Canadian Journal of Cardiology 29 (2013) 849–857

Clinical Research

Transforming Growth Factor β1 and Coronary Intimal Hyperplasia in Pediatric Patients With Congenital Heart Disease

Roberto A. Guerri-Guttenberg, MD, PhD,[a] Rocío Castilla, ScD, PhD,[a] Gabriela C. Francos, MD,[b] Angélica Müller, MSc,[a] Giuseppe Ambrosio, MD, PhD,[c] and José Milei, MD, PhD[a]

[a] *Instituto de Investigaciones Cardiológicas "Prof. Dr. Alberto C. Taquini," ININCA-UBA-CONICET, Buenos Aires, Argentina*

[b] *Pathology Division, Ricardo Gutierrez Children's Hospital, Buenos Aires, Argentina*

[c] *Division of Cardiology, University of Perugia School of Medicine, Perugia, Italy*

See editorial by Justino and Khairy, pages 757-758 of this issue.

ABSTRACT

Background: Congenital heart defects or the process of their repair leads to an increased risk for adult cardiovascular disease compared with the general population. Intimal hyperplasia is a preatherosclerotic lesion that may be produced as a consequence of transforming growth factor β1 (TGF-β1) pathway activation. We studied the presence of intimal hyperplasia in arteries from a pediatric population with congenital heart disease (CHD) and TGF-β1 expression to enlighten its possible role in the genesis of these lesions.

Methods: Coronary arteries from 10 controls and 98 CHD patients (54% cyanotic type, 32% surgically repaired) were stained, and the presence and degree of intimal thickening were analyzed. The expression of TGF-β1 was studied by immunohistochemistry.

Results: The difference between the presence of coronary intimal hyperplasia in patients with cyanotic (35; 66.1%) and noncyanotic CHD (29; 64.3%) was not significant. However, surgically repaired CHD presented a higher rate of coronary intimal hyperplasia (80%) than did the group without surgical intervention (47.3%), $P = 0.0002$. The immunostaining for TGF-β1 analyzed in samples of patients with cyanotic and noncyanotic CHD showed no significant differences. However, TGF-β1 expression was more intense on the intimal layer of patients with surgically repaired CHD than on that of those without surgery (intimal area positive for TGF-β1, 50.43% vs 15.91%, respectively; Mann-Whitney *U* test $P = 0.0005$).

RÉSUMÉ

Introduction : Les malformations cardiaques congénitales ou le processus de leur réparation mènent à une augmentation du risque de maladie cardiovasculaire chez l'adulte comparativement à la population générale. L'hyperplasie intimale est une lésion préathérosclérotique qui peut être la conséquence de l'activation de la voie du facteur de croissance transformant β1 (TGF-β1 : *transforming growth factor β1*). Nous avons étudié la présence de l'hyperplasie intimale dans les artères d'une population pédiatrique ayant une cardiopathie congénitale (CC) et une expression du TGF-β1 pour illustrer son rôle possible dans la formation de ces lésions.

Méthodes : Les artères coronaires de 10 témoins et de 98 patients ayant une CC (54 % de type cyanotique, 32 % ayant subi une réparation chirurgicale) ont été colorées, puis la présence et le degré d'épaississement de l'intima ont été analysés. L'expression du TGF-β1 a été étudiée par immunohistochimie.

Résultats : La différence entre la présence de l'hyperplasie intimale dans les artères coronaires chez les patients ayant une CC cyanotique (35; 66,1 %) et non cyanotique (29; 64,3 %) n'a pas été significative. Cependant, le groupe ayant des CC réparées par chirurgie présentaient un taux plus élevé d'hyperplasie intimale dans les artères coronaires (80 %) que le groupe n'ayant pas subi d'intervention chirurgicale (47,3 %), $P =$ 0,0002. L'immunocoloration du TGF-β1 analysée dans les échantillons de patients ayant une CC cyanotique et non cyanotique n'a montré

Congenital heart disease (CHD) patients represent a group at risk of premature atherosclerotic coronary artery disease.[1] Certain congenital heart defects, or the process of their repair, may lead to an increased risk for adult cardiovascular disease compared with the general population, based on 2 major mechanisms: abnormal coronary origin and obstructive lesions of the left ventricle and aorta.[1,2] Congenital anomalies of coronary origin have been reported to portend a high incidence of coronary atheromas, most probably due to abnormal blood flow patterns.[1] On the other hand, Fyfe et al.[3] reported that patients with cyanotic CHD presented a low incidence of coronary atherosclerosis because of hypocholesterolemia, upregulation of nitric oxide, hyperbilirubinemia, and low platelet count.

Of note, in 1976 Beçu et al.[4] described, in patients aged 6 days to 9 years subjected to pulmonary valvotomy for "isolated"

Received for publication October 19, 2012. Accepted November 23, 2012.

Corresponding author: Dr José Milei Instituto de Investigaciones Cardiológicas "Prof. Dr. Alberto C. Taquini" ININCA-UBA-CONICET, Marcelo T. De Alvear 2270- C1122AAJ, Buenos Aires, Argentina.

E-mail: ininca@fmed.uba.ar

See page 856 for disclosure information.

http://dx.doi.org/10.1016/j.cjca.2012.11.018

Conclusion: The high incidence of intimal hyperplasia in patients with surgically repaired CHD is correlated with TGF-β1 expression and may contribute to the development of atherosclerotic coronary artery disease in CHD patients.

aucune différence significative. Cependant, l'expression du TGF-β1 a été plus intense sur la couche intimale des patients ayant subi une réparation chirurgicale de la CC que sur celle de ceux n'ayant pas subi de chirurgie (surface de l'intima positive au TGF-β1, 50,43 % *vs* 15,91 %, respectivement; test U de Mann-Whitney $P = 0{,}0005$).

Conclusion : L'incidence élevée d'hyperplasie intimale chez les patients ayant subi une réparation chirurgicale de la CC est en corrélation avec l'expression du TGF-β1 et peut contribuer au développement de l'athérosclérose coronarienne chez les patients ayant une CC.

pulmonary valve stenosis, varying degrees of coronary lumen occlusions consisting of prominent intimal proliferation of smooth muscle cells (SMCs), media muscle disarray and fragmentation, and/or disappearance of the internal elastic lamina.

