewarmed at 37°C for 5 min. Duplicate coverslips were inserted into the perfusion chamber and the blood was then circulated through the perfusion chamber for 5 min. The studies were performed at a wall shear rate of 1000 s^{-1}. The system was rinsed with 10 mM Hepes buffered saline (HBS) after each perfusion. Platelet counts were measured with a Platelet Analyzer 810 (Allentown, PA, USA) with apertures set between 3.1 and 16 µm. Platelet counts before perfusion were between 150.000 and 300.000/µl. Microaggregate formation during recirculation of the blood was less than 30%.[28]

Evaluation of platelet deposition
The coverslips from the perfusion chamber were removed and rinsed with HBS, fixed in acetone for 5 min., and rinsed in 0.05 M Tris buffered saline (TBS). Adhered platelets and platelet thrombi were visualized by staining with a biotinylated mAb against glycoprotein Ib^{13} and an avidin-biotin-horseradish peroxidase-complex (Vector Laboratories, Burlingame, CA, USA). Cobalt chloride (0.025%) and nickel ammonium sulfate (0.020%) were added to the chromogenic substrate 3,31-diaminobenzidine (Sigma, St Louis, MO, USA) to yield a black reaction product.[29] Platelet deposition was quantified with a light microscope coupled to a computerized image analyzer (AMS 40-10, Saffron Walden, UK). Platelet deposition was expressed as the percentage of the surface covered with platelets and platelet aggregates.

Collagen synthesis of smooth muscle cells derived from atherectomy tissue
The synthesis of collagen was assessed by the incorporation of ^{3}H-proline using a modification of the method described by Peterkofsky et al.[30] which has been previously used in our laboratory.[31] Subconfluent cells of the same passages as used in perfusion experiments were incubated for 48 hours at 37°C in culture medium containing 2 µM ascorbic acid and 10 $\mu Ci.ml^{-1}$ ^{3}H-proline (specific activity 231 $mCi.mg^{-1}$). After incubation in 2 cm^2 culture wells (in triplicate), the medium was removed and the cells were washed with PBS before precipitating with 20% trichloroacetic acid (TCA)/1 mM proline (24 hours, 4°C). After centrifugation, the pellet was dissolved in 0.2 N NaOH overnight. Thereafter, collagen was quanti-

fied by a bacterial collagenase digestion method (Clostridium histolyticum type III, Calbiochem, La Jolla, CA, USA) and expressed as nmol ^{3}H-proline.µg total cell protein^{-1}. For total cell protein, cells were detached from culture wells by gentle scraping, washed in PBS and precipitated in 20% TCA (24 hours, 4°C). After centrifugation, the pellet was dissolved in 0.2 N Na OH overnight and total protein estimated according to the Pierce BCA assay (Pierce Rochford, IL, USA) using bovine serum albumin as the standard.[32]

Tissue factor (TF) activity

Smooth muscle cells were subcultured on gelatin coated Tharmanox® coverslips (Miles, Naperville, IL, USA). After growing to confluence, matrices were isolated as described under: "Cell culture". The amount of TF present in the extracellular matrices of smooth muscle cells was estimated by the rate of activated factor X (F Xa) generated after addition of factor VII and factor X. Coverslips (10.5x20 mm) with extracellular matrices were cut into 2 pieces and translated towards 24 well culture plates (Costar). After washing with a Hepes/saline buffer (25 mM Hepes, 135 mM NaCl, 4 mM KOL, 3 mM CaCL, 0.3% bovine serum albumin, pH 7.4), matrices were preincubated with 400 µl of the same buffer to which 2 nM factor VII was added (10 min., 37°C). At t = 0 min. factor X was added (160 nM). Samples were collected in duplicate at 10 and 20 min. The formation of F Xa was inhibited by an excess of EDTA(20mM). Concentrations of F Xa were estimated using the chromogenic substrate S2222 (Chromogenix, Moindal, Sweden). Hydrolysis of the substrate was measured as the change of adsorbence at 405 nm using a V-max ELISA reader (Molecular Devices, Menlo Park, CA, USA). Standard curves were prepared using purified F Xa.[33]

Factor VII and factor X were purified from freshly frozen plasma essentially as described by Bajaj et al.[34] and by Miletich et al.[35] The factor VII preparation was free of activated (two chain) F VII as judged by immunoblotting of reduced F VII with polyclonal rabbit anti-F VII antibodies (kindly provided by Dr. Mertens, CLB, Amsterdam, The Netherlands). The factor X preparation was free of activated F X after removing traces of F Xa by passing the preparation over SBTI-Sepharose (Sigma).

Statistical analysis

Results were expressed as mean ± standard error of the mean (SEM). Unpaired two-tailed Student's T test was used for comparison between groups, with Bonferroni correction applied when required. A p-value < 0.05 was considered significant.

Results

Smooth muscle cells

Cells started to grow out from explants after 7-21 days independent of cell origin (intima, media, plaque). Initial cell outgrowth was seen in 25 (83%) of 30 attempted cultures (2/3 of the small tissue pieces were involved in cell outgrowth). Only 7 of them could be subcultured and were used for perfusion experiments:

Table 2. Platelet coverage of smooth muscle cell (SMC) matrices

Source of SMC matrices	Platelet coverage (%)
U69 intima	16.1 ± 1.7 (n=8)
U85 intima	13.0 ± 0.7 (n=8)
U69 media	17.5 ± 0.8 (n=14)
U85 media	16.2 ± 0.4 (n=6)
U86 media	15.1 ± 0.6 (n=8)
U81 plaque	22.7 ± 1.4 (n=11)
U86 plaque	23.5 ± 1.4 (n=8)
R84 plaque*	23.1 ± 1.1 (n=14)
R109 plaque	21.9 ± 1.3 (n=8)
R19 restenosis†	41.9 ± 1.7 (n=12)

Platelet coverage was defined as the percentage of the surface covered by platelets and platelet aggregates. Values are mean ± SEM, n = number of coverslips evaluated. The smc matrices were tested in 2 independent experiments (blood from different donors), except * and †, which were tested in 4 (*) and 3(†) independent experiments.

U69 (intima + media), U81 (plaque), U85 (intima + media), U86 (media + plaque) (Table 1). The R-numbers in Table 1 represent cells obtained from atherectomy specimens: 2 from primary atherosclerotic lesions (R84 + R109) and 1 from a restenotic lesion (R19). Time to reach confluence was independent of its origin.

Perfusions over smooth muscle cell matrices

The results of the perfusion experiments are summarised in Table 2. Matrices derived from primary atherosclerotic lesions show a mean increase in platelet coverage of 46% compared with matrices derived from healthy intima and media. The higher platelet coverage on matrices of primary atherosclerotic lesions was observed in 4 independent experiments.

Platelets deposited on matrices of healthy intima and media formed dense aggregates with little platelet spreading (Fig. 2A). Platelet aggregates on matrices of primary atherosclerotic lesions were larger (elongated), less compact and the platelets were more spread (Fig. 2B). A large increase in platelet coverage (169%) was

observed on matrices derived from restenotic lesions (Table 2). This increase was reflected in both an increase in the number of aggregates, and particularly an increase in platelet spreading (Fig. 2C).

Collagen synthesis of smooth muscle cells derived from atherectomy tissue

The results of the synthesis of collagen by smooth muscle cells obtained from primary and restenotic atheromatous lesions is shown in Table 3. Matrices synthetised by restenotic smooth muscle cells had a significantly higher content of collagen than those from primary lesions.

Tissue factor (TF) activity of smooth muscle cell matrices

The F Xa formation showed great variation between matrices derived from various patients (404-1051 $ng.ml^{-1}min^{-1}$, Table 4), but there was no systematic difference for smooth muscle cells from primary atherosclerotic or restenotic lesions.

Table 3. Collagen synthesis of smooth muscle cells derived from atherectomy tissue

Patient No.	Plaque origin	Collagen synthesis
R19	Restenotic	0.041 ± 0.004 (n=3)
R84	Primary ath.	0.023 ± 0.001 (n=3)*
R109	Primary ath.	0.025 ± 0.001 (n=6)†

Synthesis of collagen was assessed by the incorporation of ^{3}H-proline. The amounts are expressed as $nMol.\mu g\ protein^{-1}$. Values are mean ± SEM. n = number of experiments. (*) $p < 0.05$, (†) $p < 0.005$
Primary ath. = primary atherosclerotic plaque.

Discussion

Vascular smooth muscle cell proliferation and production of extracellular matrix are phenomena important to both typical and accelerated forms of atherosclerosis.[1,8,9,36] The advent of directional atherectomy as a therapeutic tool in percutaneous coronary revascularisation has facilitated the access to fresh samples of human atherosclerotic tissue from these syndromes.[37-39] Being biologically viable, atherectomy specimens can be subject of cell culture, facilitating the study of their biological processes taking place in the atheromatous plaque from where they were obtained.[15-17,26] Likewise, in patients undergoing cardiac transplantation it is possible to use coronary material from the recipients heart for biological studies.

Table 4. Tissue factor (TF) activity of smooth muscle cell (SMC) matrices.

Source of SMC matrices	f Xa formation (ng/ml)
U85 intima	709 ± 44 (n=7)
U69 media	1051 ± 36 (n=8)
U85 media	715 ± 29 (n=7)
U86 media	1345 ± 69 (n=8)
R84 plaque	404 ±30 (n=8)
R109 plaque	990 ± 77 (n=7)
R19 restenosis	733 ± 65 (n=8)

TF activity was estimated as activated factor X (F Xa) formation ($ng.ml^{-1}.min^{-1}$) after preincubation of SMC matrices with factor VII and factor X. Values are mean ± SEM, n = number of coverslips.

Studies using atherectomy specimens to investigate aspects of the thrombogenicity of coronary atheroma are still scarce. Lucore et al.[40] measured the procoagulant activity of atherectomy specimens obtained in 10 patients with acute coronary syndromes. The rate of elaboration of fibrinopeptide A of plasma incubated with the samples was compared with the angiographic morphology of the lesion and their histological buildup. Procoagulant activity was detected in samples containing only intima, and was particularly marked when organising thrombus was present. In the present study, we focused on the extracellular matrix produced by vascular smooth muscle cells and its reactivity towards blood platelets. Blood platelets play an important role in the progression of atherosclerotic lesions by thrombus formation on damaged atherosclerotic tissue and release of growth factors.[10,41] In spite of this, the increased thrombogenicity of the neointima in animal models,[42] or in the atherosclerotic plaque in human coronary arteries[43] is mainly thought to be the result of an increased tissue factor activity and fibrin formation. We demonstrated however that increased reactivity of the connective tissue towards blood platelets also contributes to the increased thrombogenicity of the atherosclerotic plaque.[13] In the current study we show that cultured smooth muscle cells from atherosclerotic and restenotic coronary lesions produced extracellular matrices which were more adhesive for blood platelets than the extracellular matrices of cultured smooth muscle cells from healthy intima and media.

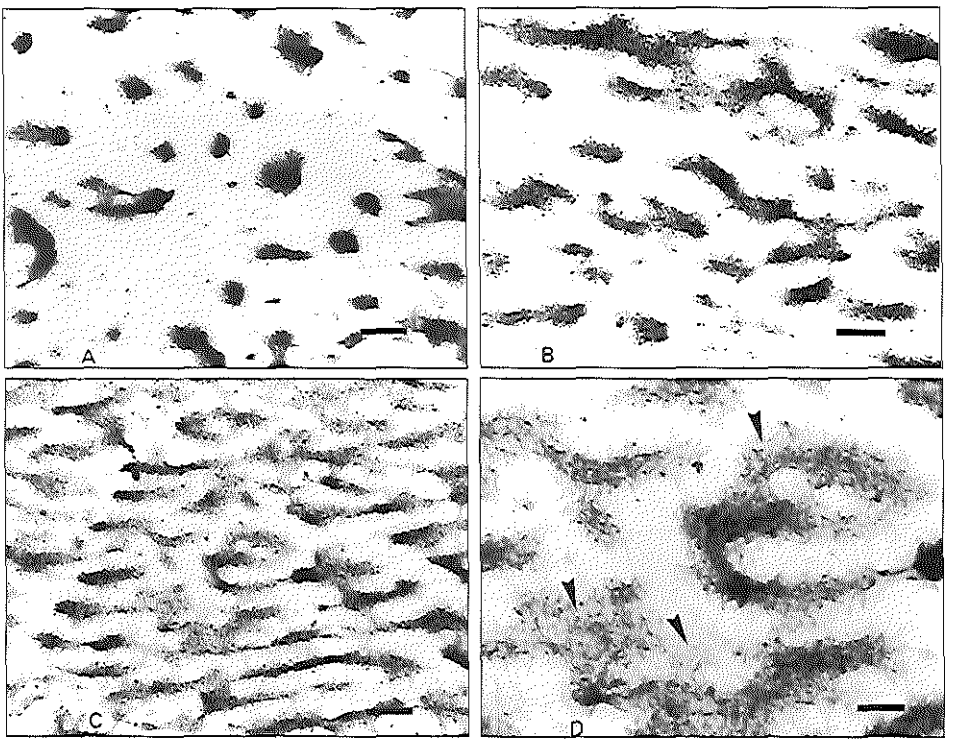

Figure 2

Platelet deposition on smooth muscle cell matrices from media (A), primary atherosclerotic lesion (B), and restenotic lesion (C), bar = 25μm. (D) Magnification of (C) showing platelet spreading (arrows), bar = 10μm.

Platelets on smooth muscle cells matrices of primary and restenotic lesions showed a more spread deposition than on matrices of normal smooth muscle cells. This observation is relevant for several physiopathological processes at the level of the arterial wall (Fig. 3). Platelet spreading facilitates better platelet vessel wall interaction; contact platelets and small thrombi with little platelet spreading are swept away more easily by the arterial blood flow. Furthermore, the amount of growth factors contained in platelets, such as platelet derived growth factor (PDGF), is strongly dependent on the surface covered by platelets, and not by the absolute number of platelets forming aggregates.[9] Platelets adhering the extracellular matrix of the subendothelium lose 97% of their α-granules in a very short period of time, while those forming aggregates do not undergo degranulation.[43]

Why do platelets adhere more to extracellular matrices derived from atheromatous plaques, and why adhesion varies with different forms of atherosclerosis? We measured the amount of collagen in extracellular matrices of cultured smooth muscle cells derived from primary atherosclerotic and restenotic lesions treated with directional atherectomy, and found that the synthesis of collagen was significantly hig-

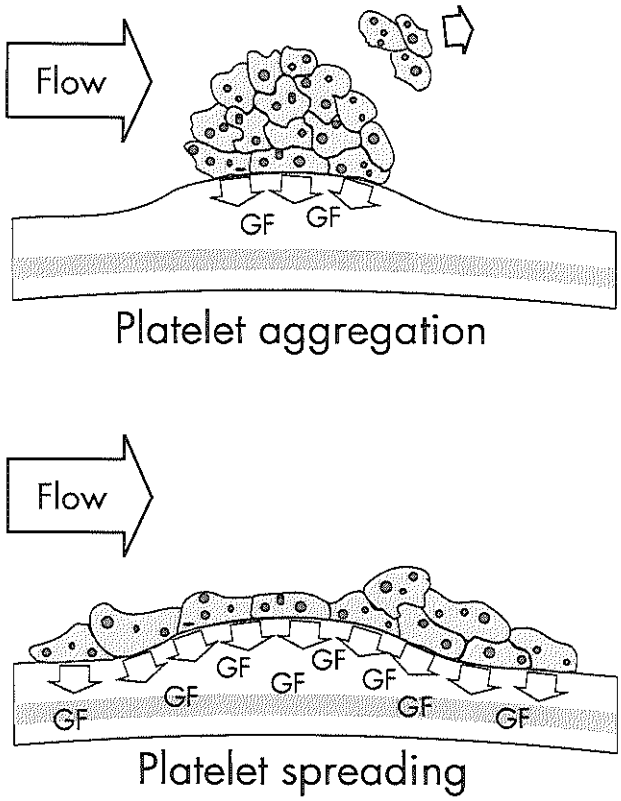

Figure 3
Implications of platelet spreading on vascular events. While contact platelets and small thrombi with little platelet spreading are swept away easily by the arterial blood flow (A), spreading facilitates better platelet vessel wall interaction (B). Furthermore, the absolute amount of growth factors (GF) contained in platelet granules delivered to the arterial wall (small arrows) is proportional to the area covered by platelets, and not by its absolute number, as in forming aggregates.

her in restenotic matrices. Although this might appear as a potential explanation for our observations, it must be remembered that an overall increase in collagen is not a prerequisite for enhancement of platelet adhesion, and in that regard probably the "quality" of matrix synthesis is a more determinant factor. Thus, increased platelet adhesion without a concomitant increase in collagen synthesis has been observed on extracellular matrices of fibroblasts cultured in the presence of ascorbic acid.[44] An increase in platelet spreading on atherosclerotic plaques was also described by Nichols et al.[45] in von Willebrand Disease pigs. At this moment it is not clear for us which proteins are involved in increased platelet deposition observed in restenotic matrices, a fact that justify that more attention should be paid to extracellular matrix production in relation with the process of vessel healing after percutaneous revascularisation.

There is evidence that the pattern of induction of tissue factor mRNA observed in vivo[46] is similar to that observed in vitro in cultured cells.[47] We did not find clear differences between smooth muscle cells matrices from healthy intima and media and matrices from atherosclerotic and restenotic lesions. Although high absolute levels of tissue factor activity were found in smooth muscle cells matrices derived from healthy intima and media, this was probably due to the presence of human

serum in the culture medium used. This observation corresponds with immunohistochemical studies on cross section of coronary arteries in which the macrophage derived foam cell was the most prominent cell type in the atherosclerotic plaque showing tissue factor antigen, which has been either undetectable or found at low levels in the media of coronary arteries.[48,49]

The present study has several limitations inherent to the use of smooth muscle cell culture as a model. First, isolation from the complex interaction that occur in vivo between smooth muscle cells and the surrounding extracellular matrix or other cell lines (eg., endothelial cells), may have caused modifications in cell phenotype.[50] Nevertheless, this should have affected equally to smooth muscle cells derived from normal media, primary and restenotic atheromatous lesions. Selection may have occurred during cell passage and subculture (which constitute the material used in our perfusion studies). Finally, limitations in the availablity of tissue from restenotic coronary lesions precluded the realisation of a similar number of experiments as those performed with the remaining sources of vascular material.

This study is the first in which platelet deposition on extracellular matrices of cultured smooth muscle cells from coronary lesions is investigated. In spite of the limitations discussed above, we believe that the results obtained justify more attention to extracellular matrix synthesis in connection with platelet adhesion.

References

1. *Fuster V, Badimon L, Badimon JJ, Chesebro JH. The pathogenesis of coronary artery disease and the acute coronary syndromes. N Engl J Med 1992;326:242-50 and 310-8.*

2. *Ross R: Atherosclerosis: A problem of the biology of arterial wall cells and their interaction with blood components. Arteriosclerosis 1981; 1:273-311.*

3. *Schwartz SM, Campbell GR and Campbell JH: Replication of smooth muscle cells in vascular disease. Circ Res 1986; 58:427-444.*

4. *Stary HC. The sequence of cell and matrix changes in atherosclerotic lesions of coronary arteries in the first forty years of life. Eur Heart J 1990; 11 (Supp E):3-19.*

5. *Ross R, Glomset J: The pathogenesis of atherosclerosis. N Engl J Med 1976; 295:369-377.*

6. *Ip JH, Fuster V, Baidmon L, Badimon J, Taubman MB and Chesebro JH: Syndromes of accelerated ahterosclerosis: role of vascular injury and smooth muscle cell proliferation. J Am Coll Cardiol 1990; 15:1667-1687.*

7. *Clowes AW, Clowes MM, Fingerie J and Reidy MA: Regulation of smooth muscle cell growth in injured artery. J Cardiovasc Pharmacol 1989; 14(Suppl.6), S12-S15.*

8. *Casscells W : Migration of smooth muscle and endothelial cells. Critical events in restenosis. Circulation 1992; 86:723—729.*

9. *Liu MW, Roubin GS, King SB. Restenosis after coronary angioplasty. Potential biologic determinants and role of intimal hyperplasia. Circulation 1989;79: 1374-87.*

10. *Davies MJ and Thomas AC: Plaque fissuring -the cause of acute myocardial infarction, sudden ischaemic death and crescendo angina (review). Br Heart J 1985; 53:363-373.*

11. *Falk E: Plaque rupture with severe pre-existing stenosis precipitating coronary thrombosis. Characteristics of coronary atherosclerotic plaques underlying fatal occlusive thrombi. Br Heart J 1983; 50:127-134.*

12. *Mizuno K, Kuita A and Imazeki N: Pathological findings after percutaneous transluminal coronary angioplasty. Br Heart J 1984; 52:588-590.*

13. *Van Zanten GH, de Graaf S, Slootweg FJ, Heynen HFG, de Groot PhG and Sixma JJ: Increased platelet deposition on atherosclerotic coronary arteries. 1992 (Submitted).*

14. *Van Zanten GH, de Graaf S, Slootweg PJ, Connolly TM, de Groot PhG and Sixma JJ: Proteins and receptors involved in incresed platelet depositions on atherosclerotic coronary arteries. 1993 (Submitted).*

15. *Bauriedel G, Windstetter U, DeMaio SJ, Kandolf R and Hofling B: Migratory activity of human smooth muscle cells cultivated from coronary and peripheral primary and restenotic lesions removed by percutaneous atherectomy. Circulation1992; 85:554-564.*

16. *Pickering GJ, Weir L, Rosenfield K, Stetz J, Jekanowski J, Isner JM. Smooth muscle cell outgrowth from human atherosclerotic plaque: Implications from the assessment of lesion biology. J Am Coll Cardiol 1992; 20:1430-9.*

17. *Escaned J, de Jong M,Violaris AG, MacLeod DC, van Suylen RJ, Umans VA, de Feyter PJ, Verdouw PD, Serruys PW. Clinical and histological determinants of smooth muscle cell outgrowth in cultured atherectomy specimens: Importance of thrombus organisation. Coronary Artery Disease 1993; 4:883-90.*

18. *Schwartz SM, Heimark RL and Majesky MW: Developmental mechanisms underlying pathology of arteries. Physiol Rev 1990; 70:1177-1209.*

19. *Robertson AL: Pathobiology of vascular cells in vitro in relation to human atherogenesis. Organ and species differences. Ann N Y Acad Sol 1990; 598:200-216.*

20. *Muller DWM, Ellis SG and Topol EJ: Experimental models of coronary artery restenosis. J Am Coll Cardiol 1992; 19:418-432.*

21. *Ferrel M, Fuster V, Gold HK and Chesebro JH: A dilema for the 90's: Choosing the appropppriate experimental animal model for the prevention of restenosis. Circulation 1992; 85:1630-1631.*

22. *Sakariassen DS, Aarts PAMM, de Groot PhG, Houdijk WPM and Sixma JJ: A perfusion chamber developed to investigate platelet interaction in flowing blood with human vessel wall cells, their extracellular matrix, and purified components. J Lab Clin Med 1983; 102:522-535.*

23. *Nieveistein PFEM, D'Alessio PA and Sixma JJ: Fibronectin in platelet adhesion to human collagen types I and III: use of nonfibrillar and fibrillar collagen in flowing blood studies. Arteriosclerosis 1988; 8:200-206.*

24. *Serruys PW, Umans VAWM, Strauss BH, van Suylen RJ and de Feyter PJ: Percutaneous directional coronary atherectomy: short-term clinical and angiographic results. Br Heart J 1991; 66:122-129.*

25. *Thomas WA and Kim DN: Biology of disease: atherosclerosis as a hyperplastic and/or neoplastic probe. Lab Invest 1983; 48:245-255.*

26. *Dartsch PC, Voisard R, Bauriedel G, Hofling B and Betz E: Growth characteristics and cytoskeletal organization of cultured smooth muscle cells from human primary stenosing and restenosing lesions. Arteriosclerosis 1990; 10:62-75.*

27. *Houdijk WPM, de Groot PhG, Nieveistein PFEM, Sakariassen KS and Sixma JJ: Subendothelial proteins and platelet adhesion: von Willebrand Factor and fibronectin, not thrombospondin, are involved in platelet adhesion to extracellular matrix of human vascular endothelial cells. Arteriosclerosis 1986; 6:24-33.*

28. *Verhoeven AJM, Mommersteeg ME and Akkerman JWN: Metabolic energy is required in human platelets at any stage during optical aggregation and secretion. Biochim. Biophys Acta 1984; 800:242-250.*

29. *Adams JC. Heavy metal intensification of DAB-based HRP reaction product. J Histochem Cytochem 1981; 29: 775.*

30. *Peterkofsky B, Diegelmann R: Use of a mixture of protein-free collagenases for the specific assay of radioactive collagen in the presence of other proteins. Biochemistry 1971;10:988-999.*

31. *MacLeod DC, Strauss BH, de Jong M, Escaned J, Umans VA, van Suylen RJ, Verkerk A, de Feyter PJ and Serruys PW: Proliferation and extracellular matrix synthesis of smooth muscle cells cultured from coronary atherosclerotic and restenotic lesions. J Am Coll Cardiol 1994 (In Press).*

32. *Redinbaugh MG and Turley RBP: Adaptation of the bicinchoninic acid protein assay for use with microtiter plates and sucrose gradient fractions. Analytical Biochem 1986; 153:267-271.*

33. *Meijers JCM, Tijburg PNM and Bouma BN: Inhibition of human blood coagulation factor Xa by alpha-2-macroglobulin. Biochemistry 1987; 26:5932-5937.*

34. *Bajaj SP, Rapaport SI and Brown SF: Isolation and characterization of human factor VII. J Bio Chem, 1981 ; 256:253-259.*

35. *Miletich JP, Jackson CM and Majerus PW: Properties of the factor Xa binding site on human platelets. J Biol Chem 1978; 253:6908-6916.*

36. *Ross R, Wight TN, Strandness E and Thiele B: Human atherosclerosis. I. Cell constitution and characteristics of advanced lesions of the superficial femoral artery. Am J Pathol 1984; 114:79-93.*

37. *Garrat KN, Edwards WD, Kaufmann UP, Vlietstra RE, Holmes DR Jr. Differencial histopathology of primary atherosclerotic and restenotic lesions in coronary arteries and saphenous vein bypass grafts: analysis of tissue obtained from 73 patients by directional atherectomy. J Am Coll Cardiol 1991: 17: 442-8.*

38. *Escaned J, van Suylen RJ, MacLeod DC, Umans VA, de Jong M, Bosman FT, de Feyter PJ, Serruys PW. Histological characteristics of tissue excised during directional coronary atherectomy in patients with stable and unstable angina pectoris.Am J Cardiol 1993; 71:1442-47.*

39. *Höfling B, Welsch U, Heimerl J, Gonschior P, Bauriedel G: Analysis of atherectomy specimens. Am J Cardiol 1993: 72:96E-107E.*

40. *Lucore CL, Winters KJ, Eisenberg PR: Procoagulant activity of athermoatous plaques. Circulation 1992; 86(suppl I): I-20.*

41. *Ross R, Masuda J and Raines EW: Cellular interaction, growth factors, and smooth muscle cell proliferation in atherogenesis. Ann N Y Acad Sci 1990; 598:102-112.*

42. *Groves HM, Kinlough-Rathbone RL, Richardson M, Jorgensen L, Moore S and Mustard JF: Thrombin generation and fibrin formation following injury to rabbit neointima. Studies of vessel wall reactivity and platelet survival. Lab Invest 1982; 46:605-612.*

43. *Baungartner HR, Mugli R" Adhesion and aggregation: Morphological demonstration and quantitation in vivo and in vitro. In Gordon JL (editor):Platelets in biology and pathoology, Amsterdam, Elsevier, 1976, p 23-60.*

44. *Hindriks GA, Sixma JJ and de Groot PhG: Ascorbic acid increases the thrombogenicity of cellular matrices. Thromb Haemost 1991; 66:505-509.*

45. *Nichols TC, Bellinger DA, Reddick RL, Read MS, Koch GG, Brinkhous KM and Griggs TR: Role of von Willebrand Factor in arterial thrombosis: studies in normal and von Willebrand Disease pigs. Circulation 1991; 83(Suppl.IV):56-64.*

46. *Marmur JD, Rossikhina M, Guha A, Fyfe B, Friedrich V, Mendlowitz M, Nemerson Y and Taubman MB: Tissue factor is rapidly induced in arterial smooth muscle after balloon injury. J Clin Invest 1993; 91:2253-2259.*

47. *Taubman MB, Marmur JD, Rosenfield CL, Guha A, Nichtberger S and Nemerson Y: Agonist-mediated tissue factor expression in cultured smooth muscle cells: role of Ca2+ mobilization and protein kinase C activation. J Clin Invest 1993; 91:547-552.*

48. *Wilcox JN, Smith KM, Schwartz SM and Gordon D: Localization of tissue factor in the normal vessel wall and in the atherosclerotic plaque. Proc Natl Acad Sci, USA 1989; 86:2839-2843.*

49. *Drake TA, Morrissey JH and Edgington TS: Selective cellular expression of tissue factor in human tissues. Am J Pathol 1989; 134:1087-1097.*

50. *Stadler E, Campbell JH and Campbell GR: Do Cultured smooth muscle cells resemble those of the arterial wall? If not, why not?. J Cardiovasc Pharmacol 1989; 14(Suppl.6):S1-S8.*

Chapter V

A Biological Paradox of Restenosis: Enhanced Smooth Muscle Cell Outgrowth from Cultured Atherectomy Specimens is Associated with Less Angiographic Luminal Loss During Follow-up

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Abstract

Background
Tissue fragments obtained during directional coronary atherectomy may be used as biopsy material from the target atheromatous lesion, providing a direct insight on the pathological substrate and the characteristics of the smooth muscle cells (SMC) present in the plaque. By combining this information with clinical or angiographic data obtained during patient follow-up, it may be possible to study prospectively the relationship between the anatomopathological substrate of the lesion and the likelihood of developing restenosis.

Aim of the study
To determine whether the degree of luminal renarrowing (loss of luminal cross-sectional area) after successful atherectomy is related to 1/ the ability of SMC present in the atherectomy specimen to grow in a cell culture model, as well as to 2/ the presence of thrombus and 3/ the maximal cell density (MCD) documented during histological examination of the specimen.

Methods
A total of 86 directional atherectomy procedures were included in the study. Quantitative angiographic analysis was performed before, after atherectomy and at follow-up in all cases. Cell culture was performed using an explant technique, choosing successful primary SMC outgrowth as a surrogate for the migratory and proliferative potential of SMC present in the specimen. In 26 cases plaque MCD was measured in a separate fragment of the cultured specimen using quantitative histology.

Results
Successful SMC outgrowth was obtained in 35/86 cultures (41%). Significantly less loss in luminal area occurred in vessels giving samples with positive SMC outgrowth (1.69±1.74 mm^2 versus 2.62±2.08 mm^2 respectively, p=0.03). A strong trend towards less luminal renarrowing was observed in 8 patients (9%) whose atherectomy specimens contained thrombus (1.75±1.10 mm^2) than in those who did not (2.46±2.23 mm^2). Mean MCD was 440±303 cells/mm^2. A significant inverse relationship was found between MCD and luminal renarrowing (Luminal renarrowing =-2.20 Log MCD + 7.97, r=0.42, $p < 0.05$).

Conclusions
We found an inverse relationship between 2 indices of biological activity of SMC in atherectomy explants and the degree of subsequent restenosis. This may indicate that the removal of tissue with enhanced proliferative potential may be associated with less propensity to restenosis.

Introduction

Since its relatively recent introduction as a therapeutic device, directional coronary atherectomy has also attracted investigators as a potential tool for obtaining information on the constitution of atheromatous plaque treated with the device.[1-6] In particular, most investigators have focussed their research on the vascular smooth muscle cell, which constitutes the kernel in the development of neointimal hyperplasia and restenosis after percutaneous intervention.[6,7]

Several groups,[8-12] including ours,[13,14] have reported on the feasibility of culturing smooth muscle cells present in atherectomy specimens using a miniaturized culture system. The use of this model has facilitated observations on the migratory[9] and proliferative[10,14] potential of smooth muscle cells from primary and restenotic lesions, and on the factors influencing this phenomenon.[13] However, it remains unknown whether information obtained from smooth muscle cell culture can be used to gain insights on the clinical problem of restenosis.

In the present study we investigate the relationship between an index of biological activity of the atheromatous plaque, namely the ability of smooth muscle cells in the retrieved atherectomy specimen to proliferate in culture conditions, and the degree of angiographic renarrowing (measured as luminal loss) observed at the site of atherectomy in the long term. In a subset of patients the cellularity of the specimen was estimated and compared with the same purpose with the degree of angiographic renarrowing.

Methods

Study population

The population in this study consisted of 86 patients who underwent directional coronary atherectomy and fulfilled the following criteria: a/ coronary angiography suitable for quantitative angiographic analysis pre-, post atherectomy and at follow-up, and (b) the procedure yielded atheromatous tissue which could be used for cell culture studies. Atherectomy was performed using the Simpson Atherocath and a conventional technique.[1] The coronary atherectomy protocol was approved by the Thoraxcenter Institutional Review Board, and informed consent was obtained in all patients prior to intervention. In all patients a number of clinical details were recorded, including sex, age, coronary syndrome (stable or unstable angina), and previous coronary intervention at the site of atherectomy. Unstable angina was defined as continuous or intermittent chest pain at rest requiring hospitalisation, associated with electrocardiographic evidence of myocardial ischaemia, but without associated increase in the serum cardiac enzymes.

Quantitative angiographic analysis. The cineangiograms obtained before-, after atherectomy and at follow-up were analysed using the Cardiovascular

Angiography Analysis System (Pie Medical, The Netherlands).[15] All measurements were performed in end-diastolic frames with optimal vessel opacification. Prior to quantitative analysis all contour positions of the contrast-free catheter tip and opacified arterial segment were corrected for pincushion distortion induced by the individual intensifiers.

Edge detection analysis was performed after selection and digitalization of a region of interest in a 512x512 pixel matrix using a high-fidelity charge couple device (CCD) videocamera. Luminal edges were then detected using a weighted sum of the first and second derivative function of the brightness profile of each vessel scanline. A diameter function was determined by computing the shortest distance between the left and right contour positions. Conversion of these measurements to absolute values was achieved by using the filmed contrast-free catheter tip as a scaling device. The diameter values are then plotted against the length of the analysed to obtain a so-called diameter function. Application of specific algorithms to the diameter function makes possible the calculation of a number of angiographic parameters, including the minimal luminal diameter. To facilitate a comparison with densitometric luminal area measurements, diameter values were transformed to area by assuming a circular morphology.

The basic principle upon which videodensitometry is based is the existing relationship between the attenuating power of the lumen filled with contrast medium, which is a function of luminal area, and the X-ray image intensity.[16] From this information a densitometric profile, which is proportional to the cross-sectional area of the lumen, is obtained. To improve the relationship between density and luminal area, subtraction of patient structure noise is applied after computing the linear regression through the background pixels located left and right of the detected luminal contours. In a similar fashion as described for edge detection, a cross-sectional area function on the analysed segment was obtained by plotting consecutive densitometric profiles in all scan-lines perpendicular to the vessel. An interpolated reference area was then calculated in a similar way to that described in the edge detection algorithm. Likewise, minimal cross-sectional area was calculated and expressed in mm^2. Conversion of individual videodensitometric profiles to absolute values was performed after a transformation of the videodensitometric profile found at the reference diameter with the corresponding geometrical area (calculated from the reference diameter and assuming a circular cross-section at that point).

Estimation of restenosis

In an attempt to calculate the area of neointimal hyperplasia developing during follow-up after atherectomy, an angiographically-derived "restenotic area" was defined as the difference between the luminal cross-sectional areas documented immediately after atherectomy and at follow-up (Fig. 1). Restenotic areas were calculated independently from both edge detection and densitometric data.

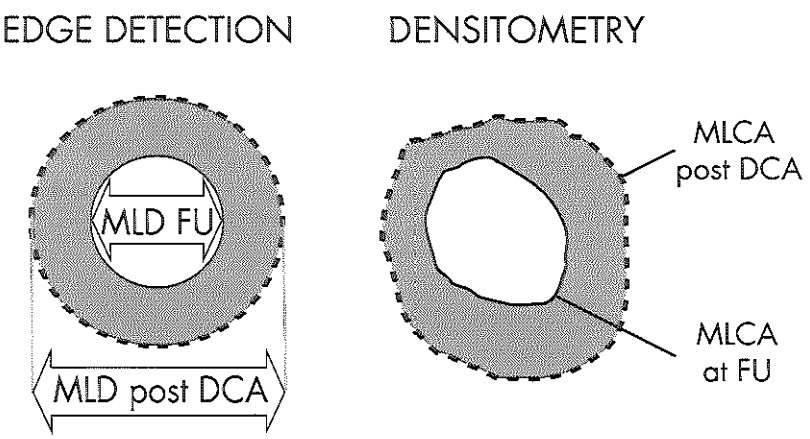


Figure 1

Calculation of restenotic area from edge detection and densitometric quantitative angiographic analysis. Dotted lines correspond to luminal boundaries immediately after atherectomy (post DCA), while solid edges correspond to those at follow-up (FU). Restenotic area was defined as the thorus of tissue comprised between both measurements (shadowed area). Areas derived from edge detection analysis were derived from luminal diameters assuming a circular luminal cross-section. Such assumption was not required with densitometry. MLD: minimal luminal diameter; MLCA: minimal luminal cross-sectional area.

