Assessment for Early Cardiovascular Risk in Pediatric Rheumatic Disease

by

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Institute of Medical Sciences
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Abstract

Objectives: 1) Evaluate the risk of atherosclerosis in rheumatic disease compared to healthy controls; 2) Assess the lipid profile of children with systemic lupus erythematosus (SLE) at presentation before treatment with corticosteroids; 3) Compare the lipid profiles of children with juvenile dermatomyositis (JDM), systemic juvenile idiopathic arthritis (SJIA), and SLE; 4) Evaluate the extent of early atherosclerosis in children with JDM, SJIA, and SLE; 5) Investigate the progression of early markers of atherosclerosis in children with SLE.

Methods. The methods include a systematic review, a cross sectional study of serum lipid levels of a cohort of children with SLE, an analysis of the first time point of a prospective study of cardiovascular disease risk factors and vascular function measures of a cohort of children with JDM, and SJIA, and SLE and a longitudinal study of vascular function measures of a prospective study of a cohort of children with SLE.

Results. Our systematic review demonstrated that carotid intima media thickness (CIMT), a surrogate marker of early atherosclerosis, was significantly increased in rheumatic disease populations. We found that newly diagnosed children with SLE before corticosteroid treatment exhibited a pattern of dyslipoproteinemia of increased triglycerides and depressed HDL-
cholesterol. When we measured the lipid profiles in children with the rheumatic diseases of JDM, SJIA, and SLE, one third of children had at least one abnormal lipid value. The most common abnormalities were found for total cholesterol and triglyceride levels and most often in children with JDM. One quarter of all patients were found to have insulin resistance. Lastly, when we considered the effects of treatment in children with SLE, we found that improvement in CIMT was possible and it correlated with a higher cumulative dose of prednisone over the study period.

Conclusions. Early markers of atherosclerosis in pediatric rheumatic disease are important for determining the risk of these children in developing heart disease as young adults. Chronic inflammation plays a significant role and should be considered an important predictor of premature atherosclerosis.
Acknowledgments

I would like to dedicate this work to my father, Myles Alan Tyrrell. I will not have the pleasure of his attendance at my convocation for he lost his fight to lung cancer years before I started this PhD. I remember my father as Mr. fix-it who was an artist and an educator in his spare time. From replacing the footings to our garage to cooking a mean stir-fry, my father taught by example and anything was possible. I owe him my strength and my determination. I would like to thank my mother Hélène, my father’s partner in crime, for her unwavering love and support for all of my endeavors. I would like to thank my three children, Aidan, Teagan, and Sabrina, for their understanding and patience. Finally, none of this would have been possible without the support, trust, and love of my beautiful wife, Jennifer.

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Figure 1. Early atherosclerosis in children with rheumatic disease.
The overall aim of this thesis was to assess the risk for early atherosclerosis in children with rheumatic disease. The specific aims were addressed with studies directed to three different pediatric rheumatic diseases: juvenile dermatomyositis (JDM), systemic juvenile idiopathic arthritis (SJIA), and pediatric systemic lupus erythematosus (pSLE).

Chapter 1 of this dissertation is an introductory chapter presenting an overview of the increased risk of cardiovascular disease in the rheumatic diseases, the importance of traditional and non-traditional cardiovascular risk factors, and the difficulties in studying atherosclerosis. The study framework along with the list of specific objectives addressed by this dissertation is presented at the end of this chapter.

Chapter 2 presents the first project of this dissertation. This study is a systematic review/meta-analysis which demonstrated that atherosclerosis, as determined by the surrogate measure of intima-media thickness by ultrasonography, is increased in patients with multiple different rheumatic diseases.

Chapter 3 is the second project of this dissertation. This study determined the frequency and pattern of dyslipoproteinemia at presentation of pSLE before treatment with corticosteroids. The association between dyslipoproteinemia and markers of disease activity and inflammatory markers at presentation of pSLE was also evaluated.

Chapter 4 is the third project of this dissertation. This study assessed the lipid profile and the role of treatment and disease activity related factors in children with JDM, SJIA and pSLE.

Chapter 5 is the fourth project of this dissertation. This study compared vascular markers of early atherosclerosis and the role of treatment and disease activity-related factors on these markers in children with JDM, SJIA, and pSLE.
Chapter 6 is the fifth project of this dissertation. This study evaluated the progression of vascular markers of premature atherosclerosis in a prospectively followed pSLE cohort and studied the role of disease activity, treatment and inflammation on premature atherosclerosis.