More recently, there has been an increasing trend to consider intimal hyperplasia as the first stage of coronary atheroma or as a preatherosclerotic lesion[5-7] and to speculate that transforming growth factor β1 (TGF-β1) might be involved in this phenomenon.[8]

TGF-β1 is a secreted multifunctional factor that modulates proliferation of many cell types, including vascular cells, and regulates their interaction with the extracellular matrix. Its signalling plays pivotal roles in SMC differentiation during vascular development and is involved in the development of many cardiovascular diseases.[9,10]

SMCs are capable of reversibly modulating their phenotype during postnatal development and can dedifferentiate into proliferative, matrix synthetic cells in response to vascular injury.[8,11] TGF-β1 regulates both SMC differentiation during embryonic development and postnatal phenotypic switching.[12] In this connection, it was observed that overexpression of TGF-β1 in normal arteries resulted in substantial extracellular matrix production accompanied by intimal and medial hyperplasia.[13]

The possibility of reviewing the original microscopic slides underlying Beçu's pioneer paper[4] encouraged us to evaluate the prevalence of coronary intimal hyperplasia in CHD in order to assess a link to accelerated atherosclerosis in patients with (1) abnormal coronary origin, (2) surgical repair, (3) obstruction of the left ventricle and aorta, and (4) cyanotic and noncyanotic heart disease and to gain insight into the possible role of TGF-β1 in the genesis of these lesions.

Methods

Population

A total of 98 autopsies from diseased patients (57 males) aged $\leq$ 17 years (range, 4 days-17 years; mean age, 2.4 years) with a diagnosis of CHD were included in the study. Ten pediatric patients aged $\leq$ 17 years who had died of causes not related to CHD were used as a control group. In patients submitted to surgical repair, after sternotomy, cardiopulmonary bypass was instituted under mild hypothermia (32°C-33°C).

The origin of the main coronary trunks was dissected with the aid of a Nikon surgical microscope (Nikon Inc, Melville, NY). The dissection included a longitudinal cut to the ascending aorta in order to visualize coronary ostia.

Histologic evaluation

On average, 4 samples of 3-mm width were taken from the left main coronary artery (LMCA), left anterior descending coronary artery, right coronary artery (RCA), circumflex (CX), and posterior descending coronary artery. Samples were embedded in paraffin, serially sectioned in both transversal and longitudinal orientations, and stained with hematoxylin eosin, blue Victoria, and Masson Trichrome.

Intimal proliferation was defined as muscle-elastic thickening characterized by (1) proliferation of SMCs; (2) scarce monocytes, and rare lymphocytes, embedded by amorphous deposits within the internal elastic membrane; and (3) endothelium above the lesion morphologically intact, with smooth surface and devoid of thrombi.[7,14] Differential diagnosis with intimal ridges responsible of vessel bifurcation was made with the aid of serial axial sections and longitudinal sections.

Immunohistochemistry

TGF-β1, a polyclonal antibody, reactive against human (Santa Cruz Biotechnology, Inc, Santa Cruz, CA), was used at a dilution of 1:200 in phosphate-buffered saline. The intensity and distribution of the antibody were analyzed with ImageJ software (National Institutes of Health, Washington, DC). Results were expressed in terms of percentage of intima area positive for the antibody.

Statistical analysis

Statistical analysis was performed with Excel (Microsoft, Henderson, NV), GraphPad Prism v5.03 (GraphPad Software, San Diego, CA), or SPSS Statistics 19 software (SPSS, Chicago, IL). Type of data distribution was assessed with the bus normality test from D'Agostino and Pearson and the normality test from Shapiro-Wilk. For nonparametric data, comparisons were performed with the Fisher exact test or the Mann-Whitney *U* test, as appropriate. To establish the relationship between 2 quantitative nonparametric variables, the Spearman correlation coefficient was used. $P < 0.05$ was considered significant.

Results

Presence of intimal thickening in arteries was studied in 350 coronary artery samples belonging to 98 patients of the CHD group and 10 controls. Sixty (61%) of the CHD cases and all control patients presented at least 1 coronary vessel with intimal hyperplasia. The most affected vessel was the

LMCA, followed by the RCA, left anterior descending coronary artery, posterior descending coronary artery, and CX.

Most patients presented complex cases with combined CHD. As an example, a patient aged 15 days, weighing 2.62 kg, and measuring 29 cm, with a heart of 25 g, showed pericarditis, perimembranous ventricular septal defect, right aortic arch, and polycystic kidney. In the histologic study, the LMCA presented coronary intimal hyperplasia with 2 components: the first, in contact with the arterial lumen, resembling a soft plaque with scarce nuclei surrounded by loose connective tissue and the second component, in contact with the media layer, characterized by SMC proliferation and dense connective tissue (Fig. 1).

Therefore, the combination of multiple structural anomalies in a single patient made it difficult to link the occurrence of intimal hyperplasia to a specific CHD.

In 81 of 98 autopsies, a correct assessment of the coronary artery origin was made; in the remaining 17 cases, a discrepancy arose among observers, given the reduced size of the hearts. Nine of 81 patients were found to have anomalous coronary artery origin. Among these patients with anomalous coronary artery origin, 8 presented at least 1 vessel with intimal hyperplasia.

Intimal hyperplasia in patients with cyanotic and noncyanotic CHD

Of CHD cases, 53 of 98 (54%) were of the cyanotic type. The difference between the presence of coronary intimal hyperplasia in patients with cyanotic CHD (35; 66.1%) and noncyanotic CHD (29; 64.3%) was not significant (Fisher exact test; $P = 0.735$).

Intimal hyperplasia in CHD patients with surgical intervention

Thirty-one cases of CHD with surgical repair (32%) were also analyzed. Eighty percent of these patients died within 1 month of operation because of complex CHD, hemodynamic impairment, and difficult surgical procedures (Fig. 2).

Surgically repaired CHD presented a higher rate of coronary intimal hyperplasia than did the group without surgical intervention. Given that some patients may have presented only 1 affected vessel while others may have presented several, 2 criteria were used for comparison: (1) percentage of coronary arteries with intimal hyperplasia and (2) percentage of patients with at least 1 coronary artery with intimal hyperplasia.

