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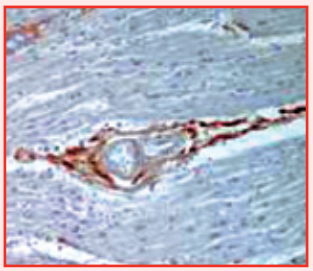

Anti-citrulline staining in rheumatoid arthritis myocardium

Searching for a Predictive Multimarker for Cardiovascular Disease in Rheumatoid Arthritis

While it is generally understood that rheumatoid arthritis (RA) is a severely disabling joint disease, what has not been recognized until the last decade is that RA, like many chronic inflammatory conditions, can accelerate heart disease.

"Heart disease and stroke are the biggest killers of RA patients," says Joan M. Bathon, MD, Chief, Division of Rheumatology, NewYork-Presbyterian/Columbia University Medical Center. "That's also true in the general population, but it's even more exaggerated in RA. The thinking is that maybe it is the chronic, unrelenting inflammation in RA that drives accelerated heart disease."

Dr. Joan M. Bathon

According to Dr. Bathon, the impact of inflammation in heart disease was originally identified in RA and systemic lupus erythematous, where the risk of heart disease is even greater. "It is a bit harder to study in lupus because these patients are treated with steroids and have other extraneous issues, such as kidney failure," she says.

Dr. Bathon and her NewYork-Presbyterian/Columbia colleagues are focusing on RA because it does not have all of the "confounding factors" associated with other inflammatory diseases. "But, in the meantime, our research has also raised the notion that maybe any inflammatory disease has a higher risk of heart disease," she says. "Atherosclerosis itself is an inflammatory condition that, in a very mild way, probably builds over decades. If you put those blood vessels in the context of a raging, systemic inflammatory process like RA, the atherosclerosis can accelerate.

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Implicating Citrullination in Heart and Lung Disease Associated with Rheumatoid Arthritis

With a decade of research on disease-related comorbidities in rheumatoid arthritis as a foundation, Jon T. Giles, MD, a rheumatologist with NewYork-Presbyterian/Columbia University Medical Center, has more recently focused his attention on understanding the role of citrullinated proteins in the development of heart and lung diseases in patients with RA.

"Citrullination is a modification of a protein that occurs over time," says Dr. Giles. "It's a change of one amino acid, called arginine, to another amino acid, called citrulline." Having already determined the potential cardiovascular consequences of citrulline on patients with RA, Dr. Giles and Joan M. Bathon, MD, Chief of Rheumatology at NewYork-Presbyterian/Columbia, began studies looking at its effects on the lung.

"We don't know a lot about what causes citrullination, but it occurs throughout the body," notes Dr. Giles. "In rheumatoid arthritis and other autoimmune diseases, autoantibodies often attack citrullinated proteins. And the presence of anti-citrullinated protein antibody, which is a standard test for rheumatoid arthritis, is associated with more severe disease."

Building on Studies in the Heart

Prior to their studies of lung disease, Drs. Giles, Bathon, and their colleagues looked at heart failure in patients with RA. "It appeared that heart failure in these patients might be different from others who developed the condition," explains Dr. Giles. "RA patients tended to have better squeeze function of their heart and preserved systolic function; however,

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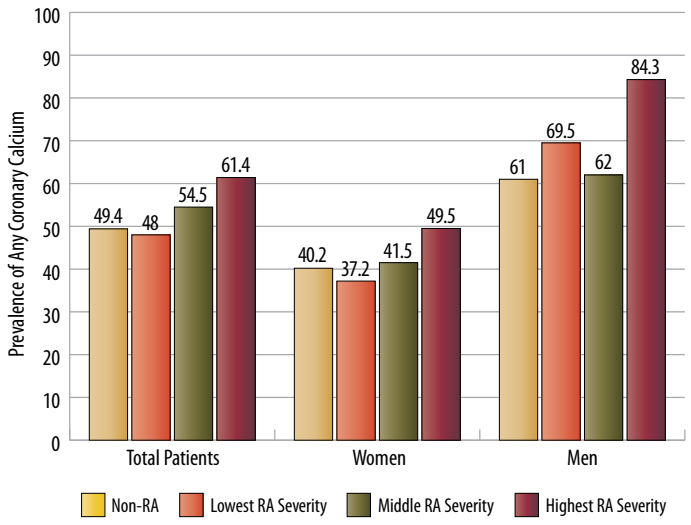
"The same has been found for other chronic, inflammatory diseases, including Crohn's disease, psoriasis, psoriatic arthritis, and ankylosing spondylitis," notes Dr. Bathon. "It's interesting how much they are similar and how much they are different, which raises some questions: Are there shared mechanisms amongst the diseases, and are their specificities from one disease to the other? They certainly share this increased risk."