Cell culture

Atherectomy specimens were cultured by a cell biologist blinded to clinical data. The technique is shown schematically in Figure 2 and has been described in detail previously.[13] After being examined macroscopically, the atherectomy specimen was divided in two representative pieces. One of these pieces was dedicated to cell cultures using an explant technique. Tissue explants were placed on human fibronectin coated (10 μg/cm^2) glass cover slips in 2 cm^2 wells (Four well plates, Nunc) and cultured in 300μl culture medium (M199 with $NaHCO_3$ (GIBCO Laboratories) supplemented with glutamine, 10% human serum, 10% fetal calf serum, penicillin 100 IU/ml, streptomycin 0.1 mg/ml and mixed in a ratio of 1:1 with conditioned medium from established smooth muscle cell lines actively growing in our laboratory). Cultures were maintained in a CO_2 incubator at 37°C in a humidified atmosphere equilibrated with 5% (v/v) CO_2 in air. The culture medium was changed every 3-4 days. Smooth muscle cell outgrowth was identified using inverted light microscopy and morphological criteria. These included a characteristic growth pattern of multiple layers of spindle or stellar shaped cells showing stress fibers and lamellipodia (Fig. 3). These morphological criteria were reinforced by positive immunostaining against smooth muscle cell α-actin (DAKO, Denmark) with human skin fibroblasts as negative controls.

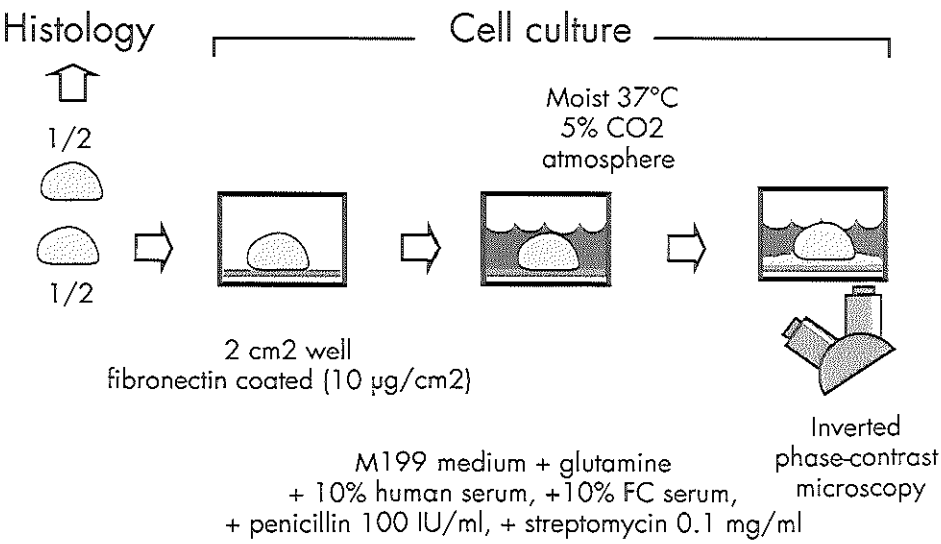


Figure 2:
Schematic representation of the techique of cell culture used in the present study (see text for details).

Since primary outgrowth represents the ability of the cells to colonize the culture environment, this was chosen as a surrogate for the proliferative and/or migratory potential of the smooth muscle cells present in the specimen. We have previously used this index since it minimizes the modifications of cell phenotype associated with enzymatic dispersion, prolonged culture, cell division and successive cell passages,[17-19] and does not abolish the influence of other components of the atheromatous plaque present in the explant that may contribute to the functional status of smooth muscle cells.[13]

Histology
The fragments of the atherectomy specimens dedicated to pathological examination were processed for light microscopy using hematoxylin-azophloxin and Verhoeff-van Giesson stains. Immunohistochemical staining for smooth muscle cell α-actin was performed in selected cases. Thrombus was identified as amorphous material, often in close apposition with atheromatous material, frequently showing collections of leucocytes between layers of fibrin. Discrimination between fibrin and dense collagen was performed using Verhoeff-van Giesson staining.

Cell counting was performed in hematoxylin-azofloxin stained sections using a computer assisted system coupled to the microscope (IBAS 2000, Kontron, Oberkochen, Germany). After estimating the number of cell nuclei present in several microscope fields, the maximal value recorded was used as an index of maximum cell density, and expressed in cells/mm^2.

Statistical analysis
Mean values and standard deviations were calculated for all continuous variables. Linear regression analysis was performed using a least squares method. Comparison of mean values was performed using two-tailed unpaired t-test. Discrete variables were compared using chi-square test, and continuity correction applied when indicated. A p value of <0.05 was considered significant.

Results

The mean age of the 86 patients included in the study was 57±10 years. There were 69 (80%) males and 17(20%) females. Fifty patients (58%) presented with stable and 36 (42%) with unstable angina pectoris. Most patients (67, 78%) underwent atherectomy in a de novo or primary atherosclerotic lesion. In 19 patients (22%) atherectomy was performed in a restenotic lesion which developed after a previous revascularisation procedure, performed a mean of 159±119 days prior to the date of atherectomy. An average of 6±3 cuts in multiple directions were made across each lesion.

Analysis of atherectomy specimens
Microscopic examination of the atherectomy fragments dedicated to histology revealed the presence of thrombus in 8 (9%) cases, more frequently in unstable than stable patients (6/36, 17% versus 2/50, 4% in stable and unstable patients respectively, $p<0.05$). Evidence of media or adventitia, indicating that resection of deep layers of the vessel had occurred, was found in 23 (27%) cases. Quantitative histological analysis revealed a mean maximal cell density in the specimens of 440±303 cells/mm^2.

Results of smooth muscle cell culture
Successful smooth muscle cell outgrowth was obtained in 35/86 cultures (41%). Smooth muscle cell outgrowth appeared as multiple layers of cells with spindle or polygonal shape and multiple stress fibers extending to lamellipodia. In the same cultures macrophages were clearly distinguished from smooth muscle cells on the grounds of a characteristic oval morphology, with eccentrically placed small, indented nuclei. Immunoperoxidase staining with macrophage specific HAM 56 was used to confirm that these cells were macrophages.

Results of quantitative angiography
Quantitative angiography revealed a mean reference diameter in the treated vessels of 3.17±0.66 mm. Minimal luminal diameters before, after atherectomy and at follow-up were 1.26±0.50, 2.41±0.48 and 1.70±0.58 mm respectively, from which luminal cross-sectional areas of 1.47±1.46, 4.81±1.83 and 2.56±1.55 mm^2 (before, after atherectomy and at follow-up respectively) were calculated by assuming a circular luminal morphology. Densitometric analysis revealed a reference area of 8.16±3.27 mm^2, and a minimal luminal cross-sectional area of 1.52±1.40,

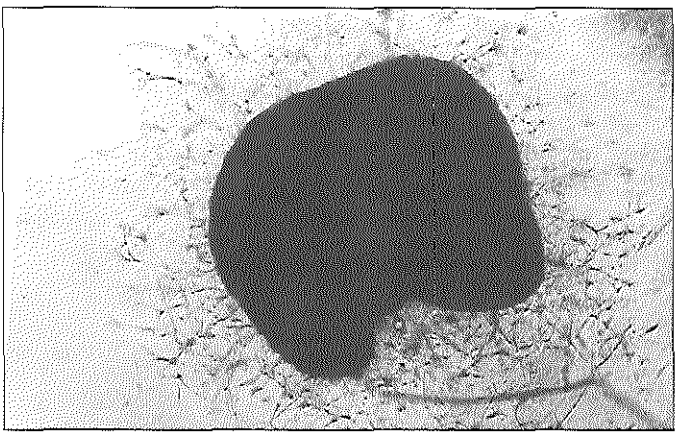
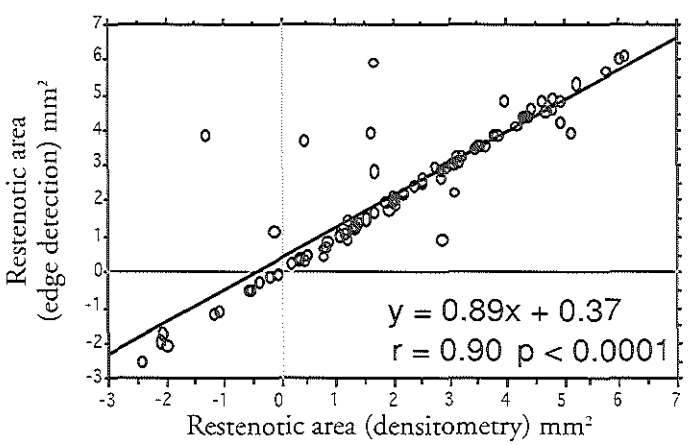


Figure 3
Fragment of retrieved tissue after 11 days in culture examined with phase-contrast microscopy. A dense population of cells migrating out of the atherectomy explant are evident. These cells fulfill the morphological criteria of myofibroblasts discussed in detail in the text. (Original magnification: x60, X120).

4.71±1.80 and 2.83±2.24 mm² before, after atherectomy and at follow-up respectively. After atherectomy, the discrepancy between edge detection and densitometric minimal luminal cross-sectional area values was higher in lesions where deep vessel resection had occurred, as judged from the presence of media±adventitia in the retrieved specimen (discrepancy of 0.27±0.97 mm² versus -0.008±0.37 mm² in cases with and without deep vessel resection respectively, p=0.06)

From the obtained angiographic data, restenotic areas of 2.23±1.99 mm² (edge detection) and 1.88±2.53 mm² (densitometry) were derived. Figure 3 shows the linear regression analysis between edge detection and densitometric restenotic areas. Correlation coefficient was 0.89. When compared with videodensitometry, edge detection tend to overestimate restenotic area (mean difference between edge detection an videodensitometry 0.14±0.91 mm², intercept +0.37). The discrepancy between edge detection and densitometry restenotic area values was more marked in lesions with evidence of deep vessel resection in the retrieved specimen (discrepancy of 1.15±2.93 versus 0.09±0.87 mm² in cases with and without evidence of media or adventitia in the retrieved specimen respectively, p=0.01).

Figure 4:
Correlation between restenotic areas derived from edge detection and densitometric quantitative angiographic analysis.



Comparison between histological, cell culture and angiographic data
A significantly smaller angiographic restenotic area was observed to have developed in cases with successful smooth muscle cell outgrowth during culture (Fig. 5). Restenotic area derived from edge detection analysis was 1.69±1.74 mm^2 and 2.62+2.08 in cases with and without documented smooth muscle cell outgrowth respectively (p=0.03). This difference was also noted in restenotic areas obtained from densitometric analysis: 1.22±2.99 mm^2 and 2.34+2.10 mm^2 in cases with and without documented smooth muscle cell outgrowth respectively (p=0.04).

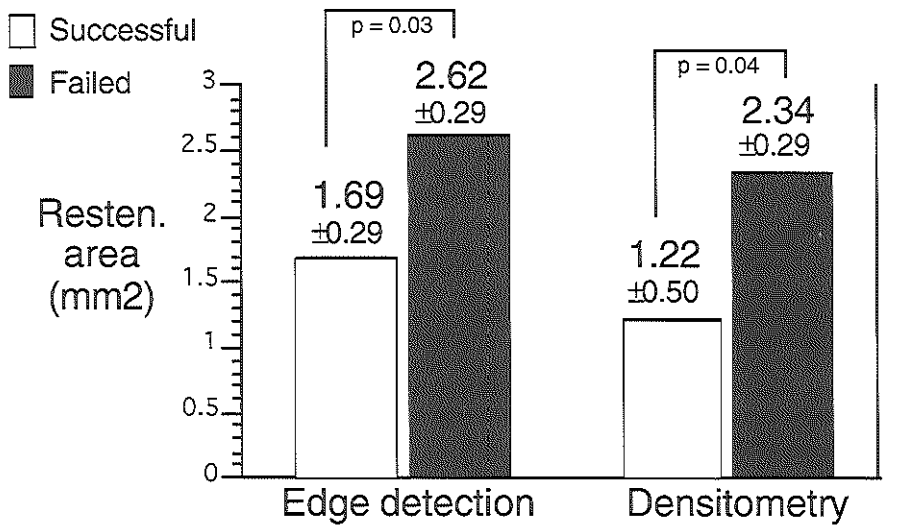


Figure 5
Comparison between restenotic areas in vessels with and without smooth muscle cell outgrowth from the retrieved specimens.

The presence of thrombus was associated with an increased outgrowth rate (6/8, 75% versus 29/78, 37%, in specimens with and without documented thrombus). There was a strong trend towards less angiographic restenotic area at follow up in patients whose atherectomy specimens contained thrombus (n=8, restenotic area=1.75±1.10 mm^2) than in those without documented thrombus (n=78, restenotic area=2.46±2.23 mm^2).

A significant inverse relationship was observed between angiographic restenotic area and the degree of cellularity documented in the retrieved atherectomy specimens. Figure 6 shows the regression analysis of cellularity and restenotic area estimated with edge detection and densitometric quantitative angiography.

No significant difference was found in restenotic area documented in vessels with and without evidence of deep resection in the atherectomy specimen. Vessels where resec-

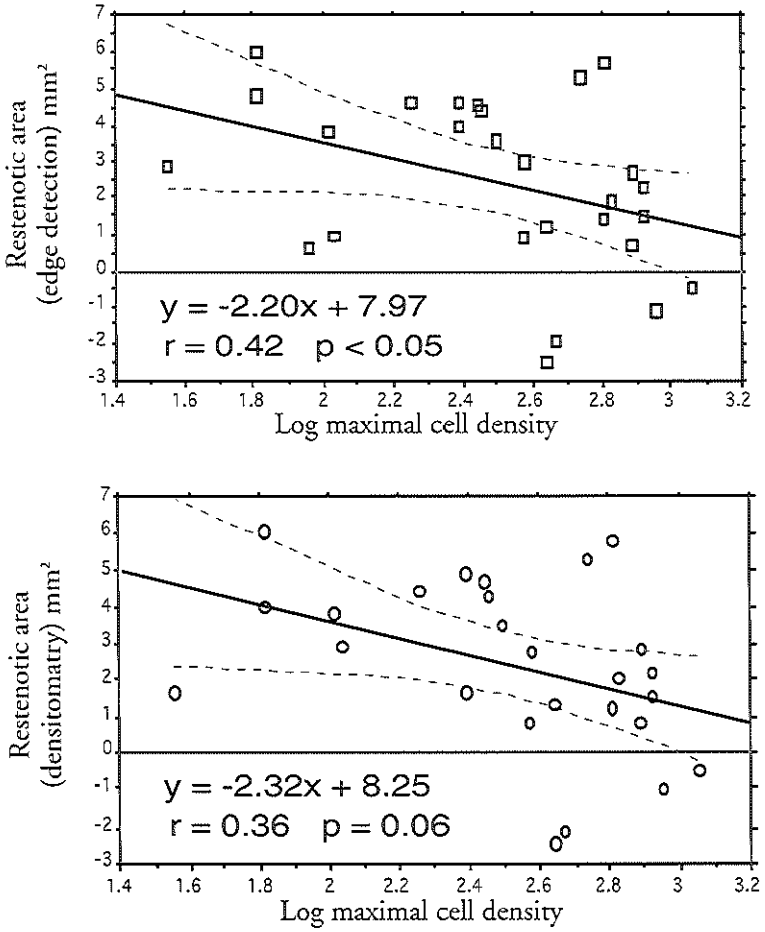


Figure 6
Linear regression analysis between the maximal cell density documented in the atherectomy specimen and the subsequent degree of restenotic area, as estimated from edge detection (squares) and densitometric (circles) quantitative angiography measurements.

tion of media ± adventitia in the retrieved specimen had mean restenotic areas of 2.43±2.09 (edge detection) and 1.88±2.56 (densitometry) mm², while in those without evidence of deep vessel resection restenotic area were 12.17±2.02 (edge detection) and 2.08±2.07 (densitometry) mm².

Comparison between clinical, cell culture and angiographic data

No significant differences in the rate of smooth muscle cell outgrowth was noted between patients with stable or unstable angina (18/50, 36%, versus 17/36, 47% in stable and unstable patients respectively, p=NS), nor between patients with restenotic or de novo lesions (9/19, 47%, versus 26/67, 38% in restenotic and de novo lesions respectively, p=NS). Likewise, quantitative angiographic analysis using edge detection revealed no significant differences in restenotic area was noted in relation with the coronary syndrome (2.36±1.91 mm² vs 2.08±2.13 mm²

in stable and unstable patients respectively, p=NS), nor between restenotic and de novo lesions (2.10±2,28 mm^2 vs 2.28±1.93 mm^2 in restenotic and de novo lesions respectively, p=NS). Densitometry revealed similar results to edge detection analysis: restenotic area was 2.04±2.16 mm^2 vs 1.68±3.03 mm^2 in stable and unstable patients respectively (p=NS), and 2.21±2.28 mm^2 vs 1.79±2.63 mm^2 in restenotic and de novo lesions respectively (p=NS).

Discussion

The vascular smooth muscle cell plays a key role in the development of restenosis after percutaneous revascularisation techniques.[7] Fibromuscular hyperplasia is recognised as the dominant pathological substrate of restenosis,[3-5] and represents a response of the vessel wall to the damage inflicted by the recanalisation procedure. Although the sequence of events leading to the development of restenosis starts after intervention and appears to be proportionally related to the degree of vessel wall injury, it is quite likely that the preexisting lesion substrate may influence the subsequent degree of smooth muscle cell proliferation in the intima. By serving as biopsy material from the target atheromatous lesion, atherectomy specimens can offer a more direct insight on the pathological substrate and the characteristics of the smooth muscle cells present in the treated lesion. In addition to this, the information obtained from the retrieved tissue can be combined with clinical or angiographic data obtained during patient follow-up, offering the unique opportunity of studying prospectively the relationship between the anatomopathological substrate of the lesion and the likelihood of developing restenosis.

The present study is the first to compare the ability of smooth muscle cells present in atherectomy specimens to grow in culture conditions with the change in luminal dimensions in the target lesion. As stated above, we believe that documentation of smooth muscle cell outgrowth serves as a simple index of the ability of smooth muscle cells in the retrieved tissue to colonize the surrounding environment, and constitutes a surrogate for their proliferative and migratory potential. Some of the limitations of cell culture as an experimental model for this purpose, such as the modification of cell phenotype associated with enzymatic dispersion, prolonged culture, cell division and successive cell passages[17-19] and lack of the influence of other components of the atheromatous plaque were minimized in the present study by using an explant technique and limiting observations to the primary culture.

Particular attention was paid to the collection and analysis of angiographic data and its correlation with biological variables. First, we used an angiographically-derived area of restenosis which appears as a direct index of the amount of neointimal tissue formed and is not influenced by vessel size. This offers the advantage of using a continuous variable which is likely to be a better estimate of the development of neointimal hyperplasia than discrete variables (eg.≥50% diameter ste-

nosis).[20,21] The second aspect relates to the modality of quantitative analysis used. Estimation of luminal cross-sectional area may be influenced by marked irregularities in luminal morphology resulting from localized excision of tissue. A lack of relationship between the volume of extracted atheromatous fragments and the change in luminal area has been reported by using edge detection quantitative angiography.[22] In that regard, densitometric analysis may constitute the technique of choice since it calculates luminal cross sectional area directly from the densitometric profile without any assumptions on luminal morphology.[16] Alternatively, it would be expected that routine performance of multiple cuts in different directions would contribute to achieve a rather circular lumen, and that the use of edge detection in estimating luminal cross-sectional area would be justified.[23] However, no firm conclusions have been derived so far as to which is the angiographic modality of choice in the assessment of luminal area after atherectomy, and a similar degree of discrepancy between luminal cross-sectional area derived from edge detection and densitometry to that found balloon angioplasty has been reported.[24] On these grounds, in the present study we report luminal area and restenotic area measurements using both approaches. In fact, concomitant use of both methods has provided new insights on the evaluation of atherectomy results with quantitative angiography. After atherectomy the discrepancy observed in luminal area (as well as in the restenotic area) calculated by both techniques was related to the presence of media or adventitia in the retrieved specimen. Opacification of deep localized cuts might have caused overestimation of both luminal dimensions and restenotic area as assessed by edge detection.

The principal finding of this study was the observed inverse relationship between smooth muscle cell outgrowth from atherectomy specimens and the degree of restenotic area developing during follow up. Although these results may appear paradoxical, a possible explanation may be that unlike balloon angioplasty, evacuation of atheroma with a high proliferative potential for smooth muscle cells may effectively remove a favorable plaque substrate for restenosis.

Several studies have suggested the existence of a relationship between the development of restenosis and the presence of histological or molecular markers in atherectomy specimens thought to identify smooth muscle cells with an enhanced proliferative potential. Leclerc et al.[25] found that smooth muscle cells present in restenotic lesions express the B isoform of nonmuscle myosin heavy chain (NMMHC) more frequently than primary lesions. The expression of NMMHC was used as an index of the activation of a gene which is involved in cellular events crucial to restenosis, such as involved in cell division, alteration of cell shape and chemotaxis. In a later work,[26] the same authors investigated prospectively a population of 20 patients undergoing directional atherectomy. The obtained specimens were hybridized with a probe for the heavy chain of NMMHC. An increase in the expression of this marker was related with a higher restenosis rate, defined as a diameter stenosis >50% at follow-up. With a similar aim, Depre et al.[27] studied the predictive value of atherectomy specimens incubated in tritiated thymidine and later stained with phosphotunstic acid haematoxylin. Thymidine labeling was observed in 75% and 25% of cases with and wit-

hout restenosis respectively, although the experiments were performed in only 9 patients. Restenosis was defined categorically, by grouping patients in 3 categories of luminal diameter loss (loss ≥0.72 mm, ≥0.36 mm and <0.36 mm respectively). The authors found that the presence of abnormal fibromyocytes with stellar shape correlated with increased thymidine labeling and luminal loss ≥0.72 mm. Based on the hypothesis that the identified cells were activated smooth muscle cells, the same authors have recently tested their initial findings in a retrospective fashion[28] using histologic and angiographic data from 105 patients enrolled in the CAVEAT study.[29] In this larger population 3 continuous angiographic variables (luminal diameter loss, relative loss and % diameter loss) were used to assess restenosis. Contrary to the preliminary results, this study demonstrated that restenosis was not increased in patients with documented stellar-shaped fibromyocytes in the retrieved specimens. In fact, a trend towards less luminal loss in those cases where such cells were present was noted.

In a recent report by Isner et al.[31] on the histological samples obtained during the CAVEAT study an inverse relationship between the presence of thrombotic material in atherectomy specimens and the subsequent degree of luminal diameter loss was also noted. This finding is supported by our data, where the angiographic restenotic area was substantially less in atherectomy specimens containing thrombus. Such findings also appear paradoxical since development of mural thrombus has been proposed as a key factor in the development of neointimal hyperplasia.[7,32-35] Thrombin exerts a chemotactic and mitogenic effect on vascular smooth muscle cells and stimulates the expression of both growth factors and their receptors.[36,37] Our group has demonstrated a link between mural thrombosis and enhanced proliferative potential of the smooth muscle cells present in the atheromatous plaque, using the same cell culture model as in the present study.[13] The presence of thrombus in cultured coronary atherectomy specimens was the only clinical or histological variable with a positive influence on smooth muscle cell outgrowth. The explanation of this paradox is again that removal of the thrombus containing plaque may in some way diminish the propensity to restenosis.

We also found an inverse relationship between the angiographic restenotic area and the maximal cellularity documented in the atherectomy specimen. To our knowledge only one previous study has correlated the cellularity of atherectomy specimen with the development of restenosis. Suarez de Lezo et al.[38] have reported that the degree of luminal loss was proportionally related to the cellularity of the atherectomy specimen. In that study restenosis was defined as a loss ≥50% of the gain in luminal diameter documented after atherectomy. Using this categorical definition the authors found that the nuclear content (defined as percent area of the histological cut covered by cell nuclei) in the resected material was higher in those patients who later develop restenosis. At first glance these results are in disagreement with those from the present study, where the existence of an inverse relationship between restenotic area and cellularity was disclosed. However, this may be attributable to major differences in the methodology, such as the use of computerised quantitative angiography and analysis of restenosis as a continuous variable in our study, as well as the application of a different quantitative histological analysis. On the other hand, support for a negative relationship between cellularity in

atherectomy specimens and restenosis can be found in the work of Depre et al. [28] discussed above, where a trend towards an inverse relationship between restenosis and the presence of stellar shape fibromyocytes (typically found in hypercellular lesions)[39] in the resected tissue was noted.

What are the potential implications of these findings for the clinical application of directional atherectomy? Should this proposed hypothesis receive further support in larger series, identification of lesions with a high potential for smooth muscle cell proliferation might lead to selection of lesions which would benefit from the use of directional atherectomy in reducing restenosis in the long-term. Information might be derived from intracoronary imaging techniques. Angioscopy is a sensitive technique in detecting intracoronary thrombus. Likewise, further developments in fluorescence spectroscopy may allow identification of hypercellular lesions. Furthermore, the considerations giving origin to the hypothesis used in our study have major methodological implications for longitudinal studies using atherectomy specimens to assess baseline characteristics of the atheromatous plaque. Researchers should be aware that the extraction of atheromatous tissue imposes a significant modification of plaque substrate, which is likely to result in a modification of plaque evolution.

Study limitations

Although an angiographically-derived area of restenosis was used in an attempt to get an optimal correlation between the biological phenomenon of restenosis, other non-proliferative components of the process of restenosis cannot be excluded. By comparing the actual volume of tissue retrieved and the angiographic change in luminal volume Penny et al. have suggested that atherectomy may be usually associated with Dotter or "facilitated mechanical angioplasty" effect.[22] Whether a recoil phenomenon similar to that observed after balloon angioplasty may occur is uncertain, but remains a potential interference with our methodology, based upon the premise that restenotic area correlates well with the development of neointimal hyperplasia. However, we cannot find any reason for which such effect would be unevenly distributed between the groups considered in this study.

Although the samples were meticulously studied to ensure that the tissue fragment dedicated to histology and cell culture sample was, at least from a macroscopic point of view, representative of the whole specimen, the possibility that the two pieces were significantly different cannot be ruled out. However, we and other authors[6] feel that the benefit derived from the combined information obtained from histopathology and cell culture justifies this methodological approach.

Two major randomised multicenter clinical trials have demonstrated that the restenosis rate associated with the use of directional atherectomy is similar to that of balloon angioplasty.[30,40] It is beyond the scope of the present study to compare the two subsets of patients identified by smooth muscle cell culture with a comparable population of patients treated with balloon angioplasty in terms of restenosis development.

References

1. *Johnson DE, Hinohara T, Selmon MR, Braden LJ, Simpson JB. Primary peripheral arterial stenoses and restenoses excised by transluminal atherectomy: a histopathologic study. J Am Coll Cardiol 1990; 15: 419-25*

2. *Safian RD, Gelbfish JS, Erny RE, Schnitt SJ, Schmidt DA, Baim DS. Coronary atherectomy. Clinical, angiographic, and histological findings and observations regarding potential mechanisms. Circulation 1990; 82: 69-79.*

3. *Garrat KN, Edwards WD, Kaufmann UP, Vlietstra RE, Holmes DR Jr Differencial histopathology of primary atherosclerotic and restenotic lesions in coronary arteries and saphenous vein bypass grafts: analysis of tissue obtained from 73 patients by directional atherectomy. J Am Coll Cardiol 1991; 17: 442-8.*

4. *Escaned J, van Suylen RJ, MacLeod DC, Umans VA, de Jong M, Bosman FT, de Feyter PJ, Serruys PW. Histological characteristics of tissue excised during directional coronary atherectomy in patients with stable and unstable angina pectoris. Am J Cardiol 1993; 71:1442-47.*

5. *Waller BF, Johnson DE, Schnitt SJ, Pinkerton CA, Simpson JB, Baim DS. Histological analysis of directional coronary atherectomy samples. A review of findings and their clinical relevance. Am J Cardiol 1993; 72: 80E-87E*

6. *Hofling B, Heimerl J, Gonschior P, Bauriedel G. Analysis of atherectomy specimens. Am J Cardiol 1993; 72: 96E-107E.*

7. *Ip JH, Fuster V, Badimon L, Badimon J, Taubman MB, Chesebro JH. Syndromes of accelerated atherosclerosis: Role of vascular injury and smooth muscle cell proliferation. J Am Coll Cardiol 1990; 15:1667-87.*

8. *Bauriedel G, Dartsch PC, Voisard R, Roth D, Simpson JB, Hofling B, Betz E. Selective percutaneous "biopsy" of atheromatous plaque tissue for cell culture. Basic Res Cardiol 1989; 84: 326-31.*

9. *Bauriedel G, Windstetter U, DeMaio SJ, Kandolf R, Hofling B. Migratory activity of human smooth muscle cells cultivated from coronary and peripheral primary and restenotic lesions removed by percutaneous atherectomy. Circulation 1992; 85: 554-564.*

10. *Dartsch PC, Voisard R, Bauriedel G, Hofling B, Betz E. Growth characteristics and cytoskeletal organization of cultured smooth muscle cells from human primary stenosing and restenosing lesions. Arteriosclerosis 1990; 10: 62-75.*

11. *Chao K, Ko YL, Cheng JJ, Lien WP. Cell cultures of coronary atherectomized target lesions. Eu-Heart-J 1991; 12 (Abstr. Suppl.): 291.*

12. *Pickering GJ, Weir L, Rosenfield K, Stetz J, Jekanowski J, Isner JM. Smooth muscle cell outgrowth from human atherosclerotic plaque: Implications from the assessment of lesion biology. J Am Coll Cardiol 1992; 20:1430-9.*

13. *Escaned J, de Jong M, Violaris AG, MacLeod DC, van Suylen RJ, Umans VA, Verdouw PD, de*

Feyter PJ, Serruys PW. Clinical and histological determinants of smooth muscle cell outgrowth in cultured atherectomy specimens: Importance of thrombus organisation. Coronary Artery Disease 1993; 4:883-90.

14. *MacLeod DC, Strauss BH, de Jong M, Escaned J, Umans VA, van Suylen RJ, Verkerk A, de Feyter PJ, Serruys PW. Proliferation and extracellular matrix synthesis of smooth muscle cells cultured from human coronary therosclerotic and restenotic lesions. J Am Coll Cardiol 1993 (In press).*

15. *Reiber JHC, Serruys PW, Kooijman CJ et al. Asessment of short-, medium- and long-term variations in arterial dimensions frok computer assissted quantification of coronary cineangiograms. Circulation 1985; 71:280-88.*

16. *Escaned J, Haase J, Foley DP, Di Mario C, den Boer A, Montauban van Swindregt EJ, Serruys PW. Videodensitometry in percutaneous coronary interventions: a critical appraisal of its contributions and limitations. In Serruys PW, Foley DP, de Feyter PJ: Quantitative angiography in clinical practice. Kuwler Academic Press, Dordrecht- New York.*

17. *Stadler E, Campbell JH, Campbell GR. Do cultured smooth muscle cells resemble those of the arterial wall? If not, why not? J Cardiovasc Pharmacol 1989; 14 (Suppl. 6): S1-S8.*

18. *Campbell G, Campbell J, Manderson J, Horrigan S, Rennick R. Arterial smooth muscle cell. A multifunctional mesenchimal cell. Arch Pathol Lab Med 1988; 112:977-86.*

19. *Campbell GR, Campbell JH. Phenotypic modulation of smooth musce cells in primary culture. In: Campbell JH, Campbell GR, eds. Vascular smooth muscle cell in culture. Boca Ratón, FL: CRC Press, 1987:39-56.*

20. *Foley DP, Hermans WM, Rensing BJ, de Feyter PJ, Serruys PW. Restenosis after percutaneous transluminal coronary angioplasty. Herz 1992; 17:1-17.*

21. *Serruys PW, Foley PW, de Feyter PJ. Restenosis after coronary angioplasty: a proposal of new comparative approaches based on quantitative angiography. Br Heart J 1992; 68: 417-24.*

22. *Penny WF, Schmidt DA, Safian RD, Erny RE, Baim DS. Insights into the mechanism of luminal improvement after directional coronary atherectomy. Am J Cardiol 1991;67:435- 7.*

23. *Umans V, Haine E, Renkin J, de Feyter PJ, Wijns W, Serruys PW. One hundred and thirteen attempts at directional coronary: the early and combined experience of two European centres using quantitative angiography to assess their results. Eur Heart J 1992; 13: 918-24.*

24. *Umans VA, Strauss BH, de Feyter PJ, Serruys PW. Edge detection versus videodensitometry for quantitative angiographic assessment of directional coronary atherectomy. Am J Cardiol 1991;68:534-9.*

25. *Leclerc G, Isner JM, Kearney M, Simons M Safian RD, Baim DS, Weir L. Evidence implicating nonmuscle myosin in restenosis. Use of in-situ hybridization to analyse human vascular lesions obtained by directional atherectomy. Circulation 1992; 85:543-53.*

26. *Simons M, Leclerc G, Safian MD, Isner JM, Weir L, Baim DS. Relation between activated smooth-muscle cells in coronary-artery lesions and restenosis after atherectomy. N Eng J Med 1993; 328: 608-13.*

27. *Depre C, Wijns W, Haine E, Renkin J, Hanet C, Havaux X. Risk of restenosis after directional coronary atherectomy: Predictive value of structural analysis and cell labelling of atheromatous fragments. Circulation 1992; 86(suppl I):I-225.*

28. *Depre C, Wijns W, Havaux X on behalf of the CAVEAT study. Morphological analysis of atheromatous fragments removed after atherectomy: A predicitive index for later restenosis? Circulation 1993. 88(suppI):I-651.*

30. *Topol EJ, Leya F, Pinkerton CA et al. on behalf of the CAVEAT Study Group. A comparison of directional coronary atherectomy with coronary angioplasty in patients with coronary artery disease. N Eng J Med 1993; 329: 221-7.*

31. *Isner JM, Kearney M, Berdan LG, Keeler G, Califf RM, Topol EJ for the CAVEAT investigators. Core pathology lab findings in 425 patients undergoing directiona atherectomy for a primary coronary artery stenosis and relationship to subsequent outcome: The CAVEAT STUDY 1993. J Am Coll cardiol 1993; 21:380A (Personal communication)*

32. *Naito M, Hayashi T, Kuzuya M, Funaki C, Asai K, Kuzuya F. Effects of fibrinogen and fibrin on the migration of smooth muscle cells in vitro. Atherosclerosis 1990; 83:9-14.*

33. *Poole JCF, Cromwell BS, Benditt EP. Behaviour of smooth muscle cells and formation of extracellular structures in the reaction of arterial walls to injury. AmJ Pathol 1971; 62:391-404.*

34 *Jorgensen L, Rowsell HC, Hovig T, Mustard JF. Resolution and organisation of platelet-rich mural thrombi in carotid arteries of swine. Am J Pathol 1967; 51:681-719.*

35. *Schwartz RS, Holmes DR, Topol EJ. The restenosis paradigm revisited: An alternative proposal for cellular mechanisms. J Am Coll Cardiol 1992; 20:1284-93.*

36. *Thyberg J, Hedin U, Sjolund M, Palmberg L, Bottger BA. Regulation of differentiated properties and proliferation of arterial smooth muscle cells. Arteriosclerosis 1990; 10:966-90.*

37. *Casscells W. Smooth muscle cell growth factors. Prog Growth Factor Res 1991; 3:177-206.*

38. *Suarez de Lezo J, Romero M, Medina A et al. Intracoronary ultrasound assessment of directional coronary atherectomy: Immediate and follow-up findings. J Am Coll Cardiol 1993; 21: 298-307.*

39. *Miller MJ, Kuntz RE, Friedrich SP, Leidig GA, Fishman RF, Schnitt SJ, Baim DS, Safian RD. Frequency and consequances of intimal hyperplasia in specimens retrieved by directional coronary atherectomy of native primary coronary stenoses and subsequent restenosis. Am J Cardiol 1993; 71:652-58.*

40. *Adelman AG, Cohen EA, Kimball BP et al.A comparison of directional coronary atherectomy with baloon angioplasty for lesions of the left anterior descending coronary artery. N Eng J Med 1993; 329: 228-33.*

Chapter VI

Restenosis Following Directional Coronary Atherectomy in Cardiac Transplant Patients

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Submitted for publication.

Abstract

Two methods of percutaneous coronary recanalisation, balloon angioplasty and directional atherectomy, have been reported for the non-surgical treatment of focal atherosclerotic lesions in transplanted hearts. Little is known on the changes that take place at the site of intervention in these patients, who develop accelerated atherosclerosis and are under immunosupressive therapy. In the present study we present the clinical and angiographic evolution of 3 patients with atherosclerosis of the cardiac allograft in whom directional atherectomy was used to treat a focal stenosis. Histological evidence on the histopathological characteristics of the stenoses was obtained by studying first the retrieved atherectomy specimens, and later (530±114 days) the cardiac allograft, obtained during autopsy in 2 cases and after retransplantation in 1 case.

Introduction

Atherosclerosis following heart transplantation that constitutes the main cause of death in patients surviving the first postoperative year. Although limited by the characteristic diffuse involvement of the coronary tree, percutaneous recanalisation using balloon angioplasty[1-5] or directional coronary atherectomy[6-8] constitute helpful therapeutic alternatives in the treatment of focal stenoses. However, virtually no information is available on the histological changes taking place at the site of percutaneous intervention in these patients, in whom the biological substrate of accelerated atherosclerosis and the concomitant use of immunosuppressive therapy can modify the development of restenosis. We present three cases of orthotopic cardiac transplantation and new focal atherosclerosis treated with directional coronary atherectomy in which a histopathological study of the of the transplanted heart was available in the long-term.

Methods

Clinical and procedural characteristics.

Directional coronary atherectomy using the technique described by Simpson et al.[9] was performed in 3 patients who developed focal coronary stenoses in a cardiac transplant allograft. Two of these procedures were performed at the Academic Hospital Dijkzigt in Rotterdam, The Netherlands, and 1 at the Cardiac Center in San Diego, California. In both institutions the procedure was performed according with procedural protocols approved by the respective ethical committees and after obtaining written consent. All three patients underwent recanalisation with a 6 Fr atherocatheter without adjunctive balloon angioplasty. Aspirin and dypiridamol was given in the 3 cases following intervention. Patient follow-up was performed prospectively as part of the cardiac transplant program in which the patients were enrolled. In the long term, two of the patients died and one underwent cardiac retransplantation. This made possible the documentation of the pathological changes that took place in the treated coronary segment from the time of atherectomy (mean ± SD days).

