Role of ageing and coronary atherosclerosis in the development of cardiac fibrosis in the rabbit

Augusto Orlandi, Arianna Francesconi, Marcella Marcellini, Amedeo Ferlosio, Luigi Giusto Spagnoli

Department of Biopathology and Image Diagnostics, Anatomic Pathology Institute, Tor Vergata University of Rome, Via della Ricerca Scientifica, 00133 Rome, Italy
Sigma-Tau Research Laboratories, Pomezia (Rome), Italy

Received 14 May 2004; received in revised form 20 July 2004; accepted 30 July 2004
Time for primary review 22 days

Abstract

Objective: Myocardial fibrosis contributes to the impairing of cardiac function and characterizes ageing, but is also a consequence of atherosclerotic ischemic disease. Since atherosclerosis is a slow progressive disease, which prevails in elderly populations, the aim of this study was to distinguish the contribution of ageing and atherosclerosis to cardiac fibrosis.

Methods: Coronary atherosclerosis was induced in 5–6-year-old rabbits by a hyperlipemic diet for 9 months. Left ventricular (LV) collagen was quantified by densitometric analysis after Sirius-Red staining; an immunohistochemical investigation of the interstitium was also performed.

Results: Atherosclerosis was associated to a marked increase of left ventricular interstitial collagen with the appearance of fibrotic foci and a decrease of coronary vessel endothelial nitric oxide synthase (eNOS) expression. In fibrotic foci, abundant macrophages co-localized with transforming growth factor beta-1 (TGFβ-1)-positive myofibroblasts and vascular cell adhesion molecule-1 (VCAM-1) positive microvessels (52.3±3.9%). In normocholesterolemic rabbits, ageing resulted in a fourfold increase of myocardial interstitial collagen, with alpha-smooth muscle actin and TGFβ-1 negative fibroblasts and VCAM-1 positive microvessels (19.4±1.2%) without macrophages, suggesting a role of endothelial dysfunction in age-related fibrosis.

Conclusions: There is a distinct difference between ageing and coronary atherosclerosis-induced cardiac fibrosis, although the effects may be cumulative. In the cascade of events leading to myocardial remodeling, reparative fibrosis with TGFβ-1-positive myofibroblasts and interstitial inflammation were the major findings in atherosclerotic old rabbits, whereas with ageing alone, interstitial fibrosis with TGFβ-1 negative fibroblasts and VCAM-1 positive microvessels prevailed.

Keywords: Ageing; Atherosclerosis; Extracellular matrix; Remodeling; Endothelial function

1. Introduction

Fibrosis, a disproportionate accumulation of fibrillar collagen, adversely increases myocardial stiffness [1] and impairs cardiac function [2]. Myocardial fibrosis is one of the consequences of ageing [3,4], together with a reduction of capillary density [5] and of energy available for ventricular pump function [6]. Although cardiac interstitium changes during development and normal growth have been extensively investigated [7], the mechanisms through which ageing excessively stimulates cardiac fibroblasts in order to synthesize collagen remain unclear. Moreover, cardiac myocyte and non-myocyte cell growth are independent each other [8]. Fibrosis in non-infarcted regions of the heart also characterises left ventricular (LV) remodeling of patients with ischemic cardiomyopathy following severe coronary atherosclerosis [9]. The development of advanced coronary lesions responsible for the clinical outset of cardiac ischemic disease is a long process and fibroatheromatous plaques are generally...
encountered after the 4th–5th decade of life [10]. Nevertheless, this prevalence among aged people makes it difficult to discriminate the remodeling of cardiac interstitium induced by coronary atherosclerosis from that deriving from intrinsic age-related cardiac modifications. Consequently, data about age-related myocardial changes remain incongruent and occasionally controversial [11]. Aged New Zealand rabbit age-related cardiac modifications. Consequently, data about by coronary atherosclerosis from that deriving from intrinsic

