A Comparison of the Rate of Arteriosclerosis in Patients with the Marfan Syndrome and the General Population

Biren P. Modi

YALE UNIVERSITY

2002
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Date
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A Thesis Submitted to the Yale University School of Medicine
In Partial Fulfillment of the Requirements for the Degree of Doctor of Medicine

By

Biren P. Modi

2002
Abstract

A COMPARISON OF THE RATE OF ARTERIOSCLEROSIS IN PATIENTS WITH THE MARFAN SYNDROME AND THE GENERAL POPULATION. Biren P. Modi and John A. Elefteriades. Section of Cardiothoracic Surgery, Department of Surgery, Yale University School of Medicine, New Haven, CT.

The purpose of this study was to determine the prevalence of arteriosclerosis in patients with the Marfan syndrome in comparison to control individuals from the general population. The study arm consisted of 8 patients with Marfan syndrome who had available preoperative computed tomography (CT) scans of the chest without intravenous contrast. The control arm consisted of 8 age and gender matched patients who also had available CT scans of the chest. The CT scans were evaluated for the presence of calcium in the thoracic aorta and the coronary arteries using a semi-quantitative scale score of 0-3 for each artery. For all patients, a thorough chart review was conducted to search for body mass index and any history of hypertension, hypercholesterolemia, diabetes, prior stroke, prior myocardial infarction, or smoking. Though the differences were not significant, the Marfan group was younger, leaner, more likely to qualify as hypertensive due to beta-blocker administration, and less likely to be diabetic. The mean aortic calcium scores for Marfan patients and control patients were 0.000 and 0.125, respectively (p=0.5). The mean coronary calcium scores for Marfan patients and control patients were 0.000 and 0.125, respectively (p=0.5). These results suggest that some trends do exist which could indicate that and explain why Marfan patients have less advanced arteriosclerosis. Future studies will bear out these trends.
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Dr. Esther Choo and Dr. Catherine (Kitty) Boots for moral support.
Introduction

Background on Arteriosclerosis

Definition

Arteriosclerosis, literally translated as the hardening of arteries, is a broad term encompassing several disease states within the arterial circulation which have in common the thickening and loss of elasticity of the arterial wall. The majority of cases of arteriosclerosis consist of atherosclerosis, the presence of atheromatous plaques within the vessel walls of large elastic arteries (e.g., aorta) and medium-sized muscular arteries (e.g., coronary arteries) (1-3). In addition, arteriosclerosis may include Monckeberg calcification of the arterial media, which can occur in conjunction with advanced atherosclerosis, and arteriolosclerosis, which is a similar process involving the smaller arterioles of the arterial system (2). The most clinically significant, and the pattern which is under discussion in this study, is atherosclerosis.

Pathophysiology

There has been a tremendous growth in the understanding of the mechanisms underlying atherosclerosis in the past decades, but many uncertainties still exist. It is now generally accepted that atherosclerosis is a dynamic process which evolves through the interplay of many factors. These factors include the endothelial and smooth muscle
cells of the arterial wall, inflammatory cells and molecular mediators from the
circulation, and the extracellular matrix. The following is a brief description of the
macroscopic evolution of atherosclerosis and the microscopic players in the process.

Atherosclerosis is a generalized process involving many arterial territories,
including the coronary circulation, the head vessels, the aorta, and the peripheral
circulation (1, 4-7). This fact is useful in understanding the pathophysiology of the
process as well as in applying techniques for molecular study, imaging, and therapy.
Since the risk factors for atherosclerotic disease are the same regardless of location, it is a
natural extension that the mechanisms of pathogenesis are similar in all vascular
territories and that treatment or prevention for one clinical manifestation would likewise
treat and prevent all atherosclerotic disease (4). In descending order, the arteries most
affected by atherosclerosis are the abdominal aorta, the coronary arteries, the popliteal
arteries, the descending thoracic aorta, the internal carotid arteries, and the arteries of the
circle of Willis (2). In all of these vessels, there exists a characteristic pattern of
distribution of atherosclerotic plaques, with most lesions located at the ostia of branches
(2, 8).

Atheromas, or atherosclerotic plaques, consist of varying proportions of cells
(e.g., smooth muscle cells, macrophages, and leukocytes), connective tissue extracellular
matrix (e.g., collagen, elastins, and proteoglycans), and lipid deposits (e.g., intracellular
and extracellular cholesterol or cholesterol esters) (1-3). These components collect
within the intima of the arterial wall, leading to intimal thickening followed sequentially
by development of a fatty streak, atheroma, and fibrous plaque (3, 7). In addition, as they advance in age and complexity, atheromas develop a fibrous cap composed of cells and dense connective tissue, all within the intima and covered by the endothelium of the artery.

This process of atheroma evolution is believed to begin very early in life as fatty dots, less than 1mm in diameter, which then coalesce to form fatty streaks. These streaks form the precursors of fully developed atherosclerotic plaques, though the exact mechanisms are not fully known. Evidence for this relationship comes from autopsy data which shows that fatty streaks, which are nearly universal in children, occur at the same anatomic locations that are later prone to have plaques. In addition, as children grow older and develop more plaques, the number of fatty streaks decreases, suggesting that they may be evolving into plaques. Risk factors known to be associated with atherosclerosis are also associated with fatty streaks early in life. It is important to note, however, that though atherosclerotic plaques may develop from fatty streaks, not all fatty streaks will develop into plaques or more advanced lesions (2, 9).

As these fatty streaks progress, they become atheromas, with varying amounts of cells, lipids, and extracellular matrix. This progression is described by the American Heart Association classification of atherosclerotic lesions (Table 1). As these atheromas evolve and become more numerous, they can lead to a number of complications. Possible complications include occlusion of smaller arteries, distal embolization, hemorrhage into the plaque, and destruction of larger arteries with invasion of the arterial
media. This latter complication can cause disruption of normal wall mechanical properties leading to thrombosis, aneurysm, or rupture. Perhaps the most clinically important complication is focal rupture or ulceration of the plaque with precipitation of thrombosis of the vessel. A “complicated” atherosclerotic plaque can also possess variations from the generic structure. These variations include calcification, which can be used as a marker for advanced disease as described below (2).

<table>
<thead>
<tr>
<th>AHA Grade</th>
<th>Criteria</th>
<th>Comments and Corresponding Gross Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal artery with or without adaptive intimal thickening; no hold</td>
<td>Normal tissue</td>
</tr>
<tr>
<td>1</td>
<td>Isolated MFCs containing lipid; no extracellular lipid; variable adaptive intimal thickening grossly with lipid staining</td>
<td>Initial atherosclerotic lesion, sometimes visible grossly with lipid staining</td>
</tr>
<tr>
<td>2</td>
<td>Numerous MFCs, often in layers, with fine particles of extracellular lipid; no distinct pools of extracellular lipid; variable adaptive intimal thickening</td>
<td>Fatty streak, visible grossly with III staining</td>
</tr>
<tr>
<td>3</td>
<td>Numerous MFCs with 2 pools of extracellular lipid; no well-defined core of extracellular lipid</td>
<td>Fatty plaque, raised fatty streak, intermediate lesion, or transitional lesion</td>
</tr>
<tr>
<td>4</td>
<td>Numerous MFCs plus well-defined core of extracellular lipid, but with luminal surface covered by relatively normal intima</td>
<td>Atheroma, fibrous plaque, or raised lesion</td>
</tr>
<tr>
<td>5</td>
<td>Numerous MFCs, well-defined core or multiple cores of extracellular lipid, plus reactive fibrotic cap, vascularization, or calcium</td>
<td>Fibroatheroma, fibrous plaque, or raised lesion</td>
</tr>
<tr>
<td>6</td>
<td>All of the above plus surface defect, hematoma, hemorrhage, or thrombosis</td>
<td>Complicated lesion</td>
</tr>
</tbody>
</table>