Twenty-six (84%) of surgically repaired CHD patients presented at least 1 coronary artery with intimal hyperplasia vs 47.3% of the nonsurgical group (2-tailed Fisher exact test $P = 0.0002$). In addition, 68% of coronary arteries belonging to the surgically repaired group presented intimal hyperplasia in more than 1 artery, compared with 25% in the CHD group without intervention (Fisher test $P < 0.0001$).

Intimal hyperplasia in CHD patients with obstruction of left ventricle or aorta

The group (n = 13) included aortic coarctation (n = 4), subaortic stenosis (n = 1), and left ventricular hypertrophy (n = 8). All the patients presented coronary intimal hyperplasia compromising at least 1 vessel. The rest of the congenital cardiopathies presented this condition in 34 patients (61%).

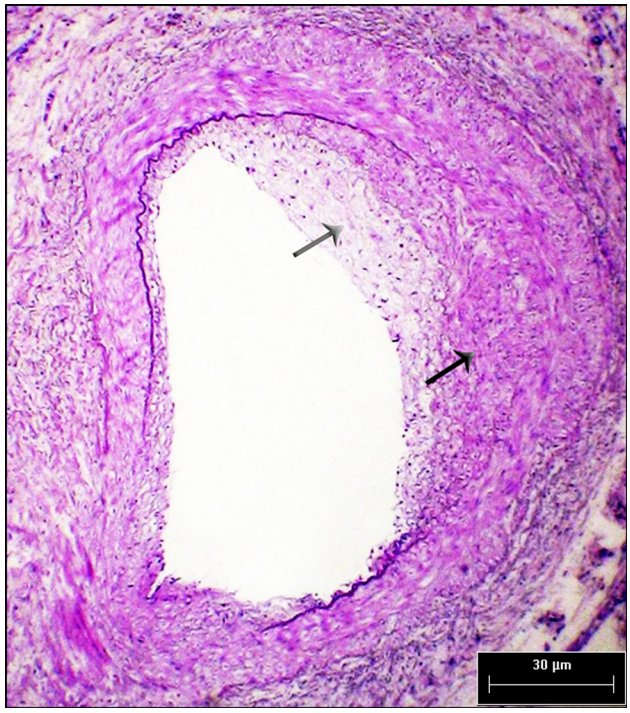

Figure 1. Soft plaque in a congenital heart disease patient. Left coronary trunk from a girl aged 15 days presenting an interventricular communication type I, pericarditis and polycystic kidney. A plaque with 2 components can be seen; the first is in contact with the lumen and resembles a soft, hypocellular plaque, with few nuclei belonging to mononuclear cells surrounding the loose connective tissue (**grey arrow**). The second, in contact with the media layer, is characterized by smooth muscle cell proliferation and dense connective tissue (**black arrow**). The interruption and duplication of the limiting membrane, due to a severe media layer distortion and smooth muscle cell proliferation, should also be noted (hematoxylin and eosin staining).

The difference between both groups was significant (2-tailed Fisher test $P = 0.0039$). A boy aged 10 years presenting subaortic stenosis, left ventricular hypertrophy, and moderate mitral insufficiency deserves special mention. The coronary tree showed intimal hyperplasia in each main vessel, with the exception of the CX. The LMCA presented a diffuse and incomplete intimal thickening that occluded 55.4% of the arterial lumen (Fig. 3).

Immunohistochemistry for TGF-β1

A semiquantitative analysis of TGF-β1 expression was made in samples of CHD patients (Fig. 4). No significant differences were found between cyanotic and noncyanotic CHD patients (percentage of reactive area was 37.2 ± 12.2 and 25.9 ± 4.9, respectively). Furthermore, TGF-β1 expression was almost undetected in any of the 10 children that had no structural heart disease (Fig. 4).

On the contrary, when immunostaining for TGF-β1 was analyzed in patients with or without surgical repair, a very significant difference between them was observed (mean intimal area positive for TGF-β1, 50.43% vs 15.91%, respectively; 2-tailed Mann-Whitney U test $P = 0.0005$; Fig. 5).

