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CONTENTS

Original Articles

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Using C-Reactive Protein as a Marker of Bacterially Aggravated Atherosclerosis in Acute Coronary Syndromes

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Background— Inflammatory and infectious markers such as C-reactive protein (CRP) have been proposed as indicators of the development or progression of coronary artery disease resulting from bacterial invasion of atherosclerotic plaques. The incidence of such bacterial involvement and its treatment response has not been clearly elucidated in the clinical setting. This study prospectively looked at individuals with newly diagnosed coronary artery disease (CAD), the incidence of elevated CRP levels, the association with acute phase antibodies (APA), detectability of the thymus gland, and macrolide antibiotic treatment response in individuals with bacterially aggravated atherosclerosis (BAA).

Methods—In part 1 of this study, 132 patients (44 women and 88 men), were studied via myocardial perfusion imaging (MPI) using high-dose dipyridamole (HDD) and Sestamibi injection following standardized single photon emission computed tomography (SPECT) and anterior (thymus) planar imaging. All 132 had blood drawn to measure CRP concentrations. When CRP levels were elevated, APAs for *H. pylori, C. pneumoniae,* and *S. pneumoniae* were measured. Results of CRP, APAs, and MPI and thymus imaging (TI) were compared. Thymus imaging was repeated following MPI. In part 2, individuals with elevated CRP and APA were treated for 2 weeks with macrolide antibiotic therapy and then restudied 2 weeks following completion of successful antibiotic therapy.

Results— In part 1, of this study, 34 of the 132 patients with newly diagnosed CAD had elevated CRP levels. Of these, 32 (94%) of the 34 had APA for *H. pylori* and/or *C. pneumoniae*. No patients had APA for *S. pneumoniae*. The remaining 2 individuals with elevated CRP levels had ulcerative colitis. Thymus gland activity was present during the anterior planar imaging period in 100% (34 of 34) of those with elevated CRP levels. Efforts to measure thymus activity 40 minutes later, following MPI, were unsuccessful. In part 2, 32 of the original 132 patients (24%) with elevated CRP and APA levels were restudied 2 weeks after completion of their 2 weeks of macrolide antibiotic therapy. In 100% of them (11 women and 21 men), the CRP normalized, and APAs were no longer elevated. Improvement in CAD was also seen on MPI.

Conclusion—Bacterially aggravated atherosclerosis (BAA) was present in 24% (32 of 132) of patients with newly diagnosed CAD. In each, APAs for *H. pylori* and/or *C. pneumoniae* were detected in addition to thymus activity on planar imaging acquired immediately before MPI. In each instance, treatment with 2 weeks of macrolide antibiotic therapy resulted in normalization of CRP levels, absence of elevated APAs, and failure to detect thymus activity on follow-up imaging. Concomitant MPI demonstrated improvement in coronary blood flow following macrolide therapy, although to varying degrees for each individual, consistent with varying significance. Not all patients with elevated CRP levels had BAA, and care must be taken to distinguish which ones do, to avoid the indiscriminate use of antibiotics. When patients have BAA, macrolide treatment provides an additional treatment for CAD.

Key Words: •C-RP •Acute Coronary Syndromes • Bacterially aggravated atherosclerosis(BAA)

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Introduction

C-reactive protein (CRP) has been proposed as a marker for heart disease,[1] suggesting a possible bacterial infection of the coronary arteries in the presence of cholesterol-laden plaques. Prior research[2-4] has demonstrated that CRP plays a part in antigen-mediated T-lymphocytes, which have a proposed role, along with interleukins,[1,5] in the pathogenesis of vascular disease. Both interleukins[1] and cellular adhesion molecules (CAMs) have been associated[6,7] with thymus activity. The thymus gland, typically not visualized after adolescence, has been identified[8-11] by use of nuclear and magnetic resonance imaging.