Chapter 7 is the final, concluding chapter. It presents the synthesis of each project along with conclusions and future research directions.
Chapter 1. Introduction

1.1 The rheumatic diseases.

Rheumatic diseases are some of the most prevalent chronic health conditions in Canada and a major cause of morbidity, disability and health care utilization. Sixteen percent of Canadians aged 15 years and older are affected and the prevalence of arthritis/rheumatic diseases increases with age. Two-thirds of those affected with arthritis are women, in whom the prevalence is almost twice that of men (19% versus 11%, respectively). Rheumatic diseases may also present during childhood (e.g. approximately 1 in 1000 Canadian children are affected by juvenile idiopathic arthritis) (1).

There are over 100 different types of rheumatic diseases that can be grouped into two broad categories: degenerative and inflammatory. As we age the cartilage of our joints begins to wear away and accumulate irreversible damage from normal daily use and the bone underneath thickens. This is known as degenerative arthritis or osteoarthritis and occurs mainly in older adults. Inflammatory arthritis involves a large group of disorders affecting the joints, ligaments, tendons, bones and other components of the musculoskeletal system. It differs from osteoarthritis in that, by definition, patients suffer from inflammation (redness, pain, heat, and swelling) of some part of their musculoskeletal system. Many of the more serious disorders such as rheumatoid arthritis and systemic lupus erythematosus are autoimmune in nature and require immunosuppression in order to reduce the chronic inflammation associated with these diseases. Typically, once started, these autoimmune/-inflammatory conditions last for the rest of one’s life but may have a course that fluctuates between exacerbations and remissions, or may possibly lead to permanent remission(2).

This study focuses on three childhood rheumatic diseases: juvenile dermatomyositis (JDM), systemic juvenile idiopathic arthritis (SJIA), and pediatric systemic lupus erythematosus (pSLE). JDM is a rare disease with an incidence of about two to three cases per million children per year. JDM is the most common form of inflammatory myopathy in children with a median age of diagnosis of 7 years. More girls are affected than boys (2:1) and JDM generally presents with skin and muscle manifestations. Autoimmune in nature, JDM has an underlying
systemic inflammation and is often characterized by small vessel vasculitis. Abnormalities in lipid processing and fat storage are a well-recognized feature of JDM and these children are at risk for both insulin resistance and partial lipoatrophy. These children are also at risk for hyperlipidemia in the setting of insulin resistance(3).

SJIA is one of seven types of juvenile idiopathic arthritis (JIA) and accounts for about 10-20% of the 8-150 per 100 000 affected children with JIA. SJIA is the most severe form of JIA with frequently high spiking fevers, rash, hepatosplenomegaly, diffuse lymphadenopathy, and serositis. Interestingly, age, gender, and HLA are not strongly associated with this rheumatic disease. However, these patients have markedly increased markers of inflammation and pro-inflammatory cytokines. SJIA typically has a variable disease course with 60% of patients going into remission or quiescence and up to 37% developing a severe, destructive chronic course (3).

Pediatric SLE is a multi-system autoimmune disease which may present very differently between patients. Fifteen to twenty percent of all SLE begins in childhood at a median age of 12-14 years. The incidence rate of 6-19 per 100 000 children comprises mostly girls (approximately 80%) and is higher in Hispanics, Blacks, Native North Americans, and children from Southeast and South Asia. Skin, musculoskeletal, renal, and the central nervous system are the most common organ systems involved in pSLE. The production of autoantibodies is one of the hallmark characteristics of this disease and often leads to serious complications such as immune deposits in the kidney. Furthermore, the presence of antiphospholipid antibodies has been associated with an increased risk of thrombosis and accelerated atherosclerosis(4).

These three pediatric rheumatic diseases are chronic inflammatory disorders that are share the common features of characterized by systemic inflammation and vasculitis, as well as lipid and metabolic abnormalities which, in the long-term, may increase the risk of cardio- and cerebrovascular disease. Earlier diagnosis and rapid introduction of aggressive immunosuppressive treatment has led to improved disease- specific outcomes(3).

1.2 Cardiovascular disease.
Cardiovascular and cerebrovascular disease (CVD) is one of the leading causes of death in industrialized countries(5) and according to Statistics Canada coronary artery disease and stroke accounted for about 30% of all deaths in Canada(6). With the ever increasing prevalence of obesity, diabetes, and metabolic syndrome, worsening of these mortality figures and their economic burden on society are of serious concern.

1.2.1 Atherosclerosis

Atherosclerosis is the medical term used to describe the build-up of cholesterol-filled fatty streaks and then plaques in the walls of arteries. When arteries that supply blood to the heart and brain are affected, the risk of cardiovascular disease is significantly increased.