2. Material and methods

2.1. Experimental design

The experimental protocols as well as other analyses of these animals have been the subject of previous publications [12–14]. Shortly, aged (5–6-year-old) white New Zealand male rabbits were fed a standard diet either alone or enriched with a low dose (0.2%) of cholesterol (Merck, Darmstadt, Germany) dissolved in olive oil. Available data suggest a usual life span of 7–8 years for this strain in captivity [15]. Six animals, randomly selected among those whose plasma cholesterol levels were higher than the overall mean after 1 month of the hyperlipemic diet were considered responders and enrolled in the study, as previously reported [13]. This group (AH) was kept on the hyperlipemic diet for further 8 months. A second group of aged normocholesterolemic rabbits (AN, n=7) received the standard diet alone. These two groups were homogeneous for initial plasma cholesterol, triglycerides and arterial pressure values (not shown). At the end of the experiment, a group of young (2–3 months old) normocholesterolemic rabbits (YN, n=6) was also enrolled. At the time of chemical analysis, the standard chow contained 11.2% water, 14.8% fiber, 17.9% proteins, 8.2% ash and 1.8% lipids by weight. The hyperlipemic diet also contained 0.2% cholesterol, 5.9% triglycerides and 0.25% phospholipids by weight. Systemic arterial pressure was measured at the beginning and regularly throughout the experiment using a 3.5 cm paediatric cuff fitted around the upper hind limb and connected to a mercury manometer [12]. All experiments were performed according to guidelines compatible with the Guide for the Care and Use of laboratory Animals (NIH Publication, 1996).

2.2. Killing procedure and sampling

After 9 months, rabbits were pre-anesthetized with 5 mg/kg body weight i.m. Ketamine hydrochlorate (Ketalar, Parke-Davis, Milan, Italy) then killed by an intraperitoneal injection of 35 mg/kg body weight of sodium thiopental (Pentothal, Abbott Spa, Aprilia, Italy). Tissues were harvested as previously reported [12]. Cardiac sections were cut perpendicularly to the axis from base to apex and fixed in 4% formalin; zinc-buffered fixative was used for endothelial nitric oxide synthase (eNOS) and vascular endothelial cell adhesion molecule-1 (VCAM-1) immunodetection.

2.3. Morphologic and morphometric analysis

To evaluate the extent of coronary atherosclerosis, 4-μm-thick sections were deparaffinized and stained with Hematoxin–Eosin or Wehroff–Van Gieson. The percentages of involved vessels (prevalence of lesions) and lumen stenosis have been also evaluated by morphometric methods using a Quantimet 920 Image Analyzer (Cambridge Instruments, UK) as previously reported [16]. The coronary area was calculated using outer elastic lamina as the external limit reference. Coronaries were classified as follows: large subepicardial (area more than 0.5 mm²), large intramyocardial (area between 0.5 and 0.2 mm²) and small intramyocardial vessels (area less than 0.2 mm²).

Types I and III collagen accumulation was calculated by morphometric analysis on Sirius Red-stained LV sections at 25× magnification using polarization microscopy, that supplies stectiometric data [17] and correlates with hydroxypoline content [18]. Relative concentration of total and interstitial collagen was calculated as the ratio of the integrated optical density (IOD) to the field projected area at 100× magnification. Background intensity was subtracted from the IOD value. For each heart, the densitometric analysis was performed in at least five fields of each LV section. The total number of fields was calculated according to stereological formulae [19]. The mean of large LV fibrotic foci was calculated for each animal at 40× magnification from two independent researchers, with intervariability less than 5%. Immunohistochemical evaluation of eNOS expression in subepicardial and intramyocardial vessels was performed using a computerized image analyzer [14] and expressed as percentage of positive endothelial cells within CD31 positive endothelium. Preliminary studies showed that endothelial CD31 expression was unchanged with ageing and hypercholesterolemia (not shown). In addition, VCAM-1 expression in myocardial microvasculature was assessed quantitatively according to the method of Iwata et al. [20]. Briefly, the vessels with any positive endothelial staining were divided by the total number of CD31 positive vascular profiles.

2.4. Immunohistochemistry and TUNEL

Immunohistochemistry was performed by deparaffinizing, rehydrating and treating serial sections in sequence with 3% H₂O₂, normal rabbit serum and then incubating them with RAM-11, a monoclonal antibody to rabbit macrophages (a gift of Dr. Allen Gown), anti-α smooth muscle actin (α-actin, DAKO), anti-transforming growth factor β-1 (TGFβ-
1; Santa Cruz), anti-CD31 (DAKO), anti-eNOS (Transduction Laboratories) and anti-VCAM-1 (Santa Cruz). All immunostainings were performed at room temperature. Biotinylated rat-absorbed anti-Mouse anti-IgG (Vector), StreptABC–POD-complex (Ylem) and diaminobenzidine (Sigma) were used respectively as secondary antibody, revelation complex and final chromogen. Negative controls without the primary antibody or with an unrelated antibody were used to check for non-specific staining. Apoptotic nuclei were revealed by TdT-mediated dUTP-biotin nick-end labelling (TUNEL), as previously reported [14].