Based on the premise that the thymus gland should not be detectable in the postpubescence period, unless activated by an inflammatory or infectious response, the detectability of the thymus gland by nuclear imaging could suggest the possibility of a bacterially aggravated atherosclerosis (BAA) in individuals with coronary artery disease (CAD). To determine the relationship between elevated CRP levels, thymus detection by nuclear imaging, and the possible role that bacterial infection may play in coronary artery disease, 132 individuals with newly diagnosed CAD were studied. In part 1 of the study, each person was examined for possible evidence of BAA including elevated CRP, acute-phase antibody (APA) levels, and the presence or absence of a detectable thymus, along with CAD on myocardial perfusion imaging (MPI) of the heart. During part 2, 32 subjects identified as having BAA were treated with macrolide antibiotic therapy for 2 weeks and then restudied 2 weeks later. The results of postmacrolide treatment were then compared with pretreatment findings.

Methods

Subject Enrollment

One hundred thirty-two patients (44 women and 88 men between the ages of 29 and 81) were enrolled from February 1999 to February 2001 after signing institutional consent forms. Patients were enrolled within 2 weeks of the diagnosis of CAD and before starting any antianginal therapy. Patients were excluded from the study if they had undergone interventional therapy. They were also excluded if they were pregnant, breast feeding, had "severe" aortic stenosis, were allergic to macrolide antibiotics, or had been diagnosed with or suspected of having cancer.

Subject enrollment in the part 2 was dependent on finding at least 1 of the following (1) elevated CRP; (2) elevated APA for *H. pylori*, *C. pneumoniae*, or *S. pneumoniae*; or (3) thymus detection on planar imaging, during the initial phase of the study. Those enrolled in the part 2 of the study underwent repeat analysis 2 weeks after the completion of macrolide antibiotic therapy. Repeat analysis included measurement of CRP, APAs, and nuclear imaging including MPI and thymus (TI) imaging.

Venous Blood Work

Measurement of CRP and of APA for *H. pylori* (IgM), *C. pneumoniae* (IgA, IgM), and *S. pneumoniae* (IgM) were performed at an independent certified CLIA laboratory. Results were reported after nuclear imaging was acquired and reported.

Myocardial Perfusion Imaging

Myocardial perfusion imaging (MPI) with high-dose dipyridamole[12-15] and sestamibi was used to determine coronary blood flow in each individual in the fasting state. An intravenous catheter was introduced into the antebrachium of either the right or left upper limb. Through this catheter, the venous blood work (see above) was obtained and sent for laboratory evaluation. With the protocol described in Figure 1, a resting study (MPI Rest Acquisition) to look for evidence of prior myocardial injury was completed. Approximately 4 hours and 20 minutes later the coronary blood flow component of the study was started using high-dose (HDD) dipyridamole (Pharmacologic Stress) followed by a second infusion of sestamibi. Thirty-one minutes after the HDD was started, "Thymus Imaging" began. Sixty-five minutes after HDD was started, coronary blood flow (Stress Image) imaging was initiated. Immediately following the "Stress Image" a second attempt was made to visualize the thymus.