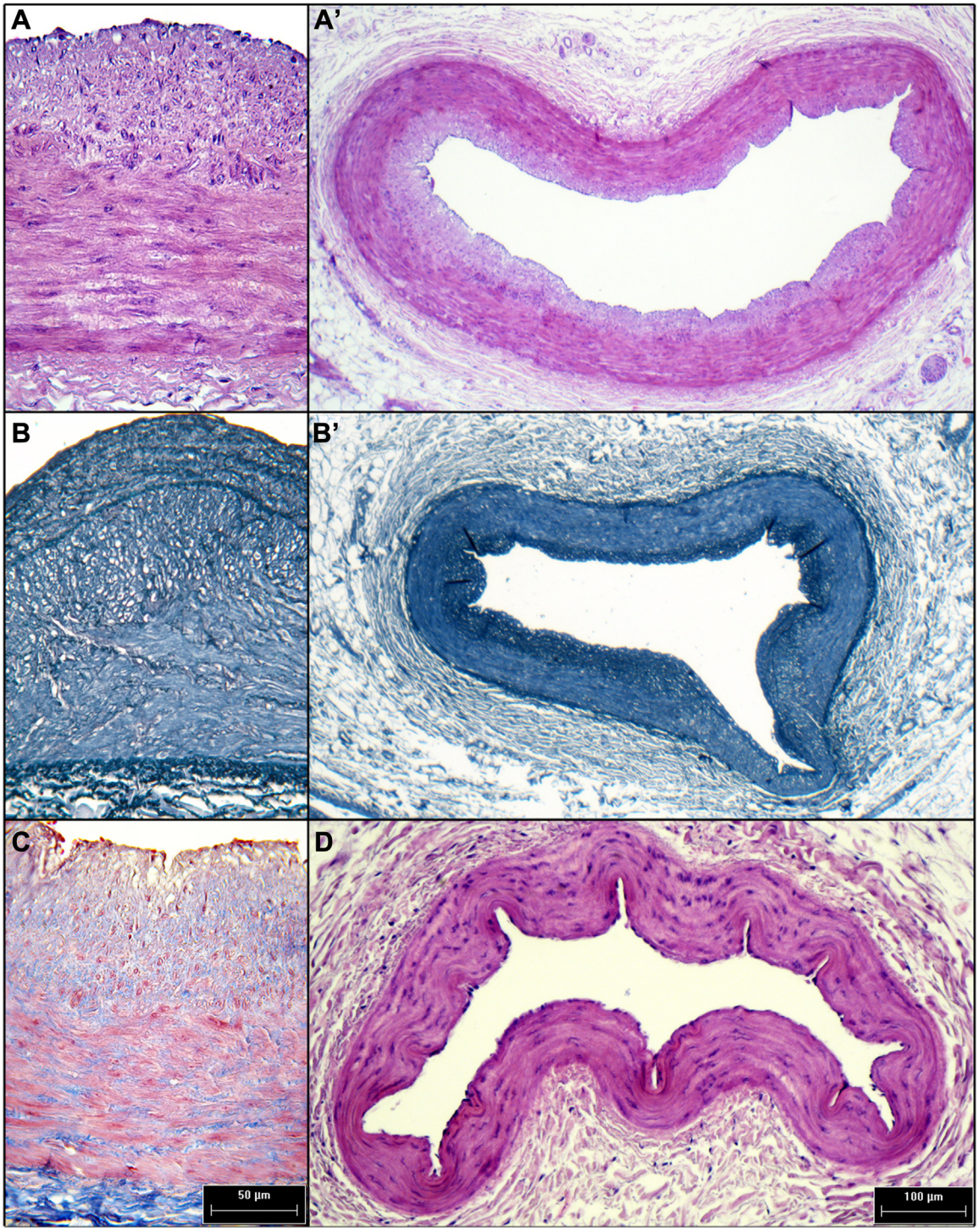

Figure 2. Diffuse intimal thickening in artery of a congenital heart disease patient. Surgically corrected tetralogy of Fallot in a boy; (**A**) diffuse intimal hyperplasia in the right coronary artery (**A** ×25, **A'** ×3.5), (**B**) left anterior descending coronary artery (**B** ×25, **B'** ×3.5), (**C**) left main coronary artery, and (**D**) with a normal circumflex artery. (**A, C, D**) Hematoxylin and eosin staining; (**B**) Victoria Blue staining.

The nonparametric comparison (Kruskal-Wallis test) of repaired CHD, nonrepaired CHD, and controls without CHD revealed that the difference was significant ($P < 0.0001$; Fig. 6).

Although the presence of intimal hyperplasia and TGF-β1 expression was more evident in LMCA and RCA compared with the remaining coronary arteries, the difference was not significant.

No correlation between degree of intimal hyperplasia (intima/media index) and percentage of intimal area stained with TGF-β1 was found (Spearman $\rho = -0.2955$; 95% confidence interval, −0.61 to 0.11, $P = 0.134$).

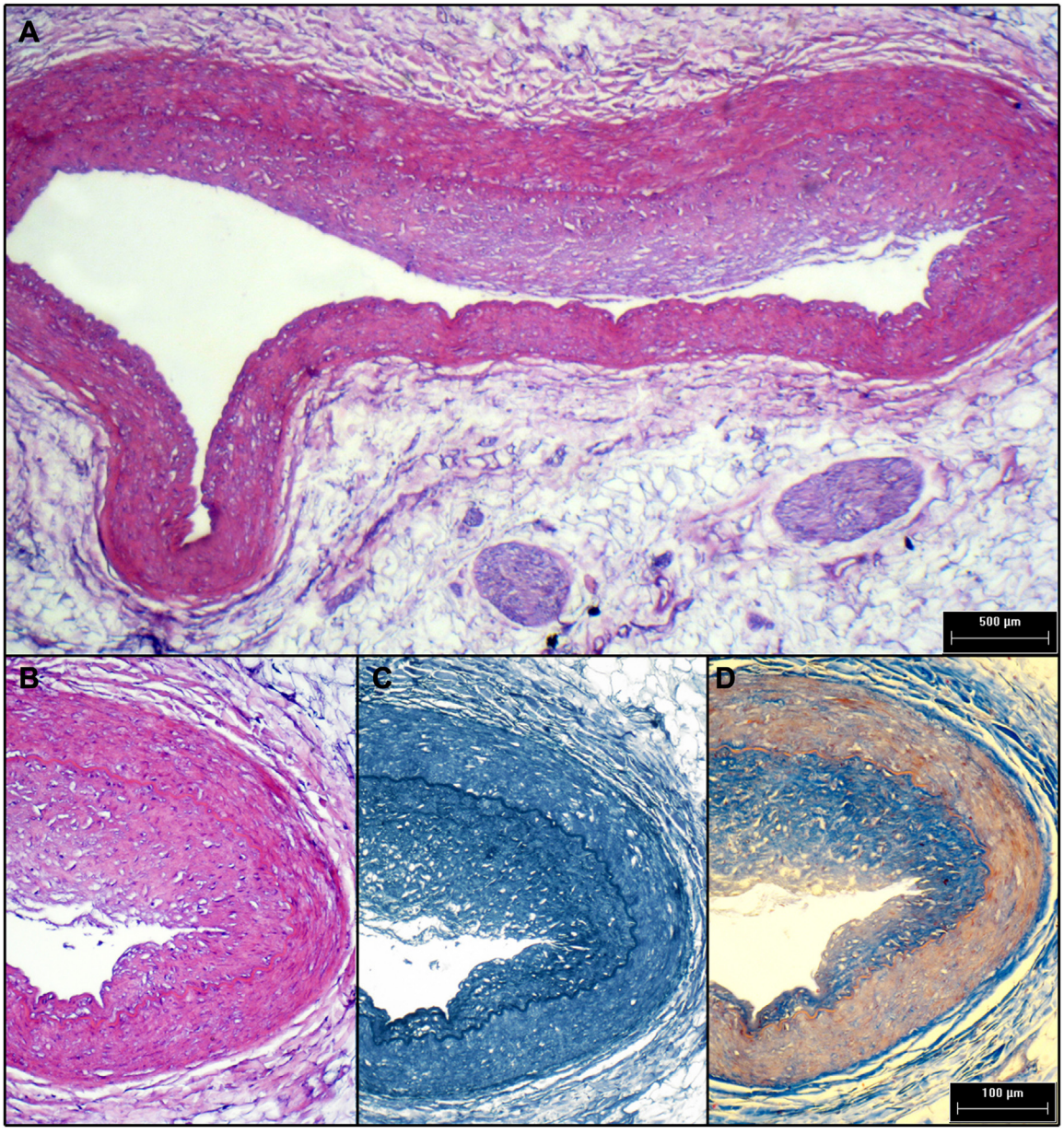

Figure 3. Focal intimal thickening in artery of a congenital heart disease patient. (**A**) Left anterior descending coronary artery of a patient, aged 10 years, with Ebstein's anomaly and a surgically corrected ventricular septal defect. Focal intimal hyperplasia can be observed on this vessel. The elastic internal membrane remains intact (Type I). The thickened intima occludes 55.4% of the arterial lumen. Images (**B**-**D**) represent the right quadrant from image. (**A**, **B**) Hematoxylin and eosin staining; (**C**) Victoria Blue staining; (**D**) Masson trichrome staining.