The number of cardiomyocytes, RAM-11, α-actin and TUNEL positive cells per mm² were calculated at 400× magnification in at least 20 gridded fields including fibrotic areas from all various LV samples. Two different pathologists who were without knowledge of the corresponding groups repeated measurements independently, with an inter-variability less than 5%.

Statistical analysis was performed using SPSS (5th ed., MJ Norusis/SPSS, Chicago, IL). Data were expressed as standard error of mean (s.e.m.). Differences were evaluated using the t-test and non-parametric Mann–Whitney tests. Values with $P<0.05$ were considered statistically significant.

3. Results

3.1. Pathophysiological findings

As reported in Fig. 1 A and B, heart and body weight increased with ageing ($P<0.0001$), with no difference between experimental aged groups. The age-related mean increase of heart weight was comparable to that of total body. In AH, long-term hyperlipemic diet was associated to a slight decrease in body weight as compared to initial value (4375±108 g, $P<0.05$) as a consequence of reduced chow intake and without plasma albumin and transaminase level increases, as previously reported [12]. In addition, arterial systolic pressure was slightly increased in AN compared to YN (Fig. 1C; $P<0.05$), according to what has been previously reported [12]. No differences were observed among pressure values of aged experimental groups at either the beginning or end of the experiment (data not shown).

As previously reported in this cohort of animals [12,13], mean plasma total cholesterol (Fig. 1D), triglyceride, phospholipids and HDL-cholesterol levels (not shown) were markedly increased in AH compared to AN rabbits ($P<0.0001$); AN and YN plasma lipid profile did not change during the course of the experiment (data not shown).

As reported in Table 1, raised fibroatheromatous plaques were detected in large subepicardial coronary vessels in AH and prevailed on other lesions ($P<0.01$). In some cases, small superficial thrombi were detected (Fig. 2A). In these plaques, fibrous cap was variably reduced in thickness but no evidence of rupture was observed. In large and small
### Table 1
Prevalence of lesions and lumen stenosis in coronary vessels (values±S.E.M.)

<table>
<thead>
<tr>
<th></th>
<th>Large subepicardial vessels (&gt;0.5 mm²)</th>
<th>Large intramyocardial vessels (0.2&gt;x&gt;0.5 mm²)</th>
<th>Small intramyocardial vessels (&lt;0.2 mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AH</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibroatheromatous plaques</td>
<td>32.9±4.0*</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fatty streaks</td>
<td>8.7±0.5</td>
<td>52.9±4.0*</td>
<td>29.6±2.5*</td>
</tr>
<tr>
<td>Myointimal thickening</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lumen stenosis</td>
<td>22.8±4.1*</td>
<td>85.5±4.8*</td>
<td>90.4±1.6*</td>
</tr>
<tr>
<td><strong>AN</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibroatheromatous plaques</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fatty streaks</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Myointimal thickening</td>
<td>35.5±3.0†</td>
<td>30.3±2.5†</td>
<td>0</td>
</tr>
<tr>
<td>Lumen stenosis</td>
<td>0.08±0.04</td>
<td>0.5±0.2</td>
<td>0</td>
</tr>
<tr>
<td><strong>YN</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibroatheromatous plaques</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fatty streaks</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Myointimal thickening</td>
<td>2.5±0.9</td>
<td>1.9±0.5</td>
<td>0</td>
</tr>
<tr>
<td>Lumen stenosis</td>
<td>0.01±0.005</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

AH=aged hyperlipemic rabbits; AN=aged normolipemic rabbits; YN=normolipemic young rabbits; prevalence of lesions as percentage of involved vessels and lumen stenosis as percentage of stenosis.

*AH vs. AN: P<0.001.
†AN vs. YN: P<0.01.