High-Dose Dipyridamole (HDD) Myocardial Perfusion & Thymus Imaging

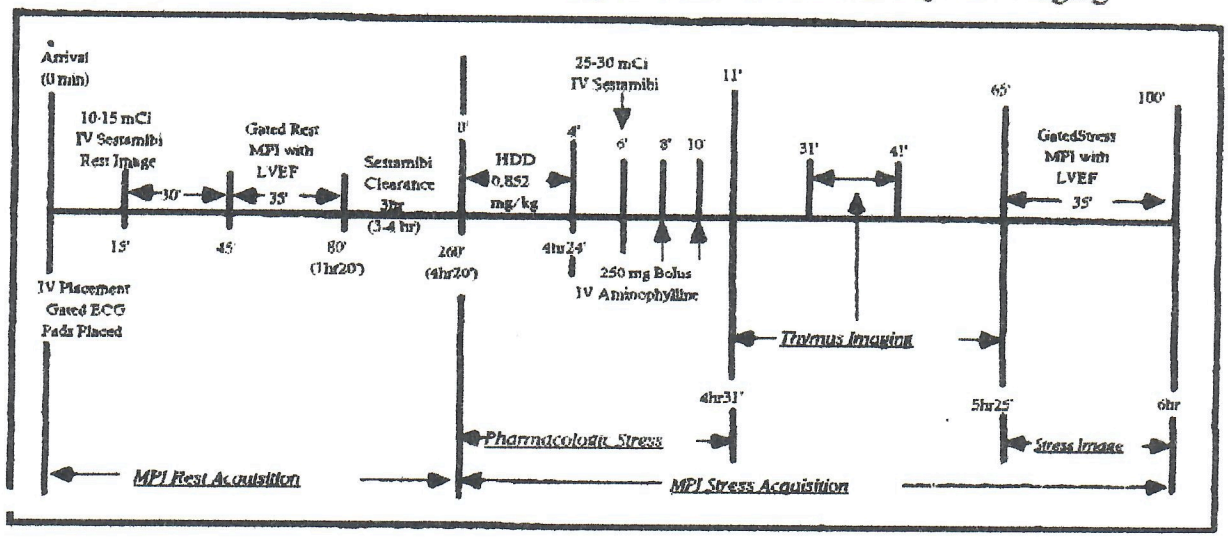

Figure 1. Protocol for imaging of the heart and thymus. Myocardial perfusion imaging begins with a resting study that looks for evidence of myocardial injury. Four hours and 20 minutes later, MPI stress acquisition begins and is divided into 3 segments. The first segment is "Pharmacologic Stress" where high-dose dipyridamole (HDD) is infused through the intravenous catheter. Thirty-one minutes later (20 minutes after the "stress" period), "Thymus (TI) Imaging" is started and lasts for 10 minutes. The "Stress Image" component of the study measures coronary blood flow.

Thymus Imaging

As shown in Figure 1, "Thymus Imaging" (TI) was completed during the "MPI Stress Acquisition" component of MPI. A second attempt to image the thymus gland was made at the completion of "Stress Image(ing)."

MPI and Thymus Image Interpretation

Following acquisition and reconstruction, the images were first read from the computer monitor and then printed on hard copy color film to determine the presence or absence of the thymus and coronary artery disease. Quantification and assessment of both the thymus and coronary blood flow were determined before information regarding blood work results. The methods of quantification and reading of both thymus and cardiac images are described in detail[7-18] elsewhere.

Macrolide Antibiotic Treatment

Individuals with elevated CRP levels and elevated acute-phase antibodies for 1 or more of the 3 bacterial species were started on clarithromycin, 500 mg bid for 14 days. One week following completion of antibiotic therapy, venous blood samples were redrawn and sent for CRP and APAs analysis. Treatment was considered successful if the subsequent CRP and APA levels were "normal."

Statistical Analysis

The results of MPI and TI were reported for each of the 132 patients and compared with the CRP and APA levels. Using the accepted standards of the clinical laboratory where the CRP and APA were determined, each individual's results were designated as either positive or negative and compared with their MPI and TI findings. This same approach was used to compare subjects in the second part of the study.

Results

Of the 132 newly diagnosed patients with coronary artery disease, 24% (32 of 132) had evidence of BAA based on the presence of (1) an elevated CRP, (2) an elevated APA to at least 1 bacterial species, and (3) improvement in coronary blood flow following completion of antibiotic therapy for the infection. Of these 32 cases, 30 (94%) were positive for *H. pylori*, 8 (25%) were positive for *C. pneumoniae*, and 6 (19%) were positive for both *H. pylori* and *C. pneumoniae*. None of these 32 individuals had evidence of *S. pneumoniae* infection associated with CAD. There were no statistical differences between type of infection and patient gender.

In every case where the CRP was elevated, the thymus was seen on nuclear imaging regardless of whether APAs were present or not. This included 2 patients with ulcerative colitis. Nuclear imaging demonstrated the thymus gland only during the initial "Thymus Imaging" component of the study as described in Figure 1. The thymus gland was not detected following the "Stress Image" acquisition.

All 32 individuals with elevated CRP and APAs tolerated the 2-week course of antibiotics without major complications. Two of the individuals reported gastrointestinal irritation but none reported diarrhea. No elevations in liver function test results or renal function were seen. All 32 (100%) demonstrated improvement with normalization of CRP levels and resolution of APAs as measured 1 week after completion of antibiotic therapy. On repeat MPI and TI evaluation, none of the 32 individuals had detectable thymus glands and MPI demonstrated improvement in coronary blood flow. These improvements were variable from patient to patient. None of the patients demonstrated normal coronary blood flow following completion of antibiotic therapy alone.