Adjusted Associations of Rheumatoid Arthritis Severity with Prevalence of Coronary Artery Calcification



Dr. Bathon and her team are pursuing research in the development of a predictive multimarker algorithm for cardiovascular disease in RA. Current cardiovascular risk assessment tools do not predict cardiovascular disease well in RA patients. "The goals of our research are to identify a group of proteins in the blood that are able to distinguish RA patients who have advanced atherosclerosis from those who do not," she says. "This will potentially allow earlier detection of atherosclerosis in this population with higher overall risk of cardiovascular disease."

The Framingham Risk Score is an effective tool in calculating blood pressure, family history, cholesterol, and various factors to provide the 10-year cardiovascular risk of an individual. "You can separate low risk from high risk from moderate risk patients and treat them with a statin or not," says Dr. Bathon. "But with rheumatic diseases, the Framingham index isn't very helpful because these patients often don't have any of those risk factors. Yet we know from studies where we're looking at subclinical atherosclerosis that a lot of these patients have advanced atherosclerosis. Framingham is a great predictor – at least in the general population. If we could add something to that for patients with inflammatory disease, in terms of a biomarker, that would help us to identify folks with advanced atherosclerosis who are likely to have an acute cardiac event."

Over the last three years through a grant from the American College of Rheumatology, as well as funding from the National Institutes of Health, Dr. Bathon and her team have assembled 600 blood and DNA tissue samples of people from around the country who had a measure of coronary artery calcium, a sensitive measure of coronary heart disease.

"The goals of our research are to identify a group of proteins in the blood that are able to distinguish RA patients who have advanced atherosclerosis from those who do not."
— Joan M. Bathon, MD

"We've been looking at about 50 different proteins; some are by routine lab assays and some are by very sophisticated mass spectrometry," says Dr. Bathon. "If we didn't have a commercial antibody available, we then investigate their levels by mass spectrometry. We're now just completing the measurements of all 50 proteins and starting to look at the data. There are some initial very interesting hits. Several of the proteins are actually proteins that we associate more with the joint and not so much with the heart."

The identification of a biomarker is important "to mark people who might have bad joint disease and who are also at risk for significant heart disease," Dr. Bathon says. "We could, theoretically, construct a modified risk score, where we might have the Framingham as the basis and then add not just one biomarker, but hopefully a panel of biomarkers that could indicate risk. We could then modify treatment in higher risk individuals by being more aggressive with statins, blood pressure control, and better management of the RA, which probably drives it."

The next step, says Dr. Bathon, will be to study more patients who have had events, such as myocardial infarction and strokes, versus people who have not. "Hopefully these are people who have been in registries and have had blood drawn at the start of their care," she says. "We can then look at these biomarkers at the beginning and at different points over the years before their heart attacks occurred to see if they would have predicted their MIs or strokes. We are assembling these cohorts by talking to research collaborators across Europe, as well as in the U.S., hoping to study up to 10,000 patients to identify MIs and non-MIs within these numbers."

Understanding predisposition to heart disease is not only a huge area of interest to researchers, but also to patients. "They ask, 'Am I going to have a heart attack or not?'" says Dr. Bathon. "When we offer our heart studies to patients they enroll avidly because they want to know."

Reference Article
Giles JT, Szklo M, Post W, Petri M, Blumenthal RS, Lam G, Gelber AC, Detrano R, Scott WW Jr, Kronmal RA, Bathon JM. Coronary arterial calcification in rheumatoid arthritis: comparison with the Multi-Ethnic Study of Atherosclerosis. *Arthritis Research & Therapy.* 2009;11(2):R36.

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their diastolic function was reduced. This suggested that something about the myocardium has made it stiffer. We also found that the heart was smaller in RA patients, which differs from the general natural history of heart failure in which the heart becomes very enlarged and the heart muscle becomes very thick."

The researchers investigated the presence and localization of myocardial citrullination in samples from RA patients compared to rheumatic and non-rheumatic disease control groups. The results revealed an association of a higher concentration of serum anti-cyclic citrullinated peptide (anti-CCP) antibodies with lower myocardial mass and smaller left ventricular chamber volumes in RA patients without known cardiovascular disease. Staining for citrullination was higher in the myocardial interstitium of RA compared to other disease states, potentially linking autoimmunity to the known increase in myocardial dysfunction and heart failure in RA.

"We found that there was actually citrullination in a particular area of the heart called the interstitium – the supporting structure of the heart – and not the heart muscle cells themselves," says Dr. Giles. "Everybody has a certain amount of citrullination, but in RA it was quantitatively higher – about 50 percent more in the RA hearts compared to the non-RA hearts. There was also a stronger association of scarring in the heart with the citrullination. This raised the possibility that RA-specific autoimmunity against citrullinated proteins might trigger changes to the myocardial structure that, in turn, may affect myocardial function."

Working on non-RA hearts, Drs. Giles and Bathon also looked for the enzymes that catalyzed the change to citrulline. They found that they were present in the heart but not in the interstitium. In fact, they were geographically distinct from each other in the heart. "That was our first look at this," says Dr. Giles. "We then asked if there are citrullinated proteins in the heart, which proteins are they? Our collaborators at Johns Hopkins, using mass spectrometry, were able to identify these protein changes raising the question of whether these patients carry an antibody against the citrullinated protein, producing a pathogenic antibody response. If you can identify a direct pathogenic relationship between an antibody and its target you can potentially block that as a therapeutic option."