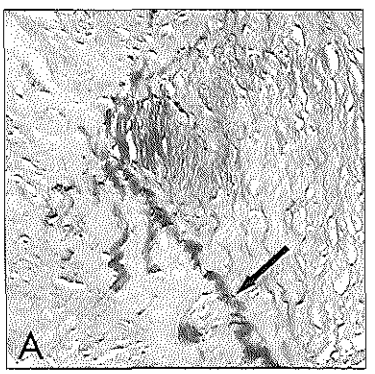
Pathological examination

The coronary arteries were removed once the site where atherectomy took place was identified with the help of angiographic frames obtained at the time of the procedure. In two cases (1 and 3) fixation was performed under pressure. Multiple sections at 2 mm intervals were obtained and processed for light microscopy. Sample staining was performed using hematoxylin-azophloxin and Verhoeff-van Giesson techniques, as well as immunohistochemical staining for smooth muscle cell α-actin (Sigma, St. Louis, USA).

Results

Case 1

A 28 year-old caucasian female underwent orthotopic heart transplantation for the treatment of peripartum cardiomyopathy. She experienced recurrent episodes of acute and ongoing cardiac rejection which were treated with methylprednisolone and anti-T cell antibodies initially, and chronic azathioprine therapy thereafter. Systemic hypertension and mild chronic renal failure developed gradually. At the 3rd year angiographic follow-up, a focal 50% stenosis was disclosed in the mid segment of the left anterior descending coronary artery. The following year the lesion had progressed to an 80% stenosis. Stress scintigraphy was performed, showing a reversible anterior perfusion defect. Directional atherectomy was performed successfully and uneventfully. Follow-up angiography 10 months later disclosed an acceptable long-term result, with <30% residual narrowing, although a new focal 40% diameter stenosis was evident in the proximal segment of the right coronary artery. This new stenosis was judged as not requiring recanalisation. Twelve months after atherectomy the patient presented with left ventricular failure due to a silent inferior myocardial infarction, which was treated conservately. Sixteen months after atherectomy the



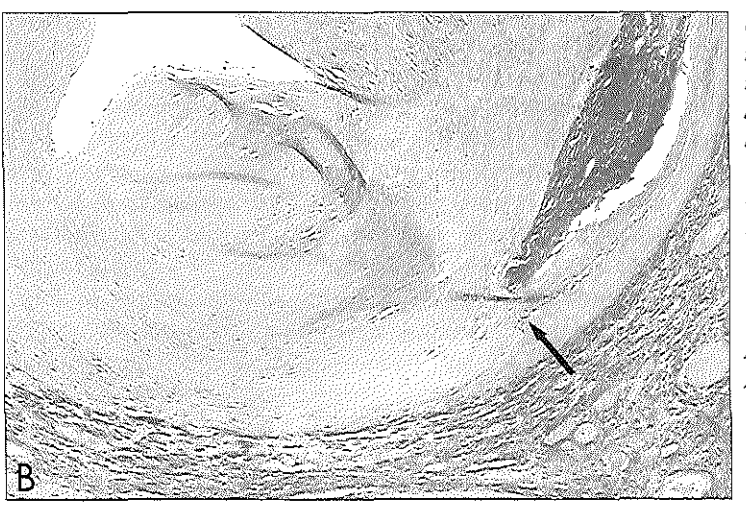


Figure 1
Histological findings in patient 1.

(A) Atherectomy specimen showing a predominantely fibrous tissue with a fragment of internal elastic lamina (arrow) and media. (Elastic stain).

(B) During post-mortem examination the site of atherectomy could be clearly identified by the discontinuation of internal elastic lamina (arrow) and media in the vascular wall. There was extensive neointimal proliferation protruding in the vascular lumen. (Elastic stain).

patient was admitted with hypotension and acute renal failure. An ECG showed an acute myocardial infarction in the anterolateral territory. Although her condition was initially controlled with inotropes, the patient died as a consequence of asystole refractory to cardiopulmonary resuscitation.

Pathological findings
Two tissue fragments were obtained. Serial sections of one of the specimens showed fragments of the internal elastic lamina, media and adventitia, providing evidence of deep vessel resection (Fig 1A). No evidence of foam cells or cholesterol crystal clefts was found.

During examination of the coronary vessel during autopsy the site corresponding to the atherectomy could be clearly identified in consecutive transverse sections by the absence of the deep vessel layers which were previously noted in the atherectomy specimens (Fig 1B). A large area of neointimal hyperplasia originating from this point and causing severe compromise of the vascular lumen was clearly distinguishable from the denser fibrous tissue of the older plaque. This area of newer tissue was significantly more cellular than pre-existing tissue (as documented in the specimen and in remanant plaque) (Fig. 1C). No lipid deposits were found. Immediately distal to the site of atherectomy, the coronary vessel was occluded by a thrombus with areas of platelet rich and red constitution. An area of myocardial infarction was evident in the distribution territory of the vessel. Areas of old myocardial infarction were identified in the inferior ventricular wall.

Case 2
A 51 year-old caucasian male underwent cardiac transplantation for end-stage ischemic cardiomyopathy. Post-operative recovery was complicated by necrotizing pseudomonas pneumonia requiring right upper lobe resection. Cytomegalovirus viremia was diagnosed five months after transplantation but resolved without treatment. No acute moderate rejection or significant infection followed. During the 4th annual arteriogram, a 50% mid right coronary and 80% posterior descending artery stenoses were noted. Intravascular ultrasound imaging of the mid right coronary lesion revealed homogeneous echogenicity consistent with a fibrous, non-calcified, plaque buildup . Left ventricular angiography show a 65% ejection fraction and no significant ventricular wall motion abnormalities. Over the next two months increasing exertional dyspnea and intermittent ankle edema developed. Repeat angiography showed further progression of the mid right coronary and posterior descending artery stenoses (75% and 85% diameter stenosis respectively). Stress scintigraphy showed a large, reversible inferior ischemic zone. Percutaneous revascularisation of both lesions was performed using directional atherectomy in the mid right coronary and balloon angioplasty in the posterior descending stenoses. Both procedures were uncomplicated and the patient was discharged. However, 48 hours after the procedure had a pre-syncopal episode accompanied by profuse sweating but did not seek medical advice. Several weeks later the patient was examined during routine follow-up. Resting electrocardiogram revealed

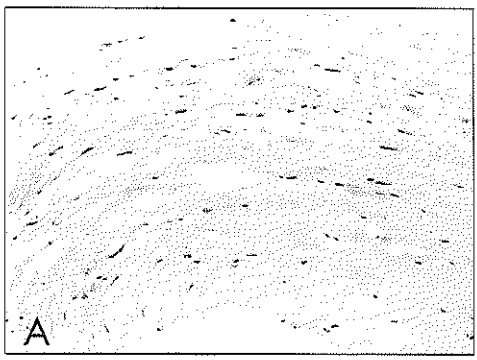


Figure 2 A
Histological findings in Case 2.
A: Atherectomy specimen showing dense fibrous tissue.

new, small Q waves and inverted T waves in the inferior leads with non-specific ST changes. Thallium exercise treadmill demonstrated a large, fixed perfusion defect. The study was prematurely terminated after AV block resulting in syncope developed. Ambulatory electrocardiographic monitoring was performed, showing long sinus pauses leading to an idioventricular rhythm associated with exertional dyspnea. A new coronary arteriogram disclosed a 90% mid right coronary stenosis and occlusion of the posterior descending artery, as well as diffuse progression of atherosclerosis in the proximal segment. Further narrowing was also noted in other epicardial branches. Ventriculography showed an 39% ejection fraction with posterobasal and inferior akinesis. The patient was evaluated for retransplantation which was successfully performed a year later.

Pathological findings.
Four small pieces of whitish-gray tissue were recovered, consisting of moderately dense fibrous tissue (Fig. 2A). Some cholesterol crystal clefts were also noted. A fragment of media was also evident in one of the specimens.

Examination of coronary sections obtained from the heart after re-transplantation showed a virtual occlusion of the vessel at the level where atherectomy had taken place. Around 40% of the area comprised within the internal elastic lamina corresponded to mixed atheromatous plaque containing both fibrous tissue and cholesterol crystal clefts. The remaining lumen was filled by neointimal proliferation showing multiple multiluminal channels, suggesting organisation of a previous episode of thrombotic occlusion (Fig. 2B). At the site of occlusion the interphase between pre-existing cholesterol crystal clefts and newly formed neointimal hyperplasia showed numerous multinucleated giant cells (Fig. 2C), which are likely to represent a foreign body reaction against the lipidic plaque core after being exposed to the bloodstream by atherectomy. Distal to this segment the depending myocardium showed patchy fibrous scarring consistent with an old sub-endocardial infarction of the infero-posterior wall.

Case 3
Patient 3 was a 37-year-old caucasian male transplanted for the treatment of dilated cardiomyopathy. He had one episode of acute rejection after transplantation. He resumed smoking. Average cholesterol during follow-up was 5.9 mmol/L and serum triglyceride 1.8 mmol/L. A focal eccentric lesion was identified during the 5th year

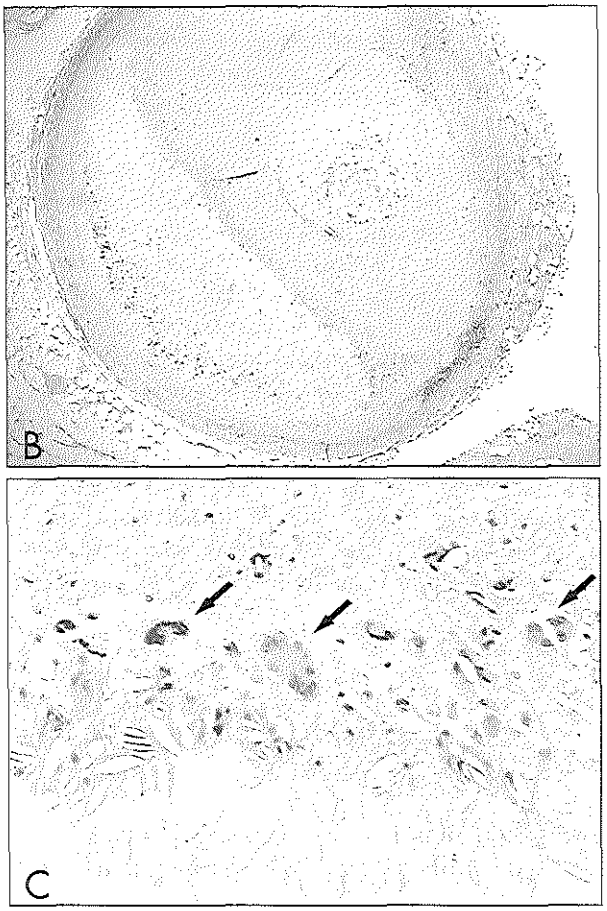


Figure 2 B and C
Histological findings in Case 2 (continuation).

B: Cross section of the vessel at the site of atherectomy obtained in the transplanted heart after retransplantation, showing total vessel occlusion by fibrous tissue with multiluminal channels, the latter suggesting a prior episode of thrombotic occlusion with partial recanalisation. The remnant plaque shows dense fibrous tissue with areas of cholesterol crystal clefts.

C: At the interphase between the occluding fibrous tissue and the old plaque numerous multinucleated giant cells (arrows) were visible, probably in relation with the exposure of lipidic plaque material to the bloodstream after

control angiogram in the left circumflex coronary artery. Reversible ischaemia in the left ventricular posterior wall was demonstrated during stress scintigraphy. Directional atherectomy was performed successfully and uneventfully. Coronary angiography 7 months later revealed no significant restenosis. He subsequently underwent laparotomy twice because of a perforated peptic ulcer and peritonitis.The post-operative course was complicated by adult respiratory distress syndrome, multi-organ failure and pneumonia. After this hospital admission, the patient was started on haemodialysis for chronic renal insufficiency secondary to cyclosporin treatment. Twenty-two months after atherectomy the patient suffered circulatory arrest. The patient was resuscitated, although did not regain consciousness and died 3 days later.

Pathological findings.
Three pieces of whitish tissue with brownish areas were obtained. These were composed mainly of poorly cellular fibrous tissue. Evidence of internal elastic lamina and media were also found. At the site of atherectomy, the circumflex artery obtained during autopsy showed evidence of the prior excision of deep vessel layers, reaching the adventitia. A large area of neointimal hyperplasia filled the resected area of plaque

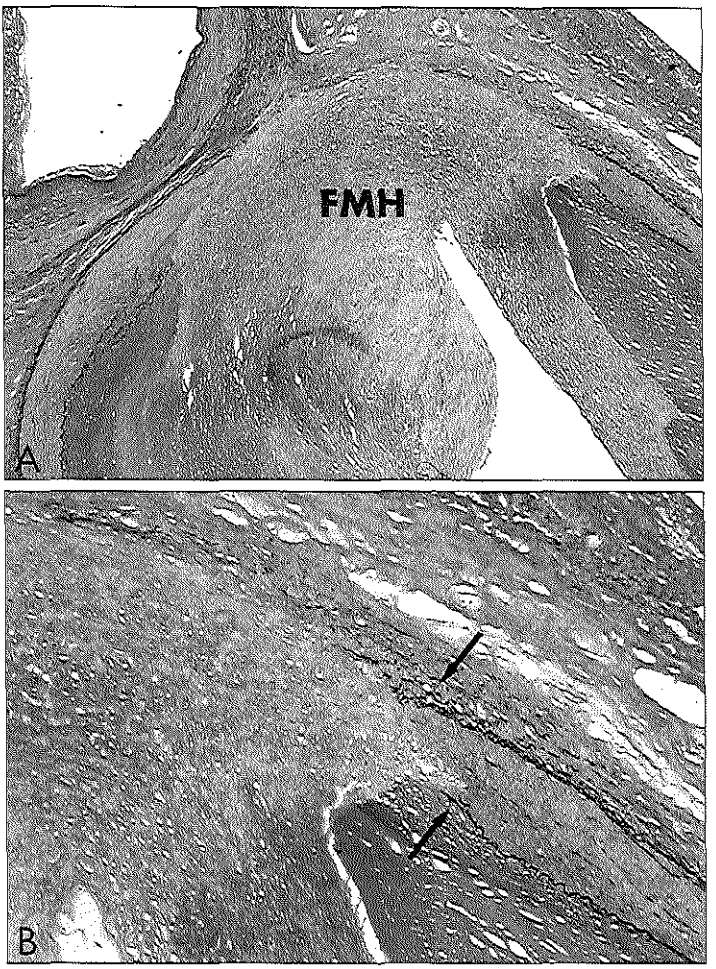


Figure 3
Histological findings in patient 3. (A) In the post-mortem coronary specimen a large area of fibromuscular hyperplasia (FMH) originates from the site of atherectomy, which can be identified (B) by the missing internal and external elastic lamina (arrows), media and adventitia in the vascular wall. (Elastic stain).

and deep vessel wall, extending to the remaining lumen. The newly formed tissue was clearly more cellular and had a looser extracellular matrix than that forming the old plaque, and contained numerous plasma cells. No cholesterol crystal clefts or foam cells were identified.

Discussion

Cardiac allograft atherosclerosis has a reported prevalence of 2% and 25% in the first year, and as high as 50% at 5 years after transplantation.[10-12] It constitutes the predominant cause of death of cardiac transplant patients following the first post-toperative year. Retransplantation does not constitutes a practical solution to this problem since it is currently associated with an increased mortality,[13] while puts in

jeopardy the higher chances of survival of patients waiting for their first cardiac transplantation. Coronary angioplasty has been proposed as a potential alternative for the treatment of focal occlusive disease in transplanted patients,[1-5] and preliminary results from a multicenter study suggest that although its procedural success and complication rate are similar to that associated with routine coronary angioplasty, it is associated with a high restenosis rate. The feasibility of directional coronary atherectomy as a recanalisation technique in these patients has also been reported.[6-8] The potential benefit derived from the use of percutaneous coronary revascularisation is emphasised by recent evidence that the presence of focal stenoses in the proximal and mid-epicardial vessels drecrease actuarial survival in heart transplanted patients.[9]

The cause of post-transplant accelerated atherosclerosis remains unclear. Several causes have been proposed, including ongoing immune-mediated vascular injury,[15,16] endothelial injury during the pre-implantation period,[16] injury associated with viral infection,[17,18] cardiac denervation, and a high prevalence of risk factors for coronary artery disease, particularly hyperlipidaemia and hypertension which were either preexisting or develop as a result from cyclosporin and corticosteroid therapy.[16, 19] Transplant vasculopathy presents histological differences with classic forms of atherosclerosis, including the absence of medial thinning, a predominantly concentric distribution of atheroma.[13,20,21] Indeed, early transplant atherosclerosis resembles post-angioplasty restenosis in the development of neointimal hyperplasia as the dominant pathological feature of the disease, having been documented as early as 1 week after transplantation.[11] Late development of more complex atheroma in the transplanted heart, with extensive lipid deposits, appears to be related to a number of concomitant pro-atherogenic factors that are nearly universal to all transplanted patients, including the development of unfavorable lipid profiles and systemic hypertension associated with the use of immunosuppressive drugs.[19] A relation between these factors is becoming clear. In a recent report using combined angioscopy and intravascular ultrasound imaging, Ventura et al.[22] has demonstrated that xanthomatous atherosclerotic plaques, which suggest the presence of lipid deposits in the plaque, were associated with a longer time interval from transplantation, higher serum cholesterol levels and more severe obstructions than those atheromatous plaques with white surface. This is in agreement with the findings of Johnson et al.[20] who found that, in contrast with diffuse lesions, focal coronary stenoses present in transplanted patients are frequently rich in lipid deposits. In this regard, the characteristic extracellular matrix of neointimal hyperplasia, which is rich in glycosaminoglycans,[23] may favor the uptake of lipids as during the early stages of coronary atherosclerosis.[24]

The consequences that plaque disruption secondary to percutaneous intervention has on subsequent pathobiological events of this particular type of accelerated atherosclerosis are unknown. Potentially, the development of a restenotic neointima could be either potentiated by the biological substrate (enhanced reactivity towards blood platelets, presence of activated cells[25,26]) or reduced by the adminis-

tration of concomitant immunosuppressive therapy. Information on the long-term pathological changes found at the site of angioplasty in transplanted hearts is extremely limited. Shandu et al.[9] make a short reference to the autopsy findings in 2 heart transplant patients after balloon angioplasty included in his series. Neointimal hyperplasia was the dominant pathological finding. No significant differences were noted between the angioplasty site and neighbor coronary segments. At a difference with these observations, in our 3 patients we could identify the exact location of the treated site not only by comparing the angiogram with the coronary anatomy while dissecting the heart, but also by the absence of internal elastic lamina and media resulting from atherectomy. Our observations suggest not only that neointimal hyperplasia is found in response to percutaneous intervention in transplanted hearts, but also that it presents different morphological features than the preexisting fibrous tissue existing in the plaque.

All 3 cases presented in this work fall in the angiographic category of type A lesions.[13,20] Type A lesions have been shown to contain lipid deposits in 67% of cases, in contrast with 14% of type B.[20] In the present study, lipid deposits in the atherectomy specimen or at the treated segments could be demonstrated only in 1 patient, although the small number of patients studied precludes any conclusions. It is interesting to note that atherectomy in the stenosis containing a lipidic core was associated with subsequent occlusive thrombosis. Figure 2C shows the interphase between the preexisting atherosclerotic material in the plaque and that formed after directional coronary atherectomy. Numerous multinucleated giant cell are visible in the transition between the deposits of cholesterol crystal cleft and neointimal hyperplasia, which are likely to represent a foreign body reaction against the lipidic core when exposed to the bloodstream. The high thrombogenicity of lipid deposits[27] may be related with the thrombotic event that followed directional atherectomy in this patient.

Clinical consequences

The three cases also illustrate that coronary events following coronary intervention in transplanted patients manifest atypically when compared with non-transplanted patients. In the first patient heart failure was the only manifestation of acute myocardial infarction, which during post-mortem examination appeared to be related to the previously intervened site. In the second patient dizziness and malaise was the only manifestations of myocardial infarction resulting from subacute vessel closure after DCA. The remaining patient died suddenly presumably due to an unnoticed ischemic event triggering ventricular arrhythmia, and no evidence suggesting a direct link between intervention and cardiac death was found.

Although no coronary angiography was performed besides the fixed annual evaluations, the degree of luminal obstruction found in 2 of the treated lesion appeared to be significantly higher than that suspected in the 6 month angiographic

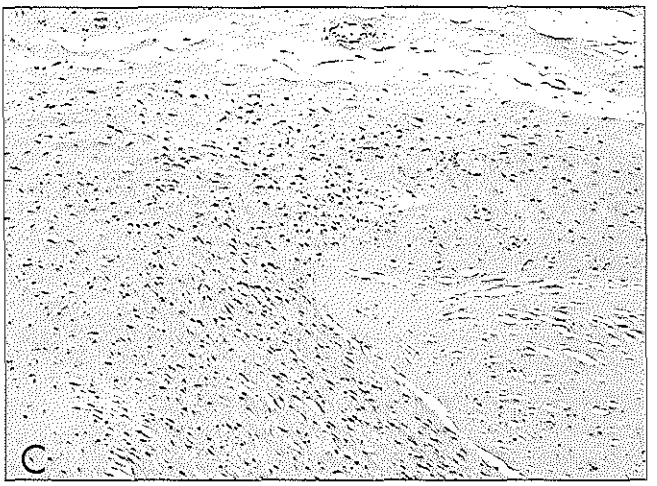


Figure 3 C
Hematoxilin-azophloxin stain of Figure B revealing a substantial diference in the cellularity of the old and newly formed neointima.

follow-up after atherectomy. This fact poses the question that in these patients neointimal proliferation may have continued beyond the classical 6 month period reported in multiple longitudinal studies on restenosis after balloon angioplasty.[28] In a larger series reported on transplanted patients undergoing balloon angioplasty (n=35), only 43% were free of the combined end-point myocardial infarction, death, retransplantation or repeat angioplasty at 8±1 months of follow-up. Unfortunately, no clear information can be obtained from that study on whether myocardial infarction was a direct result of a cardiac event in the previously dilated artery, nor on the histopathological substrate of such events.[5] However, it is a matter of concern that a previous report[29] fatal cardiac events occurring subacutely after directional coronary atherectomy occurred with extensive development of neointimal hyperplasia. This might be due to intrinsic differences in the mechanism of action of directional atherectomy and balloon angioplasty, and its further study merits more attention if future application of the technique to cardiac transplant patients is planned.

References

1. *Gammage MD, Shiu MF, English TA. Percutaneous coronary angioplasty in a cardiac transplant recipient. Br-Heart-J. 1988; 59: 253-5.*

2. *Hastillo-A, Cowley-MJ, Vetrovec-G, Wolfgang-TC, Lower-RR, Hess-ML. Serial coronary angioplasty for atherosclerosis following heart transplantation. J-Heart-Transplant. 1985; 4: 192-5.*

3. *Wohlgelernter D, Stevenson LW, Brunken R. Reversal of ischemic myocardial dysfunction by PTCA in a cardiac transplant patient. Am-Heart-J. 1986 ; 112: 837-9.*

4. *Shandu JS, Uretsky BF, Reddy PS, Denys BG, Ruffner RJ, Breisblatt WM, Zerbe TR, Kormos RL, Armitage JM, Hardesty RL, Griffith BP. Potential limitations of percutaneous transluminal coronary angioplasty in heart transplant recipients. Am J Cardiol 1992; 69:1234-37.*

5. *Halle AA, Wilson RF, Massin EK et al. Coronary angioplasty in cardiac transplant patients. results of a multicenter study. Circulation 1992; 86:458-62.*

6. *Strikwerda S, Umans VA, van der Linden MM, van Suylen RJ, Balk AH, de Feyter PJ, Serruys PW. Percutaneous directional atherectomy for discrete coronary lesions in cardiac transplant patients. Am Heart 1992; 6:1686-90.*

7. *Halle AA, DiSciascio G, Wilson RF et al. PTCA, directional atherectomy and coronary artery bypass surgery in cardiac transplant patients: Multicenter findings. J Am Coll Cardiol 1993; 21 (suppl): 333A.*

8. *Jain SP, Ventura HO, Ramee SR, Collins TJ, Isner JM, White CJ. Directional coronary atherectomy in heart transplant recipients. J Heart Lung Tranplant 1993; 12:819-23.*

9. *Johnson DE, Hinohara T, Selmon MR, Braden LJ, Simpson JB. Primary peripheral arterial stenoses and restenoses excised by transluminal atherectomy: a histopathologic study. J Am Coll Cardiol 1990; 15: 419-25.*

10. *Pahl E, Fricker FJ, Armitage J, Griffith BP, Taylor S, Uretsky BF, Beerman LB, Zuberbuhler JR. Coronary arteriosclerosis in pediatric heart transplantation survivors: Limitation of long-term survival. J Pediatr 1990; 116:177-83.*

11. *Uretsky BF, Murali S, Reddy PS et al. Development of coronary artery disease in cardiac transplant patients receiving immunosuppressive therapy with cyclosporin and prednisone. Circulation 1987; 76:827-34.*

12. *O'Neill BJ, Pflugfelder PW, Singh NR, Menkis AH, McKenzie FN, Kostuk WJ. Frequency of angiographic detection and quantitative assessment of coronary arterial disease one and three years after cardiac transplantation. Am J Cardiol 1989; 63:1221-26.*

13. *Gao SZ, Schroeder JS, Hunt S, Stinson EB. Retransplantation for severe accelerated coronary vascular disease in heart transplant recipients. Am J Cardiol 1988; 62:867-81.*

14. *Keogh AM, Valantine HA, Hunt SA et al. Impact of proximal or midvessel discrete coronary stenoses*

on survival after heart transplantation. J Heart Lung Transplant 1992; 11:892-901.

15. Hess ML, Hastillo A, Mohanakumar T et al. Accelerated atherosclerosis in cardiac transplantation: role of cytotoxin B cell antibodies and hyperlipidemia. Circulation 1983; 68(suppl II):II-94-101.

16. Ip JH, Fuster V, Badimon L, Badimon J, Taubman MB, Chesebro JH. Syndromes of accelerated atherosclerosis: Role of vascular injury and smooth muscle cell proliferation. J Am Coll Cardiol 1990; 15:1667-87.

17. Mc Donald K, Rector T, Braunlin E, Olivari MT. Cytomegalovirus infection in cardiac transplant recipients predicts the incidence of allograft atherosclerosis. J Am Coll Cardiol 1989; 13 (suppl A):213A.

18. Skowronski EW, Mendoza A, Smith SC, Jaski BE: Detection of cytomegalovirus in paraffin-embedded postmortem coronary artery specimens of heart transplant recipients by the polymerase chain rection: Implications of cytomegalovirus association with graft atherosclerosis. J Heart Lung Trasplant 1993: 12:717-23.

19. Hosenpud JD, Shipley GD, Wagnerr CR. Cardiac allograft vasculopathy: Current concepts, recent developments, and future directions. J Heart Lung Transplant 1992; 11:9-23.

20. Johnson DE, Alderman EL, Schroeder JS, Gao S, Hunt S, DeCampli WM, Stinson E, Billingham M. Transplant coronary artery disease: Histological correlations with angiographic morphology. J Am Coll Cardiol 1991; 17:449-57.

21. Johnson DE, Gao SZ, Schroeder J, DeCampli WM, Billingham M. The spectrum of coronary artery pathologic findings in human cardiac allografts. J Heart Lung Transplant 1989; 349-59.

22. Ventura HO, Jain A, Collins TJ, White CJ, Ramee SR, Smart FW, Stapleton DD. Angioscopic surface morphology of early allograft coronary artery disease in cardiac transplant recipients with atherosclerosis documented by intravascular ultrasound. J Am Coll Cardiol 1993; 21 (suppl): 62A.

23. Waller BF, Johnson DE, Schnitt SJ, Pinkerton CA, Simpson JB, Baim DS. Histological analysis of directional coronary atherectomy samples. A review of findings and their clinical relevance. Am J Cardiol 1993; 72: 80E-87E

24. Hurt E, Camejo G. Effect of arterial proteoglycans on the interaction of LDL with human monocyte-derived macrophages. Atherosclerosis 1987; 67:115-21.

25. Salomon RN, Hughes CCW, Schoen FJ, Payne DD, Pober JS, Libby P. Human thansplantation-associated atherosclerosis. Evidence for a chronic immune reaction to activated graft endothelial cells. Am J Pathol 1991; 138:791-98.

26. Libby P, Salomon RN, Payne DD, Schoen FJ, Pober JS. Function of vascular wall cells related to development of transplantation associated coronary atheromatous disease. Transplant Proc 1989; 21:3667-84.

27. Stuart MJ, Gerrard JM, White JG. Effect of cholesterol on production of thromboxane B2 by platelet in vitro. N Eng J Med 1980; 302:6-10

28. *Serruys PW, Luijten HE, Beatt KJ, et al. Incidence of restenosis after successful coronary angioplasty: a time-related phenomenon. A quantitative angiographic study in 342 consecutive patients at 1, 2, 3, and 4 months. Circulation 1988;77:361-71.*

29. *Garrat KN, Edwards WD, Vliestra RE, Kaufmann UP, Holmes DR. Coronary morphology after percutaneous directional coronary atherectomy in humans: Autopsy analysis of three patients. J Am Coll Cardiol 1990; 16:1432-6.*

Part II

The Role of Intracoronary Imaging in the Study of the Pathophysiological Substrate of Coronary Syndromes

Chapter VII

The Use of Angioscopy in Percutaneous Coronary Interventions

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Introduction

Although coronary angiography is still the gold standard coronary imaging modality for the interventional cardiologist, more information is emerging on the specific limitations that percutaneous recanalisation poses to the interpretation of angiographic images. Coronary angioscopy, which can be used during percutaneous coronary interventions and provides a more accurate picture of the luminal aspect of the vessel, has been shown to be complementary to angiography.[1] We review some of the contributions that coronary angioscopy has made to interventional cardiology during its short existence, as well as the current trends for its application in a wider spectrum of clinical situations.

Contributions of angioscopy to the study of acute coronary syndromes

One of the strengths of coronary angioscopy over other imaging techniques such as angiography and intravascular ultrasound is its high sensitivity for the detection of coronary thrombosis[2,3] which stems from the marked differences in colour between thrombus and the normal arterial wall or atheroma. Using intra-operative angioscopy during bypass grafting, Sherman et al.[4] found that coronary thrombus is common in patients with unstable angina, and that frequently are not identifiable by coronary angiography. Although this work was limited by patient selection and the performance of retrograde visualization of the lesion from the arteriotomy site, it set a landmark in the in-vivo study of the substrate of unstable syndromes. The development of more flexible angioscopes that can be used percutaneously and allow antegrade visualization of the culprit lesion has facilitated further progress in this field.[5-7] Differences in the visual characteristics of thrombus have been reported in unstable angina and myocardial infarction. Mizuno et al.[6] reported that thrombus in unstable angina is predominantly grayish and non-occlusive, while thrombus associated with myocardial infarction is predominately red and causes complete occlusion. The former was though to correspond to platelet rich thrombus, while the latter was identified as rich in red blood cells and poorer in fibrin. Conclusions on the pathological substrate giving origin to thrombosis have also been drawn from angioscopic examination. Xantomatous plaques have been identified particularly in patients with myocardial infarction,[5] providing additional support to the hypothesis that plaque rupture leading to myocardial infarction often happens in weakened lipid-rich plaques.[8]

Although coronary angioscopy has played a significant role in facilitating the study of acute coronary syndromes in-vivo, most studies have been biased by translation of visual findings to pathological terms by the cardiologist, without the assistance of a cardiovascular pathologist. As discussed above, the sensitivity of angioscopy in detecting thrombus probably relies on the existence of substantial chromatic differences between red thrombus and the arterial wall (Fig. 1).

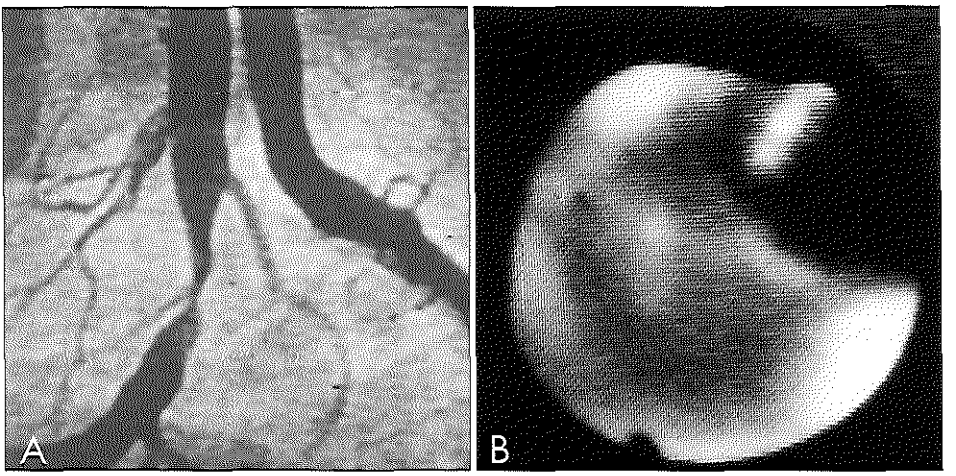

Figure 1

Angioscopy of a stenosis in the mid left anterior descending coronary artery in a patient with post-infarction angina pectoris. Although the angiographic morphology of the lesion (A) does not suggest the presence of coronary thrombus, angioscopy (B) reveals a large area of ulceration and thrombus which can be clearly differentiated from the white vascular wall. Note the heterogeneity of the thrombus, with white areas that may correspond to fragments of the fibrous cap, deposits of platelet-rich thrombus or areas of thrombotic organisation.

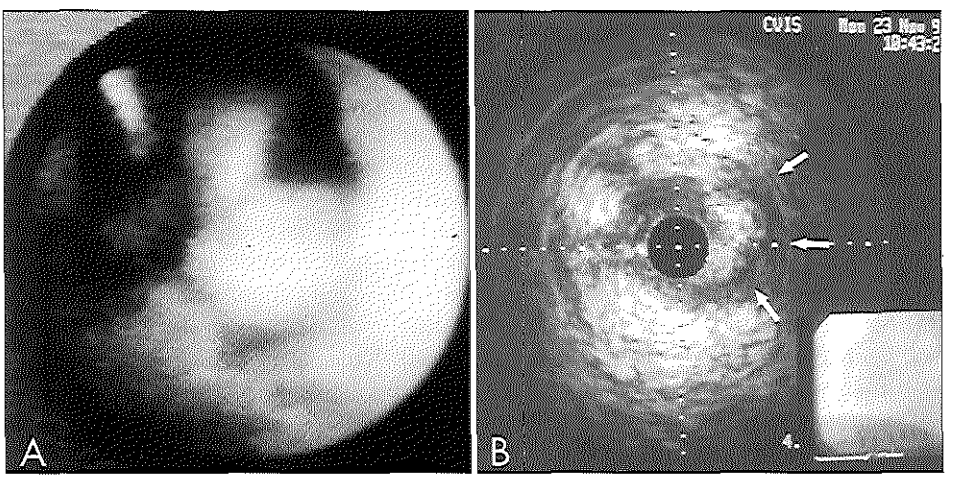

Figure 2

Intracoronary imaging in a patient with unstable angina pectoris. A globular mass that resembles a platelet rich thrombus was evident during angioscopic examination (A). However, concommitant examination with intravascular ultrasound suggests that this structure had a moderately high echogenicity, suggesting that visualised structure was more likely to have a fibrocalcific substrate (B).

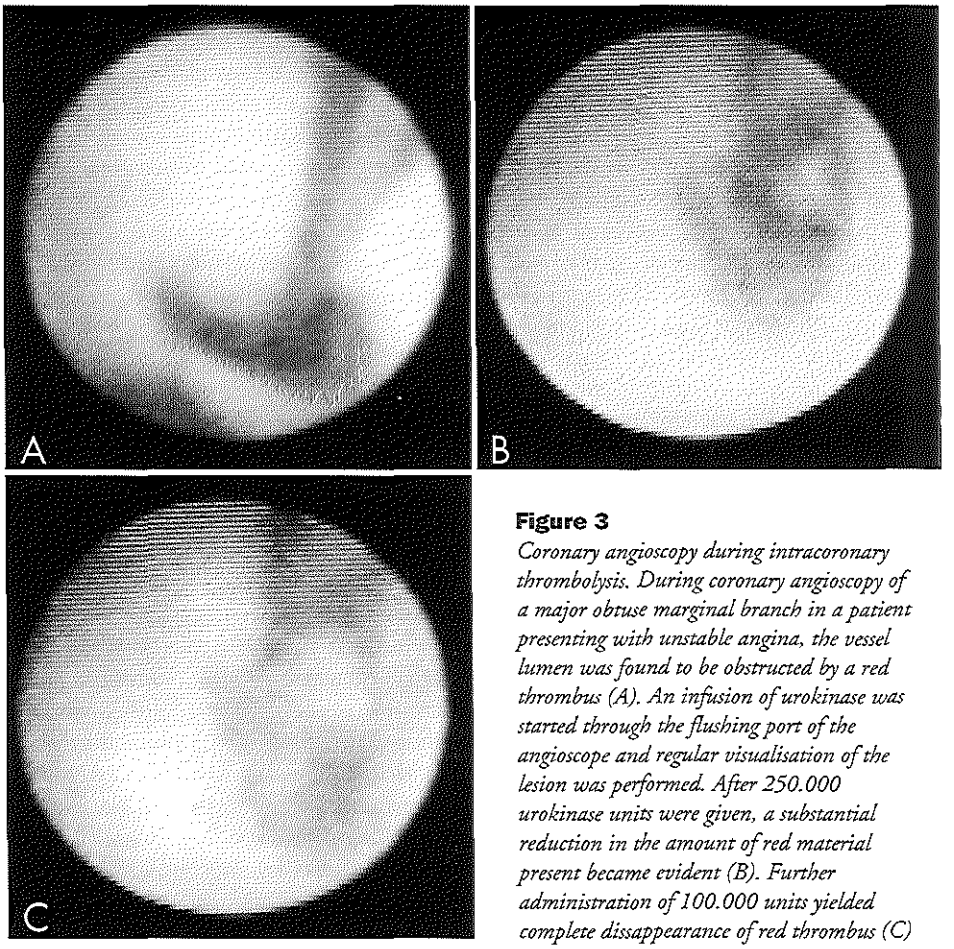

Figure 3

Coronary angioscopy during intracoronary thrombolysis. During coronary angioscopy of a major obtuse marginal branch in a patient presenting with unstable angina, the vessel lumen was found to be obstructed by a red thrombus (A). An infusion of urokinase was started through the flushing port of the angioscope and regular visualisation of the lesion was performed. After 250.000 urokinase units were given, a substantial reduction in the amount of red material present became evident (B). Further administration of 100.000 units yielded complete dissappearance of red thrombus (C) and marked angiographic improvement. The underlying lesion was a grayish lesion with irregular borders suggesting plaque ulceration (Courtesy of Dr Antoni Serra, Hospital Clinic, Barcelona, Spain).