Figure 2 shows the results of 1 patient with elevated CRP and APA to *C. pneumoniae* before and after macrolide treatment. The thymus is marked in the image acquired before antibiotic treatment and is absent in the posttreatment planar image of the chest. The lower panel

of images demonstrates decreased coronary blood flow in the regions (green) marked with arrows. Improvement in coronary blood flow is seen in the next image following a 2-week course of antibiotics as described in Figure 2.

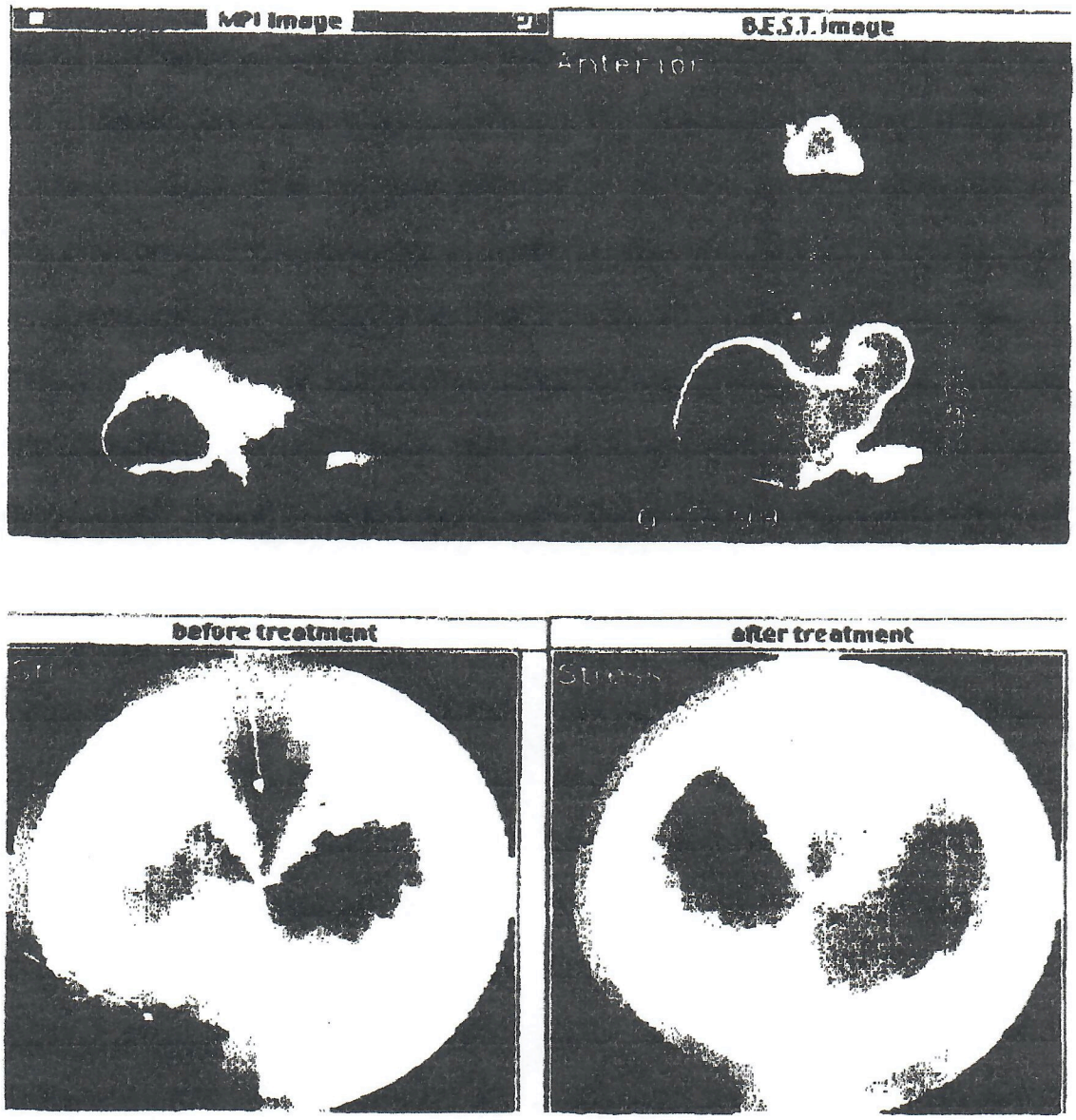

Figure 2. Nuclear images of the thymus and heart.The top 2 images represent the "Thymus Image" before (left) and after (right) macrolide antibiotic treatment of bacterially aggravated atherosclerosis (BAA). The arrow in the before image shows thymus activity, which is absent in the after treatment image. The bottom row of images show the coronary blood flow for the same patient seen in the top row. The image to the left shows coronary artery disease with reduced coronary blood flow (green areas) marked with arrows before antibiotic treatment. The image on the right shows improvement in coronary artery disease (reduction in green) following treatment.

Discussion

The pathogenesis of vascular disease[1] is the result of many independent variables playing differing roles in different patients. Conflicting reports about the importance of bacterial infections have resulted in disagreement by many individuals about the role bacteria play in coronary and carotid artery disease. Additional reports about CRP have likewise been confusing, with patients being treated either as having an infection requiring antibiotic treatment or as having an inflammatory process requiring appropriate anti-inflammatory therapy.

In this prospective study of 132 individuals with newly diagnosed CAD, approximately 26% had elevated CRP levels. However, 2 of these patients had other causes for elevated CRP levels and would not have been appropriate candidates for antibiotic treatment. The remaining 32 patients, representing 24% of the patients with newly diagnosed CAD, did have evidence of an infectious process that we have termed "bacterially aggravated atherosclerosis" (BAA). The diagnosis of BAA required (1) the detection of an elevated CRP, (2) the detection of elevated APAs to 1 of the bacterial species tested for, (3) the presence of thymus detection on nuclear imaging, (4) the presence of coronary blood flow