Atherosclerosis has been described as a chronic inflammatory disease (7). Primary inflammation is protective in nature with aims to neutralize any injurious foreign body and to initiate the process of tissue repair and healing. Unfortunately, left uncontrolled, inflammatory cell infiltrates can also cause damage. The atherogenic process can start as early as childhood (8-11). Although the risk of developing atherosclerosis increases with age and is worsened by traditional risk factors such as smoking, hypertension, increased body mass index, and the presence of diabetes mellitus, the main lipid determinants of atherosclerotic risk in the general population remain elevated concentrations of serum low density lipoprotein (LDL) and reduced high density lipoprotein (HDL) cholesterol concentrations(12).

1.2.2 Framingham Heart Study

The concept of CVD risk evolved in the 1960s from the evaluation of long-term epidemiological studies in which individual characteristics were related to the subsequent incidence of CVD(13). The Framingham Heart Study is widely acknowledged as one of the most influential longitudinal studies from that time(14). Following the Second World War, infectious disease was soon replaced by cardiovascular disease as a major cause of death in the population. In April 1950, the Framingham Study was formally underway with a focus on arteriosclerotic (e.g. coronary heart disease, angina pectoris and stroke) and hypertensive CVD. A random sample of subjects (from the small town of Framingham, Michigan, USA) in the age group where these forms of CVD were known to develop was selected. Based on a complete examination, those subjects free from definite signs of the disease were then selected for
reexamination at periodic internals and observation. This continued over a period of years until a sizable number were found to have acquired the disease. At that time a search was made to identify the factors which influenced the development of the disease. One of the more important contributions from this study was the development of risk scores to calculate “risk prediction estimates” for the risk of various cardiovascular disease outcomes in different time horizons.

The Framingham study and other similar major epidemiological studies that followed(15-18) established the major “traditional” risk factors that can be grouped into two broad categories: non-modifiable risks such as age, sex, family history of CVD, previous personal history of CVD, and ethnic origin; and the modifiable risks such as plasma lipids, hypertension, smoking, diabetes, obesity, physical inactivity, and alcohol consumption. More recently “novel” risk factors have been described and simply represent risk factors that have come after the description of traditional risk factors. They include thrombogenic factors, homocysteinemia, and inflammatory mediators(19-21).

1.2.3 Non-modifiable risk factors.

1.2.4 Potentially Modifiable risk factors.

The most important modifiable risk factor for CVD is smoking, followed by high blood pressure, alcohol consumption, high cholesterol and obesity(5). Smoking is an interesting risk factor in that it accounts for one fifth of all cardiovascular deaths and one quarter of all deaths due to other co-morbidities such as cancer. It is believed that exposure to tobacco smoke contains large amounts of free radicals, is dose dependent, and results in endothelial dysfunction. It has also been shown to reduce HDL cholesterol levels and increase triglyceride levels(22,23). Both systolic and diastolic blood pressures have been found to be positively associated with atherosclerotic events. The major cause of death attributable to hypertension is stroke(12).

High cholesterol and obesity are modifiable risk factors most prevalent in children (24-27). The positive relationship between CVD and LDL cholesterol levels is strong and continuous, and applies to both sexes. Furthermore, the risk becomes increasingly steep as cholesterol concentrations increase and is considerably modified by the presence of other risk factors(28).
HDL cholesterol on the other hand has a negative relationship with CVD and has been termed “good cholesterol”. The Framingham data has shown that low levels of HDL cholesterol are associated with the risk of CVD. The beneficial actions of HDL cholesterol are mainly due to reverse cholesterol transport and its antioxidant effects on LDL cholesterol. This is important for reducing the risk of formation of fatty streaks within the intima of vessels considered to be an early step in the development of atherosclerosis(19,29).

Although serum triglyceride levels do not play as dominant a role in the development of CVD as cholesterol, meta-analyses have suggested that increased triglyceride levels predict the risk of CVD(30-33). It has been suggested that it is not only the triglyceride-rich lipoproteins on their own that are the problem but the atherogenic changes that often accompany hypertriglyceridaemia such as low HDL cholesterol levels, increased VLDL cholesterol, and increased small, dense LDL cholesterol levels.

Diabetes is one of the most common chronic diseases worldwide, affecting up to 8% of Western populations. The prevalence of both type 1 (autoimmune destruction of pancreatic beta cells) and type 2 (insulin resistance associated with glucose intolerance and failing insulin secretion) are on the rise. Type 2 represents 90% of all diabetes and is associated with obesity and reduced physical activity. Results from the Bogalusa Heart Study suggest that childhood obesity is associated with a greater prevalence of cardiovascular risk factors and that this problem tracks into adulthood(34). The mechanism of increased CVD risk remains unclear as there is often overlapping increasing risks of insulin resistance and type 2 diabetes, hypertension, lipid abnormalities, and reduced physical activity. Importantly, virtually every lipid and lipoprotein is affected by all of these factors with hypertriglyceridaemia a common feature. Much of the risk of these factors can be attributed to the resulting dyslipidaemia(35).