Discussion

This large autopsy study reports on several potentially important findings: (1) intimal hyperplasia is a common finding in the coronary tree of young patients with CHD, (2) TGF-β1 seems to play a major role in this phenomenon, and (3) surgical correction of CHD is associated with further coronary vascular remodelling.

Obstructive lesions of the left ventricle and aorta

All patients with congenital subaortic stenosis, coarctation of the aorta, and left ventricular hypertrophy presented coronary intimal hyperplasia involving at least 1 vessel. This finding may lend support to epidemiologic studies revealing that this group has a higher risk for developing coronary atherosclerosis.[1] Coarctation of the aorta is linked to systemic hypertension,[15] and left ventricular hypertrophy is an independent risk factor for cardiovascular disease morbidity and mortality in adults.[2,16] The association between intimal hyperplasia in these patients and the high risk of accelerated atherosclerosis described in epidemiologic studies[1] are consistent with the hypothesis that intimal hyperplasia can be observed as the first atherogenic event.[5-7]

Cyanotic CHD

No differences between cyanotic and noncyanotic heart disease, regarding the incidence of coronary intimal hyperplasia, were found in this study.

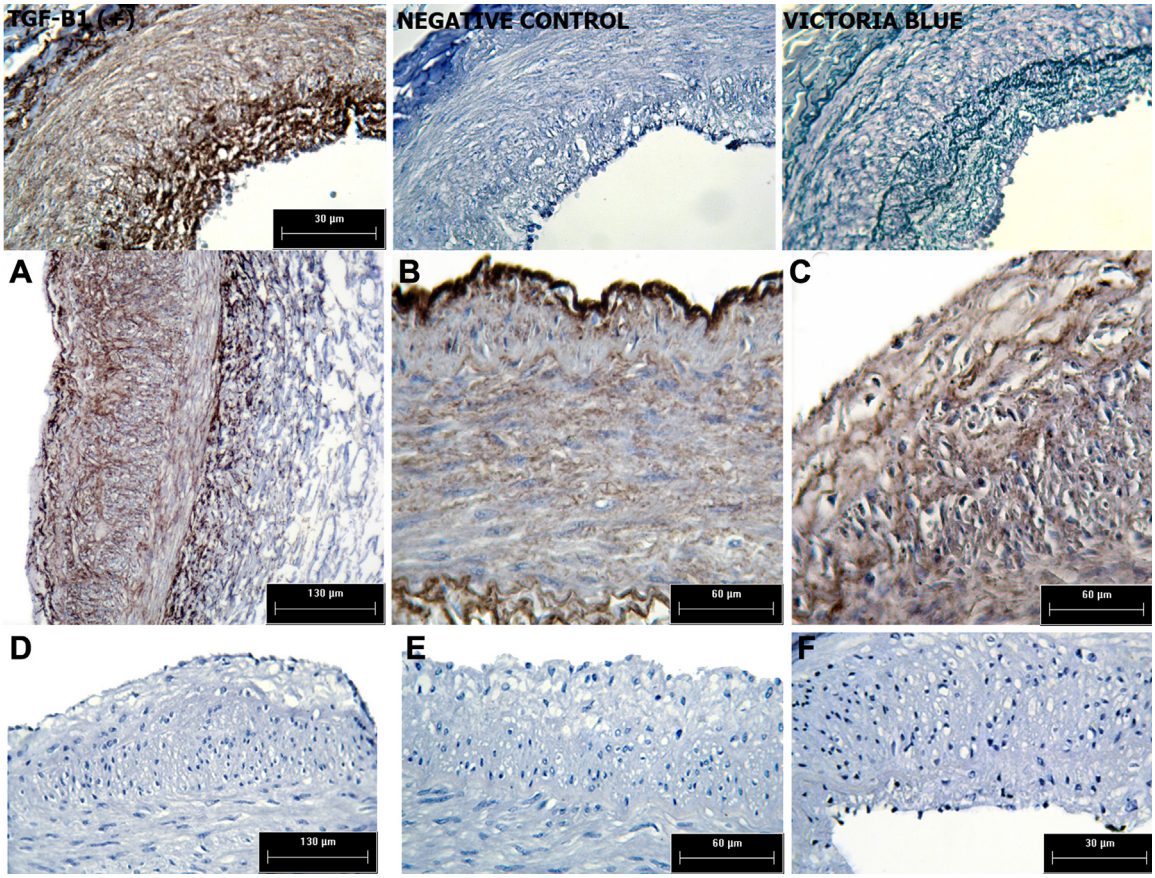

Figure 4. Immunohistochemical staining for transforming growth factor β1 (TGF-β1). Different patterns are shown. On the **top row**, we show immunostaining for TGF-β1, its negative control stained with hematoxylin and eosin to corroborate the absence of nonspecific stain, and Victoria Blue staining for elastic fibres performed on the proximal segment of the right coronary artery of a patient with transposition of the great vessels. In the **middle row**, different patterns of TGF-β1 can be observed. (**A**) Left coronary trunk from a boy aged 4 days, measuring 48 cm and weighing 3185 g. The autopsy revealed a 30-g heart with a truncus arteriosus and interatrial communication. (**B**) Right coronary artery from a girl aged 2 months, 50 cm long, weighing 2200 g, who presented coarctation of the aorta and biventricular hypertrophy. (**C**) Left coronary trunk from a patient with truncus arteriosus. In bottom, proximal segment from the right coronary artery is shown. In the **bottom row**, the absence of TGF-β1 expression can be observed in the population with no congenital heart disease. (**D**) Right coronary artery from a boy aged 5 years who died of intracranial hypertension. (**E**) Right coronary artery from a boy aged 9 years who died of meningitis. (**F**) Right coronary artery from a boy aged 7 years who died of acute hydrocephalus.