---

Fig. 2. Fibroatherosclerotic plaque in (A) large subepicardial coronary vessel and (B) large intramyocardial vessel of aged hyperlipemic rabbit; (C) a foamy fatty streak in a small intramyocardial vessel of aged hyperlipemic rabbit; (D) at low magnification, large fibrotic area replacing myocardial tissue; (E) an intramyocardial fibrotic area associated to vessels with fatty streaks narrowing the lumen (head arrows); (F) at high magnification, interstitial myocardial fibrosis with inflammatory cells in aged hyperlipemic rabbit; (*) a superficial thrombus.
intramyocardial vessels of AH rabbits, raised fatty streaks (Fig. 2B and C) from variable accumulations of rounded foamy or elongated myocyte-like cells and extracellular matrix with scarce accumulation of amorphous material were detected. These lesions induced a more marked stenosis than that observed in large subepicardial vessels ($P<0.01$). In large subepicardial and intramyocardial vessels of AN rabbits, an eccentric myointimal thickening was detected. Intimal thickening did not contain foamy or RAM-11 positive cells and was absent in YN coronary vessels at working magnification.

3.3. Myocardial fibrosis and cardiac fibroblast phenotype

Densitometric evaluation of Sirius Red-stained LV sections (Fig. 1E and F) showed a marked collagen accumulation in the interstitium and in the perivascular site of AH (Fig. 3A and B), greater than that observed in AN rabbits (Fig. 3C and D; $P<0.0001$). LV collagen accumulation in AN was mainly interstitial and greater than in YN rabbits (Fig. 3E and F; $P<0.0001$). Large fibrotic foci (Fig. 2D) could also be observed at low magnification, more frequently in AH (Fig. 1G; $P<0.0001$) and almost absent in AN. In AH, large fibrotic foci originated from the LV subendocardial region (3±0.4), but were also intramyocardial (2.5±0.2); in many fibrotic foci, atherosclerotic vessels with narrowed lumen were present (Fig. 2E). Serial sections revealed that RAM-11 positive cells co-localized in LV areas characterized by increased Sirius Red positive collagen accumulation compared to the surrounding myocardium (Fig. 4A and B). In these fibrotic areas, $\alpha$-actin-positive myofibroblasts were also co-localized (Fig. 4C). RAM-11 positive macrophages were also observed in the perivascular and interstitial space of hyperlipemic rabbits (Fig. 4D). No RAM-11 positive cells were detected in myocardial sections of AN and YN rabbits (Fig. 1I). In fibrotic foci, cardiac fibroblasts were also partially TGF$\beta$-1 positive (Fig. 4E) as well as in perivascular spaces (Fig. 4F). In AN and YN rabbits, LV interstitial fibroblasts were $\alpha$-actin and TGF$\beta$-1 negative.

3.4. Myocardial cell number and apoptosis

Morphologic examination of LV sections in old rabbits revealed the presence of hypertrophic myocytic cell foci with exceptional myocytolysis. As reported in Fig. 1K, the number of LV cardiomyocytes per mm$^2$ was less in AN compared to YN and further decreased in the AH rabbits ($P<0.0001$). As reported in Fig. 1L, TUNEL positive myocardial apoptotic cells were practically absent in YN. Apoptotic cells increased in AN and further in AH rabbits.

Fig. 3. Sirius Red histochemical staining examined to polarized light microscope with contrast phase shows at different magnifications (A,C,E) perivascular and (B,D,F) interstitial collagen accumulation in left ventricular myocardium increased in (A,B) aged hyperlipemic rabbit compared to (C,D) aged normolipemic and (E,F) young normolipemic rabbit.
myocardial vessels were VCAM-1 negative (Fig. 4I). Moreover, Fig. 1M shows that the percentage of eNOS positive endothelial cells in AH coronary vessels (Fig. 4J) was reduced compared to that of AN rabbits (Fig. 4K, \( P<0.0001 \)). This reduction did not significantly differ comparing AH atherosclerotic and non-atherosclerotic vessels (not shown). In YN coronary vessels, e-NOS positive endothelial cells (Fig. 4L) resulted more than in AN (\( P<0.0001 \)).