However, it is a matter of concern that structures that are not "red" are identified as "white" or platelet thrombus. The various steps leading to the development of red occlusive thrombus in the injured vessel have been documented by angioscopy in animal models.[3,9] The first step of this process consists of adhesion of fibrinous network-like material and white components of thrombus (platelet aggregates), followed by the apposition of mixed red and white components that finally occlude the vessel lumen. Analysis of the white components of the observed thrombus has demonstrated that it was composed of platelet aggregates and strands of fibrin, while the red thrombus was rich in red blood cells trapped in a fibrin network.[3] It has not been established that in clinical practice angioscopy may be equally sensitive to the detection of white components of thrombus, since the latter may not

differ significantly in colour from other structures. In addition to this, it is well known that thrombus organisation modifies red thrombus to a wide range of hues, ranging from opaque red or pink (after endothelialization of the thrombotic surface) to gold (as macrophages are transformed into foam cells by digesting cholesterol from red blood cell membranes) and white (infiltration by myofibroblasts).[10-12] The use of directional atherectomy as a sampling tool in acute coronary syndromes has led to the suggestion that a higher prevalence of these changes are to be expected in-vivo than in post-mortem studies.[13,14] In this regard, it is foreseeable that the validation of in-vivo angioscopic observations will emerge from the concomitant use of other imaging devices and the study of atherectomy samples obtained during the same study (Fig. 2).

Angioscopy in stratification and assessment of patients with unstable angina

The contribution of angioscopy to the study of acute coronary syndromes goes well beyond the pure study of natural history and may contribute to unveil several management dilemmas posed by patients with unstable angina. The first of these has been precipitated by the failure of thrombolytic agents in reducing mortality or morbidity in that syndrome. Although the current paradigm of the syndrome postulates that mural thrombosis is the key cause of myocardial ischaemia in unstable angina through several mechanisms (thrombotic occlusion, enhanced vasoreactivity),[15,16] multiple randomized trials have failed in showing any clinical benefit of thrombolytic treatment.[17] The reasons for this failure are unknown. A lower prevalence of thrombus than expected from coronary angiography and post-mortem studies, or an enhanced resistance of thrombus to lytic therapy, secondary to organisation or protection from circulating lytic agents by mechanical barriers, have been proposed as possible explanations. It is clear that angioscopy could provide further insights on whether these hypothesis have a real basis. Furthermore, it is foreseeable that the combination of angioscopy and thrombolysis, which has been reported in experimental models,[9,18] will be more frequently used in the catheterisation laboratory in the near future. This might facilitate not only the selection of candidates for thrombolysis based on a more specific detection of thrombus, but also a more objective assessment of the success or failure of thrombolytic therapy. In a recent work, Inoue et al.[19] have reported on the success of systemic thrombolysis based on angiographic and angioscopic criteria. Residual thrombus was disclosed by angioscopy in 50% and 100% cases with TIMI reperfusion grades III and II respectively. In addition to the assessment of its efficacy, angioscopy may play a more participative role in coronary thrombolysis, facilitating the delivery of high concentrations of thrombolytic agents through the flushing port of the angioscope while assessing lysis (Fig. 3). Likewise, the effect of novel non-pharmacological thrombolysis such as that performed with ultrasonic devices,[20] which can that theoretically overcome some of the limitations of the pharmacological agents outlined above, could be also tested.

Angioscopy may also be useful in investigating the pathological substrate for the high complication rate of percutaneous coronary interventions in unstable patients. Again, the presence of intraluminal thrombus has been proposed as a key factor, since its disturbance may potentiate further episodes of thrombosis.[21,22] However, the use of thrombolytic agents prior to intervention in vessels with angiographically suspected thrombus has failed to reduce procedural complications, even showing a trend toward increase.[23] As discussed above, the use of angioscopy may contribute to a more selective stratification of patients based on the presence or extent of coronary thrombosis than can be achieved by applying angiographic criteria.[24] On the contrary, the detection of other mechanisms leading to sudden change in plaque morphology, such as plaque disruption, aggressive atherosclerosis or intramural hemorrhage may favor the selection of specific recanalisation techniques, such as balloon angioplasty, coronary stenting or atherectomy .

Optimizing and assessing the results of coronary intervention with angioscopy.

Angioscopy has been used to document the changes undergone by the vessel wall immediately after balloon angioplasty[18,25-27] and new interventional devices.[18,28-30] These studies have similar findings than those reported in post-mortem examination of vessels that had undergone percutaneous recanalisation and that frequently are undetected by angiography, such as intimal flaps, major vessel wall disruption, thrombus and subintimal hemorrhages.[31] In many of these instances, the information obtained with angioscopy can be comparable to that obtained with intravascular ultrasound. Figure 4A shows a major plaque dehiscence originating after balloon dilatation. The concomitant use of intravascular ultrasound imaging makes possible to document a deep extension of the dissection to medial layers (Fig. 4B) , similar to that found in a post-mortem examination of a dilated arterial segment (Fig. 4C).

The consequences of vessel wall laceration are twofold. Firstly, vessel wall dissection can lead to the development of acute vessel closure, as demonstrated in angiographic studies.[32] Several reports based on experimental[18,33] and clinical[34,35] work suggest that the use of angioscopy can be superior to angiography in detecting intimal flaps and filling defects originating from wall injury (Fig. 5). Using percutaneous angioscopy, Jain et al.[36] identified the primary cause of acute occlusion during PTCA in 10 patients as occlusive thrombus in 2 cases and vessel dissection in 8.

A second aspect of the consequences of vessel wall laceration during intervention is its impact on the long-term procedural outcome. Although reports on the use of angioscopy to study the substrate of restenosis are scarce,[29,37] it is likely that this application of angioscopy will be implemented in the design of future clinical stu-

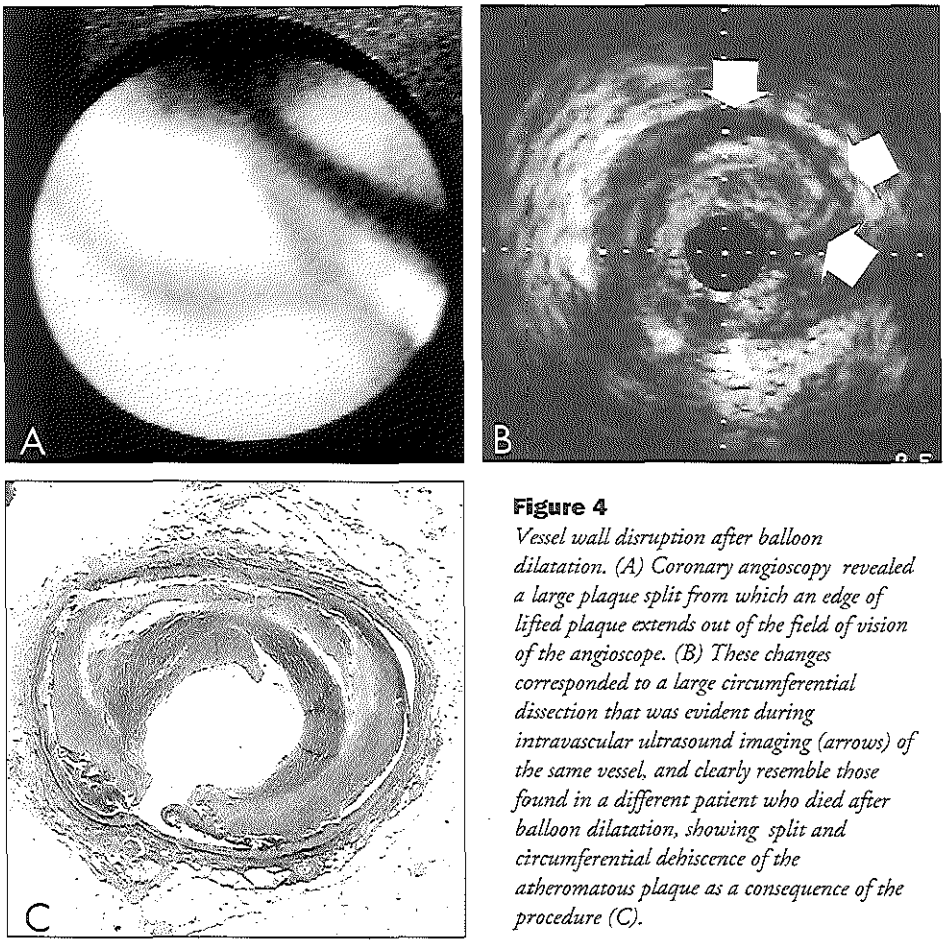

Figure 4
Vessel wall disruption after balloon dilatation. (A) Coronary angioscopy revealed a large plaque split from which an edge of lifted plaque extends out of the field of vision of the angioscope. (B) These changes corresponded to a large circumferential dissection that was evident during intravascular ultrasound imaging (arrows) of the same vessel, and clearly resemble those found in a different patient who died after balloon dilatation, showing split and circumferential dehiscence of the atheromatous plaque as a consequence of the procedure (C).

dies for the prevention of restenosis. The first and perhaps more unexplored area of restenosis consist in the identification of suboptimal procedural results that escape angiographic detection and that can lead to a phenomenon of "pseudorestenosis". This is particularly important since although coronary angiography has been the only method used in assessing restenosis post intervention, the complex vessel morphology associated with several recanalisation techniques makes difficult the interpretation of the angiographic image as to the gain in true luminal dimensions achieved, a fact that may explain why the reliability of quantitative coronary angiography decreases significantly after percutaneous interventions.[38,39]

A proportional relation between the extent of vessel wall injury and the subsequent loss of luminal dimensions has been suggested.[40-42] Angioscopic quantification of vessel damage may found a better predictive value than angiography, allo-

wing a more direct assessment of the results obtained with different recanalisation techniques.[20,26-30] The relation between wall injury and lining coronary thrombosis has also been proposed as a key factor in the development of neointimal proliferation,[43] and has been suggested as the cause for the high restenosis restenosis rate associated to percutaneous interventions performed in unstable patients.[44] As shown by den Heijer et al.,[29] angioscopy demonstrates coronary thrombus progression during the first hour after balloon angioplasty that is otherwise undetected by angiography. A wider application of this concept might provide information on the relationship between mural thrombosis and restenosis, as well as constitute a tool in assessing the efficacy of pharmacological strategies directed towards the avoidance of this phenomenon.

Although the use of angioscopy as a quantitative technique has been neglected, new methods are currently being applied to obtain an angioscopic estimation of

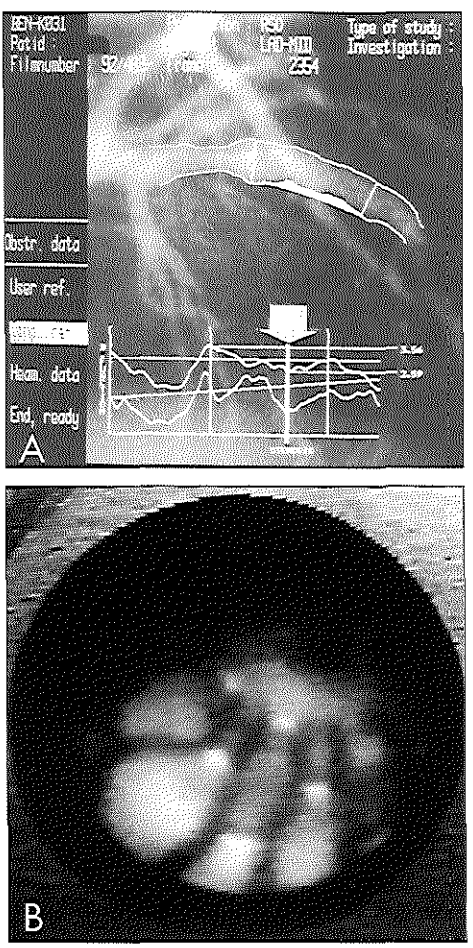

Figure 5

Quantitative angiographic analysis (A) after deployement of a Palmar-Schatz stent in the mid left anterior descending lesion, showing a large discrepancy (arrow) between luminal area obtained with edge detection (upper curve) and videodensitometric analysis (lower curve). During angioscopy a good result was noted in the distal subunit of the stent (B). However, a large area of disrupted vessel wall (C, arrows) was evident at the point where the two subunits of the stent are articulated by a single strut, failing to provide adequate scaffolding.

luminal size. Preliminary results with the use of a light wire have recently been reported by Spears et al.[44] Other authors have proposed a semi-quantitative approach, using the width of the guidewire as a scaling device. Several outstanding issues that may affect the reliability of the measurements obtained have yet to be solved. The distending coronary pressure during imaging may differ significantly from normal. Furthermore, a coaxial placement of the angioscope is not always possible to ensure complete visualization of the coronary lumen.

State-of-the-art and future developments in coronary angioscopy

From the first coronary angioscopy performed with a 1.8 mm thick fiber optic angioscope and reported by Spears et al. in 1983, the technique has undergone major changes. Today's angioscopes are highly sophisticated system that can be used without difficulty in most routine cases performed in the interventional catheterisation laboratory. In the following paragraphs we review some of the advantages and limitations of the state-of-the-art angioscopy, as well as some of the research trends in new applications.

Current angioscopy systems are built using bundles with a high number of independent fibers, tipped with highly regular epoxy lenses of less than 0.3 mm in diameter, have a high flexibility and provide excellent image quality. Charge couple device (CCD) cameras which are small and can be easily handled are used. Full compatibility with conventional over-the-wire equipment is now the rule. Better visualization is facilitated by a new generation of delivery catheters with low-pressure inflatable cuffs to temporarily interrupt antegrade blood flow. These systems are difficult to use in very proximal arterial segments or when cuff inflation compromises blood supply to more than one large epicardial vessel. A variety of dedicated irrigation pumps is used to flush transparent medium during angioscopy. The use of Ringer's lactate is preferred over saline by some operators since it has been suggested to have less arrhythmogenic potential. Oxygen-carrying solutions may facilitate longer visualization times during angioscopy, and its performance in patients with moderately impaired left ventricular function, or a large area of dependent myocardium, but their use has been limited thus far by high viscosity. Establishing the spatial location of the angioscope in the vessel during on-line and off-line image analysis can be facilitated by simultaneous recording of angioscopic and fluoroscopic images with a videomixer during the procedure. As progressive refinements and improvements are made, steerability of the catheter tip is now the main limitation to collecting adequate information, particularly in tortuous vessels. Several systems to correct for misalignment of the angioscope and to facilitate more selective visualization are under development.

Many of the strengths of coronary angioscopy discussed in previous sections of this article are related to the capability for retrieving chromatic information of the structures present in the luminal aspect of the vessels. Most available systems have

automatic white balance systems aimed to ensure a faithful reproduction of luminal colours. However, the angle of incidence of the light beam on the visualized surface and manual adjustment of light intensity, which is frequently needed to optimize visualization, may significantly alter colours in the obtained images. Although no studies are yet available on the interobserver variability associated with this type of observations, the fact that in a normal population the ability to distinguish between colours varies significantly from one individual to other makes probable that it is significantly high. Automated colour analysis can possible contribute to the solution of this limitation, as suggested by preliminary results obtained by our group. Other factors may interfere with colour characterization, including the chromatic aberration resulting from the absorption of particular wave-lengths by optic fibers and the modifications due to magnetic storage in videotapes and their subsequent off-line analysis.

Further technological developments in image processing may allow interpretation of angioscopically obtained images to provide objective information on the constitution of the atherosclerotic vessel wall, which does not rely on subjective evaluation. Based on the characteristic absorption patterns of the constituents of the atheromatous plaque, several groups have reported in the possibility of applying laser-induced fluorescence to the diagnosis of atherosclerosis.[45-47] Although still in its developmental stages, this "spectroscopic angioscopy"[46] might provide insights to the structure of the vascular wall which have so far been confined to the realm of intravascular ultrasound imaging.

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References

1. *Shapiro TA, Herrmann HC . Coronary angiography and interventional cardiology. Curr Opin Radiol; 1992; 4: 55-64.*

2. *Siegel RJ, Ariani M, Fishbein MC, Chae JS, Park JC, Maurer G, Forrester JS. Histopathologic validation of angioscopy and intravascular ultrasound. Circulation; 1991; 84: 109-17.*

3. *Mizuno K, Miyamoto A, Isojima K, Kurita A, Senoo A, Arai T, Kikuchi M, Nakamura H. A serial observation of coronary thrombi in vivo by a new percutaneous transluminal coronary angioscope. Angiology 1992; Feb:91-99.*

4. *Sherman CT, Litvack F, Grundfest W et al. Coronary angioscopy in patients with unstable angina pectoris. N Eng J Med 1986; 315:913-9.*

5. *Mizuno K, Miyamoto A, Satomura K, Kurita A, Arai T, Sakurada M, Yanagida S, Nakamura H. Angioscopic coronary macromorphology in patients with acute coronary disorders. Lancet 1991; 337:809-12.*

6. *Mizuno K, Satomura K, Miyamoto A, Arakawa K, Shibuya T, Arai T, Kurita A, Nakamura H, Ambrose JA. Angioscopic evaluation of coronary-artery thrombi in acute coronary syndromes. N Eng J Med 1992; 326:287-91.*

7. *Hombach V; Hoher M; Kochs M; Eggeling T; Schmidt A; Hopp HW; Hilger HH . Pathophysiology of unstable angina pectoris—correlations with coronary angioscopic imaging. Eur-Heart-J 1988; 9 (Suppl N):40-5.*

8. *Fuster V, Badimon L, Badimon JJ, Chesebro JH. The pathogenesis of coronary artery disease and the acute coronary syndromes. N Engl J Med 1992;326:242-50 and 310-18.*

9. *Tomaru T, Uchida Y, Sugimoto T. Fiberoptic study on the effects of transluminal angioplasty in experimental occlusive arterial thrombosis. Am Heart J 1988; 115:312-17.*

10. *Hand RA, Chandler AB: Atherosclerotic metamorphosis of autologous pulmonary thromoemboli in the rabbit. Am J Pathol 1962; 40:469-86.*

11. *Dible JH: Organisation and canalisation in arterial thrombosis. J Path Bact 1958; 75: 1-7.*

12. *Pearson TA, Dillman J, Solez K, Heptinstall RH: Monoclonal characteristics of organising arterial thrombi: Significance in the origin and growth of human atherosclerotic plaques. Lancet, 1979; 1:7-11.*

13. *Isner JM, Brinker JA, Gottlieb RS, Leya F, Masden RR, Shani J, Kearney M, Topol EJ ,for CAVE-AT. Coronary thrombus: Clinical features and angiographic diagnosis in 370 patients studied by directional coronary atherectomy. Circulation 1992 (Suppl. I); 86:I-648.*

14. *Escaned J, van Suylen RJ, MacLeod DC, Umans VA, de Jong M, Bosman FT, de Feyter PJ, Serruys PW: Histological characteristics of tissue excised during directional coronary atherectomy in stable*

and unstable angina pectoris. Am J Cardiol 1993 (In press).

15. Ambrose JA: Plaque disruption and the acute coronary syndromes of unstable angina and myocardial infarction: If the substrate is similar, why is the clinical presentation different? J Am Coll Cardiol 1992; 19:1653-8.

16. Fuster V, Badimon L, Cohen M, Ambrose JA, Badimon JJ, Chesebro J: Insights into the pathogenesis of acute ischemic syndromes. Circulation 1988; 77:1213-20

17. Scrutino D, Biasco MG, Rizzon P. Thrombolysis in unstable angina: Results of clinical studies: Am J Cardiol 1991; 68:99B-104B.

18. Beck A, Reinbold WD, Blum U, Nanko N, Milic S, Papacharalampous X . Clinical application of percutaneous transluminal angioscopy. Comparison of findings in percutaneous transluminal angioplasty, thrombolysis, thrombus-extraction and stent-application. Herz 1988; 13:392-9.

19. Inoue K, Kuwaki K, Ochiai H, Mukaiyma Y. Plaque morphology underlying occlusive thrombus in acute myocardial infarction as encountered using percutaneous angioscopy. J Am Coll Cardiol 1993; 21:195A.

20. Ariani M, Fishbeim MC, Chae JS, Sadeghi H, DonMichael A, Dubin SB, Siegel RJ. Disolution of peripheral arterial thrombi by ultrasound. Circulation 1991; 84: 1680-88.

21. Myler RK, Shaw RE, Stertzer SH, Bashour TT, Ryan C, Hecht HS, Cumberland DC. Unstable angina and coronary angioplasty. Circulation 1990; 82 [supp II]:88-95.

22. Badimon L, Lasila R, Badimon J, Fuster V. Residual thrombus is more thrombogenic than severely damaged vessel wall. Circulation 1988; 78-suppl II: II-119.

23. Haine E, Urban P, Verine V, Mehan K, Dorsaz PA, Meier B. Lack of immediate benefit of urokinase prior to angioplasty for unstable angina. A double-blind, randomized study. J Am Coll Cardiol 1993; 21:435A.

24. den Heijer P, van Dijk RB, Pentinga ML, Lie KI. Serial angioscopy during the first hour after successful PTCA. Circulation 1992; 86 (suppl I): I-458.

25. Siegel RJ, Chae JS, Forrester JS, Ruiz CE . Angiography, angioscopy, and ultrasound imaging before and after percutaneous balloon angioplasty. Am Heart J, 1990. 120:086-90.

26. Uchida Y, Hasegawa K, Kawamura K, Shibuya I. Angioscopic observations of the coronary luminal changes induced by percutaneous transluminal coronary angioplasty. Am Heart J 1989; 117:769-76.

27. Ramee SR, White CJ, Collins TJ, Mesa JE, Murgo JP . Percutaneous angioscopy during coronary angioplasty using a steerable microangioscope. J Am Coll Cardiol 1991; 17:100-5.

28. Bergeron P, Rudondy P, Poyen V, Pinot JJ, Alessandri C, Martelet JP. Long-term peripheral stent evaluation using angioscopy. Int Angiol, 1991; 10:82-6.

29. Resar JR, Brinker J. Early coronary artery stent restenosis: Utility of percutaneous angioscopy.

Catheterisation Cardiovasc Diagn 1992; 27:276-79.

30. Nakamura F, Kyasnicka J, Uchida Y, Geschwind R. Percutaneous angioscopic evaluation of luminal changes induced by excimer laser angioplasty. Am Heart J 1992; 124:1467-72.

31. Waller BF: Pathology of coronary angioplasty and related topics. In: Topol EJ, ed. Textbook of Interventional Cardiology. Philadelphia. WB Saunders Company, 1990: 395-451

32. Black AJ, Namay DL, Niederman AL, Lembo NJ, Roubin GS, Douglas JS Jr, King SB. Tear or dissection after coronary angioplasty. Morphologic correlates of an ischemic complication. Circulation 1989; 79: 1035-42.

33. Neville RF Jr, Yasuhara H, Watanabe BI, Canady J, Duran W, Hobson RW 2d. Endovascular management of arterial intimal defects: an experimental comparison by arteriography, angioscopy, and intravascular ultrasonography. J Vasc Surg,1991; 3:496-502.

34. Richens D, Renzulli A, Hilton CJ . Dissection of the left main coronary artery: diagnosis by angioscopy. Ann Thorac Surg 1990; 49:469-70.

35. Siegel RJ, Fishbein MC, Chae JS, HelfAnt RH, Hickey A, Forrester JS. Comparative studies of angioscopy and intravascular ultrasound for the evaluation of coronary artery disease. Echocardiography 1990; 7:495-502.

36. Jain SP, White CJ, Collins TJ, Escobar A, Ramee SR. Etiologies of acute occlusion after PTCA: Angioscopic morphology. J Am Coll Cardiol 1993; 21:484A.

37. White CJ, Ramee SR, Mesa JE, Collins TJ . Percutaneous coronary angioscopy in patients with restenosis after coronary . J Am Coll Cardioll 1991 May; 17(6 Suppl B); P 46B-49B

38. Katristsis D, Webb-Peploe M. Angiographic quantitation of the results of coronary angioplasty: Where do we stand? Cath Cardiovasc Diagn 1990; 21:65-71.

39. Sanz ML, Mancini J, LeFree MT, Mickelson JK, Starling MR, Vogel RA, Topol EJ: Variability of quantitative digital subtraction coronary angiography before and after percutaneous transluminal coronary angioplasty. Am J Cardiol. 1987; 60: 55-60.

40. Schwartz RS, Huber KC, Murphy JG, Edwards WD, Camrud AR, Vlietstra RE, Holmes DR. Restenosis and the proportional neointimal response to coronary artery injury: results in a porcine model. J Am Coll Cardiol 1992;19:267-74.

41. Foley DP, Hermans WR, de Jaegere PP et al. Is "bigger" really "better"? A quantiative angiographic study of immediate and long term outcome following balloon angioplasty, directional atherectomy and stent implantation. Circulation 1992; 86(Suppl. 4): I-530.

42. Kuntz RE, Gibson CM, Nobuyoshi M et al. Generalised model of restenosis after balloon angioplasty, stenting and directional coronary atherectomy. JACC 1993; 21:15-25.

43. Ip JH, Fuster V, Badimon L, Badimon J, Taubman MB, Chesebro JH. Syndromes of acelerated atherosclerosis: Role of vascular injury and smooth muscle cell proliferation. J Am Coll Cardiol 1990; 15:1667-87.

44. Spears JR, Raza SJ, Ali M, Iyer GS, Cheong WF, Crilly RJ. Quantitative angioscopy: A new method for measurement of coronary dimensions by use of a "lightwire". J Am Coll Cardiol 1993; 21:133A.

45. Kittrell C, Willett RL, Santos-Pacheo C et al. Diagnosis of fibrous arterial atherosclerosis using fluorescence. Appl Optics 1985; 24:2280-1.

46. Fitzmaurice M, Bordagaray JO, Engelmann GL et al. Argon ion laser-excited autofluorescence in normal and atherosclerotic aorta and coronary arteries: Morphological studies. Am Heart J 1989; 118:1028-38.

47. Leon MB, Lu DY, Prevosti LG, Macy WW, Smith PD, Garnowsky M, Bonner RF, Alaban RS. Human arterial surface fluorescence: Atherosclerotic plaque identification and effects of laser atheroma ablation. J Am Col Cardiol 1988; 12:94-102.

Chapter VIII

Intracoronary Ultrasound and Angioscopic Imaging Facilitating the Understanding and Treatment of Postinfarction Angina

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Abstract

We report on the use of intravascular ultrasound, coronary angioscopy and on-line quantitative angiography in a unstable patient soon after myocardial infarction. Combined intracoronary imaging made possible to solve the therapeutic problem posed by an unusual angiographic appearance secondary to intracoronary thrombolysis during coronary recanalisation. The concomitant use of directional atherectomy made possible the pathological validation of the observations performed with angioscopy and intravascular ultrasound.

Introduction

We present the case of a patient with postinfarction angina where the combined use of on-line quantitative analysis, intravascular ultrasound and angioscopic imaging greatly facilitated the therapeutic strategy and the understanding of the pathological substrate of a patient with an unusual angiographic morphology after intracoronary thrombolysis.

Case report

We report on a 49 year old white caucasian male with post-infarction angina pectoris was referred to our Institution as a candidate for percutaneous coronary angioplasty. The patient had been admitted 9 days before in a different Hospital with chest pain at rest lasting for more than 48 hours. An ECG disclosed sinus rhythm with Q waves in leads II, III and aVF. Physical examination, chest X ray, blood biochemistry (CPK 120 U/l) and hematology were normal. Blood pressure was 135/70 mm Hg. Coronary risk factors included smoking and a family history of coronary artery disease. After admission he had intermittent episodes of chest pain at rest. Intravenous nitroglycerine and heparin were started. Betablockers, calcium antagonists and aspirin were also given. Coronary angiography performed at the referring Hospital revealed a severe eccentric stenosis with smooth borders in the mid right coronary artery which was identified as the culprit lesion. Left ventricular angiography disclosed an ejection fraction of 52% and an area of posterobasal hypokinesis.

The following day the patient was transferred to our Institution for percutaneous coronary intervention. Repeat angiography of the right coronary disclosed a new overhanging, moving filling defect originating from one of the borders of the stenosis (Fig. 1A). Some minor irregularities were noted in the proximal segment of the vessel, which were judged to correspond to a separate, non-significant stenosis. Quantitative angiography was performed on-line from the digital angiogram (ACA, Philips DCI, Eindhoven, The Netherlands). Stenosis severity in the mid segment was 76%, obstruction diameter was 0.84 mm and the interpolated reference diameter was 3.55 mm. Interpolation of the reference dimensions showed inverted tapering of the vessel, which was thought to indicate the existence of post-stenotic dilatation (Fig. 2A). The moving defect was identified as a tail of thrombus and intracoronary thrombolysis was performed with rTPA (50 mg over 30 minutes). Repeat angiography 30 minutes later revealed the disappearance of this overhanging filling defect and an obstruction diameter of 0.82 mm, but was followed by the appearance of a new large contrast opacification protruding outside the luminal borders identified in previous views (Fig. 1B). The nature of this new opacification could not be ascertained, and the possibilities considered included major intraplaque bleeding, extravasation of contrast medium or vessel dissection, all of them posing a serious problem to continuing thrombolytic treatment.

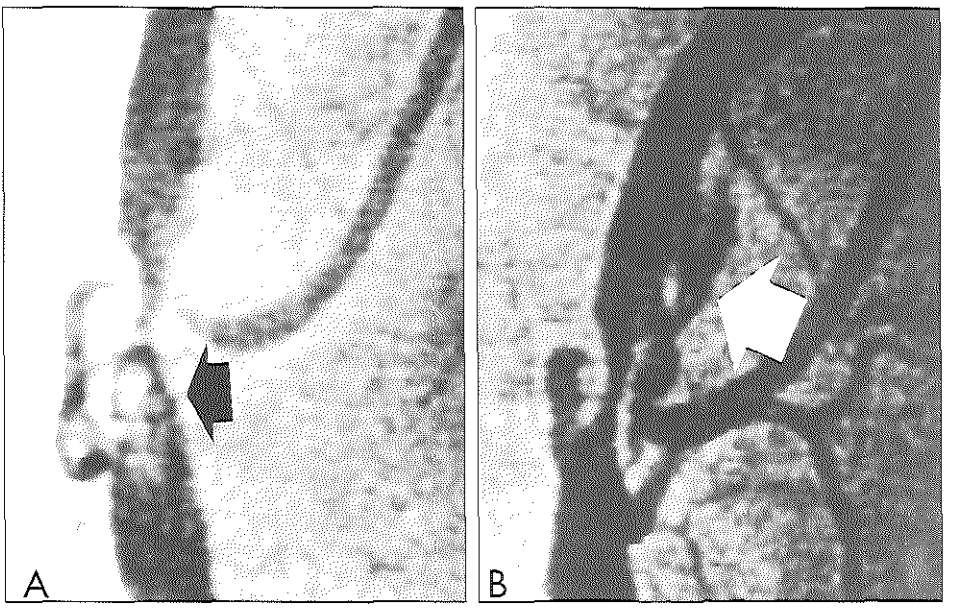

Figure 1

A: Coronary angiography showing an overhanging, mobile filling defect (black arrow) distal to the mid right coronary artery stenosis that was identified as a tail of thrombus. Minor irregularities in the proximal segment were also noted but thought to correspond to a separate non-significant stenosis. B: Repeat angiography 30 after intracoronary thrombolysis disclosed complete disappearance of the filling defect. However, a new large contrast opacification protruding out the original luminal borders (white arrow) became evident.

In an attempt to obtain complementary information on the changes taking place within the arterial wall, two-dimensional ultrasound imaging of the proximal and stenotic segment was performed with a 4.3 Fr 30 MHz intracoronary probe (Cardiovascular Imaging Systems, Inc., California). Proximal to the stenosis the vessel showed a well-defined ultrasonic 3-layer appearance (Fig. 3). The area comprised within the ultrasound-defined internal elastic lamina was 25.5 mm^2, much larger than expected from the angiographically-derived reference area (9.92 mm^2). Only 7.9 of the total 25.5 mm^2 (31%) corresponded to vessel lumen, the remaining corresponding to occluding material presented low echodensity and speckling. Proximal to the stenosis several luminal channels, that were best visualized during the injection of saline , were seen (Fig. 3). The penetration of contrast medium in these newly formed channels or lacunar cavities was identified as the cause for the protruding opacification. Based on this information, a large mass of remaining thrombus was judged as the underlying substrate of the images, in spite that intravascular ultrasound has a low sensitivity in detecting thrombotic material.[1] Since any contraindication for thrombolysis was ruled out, an intravenous infusion of streptokinase (1.500.000 units) was started and continued overnight. The patient was reinvestigated the following day. Repeat quantitative angiography

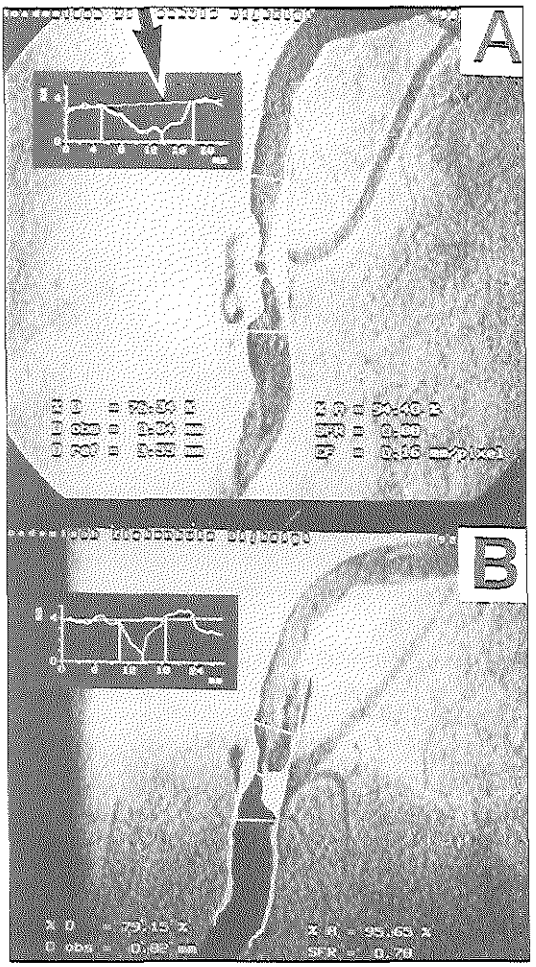

Figure 2

A: On-line quantitative angiographic analysis performed prior to thrombolysis. An inverted tapering of the vessel is noted in the diameter function (arrow). B: Quantitative analysis after thrombolysis. Although there is no substantial change in obstruction diameter, significant remodeling of the plaque is evident (white plaque area), with normalization of the inverted vessel tapering found the day before.

revealed significant remodeling of the stenosis, with marked disappearance of the plaque bulk and considerable expansion of the extraluminal opacification. The obstruction diameter remained virtually unchanged (0.82 mm). The reference diameter was 3.94 mm (0.49 mm increase from the previous day). Marked attenuation of the inverted vessel tapering demonstrated during quantitative analysis the day before was evident(Fig. 2B). Coronary angioscopy (Baxter Edwards, Irvine, California) was performed to discriminate between the components of the stenosis on the grounds of their chromatic characteristics. This angioscopy system used includes a proximal compliant occlusive cuff that abolishes antegrade blood flow to facilitate visualization of the lumen while Ringer's lactate is flushed. A mobile optical bundle with 5 cm capacity was manipulated to obtain images over the coronary segment of interest. The luminal surface showed a very irregular morphology, with wall disruption, intraluminal flaps and large areas of red material extending from the proximal right coronary segment to the site of the stenosis (Fig. 4), confirming that the occluding material identified by intravascular ultrasound was thrombus. Vessel disruption and areas of yellowish material were also seen. Directional atherectomy was performed using a 7 Fr atherocatheter (DVI, San Diego, California). Three passages were done at the stenosis site. The retrieved material showed areas of dense fibrous tissue and thrombus, the latter containing cholesterol crystal clefts and necrotic debris (Fig. 5).