abnormalities on MPI, and (5) improvement in coronary blood flow, CRP and APA and resolution of thymus detection following treatment with macrolide antibiotics. In the absence of a nuclear laboratory specializing in diagnostic blood flow and thymus imaging, the diagnosis of BAA may hinge on the detection of CAD, elevated CRP, and elevated APAs, which, following antibiotic treatment, improve.

While this study examined individuals with newly diagnosed coronary artery disease, the incidence and response to treatment of BAA in individuals with CAD of longer duration is not known. Furthermore, the 3 bacterial species we investigated in this study are probably not the only potential causes of infection for diseased arteries with plaque deposition, regardless of whether we are talking about the coronary, carotid, or other arteries. It has long been recognized that abdominal aortic aneurysms may be associated with *Salmonella* infections and there is no reason to believe that other bacteria or viruses could not be associated with a worsening of CAD. Finally, treatment of BAA improved coronary blood flow but did not result in the restoration of normal blood flow. Clearly, BAA is not the causative factor in CAD but an opportunistic infection that appears to respond to appropriate antibiotic treatment. Whereas bacterial infections were the leading cause of death at the beginning of the 20th century, a different kind of bacterial infection is present at the dawn of the 21st century.

Conclusion

In patients with newly diagnosed CAD, appropriate testing to look for evidence of BAA may result in treatment that could potentially improve CAD and aid in the stabilization of atherosclerotic plaque. Such infections may represent up to one quarter of newly diagnosed patients and can be easily determined by checking an elevated CRP level. If the CRP level is elevated, APAs should be checked to look for evidence of BAA. If APAs are present, a 2-week course of macrolide antibiotics (or appropriate antibiotic if the patient is allergic to macrolide antibiotics) could improve coronary blood flow and augment treatment of CAD. If APAs are normal, in the presence of elevated CRP levels, other causes should be looked for. If other causes are not identified outside of the coronary arteries, a trial of anti-inflammatory medication may reduce an inflammatory response in the coronary arteries.

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