MFC indicates macrophage foam cell; AHA, American Heart Association

The clinical importance of atherosclerosis depends upon the chronic and acute impedance of blood flow via stenosis of the vessel lumen or via distal embolization. The original response of the arterial vessel to the atherosclerotic plaque is positive remodeling, a process which preserves the arterial lumen by outward growth of the vessel. As the capacity of the vessel to remodel outward is exceeded, the process of negative remodeling, which encroaches on the vessel lumen, begins to interfere with normal blood flow (3, 7). Thus, a plaque is quite large before it starts to impinge on the vessel lumen. Positive and negative remodeling are depicted in Figure 1. Plaque growth can occur via inclusion of more cells and extracellular matrix as well as by repetitive cycles of superficial plaque erosion with subsequent thrombosis and healing. The manifestations of atherosclerotic disease generally affect the coronary, cerebral, lower extremity and intestinal vasculature. As the plaque grows larger, over the course of many decades, the plaque may cause decreased blood flow during times of increased demand, resulting in symptoms such as angina pectoris or claudication. However, in many cases, acute coronary events such as myocardial infarction occur without prior symptoms, with the likely mechanism being acute rupture of the fibrous cap or erosion into the plaque with subsequent thrombus formation occluding the vessel lumen. In these cases, the disease is often deadly. For example, 1 out of every 3 initial coronary attacks is fatal (3-4). Distal embolization of plaque or thrombus can also result in clinical symptoms such as transient ischemic attacks, stroke, or intestinal ischemia.

Molecular and Cellular Biology

The molecular pathology and pathogenesis of these lesions is summarized in the "response to injury" hypothesis, which states that atherosclerosis is a chronic inflammatory process within the arterial wall in response to injury to the endothelium (2). The hypothesis consists of the following steps: focal chronic injury to the endothelium with resultant endothelial dysfunction, insudation and modification of lipoproteins, immigration of monocytes and leukocytes with subsequent conversion of monocytes into macrophages and foam cells, release of molecular factors causing migration of smooth muscle cells from the media into the intima, proliferation of smooth muscle cells and elaboration of extracellular matrix, and continued accumulation of lipids both intracellularly, within macrophages and smooth muscle cells, and extracellularly. These steps and their molecular and cellular participants are described in detail below and summarized in Table 2 and Figure 2.
<table>
<thead>
<tr>
<th>Event</th>
<th>Mechanism</th>
<th>Molecular Players</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Chronic endothelial injury causing increased endothelial permeability and leukocyte adhesion</td>
<td>--Hemodynamic disturbances</td>
<td>ICAM-1, VCAM-1, E-selectin, P-selectin</td>
</tr>
<tr>
<td></td>
<td>--Hypercholesterolemia</td>
<td></td>
</tr>
<tr>
<td>2. Lipoprotein insudation and modification</td>
<td>--Oxidative injury</td>
<td>Oxidized LDL, superoxide, scavenger receptor</td>
</tr>
<tr>
<td></td>
<td>--Monocyte/Macrophage recruitment</td>
<td></td>
</tr>
<tr>
<td></td>
<td>--Foam cell formation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>--Direct toxicity</td>
<td></td>
</tr>
<tr>
<td>3. Recruitment of monocytes with formation of foam cells</td>
<td>--Chemotaxis and cell adhesion</td>
<td>IL-1, TNFα, MCP-1</td>
</tr>
<tr>
<td></td>
<td>--Elaboration of cytokines, chemokines and growth factors leading to growth of lesion and immigration of smooth muscle cells and leukocytes</td>
<td></td>
</tr>
<tr>
<td>4. Immigration and proliferation of smooth muscle cells</td>
<td>--Continued smooth muscle cell proliferation</td>
<td>PDGF, FGF, TGFα, TGFβ, collagen, elastin, proteoglycans</td>
</tr>
<tr>
<td></td>
<td>--Elaboration of extracellular matrix</td>
<td></td>
</tr>
<tr>
<td>5. Continued progression</td>
<td>--Formation of complicated plaque</td>
<td>Calcium, bone morphogenetic protein-2a, osteopontin, osteonectin, osteocalcin, matrix metalloproteinases, Gla-proteins, IL-6, estradiol</td>
</tr>
<tr>
<td></td>
<td>--Calcification</td>
<td></td>
</tr>
<tr>
<td></td>
<td>--Rupture</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. A summary of the steps in the “response to injury” hypothesis and some of the key molecular mediators. (Abbreviations: ICAM-1=intercellular adhesion molecule-1, VCAM-1=vascular cell adhesion molecule-1, E-selectin=endothelial selectin, P-selectin=platelet selectin, LDL=low density lipoprotein, IL=interleukin, TNFα=tumor necrosis factor-α, MCP-1=monocyte chemoattractant protein-1, PDGF=platelet derived growth factor, FGF=fibroblast growth factor, TGF=transforming growth factor, Gla-protein=gamma carboxyglutamate containing protein)
1. Chronic endothelial injury:
   - Hyperlipidemia
   - Hypertension
   - Smoking
   - Homocysteine
   - Hemodynamic factors
   - Toxins
   - Viruses
   - Immune reactions

2. Endothelial dysfunction (e.g., increased permeability, leukocyte adhesion)
   Monocyte adhesion and emigration.


4. Macrophages and smooth muscle cells engulf lipid

5. Smooth muscle proliferation, collagen and other ECM deposition, extracellular lipid

Response to injury

Endothelium
Intima
Media
Adventitia

Fatty streak

Lymphocyte

Fibrofatty atheroma

Lymphocyte
Collagen
Lipid debris
The process of atherosclerosis in this model begins with endothelial dysfunction resulting from chronic injury to focal areas of endothelium. The dysfunction is manifest in increased endothelial permeability and enhanced leukocyte adhesion through endothelial cell expression of intercellular adhesion molecules of the immunoglobulin superfamily (e.g., intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1)) and the selectins (e.g., endothelial selectin (E-selectin) and platelet selectin (P-selectin)) (2-3). This dysfunction is distinct from normal arterial endothelium, which is resistant to adhesive interactions with circulating cells, and from inflamed tissue, in which the process of inflammatory cell recruitment occurs in the postcapillary venule, not the artery or arteriole (3). Many initiating factors for endothelial injury have been suggested, including endotoxins and infectious agents, hypoxia, products contained in cigarette smoke, and endothelial toxins such as homocysteine (2, 7), but the concept of endothelial injury in this hypothesis relies on two main mechanisms of injury. One stems from hemodynamic disturbances while the other stems from the adverse effects of hypercholesterolemia. The finding that most atherosclerotic plaques in large arteries occur in the area of ostia to branch arteries is well defined and lends support to the first mechanism (2, 8). In addition, it is known that disturbed flow induces proatherogenic activities in the endothelium, including the adhesion molecules mentioned above, while smooth laminar flow actually induces antiatherogenic, or atheroprotective, gene products such as the antioxidant superoxide dismutase (2-3).
Hyperlipidemia, specifically hypercholesterolemia, also contributes to atherogenesis. The mechanisms underlying this activity include production of oxygen free radicals leading to endothelial injury and insudation of lipoproteins within the intima at sites of increased endothelial permeability, thus beginning the formation of the fatty streak. This leads to the modification of these lipoproteins via oxidation by oxygen free radicals (2-3). These modified lipoproteins are then ingested via the scavenger receptor of macrophages which, unlike the normal LDL receptor, is able to ingest modified LDL (3). This process leads to foam cell formation, chemotaxis of circulating monocytes, monocyte adhesion, recruitment and retention within the lesion, release of cytokines and growth factors, and direct injury to endothelial and smooth muscle cells (2). It is at this level that the beneficial effects of antioxidants such as vitamin E and betacarotene may be manifest (7).