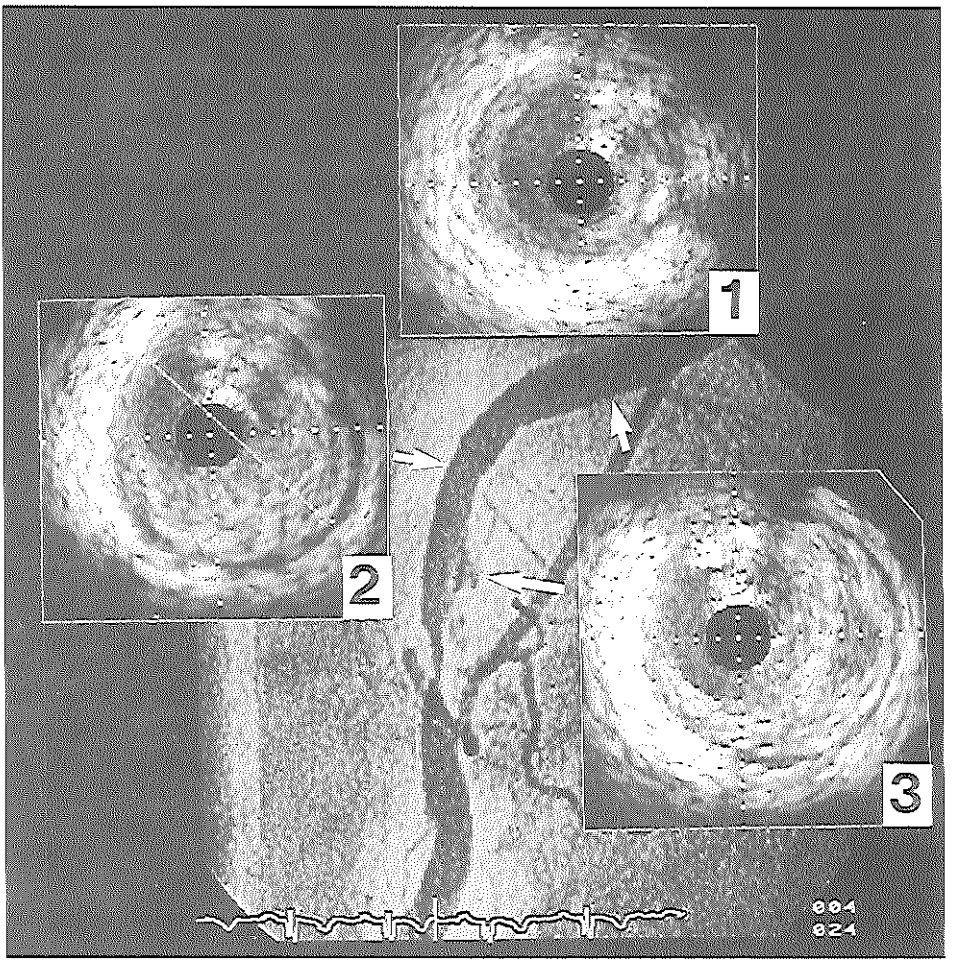

Figure 3

Intravascular ultrasound imaging immediately after intracoronary thrombolysis. In the proximal segment of the vessel only minimal atheroma was disclosed following a concentric distribution (1). However, further advance of the probe revealed a larger vessel than expected from coronary angiography, occluded by homogeneous material of moderate echogenicity with a concentric lumen in eccentric position (2). At the level of the protruding angiographic opacification (3), the occluding material showed areas of low echogenicity suggestive of newly formed vascular channels or lacunar spaces.

The immediate result of atherectomy was good as judged from the images obtained with the 11 Fr atherectomy catheter, and the atherectomy system, including the guidewire, was removed. However, repeat angiography performed with a conventional 7 Fr diagnostic coronary catheter revealed an major ostial dissection of the right coronary artery which rapidly lead to acute vessel occlusion. The dissection could not be recrossed with the guiding wire and the procedure was abandoned.

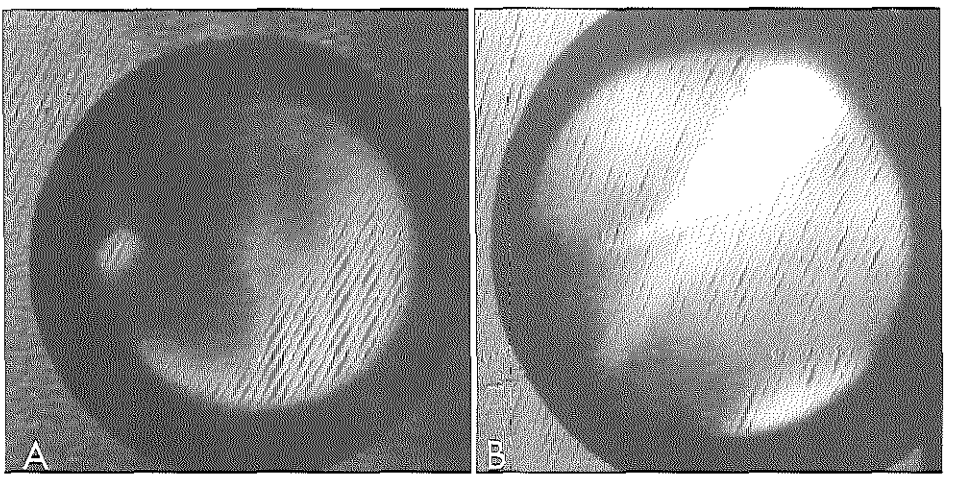

Figure 4

A: Coronary angioscopy showing red material with large disruption of the vessel wall. B: The true vascular lumen can be identified by the position of the guidewire (W) used to cross the lesion.

No emergency surgery was performed. The patient developed transient 1 mm ST elevation in leads II, III and aVF and ST depression in the anteroseptal leads without concomitant hemodynamic embarrassment. The CPK raised to 492 U/l. After this episode of reinfarction the patient followed an uncomplicated evolution and was discharged 8 days later.

Discussion

It is well known that the complication rate of coronary angioplasty in unstable patients is higher than in stable patients, particularly in the presence of coronary thrombus. In those cases, intracoronary thrombolysis prior to coronary intervention has been used in attempt to decrease procedural risk.[2] However, the induction of a lytic state may facilitate the extension of plaque fissures to deep layers of the vessel wall, leading to the formation of an intramural or extravascular haematoma and causing subsequent vessel dissection or lumen compression.[3] This was suspected from the angiographic image shown in Figure 1B. The additional information obtained with intravascular ultrasound imaging excluded such potential complication and suggested continued thrombolytic treatment for the dissolution of an otherwise unsuspected large thrombotic mass. The criteria used for the ultrasonic characterization of the occluding material were later reinforced by angioscopic examination.

Other observations made in this case illustrate several interesting aspects of the pathophysiology of myocardial infarction and post-infarction angina. Plaque rup-

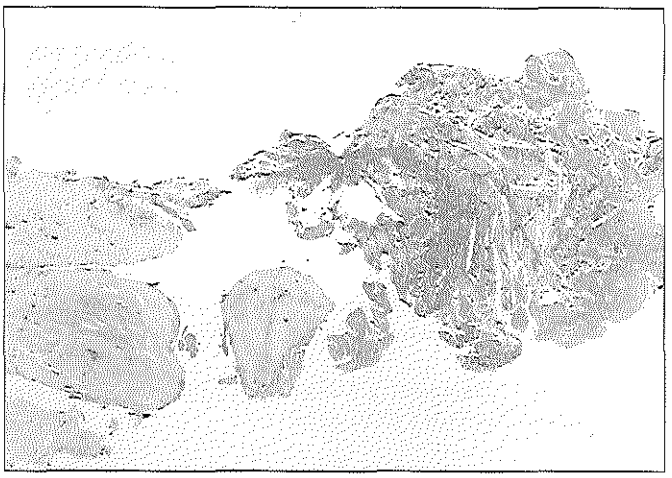

Figure 5
Atherectomy specimen obtained from the lesion showing dense fibrous tisue (left) and fresh thrombotic material (right) in association with areas of cholesterol crystal clefts and inflammatory cells.

ture and exposure of plaque material to the bloodstream has been suggested as a common initial event of acute coronary syndromes.[3] During plaque rupture embolisation of atheromatous material has been shown to occur.[4] This is consistent with the histopathological findings in the atherectomy retrieved tissue of the present case showing cholesterol crystal clefts isolated from plaque material and embedded in the retrieved thrombus. The fresher tail of thrombus that had developed at the narrowest stenotic point in the interval from the diagnostic angiogram and that was first lysed during the administration of intracoronary thrombolytics was probably the combined result of preexisting thrombus and high shear stress at that point of the vessel.[5] As observed by other authors, remodeling of stenosis morphology secondary to the lysis of older thrombus was observed during angiography after sustained thrombolysis and anticoagulation.[6] However, the present case illustrates how visual angiographic assessment may underscore the degree of remnant coronary thrombosis. The concomitant use of on-line computerized angiographic analysis can improve the sensitivity of coronary angiography and offer additional data that otherwise might be missed by the observer, such as changes in tapering characteristics of the vessel or modifications in luminal dimensions secondary to thrombolysis (Fig. 2).

Angioscopic and intravascular ultrasound findings have been previously validated in vitro,[1] suggesting that the information obtained with both methods can be complementary in the study of coronary artery disease. The present case shows how different imaging techniques can contribute to the solution of a specific problem found during coronary intervention, and how tissue retrieved by directional atherectomy can facilitate the validation in-vivo of such observations. Although we feel that the unfortunate ostial dissection that complicated this case was related to the withdrawal of the atherectomy catheter, the possibility that this could be related to the use of concomitant intracoronary imaging cannot be ruled out. Should further studies demonstrate the safety of intracoronary ultrasound and

angioscopic imaging, the harmonic integration of data obtained by these two techniques may contribute to the understanding of the anatomopathological substrate of coronary syndromes, and subsequently influence the therapeutic management.

References

1. *Siegel RJ, Ariani M, Fishbein MC, Chae JS, Park JC, Maurer G, Forrester JS. Histopathologic validation of angioscopy and intravascular ultrasound. Circulation: 1991; 84: 109-17.*

2. *Pavlides GS, Schreiber TL, Gangadharan V, Puchrowicz S, O'Neill WW. Safety and efficay of urokinase during elective coronary angioplasty. Am Heart J 1991; 121:731-7.*

3. *Davies MJ. Successful and unsuccessful coronary thrombolysis. Br Heart J 1989; 61:381-4.*

4. *Falk E. Morphologic features of unstable atherothrombotic plaques underlying acute coronary syndromes. Am J Cardiol 1989; 63:114E-120E.*

5. *Badimon L, Badimon JJ. Mechanisms of arterial thrombosis in non-parallel streamlines: Platelet thrombi grow on the apex of stenotic severely injured vessel wall. Experimental study in the pig model. J Clin Invest 1989; 84:1134-1144.*

6. *Davies SW, Marchant B, Lyons JP, Timmis AD, Rothman MT, Layton CA, Balcon R. Coronary lesion morphology in acute myocardial infarction: Demonstration of early remodeling after streptokinase treatment. J Am Coll Cardiol 1990; 16:1079-86.*

Chapter IX

The Cause of Coronary Luminal Obstruction in Unstable Angina Refractory to Medical Treatment: Insights from Angioscopy and Directional Atherectomy

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Abstract

Background

Coronary angioscopy constitutes a potential tool in determining which is the predominant cause of coronary obstruction in unstable patients refractory to medical treatment.

Aim of the study

To investigate with intracoronary angicoscopy the characteristics of the culprit lesion of primary or post-infarction unstable angina pectoris refractory to medical treatment, and to establish the relationship between the obtained visual findings with coronary angiography and histopathological characteristics of retrieved atherectomy specimens.

Methods

Angioscopy was performed in 41 patients at the time of percutaneous recanalisation. In all cases visual findings were classified objectively, without making a translation to histological terms. In 9 (22%) examination of tissue samples obtained during directional atherectomy possible the correlation between histopathological and angioscopic features. Quantitative angiography and classification of angiographic morphology was performed in all cases and correlated with angioscopic findings.

Results

Red material was seen in 25 (60%) cases, more frequently in patient with post-infarction angina (13/17, 76%) than in primary unstable angina (12/ 24, 50%), and protruded significantly in the lumen in 7 (41%) patients with postinfarction and in 3 (12%) with primary unstable angina. Atherectomy specimens revealed the presence of red thrombus. The protrusion of red material correlated significantly with a lower minimal luminal diameter (0.55±0.56 mm versus 1.11±0.42 mm in stenoses with and without protruding red material, p=0.009) and greater percent diameter stenosis (79±19 % versus 64 ±15% in stenoses with and without protruding red material, p=0.04). Conversely, the angiographic morphology correlated poorly with the presence of red material during angioscopy (15/21, 71%, with and 10/20, 50%, without complex angiographic morphology (p=NS). Xanthomatous plaques presented more frequently in patients with postinfarction (10/17, 58%) than in unstable patients (5/24, 21%) (p = 0.02), frequently in association with red material (13/15, 87%, versus 2/15, 13%, in stenosis with and without red material respectively, p=0.02), and were associated with deposits of cholesterol crystal clefts in 2 atherectomy specimens. Pink areas were identified in 12 (50%) and 2 (12%) patients with unstable and post infarction angina respectively (p = 0.03). White-gray protruding masses were observed more frequently in unstable (8/24, 33%) than postinfarction patients (3/17, 19%), with 2 atherectomy specimens revealing fibrin-rich and organising thrombus. Finally, an stenotic vessel wall with normal coloration was found in 4 (10%) cases, 3 of which showing an smooth surface and 1 evidence of wall disruption.

Conclusions

These observations provide information as to the cause of luminal obstruction in unstable patients refractory to medical treatment that may be relevant for the formulation of therapeutic strategies.

Introduction

The understanding of the pathological substrate of acute coronary syndromes has been based largely on post-mortem studies of coronary arteries.[1-4] These studies have suggested that sudden disruption of the atheromatous plaque is the common initiating factor of myocardial infarction, primary unstable angina and sudden death, through the onset of several pathogenic mechanisms such as platelet activation, adhesion and aggregation, vasoconstriction and thrombus formation.[5,6]

In spite of the considerable knowledge accumulated on these pathophysiological mechanisms, the management of primary unstable and post-infarction angina pectoris still presents a major therapeutic problem.[7,8] Conventional diagnostic tools provide no direct insight on which pathogenic mechanism is operative or prevails in an individual patient presenting with unstable symptoms. This may partly explain the refractoriness to conventional treatment. Furthermore, it is possible to speculate that some of the pitfalls in the pharmacological treatment of unstable angina are due to incomplete understanding of a continuously evolving pathological substrate.

Percutaneous angioscopy constitutes a unique tool in the study of the coronary syndromes, by facilitating direct visualization of changes taking place in the luminal environment of the culprit stenosis.[9-13] Previous angioscopic studies[11,12] have identified marked differences in the characteristics of the culprit lesion in stable and unstable patients. However, these studies have been focused on changes at the time of the initial clinical presentation, which may be substantially different from those found in patients in whom medical treatment has proved ineffective. Furthermore, a common bias of these studies has been the direct translation of visual findings to pathological terms by the investigating cardiologist.

In the present study we investigated the angioscopic substrate of patients presenting with refractory unstable angina with or without a history of recent myocardial infarction. Visual findings were objectively classified according to chromatic and morphological characteristics, and conclusions on the corresponding pathological substrate were drawn from histological study of atherectomy specimens obtained in a representative sample of the study population.

Patients and methods

During the period between September 1992 and May 1993 percutaneous coronary angioscopy was performed in 41 patients presenting initially with primary unstable angina (class IIB and IIIB in the classification proposed by Braunwald[14]) or postinfarction angina (class IIIC in the same classification).[14] In all cases the patients were receiving intravenous heparin and nitrates since the date of admission. In 6 cases (15%) systemic thrombolysis had been performed. In addition, all

patients were receiving oral antianginal medication, consisting of beta blockers, calcium antagonists or a combination of both. Catheterization was performed with a view to coronary intervention due to persistent clinical evidence of myocardial ischaemia. The investigations were approved by the Institutional Review Board of the Cardiology Department of the Dijkzigt Ziekenhuis and the patients were studied only after giving informed consent.

Table I. Baseline characteristics of 41 patients

n= 41	
Age (years)	59±10
Male sex	33 (80%)
Time from onset of syndrome (days):	
-Unstable angina	18±16
-Post-infarction angina	15±15
Ischemia-related vessel :	
-RCA	17 (41%)
-LAD	15 (36%)
-LCX	9 (23%)
Reference diameter (mm)	3.15±0.71
Minimal luminal diameter (mm)	0.98±0.51
% diameter stenosis (%)	67±16
Complex angiographic morphology	21 (51%)
Multivessel disease	9 (23%)
Prior coronary intervention	3 PTCA, 2 DCA
Thrombolysis	6 (35 % of all post MI)

RCA: right coronary artery; LAD: left anterior descending coronary artery; LCX: left circumflex coronary artery; MI: myocardial infarction.

Procedures

Selective coronary angiography in multiple projections was performed before and after intervention. All patients received aspirin (250 mg) and i.c. nitroglycerin before the procedure and were on optimal anticoagulation with heparin, with an activated clotting time was over 300 seconds. During the procedure patients received intravenous diazepam. After passage of a 0.014 in. guide wire across the culprit lesion, coronary angioscopy was performed. In all cases an attempt was made to cross the lesion with the angioscope to obtain information from both the proximal and distal aspect of the lesion. Following angioscopy, balloon angioplasty (n=32) or directional coronary atherectomy (n=9) were performed according to standard practice.

Selection of ischemia-related lesion
Culprit lesion was selected as follows: in cases with single vessel disease the most severe lesion within that vessel was selected; in cases with multivessel disease the selection was determined using angiographic and electrocardiographic criteria, including the location of transient ST-T segment changes in the electrocardiographic leads occurring during ischaemia at rest.

Angiography
Luminal dimensions at the stenotic and reference segment were obtained with quantitative coronary angiographic analysis, which was performed using the CAAS Mark II system,[15,16] which constitutes the latest generation of a previously validated system.[17] Qualitative information was also recorded according to a modification of the angiographic classification of lesion morphology proposed by Ambrose et al.[18] Complex lesion morphology was recorded when eccentric lesions with ragged or overhanging edges were present, or when intraluminal filling defects were noted.

Angioscopic device
The angioscope used was a 4.5 Fr polyethylene monorail catheter (Baxter-Edwards, Irvine, California). The catheter features a compliant occlusive cuff, a flush port, guidewire compatibility and a movable optical bundle with a depth of field > 1 mm and an excursion capability of 5 cm. In order to keep a uniform color temperature in the light source, light intensity is modified by the use of a diaphragm. Thus, artificial variations in the observed colors were minimized. To facilitate the review process of the obtained images, real-time fluoroscopy or cine-angiography was recorded simultaneously with angioscopic images using a digital videomixer. This provides a better estimation of the location of the angioscope within the coronary tree.

Analysis of angioscopic images
All images were analysed off-line by three independent interventional cardiologists familiar with coronary angioscopy. In addition, the films were reviewed by an independent cardiovascular pathologist blinded to clinical, angiographic and pathological data. At the site of the stenosis the arterial wall was classified as disrupted (loss of continuity in the arterial wall), irregular (rough surface with wall integrity preserved), or predominantly smooth surface. The presence of red material was recorded, and classified as mural (lining the arterial wall but without significant protrusion in the lumen) or occlusive (accounting for a significant obliteration of the arterial lumen during angioscopy). White protruding masses were recorded independently. The presence of pink areas in the arterial wall or on the surface of a white protruding mass was also recorded. Yellow plaques were defined as areas of homogeneous yellow color clearly identifiable from a neighboring area of normal white wall.

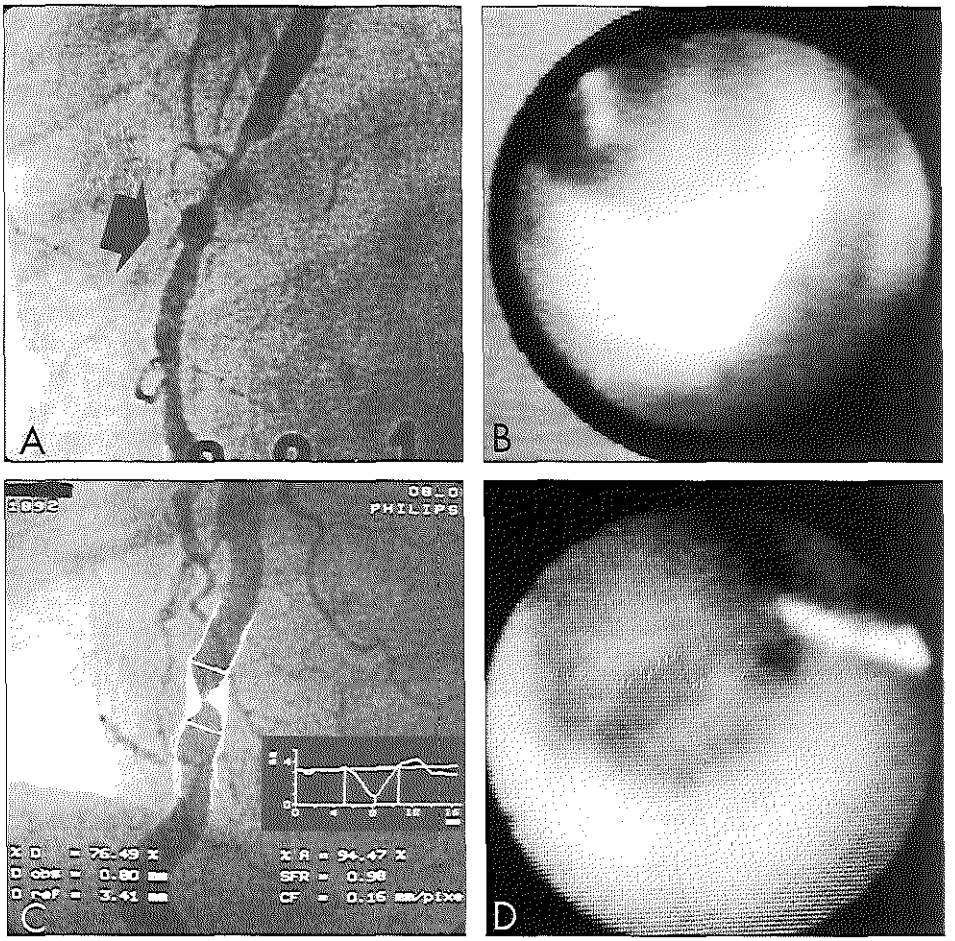


Figure 1

Red material as visualised by angioscopy in 2 patients with primary unstable angina and culprit stenoses located in the mid right coronary artery. A: Coronary arteriogram showing an eccentric lesion with an overhanging edge in its distal aspect (arrow). B: Angioscopy revealed that the latter was due to vessel wall disruption, with underlying mural red material. C: On-line quantitative angiographic analysis of a concentric stenosis. D: A protruding reddish mass, clearly distinguishable from the vessel wall, became evident during angioscopy.

Histopathological studies of atherectomy specimens

In the 9 cases undergoing directional coronary atherectomy the obtained specimens were removed from the atherocatheter, flushed with saline, examined macroscopically and then fixed in 10% formalin. Routine processing for light microscopy and haematoxylin-azophloxin and Verhoeff-van Giesson staining was performed. All specimens were reviewed by two independent observers who were blinded to clinical data. The recommendations layed out in the American Heart Association Medical/Scientific Statement on the Definition of the Intima of

Human Arteries and of its Atherosclerosis-Prone Regions[19] were followed in collecting information regarding intimal constituents. Fibrous tissue was classified as dense when composed of acellular or poorly cellular connective tissue formed predominantly by dense collagen, and classified as loose when the tissue fragments showed a moderate cellularity and collagen bundles separated by accumulations of extracellular matrix. Neointimal hyperplasia was defined as fibromuscular connective tissue showing a random orientation of spindle shaped and stellate cells embedded in abundant extracellular matrix. Calcifications and lipid deposits such as cholesterol crystal clefts or foam cells were separately recorded independently. No special staining was used to identified calcium. Thrombus and / or intraplaque hemorrhage were identified as amorphous material, in close apposition with atheromatous material, frequently showing collections of leucocytes between layers of fibrin. Large masses of fibrin that might correspond to platelet-rich thrombus and were unlikely to be solely related to the atherectomy procedure were recorded. The Verhoeff-van Giesson staining was used to discriminate between fibrin and dense collagen. Organisation was judged when infiltration by cellular elements, e.g. smooth muscle cells, fibroblasts, capillary sprouts, was observed.

Statistical analysis

Mean values and standard deviations are presented for continuous variables. Comparison of mean values was performed using two-tailed unpaired Student's t-tests. Discrete variables were compared using chi-square tests, and Yates' continuity correction applied when indicated. Statistical significance was accepted at the 5% level.

Results

The baseline characteristics of the 41 patients included in the study are shown in Table I. In 35 (85%) cases the entire target stenosis was satisfactorily visualised by angioscopy, while in the remaining 6 (15%) the stenosis was not crossed with the angioscope and only the proximal aspect of the stenosis was visualised. The introduction of the angioscope at the ischemia-related lesion was associated with chest pain and electrocardiographic ST-T segment changes in 33 cases (80%). These changes were quickly reversible after cuff deflation and withdrawal of the catheter, with the exception of two patients (4%) in whom abrupt coronary occlusion developed. This was effectively treated by balloon angioplasty, without adverse sequelae. In a third patient a small non-occlusive dissection was noted immediately after angioscopy. Subsequent balloon angioplasty resulted in occlusive dissection, which was effectively managed by stent implantation. Finally, in a fourth patient with post-infarction angina, an occlusive dissection in the right coronary ostium, unrelated to angioscopy, was caused during the removal of the atherectomy guiding catheter, following a technically successful procedure in a mid right coronary artery stenosis. This was complicated by re-infarction (max. CK 600 U/l) which was treated conservatively.

Angioscopic characteristics of the culprit lesion.
In all cases visualization of ≥2/3 of the circumference of the inner vascular wall was achieved. Vessel wall disruption, suggesting ulceration, was identified in 27 (66%) patients. The stenosis presented an irregular surface in a further 8 (19%) patients. In the remaining 6 (15%) patients the stenotic wall was smooth, without disruption or marked irregularities.

Red material (Fig. 1) was seen in 25 (60%) of all cases, more frequently in postinfarction (13/17, 76%) than in primary unstable angina (12/ 24, 50%), although this difference did not reach statistical significance. The presence of red material was not influenced by the time elapsed from the beginning of the syndrome to angioscopy: 15±17 days versus 18±13 days in stenosis with and without red material, respectively. Red material was occlusive in 7 (41%) patients with postinfarction and in 3 (12%) with primary unstable angina (p=NS). The protrusion of red material correlated significantly with a lower minimal luminal diameter (0.55±0.56 mm versus 1.11±0.42 mm in stenoses with and without protruding red material, p=0.009) and greater percent diameter stenosis (79±19 % versus 64 ±15% in stenoses with and without protruding red material, p=0.04) measured with quantitative angiography. The prevalence of red material was significantly related to the angioscopic characteristics of the vessel wall, being present in 19 (70%) of the cases with disrupted, 5 (62%) of the lesions with irregular , and 1 (17%) of the lesions with smooth vessel wall (p=0.05). Conversely, there was no statistical relationship between the existence of a complex angiographic morphology and the presence of red material during angioscopy, the latter observed in 15/21 (71%) lesions with and 10/20 (50%) without complex angiographic morphology (p=NS).

Xanthomatous plaques presented typically as discrete raised plaques with a marked homogeneous yellow color, suggesting the existence of a very thin fibrous cap (Fig. 2A). They were identified in 15 (37%) of all patients, and presented more frequently in patients with postinfarction (10/17, 58%) than in unstable patients (5/24, 21%) (p = 0.02). Yellow plaques were found more frequently in stenoses containing red material (13/15, 87%, versus 2/15, 13%, in stenosis with and without red material respectively, p=0.02).

Pink areas were identified in 12 (50%) and 2 (12%) patients with unstable and post infarction angina respectively (p = 0.03). White-gray protruding masses (Fig. 1B) were observed more frequently in unstable (8/24, 33%) than postinfarction patients (3/17, 19%), although the difference was not statistically significant. In 4 cases (10%) a white protruding mass was seen in a stenosis containing also red material (3 unstable and 1 post-infarction patients). Finally, stenotic vessel wall with normal coloration was found in 4 (10%) cases, of which 3 showed smooth surface and 1 showed evidence of wall disruption.

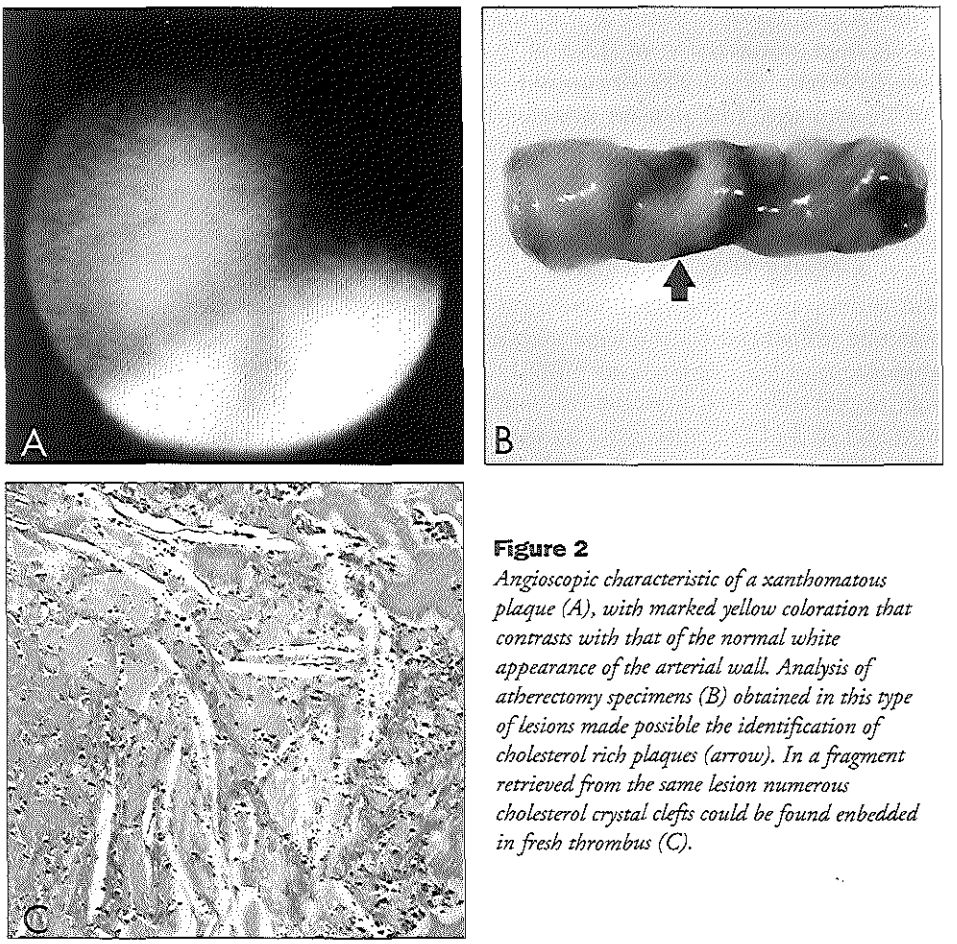


Figure 2
Angioscopic characteristic of a xanthomatous plaque (A), with marked yellow coloration that contrasts with that of the normal white appearance of the arterial wall. Analysis of atherectomy specimens (B) obtained in this type of lesions made possible the identification of cholesterol rich plaques (arrow). In a fragment retrieved from the same lesion numerous cholesterol crystal clefts could be found enbedded in fresh thrombus (C).

Prevalence of red material after thrombolysis

In the postinfarction angina group, 6 patients had received systemic thrombolysis 9±7 days before angioscopy. No significant differences with regard to the presence of red material were noted when this group was compared to the other 11 patients with postinfarction angina who did not receive thrombolysis, showing a prevalence of 4/6 (66%) in patients with and in 9/11 (82%) without prior thrombolysis. Likewise, thrombolysis did not influence the degree of luminal occlusion caused by red material, which was occlusive in 2/4 (33%) and 5/11 (45%) cases with and without previous thrombolysis respectively (p=NS). In one additional patient intracoronary thrombolysis was performed after coronary angioscopy demonstrated total luminal obstruction by red material. Using the distal lumen of the angioscope, the thrombolytic agent (rTPA) was introduced to the space existing between the occlusion and inflated proximal cuff, in an attempt to increase the concentration of lytic agent in contact with the thrombus (Fig. 4A). After 20

minutes, coronary angiography revealed incomplete coronary recanalisation, while angioscopy showed a substantial change in the color of the previously red material, which now presented several mobile structures of pinkish color which had been covered by the red occlusive mass and which remained unlysed during further observations (Fig. 4B). Subsequent balloon angioplasty yielded an optimal result, with complete restoration of antegrade blood flow through the stenosis.

Analysis of atherectomy specimens

Directional atherectomy was performed in 9 cases (22%), each yielding pathological specimens. Macroscopic examination of the specimens allowed identification of several features visualized during angioscopy. The correlation between histopathological and angioscopic findings in these 9 patients is summarized in Table II. Histopathological analysis of the material retrieved in all 5 stenosis containing red material showed fresh red thrombus (Fig. 2). In three of these patients the presence of a xantomatous plaque had been reported by angioscopy (Fig. 2A). The retrieved material demonstrated the presence of numerous cholesterol crystal clefts with inflammatory cells, associated with fresh red thrombus (Fig. 2C) in two cases, suggesting that extrusion and exposure of lipid material to the bloodstream was the precipitant cause for coronary thrombosis. Directional atherectomy was not performed in any of the cases where pink areas had been visualized. Coronary samples were obtained from two lesions with white-gray protruding masses. Microscopic examination revealed the presence of a large fibrinous mass in one case, and organizing thrombus in the other (Fig. 3B). Finally, atherectomy was performed in one case with a normal-coloured stenotic wall, with the specimen showing the presence of dense fibrous tissue and neointimal hyperplasia.

Discussion

The information accumulated during the last decade on the pathophysiological mechanisms involved in the genesis of acute coronary syndromes[5,6] has enabled the development of specific therapeutic strategies targeted against particular pathophysiological processes.[20-22] Some of these approaches have been shown to reduce significantly the mortality and morbidity associated with the syndrome. However, the management of unstable patients is still far from being straightforward. Although a thrombotic origin in primary unstable angina seems well established,[1-5,23] the syndrome is frequently refractory to thrombolysis, platelet antiaggregants and systemic anticoagulation.[8,24] Likewise, the advent of the thrombolytic era has not diminished the number of patients presenting with unstable angina in the weeks following acute myocardial infarction.[25]

Part of this therapeutic problem may be related to the lack of knowledge on the evolution of the pathological substrate from the initial stages of the acute coronary syndrome. While post-mortem studies have contributed substantially to the understanding of the underlying pathology, it is obvious that this source of know-

Table II. Correlation between angioscopic and histological findings in patients treated with directional coronary atherectomy.

Pat. no.	Angioscopic findings	Histological findings
1	Red (protruding) material, xanthomatous plaque	Dense fibrous tissue, fresh thrombus, cholesterol crystals
2	Red (mural) material, xanthomatous plaque	Dense fibrous tissue, fresh red thrombus, cholesterol crystals
3	Red (occlusive) material	Dense fibrous tissue, fresh red thrombus, neointimal hyperplasia
4	Red (mural) material	Dense fibrous tissue, fresh thrombus, media + advent.
5	Red (mural) material	Dense + loose fibrous tissue, fresh red thrombus
6	Red (mural) material, xanthomatous mass	Dense fibrous tissue, fresh thrombus.
7	White mass	Dense fibrous tissue, fibrin rich thrombus.
8	White mass	Dense fibrous tissue, organizing thrombus
9	Normal vessel wall	Dense fibrous tissue, neointimal hyperplasia

ledge is biased by case selection, presumably reflecting more acute and extensive changes than those existing in the majority of patients.

Coronary angioscopy may serve as a useful tool in visualizing the ischemia-related vessel in unstable patients, providing insight not only on the genesis but on further evolution of the changes taking place in the environment of the atheromatous plaque. Previous studies using angioscopy have suggested a high frequency of coronary thrombosis in patients with unstable angina and myocardial infarction.[10-13] Grayish, non-occlusive, presumably platelet rich thrombus has been described as a common substrate of patients presenting with unstable angina, while red, occlusive

thrombus was found mainly in patients with myocardial infarction.[12] In the latter group, a higher prevalence of xanthomatous plaques was also found.[12] Although these studies have contributed significantly to the in-vivo study of acute coronary syndromes, they share the common limitation that translation of the visual findings to pathological terms was performed automatically. This is particularly relevant since pathological validation of angioscopic studies has never been performed in-vivo, and in-vitro studies are scarce. These include the work of Tomaru et al.[26] and Mizuno et al.,[27] reporting on the formation of thrombus and subsequent action of thrombolysis in two different experimental models, and that of Siegel et al.[28] validating angioscopic and intravascular ultrasound observations obtained in-vitro in peripheral human arteries. Although in these studies pathological examination of the imaged vascular substrate was performed, it is likely that the models used may be quite far from being representative of those changes found in the culprit lesion of of patients with unstable angina.

Our work differs from all these previous studies in several aspects. First, an objective approach to evaluation of the visual findings was followed to classify the chromatic and morphological characteristics of the culprit stenosis as visualized during angioscopy. To reduce the potential interobserver variability associated with angioscopy we performed panel review of the images, a method which has previously proved useful for this purpose in studies using coronary angiography.[29] A translation to pathological findings was attempted only after concomitant directional atherectomy was performed, yielding histological specimens that were compared with visual findings. The independent contribution of a cardiovascular pathologist was also sought in an attempt to get an expert opinion on the changes seen during angioscopy. Second, the study population was a representative sample of unstable patients with or without previous myocardial infarction, with the common denominator of being refractory to medical treatment and referred for percutaneous recanalisation. This implied a longer time interval (16±15 days) between the onset of the syndrome and the time of angioscopy. Finally, patients with prior thrombolysis were also included in the study.