4. Discussion

The present study demonstrates that coronary atherosclerosis and ageing contribute to the increase of cardiac fibrosis as two distinct processes, although their effects can be partially cumulative. Fibrosis is a frequent alteration of myocardium that characterizes cardiac hypertrophy [18] and is associated to a reduced left ventricular compliance and increased risk of arrhythmias [21]. In the myocardium, the non-myocyte compartment is a complex and non-secondary structure [22]. Under certain circumstances, structural abnormalities of the interstitium may be a primary mechanism resulting in cardiac failure [23]. Cardiac fibroblasts are the interstitial cells responsible for collagen synthesis [23]. During normal growth, fibrillar collagen accumulates in relationship to the increase of myocyte size [24] and there is a balanced hyperplasia of myocytes and fibroblasts with an unaltered relative collagen content [25]. During cardiac failure, fibroblast proliferation appears independent from myocytes and its deregulation is associated to a progressive interstitial and, successively, endomyocardial fibrosis [26].

4.1. Collagen deposition and coronary atherosclerosis

In the presence of coronary atherosclerosis, we observed a marked increase of LV fibrosis with the outset of variably sized fibrotic foci. The largest of these foci were generally associated to the presence of stenotic atherosclerotic intramyocardial coronary vessels. Massive myocardial infarctions were not observed, but they are a very rare event in old rabbits even after a very prolonged hypercholesterolemic diet [12,27]. This is likely related to the lower stenosis induced in large subepicardial coronaries by a hyperlipemic diet compared to that in intramyocardial vessels. Intramyocardial stenosis impairs blood flow haemodynamics and induces chronic hypoxia with myocyte loss that stimulates " reparative" collagen synthesis in cardiac fibroblasts [28]. Fibroblasts cultured in hypoxic conditions show an increased proliferation and an upregulation of collagen synthesis [29]. We also observed abundant macrophagic accumulations in large fibrotic foci, confirming their scarring nature [28]. The finding of \( \alpha \)-actin positive fibroblasts suggests that the presence of myofibroblasts is a prerequisite for the outset of " reparative" fibrotic foci.
These data are in agreement with those reported in human hearts, where interstitial fibrosis with α-actin and TGF-β1 positive myofibroblasts is observed during post-infarctual scarring process, whereas fibroblasts associated to interstitial fibrosis during dilated cardiomyopathy are generally α-actin and TGFβ-1 negative [30]. In healing fibrosis, myofibroblasts synthesize collagen more actively, express high levels of TGFβ-1 and show an increased susceptibility to TGFβ-1-mediated inflammatory stimuli [31]. Reparative fibrosis greatly contributes to LV stiffness and diastolic dysfunction [31]. All these findings indicate that cardiac fibroblasts display phenotypic changes according to the pathological condition and also suggest that distinct pathways of remodelling of cardiac interstitium occur during the different pathologic conditions.

4.2. Inflammatory cellular infiltration and interstitial fibrosis

In addition to RAM-11 positive macrophages being observed in foci of “reparative” fibrosis, they were also found in the LV interstitium of AH rabbits, even where evident fibrotic areas were not present. This confirms that inflammatory cellular infiltration also plays a pivotal role in “reactive” fibrosis [28] as also reported by Yu et al. [32] in non-infarct zones of rat hearts. An acute and chronic inflammatory reaction also characterizes the myocardium surrounding intracoronary stents [33]. Our results documented a dramatic reduction of coronary endothelial cell eNOS activity in aged atherosclerotic rabbits. The decrease of eNOS reduces NO bioavailability [34] and suggests that an endothelial dysfunction predisposes blood vessels to atherosclerosis by increasing adherent monocytes [14] but also favours the increase of myocardial interstitial inflammatory cells. Interstitial inflammation is followed by the local delivering of cytokines and growth factors [35], in particular TGF-β1 that stimulates phenotypic changes [36] and collagen synthesis in cardiac fibroblasts [35]. In addition, endothelial dysfunction induced by hypercholesterolemia favours the interstitial and perivascular accumulation of plasmatic substances [14]. Triglyceride-rich lipoproteins diffuse across arterial and capillary walls and accumulate in the interstitium, where further stimulate inflammatory cell recruitment [37]. In human coronary vessels, inflammation is characteristic of the entire coronary wall and it extends beyond the site of the culprit lesion responsible for the acute ischemic myocardial infarction [38]. It is likely that an interstitial “atherosclerotic myocarditis” deriving from hyperlipemia and coronary atherosclerosis, contributes to collagen accumulation in the interstitium.