In conjunction with the role of lipoproteins, circulating monocytes are recruited to the area of the lesion via the expression of the specific adhesion molecules discussed above. The selectins contribute to the saltatory motion of circulating cells while the immunoglobulin family molecules contribute to binding of the cells to the endothelium. Chemotaxis due to oxidized LDL and the resultant expressed cytokines then leads to the diapedesis of these cells into the intima, the ingestion of oxidized LDL via the scavenger receptor, and the formation of foam cells (2-3). These cells now play a significant role in the progression of the plaque. They release the important cytokines interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-α), and the chemokine monocyte chemoattractant
protein-1 (MCP-1), all of which further the recruitment of monocytes and leukocytes. In addition, they elaborate chemokines and growth factors which lead to the migration of smooth muscle cells from the media into the intima and their subsequent proliferation (2-3). With the continuation of these processes through continued endothelial injury and persistent hypercholesterolemia, these lesions grow and aggregate, forming fatty streaks. If the hypercholesterolemia is treated, the lesions up to this point may regress without progression (2-3).

The next steps in the progression of the atherosclerotic lesion are important to the conversion from fatty streak to mature atheroma and subsequent complicated plaque (2). As smooth muscle cells, normally quiescent cells within the media of the arterial wall, migrate into the intima and proliferate, they synthesize an extracellular matrix composed of collagen, elastin, and glycoproteins (2-3). This process is governed by the cytokines and growth factors elaborated by the adherent platelets and leukocytes, macrophages and foam cells, endothelial cells, and the smooth muscle cells themselves. These factors include platelet derived growth factor (PDGF), fibroblast growth factor (FGF), and transforming growth factor-alpha (TGF-α) and beta (TGF-β) (2-3).

Progression of the plaques from these early lesions involves maturation of the core from a simple aggregation of foam cells derived from macrophages and smooth muscle cells to a cellular-fatty atheroma consisting of surviving foam cells, dead cells with cellular debris and extracellular lipids, and deposited collagen and proteoglycans. In addition, the connective tissue on the intimal aspect is organized into a fibrous cap. As
they age, these fibrofatty atheromas can undergo further connective tissue proliferation to become fibrous plaques. The larger plaques, like neoplasms, also stimulate adjacent angiogenesis through mediators such as vascular endothelial growth factor (VEGF) (3). In addition, these lesions can progress to “complicated” plaques as described in the section on pathogenesis. For example, the important event of rupture and thrombus formation is thought to originate with the digestion of the extracellular matrix and fibrous cap by matrix metalloproteinases released by resident macrophages (1, 3, 9).

Another feature, calcification of the plaque, is of particular interest to this study and is therefore described here in some detail. One of the modifications which defines a plaque as “complicated” is mineralization with calcium. The process is likely a dynamic interaction of cells and mediators with regulation similar to the process of bone formation (3, 9, 10). Important mediators of this regulation are intercellular messenger molecules, such as interleukin-6 (IL-6) and estradiol (11). In addition, the plaques contain bone morphogenetic protein-2a, a potent factor influencing osteoblast differentiation, and often contain fully formed bone tissue, including marrow (9, 12). Other factors involved in the process are osteopontin, osteonectin, and osteocalcin, all known to be involved in bone mineralization, and proteins containing gamma carboxyglutamate (Gla), a rare amino acid whose only known function is to bind calcium (9). Also, cells that are known to be capable of osteoblastic differentiation and which may be the predecessors of vascular calcifying cells are found in atherosclerotic plaques (9). Though calcification does define a plaque as being advanced and complicated, the presence of calcification in itself does not predispose to complications of atherosclerosis. In fact, some studies suggest that
calcification of the arteries due to atherosclerosis, though a useful marker of atherosclerosis (see below), may actually strengthen a plaque, thus preventing it from undergoing complications leading to clinical events (9 13, 14).

Epidemiology

The social importance of arteriosclerosis is well known, and can be defined by examining the epidemiological and health-care cost data available. All atherosclerotic cardiovascular disease was estimated to account for $259 billion dollars of health care costs in 1997, up from $210 billion dollars in 1993. The biggest portion of this amount consisted of $51 billion dollars for coronary heart disease alone, followed by $35.5 billion dollars for congestive heart failure and $19 billion dollars for stroke (4). In 2002, the estimated direct costs of major atherosclerotic disease, consisting of coronary heart disease, stroke, and congestive heart failure, are $110.4 billion dollars. This estimate does not include the indirect costs of these diseases from lost productivity and does not include other atherosclerotic diseases such as lower extremity gangrene (15).

The clinical importance of arteriosclerosis is evident when examining the epidemiological data on the prevalence and associated mortality and morbidity of atherosclerosis. Although atherosclerosis is a diffuse process capable of involving any vascular territory in the body, symptomatic atherosclerotic disease most often involves the heart, brain, kidneys, lower extremities and intestines. For this reason, data about incidence and prevalence of atherosclerotic disease are generally expressed in reference
to ischemic coronary heart disease (CHD) and its consequences (e.g., angina, myocardial infarction, congestive heart failure), cerebrovascular accidents (CVA), end organ infarction and ischemia, and gangrene of the limbs (2). Atherosclerotic disease and its clinical consequences account for over half of all deaths in the developed world (2), and have been the number one killer in the United States since 1900 (15). Fatty streaks are nearly ubiquitous, with one study demonstrating that all teenagers in the United States who were sampled had fatty streaks somewhere in their arterial system (16). The prevalence of more advanced atherosclerosis increases as the patient ages, with atherosclerotic plaque present in 50% of individuals aged 20-29 years but in 80% of individuals aged 30-39 (9). Although on the decline in recent years due to prevention and advances in medical care, coronary heart disease (CHD) independently remains the leading cause of death in the United States (2, 17-19). Approximately 12 million people in the United States have CHD (Figure 3), resulting in over 500,000 deaths per year (4, 15, 18, 20).

Some data do exist for the presence of atherosclerotic calcification in the population. Calcification of the vasculature is present in 50% of individuals aged 40 to 49 years, and 80% of individuals aged 60 to 69 (9). In one study, calcified plaques specifically in the thoracic aorta were present in 8.5% of men and 3.9% of women aged 40 to 44 years, with equalization of the sexes by age 60 and more than 80% in both sexes at age 75 to 80 (6, 21-22).

Risk Factors

Several large prospective clinical trials have identified many risk factors, both constitutional and acquired, which contribute to the pathogenesis and evolution of atherosclerosis. The major risk factors are described in more detail below and are shown
in Table 3 along with some minor and uncertain risk factors. Age, sex, family history and genetics are the constitutional factors (2, 9, 15).

<table>
<thead>
<tr>
<th>Major</th>
<th>Lesser, Uncertain, or Nonquantitated</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Constitutional</strong></td>
<td>Obesity</td>
</tr>
<tr>
<td>Increasing age</td>
<td>Physical inactivity</td>
</tr>
<tr>
<td>Male gender</td>
<td>Stress</td>
</tr>
<tr>
<td>Genetics</td>
<td>Homocysteine</td>
</tr>
<tr>
<td><strong>Potentially Controllable</strong></td>
<td>Postmenopausal estrogen deficiency</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>High carbohydrate intake</td>
</tr>
<tr>
<td>Hypertension</td>
<td>Alcohol</td>
</tr>
<tr>
<td>Cigarette smoking</td>
<td>Lipoprotein (a)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>Hardened (trans) unsaturated fats</td>
</tr>
<tr>
<td></td>
<td><em>Chlamydia pneumoniae</em></td>
</tr>
<tr>
<td></td>
<td>Herpes viruses</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Age (y)</th>
<th>Probability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Women</td>
</tr>
<tr>
<td>30-34</td>
<td>&lt;1</td>
</tr>
<tr>
<td>35-39</td>
<td>&lt;1</td>
</tr>
<tr>
<td>40-44</td>
<td>2</td>
</tr>
<tr>
<td>45-49</td>
<td>5</td>
</tr>
<tr>
<td>50-54</td>
<td>8</td>
</tr>
<tr>
<td>55-59</td>
<td>12</td>
</tr>
<tr>
<td>60-64</td>
<td>13</td>
</tr>
<tr>
<td>65-69</td>
<td>9</td>
</tr>
<tr>
<td>70-74</td>
<td>12</td>
</tr>
</tbody>
</table>