We found that the chromatic characteristics of the stenotic wall found in the study population were similar to those reported by Mizuno et al.[12] in the very early stages of the syndrome. Red intraluminal material was a frequent finding in unstable and postinfarction patients, and the study of atherectomy specimens revealed that its histological substrate was in fact red thrombus. Thrombus was frequently present in spite of relatively prolonged treatment with therapeutic doses of intravenous heparin, in agreement with the work of Badimon et al demonstrating that anticoagulation with heparin does not abolish thrombus growth.[30] In the post-infarction group previous treatment with thrombolytic agents did not appear to reduce the prevalence of red thrombus.

Likewise, we found that white protruding masses are relatively common in unstable patients, and occur mainly in patients with primary unstable angina. Insight

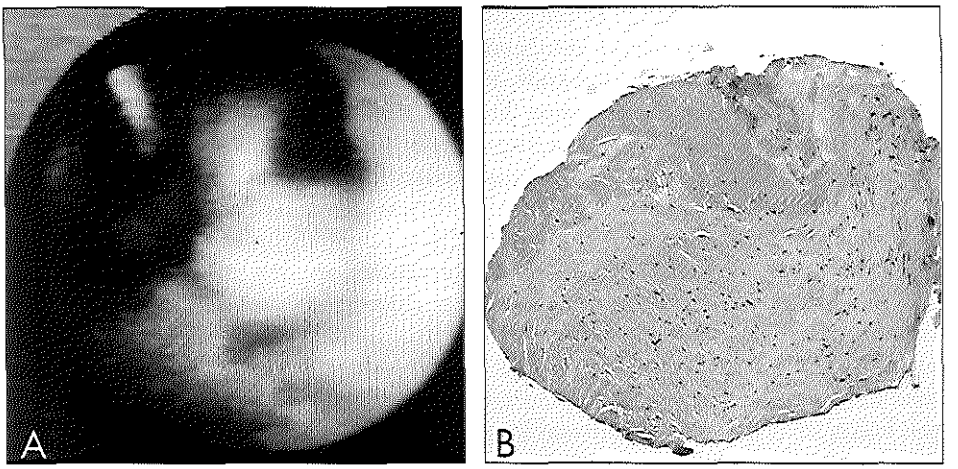
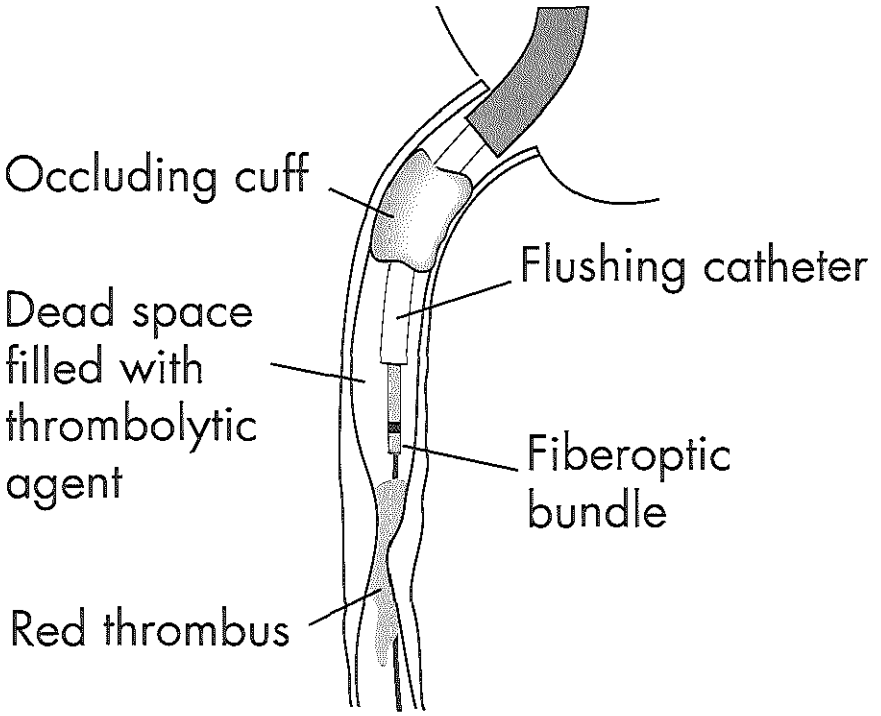


Figure 3

A: Intracoronary angioscopy in a patient with unstable angina pectoris showing a globular white mass that protrudes in the lumen. B: Histological examination of a similar mass observed in a patient undergoing atherectomy revealed moderately cellular fibrous tissue with evidence of thrombotic organisation.

to the pathological nature of this observation was obtained in two cases. A fibrin-rich thrombus was found in an atherectomy specimen obtained from a lesion with a white-grayish protruding mass. In the second specimen, the underlying substrate was identified as fibrous tissue in association with organising thrombus. In previous studies, we and others found that thrombus organisation is common in atherectomy specimens obtained in patients with primary unstable angina pectoris, even in the early stages of the clinical syndrome.[31,32] It is likely that modification of the color of thrombus which occurs during the process of organisation,[33] with a shift from red to a pinkish, color, can make it undistinguishable from platelet rich thrombus. We also observed a statistically significant preponderance of pink areas in the vessel wall in patients with unstable angina, although its pathological nature remains unclear since no atherectomy was performed in any of these cases. It is possible, however, to speculate that these may correspond to mural thrombus being organised and integrated in the arterial wall, or to the presence of haematic material in the subintima.

Several observations relevant to the vascular substrate underlying thrombus formation were drawn from the angioscopic images. We found a significant gradation in the presence of red material in stenoses with disrupted, irregular and smooth vessel wall. It is likely that these stenosis characteristics imply a variable thrombogenic potential of the stenotic wall. Exposure of deep components of the plaque (type III vascular injury in the classification suggested by Ip et al.)[34] may cause thrombosis more frequently than in areas with merely absent or dysfunctional endothelium (vascular injury type II in the same classification), promoted by shear



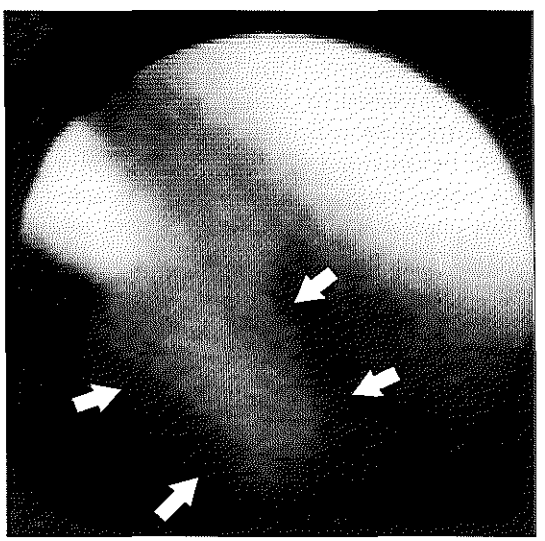


Figure 4

A: Coronary angioscopy can be used as an effective method for thrombolytic drug delivery in selected patients. By inflating the proximal occluding cuff of the angioscope, which is normally used to interrupt antegrade blood flow and facilitate visualisation, thrombolytic agents can be delivered at the site of occlusion, reaching high local concentrations that may facilitate thrombolysis.

B: This technique was successfully applied in a mid righ coronary occlusion by red material, suggestive of fresh thrombus.

During thrombolysis, angioscopy demonstrated marked remodelling of the occluding red material. A mobile, whitish core (arrows) which was underlying the original occluding mass became evident and remained unchanged during the rest of the examination.

stress in segments presenting an irregular lumen.[35] Our study supports a previous report on a higher prevalence of xanthomatous plaques in patients who developed myocardial infarction. The presence of red material and xantomatous plaques were strongly correlated. Concomitant study of atherectomy specimens obtained from xanthomatous lesions revealed the presence of cholesterol crystal clefts, sometimes in close association with inflammatory cells and embedded in red thrombus, suggesting that, in accordance with previous postmortem studies, extrusion and dispersion of plaque material secondary to plaque rupture had preceded the episode of thrombosis[1,3,4,36] (Fig. 2). Xanthomatous plaques appeared as discrete, raised plaques where a thin, transparent fibrous cap is assumed since the underlying yellow lipid material can be seen. These observations support current views on the genesis of myocardial infarction, precipitated by rupture of lipid-rich plaques[5,37] that promote thrombus formation.[38,39]

Which is the main cause of persistent luminal obstruction in unstable patients refractory to medical treatment? As judged from angioscopy in the present study, red material protruded significantly in the coronary lumen only in 24% of the cases. Even if patients with a protruding white mass are added to this figure (9 patients, since 1 concomitantly presented a red mass), obstruction by an intraluminal mass occurred in only 46% of all cases. Since the primary aim of thrombolysis is to tackle coronary thrombus as the main cause of intraluminal obstruction, this observation provides clear evidence as to why thrombolytic treatment has previously not been helpful in the treatment of unstable patients. Interestingly, in this study the only angiographic characteristic of lesions showing a significant obliteration of the lumen by red material was the presence of significantly greater stenosis severity by quantitative angiographic analysis. This finding may provide an unexpected explanation of the findings reported by De Zwaan et al.[10] in the sense that only unstable patients with total or subtotal coronary occlusion demonstrated benefit from thrombolytic treatment. In the light of the observations of the present study, such benefit would be derived from selecting cases for treatment where occluding red thrombus plays a considerable aetiological role. As in the present study, less severe stenoses probably present a lower contribution of thrombus to luminal obstruction. These findings suggest that angioscopy may be more useful than angiograhy in reliably and specifically identifying the presence of intraluminal thrombus in order to perform a more selective use of thrombolytic agents.

This applies also to patients with unstable postinfarction angina, as suggested by a pilot study from the John Hopkins Hospital.[9] In that work, intracoronary streptokinase was given in postinfarction patients with a persistently occluded vessel. Coronary reperfusion was achieved in 9/15 (60%) of patients, who had a better clinical outcome than patients not receiving thrombolysis, or in whom coronary reperfusion was not achieved. We could document the modification of the angioscopic characteristics of occlusive red material in a postinfarction patient following exposure to a highly concentrated dose of thrombolytic agent. An underlying whi-

tish, mobile core which remained unlysed (Fig. 4B) became evident. This observation fits nicely with that of Uchida et al.[10] in their in-vitro angioscopic study on the effect of thrombolysis, where pale material was later identified histologically as a fibrin-rich thrombotic core. It remains unknown whether a similar effect of thrombolysis is to be expected in cases where an intraluminal white protruding mass rich in fibrin predominates. Likewise, the presence of some of the other characteristics of thrombus found in our study, such as the presence of extruded cholesterol crystal clefts and cellular infiltration within a formed thrombus, may interfere with the action of thrombolytic agents.[42]

It has been emphasized that in the acute coronary syndromes thrombosis is a dynamic process[2,3,5,43] with waxing and waning of the cause of coronary occlusion (either red or platelet-rich thrombus). The presence of protruding thrombus may also stimulate thrombus progression due to a combination of rheological factors and the high thrombogenic activity of the actual surface of the thrombus itself.[34] Likewise,the resulting increase in shear stress may lead to new episodes of thrombosis if intermittent fissuring or exposure of its core to the bloodstream occurs later during thrombus organisation, where thrombin remains protected from circulating antithrombin.[34]

It is important to keep in mind that the information provided by angioscopy is limited to the luminal aspect of the vessel, and that therefore changes in plaque geometry as a consequence of the development of intraintimal hemorrhage or haematoma formation can be missed, or underscored by visualization of a mural thrombus at the site of a fissure in the fibrous cap (a "tip of the iceberg" phenomenon) (Fig. 1 A and B). It would be expected that the presence of hemorrhage or haematoma within the intima would constitute a major barrier to the action of thrombolytic agents.[42] Alternatively, and since resolution and organisation of a thrombotic episode seems to be associated with enhanced smooth muscle cell proliferation[45] and development of neointimal hyperplasia,[46-48] changes in plaque geometry may also result from accelerated formation of fibrous tissue which had been initially triggered from a clinically silent episode of thrombosis.

The results of this study reinforce the concept that coronary angioscopy constitute an useful research tool in the study of different coronary syndromes, and that such application is enhanced through concomitant use of directional atherectomy. The technique of angioscopy may additionally allow a more selective application of thrombolytic treatment in those patients with occluding thrombus. Likewise, it may play a important role in the context of clinical trials aimed at reducing coronary thrombosis, such as in the control of restenosis post intervention, or in testing new pharmacological or mechanical thrombolytic approaches.

Our experience shows that the performance of angioscopy in unstable patients can cause abrupt occlusion, which is probably due to the manipulation of the guidewire and a intracoronary device in an already disrupted vascular segment. In this

respect, it must be noted that, in contrast with previous studies, we attempted a complete angioscopic examination of the proximal and distal aspects of the stenosis. Despite the occasional local complications, our study shows that angioscopy with the system described remains a safe technique in unstable patients during percutaneous intervention.

Limitations

Although the capability of angioscopy for providing chromatic information on the vascular lumen constitutes one of its main advantages in the study of acute coronary syndromes, this aspect remains also its main limitation. No studies on the intraobserver and interobserver variability of colorimetric observations are available. However, in a normal population the ability to distinguish between colors varies significantly from one individual to another. We attempted to reduce such variability by panel review of the images, a method which has previously been validated in coronary angiography.[29] Our population was biased by the fact that the patients studied were suitable for percutaneous recanalisation. Thus, information of patients presenting triple vessel disease may have been lost.

Our patients were somewhat selected, since they were refractory to medical therapy and the findings may not be representative of the luminal environment early after the onset of the syndrome. In addition all our patients were suitable for percutaneous recanalisation and clearly had already survived the early acute phase of the syndrome and thus may not be representative of patients who suffer a precipitous clinical deterioration resulting in death. Nevertheless, we believe the combination of angioscopic, quantitative angiographic and histological findings in this study at least demonstrates the exciting possibility that comprehensive study of the elusive cause of unstable angina is clearly feasible. Thus, it is clear that we can anticipate further and rapid progress in this resistant and difficult area.

References

1. *Davies MJ, Thomas AC. Plaque fissuring - the cause of acute myocardial infarction, sudden ischaemic death and crescendo angina. Br Heart J 1985;53:363-73.*

2. *Davies MJ. A macro and micro view of coronary vascular insult in ischemic heart disease. Circulation 1990; 82(suppl II): II-38 - II-46.*

3. *Falk E. Plaque rupture with severe pre-existing stenosis precipitating coronary thrombosis: characteristics of coronary atherosclerotic plaques underlying fatal occlusive thrombi. Br Heart J 1983; 50:127-34.*

4. *Friedman M, Van den Bovenkamp GJ. The pathogenesisof a coronary thrombus. Am J Pathol 1966; 48:19-44.*

5. *Fuster V, Badimon L, Badimon JJ, Chesebro JH. The pathogenesis of coronary artery disease and the acute coronary syndromes. N Engl J Med 1992;326:242-50 and 310-8.*

6. *Bashour TT, Myler RK, Andreae GE, Stertzer SH, Clark DA, Ryan CJM. Current concepts in unstable myocardial ischaemia. Am Heart J 1988; 115: 850-61.*

7. *Mulcahy R. Natural history and prognosis of unstable angina. Am Heart J 1985;109:753-8.*

8. *Betriu A, Heras M, Cohen M, Fuster V. Unstable angina: Outcome according to clinical presentation. J Am Coll Cardiol 1992; 19:1659-63.*

9. *Sherman C, Litvack F, Grundfest W, Lee M, Chaux A, Kass R, Blanche C, Matloff J, Morgenstern L, Ganz W, Swan H, Forrester J. Demonstration of thrombus and complex atheroma by in-vivo coronary angioscopy in patients with unstable angina pectoris. New Eng J Med 1986; 315:913-19.*

10. *Uchida Y, Masuo M, Tomaru T, Kato A, Sumigoto T. Fiberoptic observation of thrombosis and thrombolysis in isolated human coronary arteries. Am Heart J 1986; 112:691-6.*

11. *Mizuno K, Satomura K, Miyamoto A, Arakawa K, Shibuya T, Arai T, Kurita A, Nakamura H, Ambrose JA. Angioscopic evaluation of coronary-artery thrombi in acute coronary syndromes. N Eng J Med 1992; 326:287-91.*

12. *Mizuno K, Miyamoto A, Satomura K, Kurita A, Arai T, Sakurada M, Yanagida S, Nakamura H. Angioscopic coronary macromorphology in patients with acute coronary disorders. Lancet 1991; 337:809-12.*

13. *Hombach V; Hoher M; Kochs M; Eggeling T; Schmidt A; Hopp HW; Hilger HH . Pathophysiology of unstable angina pectoris—correlations with coronary angioscopic imaging. Eur-Heart-J 1988; 9 (Suppl N):40-5.*

14. *Braunwald E. Unstable angina: a classification. Circulation 1989;80:410-4.*

15. *Gronenschild E, Janssen J. A compact system for quantitative cardiovascular angiography analysis. Medinfo. KC Lun en al. (editors). Amsterdam, New York;Elsevier Science Publishers, 1992; 795-*

800.

16. Haase J, Escaned J, Montauban van Swijndregt E, Ozaki Y, Gronenschild E, Slager C, Serruys PW. Experimental validation of geometric and densitometric coronary measurements on the new generation Cardiovascular Angiography Analysis System (CAAS II). Cathet Cardiovasc Diagn 1993; 30:104-14.

17. Reiber JHC, Serruys PW, Kooijman CJ et al. Asessment of short-, medium- and long-term variations in arterial dimensions frok computer assissted quantification of coronary cineangiograms. Circulation 1985; 71:280-88.

18. Ambrose JA, Winters SL, Arora RR, et al. Coronary angiographic morphology in myocardial infarction: a link between the pathogenesis of unstable angina and myocardial infarction. J Am Coll Cardiol 1985;6:1233-8.

19. Stary HC, Blackenhorn DH, Chandler B, Glagov S, Insull W, Richardson M, Rosenfeld ME, Schaffer SA, Schwartz CJ, Wagner WD, Wissler RW. A definition of the intima of human arteries and of its atherosclerosis-prone regions. Circulation 1992; 85:391-405.

20. Lewis DH, Davis JW, Archibald DG, et al. Protective effects of aspirin against acute myocardial infarction and death in men with unstable angina. N Engl J Med 1983;309:396-403.

21. Cairns JA, Gent M, Singer J, et al. Aspirin, sulfinpyrazone or both in unstable angina: results of a Canadian multicenter trial. N Engl J Med 1985;313:1369-75.

22. Théroux P, Ouimet H, McCans J, et al. Aspirin, heparin or both to treat acute unstable angina. N Engl J Med 1988;319:1105-11.

23. Ambrose J. Plaque disruption and the acute coronary syndromes of unstable angina and myocardial infarction: If the substrate is similar, why is the clinical presentation different? J Am Coll Cardiol 1992; 19:1653-8.

24. Brunelly C, Spallarossa P, Ghigliotti G, Iannetti M, Caponneto S. Thrombolysis in unstable angina. Am J Cardiol 1991; 68:110B-118B.

25. Ouyang P, Shapiro EP, Gottlieb SO. Thrombolysis in postinfarction angina. Am J Cardiol 1991; 68:119B-124B.

26. Tomaru T, Uchida Y, Sugimoto T. Fiberoptic study on the effects of transluminal angioplasty in experimental occlusive arterial thrombosis. Am Heart J 1988; 115:312-17.

27. Mizuno K, Miyamoto A, Isojima K, Kurita A, Senoo A, Arai T, Kikuchi M, Nakamura H. A serial observation of coronary thrombi in vivo by a new percutaneous transluminal coronary angioscope. Angiology 1992; Feb:91-99.

28. Siegel RJ, Ariani M, Fishbein MC, Chae JS, Park JC, Maurer G, Forrester JS. Histopathologic validation of angioscopy and intravascular ultrasound. Circulation; 1991; 84: 109-17.

29. Sanmarco ME, Brooks SH, Blankenhorn DH. Reproducibility of a consensus panel in the interpreta-

tion of coronary angiograms. Am Heart J 1978; 96:430.

30. Badimon L, Badimon JJ, Lassila R, Merino A, Chesebro JH et al. Re-thrombosis on an evolving thrombus is mediated by thrombus-bound thrombin that is not inhibited by systemic heparin. Thrombosis and Haemostasis 1991; 65:760.

31. Isner JM, Brinker JA, Gottlieb RS, Leya F, Masden RR, Shani J, Kearney M, Topol EJ on behalf of CAVEAT. Coronary thrombus: Clinical features and angiographic diagnosis in 370 patients studied by directional coronary atherectomy. Circulation 1992 (Suppl. I); 86:I-648.

32. Escaned J, van Suylen RJ, MacLeod DC, Umans VA, de Jong M, Bosman FT, de Feyter PJ, Serruys PW. Histological characteristics of tissue excised during directional coronary atherectomy in patients with stable and unstable angina pectoris.Am J Cardiol 1993; 71:1442-47.

33. Pearson TA, Dillman J, Solez K, Heptinstall RH: Monoclonal characteristics of organising arterial thrombi: Significance in the origin and growth of human atherosclerotic plaques. Lancet, 1979; 1:7-11.

34. Ip JH, Fuster V, Badimon L, Badimon J, Taubman MB, Chesebro JH. Syndromes of acelerated atherosclerosis: Role of vascular injury and smooth muscle cell proliferation. J Am Coll Cardiol 1990: 15:1667-87.

35. Gertz SD, Roberts WC. Hemodynamic shear force in rupture of coronary arterial atherosclerotic plaques. Am J Cardiol 1990; 66:1368-72.

36. Chapman I. Morphogenesis of acute coronary thrombosis. Archs Pathol 1965; 80:256-61.

37. Richardson PD, Davies MJ, Born GVR. Influence of plaque configuration and stress distribution on fissuring of coronary atherosclerotic plaques. Lancet 1989; ii:941-44.

38. Stuart MJ, Gerrard JM, White JG. Effect of cholesterol on production of thromboxane B2 by platelet in vitro. N Eng J Med 1980; 302:6-10.

39. Tracy R, Devaney K, Kissling G. Characteristics of the plaque under a coronary thrombosis. Virchows Arch 1985;405:411-27.

40. De Zwaan C, Bar FW, Janssen JHA, de Swart HB, Vermeer F, Wellens HJJ. Effects of thrombolytic therapy in unstable angina: Clinical and angiographic results. J Am Coll Cardiol 1988; 12: 301-9.

41. Shapiro EP, Brinker JA, Gottlieb SO, Guzman PA, Bulkley PH. Intracoronary thrombolysis3 to 13 days after acute myocardial infarction for postinfarction angina pectoris. Am J Cardiol 1985; 55:1453-58.

42. Davies MJ. Successful and unsuccessful coronary thrombolysis. Br Heart J 1989; 61:381-4.

43. Hackett D, Davies G, Chierchia S. Intermitent coronary occlusion in acute myocardial infarction. Value of combined thrombolytic and vasodilatory therapy. N Eng J Med 1987; 317: 1055-59.

44. Badimon L, Badimon JJ. Mechanisms of arterial thrombosis in nonparallel streamlines: platelet thrombi grow on the apex of stenotic severely injured vessel wall. Experimental study in the pig model. J Clin Invest 1989;84:1134-44.

45. Escaned J, de Jong M,Violaris AG, MacLeod DC, van Suylen RJ, Umans VA, de Feyter PJ, Verdouw PD, Serruys PW. Clinical and histological determinants of smooth muscle cell outgrowth in cultured atherectomy specimens: Importance of thrombus organisation. Coronary Artery Disease 1993; 4:883-90.

46. Jorgensen L, Rowsell HC, Hovig T, Mustard JF. Resolution and organisation of platelet-rich mural thrombi in carotid arteries of swine. Am J Pathol 1967; 51:681-719.

47. Schwartz RS, Holmes DR, Topol EJ. The restenosis paradigm revisited: An alternative proposal for cellular mechanisms. J Am Coll Cardiol 1992; 20:1284-93.

48. Ip JH, Fuster V, Israel D, Badimon L, Badimon J, Chesebro JH. The role of platelets, thrombin and hyperplasia in restenosis after coronary angioplasty. J Am Coll cardiol 1991; 17: 77B-88B.

Chapter X

Ischemia-related Lesion Characteristics in Patients with Unstable and Postinfarction Angina Pectoris: A Study with Combined Intracoronary Angioscopy and Ultrasound Imaging

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Abstract

Background

The paradigm of acute coronary syndromes is sudden disruption of the fibrous cap, overlying a soft, lipid-laden plaque followed by thrombus formation. Current percutaneous catheter-based imaging technology enables characterization of the coronary plaque and thus may confirm the paradigm in patients with unstable angina or postinfarction angina.

Aim of the study

To determine in patients with unstable angina using angioscopy the frequency of thrombus, and whether the lesion is complex or xanthomatous and to assess using ultrasound the composition of the ischemia-related lesion.

Methods

In 31 patients, 24 males, 7 females, mean age 60±10 years either presenting with unstable angina (19) or postinfarction angina (12), intracoronary angioscopy and ultrasound imaging of the ischemia-related lesion was performed before coronary intervention. Angioscopic derived images of the lesions were classified as either stable, (smooth surface, no thrombus) or complex (ulcerated surface with or without thrombus). The presence of thrombus (red intraluminal mass) and xanthomatous plaque (yellow colour) was recorded. The ultrasound-derived images of the lesion were classified as predominantly calcified (highly echo-reflective with shadowing representing calcium), hard (highly echo-reflective representing dense fibrous tissue), soft (poorly echo-reflective representing loose fibrous tissue or lipid) or mixed.

Results

Angioscopy: the lesion was stable in 26%, complex 74%, and yellow in 36%. A thrombus was present in 68% of the lesions.

Ultrasound imaging: the composition of the ischemia-related plaque was predominantly soft in 41%, hard in 6%, and none was extremely calcified. The remaining 52% were mixed lesions: all of them were composed of soft tissue in combination with hard or calcified tissue.

Conclusions

Combined imaging with angioscopy and ultrasound demonstrated a preponderance of complex ischemia-related lesions, frequently with associated thrombosis. Intravascular ultrasound revealed characteristics of soft atheromatous plaques, and one-third of the lesions were yellow during angioscopy, suggesting the presence of subintimal lipid deposits. Finally, 25% of all lesions did not presented associated thrombus or plaque disruption. In these patients other mechanisms to those proposed by the current paradigm of the syndrome may play an etiological role, and other treatment strategies may be required.

Introduction

The cause of unstable anginal syndromes is considered to be sudden disruption of the atheromatous plaque, setting into action several pathogenic mechanisms such as platelet activation, adhesion and aggregation, increased vasoconstriction and thrombus formation.[1] This may result in an abrupt transient reduction in coronary flow. The presence of local and systemic thrombogenic risk factors at the time of plaque disruption may modify the extent and duration of thrombus formation and account for the waxing and waning of pathologic and ensuing clinical manifestations.[1] Current available diagnostic tools do not provide direct information about the underlying processes and it remains conjectural which major pathogenic mechanism is operative or prevails in an individual patient at a certain time. This precludes tailored treatment and may partly explain why patients become refractory to established treatment or progress to myocardial infarction or death which occurs respectively in 3.5% to 14% and 0.5% to 6% within 1-3 months of hospitalization.[2-7] Clearly, additional information is required that provides better insights into the underlying disease mechanism(s) to improve patient management and prognosis.

Two recently developed diagnostic tools which have the potential to yield insights into the pathogenesis of unstable angina are currently available for clinical use. Intracoronary ultrasound imaging provides information about size and composition of the plaque[8-13] and intracoronary angioscopy can accurately detect presence of intracoronary thrombus and complex plaque.[14-18] The purpose of this study was to determine the composition of the ischemia-related lesion and to assess the frequency of complex lesions, yellow lesions and thrombi with the sequential use of intracoronary angioscopy and ultrasound imaging in patients with primary unstable and early postinfarction angina before undergoing percutaneous intervention.

Patients and methods

Between September 1992 and March 1993 thirty-five patients with unstable angina were studied who were classified as having primary angina at rest class IIB and IIIB or postinfarction unstable angina class IIIC according to the recent Braunwald classification[19] and who were scheduled for coronary intervention.

In one patient the procedure was aborted because of development of severe ischemic chest pain after introduction of the angioscope in the ischemia related coronary artery. Immediately performed PTCA was successful without adverse sequelae. In 3 patients the angioscope was introduced but the obtained images were of insufficient quality. These 4 patients were excluded from further analysis. Thus, the study population comprised 19 patients with unstable angina and 12 patients with postinfarction angina. The clinical and angiographic data of these patients are presented in Table 1. The investigations were approved by the Institutional

Table 1. Baseline patient and angiographic characteristics

Total number patients	31
Male/female (N)	24/7
Age, mean±SD (years)	60 ± 10
Multivessel disease (N)	9
Premedication:	
-Heparin/Aspirin (N)	31/25
-Thrombolysis (N)	6 (all post MI)
-Nitroglycerin i.v. (N)	27
-Ca2+ channel antagonists (N)	23
-Beta-blockers (N)	27
Days from onset instability	14 ± 14
Ischemia-related lesion:	
-LAD	13
-RCA	13
-CX	5
Minimum luminal diameter (mm)*	1.05 ± 0.50
Reference diameter (mm)*	3.11 ± 0.72
Complex angiographic morphology (N)	16

* mean ± 1 SD, determined with quantitative coronary angiography. MI : myocardial infarction, i.v. : intravenous, LAD : left anterior descending artery, RCA : right coronary artery, CX : circumflex artery.

Review Board of the Cardiology Department of the Dijkzigt Ziekenhuis and the patients were studied after giving informed consent.

Procedures

Selective coronary angiography in multiple projections was performed before and after angioplasty. All patients received aspirin (250 mg) and intracoronary nitroglycerin before the procedure. They were all on full anticoagulation with heparin, such that activated clotting time was over 300s. During the procedure patients received a combination of diazepam and thalamonal intravenously to relieve anxiety and to promote comfort.

After passage of a 0.014 in. guide wire across the lesion intracoronary imaging was performed before dilation. Angioscopy was always performed first, and there after intracoronary ultrasound imaging was carried out. The coronary segment proximal to the ischemia-related lesion was imaged with the ultrasound catheter. In all cases an attempt was made to cross the lesion with both devices to obtain information about the entire lesion. Coronary angioplasty or other interventional techniques were used according to standard practise.

Selection of ischemic-related lesion
The selection of the ischemic-related lesion for analysis with angioscopy and ultrasound imaging was determined as follows. In cases with single vessel disease the most severe lesion within that vessel was selected. In cases with multivessel disease the selection was determined by the combination of the electrocardiographic area indicated by transient ST-T segment changes occurring during ischemia at rest and the closely corresponding coronary vessel containing the most severe lesion.

Angiography
A modified classification of angiographic morphology proposed by Ambrose et al.[20] was used to categorize each target lesion as non complex (concentric or eccentric with smooth borders) and complex (eccentric with irregular borders or overhanging edges; multiple irregularities or intraluminal filling defects). Segments proximal to the ischemia-related lesion were classified as having an angiographically smooth normal lumen, or minimally to moderately severe lumen irregularities.

Quantitative coronary angiography was performed with the CAAS-2 system (PIE Data, Maastricht, The Netherlands) using the non contrast-filled catheter as calibration.[21]

Angioscopic device
The percutaneous coronary angioscopic device is a monorail type polyethylene catheter device, which has a size of 4.5 F and which is accommodated by a 8 F guiding catheter (Baxter-Edwards; Irvine California). The catheter features a compliant occlusive cuff, a flush port, guidewire compatibility and a movable optical bundle. The depth of field is > 1 mm and the minimum resolution 3.5 lp/mm. During angioscopic imaging the distal coronary artery was flushed with saline injected with a flow of 30 to 40 cc per minute.

To facilitate the review process a real-time fluoroscopy or cineangiography is combined with real-time angioscopy and ultrasound imaging by using split screen videotaping. This provides a better orientation of where the angioscopic and ultrasound images were derived from within the coronary tree.

Ultrasound imaging device
This study was performed with a commercially available intracoronary ultrasound device (Cardiovascular Imaging Systems Inc., Sunnyvale, Calif). Images were obtained with a 4.3 F, 30 MHz ultrasound catheter. The device produces real-time images of vessel cross sections via the rotation of a metallic mirror at 30 revolutions/s with an axial resolution of 75 μm and lateral resolution of approximately 150 μm.[15] After optimization of the time-gain compensation, reject and compression settings images, were recorded on high-quality videotape (sVHS) for off-line review and quantification.

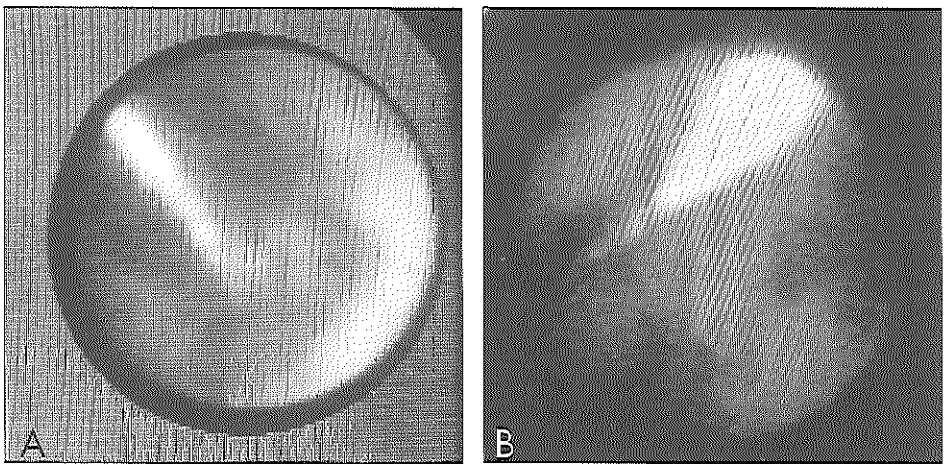

Figure 1
Angioscopic images of occlusive (A) and non-occlusive (mural) red thrombus.

Angioscopic images

Thrombi were defined as a red, intraluminal masses adherent to the intima (Fig. 1). Thrombi were categorised as non-mobile-mural (closely adherent to the vessel wall) or mobile-protruding into the lumen or totally occlusive. Also noted was the colour of the lesion: grey/white and yellow suggesting underlying lipid pool.

Wall surface was classified as ulcerated when major disruption with lack of continuity was found. When ulceration was absent but wall irregularities were noted, the surface was classified as irregular. Finally, when none of these alterations was present and the wall presented the characteristic pattern noted in normal non-stenotic segments the surface was classified as smooth.

Angioscopic images of lesions were classified into a complex lesion if they had a irregular, ulcerated raised surface with or without presence of thrombus or into a stable lesion if the raised surface was regular and smooth, without thrombus.[26]

Reddish streaks were defined as non-raised reddish-pink areas within the coronary artery wall. White masses were defined as white mobile protruding masses attached to the wall or in close association with a red thrombus.

Ultrasound images

Qualitative analysis was performed by consensus of three observers. The composition of the ischemia-related lesion was classified as calcified, hard, or soft (Fig. 2), concentric or eccentric and as homogeneous or mixed (Fig. 2).

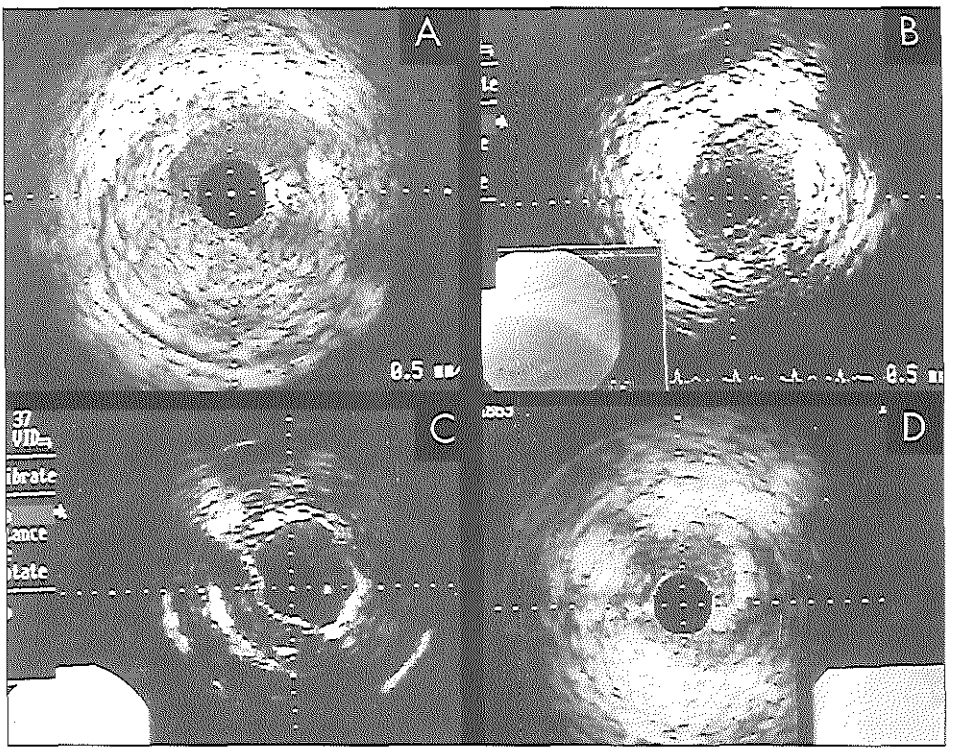

Figure 2

Types of ultrasound-derived lesions. A : soft plaque : poorly echoreflective tissue predominantly between 4 and 9 o'clock. B : hard plaque: highly echoreflective tissue with eccentric localization between 1 and 4 o'clock. C: calcific plaque: highly echoreflective tissue with shadowing: almost entirely calcified lesion. D: mixed plaque: composed of soft plaque at 9-2 o'clock, hard plaque at 5-8 o'clock and calcific plaque with shadowing at 2-3 o'clock

The following definitions were used, which were a reflection of previous studies:[22-25]
Calcific plaque: highly echo-reflective intimal thickening with acoustic shadowing representing the presence of calcium.
Hard plaque: highly echo-reflective intimal thickening without shadowing representing dense fibrous tissue.
Soft plaque: poorly echo-reflective intimal thickening representing soft structures such as loose fibrous tissue, lipid, thrombus.