> **"To our knowledge, this is the first report of ACPA specificity with radiographic indicators of pulmonary restriction and/or impaired diffusion commonly associated with RA-associated interstitial lung disease."**
>
> **— Jon T. Giles, MD**

Pursuing Parallel Work in the Lung

Pulmonary complications and, in particular, interstitial lung disease, also represent a leading contributor to mortality in patients with rheumatoid arthritis. Also known as diffuse parenchymal lung disease, interstitial lung disease is an infiltration of lung tissue with inflammatory cells that can lead to varying amounts of scarring in the lung. "Citrullinated proteins have been observed in RA lung tissues," says Dr. Giles, "but the association of specific anti-citrullinated peptide antibodies with interstitial lung disease is unknown."

Dr. Jon T. Giles

About 10 to 15 percent of RA patients will develop lung inflammation and interstitial lung disease "This can make it difficult to breathe and can also lead to early mortality," says Dr. Giles. "It's not an insignificant consequence of RA to have interstitial lung disease. Interestingly, we have actually seen some people who've had interstitial lung disease that eventually turns out to be RA interstitial lung disease because they developed RA later. So its development doesn't necessarily have to be preceded by joint disease."

In their recent research, Dr. Giles and his colleagues sought to explain the association of anti-citrullinated peptide antibodies (ACPA) with interstitial lung disease in patients with RA. To accomplish this, the researchers reviewed multidetector CT scans of the chest of 177 patients and, on the same patients, tested a very broad array of citrullinated antibodies. "We found that the more of these antibodies that were present, the more likely patients were to have features of interstitial lung disease," says Dr. Giles. "To our knowledge, this is the first report of ACPA specificity with radiographic indicators of pulmonary restriction and/or impaired diffusion

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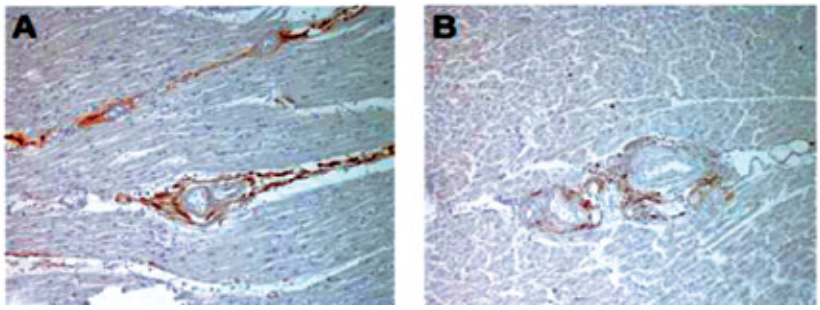
Anti-citrulline Staining in Rheumatoid Arthritis Myocardium (A) and Control Myocardium (B)



Citrullination was restricted to the myocardial interstitium in both groups, with staining intensity qualitatively higher in the RA group.

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commonly associated with RA-associated interstitial lung disease. These findings of a broader ACPA repertoire suggest a possible role for ACPA in the pathogenesis of interstitial lung disease in patients with RA. Whether similar processes targeting citrullinated, rather than native, lung proteins mediate this, warrants additional study."

According to Dr. Giles, citrullination is also present in the lungs of patients with other types of lung disease, including those of heavy smokers. "This could represent a consequence of either smoking or an environmental exposure that increases this modification, which, in turn, incites an antibody response in these patients that by itself may cause this particular type of lung disease." But, he says, there is definitely a tie-in with smoking and interstitial lung disease in RA patients.

"We are trying to work in parallel to identify citrullinated proteins in both the heart and the lung," says Dr. Giles. "We want to be able to identify whether or not the antibodies are just markers of the disease or if they have some pathogenic effect. Also, we want to see what role this protein modification may have on the way that inflammatory cells work in the lung. Do they activate inflammatory cells? Do they cause more scarring in the tissue? Do they cause more inflammatory cells to be recruited to the area and increase the level of inflammation? These are among the many questions that are driving our research in this area, with the goal of reducing the incidence of heart and lung comorbidities."

Reference Articles

Giles JT, Fert-Bober J, Park JK, Bingham CO 3rd, Andrade F, Fox-Talbot K, Pappas D, Rosen A, van Eyk J, Bathon JM, Halushka MK. Myocardial citrullination in rheumatoid arthritis: a correlative histopathologic study. *Arthritis Research & Therapy.* 2012 Feb 24;14(1):R39.

Giles JT, Danoff SK, Sokolove J, Wagner CA, Winchester R, Pappas DA, Siegelman S, Connors G, Robinson WH, Bathon JM. Association of fine specificity and repertoire expansion of anticitrullinated peptide antibodies with rheumatoid arthritis associated interstitial lung disease. *Annals of the Rheumatic Diseases.* 2013 May 28. [Epub ahead of print]

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