As is evident from the description of pathogenesis and molecular biology, atherosclerosis begins early in life and becomes progressively worse over the course of the lifespan to the point of becoming clinically relevant after middle age. One possible mechanism of this correlation may be a decrease in elasticity as the artery ages (8). The prevalence of CHD is age-dependent (Table 4), with an increase from 86 per 1000 men at ages 45 to 64 up to 169 per 1000 men at ages 65 and older suffering from CHD. These numbers in women, though slightly lower, are substantial and on the rise (4). In addition, 1 in every 3 males and 1 in every 10 females in the United States suffers a major atherosclerotic cardiovascular event before the age of 60 years. This risk for these events is also age-dependent, with the annual rate increasing from 5 per 1000 in the 35 to 44 age range to 59 per 1000 in the 85 to 94 age range (4). An additional example is the five-fold increase in the incidence of myocardial infarction from age 40 to age 60 (2). Some of these data are presented in a different form in Figure 4.

Additional data show that the male sex is also an independent factor in the prevalence of atherosclerosis (see again, Table 4), though the distinction between sexes becomes insignificant after the age of menopause as the occurrence of atherosclerotic events accelerates in women (Figure 4). There is some data supporting the idea that this trend is due to a favorable lipid profile and better endothelial function associated with higher levels of estrogen (2, 9).

Figure 4 (on following page). The incidence major cardiovascular events in 36-year follow-up of the Framingham Study (Reproduced from Kannel, W.B. 1998. Overview of atherosclerosis. Clinical Therapeutics. 20, Supplement B: B2-B17)
The final constitutional factor is genetics. Familial clustering of atherosclerosis certainly exists. The process is likely polygenic, and often linked to other risk factors such as diabetes or hypertension. There also exist well-defined familial diseases of lipid metabolism leading to hyperlipidemia with and without hypercholesterolemia (2).

Many of the major risk factors for atherosclerotic disease are acquired or controllable with therapy or lifestyle modification. These include hyperlipidemia, hypertension, smoking, and diabetes (2, 4, 15, 23-24). It is at the level of these factors that the greatest strides have been made in reducing the incidence of atherosclerosis related disease over the past several decades with the use of primary and secondary prevention (2, 15).
Hyperlipidemia is an obvious offender when one analyzes the molecular biology of atherosclerosis as described above. The proof for the relationship lies in the high incidence of atherosclerotic disease present in high lipid states such as familial hypercholesterolemia and diabetes. In addition, it has been proven that the higher the level of cholesterol, the greater the severity of atherosclerosis, with an exponential increase after exceeding 200mg/dl of total cholesterol. Many studies have also shown that a decrease in serum cholesterol with diet and lipid-lowering drugs leads to a decrease in atherosclerotic disease (2, 9).

Hypertension is also a controllable risk factor, and is actually a stronger risk factor for atherosclerosis than hypercholesterolemia after age 45 years. Both systolic and diastolic pressures are important, and control of blood pressure with antihypertensive therapy reduces the incidence of atherosclerotic disease (2).

Cigarette smoking is a well-established risk factor for both men and women, and is believed to have contributed to a recent increase in the incidence of atherosclerosis in women (2, 9). As with aging, smoking has been shown to decrease the elasticity of arteries, thereby leading to flow changes which may lead to hemodynamic endothelial injury (8). Smoking can cause a 200% increase in the death rate from ischemic heart disease when one or more packs are smoked per day for several years. This risk is partially reversible, as cessation of smoking halves this increase in risk (2).

The last major risk factor is diabetes mellitus. Diabetes can induce
hypercholesterolemia and has been shown to nearly double the risk of myocardial infarction as compared to nondiabetics. The most prevalent atherosclerotic disease in diabetics is atherosclerosis-induced gangrene of the lower extremities, the risk of which is increased by a factor of 100 in diabetics versus nondiabetics (2).

Many other minor and uncertain risk factors exist, as described in Table 3. One important facet of this data is that atherosclerosis can develop in the absence of any known or hypothesized risk factor, emphasizing the fact that this field is still actively evolving and its true pathogenesis remain highly speculative (2). Another important fact, summarized in Figure 5, is that the risk factors are not merely additive when compounded. They are somehow synergistic so that two risk factors increase the risk of atherosclerotic disease fourfold, while three risk factors increase the risk sevenfold (2).
Imaging

Imaging of atherosclerosis in vivo is an important method of identifying and following patients with atherosclerotic disease. Imaging of the coronary arteries by noninvasive means is difficult but possible with certain modalities. Another potential target for imaging is the aorta. Many studies demonstrate that atherosclerosis of the aorta correlates well, positively and negatively, with the presence of the generalized atherosclerotic process (1, 5, 9, 25). This relationship seems especially useful for younger patients (6, 26).

Imaging of atherosclerosis can be accomplished by a variety of modalities. The list of modalities continues to grow with the advent of newer technologies and imaging techniques such as electron-beam computed tomography (EBCT) and “black-blood” magnetic resonance imaging (MRI) (1, 13, 27-28). The primary modalities are echocardiography, fluoroscopy, and computed tomography. These modalities, as they pertain to this study, will be discussed below.

Echocardiography, specifically transesophageal echocardiography (TEE), is a noninvasive modality ideal for the detection, measurement and characterization of
atherosclerotic plaques of the aorta. It is able to detect intimal thickening, often the first
detectable sign of atherosclerosis, ulceration, and calcification, and has the ability to
grade plaques based on these characteristics (13, 26, 29). There are limitations of TEE,
however, for the purposes of this study. For example, TEE cannot fully visualize the
ascending aorta and arch due to its anatomic relationship to the trachea (13, 30). TEE can
also visualize only the proximal coronary circulation with a good degree of resolution,
and does not provide adequate imaging for the detection of coronary calcium. In
addition, without advance specification requesting characterization of the aorta, most
echocardiographers do not focus on obtaining adequate images of the descending aorta.
Although a versatile tool in the detection of atherosclerosis of the aorta, TEE was not
considered a useful imaging modality in this retrospective study due to these limitations.

Fluoroscopy is an excellent imaging modality for the detection of significant
atherosclerotic disease by its virtue of being able to detect lesions which impinge on the
lumens of the coronary arteries or the aorta. This modality is also useful in detecting
moderate to large levels of coronary and aortic calcification, though its sensitivity for
lesser amounts of calcium is limited (9). In addition, coronary angiography and
aortography are invasive procedures which not all patients qualify for due to risks
inherent in the procedure and the use of fluoroscopic dye. For the purposes of this study,
not all patients in the Marfan group received coronary angiography or aortography. In
addition, no valid control group of patients exists which adequately represents the general
population.
Conventional computed tomography (CT) is extremely useful for the specific purpose of detecting vascular calcification (9). The specificity of coronary calcification seen on CT for significant coronary artery disease are 78% to 100% in studies comparing CT to angiographically significant stenosis, with a positive predictive value of 83% to 100%, suggesting that if coronary calcium was seen on CT, significant coronary artery disease was likely to be present (9). In addition, computed tomography is also useful in assessing the entire thoracic aorta, allowing for even better sensitivity and specificity for significant atherosclerotic disease (9, 13). Some studies suggest that the presence of atherosclerotic lesions in the thoracic aorta possesses both a strong positive predictive value and a high negative predictive value (as high as 79% in one study) for coronary artery disease, suggesting that noninvasive detection of aortic atherosclerosis is a useful and reliable marker for significant atherosclerotic disease in the patient (5, 25). Electron beam CT, a novel technique which allows for very exact quantification of coronary calcium, is extremely useful for assessing the degree of atherosclerotic disease (1, 9, 13). This new technique, however, is not universally available and is too recent to be available for this retrospective study. Conventional CT, however, is universal and has been used in assessing the severity of atherosclerotic disease by use of a semi-quantitative calcification grading scale which correlates well with the exact quantification of electron beam CT (1, 10, 22, 30-32). This can be accomplished within the coronary arteries, and more so within the aorta, the larger size of which makes it an easier subject of imaging. CT is also useful for the purposes of this study because many Marfan patients are followed with serial CT scans for assessment of aortic aneurysm growth (see details of Marfan and quantitative method below).
Calcification of vessels has been shown to be a definite marker of atherosclerotic disease. Detection of atherosclerosis of the coronary arteries and aorta via identification of calcification within these arteries is a reliable indicator of presence and severity of atherosclerotic disease (9, 14). For example, calcification of the coronary arteries occurs exclusively in atherosclerotic arteries, sparing normal vessels (9). Thus, calcification of the aorta and coronary arteries, visualized with CT, is a marker for atherosclerosis of these vessels. In addition, atherosclerosis of the aorta itself is a significant marker for coronary artery disease (1, 27, 33-37). To extend this relation farther, since atherosclerosis is a diffuse process, the calcification of these vessels is also an indicator of generalized atherosclerotic disease (1, 9). Since the aorta and coronary arteries are among the most frequently affected vessels, as described above, their use as markers for atherosclerotic disease is also extremely sensitive. In addition, this relationship is most significant when studying patients younger than 65 years of age (6, 26, 34). Calcification of the coronary arteries and aorta is thus ideal for the study of the relatively young Marfan patient population in this study.