Quantitative measurements included measurement of plaque area. Total vessel area was defined as the area central to the ultrasound-defined medial-adventitial boundary. Lumen area was defined as the area central to lumen-intimal boundary. Plaque area was calculated as the difference between total vessel area and lumen area.

According to the distribution of atheroma, lesions were categorized as:
Concentric lesions: atheroma distributed along the entire circumference of the vessel wall in a cross-section, or as
Eccentric lesions: atheroma involving part of the circumference of the vessel wall in a cross-section leaving a disease-free or minimal diseased part of the vessel wall.

The eccentricity of the plaque was determined using the ratio between thickest and thinnest part of the intimal thickening. Eccentricity was defined by a ratio of more than 1.5. A soft or hard lesion was considered homogeneous if the plaque consisted of > 75% of one type of lesion determined from an integrated pull-back image of the entire lesion. A calcified lesion was considered homogeneous if the intimal thickening occupied > 180 degrees of the vessel circumference. A mixed soft/hard lesion was defined if the lesion contained both types of tissue. A mixed calcific lesion was defined as calcified intimal thickening more than 90 degrees of the vessel circumference in combination with a soft or hard lesion.

Statistical analysis
All measured values are represented as mean ± standard deviation. Analysis of variance was performed by using the chi-square test with Yates correction applied when required. A p value < 0.05 was considered statistically significant.

Results

The introduction of the imaging catheters at the ischemia-related lesion was invariably blood flow obstructive and in the majority of the cases (25/31) associated with chest pain and electrocardiographic ST-T segment changes. These changes were quickly reversible after withdrawal of the catheter, although in two patients abrupt occlusion occurred at the site of the ischemia related lesion. This was effectively treated with balloon angioplasty, without adverse sequelae. The angioscope caused a small non-occlusive dissection in one patient. Subsequent balloon angioplasty resulted in an occlusive dissection which was effectively managed by stent implantation. In another patient the atherectomy guiding catheter caused an occlusive ostial dissection after a technically successful procedure of a lesion in the RCA. A non-q-wave infarction developed (max. CK 600 U/l); the recovery was uneventful.

In 4 cases the culprit lesion was not crossed with the angioscope, and the observations were restricted to the proximal aspect of the stenosis. In the 2 cases where the lesion could not be crossed with the ultrasound probe, the plaque composition and size were taken from post angioplasty examination.

Combined imaging of ischemia-related lesion and coronary segment proximal to the lesion

The data obtained with intracoronary angioscopy and ultrasound are shown in Table 2 and 3. The prevalence of red thrombus was not different in patients with unstable angina (12/19;63%) or early postinfarction angina (9/12;75%). White masses were found in 10 (32%) and reddish streaks in 6 (19%) of the patients.

Table 2. Angioscopic characteristics of ischaemia related lesions (N=31)

	N (%)
Thrombus	21 (68)
-*Occlusive*	5 (24)
-*Mural*	14 (67)
-*Protruding*	2 (10)
Surface lesion	
-*Ulcerated*	19 (62)
-*Irregular*	6 (19)
-*Smooth*	6 (19)
Yellow plaque	11 (36)
Stable plaque	8 (26)
Complex plaque	23 (74)

Twenty-nine of the ischemia-related lesions were either predominantly soft (13) or did have significant soft areas (16); only 2 patients had a predominantly fibrous plaque without soft areas. The segments proximal of the ischemia-related lesion were diseased in all patients, although these segments were angiographically normal in 50% of the cases. The characteristics of these plaques are tabulated in Table 3.

The relationship between the angioscopic-derived presence of a thrombus, the angioscopic-derived surface characteristics of the lesion and the ultrasound-derived composition of the plaques is shown in figure 3. An ulcerated surface was associated with the presence of thrombus, whereas a smooth surface was noted more frequent in lesions without thrombus.

Relation between coronary angiography and intracoronary angioscopy

In figure 4 is shown that an angiographically-determined complex lesion is either almost always associated with an angioscopic determined ulcerated or irregular surface or is often associated with the presence of a angioscopic determined thrombus. A non-complex lesion is neither predictive of a smooth surface nor of absence of thrombus.

Discussion

Morphology of ischemia-related lesion

Angiographic, morphologic and angioscopic studies have described the degrees of luminal narrowing, the angiographic characteristics of the lesions, and the presence of thrombus in patients with acute ischemic syndromes.[14-18,20,27-32] Limited morp-

Table 3. Intracoronary ultrasound derived composition ischemia-related lesions and segments proximal to the lesion

	Stenoses N (%)	Proximal Segments N(%)
Total number	31	31
Composition plaque		
-*Soft*	13 (87)	18 (95)
-*Hard*	2 (13)	1 (5)
-*Calcific*	0 (0)	0 (0)
-*Mixed*	16 (52)	12 (39)
-*Soft/hard*	5 (31)	1 (8)
-*Soft/calcific*	1 (6)	3 (25)
-*Hard/calcific*	0 (0)	5 (42)
-*Soft/hard/calcific*	10 (63)	3 (25)
Eccentric	27 (87)	19 (61)
Extent plaque (mm^2)	14.48 ± 4.20	9.64 ± 3.09

hologic information is available on the composition of the individual coronary arterial atherosclerotic plaque in patients with unstable angina[31,32] and until now very limited information is available on the ultrasound derived composition of the plaque in living humans with unstable angina.[13] Recently, Kragel et al.[33] have described the composition of all atherosclerotic plaques in patients with unstable angina, shortly before death. They found that the major component of the plaques consisted of a combination of dense acellular and cellular fibrous tissue (82% to 86%) whereas much smaller portions of the plaques were composed of pultaceous debris, calcium, foam cells and inflammatory infiltrates. No attempt was made to identify the "culprit" lesion and whether the composition of this individual lesion was different from the "stable" lesions. This may explain their findings that the volume of lipid deposition apparently did not play a significant role in unstable patients, which is contrary to the opinion that unstable plaques are plaques which often contain an extensive volume of extracellular lipid.[31,34]

Hodgson et al.[13] performed a morphologic analysis of the ultrasound images obtained from ischemia-related lesions in patients with unstable angina. They found that in these patients soft lesions were present in 74%, and calcified or fibrocalcific lesions in 25%. There were only few intralesional calcium deposits (16%).

In our study we confirmed that the majority of the ischemia related lesions were either predominantly soft lesions or contained areas with soft material, as defined

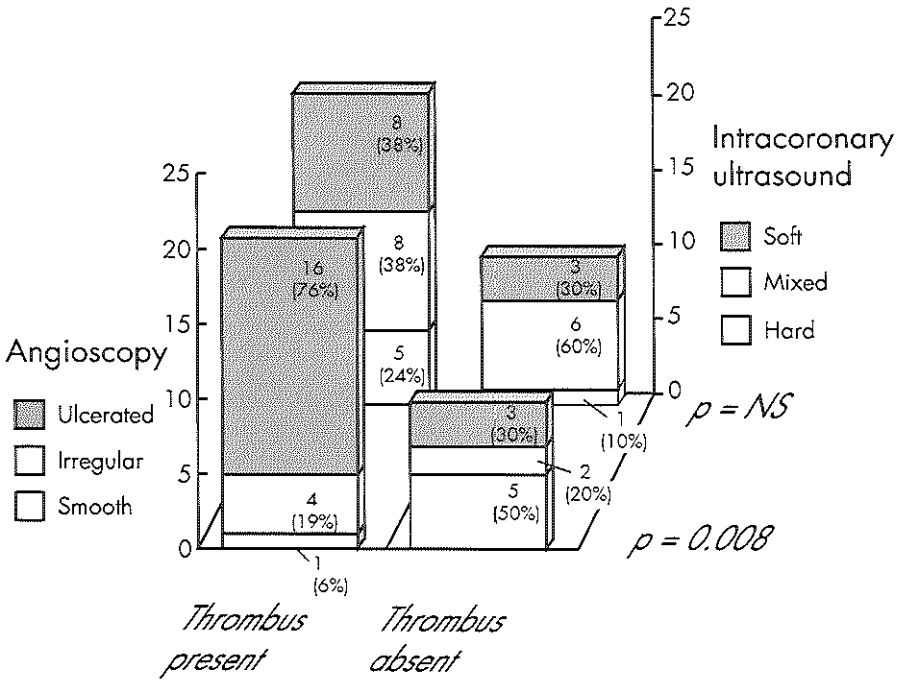

Figure 3

Relationship between angioscopic-derived presence of thrombus, the angioscopic-derived surface characteristics of the lesion and ultrasound-derived composition of the plaque.

above . However, these findings should be interpreted with caution because, although soft lesions are thought to represent lipid-containing lesions, ultrasound images cannot distinguish between loose fibrous tissue, lipid-rich lesions and thrombus.

We confirm previous angioscopic studies[14-18] which also demonstrated that ulcerated plaques with some degree of associated thrombus were present in the majority of the cases, and that components of the lesion were yellow coloured in one-third to about half of the cases.[16] However, in 23% of the patients the ischemia-related lesion was smooth, and not associated with thrombus suggesting that possibly other mechanisms than plaque disruption and thrombus formation may play a role. In our study we found that approximately one-third of the lesions was yellow. This rather low frequency may be related to the insensitivity of angioscopy to discern yellow components of the plaque, and to the criteria used in our study, where, in order to be avoid pitfalls such as colour distortion, an adjacent area of normal (white) arterial wall was requiered to qualify as xanthomatous plaque. Another possibility is that the yellow components of the plaque are covered by the overlying red thrombus and thus escape detection or the fibrous cap is too thick rendering the lipid-pool invisible. Angioscopy also revealed the presence of white

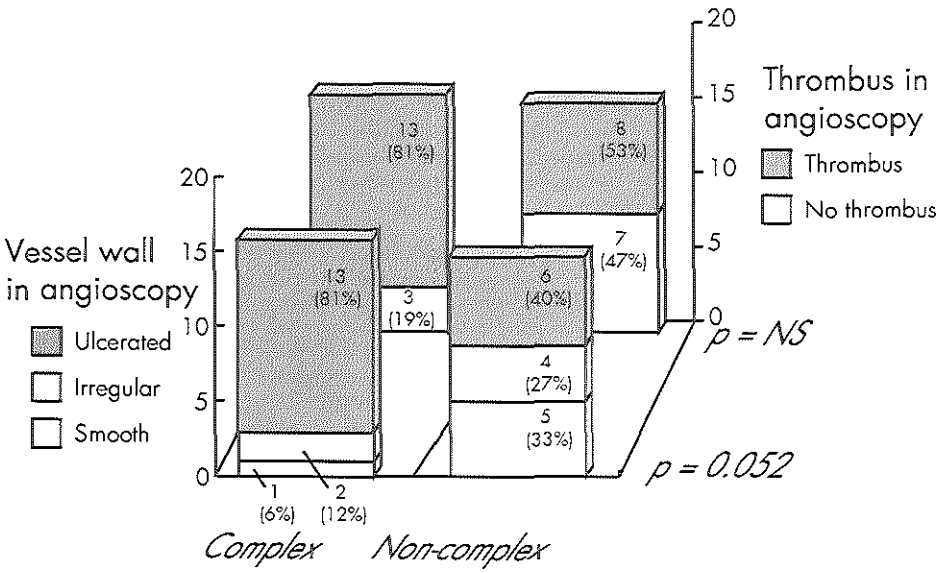

Figure 4

Relationship between angiographically-determined complex lesion, angioscopic-derived surface characteristics of the lesion and angioscopic-derived presence of red thrombus.

masses at the site of the ischemia-related lesion. These white masses possibly may represent early platelet thrombi, organized thrombi or intimal flaps covered with a thick layer of fibrin. Since we were not certain of their nature, we decided to classify them objectively as white masses. In 6 patients we found reddish streaks in the wall of the coronary artery. These streaks may represent areas of bleeding within the wall.

The data provide further evidence for the theory that plaque disruption of a fibrous cap overlying a lipid-rich lesion which is followed by non-occlusive thrombus formation occurs frequent in patients with unstable angina pectoris or early postinfarction angina.

Possible consequence for the management unstable angina

The major recognised physiopathologic mechanisms underlying unstable angina include a) plaque fissuring, which sets into action a complex interplay of b) platelet activation, adhesion and aggregation c) activation of coagulation system with formation of a transient, labile intraluminal thrombus and d) increased vasoconstriction by the platelet release of vasoactive products, serotonin and thromboxane and production of thrombin in combination with abnormal endothelial derived

relaxing factor modulated vasomotor response caused by endothelial dysfunction. All these events culminate into transient vessel occlusion and myocardial ischemia.[1] In the clinical setting of unstable angina it is largely speculative which of these mechanisms is dominant at a certain period. This precludes the employment of a more specific causal-related treatment. In an individual patient one assumes that all these mechanisms are operative at the same time and pragmatically treatment is directed to counteract all these mechanisms. Thus treatment includes antiplatelet aggregation, anticoagulation, and vasodilation in combination with treatment directed towards reduction in myocardial oxygen demand such as bedrest, reduction in both heart rate (β-blockers), and preload or afterload (systemic and arterial vasodilators). In addition thrombolytic treatment has also been used, but a large body of evidence is available indicating that thrombolytic treatment is, surprisingly, not efficacious in the setting of unstable angina.[35-37] This is the more surprising because of the expected high frequency of thrombi in these patients. It could be argued that these thrombi are resistent to thrombolytic treatment because they represent older organized thrombi.

The majority of the patients with unstable angina can be stabilized, and the presence and extent of remaining ischemia dictate further management. However, despite our increased knowledge, the mortality and infarction incidence in these patients remains high. Knowledge of the composition of the plaque and vessel wall, in combination with information about the presence of thrombus or complex plaque as can be offered by intracoronary angioscopy and ultrasound may increase our understanding and may allow better individualisation of patient management.

A severe, concentric lesion, composed of "hard" tissue (fibrocalcific or predominantly calcific) may be indicative of low likelihood of a beneficial response to pharmacological therapy and may require early revascularisation. A very soft lesion may be more amenable to remodelling and initial pharmacologic treatment, including cholesterol lowering drugs, may stabilize or reduce this lesion. An eccentric lesion with a opposite normal disease free vessel wall capable of dilatation may suggest that vasomotion does play a significant role and may require intensive use of vasodilator treatment.[38] The presence of an angioscopically determined smooth stable lesion may be a significant clue to suspect vasomotion as a major pathogenic mechanism. An occlusive red thrombus or the presence of abundant non-occlusive thrombus, easily detected by the angioscope, may suggest that adjunctive thrombolytic treatment is useful, whereas mural, non-occlusive thrombus, often seen in these patients may not require adjunctive thrombolytic therapy.

Information about the location of the thrombus in the lesion may enhance treatment. At the apex of the lesion a high-shear rate induced white platelet thrombus is formed[39] which is resistent to aspirin and probably requires therapy directed against von Willebrand Factor specific domain on GPIIb-IIIa and GPIb.[40,41] Proximal and distal of the lesion a low shear rate red coagulation thrombus is for-

med; the latter is often angiographically visible as a free floating tail. Aspirin, heparin or thrombolytics may be required in these situations.[1,29]

Knowledge of the composition of the plaque may optimize the selection of an appropriate intervention technique. Calcific lesions may be more amenable to rotablation techniques or non-calcified eccentric lesions may be better suitable to directional atherectomy. Calcified target lesions have a higher likelihood of dissection after balloon angioplasty.

Limitations of this study

This study was performed in unstable patients having angina at rest class IIIB or early postinfarction angina class IIIC according to the Braunwald classification and selected for balloon angioplasty. They represent only a sample from the broad spectrum of patients with unstable angina and thus precludes generalisation of the findings.

Unstable angina pectoris is a dynamic process with different pathophysiologic mechanisms which waxe and wane over time. In this study the images were obtained only once, and the presence of certain processes may have been missed. Only longer-term monitoring would resolve this problem.

The current available imaging devices are yet still bulky and stiff. Sometimes the ischemia-related lesions could not be crossed, so that entire interrogation of the lesion was not always possible. Even, after crossing the lesion, certain aspects may escape detection because the current angioscopic design does not include a flexible, steerable feature so that the entire surface area cannot always be inspected. Also, structures lying behind calcific lesions cannot be detected with ultrasound because the plaque prevents penetration of the ultrasonic beam.

Certain features may have been missed because the device may have detached thrombi or other structures or during angioscopy loosely attached thrombi may have been flushed away.

Currently, assessment of angioscopy and ultrasound images, is qualitative, and thus subject to differences in interpretation. In the near future 3-dimensional reconstructed longitudinal display of ultrasound images of lesions may provide a more accurate delineation of extent and composition of the plaque and may allow quantification of the lesions.[42]

Final remarks

Sequential imaging of the ischemia-related lesion with intracoronary angioscopy and ultrasound is feasible, and currently only safe in the setting of patients who are scheduled for coronary intervention. Inadvertent damage to the lesion can be reversed by immediate angioplasty. The obtained information is complementary and adjunctive to coronary angiography. Angiography defines the contour of the

lumen of the vessel, provides information about extent and severity of coronary atherosclerosis along the entire coronary tree and provides rather crude qualitative information about antegrade blood flow (TIMI 0 to 3) or presence of collateral flow. In addition it serves as the necessary road map for angioscopic and ultrasound catheters. Angioscopy provides direct information about the endovascular surface that is relatively easily interpretable. Ultrasound imaging provides information about the wall thickness and composition of the lesion.

The integrated information substantiates some of the suggested mechanisms in the pathogenesis of unstable angina. Soft, lipid-rich plaques are prone to rupture and subsequent thrombus formation. However, in some patients the dominant paradigm of the syndrome could not be confirmed. It remains speculative to propose that this happened because the fissuring or thrombus formation were too transient and too subtle to be detected, or because other pathogenetic mechanisms may have played a role in these patients. Further miniaturization of these devices will allow investigation of the lesions without the necessity for subsequent coronary intervention and is expected to provide useful additional information to improve patient management and prognosis.

References

1. *Fuster V, Badimon L, Badimon JJ, Chesebro JH. The pathogenesis of coronary artery disease and the acute coronary syndromes. N Engl J Med 1992;326:242-50 and 310-8.*

2. *Mulcahy R. Natural history and prognosis of unstable angina. Am Heart J 1985;109:753-8.*

3. *Lewis DH, Davis JW, Archibald DG, et al. Protective effects of aspirin against acute myocardial infarction and death in men with unstable angina. N Engl J Med 1983;309:396-403.*

4. *Cairns JA, Gent M, Singer J, et al. Aspirin, sulfinpyrazone or both in unstable angina: results of a Canadian multicenter trial. N Engl J Med 1985;313:1369-75.*

5. *Théroux P, Ouimet H, McCans J, et al. Aspirin, heparin or both to treat acute unstable angina. N Engl J Med 1988;319:1105-11.*

6. *Muller JE, Turi ZG, Pearle, et al. Nifedipine and conventional therapy for unstable angina pectoris: a randomized double-blind comparison. Circulation 1984;69:728-39.*

7. *Holland Interuniversity Nifedipine/Metoprolol Trial (HINT) Research Group: early treatment of unstable angina in the coronary care unit: a randomized, double blind, placebo controlled comparision of recurrent ischemia in patients treated with nifedipine or metoprolol or both. Br Heart J 1986;56:400-13.*

8. *Nissen SE, Gurley JC, Grines CL, et al. Intravascular ultrasound assessment of lumen size and wall morphology in normal subjects and patients with coronary artery disease. Circulation 1991;84:1087-99.*

9. *Tobis JM, Mallery JA, Mahon D, et al. Intravascular ultrasound imaging of human coronary arteries in vivo. Analysis of tissue characterizations with comparison to in vitro histologic specimens. Circulation 1991;83:913-26.*

10. *Coy KM, Maurer G, Siegel RJ. Intravascular ultrasound imaging: a current perspective. J Am Coll Cardiol 1991; 18: 1811-23.*

11. *Mintz GS, Douek P, Pichard AD, et al. Target lesion calcification in coronary artery disease: an intravascular ultrasound study. J Am Coll Cardiol 1992;20:1149-55.*

12. *Tenaglia AN, Buller CE, Kisslo KB, Phillips HR, Stack RS, Davidson CJ. Intracoronary ultrasound predictors of adverse outcomes after coronary artery intervention. J Am Coll Cardiol 1992;20:1385-90.*

13. *Hodgson J, Reddy KG, Suneja R, Nair RN, Lesnefsky EJ, Sheehan HM. Intracoronary ultrasound imaging: correlation of plaque morphology with angiography, clinical syndrome and procedural results in patients undergoing coronary angioplasty. J Am Coll Cardiol 1993;21:35-44.*

14. *Sherman CT, Litvack F, Grundfest W, et al. Coronary angioscopy in patients with unstable angina pectoris. New Engl J Med 1986;315:913-9.*

15. *Mizuno K, Arai T, Satomura K, et al. New percutaneous transluminal coronary angioscope. J Am Coll Cardiol 1989;13:363-8.*

16. *Mizuno K, Miyamoto A, Satomura K, et al. Angioscopic coronary macromorphology in patients with acute coronary disorders. Lancet 1991;337:809-12.*

17. *Ramee SR, White CJ, Collins TJ, Mesa JE, Murgo JP. Percutaneous angioscopy during coronary angioplasty using a steerable microangioscope. J Am Coll Cardiol 1991;17:100-5.*

18. *Mizuno K, Satomura K, Miyamoto A, et al. Angioscopic evaluation of coronary artery thrombi in acute coronary syndromes. N Engl J Med 1992;326:287-91.*

19. *Braunwald E. Unstable angina: a classification. Circulation 1989;80:410-4.*

20. *Ambrose JA, Winters SL, Arora RR, et al. Coronary angiographic morphology in myocardial infarction: a link between the pathogenesis of unstable angina and myocardial infarction. J Am Coll Cardiol 1985;6:1233-8.*

21. *Haase J, van der Linden MMJM, Di Mario C, van der Giessen WJ, Foley DP, Serruys PW. Can the same edge-detection algorithm be applied to on-line and off-line analysis system? Validation of a new cinefilm-based geometric coronary measurement software. Am Heart J 1993: accepted for publication.*

22. *Gussenhoven EJ, Essed CE, Roelandt JRTC, et al. Arterial wall characteristics determined by intravascular ultrasound imaging: an in vitro study. J Am Coll Cardiol 1989;14:947-52.*

23. *Potkin BN, Bartorelli AL, Gessert JM, et al. Coronary artery imaging with intravascular ultrasound. Circulation 1990;81:1575-85.*

24. *Nishimura RC, Edwards WD, Warnes CA, et al. Intravascular ultrasound imaging in vitro validation and pathologic correlation. J Am Coll Cardiol 1990;16:145-54.*

25. *Di Mario C, The SK, Madrestma S, et al. Detection and characterization of the atherosclerotic plaque. J Am Soc Echocardiogr 1992;5:135-46.*

26. *Siegel RJ, Ariani M, Fishben MC, et al. Histopathologic validation of angioscopy intravascular ultrasound. Circulation 1991;84:109-17.*

27. *Davies MJ, Thomas AC. Plaque fissuring - the cause of acute myocardial infarction: sudden ischaemic death and crescendo angina. Br Heart J 1985;53:363-73.*

28. *Hangarter JWR, Charleston AJ, Davies MJ, Thomas AC. Morphological characteristics of clinically significant coronary artery stenosis in stable angina. Br Heart J 1986;56:501-8.*

29. *Falk E. Morphologic features of unstable atherothrombotic plaques underlying acute coronary syndromes. Am J Cardiol 1989;63:114E-20E.*

30. *Tracy R, Devaney K, Kissling G. Characteristics of the plaque under a coronary thrombosis. Virchows*

Arch 1985;405:411-27.

31. Davies MJ, Woolf N, Katz DR. Structure and cellular composition of aortic atherosclerotic plaques undergoing ulceration. Br Heart J 1991;66:92 (abstr).

32. Kragel AH, Gertz SD, Roberts WC. Morphologic comparison of frequency and types of acute lesions in the major epicardial coronary arteries in unstable angina pectoris, sudden coronary death and acute myocardial infarction. J Am Coll Cardiol 1991;18:801-8.

33. Kragel AH, Reddy SG, Wittes JT, Roberts WC. Morphometric analysis of the composition of coronary arterial plaques in isolated unstable angina pectoris with pain at rest. Am J Cardiol 1990;66:562-7.

34. Richardson P, Davies M, Born G. Influence of plaque configuration and stress distribution on fissuring of coronary atherosclerotic plaques. Lancet 1989;2:941-4.

35. Williams DO, Topol EJ, Califf RM, et al. and Coinvestigators. Intravenous recombinant tissue-type plasminogen activator in patients with unstable angina pectoris. Results of a placebo-controlled, randomized trial. Circulation 1990;82:376-83.

36. Freeman MR, Langer A, Wilson RF, Morgan CD, Armstrong PW. Thrombolysis in unstable angina. Randomized double-blind trial of t-PA and placebo. Circulation 1992;85:150-7.

37. Bär FW, Verheugt FW, Col J, Materne P, et al. Thrombolysis in patients with unstable angina improves the angiographic but not the clinical outcome. Results of UNASEM, a multicenter, randomized, placebo-controlled, clinical trial with anistreplase. Circulation 1992;86:131-7.

38. Saner HE, Gobel FL, Salomonowitz E, Erlien DA, Edwards JE. The disease-free wall in coronary atherosclerosis: its relation to degree of obstruction. J Am Coll Cardiol 1985;6:1096-9.

39. Badimon L, Badimon JJ. Mechanisms of arterial thrombosis in nonparallel streamlines: platelet thrombi grow on the apex of stenotic severely injured vessel wall. Experimental study in the pig model. J Clin Invest 1989;84:1134-44.

40. O'Brien JR. Shear-induced platelet aggregation. Lancet 1990;335:711-3.

41. Ruggeri ZM. Von Willebrand factor as a target for anti-thrombotic intervention. Circulation 1992;86:(suppl III)III26-III9.

42. Rosenfield K, Losordo DW, Ramaswamy K, et al. Three-dimensional reconstruction of human coronary and peripheral arteries from images recorded during. Two-dimensional intravascular ultrasound examination. Circulation 1991;84:1938-56.

Chapter XI

The Significance of Automated Stenosis Detection During Quantitative Angiography: Insights Gained from Intracoronary Ultrasound Imaging

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Abstract

Background

Automated stenosis analysis is a common feature of commercially available quantitative angiography systems (QCA), allowing automatic detection of the proximal and distal boundaries of the stenosis, interpolation of the expected dimensions of the coronary vessel at the point of obstruction, and angiographically-derived estimation of atheromatous plaque size. However, the ultimate meaning of this type of analysis as to the degree of underlying atherosclerotic disease (AD) remains unclear.

Aim of the study

We investigated the relationship between stenosis analysis performed with the CAAS system and the underlying degree of atherosclerotic disease as judged from intracoronary ultrasound (ICUS) imaging.

Methods

In 40 coronary stenoses QCA was used for automated identification of the sites of maximal luminal obstruction and start of the stenosis using curvature analysis of the diameter function. Plaque size was measured using ICUS at both locations, with an additional ICUS measurement immediately proximal to the start of the stenosis. Crescent-like distribution of plaque, indicating an AD-free arc of the arterial wall, was recorded.

Results

At the site of the obstruction total vessel area measured with ICUS was 16.65±4.04 mm^2, while an equivalent measurement obtained from interpolated reference dimensions was 7.48±3.30 mm^2, (p=0.0001). Plaque area derived from angiographic data was significantly lower than that calculated from intravascular ultrasound data, (6.32±3.21 and 13.29±4.22 mm^2, respectively, mean difference 6.92±4.43 mm^2, p=0.0001). At the site identified as the start of the stenosis by automated analysis, ICUS luminal cross-sectional area was 9.38±3.17 mm^2, and total vessel area was 18.77±5.19 mm^2, (50±11% total vessel area stenosis). The arterial wall presented a disease-free segment in 28 (70%) of proximal locations, but only in 5 (12%) sites corresponding to the start of the stenosis and no at the obstruction (p=0.0001). At the site of obstruction all vessels showed a complete absence of a disease free segment, and the atheroma presented a cuff-like or all-around distribution with a variable degree of eccentricity.

Conclusions

1/ AD was consistently present at the start of the stenosis used as a reference site by automated stenosis analysis. 2/ The mean degree of AD involvement at the start of the stenosis was 50% total area stenosis. 3/ A significant change from crescent to all-around distribution of AD was found at the start of the stenosis. These findings suggest that the start of the stenosis identified by automated stenosis analysis represents the point where compensatory vessel enlargement fails to preserve luminal dimensions, and provide insights on the mechanisms involved in this phenomenon.

Introduction

During its relatively short history, the role of coronary angiography as a standard in the assessment of coronary artery disease has been challenged by two types of limitations. First, visual assessment of stenosis severity from the cineangiogram is associated with a high intra- and interobserver variability.[1-4] Second, major discrepancies between the appearance of the opacified vascular lumen and the actual degree of underlying atherosclerosis have been reported.[5-9] These can be due to the presence of extensive diffuse disease which affects the whole length of the opacified coronary tree, without a remnant "healthy" reference segment. More importantly, underestimation of the extent of atherosclerotic disease may occur due to the fact that during the development of both diffuse and focal atherosclerotic lesions coronary arteries undergo compensatory enlargement.[10,11]

The advent of quantitative angiography has reduced significantly the first limitation. Several quantitative angiography systems, including the Cardiovascular Angiography Analysis System (CAAS) which was developed at our Institution, are capable of performing automated stenosis detection in a given coronary segment.[12,13] Using information obtained from computerised analysis of the entire segment, automated analysis detects not only the proximal and distal boundaries of the stenosis, but also an interpolation of the expected dimensions of the coronary vessel at the point of obstruction (a so-called interpolated reference). The angiographic estimation of the amount of atheromatous plaque derived from this data is also a common feature of commercially available quantitative angiography packages, which are likely to become more widely used since they are now built-in features of many modern digital angiographic systems. In spite of this, it remains unknown whether the data calculated from automated stenosis analysis can provide reliable information on the degree or presence of underlying atherosclerotic disease. The use of automatic stenosis detection techniques may reduce the variability associated with the arbitrary selection of a reference segment, being useful in longitudinal angiographic studies. However, its basic premise, that is, that computerised analysis of a large coronary segment encompassing the stenosis can identify the actual boundaries of the stenosis, has never been tested.

Intracoronary ultrasound can provide information on the characteristics of the arterial wall.[14,15] This characteristic justifies its growing application in the study of atherosclerotic coronary artery disease,[15-22] and its proposal as an alternative gold standard to coronary angiography.[14] However, comparisons between intracoronary ultrasound and quantitative angiography have been confined only to its ability to measure luminal dimensions, and never used to investigate the significance of other findings obtained during automated stenosis analysis.[14]

The objective of this study was twofold. First, we wanted to investigate with intracoronary ultrasound the characteristics of the arterial wall at the site identified by automated stenosis analysis as the proximal boundary of the stenosis, since pre-

vious studies with quantitative angiography have assumed the absence of atherosclerotic disease at this location for the calculation of interpolated refernce dimensions.[23] Secondly, we were interested in assessing whether, at the site of maximal luminal obstruction, the amount of atheroma derived from automated stenosis analysis reflects the degree of atherosclerotic involvement as judged by intracoronary ultrasound.

Methods

Patient population

The study population consisted of forty patients (31 male and 9 female) with de novo coronary stenosis undergoing cardiac catheterisation immediately prior to percutaneous revascularisation. Mean age was 61±10 years. All investigations were approved by the Institutional Review Board of the Thoraxcenter, and patients were studied after giving informed consent.

Quantitative angiography

In this study both on line and off-line quantitative coronary angiography was performed. On line measurements were performed immediately prior to intravascular ultrasound examination using a Philips DCI angiography system in conjunction with a commercially available quantitative angiography package (ACA, Philips, Eindhoven, The Netherlands). The results of the analysis, including the location of the beginning and end of the stenosis, as well as the point of maximal luminal obstruction identified by the computerized analysis, were permanently displayed in a video monitor, serving as a guide for the operator during the ultrasound study. Coronary cineangiograms were also obtained and later analysed off-line in a 3rd generation edge detection quantitative angiography system (CAAS 2, Pie Data, Maastricht, the Netherlands),[24,25] which uses a similar algorithm as the ACA for the purpose of stenosis identification and reference diameter interpolation.[26] A description of the consecutive steps followed during the analysis of the cineangiogram is given below:

1/ *Image acquisition*: End-diastolic angiographic frames showing the stenosed vessel were selected. Using a CCD camera, a region of interest of 512x512 pixels encompassing a wide vascular segment proximal to the stenosis was selected in the cineframe and digitized for subsequent analysis (Fig. 1A).

2/ *Identification of luminal edges*: Following the identification of the vessel centerline by the computer algorithm, a number of scanlines perpendicular to it were obtained. Luminal edges were detected on the basis of a weighted sum of the first and second derivative function of the brightness profile of each of these scanlines (Fig. 1B).

3/ *Diameter function*: Vessel diameters were determined by computing the shortest

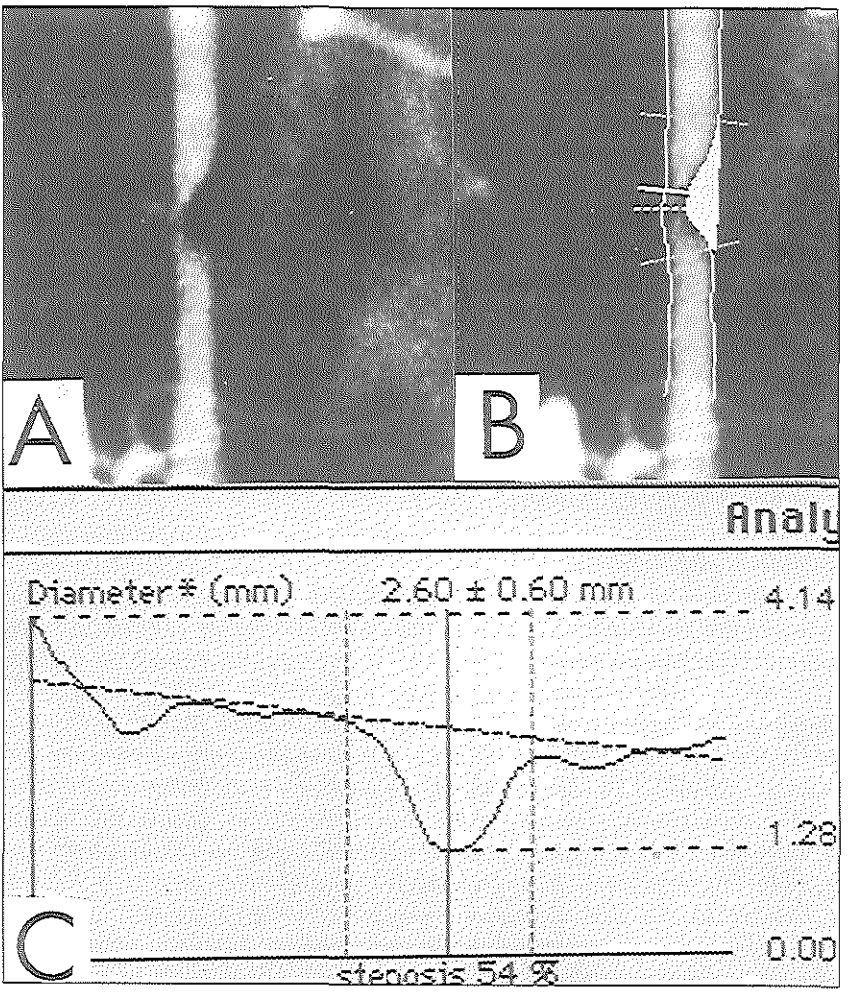

Figure 1

Quantitative angiographic analysis as performed in the present study. After a region of interest in the coronary angiogram showing a wide vascular segment encompassing the stenosis was digitised (A), luminal edges were identified using a contour detection algorithm (B). After calibration using the coronary actehter as a scaling device was performed plotting, the consecutive vessel diameters were plotted to create a so-caled vessel diameter function (C). Application of specific algorithms to this function made possible the identification of the poitn with minimal luminal diameter (solid bar) as well as the proximal and distal boundaries of the stenosis (dotted bars). Based in the diameter function, the expected dimensions of the vessel at the site of the obstruction (interpolated reference diameter) were calculated. (See text for details)

distance between the left and right edge positions. These measurements were converted to absolute values using the coronary guiding catheter as a scaling device. By plotting all consecutive diameter values obtained at approximately 0.2 mm intervals over the analysed vessel length, a so-called diameter function was created (Fig. 1C).

4/ *Identification of the start of the stenosis.* Application of specific algorithms to the diameter function made possible the identification of vessel location where critical change in the diameter values occurred. In any coronary segment subject of analysis it is possible to observe dips in the diameter function resulting from changes in luminal diameter or image noise. To discriminate between these artifactual changes and the actual change in luminal diameter associated with the start of a stenosis, the diameter function is analysed in the CAAS II system using a curvature detection algorithm which identifies maxima in curvature using variable degrees of smoothing. The algorithm is nearly identical to that described by Rosenfeld and Johnston.[27] The proximal and distal boundaries of the obstruction are defined by the positions featuring the first local maximum in curvature in proximal and distal directions respectively with respect to the minimal diameter position. The extent of the stenosis is indicated in the diameter function by two dotted lines as is represented as a shaded area superimposed on the artery (Fig. 1C)

5/ *Identification of the site of obstruction.* From the diameter function, the site of obstruction is identified as that corresponding to the lowest diameter value in the segment encompassed between the start and end of the stenosis.