Fyfe et al.[3] described by angiography a low incidence of atherosclerosis occurring in cyanotic CHD, although it must be taken into account that this method is not well suited for visualizing nonraised lesions.

The role of surgical repair in coronary intimal thickening

In the present study, surgery was the variable with the greater impact over the prevalence (but not the degree) of intimal hyperplasia. Age and sex were discarded as possible confounding variables. Given that, because of severity of the CHD, most patients died within a month of the surgical intervention, it is difficult to assess whether lesions found where stable or transient.

The role of TGF-β1 in intimal hyperplasia

Migration of SMCs from the media to the intima and SMC proliferation are of utmost importance in the genesis of arterial intimal thickening.[5,6] TGF-β1 is a secreted factor that modulates the proliferation of many cell types, including vascular cells, and regulates their interaction with the extracellular matrix.[17]

We describe for the first time a significant increase in TGF-β1 expression in patients with surgical correction of CHD compared with patients without surgical intervention and those without CHD.

TGF-β1 has been identified as an underlying factor in the reparative process after injury in various organs,[18] and the overproduction of this growth factor has been implicated as a causative agent in tissue repair processes characterized by increased production of extracellular matrix and fibrosis.[19]

The reasons that patients with CHD subjected to surgical repair present significantly higher TGF-β1 levels are not completely clear. It may be speculated that cardiac surgery is a cause of vascular injury that leads to activation of this pathway. Thus, a "stressor" during the surgical procedure induces the production of TGF-β1, which in turn leads to intimal hyperplasia. A stressor could be endothelial hypoxia-ischemia. This occurs during arterial clamping,[20] external compression of the vessels, extracorporeal circulation, or cardiac preservation before

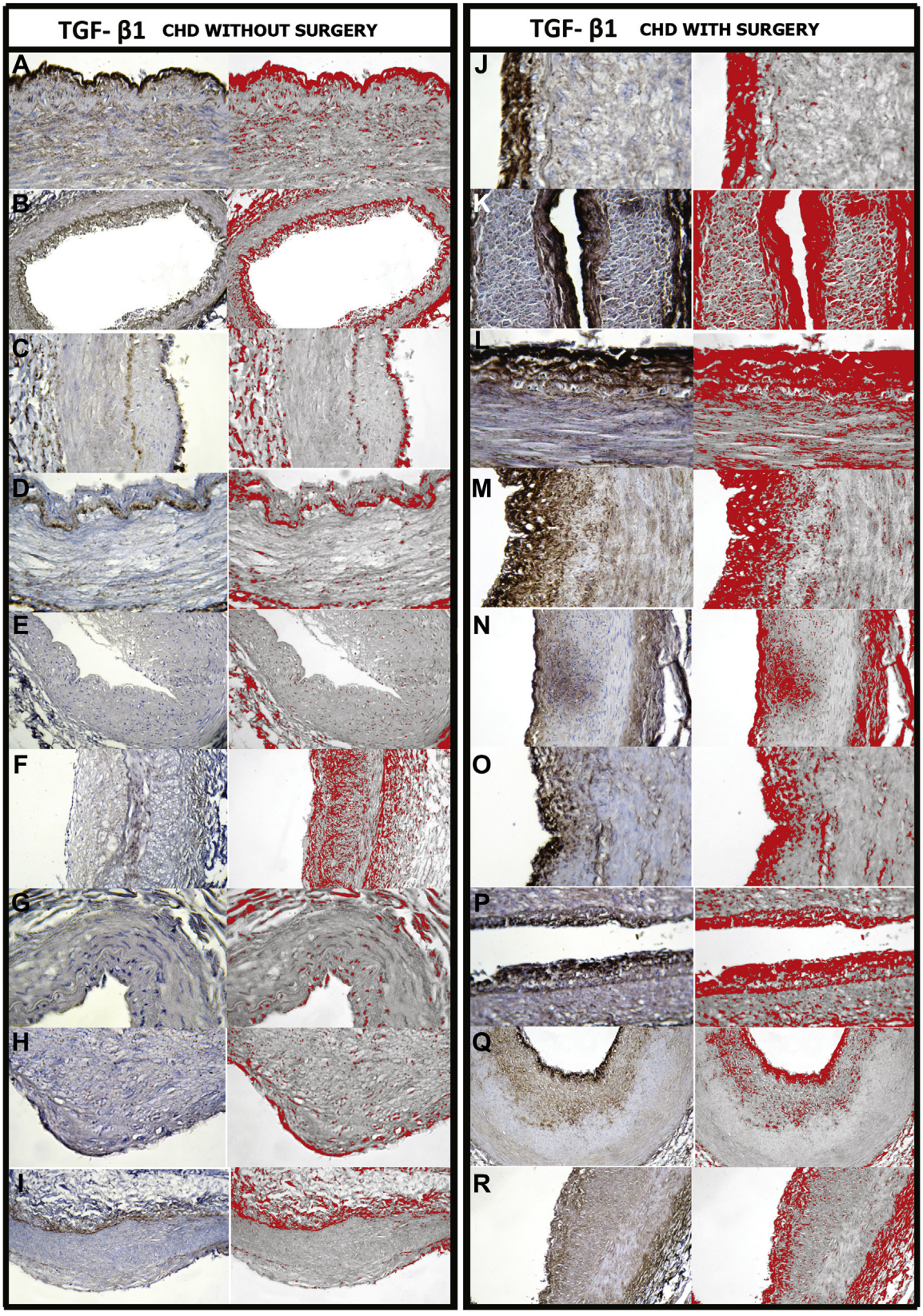

Figure 5. Immunostaining for transforming growth factor β1 (TGF-β1) in surgically repaired and non—surgically repaired patients. (**A-I**) Non—surgically repaired congenital heart disease (CHD) patients. (**J-R**) Surgically intervened CHD patients. ImageJ software analysis is shown in **red**.