Background on the Marfan Syndrome

Definition

First described in 1896 by the Parisian pediatrician Auguste Marfan, the Marfan syndrome has become a prototypical example of the group of syndromes collectively
known as the disorders of connective tissue. Marfan syndrome (MFS), with an incidence of approximately 1 in 10,000 to 20,000 live births and a prevalence of 1 in 3,000 to 5,000 individuals, is an autosomal dominant condition caused by mutations in the FBN1 gene encoding the extracellular molecule fibrillin-1 (38-43). The lifespan of patients with MFS has increased steadily over the past decades as treatment modalities evolve. In 1972, the average lifespan for a patient with MFS was 32 years. This number increased to 41 years in 1993 and 61 years in 1996, and now approaches the normal lifespan of the population. For as yet unknown reasons, the lifespan of patients with MFS is significantly lower in men than in women (38, 41, 43-44).

Features and Diagnosis

MFS lies at the extreme end of a spectrum of disease conditions, the fibrillinopathies, ranging from variations of normal to individual features of MFS to MFS (41, 44). MFS is a pleiotropic disease involving abnormalities in multiple organ systems, most notably the ocular, musculoskeletal, and cardiovascular systems (38, 39, 41, 44). The diagnostic criteria for MFS were recently updated by the group from Gent, Belgium (39), and are listed in Table 5, while the differential diagnoses of MFS are listed in Table 6. The classic, diagnostic features of MFS and some of their treatments are described here, with particular emphasis on the cardiovascular manifestations of the disease. In this revised scheme, “major” criteria are those which carry high specificity for MFS due to relatively low frequency in other conditions and the general population (39).
Ocular manifestations of MFS include ectopia lentis, with upward dislocation of the lens unilaterally or bilaterally, myopia secondary to the increased length of the globe and flattening of the cornea, retinal tears and detachment, and glaucoma (38-39, 41-42). Dislocation of the lens, one of the most recognized ocular abnormalities of MFS, occurs in approximately 60% of patients with the disease. Conservative correction of vision with ectopia lentis in MFS is usually attempted prior to surgical correction with lens removal and replacement. Though a major diagnostic manifestation of MFS, ectopia lentis does occur as an isolated disease in some patients with FBN1 mutations who do not meet diagnostic criteria for MFS (38, 41). This underscores the importance of the Gent criteria and will be discussed in more detail in the section on molecular biology and genetics.

<table>
<thead>
<tr>
<th>Major criteria</th>
<th>Minor criteria</th>
<th>Involvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family history</td>
<td>First-degree relative who meets diagnostic criteria</td>
<td>None</td>
</tr>
<tr>
<td>Marfan-causing FBN1 mutation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haplotype around FBN1 associated with confirmed Marfan inherited by descent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skeletal</td>
<td>Presence of any four of the following:</td>
<td></td>
</tr>
<tr>
<td>Pectus carinatum</td>
<td>Pectus excavatum</td>
<td></td>
</tr>
<tr>
<td>Pectus excavatum requiring surgery</td>
<td>Joint hypermobility</td>
<td></td>
</tr>
<tr>
<td>Reduced upper segment-lower segment ratio or spanheight &gt;1.05</td>
<td>High arched palate with dental crowding</td>
<td></td>
</tr>
<tr>
<td>Positive wrist and thumb signs</td>
<td>Facial appearance</td>
<td></td>
</tr>
<tr>
<td>Scoliosis &gt;20 degrees or spondylolisthesis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extension &lt;170 degrees at elbow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medial displacement of the medial malleolus causing pes planus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protrusio acetabuli</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ocular</td>
<td>Ectopia lentis</td>
<td></td>
</tr>
<tr>
<td>Dilatation of the ascending aorta with or without aortic regurgitation involving at least the sinuses of Valsalva</td>
<td>Flat cornea (by keratometry)</td>
<td>One major or one minor criterion</td>
</tr>
<tr>
<td>Dissection of the ascending aorta</td>
<td>Increased globe length (by ultrasound)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hypoplastic iris or hypoplastic ciliary muscle causing decreased miosis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mitral valve prolapse with or without mitral regurgitation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dilatation of main pulmonary artery without other cause at age 40 years or younger</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Calcification of the mitral annulus at age 40 years or younger</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dilatation or dissection of the descending thoracic or abdominal aorta at age 50 years or younger</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spontaneous pneumothorax</td>
<td>One minor criterion</td>
</tr>
<tr>
<td></td>
<td>Apical blebs (radiographically)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Striae atrophicae not associated with marked weight changes, pregnancy, or recurrent stress</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Recurrent or incisional hernia</td>
<td>One major or one minor criterion</td>
</tr>
<tr>
<td>Pulmonary</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Skin and central nervous system</td>
<td>Lumbosacral dural ectasia</td>
<td></td>
</tr>
</tbody>
</table>

*To make a diagnosis of Marfan syndrome in an individual with no family history, requires major criteria in two organ systems and involvement of a third. If there is a family history, major criteria must be met in one organ system and another must be involved.*

<table>
<thead>
<tr>
<th>Common Skeletal Manifestations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classic Ehlers-Danlos syndrome (MIM 130000, 130010, 130020)</td>
</tr>
<tr>
<td>Sickle-cell disease (MIM 141900.0243)</td>
</tr>
<tr>
<td>Multiple endocrine neoplasia type III (MIM 162300)</td>
</tr>
<tr>
<td>Trisomy 8</td>
</tr>
<tr>
<td>Klinefelter syndrome</td>
</tr>
<tr>
<td>Congenital contractural arachnodactyly (MIM 121050)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Common Ocular Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ehlers-Danlos syndrome, kyphoscoliosis form (type VI; MIM 225400)—retinal detachment</td>
</tr>
<tr>
<td>Weill-Marchesani syndrome (MIM 277600)—ectopia lentis</td>
</tr>
<tr>
<td>Autosomal dominant ectopia lentis (MIM 129600)</td>
</tr>
<tr>
<td>Autosomal recessive ectopia lentis with (MIM 225200) and without (MIM 225100) ectopic pupils</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Common Cardiovascular Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitral valve prolapse</td>
</tr>
<tr>
<td>MASS phenotype (familial mitral valve prolapse; MIM 157700).</td>
</tr>
<tr>
<td>Dilatation of the ascending aorta</td>
</tr>
<tr>
<td>Tertiary syphilis</td>
</tr>
<tr>
<td>Ankylosing spondylitis</td>
</tr>
<tr>
<td>Relapsing polychondritis</td>
</tr>
<tr>
<td>Reiter syndrome</td>
</tr>
<tr>
<td>Familial aortic aneurysm (MIM 132900)</td>
</tr>
<tr>
<td>Bicuspid aortic valve, coarctation of the aorta, and medial degeneration of the ascending aorta</td>
</tr>
</tbody>
</table>