6/ *Interpolated reference diameter.* The third parameter derived from the analysis of the diameter curve is the interpolated reference diameter. After the creation of a first degree polynomial computed through the diameter values of the proximal and distal portions of the arterial segment, a translation to the 80th percentile level was performed. Combining this information with the location of the obstruction, the expected diameter of the vessel at the site of minimal luminal diameter was calculated. In this way, a correction for the expected changes in vessel diameter between the start and end of the stenosis, such as those resulting from the origin of side branches. is introduced.

7/ *Angiographically-derived plaque area.* Based in the above discussed premises, plaque area was defined as the difference between the interpolated and luminal dimensions at the obstruction site (Fig. 2A). This is a variation of the calculation of plaque area performed in the CAAS and other commercially available systems[12,13] in the longitudinal axis (Fig. 1 B), and was chosen to facilitate its comparison with cross-sectional areas measured during intravascular ultrasound (Fig. 2B).

Intravascular ultrasound

Intravascular ultrasound was performed using a 30 MHz intravascular ultrasound system (Cardiovascular Imaging Systems, Inc., California). Collection of data was

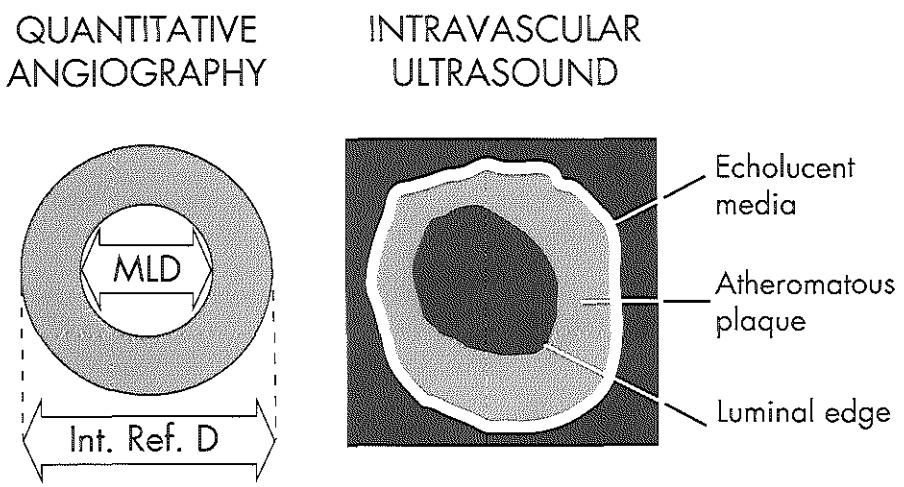

Figure 2

Method used for the calculation of atherosclerotic plaque area using quantitative angiography and intravascular ultrasound. Assuming a circular cross section, plaque area was calculated from quantitative angiography as the difference between the areas derived from the interpolated reference (Int.Ref.D) and minimal luminal (MLD) diameters. Plaque area was defined with intravascular ultrasound imaging as the difference between the areas comprised within the medial and luminal boundaries, obtained directly from planimetric measurements.

restricted to the pre angioplasty stage. The observer was free to adjust gain, magnification and other settings of the ultrasound system to obtain optimal visualization of the plaque and luminal borders. Particular attention was paid to ensure that the collection of echocardiographic data matched the sites identified by QCA as the beginning of the lesion and the obstruction site. In order to do this, on-line quantitative analysis was performed and displayed in one of the monitors to be used as a reference during the procedure. Once the stenosis was crossed with the guidewire, the operator was free to perform any contrast injection, maneuver with the guiding catheter that were required in order to advance safely the ultrasound catheter until a location distal to or wedged in the stenosis. This location was documented by contrast injection. A slow pull back of the ultrasound catheter was then performed, documenting its location with new contrast injections at the points identified by quantitative angiography as the obstruction site, beginning of the lesion and its adjacent proximal site. During the whole procedure, simultaneous recording of fluroscopy and echocardiographic images was performed using a digital videomixer. This facilitated later the correlation between ultrasound images and the location of the echo probe in the opacified vessel. Furthermore, the location of the echo probe was documented in pre-designed printed forms during the procedure, using the time counter of the echo machine (which was recorded along the images) as a temporal landmark. After the procedure, off-line area measurements in the locations of interest were performed using digital planimetry which

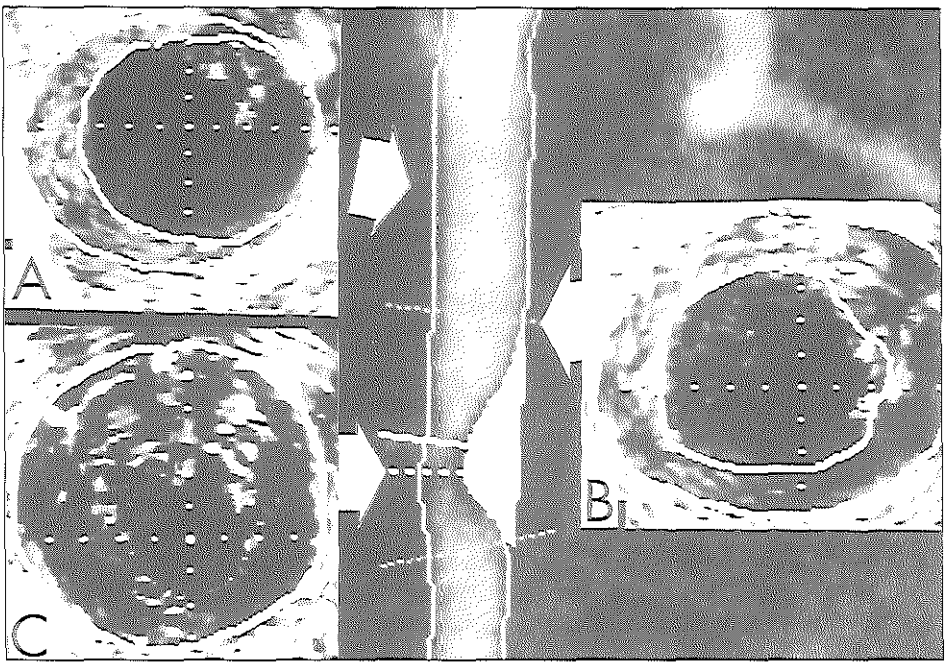

Figure 3

Intravascular ultrasound findings at the site proximal to the stenosis (A), at the start of the stenosis as defined by automated stenosis analysis (B) and at the site of maximal luminal obstruction (C), defined also by automated stenosis analysis. Note the marked change in distribution of atheroma around the lumen (centre of the cross-hair) at the three levels.

is a build-in feature of the described ultrasound system. Total vessel area was defined as that comprised within the echolucent medial layer, while luminal area as that comprised within the luminal edges. Plaque area was defines as the difference between total vessel and luminal areas. All measurements were performed independently in two separate sessions by two observers with expertise in intravascular ultrasound.

Exclusion criteria

Vessels with anatomical features that interfere with computerized stenosis analysis, including ostial lesions where a proximal segment of the vessel was not present, and total or functional occlusions with incomplete opacification of the coronary segment distal to the stenosis,were excluded from the study.

Statistical analysis

Mean ± standard deviation were calculated for all continuous variables. Least squares linear regression analysis was performed and correlation coefficients calculated. Continuous variables were compared using two-tailed paired and unpaired Student's t test as required. Bonferroni correction was applied when comparison

between more than 2 groups were done. A p value less than 0.05 was considered statistically significant.

Results

Findings at the site of obstruction.
In the 40 patients studied, quantitative angiography revealed a minimal luminal cross-sectional area of 1.24±1.12 mm^2. At the site of obstruction, intravascular ultrasound yielded a luminal area of 2.80±1.64 mm^2. Wedging of the catheter was observed in 24 cases (60%). In the 16 cases where the ultrasound catheter was not wedged a good correlation between angiographic and intravascular ultrasound luminal measurements was observed (r=0.78, p=0.0002).

At the site of the obstruction intravascular ultrasound revealed a total vessel area of 16.65±4.04 mm^2 (83±10% total vessel area stenosis). This was significantly larger than that calculated from the interpolated reference dimensions obtained with quantitative angiography (7.48±3.30 mm^2, p=0.0001). Thus, quantitative angiography underestimated the dimensions of the original vessel as assessed with intravascular ultrasound. As a result of this differences, plaque area derived from angiographic data was significantly lower than that calculated from intravascular ultrasound data, (6.32±3.21 and 13.29±4.22 mm^2 respectively, mean difference 6.92±4.43 mm^2, p=0.0001). Regression analysis yielded a correlation coefficient between both estimates of plaque size of 0.23 (R-squared =0.05, p=NS).

Findings at the start of the stenosis and in the proximal vessel.
At the site identified as the start of the stenosis by automated analysis, intravascular ultrasound cross-sectional area was 9.38±3.17 mm^2, and total vessel area was 18.77±5.19 mm^2 (50±11% total vessel area stenosis). Significant differences were found in the distribution of the atheromatous plaque around the lumen in the proximal vessel, start of the stenosis and site of the obstruction. Thus, the arterial wall presented a disease-free segment in 28 (70%) of proximal locations, but only in 5 (12%) sites corresponding to the start of the stenosis and no at the obstruction (p=0.0001)(Fig. 2). At the site of obstruction all vessels showed a complete absence of a disease free segment, and the atheroma presented a cuff-like or all-around distribution with a variable degree of eccentricity

Discussion

From a historical point of view, the reason for the development of computerized analysis of the stenosis was to reduce the variability associated with arbitrary selection for the user of a reference segment, since atherosclerotic involvement could be demonstrated at that location.[28,29] Coronary angiography represents a "luminogram" or "shadowgram" of the vessel, and its visual interpretation conveys little or

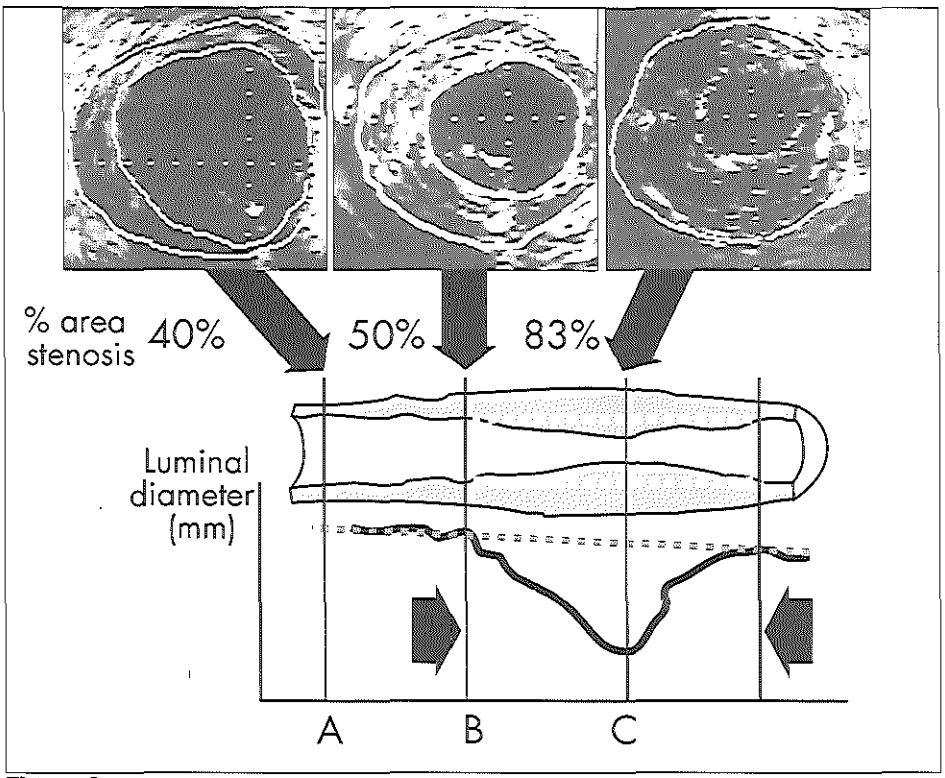

Figure 4

Schematic representation of the findings in the present study. Proximal to the stenosis (A), as defined by automated stenosis analysis (solid arrows in the diameter function), atheroma caused a mean vessel stenosis of 40%. At the site identified as the proximal boundary of the stenosis mean vessel (B) stenosis was 50%, progressing to 83% at the site of maximal obstruction (C). The discrepancy with plaque size calculated from the interpolated reference diameter (dotted line) may be related either to the incorrect assumption that at the start of the stenosis no disease was present, or to outward expansion of the plaque due to compensatory enlargement.

no information as to the extent of atherosclerotic disease in the arterial wall. Studies comparing angiographic and pathological data have demonstrated that visual interpretation of the angiogram underscores the degree of underlying disease both at the site of the obstruction and in segments which are apparently free of disease.[5-9] Thus, although the advent of early quantitative angiographic systems had facilitated a more accurate assessment of luminal obstruction, choosing a reference coronary segment for clinical purposes such as the calculation of relative measurements of stenotic severity remained associated with high variability and inaccuracy.

In an attempt to find a solution to these problems, several automated methods of analysis of the luminal dimensions have been developed[12,13] with the aims of provi-

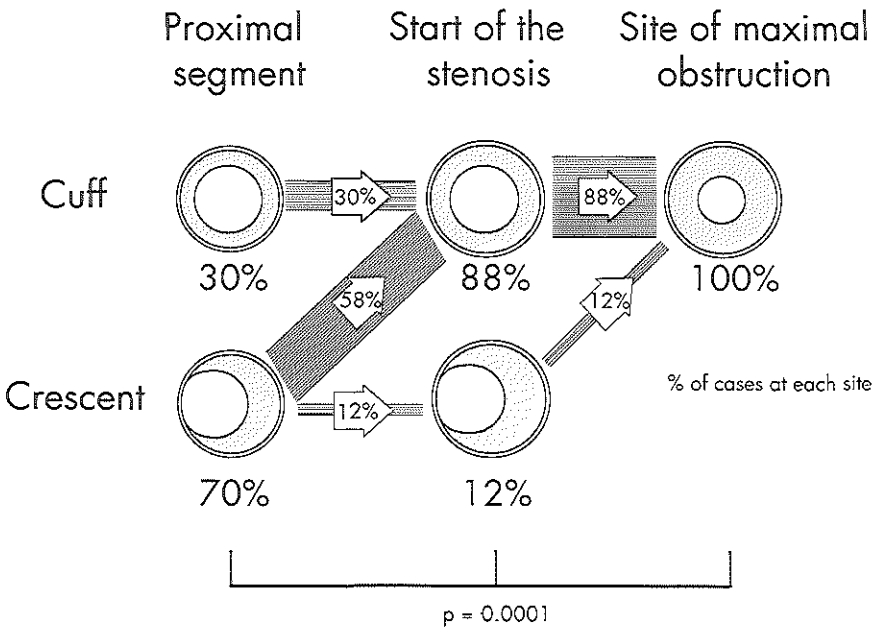

Figure 5

Change in the distribution pattern of atheroma in the analysed vessel. Proximal to the start of the stenosis (as defined by automated analysis) most vessels presented a characteristic crescent-like distribution of atheroma, with an apparently disease-free arc of the vessel wall. A significance change from this pattern to a cuff-like or all-around distribution of atheroma at the sites defined as the start of the stenosis and maximal obstruction was noted. The dissapearence of the disease-free arc in the vascular wall may constitute the landmark for the failure of compensatory mechanisms of vessel enlargement that preserve tha vascular lumen during the early stages of atherosclerosis progression.

ding objective identification of the boundaries and length of the stenosis and identifying a segment apparently free of atherosclerotic disease to be used as a reference during the calculation of relative measurements, and for the application of interpolated reference techniques. As we have described above, the approach followed by the CAAS system consists in applying specific algorithms to a so-called diameter function, obtained by plotting all consecutive luminal diameters in the vascular segment encompassing the stenosis. However, the relationship between computerized analysis of the luminal dimensions in a segment that encompasses a stenosis and the underlying degree of atherosclerosis has not been previously studied.

The possibility of inferring information on the degree of underlying atherosclerotic disease from such computerized analysis of luminal dimensions would be supported by the concept that atherosclerosis is a focal[30] and not diffuse[31,32] process. In

this regard, Baroldi et al.[33] found in a pathological study of 565 atherosclerotic coronary vessels that the length of coronary lesions was less than 5 mm in 13%, between 5 and 20 mm in 38%, and more than 20 mm in 49% of cases . Thus, a substantial number of coronary lesions have a length covered by conventional computerized angiographic analysis of a vascular segment.

Our observations with intravascular ultrasound indicate that atherosclerotic involvement at the site identified by QCA as the start of the lesion is common. Therefore, the site identified by the curvature detection algorithm in the diameter function does not correspond, as first thought, to a coronary location free of atherosclerotic disease. Interestingly, we found that at this level total vessel area stenosis is quite similar to that found by Glagov et al.[10] to be related with the failure of compensatory mechanisms of vessel enlargement, which is observed during the early phases of atherosclerosis progression.[10] The observations reported by other authors in coronary segments with minimal or no luminal narrowing as assessed by angiography support our findings. In such segments total vessel area was occupied by atheroma in 35±23% and 45±15% in the work of Tobis[17] and Hodgson[18] respectively. Although these observations were performed with a different aim, and therefore the collection of data was not matched with computer analysis of the stenosis, it is fair to conclude that protrusion of atheroma into the lumen probably does not occur below a atherosclerotic involvement causing 50% reduction in total vessel area, which is located at the start of the angiographic stenosis. Based on these observations, we can formulate the first conclusion of our study by saying that the proximal boundary of the stenosis identified during automated stenosis analysis with the CAAS system does not correspond to a location free of atherosclerotic disease but, presumably, where the compensatory mechanisms of vessel enlargement fail in preserving luminal dimensions.

Some qualitative observations with intracoronary ultrasound in performed in the present study may provide new insights on the mechanisms underlying compensatory vessel enlargement. We found that at the level of the proximal boundary of the stenosis there was a significant change in the distribution of the atheroma around the coronary lumen when compared with more proximal locations. This consisted of a change from a crescent-like pattern of atheroma, which was the dominant pattern in a proximal location , to a cuff-like pattern at the start of the stenosis. At the point of maximal luminal obstruction, only this latter pattern of atheroma distribution was observed, an observation that fits with the findings of Hangartner et al.[34] in a pathological study. The loss of an arc of disease-free wall, characteristic of

the crescent-like distribution of atheroma, may, as first proposed by Glagov,[10] be critical in the loss of compensatory vessel enlargement, a phenomenon that, as the present study suggests, can be observed at the start of the stenosis as defined from computerized analysis of the angiogram. Previous work has demonstrated that in the presence of a normal arterial wall, vessel enlargement occurs in circumstances of increased shear rate, a phenomenon that appears to be endothelium-mediated.[35] During the progression of coronary artery disease, reactive expansion to the increased shear stress may constitute the basis of compensatory vessel enlargement, but it would be expected that would disappear when a complete loss of normal reactive wall occurs.[36] The abolishment of such compensatory response might be due to complete encroachment of the lumen by atheroma, leading to a rapid decrease in luminal dimensions caused by inward growth of the atheromatous plaque. This phenomenon might explain the disproportionately larger degree of progression in the reference diameter found in a major study on atherosclerosis regression,[37] since a relatively small progression in the disease process propitiated by disturbances in flow caused by the neighbor narrowing[36] may have led to the critical loss of a remnant arc of reactive vessel wall. These findings complement previous intravascular ultrasound [16] and pathological observations[2-4] on the presence of multiple crescents of atheroma in "angiographically normal" coronary segments, representing foci of atheroma progression that have not caused luminal obliteration due to ongoing compensatory enlargement.

Finally, we also observed that interpolated techniques are of no use in obtaining a reliable estimate of the underlying plaque area. The basic premise of this principle could be stated by saying that at the point of minimal luminal narrowing the interpolated reference area should be representative of that comprised within the internal elastic lamina. However, we found that the interpolated reference area calculated by quantitative angiography was significantly smaller than total vessel area observed with intravascular ultrasound. This may be partly due to the initial failure of the curvature detection algorithm to identify a segment where no disease was present on which the interpolation could be based. A second source of error could be due to compensatory vessel enlargement may have occurred.

We believe that the observations performed during this study introduce a change in the concepts that are routinely used in quantitative coronary angiography with regard to automated stenosis analysis.

Study limitations

Since wedging of the ultrasound catheter was required in a number of cases in order to visualize vessel wall at the site of maximal obstruction, distortion of the vessel at that site may have occurred. However, since atheroma is not compressible, we believe that this would not influence substantially the measurement of plaque size nor the distribution of atheroma (which, as stated above, was distributed circumferentially around the lumen at that level). Tracing of luminal and medial boundaries was performed manually, and therefore subjectively. The calculation of plaque area from angiographic dimensions was performed assuming circular morphology for both the lumen and the internal elastic lamina.

References

1. *Cameron A, Kemp HG, Fisher LD et al. Left main coronary artery stenosis: Angiographic determination. Circulation 1983; 68:484-94.*

2. *Detre J, Wright E, Murphy ML, Takaro T. Observer agreement in evaluating coronary angiograms. Circulation 1975; 52:979-88.*

3. *DeRouen KM, Murray JA, Owen W. Variability in the analysis of coronary arteriograms. Circulation 1977; 55:324-33.*

4. *Fisher LD, Judkins MP, Lesperance J et al. Reproducibility of coronary arteriographic readings in the Coronary Artery Surgery Study (CASS). Cathet Cardiovasc Diagn 1982; 8:565-72.*

5. *Vlodaver Z, French R, van Tassel RA, Edwards JE: Correlation of the antemortem coronary angiogram and the postmortem specimen. Circulation 1973; 47:162-69.*

6. *Grondin CM, Dysda I, Pasternac A, Campeau L, Bourassa MG, Lesperance J: Discrepancies between cineangiographic and postmortem findings in patients with coronary revascularisation. Circulation 1974; 49:703-708.*

7. *Arnett EN, Isner JM, Redwood DR et al. Coronary narrowing in coronary heart disease: comparison of cineangiographic and necropsy findings. Ann Intern Med 1979; 91:350-8.*

8. *Isner JM, Kishel J, Kent KM, Ronan JA Jr, Ross AM, Roberts WC: Accuracy of angiographic determination of left main coronary narrowing. Circulation 1981;63:1056-63.*

9. *Dietz WA, Tobis JM, Isner JM. Failure of angiography to accuarately depict the extent of coronary artery narrowing in three fatal cases of percutaneous transluminal coronary angioplasty. J Am Coll Cardiol 1992; 19:1261-70.*

10. *Glagov S, Wisenberd E, Zarins CK, Stankunavicius R, Kolettis GJ: Compensatory enlargement of human atherosclerotic coronary arteries. N Eng J Med 1987; 316:1371-5.*

11. *Stiel GM, Stiel SG, Schofer J, Donath K, Mathey DG: Impact of compensatory enlargement of atherosclerotic coronary arteries on angiographic assessmment of coronary artery disease. Circulation 1989; 80:1603-9.*

12. *Hermiller JB, Cusma JT, Spero LA et al. Quantitative and qualitative coronary angiographic analysis: Review of methods, utility and limitations. Cathet Cardiovasc Diagn 1992; 25:110-31.*

13. *Reiber JHC.van der Zwet PMJ, von Land CD, Koning G, van Meurs B, Buis B, van Voorthuisen AE. Quantitative coronary arteriography: equipment and technical requirements. In: Advances in quantitative coronary arteriography., Reiber JHC, Serruys PW (editors) Kluwer Academic Publishers, Dordrecht, 1993: 75-112.*

14. *Liebson PR, Klein LW. Intravascular ultrasound in coronary atherosclerosis: A new approach to clinical assessment. Am Heart J 1992; 123:1643-60.*

15. *Di Mario, Bom N, Roelandt JRTC et al. Detection and characterization of vascular lesions by intravascular ultrasound. An in-vitro correlative study with histology. J Am Soc Echocardiogr 1992; 5:135.*

16. *Nissen SE,Gurley JC, Grines CL et al. Intravascular ultrasound assessment of lumen size and wall morphology in normal subjects and patients with coronary artery disease. Circulation 1991; 84:1087-99.*

17. *Tobis JM, Mallery J, Mahon D et al. Intravascular analysis of coronary arteries in vivo. Analysis of tissue characteristics with comparison to in-vitro histological specimens. Circulation 1991; 83:913-26.*

18. *Hodgson McJB, Reddy KG, Suneja R, Nair RN, Lesnefsky EJ, Sheehan HM. Intracoronary ultrasound imaging: Correlation of plaque morphology with angiography, clinical syndrome and procedural results in patients undergoing coronary angioplasty. J Am Coll Cardiol 1993; 21:35-44.*

19. *Gussenhoven EJ, Essed CE, Lancee CT et al. Arterial wall characteristics determined by intravascular ultrasound imaging. J Am Coll Cardiol 1989; 14:947-52.*

20. *Hermiller JB, Tenaglia AN, Kisslo KB, Phillips HR, Bashore TM, Stack RS, Davidson CJ. In vivo validation of compensatory enlargement of atherosclerotic coronary arteries. Am J Cardiol 1993; 71:665-68.*

21. *Waller BF, Pinkerton CA, Slack JD. Intravascular ultrasound: a historical study of vessels during life. Circulation 1992; 85:1305-10.*

22. *Waller BF. Anatomy, histology and pathology of the major epicardial coronary arteries relevant to echocardiographic imaging techniques. J Am Soc Echogr 1989; 2(4):232.*

23. *Rensing BJ, Hermans WRM, Deckers JW, de Feyter PJ, Serruys PW. Qhich angiographic variabe best describes functional status 6 months after successful single-vessel coronary balloon angioplasty? J Am Coll Cardiol 1993; 21: 317-24.*

24. *Gronenschild E, Janssen J. A compact system for quantitative cardiovascular angiography analysis. Medinfo. KC Lun en al. (editors). Amsterdam, New York:Elsevier Science Publishers, 1992: 795-800.*

25. *Haase J, Escaned J, Montauban van Swijndregt E, Ozaki Y, Gronenschild E, Slager C, Serruys PW. Experimental validation of geometric and densitometric coronary measurements on the new generation Cardiovascular Angiography Analysis System (CAAS II). Cathet Cardiovasc Diagn 1993; 30:104-14.*

26. *Haase J, Nugteren SK, Montauban van Swijndregt E, Slager CJ, Di Mario C, de Feyter PJ, Serruys PW. Digital geometric measurements in comparison to cinefilm analysis of coronary artery dimensions. Cathet Cardiovasc Diagn 1993; 28:283-90.*

27. *Rosenfeld A, Johnston E. Angle detection on digital curves. IEEE Trans Comput 1973; vol C-22, pp 875-78.*

28. *Blankenhorn DH, Brooks SH, Selzer RH, Barndt R. The rate of atherosclerotic change durinh hyperlipoproteinemia. Circulation 1978; 57: 355*

29. *Crawford DW, Brooks SH, Selzer RH et al. Computer densitometry for angiographic assessment of arterial cholaterol content and gross pathology in human atherosclerosis. J Lab Clin Invest 1977; 89: 368.*

30. *Vlodaver Z, Amplatz K, Burchell HB, Edwards JE. Coronary heart disease. Clinical, angiographic and pathologic profiles. New York: Springer-Verlag, 1976.*

31. *Roberts WC. The status of the coronary arteries in fatal ischemic heart disease. In: Brest A, Wenger N, Chung E, Kasparian H, eds. Innovations in the Diagnosis and Management of Acute Myocardial Infarction. Philadelphia: FA Davies, 1975.*

32. *Waller BF. The eccentric coronary plaque: morphological observations and clinical relevance. Clin Cardiol 1989; 12: 14-20.*

33. *Baroldi G. Myocardial infarction and sudden death in relation to coronary occlusion and collateral circulation. Am Heart J 1966; 71:826.*

34. *Hangartner JRW, Charleston AJ, Davies MJ, Thomas AC. Morphological characteristics of clinically significant coronary artery stenosis in stable angina. Br Heart J 1986; 56:501-8.*

35. *Marshall JJ, Kontos HA. Endothelium-derived relaxing factors. A perspective from in vivo data. Hypertension 1990; 16: 371-86.1.*

36. *Vita JA, Treasure CB, Ganz P, Cox DA, Fish RF, Selwyn AP. Control of shear stress in the epicardial coronary arteries of humans: Impairement by atherosclerosis. J Am Coll Cardiol 1989; 14: 1193-9.*

37. *Stone PH, Gibson M, Pasternak RC, McManus K, Diaz L, Boucher T, Spears R, Sandor T, Rosner B, Sacks FM. Natural history of coronary atherosclerosis using quantitative angiography in men, and implications for clinical trials of coronary regression. Am J Cardiol 1993; 71:766-72.*

Conclusion

In this thesis three novel intracoronary tools available to the interventional cardiologist were used to investigate the characteristics of coronary atheroma in different coronary syndromes. The working hypothesis was that in-vivo imaging and sampling of the atheromatous plaque might constitute complementary approaches in studying the pathobiological substrate of coronary atheroma, shedding further insights to the information which has been provided by post-mortem studies, which have been the main source of knowledge in this area. The basis for this hypotesis was that atherectomy specimens and intracoronary images provide information on an ongoing coronary syndrome, whereas the study of necropsy material is biased by restriction to the extreme of the clinical - and presumably pathological - spectrum of the syndrome.

When atherectomy specimens are used as a form of biopsy of the target coronary stenosis, the researcher can make use of cell culture and molecular biology techniques, in addition to conventional histopathological techniques, to infer information as to the ongoing biological processes in the plaque, therefore the term "pathobiology" used in the heading. On the other hand, atherectomy specimens do not provide information on the overall structure and spatial distribution of atheroma in the arterial wall, posing problems similar to those of the anthropologist using a single bony fragment retrieved in an archaeological dwelling to infer information on the skull from which it was originally derived.

Pathobiological observations in coronary atheroma from patients with unstable angina

With these principles in mind, a study of the pathological substrate of unstable angina was undertaken as one of the main subjects of this thesis to determine whether the information collected in-vivo with intracoronary techniques confirm, complement or challenge the current paradigm of the pathogenesis of primary unstable angina as a predominantly thrombotic syndrome secondary to plaque fissuring or disruption, which is largely based on post-mortem studies.

Histological and angioscopic findings

The information obtained substantiated aspects of previous autopsy studies, such as a higher prevalence of thrombotic material in unstable than in stable patients, or the presence of lipid-rich plaques in patients with post-infarction angina. More importantly, new insights on the substrate of unstable patients refractory to medical treatment were obtained. Four major clues as to the thrombotic characteristics

in the population were provided. First, the overall prevalence of histological thrombus in unstable patients was lower than previously reported in necropsy studies, an observation complemented in further studies with intracoronary imaging which demonstrated that intraluminal thrombus causes significant obliteration of the coronary lumen in a relatively low percentage of unstable patients. Second, thrombus organisation was more common than expected from post-mortem studies, probably reflecting plaque evolution not interrupted by fatal outcome. Third, the degree of organisation bore a poor relationship with the time elapsed from the first episode of angina at rest, suggesting that the original thrombotic episode was frequently subclinical. Finally, thrombotic organisation was associated with a significant development of neointimal hyperplasia.

Cell culture findings

The smooth muscle cell has been relatively ignored in the pathogenesis of unstable angina, with more attention paid towards the mechanical and other cellular factors (eg distribution of tensile stress, infiltration by macrophages) which may contribute to destabilization and subsequent fissuring and thrombosis of the atheromatous plaque. A miniturised cell culture model was set up to study the functional characteristics of smooth muscle cells present in atherectomy specimens. Thrombus organisation was associated with an enhanced ability of these cells to colonise the surrounding culture environment, suggesting a relationship between smooth muscle cell activation and plaque thrombosis which was probably causative in the disclosed development of neointimal hyperplasia.

From all the above, it is possible to speculate that some of the pitfalls in the pharmacological treatment of unstable angina are due to incomplete understanding of this evolving pathological substrate. The disappointing results of thrombolytic treatment in unstable patients, which might appear paradoxical under the current paradigm of unstable angina, are partially explained by the findings in this thesis from the comparatively low frequency of a lysable substrate causing luminal occlusion. By promoting smooth muscle cell proliferation, thrombus organisation may cause plaque remodeling which results in accelerated luminal obstruction and clinical unstability. Furthermore, the use of angiography to assess thrombolytic therapy in unstable patients may have contributed to such confounding results, as some of the observations reported in this thesis, from either histological analysis of retrieved atherectomy specimens or intracoronary angioscopy, cast doubts as to the actual relationship between complex angiographic morphology and the presence of underlying thrombus. Like in Plato's myth of the cavern, angiography provides a mere shadow of the actual changes occurring in the vascular wall and lumen, which according to the belief in the current paradigm of the syndrome, is frequently taken for real.

Pathobiology of accelerated atherosclerosis syndromes

A second angle of research was to apply the techniques described above to compare the pathobiology of de novo and restenotic atheromatous plaques. In agreement with other investigators, neointimal hyperplasia was frequently found in the latter group. Smooth muscle cell culture revealed significant differences in the growing characteristics of the cells, suggesting an enhanced potential in those present in restenotic plaques. Furthermore, a significant gradient was observed in the reactivity toward extracellular matrix produced by smooth muscle cells from normal coronary media, primary atheromatous and restenotic plaque, the latter being the most reactive and promoting extensive platelet adhesion and spreading. Contrary to pathological studies, which cannot be sequential, patients in whom atherectomy samples have been obtained may have a clinical, angiographic and, occasionally, pathological follow-up - eg, either through subsequent directional atherectomy or direct pathological study of the heart obtained at necropsy or after transplantation. Taking advantage of this possibility, two studies were undertaken. First, the development of two concomitant syndromes of accelerated atherosclerosis (restenosis and transplant allograph vasculopathy) was assessed in the long term in three cardiac transplant patients treated with directional atherectomy. Second, in a large population treated with directional atherectomy the results of smooth muscle cell culture from the retrieved specimens were correlated with the development of angiographic restenosis in the long term. A significant relationship was disclosed but, to our surprise, in an inverse fashion: smooth muscle cell outgrowth was associated with less restenosis at follow-up. This observation may have clinical relevance with regard to the use of atherectomy in selected patients. More importantly, when atherectomy is used to assess the baseline characteristics of the atheromatous plaque in longitudinal studies, the researcher should be aware that the extraction of atheromatous tissue imposes a significant modification of plaque evolution, similarly as proposed by Heisenberg in the course of his work in experimental physics in his famous uncertainty principle, stating that the actual practice of observation itself introduces modifications that invariably affect the interpretation of that observation.

Observations on a higher prevalence of neointimal hyperplasia in atherectomy specimens from primary lesions of younger patients reported in this thesis and which have received support from independent researchers, and serves as an example of a clinical entity where potentially the pathophysiological mechanisms differ from the classic form of atherosclerosis.

Comparison and validation of coronary imaging techniques

Examination of tissue samples obtained during concomitant atherectomy appears as a valuable tool in providing an in-vivo validation of angioscopic observations, which may contribute to standarisation of the terminology used in coronary

angioscopy. By the same token, intracoronary imaging can be used to validate other imaging techniques. The relationship between angioscopy and intravascular ultrasound was explored. Furthermore, valuable insights on the significance of computerised analysis of the angiogram, now widely applied widespread due to its inclusion as a built-in feature of many digital angiography systems, were performed by using intravascular ultrasound. Changes in the distribution of coronary atheroma in the vessel wall kept a significant relationship with features identified by quantitative angiographic analysis, suggesting that protrusion of atheroma in the arterial lumen happens when encroachment of the lumen by atheroma occurs. This latter phenomenon is presumably due to the loss of compensatory enlargement provided by an arc of disease-free arterial wall, and has implications for our understanding of the progression of coronary atherosclerosis.

Final remarks

It is foreseeable that generalisation in the use of intracoronary techniques for the in-vivo investigation of coronary atheroma will bring a diversification of atherosclerotic syndromes beyond the categories used currently, in accordance with different etiological and physiopathological mechanisms. Some of the limitations of techniques which have been fundamental instruments in clinical and research fields, such as coronary angiography, emerges from the use of the results of this thesis. Revision of the results obtained previously, like the use of thrombolytic agents in unstable angina, by application of intracoronary imaging techniques may be anticipated in the near future. Likewise, a close, interdisciplinary work between cardiologists, pathologists and basic scientists appears as a requierement to obtain full benefit from the new access to the coronary arteries of the living man facilitated by interventional cardiology techniques.

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Curriculum Vitae

The author was born at Vigo, Spain, on March 20, 1959. He received his basic medical training at the University of Santiago de Compostela, Spain, from which he graduated in 1985. After his pre-registration clinical rotation in the Hospital Provincial, Pontevedra, he spent 4 months of training in intensive care medicine at the Policlinico Vigo (Spain). He gained his initial experience in cardiology working from 1986 to 1988 for the National Health Service at the Servicio de Ambulatorios and Hospital Xeral, Vigo (Spain). Then he moved to the United Kingdom, where he completed his clinical training from 1988 till the end of 1992 as Research Fellow of the Department of Cardiovascular Medicine, University of Birmingham, under the supervision of Prof. W.A. Littler and Dr. M.F. Shiu, with appointments as Cardiology Registrar at the Queen Elizabeth Hospital, Birmingham, and Walsgrave General Hospital, Coventry. During this period his training was focussed on the field of interventional cardiology. He participated as co-investigator in the MERCATOR European trial for the prevention of restenosis and produced original research in coronary angioplasty and quantitative angiography. In January 1992 he was appointed as Research Fellow / Assistant Cardiologist at the Catheterisation Laboratory, Thoraxcenter, Rotterdam, The Netherlands, under the supervision of Prof. P.W. Serruys and Dr. P. J. de Feyter. His research during this period was sponsored by the Spanish Ministry of Science and Education, and covered clinical and experimental aspects of interventional cardiology. In May 1993 he was appointed as Director of the catheterisation laboratory at the Center for Thoracic and Cardiovascular Diseases, Sanatorio Modelo, La Coruña (Spain).

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