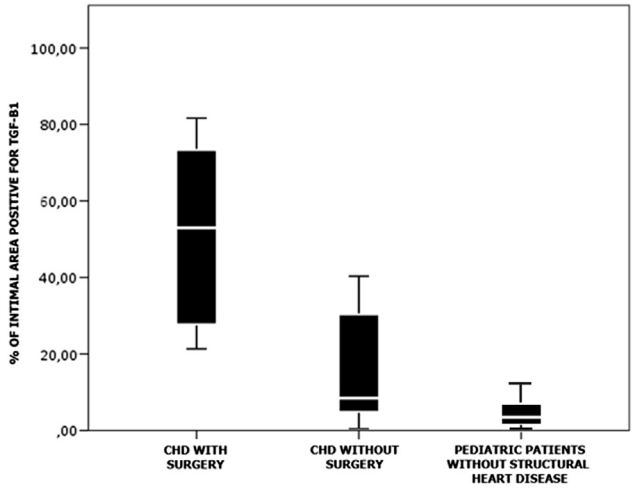

Figure 6. Quantification of immunostaining for transforming growth factor β1 (TGF-β1) in surgically repaired and non–surgically repaired congenital heart disease (CHD) patients. Difference in the percentage of intimal area positive for TGF-β1 between the surgical group, the nonsurgical group, and the pediatric population with no CHD. The horizontal line that forms the top of the box is the 75th percentile. The horizontal line that forms the bottom is the 25th percentile. The horizontal line that intersects the box is the median. **Bars** represent maximum and minimum values. The difference among these groups was significant (Kruskal-Wallis $P < 0.0001$).

transplant. Hypoxia leads to an increase of TGF-β1 in human pulmonary artery[21] and human dermal fibroblast.[22] It has also been postulated that hypoxia could stimulate TGF-β1 through changes in oxidation-reduction state.[18] On the other hand, the patients who were submitted to surgery were the most hemodynamically impaired. Because of this degree of impairment, hypoperfusion and thus low shear stress would likely contribute to inflammation more than in the nonsurgical patients. This impairment would also contribute to the increase in intimal hyperplasia.

The finding that intimal hyperplasia was also observed in our control population is not surprising, as we have already reported it in pediatric patients,[7,14] and it occurs in the absence of substantial TGF-β1 expression. However, the control group comprised children who had died of noncardiac disease. Our data seem to indicate that the mechanisms behind intimal hyperplasia are multiple: TGF-β1 is conspicuously increased in children with CHD and even more so in those who are hemodynamically unstable and/or were subjected to surgery, and hence it may contribute to development of intimal hyperplasia in those patients; on the other hand, other factors, different from TGF-β1, may operate in children who died of causes different from CHD.

Intimal hyperplasia may regress by apoptosis, may stay asymptomatic, or may retain lipids and evolve into atherosclerotic lesions.[7] However, the pathophysiology of surgical patients seems to be different because of TGF-β1 activation. A study on coronary arteries of rats that overexpressed TGF-β1 proved that this growth factor stimulates intimal hyperplasia rich in extracellular matrix with reversibility of coronary intimal hyperplasia by apoptosis after 8 weeks.[23]

Therefore, it seems that clinical implications of intimal hyperplasia in surgical patients, due mainly to TGF-β1 overexpression, depend on the reversibility of these lesions once the adverse stimulus disappears. This is not the case of intimal hyperplasia found in the pediatric population, as they do not appear to be related to activation of the TGF-β1 cascade.[7,24]

On the other hand, we found no association between the degree of intimal hyperplasia and TGF-β1 expression. However, a high incidence of mild intimal hyperplasia was observed in surgical patients with intense TGF-β1 expression, whereas severe intimal hyperplasia lesions in nonsurgical patients and in patients with no CHD were negative for TGF-β1.

A direct relationship between intimal hyperplasia and TGF-β1 is difficult to investigate in these patients because the signal transduction of TGF-β1 is very complex, and it interacts with multiple agents. For example, *in vitro*, vascular SMC growth can be stimulated or inhibited by TGF-β1, depending on cell density, cell age, coculture factors, and the concentration of TGF-β1.[25-27] The finding of a second marker together with TGF-β1 could help to clarify these discrepancies.

Moreover, an acute temporal study indicating injury time, TGF-β1 expression, and intimal hyperplasia development would be necessary but would be difficult to obtain in human beings.

Intimal hyperplasia also has an important role in restenosis after coronary stent deployment, in pulmonary hypertension, and in coronary artery lesions after cardiac transplant.[28,29] Yutani et al. described that 80% of restenosed coronary arteries were positive on their intimal layer for TGF-β1.[30] Furthermore, TGF-β1 administration before carotid balloon angioplasty resulted in more-extensive intimal hyperplasia after the procedure.[31]

The hypoxic theory also might explain the increase of TGF-β1 and posterior coronary intimal hyperplasia after stent implant or balloon angioplasty, as both interventions are associated with arterial wall hypoxia and neovessel formation in the adventitial layer.[32]

In conclusion, the high incidence of intimal hyperplasia in patients with surgically repaired CHD is probably related to an increment in TGF-β1 expression, and it should be considered as another predisposing factor for the development of atherosclerotic coronary artery disease in CHD patients.

Funding Sources

The present study was performed with a grant and a research scholarship from the National Scientific and Technical Research Council (CONICET), PIP N° 112-2000801-03167.

Disclosures

The authors have no conflicts of interest to disclose.

References

1. Kavey RE, Allada V, Daniels SR, et al. Cardiovascular risk reduction in high-risk pediatric patients: a scientific statement from the American Heart Association Expert Panel on Population and Prevention Science; the Councils on Cardiovascular Disease in the Young, Epidemiology and Prevention, Nutrition, Physical Activity and Metabolism, High Blood

Pressure Research, Cardiovascular Nursing, and the Kidney in Heart Disease; and the Interdisciplinary Working Group on Quality of Care and Outcomes Research: endorsed by the American Academy of Pediatrics. Circulation 2006;114:2710-38.

2. Levy D, Garrison RJ, Savage DD, Kannel WB, Castelli WP. Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. N Engl J Med 1990;322:1561-6.