The main musculoskeletal features of MFS are arachnodactyly, confirmed by the thumb and wrist signs, tall stature with decreased upper body to lower body ratio and arm span exceeding height, generalized joint hypermobility, protrusio acetabuli, and disorders of tubular bone overgrowth, manifest as scoliosis and pectus deformities (38-39, 41-42, 44). Since many of these musculoskeletal features are common in the population, a combination of multiple defects is required for involvement of this system (39). A positive thumb sign for arachnodactyly means that the distal phalanx or entire fingernail of the thumb protrudes beyond the fifth digit when the fist is clenched. A positive wrist sign means that the thumb and fifth digit overlap when circling the contralateral wrist. Scoliosis and pectus deformities may require surgical correction if the subsequent restrictive pulmonary disease and cor pulmonale is morbid, but these corrections must be timed to avoid recurrence due to continued bone growth (41-42).

The main cardiovascular features of MFS are aneurysmal aortic dilatation, with and without aortic regurgitation, aortic dissection and/or rupture, and mitral valve prolapse (38-39, 41, 44). Prior to the development of successful surgical intervention, these cardiovascular features accounted for over 90% of mortality in MFS (41-42). Dilatation of the aorta is found in 50% of children with MFS, and progresses with time to involve 60% to 80% of adults. Many patients have involvement of other segments of the aorta (38, 43). In addition, other arteries, including the pulmonary artery, the carotids, and cerebral vessels may display dilatation (38). Aortic dissections occur more frequently as the aorta becomes progressively more dilated, with the majority being Type A dissections originating at the aortic root (38, 44).
Mitral valve prolapse is also common in MFS, but due to its high prevalence as an isolated entity, it is only a minor criterion for MFS. In MFS, mitral valve prolapse occurs with higher incidence in women and with age (38). The cause of mitral valve prolapse in MFS, as with isolated mitral valve prolapse, seems to be a redundancy of the valve leaflets with dilatation of the mitral annulus and stretching of the chordae tendineae. In addition, the annulus in these patients is often calcified (38, 41); this fact is particularly relevant to the present study in demonstrating that patients with MFS do seem to have normal, active calcification of damaged endothelial and vascular structures.

The management of aortic disease in patients with MFS has progressed dramatically in the past few decades with the development of composite graft replacement surgery of the aortic valve and ascending aorta and the demonstration of a potential prophylactic benefit with the use of beta-blockers and possibly calcium channel antagonists. (38, 41, 43-44). Elective repair of the aortic root, when the root diameter exceeds twice the upper limit of normal for an individual’s body surface area, is optimal (38, 41-42). The 30-day mortality for elective repair is significantly lower as compared to the urgent (within 1 week of surgical consultation) and emergent repairs (within 24 hours of surgical consultation) which occur as a result of dissection or impending dissection (38, 41, 45). In addition, the likelihood of requiring additional aortic surgery at other sites in the aorta is increased in patients who have a dissection at the time of their first surgery (38, 45). These statistics are especially important for the management of pregnant MFS patients, who are at increased risk for acute enlargement of the aortic root.
diameter and dissection (38, 44). Also, as treatment for the cardiovascular manifestations of MFS are improving, the resultant increase in lifespan has important implications for the more prominent role of manifestations in the other organ systems involved. In the case of mitral valve prolapse, mitral valve repair or replacement is often required if heart failure occurs (41). Another important point in the management of the cardiovascular features of MFS is antibiotic prophylaxis during procedures which may introduce bacteria into the bloodstream, as these patients are particularly prone to bacterial endocarditis (41).

Other features of MFS include dural ectasia, cutaneous striae densae, and apical pulmonary blebs with spontaneous pneumothorax (39). The dural ectasia, resulting in enlargement of the lumbosacral spinal canal with erosion of vertebral bone and potential meningoceles, occurs in up to 90% of MFS patients but has an uncertain clinical significance beyond minor nerve compression symptoms (38-39). The cutaneous striae occur in flexural areas and can often be associated with incisional and inguinal hernias (38-39).

Table 5 details which of these features qualify as minor and major diagnostic criteria for MFS, as well as the requirements for involvement of an organ system. To achieve an independent diagnosis of Marfan Syndrome by these 1996 Gent criteria (39), a patient must have major manifestations in two systems with involvement of a third system. To qualify for a major criterion in the family history or genetic history category, a patient must have a parent, sibling, or child meeting the independent criteria, or they
must possess a mutation in FBN1 known to cause MFS, or they must possess a linked haplotype around the FBN1 gene which is known to be associated with independently diagnosed MFS in the family. If a patient has one of these major criteria in the family history category, then he or she may be diagnosed with MFS if they have a major manifestation in one system with involvement of a second system. The simple presence of a mutation in the FBN1 gene is not sufficient for diagnosis due to the wide variation in phenotype from such mutations. This will be discussed in greater detail below.

*Molecular Biology and Genetics*

The FBN1 gene, now linked to MFS and other similar disease processes, produces fibrillin-1, a glycoprotein component of the connective tissue 10nm elastin-associated microfibril (44). Another fibrillin gene, FBN2, and its product fibrillin-2, have also been identified and linked to congenital contractural arachnodactyly, but not to MFS (41). FBN1 is a large gene with 65 exons located at chromosome 15q21.1. Fibrillin-1 and the connective tissue microfibril are components of both elastic and nonelastic connective tissues and are essential to normal elastic fibrillogenesis. With many cysteine-rich sequences, fibrillin-1 is homologous to epidermal growth factor or transforming growth factor-beta binding protein-1 (38, 46).

There is extensive intragenic heterogeneity, with over 100 mutations in FBN1 identified in patients with MFS. In addition, many other mutations have been identified in patients with milder phenotypes that overlap with MFS. Also, at least 25%
of patients with MFS represent new mutations not present in the family genetic history (38, 41). These facts make molecular diagnosis of MFS extremely difficult, so that the clinical diagnostic criteria mentioned above are more precise and still necessary despite knowledge of the molecular biology of the disease (41-42, 44, 47). In mutations known to cause MFS, penetrance approaches 100% but wide clinical variation (39, 41, 43-44, 47). Though no exact genotype to phenotype correlations have been defined within MFS, it seems that patients with exon skipping or in-frame mutations have a more severe phenotype than do patients with termination mutations (38, 41, 44). This fact is consistent with the thought that the MFS phenotype is the result of a dominant negative action of the mutant fibrillin-1 protein (41).

The connective tissue microfibrils are ubiquitous, and act as principal components of elastic fibers, anchoring fibers between the dermis and epidermis, and as the ocular zonules (41, 47). In the arterial wall, the changes are mainly in the media. The media of the elastic arteries in patients with MFS display fragmentation and disarray of the elastic fibers, a decreased amount of smooth muscle cells, and the separation of muscle fibers by collagen and mucopolysaccharide (41-42). The result of these defects is increased stiffness and decreased distensibility in the ascending aorta (38, 41, 43).

*Arteriosclerosis in the Marfan Syndrome*

Very little literature exists regarding the prevalence of arteriosclerosis in patients with MFS. An extensive literature search using the Medline database revealed no journal
entries focusing on both Marfan syndrome and arteriosclerosis. One study does find that
the rate of coronary artery disease detected by coronary angiography was significantly
less in patients with acute aortic dissection than in patients with abdominal aortic
aneurysms or arteriosclerosis obliterans, with a rate of 19.7%, 43.9%, and 51.5%,
respectively (48). This study, however, specifically excluded patients with the Marfan
syndrome, thus making difficult any extrapolation to this particular subset of patients
with acute aortic dissections. The experience of the cardiothoracic surgery group at Yale,
however, suggests that patients with MFS do have significantly less arteriosclerotic
disease in their large arteries than do other patients.