4.3. Ageing, microvascular dysfunction and cardiac remodeling

In old normocholesterolemic rabbits, the relative collagen content of LV was fourfold compared to that of young animals. The increase was mainly located in the interstitium, similarly to that reported in the rat and other mammalians [39]. Cardiac fibrosis in aged normocholesterolemic animals was observed in the absence of interstitial RAM-11 positive cells. We also observed a loss of myocytes and an increase of apoptosis with ageing, similar to that reported in rat and human hearts [40,41]. Therefore, other factors than inflammation seem to regulate the age-related remodeling of cardiac interstitium. Little is known about the role of cardiac microvascular endothelium in spite of its prominence. We documented an increase of VCAM-1 positivity in LV microvessels with ageing. This confirms a similar finding in the aortic endothelium of old mice [42]. VCAM-1 is an adhesion molecule, normally not expressed by endothelial cells [43]. VCAM-1 expression can be induced in response to cytokine activation and does regulate transendothelial leukocyte migration [43]. Endothelial cells elaborate substances and express surface molecules that likely influence vascular toxicity as well as the growth and metabolic behaviour of neighbouring non-endothelial cells, in particular cardiac fibroblasts [44]. In a reciprocal manner, cardiac fibroblasts influence endothelial production in a sort of cell–cell signalling [44]. In response to changes in hemodynamic forces, microvascular endothelial cells release substances that regulate cardiomyocyte function and extracellular matrix content of adjacent interstitium [45]. In normocholesterolemic rabbits, the age-increase expression of VCAM-1 in microvascular endothelium was plasma cholesterol independent, although it further increased with hypercholesterolemia. VCAM-1 expression is an early feature during fatty streak development and precedes intimal macrophagic accumulation in prone aortic areas of young rabbits [46]. An increased VCAM-1 expression may represent an early sign of age-related endothelial dysfunction of myocardial microvasculature and a possible cardiovascular risk factor, in the absence of hyperlipemia as well [42,47]. With ageing alone, coronary arterioles as well as aortic endothelial cells show a reduction in eNOS expression [14,48], confirming previous reports in rat aortas [49]. Also, the number of inflammatory cells adhering to aortic endothelium increased in normocholesterolemic old rabbits compared to young animals [14]. All these findings suggest that an age-related endothelial dysfunction may directly activate interstitial fibroblasts, possibly by releasing endothelial soluble factors [44]. We could not exclude that a slight age-related increase of systolic pressure observed in old rabbits may influence endothelium dysfunction and, consequently, contribute to the remodeling of interstitium by the present results. It has been hypothesised that hypertension represents an accelerated form of ageing [50]. In any case, our results suggest a complex interaction between cardiac microvasculature and interstitium with ageing, in which endothelial dysfunction likely precedes fibrogenetic remodelling. More in general, the possible reduction of abnormalities in ageing hearts indicates cardiac microvascular endothelial cells as a target for a possible
therapeutic intervention [51] and supports the dynamic nature of cardiac extracellular matrix [52].

In conclusion, ageing and coronary atherosclerosis can be considered two conditions that may synergistically concur to the development of cardiac fibrosis. In the cascade of events leading to myocardial remodeling, healing fibrosis with TGFβ1-positive myofibroblasts and interstitial inflammation were the major findings in old atherosclerotic rabbits, whereas with ageing alone, interstitial fibrosis characterized from TGFβ1 negative fibroblasts and VCAM-1 positive microvessels was detected.

Acknowledgments

The authors thank Prof. G.F. Bottini for statistical analysis and A. Ciucci, S. Cappelli, A. Colantoni, A. Volpe for their technical assistance. This work was partially supported by a grant from Spedali Civili of Brescia for their technical assistance. This work was partially supported by a grant from Spedali Civili of Brescia for their technical assistance. This work was partially supported by a grant from Spedali Civili of Brescia for their technical assistance. This work was partially supported by a grant from Spedali Civili of Brescia for their technical assistance. This work was partially supported by a grant from Spedali Civili of Brescia for their technical assistance.

References