3. Fyfe A, Perloff JK, Niwa K, Child JS, Miner PD. Cyanotic congenital heart disease and coronary artery atherogenesis. Am J Cardiol 2005;96: 283-90.

4. Beçu L, Somerville J, Gallo A. 'Isolated' pulmonary valve stenosis as part of more widespread cardiovascular disease. Br Heart J 1976;38:472-82.

5. Nakashima Y, Wight TN, Sueishi K. Early atherosclerosis in humans: role of diffuse intimal thickening and extracellular matrix proteoglycans. Cardiovasc Res 2008;79:14-23.

6. Nakashima Y, Fujii H, Sumiyoshi S, Wight TN, Sueishi K. Early human atherosclerosis: accumulation of lipid and proteoglycans in intimal thickenings followed by macrophage infiltration. Arterioscler Thromb Vasc Biol 2007;27:1159-65.

7. Milei J, Grana DR, Navari C, Azzato F, Guerri-Guttenberg RA, Ambrosio G. Coronary intimal thickening in newborn babies and <or=1-year-old infants. Angiology 2010;61:350-6.

8. Mosse PR, Campbell GR, Campbell JH. Smooth muscle phenotypic expression in human carotid arteries. II. Atherosclerosis-free diffuse intimal thickenings compared with the media. Arteriosclerosis 1986;6: 664-9.

9. Deng HB, Jiang CQ, Tomlinson B, et al. A polymorphism in transforming growth factor-beta1 is associated with carotid plaques and increased carotid intima-media thickness in older Chinese men: the Guangzhou Biobank Cohort Study-Cardiovascular Disease Subcohort. Atherosclerosis 2011;214:391-6.

10. Saltis J, Agrotis A, Bobik A. Regulation and interactions of transforming growth factor-beta with cardiovascular cells: implications for development and disease. Clin Exp Pharmacol Physiol 1996;23:193-200.

11. Owens GK, Kumar MS, Wamhoff BR. Molecular regulation of vascular smooth muscle cell differentiation in development and disease. Physiol Rev 2004;84:767-801.

12. Mack CP. Signaling mechanisms that regulate smooth muscle cell differentiation. Arterioscler Thromb Vasc Biol 2011;31:1495-505.

13. Nabel EG, Shum L, Pompili VJ, et al. Direct transfer of transforming growth factor beta 1 gene into arteries stimulates fibrocellular hyperplasia. Proc Natl Acad Sci U S A 1993;90:10759-63.

14. Milei J, Ottaviani G, Lavezzi AM, Grana DR, Stella I, Matturri L. Perinatal and infant early atherosclerotic coronary lesions. Can J Cardiol 2008;24:137-41.

15. Costa MA, Simon DI. Molecular basis of restenosis and drug-eluting stents. Circulation 2005;111:2257-73.

16. Verdecchia P, Porcellati C, Reboldi G, et al. Left ventricular hypertrophy as an independent predictor of acute cerebrovascular events in essential hypertension. Circulation 2001;104:2039-44.

17. Guo X, Chen SY. Transforming growth factor-beta and smooth muscle differentiation. World J Biol Chem 2012;3:41-52.

18. Sporn MB, Roberts AB. Transforming growth factor-beta: recent progress and new challenges. J Cell Biol 1992;119:1017-21.

19. Border WA, Ruoslahti E. Transforming growth factor-beta in disease: the dark side of tissue repair. J Clin Invest 1992;90:1-7.

20. Milei J, Forcada P, Fraga CG, et al. Relationship between oxidative stress, lipid peroxidation, and ultrastructural damage in patients with coronary artery disease undergoing cardioplegic arrest/reperfusion. Cardiovasc Res 2007;73:710-9.

21. Ismail S, Sturrock A, Wu P, et al. NOX4 mediates hypoxia-induced proliferation of human pulmonary artery smooth muscle cells: the role of autocrine production of transforming growth factor-{beta}1 and insulin-like growth factor binding protein-3. Am J Physiol Lung Cell Mol Physiol 2009;296:L489-99.

22. Falanga V, Qian SW, Danielpour D, Katz MH, Roberts AB, Sporn MB. Hypoxia upregulates the synthesis of TGF-beta 1 by human dermal fibroblasts. J Invest Dermatol 1991;97:634-7.

23. Schulick AH, Taylor AJ, Zuo W, et al. Overexpression of transforming growth factor beta1 in arterial endothelium causes hyperplasia, apoptosis, and cartilaginous metaplasia. Proc Natl Acad Sci U S A 1998;95:6983-8.

24. Ikari Y, McManus BM, Kenyon J, Schwartz SM. Neonatal intima formation in the human coronary artery. Arterioscler Thromb Vasc Biol 1999;19:2036-40.

25. Barnard JA, Lyons RM, Moses HL. The cell biology of transforming growth factor beta. Biochim Biophys Acta 1990;1032:79-87.

26. Owens GK, Geisterfer AA, Yang YW, Komoriya A. Transforming growth factor-beta-induced growth inhibition and cellular hypertrophy in cultured vascular smooth muscle cells. J Cell Biol 1988;107:771-80.

27. Majack RA. Beta-type transforming growth factor specifies organizational behavior in vascular smooth muscle cell cultures. J Cell Biol 1987;105: 465-71.

28. Ramzy D, Rao V, Brahm J, Miriuka S, Delgado D, Ross HJ. Cardiac allograft vasculopathy: a review. Can J Surg 2005;48:319-27.

29. Newby AC, Zaltsman AB. Molecular mechanisms in intimal hyperplasia. J Pathol 2000;190:300-9.

30. Yutani C, Ishibashi-Ueda H, Suzuki T, Kojima A. Histologic evidence of foreign body granulation tissue and de novo lesions in patients with coronary stent restenosis. Cardiology 1999;92:171-7.

31. Kanzaki T, Tamura K, Takahashi K, et al. In vivo effect of TGF- beta 1: enhanced intimal thickening by administration of TGF- beta 1 in rabbit arteries injured with a balloon catheter. Arterioscler Thromb Vasc Biol 1995;15:1951-7.

32. Cheema AN, Hong T, Nili N, et al. Adventitial microvessel formation after coronary stenting and the effects of SU11218, a tyrosine kinase inhibitor. J Am Coll Cardiol 2006;47:1067-75.