Many mechanisms could provide a reason for this observation. The reason could
be a simple age bias, since the operative MFS population tends to be younger than other
cardiothoracic surgery patients. There could be reasons why disruption of the normal
arterial media could provide protection from intimal injury or progression of intimal
lesions. As mentioned above, the defective fibrillin-1 protein is an integral component of
the 10nm connective tissue microfibril. This microfibril interacts with many different
constituents of the extracellular matrix and a defect in the fibril could create difficulties in
the normal inflammatory processes required for arteriosclerosis. For example, loss of the
normal elastic properties of the artery could prevent normal hemodynamic stresses which
cause endothelial injury. This would seem paradoxical, however, due to the increase in
atherosclerosis which occurs with age due, theoretically, to the decreased elasticity of
older vessels. Also, there could be as yet unknown functions of the microfibril, perhaps
in interactions with the thrombotic mechanism or serum lipoproteins, which could link a
defect in this microfibril to the process of arteriosclerosis. Alternatively, as mentioned above, there is a decrease in the number of smooth muscle cells in the arterial media of patients with MFS. Without these smooth muscle cells, normal progression of the atherosclerotic plaque could not occur. They could not migrate to the intima (due to decreased number as well as potentially defective migratory properties in the setting of defective microfibrils) and elaborate the extracellular matrix and intercellular mediators which build upon the early lesion.

The dearth of knowledge in this area creates an opportunity for ground level research. It is the aim of this study to probe the relationship between Marfan syndrome and arteriosclerosis. The specific hypothesis and aims of the study are given below.
Statement of Purpose

No organized and decisive studies evaluating the relationship between the Marfan syndrome and arteriosclerosis exist in the medical literature. Nevertheless, the Marfan syndrome is a disease of the connective tissue of the vessel wall, placing it in a unique position to interact extensively and by a variety of means with the processes of arteriosclerosis. It is the observational experience of the faculty of the section of cardiothoracic surgery at Yale University School of Medicine that patients with Marfan syndrome who have been operated upon at Yale-New Haven Hospital have had relatively low rates of overt arteriosclerosis.

This pilot study has been designed to probe the nature of the relationship between these two important diseases in order to 1) determine the prevalence of arteriosclerosis in the Marfan syndrome, 2) compare patients with the Marfan syndrome to the general population in this regard, and 3) begin to understand any lessons each disease may offer about the other. In time, this work will be developed into a national study with a large cohort of patients. The hypothesis of this study is that patients with the Marfan syndrome have significantly less arteriosclerotic disease, as detected by calcification in the thoracic aorta and coronary arteries on a chest CT scan, than individuals in the general population.
Methods

Institutional Review Board Approval

Approval for this study was obtained from the Human Investigation Committee of the Yale University School of Medicine prior to the initiation of the study.

Selection of Patients

The database of the Yale Center for Thoracic Aortic Disease was used to select patients for the Marfan arm of the study. The database contains 1282 patients with aortic disease who have been cared for by the section of cardiothoracic surgery at Yale University School of Medicine. Of these patients, 65 carried a confirmed diagnosis of Marfan syndrome using the Gent diagnostic criteria. These 65 patients were then cross-referenced with the databases of the department of diagnostic imaging for the availability of a pre-operative CT scan of the chest without the use of intravenous contrast, resulting in a total of 8 patients in the Marfan arm. Only pre-operative scans were included in this study due to concerns about artifact from prosthetic grafts and changes in flow dynamics which may affect local vessel injury.

For the control arm of the study, the databases of the department of diagnostic imaging were queried for patients between September, 1998, and February, 1999, and between September and December, 2000, who had undergone chest CT scanning without
the use of intravenous contrast. Patients with definite cardiac or aortic pathology were excluded, as were patients who had undergone prior thoracic surgery. These patients were then matched with the patients in the Marfan arm of the study for both age at time of CT scanning and gender. Age matching was conducted by creating categorical ranges of age as follows: \( \leq 30 \) years old, 31-35 years old, 36-40 years old, and 41-45 years old. This resulted in 8 control patients.

**Evaluation of CT scans**

All CT scans were officially interpreted on film or AutoRad by Dr. Coralie Shaw from the department of diagnostic imaging. The scans were evaluated for the presence of calcification within the walls of the coronary arteries or the thoracic aorta. The right coronary artery, left circumflex artery, and the left anterior descending artery were evaluated separately, with the sum of their individual scores forming the score for the coronary arteries. Points for calcification were given as follows: 0 points for no calcium, 1 point for minimal flecks (<1mm diameter) of calcium on single levels, 2 points for more than three minimal flecks or larger non-circumferential flecks (1-3mm diameter) on single or multiple levels, and 3 points for severe (>3mm diameter) flecks or circumferential calcification. This semi-quantitative scale for calcification has been described by others (1, 10, 22, 30-31). The total scores possible were 3 points for the thoracic aorta and 9 points for the coronary arteries (3 from each artery).
**Review of Medical History**

The hospital charts of all included patients were thoroughly reviewed for the presence of the risk factors discussed above which may have confounded the analysis of arteriosclerosis. Age and gender had already been matched in creating the control arm. The factors for which the charts were searched include the body mass index (weight in kilograms divided by height in meters squared) at the time of scanning, as well as history of hypertension, diabetes mellitus, smoking, and hypercholesterolemia. History of prior myocardial infarction or stroke was also included in the chart review. History of hypertension was considered present if the patient had chart documented history of hypertension at the time of scanning, was taking antihypertensive medication at the time of scanning, or had a chart documented blood pressure above 140 millimeters of mercury systolic or 90 millimeters of mercury diastolic on at least two separate occasions (31). History of diabetes mellitus (DM) was considered present if the patient had chart-documented history of DM at the time of scanning or was taking insulin or oral hypoglycemic medication at the time of scanning. History of smoking was considered present if the patient had chart documented history of current or remote smoking at the time of scanning. History of hypercholesterolemia was considered present if the patient had chart documented history of hypercholesterolemia at the time of scanning, was taking lipid lowering medication at the time of scanning, or had chart documented total cholesterol above 200 milligrams per deciliter on two fasting serum samples (25). History of prior myocardial infarction or stroke was considered present if the patient had chart documented history of these prior events at the time of scanning.
Data Analysis and Statistical Considerations

The data from the review of patient records and the evaluation of CT scans were analyzed for significant differences between the two groups. Continuous variables, including average age, average body mass index, average calcification score in the thoracic aorta, and average calcification score in the coronary arteries, were evaluated using the student's t-test. Since the number of records was relatively small, the dichotomous variables, which included all other confounders from the patients' records, were analyzed using the Fisher exact probability test rather than the Chi-square method. In all variables, there was one degree of freedom and differences were considered significant at a p-value less than 0.05. As the number of patients studied in this pilot study was small, no attempt at multivariate analysis was conducted to determine and eliminate the influence of the confounding factors on the level of calcification.
Results

The results of the record review and CT scan evaluation are given as a compilation of the different patients into averages and total numbers with statistical results in Table 7. In addition, the data for each patient in each group is given individually in Table 8.

With regard to confounding factors, age and gender were matched in creating the control group. However, the average age in the two groups did differ, with the Marfan group average at 26.6 years and the control group average at 30.5 years. This difference was not significant (t = 0.850, p = 0.55). For all other confounders, the differences between the two groups also did not reach statistical significance. The average body mass index was 21.9 kg/m$^2$ for Marfan patients and 25.6 kg/m$^2$ for control patients (t = 1.02, p = 0.49). There were no patients with hypercholesterolemia or prior myocardial infarction in either group. Five patients (62.5%) in the Marfan group qualified for hypertension while only three (37.5%) qualified in the control group (Fisher probability = 0.24). All five of the Marfan patients, however, qualified in this category due to beta-blocker therapy for aortic dissection prophylaxis, not for classically defined hypertension. In contrast, all three of the control patients qualified in this category due to chart-documented elevations in blood pressure in addition to anti-hypertensive therapy. No patients (0%) had chart-documented diabetes mellitus in the Marfan group, while three patients (37.5%) had diabetes mellitus in the control group (Fisher probability = 0.1). Four patients (50%) in the Marfan group and three patients (37.5%) in the control group
had a history of cigarette smoking (Fisher probability = 0.34). Finally, one patient (12.5%) in the Marfan group, but no patients (0%) in the control group, had a history of a minor prior stroke (Fisher probability = 0.5).

The evaluation of CT scans revealed only one patient in the study who had evident calcification in the thoracic aorta and/or coronary arteries. This control patient received a calcification score of 1 point for the thoracic aorta and 1 point for the left anterior descending artery (the left circumflex artery and the right coronary artery were without calcification). This resulted in an average calcification score of 0.125 for the control group and an average calcification score of zero for the Marfan group in both the thoracic aorta and the coronary arteries. These differences were analyzed with the student’s t-test and found to be not significant, with a p-value of 0.5 in both vessel distributions.

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>BMI</th>
<th>HTN</th>
<th>Chol.</th>
<th>DM</th>
<th>Smoker</th>
<th>MI</th>
<th>CVA</th>
<th>Coronaries</th>
<th>Aorta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marfan</td>
<td>26.6</td>
<td>21.9</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>30.5</td>
<td>25.6</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0.125</td>
<td>0.125</td>
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Table 7. Compilation of results for the confounding factors and calcification scores for each group with results of analysis for statistical analysis. (Abbreviations: Age=average age at time of CT scan, BMI=average body mass index in kg/m², HTN=number of patients with history of hypertension, Chol.=number of patients with history of hypercholesterolemia, DM=number of patients with history of diabetes mellitus, Smoker=number of patients with history of cigarette smoking, MI=number of patients with history of prior myocardial infarction, CVA=number of patients with history of prior stroke, Coronaries=average calcification score in the coronary arteries, Aorta=average calcification score in the aorta, p-value=Fisher probability for HTN, DM, Smoker, and CVA, and student’s t-test derived p-value for age, BMI, Coronaries, and Aorta)
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<th>HTN</th>
<th>Chol.</th>
<th>DM</th>
<th>Smoker</th>
<th>MI</th>
<th>CVA</th>
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<th>Chol.</th>
<th>DM</th>
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**Table 8.** Results of study for individual patients in each group. (Abbreviations: Age=age at time of CT scan, BMI=body mass index in kg/m², HTN=history of hypertension, Chol.=history of hypercholesterolemia, DM=history of diabetes mellitus, Smoker=history of cigarette smoking, MI=history of prior myocardial infarction, CVA=history of prior stroke, Coronaries=calcification score in the coronary arteries, Aorta=calcification score in the aorta)
Discussion

Conclusions

The results of this study indicate that the Marfan and control groups were not significantly different in their exposure to the major known risk factors for arteriosclerosis. The difference in the average levels of CT-detected calcification in the thoracic aorta and coronary arteries of the patients in these two groups also did not reach statistical significance. Thus, despite the overwhelming observational trends which led to the inception of this pilot study, the data from this study do not support the hypothesis that there is a lower level of arteriosclerosis in patients with Marfan syndrome than in the general population.

However, this lack of conclusive support for the hypothesis does not indicate that the hypothesis is false. With only eight patients eligible for each group, this pilot study has a limited data set which lacks the power to allow for substantial and conclusive determinations of scientific significance. The small data set creates difficulty in making valid conclusions for several reasons.

One reason is that the control group cannot be completely matched with the study group in regards to the confounding factors. Although the statistical analysis revealed an insignificant difference between the groups for each of the confounding factors, the lack of power of the analysis could preclude the identification of important differences which
may affect the occurrence of calcification in the patients. For example, there are potential trends in the data which indicate that the patients with Marfan syndrome are younger, thinner, and less likely to have diabetes mellitus than available controls. Though the numbers are not significant, the trend is convincing relative to the number of patients in the study, and could be a potential source for bias in the observations which served as the impetus for this study. These trends could also potentially become significant with larger numbers of patients. Also, though the number of patients who qualify as having a history of hypertension is larger in the Marfan group, in all cases these patients were on beta-blocker therapy for prevention of aortic dissection. In fact, this risk factor, as defined by this study and others, may actually be atheroprotective due to the potentially lower blood pressures of the patients on beta-blocker prophylaxis without classically defined hypertension.

In addition, only one patient in the study actually had detectable aortic or coronary calcification on the CT scan. This patient was the oldest patient included in the study and, as an individual, had several of the risk factors for arteriosclerosis (see Patient 5 under Control Group in Table 8). This fact raises many questions about arterial calcification. The data presented above from other studies regarding the prevalence of arterial calcification suggest that more of the patients in this study should have had calcification present on CT scanning.

One plausible explanation for the lack of results in this study is the young age of the included patients. With average ages of 26.6 years and 30.5 years in the Marfan and
control groups, respectively, the population studied may be too young to detect a substantial amount of calcification. This limitation may be overcome by including older patients. The difficulty with inclusion of older patients lies in the fact that most Marfan patients receive operative management of their aortic aneurysms prior to reaching middle age. In this case, it may be possible to assess post-operative CT scans of Marfan patients at an older age, with age-matched control subjects, by evaluating calcification in areas of the aorta and coronary arteries not affected by post-operative changes and artifacts. The use of post-operative scans would also drastically increase the numbers of patients studied, since many of the 65 Marfan patients were ineligible for this study due to the lack of availability of a pre-operative CT scan. This possibility will be discussed in more detail below in the section on future directions.

Another plausible explanation for the lack of calcification seen in this study is that a trend is present which is not statistically significant without a larger data set. The potential does exist that the hypothesis of this study is accurate, and will be proven to be accurate, with larger, more in-depth studies in the future. The consequences of this potential finding are remarkable. Arteriosclerosis continues to be a devastating disease with global epidemiological impact. Any further understanding about arteriosclerosis that could be gained through knowledge of the interaction of the Marfan vasculature with the atherosclerotic process would provide great insight into the pathogenesis and molecular biology of this important disease. This insight could have direct benefits by providing specific molecular targets for therapy and prevention. In addition, as patients with Marfan syndrome now survive longer, with nearly normal lifespans,
of the impact of arteriosclerosis in these patients is becoming paramount. Also, the possibility that the Marfan syndrome may be atheroprotective has significant consequences, both in the understanding of arteriosclerosis and in the potential psychological benefit this finding could provide to patients with the Marfan syndrome.

Future Directions

The limitations of this study provide insight into the potential for future studies, which may further this research and allow for more meaningful conclusions about the nature of the relationship between Marfan syndrome and arteriosclerosis. Extensions of this study have already begun here at Yale. Marfan patients with post-operative films are also being included, in order to increase the sample size and evaluate older patients in whom arteriosclerosis may be more detectable. It is probable that these scans will be useful for the evaluation of calcification and arteriosclerosis despite potential artifact and flow disturbances. In addition, with the assistance of advertisement through the publications and website of the National Marfan Foundation, a national pool of Marfan patients is being recruited for the Arteriosclerosis in Marfan Syndrome (AIMS) study, which will possess a much larger set of patients from a variety of locations throughout the United States. It is the hope of the authors that, with the results of these larger studies, relevant information will be discovered which will provide insight into the arteriosclerotic process in Marfan patients, provide an important advance in the knowledge of Marfan syndrome, and help to further the journey towards complete treatment and prevention of arteriosclerosis.
References


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