Efforts are being made to assess additional biomarkers for CVD such as lipoprotein (a), C-reactive protein, fibrinogen, and homocysteine. Homocysteine is thought to exert its action by inducing endothelial dysfunction and promoting LDL cholesterol oxidation (36,37). Though plasma concentration of homocysteine has been found to be associated with ethnicity, age, and sex it has also been thought to be strongly influenced by poor diet. Impaired fibrinolysis, as well as disordered lipid metabolism, has been recognized as risk factors for CVD.
Lipoprotein(a) is a serum lipid fraction that contains an apolipoprotein that has a structural homology with plasminogen. By acting as a competitive inhibitor of plasminogen it reduces fibrinolytic activity resulting in an increased risk of thrombosis. The predisposition to increased lipoprotein(a) levels appears to be an inherited trait and, unfortunately, does not respond to lipid lowering therapy(38). Fibrinogen is a large molecule that increases blood viscosity and platelet aggregation and has been found to be an independent risk factor for CVD. Fibrinogen is thought to contribute to atherogenesis by promoting smooth muscle cell migration and proliferation as well as deposition of fibrin in the vessel walls. Interestingly, fibrinogen is an acute phase protein whose synthesis is increased in response to pro-inflammatory stimulus. It is thought that fibrinogen may contribute to the risk of CVD by both increasing the risk of thrombosis and by playing an active role in the inflammatory process, also considered a risk for CVD(39). Cytokines also stimulate the production of acute phase proteins from the liver. C-reactive protein (CRP) is one such protein and represents a general marker of inflammation. Elevated levels of CRP have been found to be associated with the risk of CVD similar to that of elevated fibrinogen(40). It is thought that CRP participates in the atherogenic process by binding to oxidized LDL cholesterol and activating complement resulting in tissue damage and further inflammation. Other acute phase protein such as serum amyloid A protein and other markers of inflammation such as erythrocyte sedimentation rate (ESR), raised white blood cell count, and serum albumin have all been found to have weaker associations with the risk of CVD(41). However, though these novel biomarkers have been shown to be associated with an increased risk of CVD, clear demonstration of prediction beyond traditional risk factor models remains elusive(42). These biomarkers may very well not be independent from traditional CVD risk factors (collinearity). Furthermore, heterogeneity may exist for the predictive value of these biomarkers across different populations.

1.3 Rheumatic disease and CVD risk.

Atherosclerosis is inflammatory in nature. From Virchow’s postulations to more recent studies in the past few decades, the significant role of inflammation in both the initiation and the progression of arterial disease has been convincingly demonstrated(7,43,44). The release of inflammatory mediators is in response to a variety of acute (infection) and chronic stimuli (oxidized LDL cholesterol and smoking). The resulting released cytokines activate the endothelial cells stimulating the production of adhesion molecules and thrombogenic factors.
The adhesion of circulating leucocytes to the endothelium is the start to the atherogenic process which can ultimately lead to plaque development. It is the rupture of the fibrous cap of the plaque, with the resultant exposure of thrombogenic subendothelial plaque constituents, which is believed to be the critical step that leads to a thromboembolic event. Beginning in the early 1970s accelerated atherosclerosis leading to premature cardiovascular disease (CVD) began to be recognized as a significant cause of morbidity and mortality in autoimmune diseases and in particular in rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) (45-48). This was initially felt to be due to traditional Framingham risk factors for atherosclerosis and to the use of certain drugs, in particular corticosteroids. More recently however, it is has been shown that traditional Framingham risk factors alone cannot explain the extent of observed premature atherosclerosis and that the chronic inflammation seen in patients with autoimmune diseases is an important contributing factor. A recent systematic review has found that RA is associated with a 60% increase in risk of CV death(48). Furthermore, a recent comparative study between RA and diabetes has suggested that preclinical atherosclerosis appears to be of equal frequency and severity in the 2 diseases, and that CV risk factors in RA may need to be targeted as aggressively as in diabetes(49). It has become evident that the identification of subclinical atherosclerosis prior to cardiovascular or cerebrovascular events is needed to allow the clinician to design preventive therapy(50).

1.4 Children with rheumatic disease and early atherosclerosis.

The development of classic autoimmunity is typically associated with autoreactive antigen-specific T lymphocytes and high-titer autoantibodies that lead to a destructive immune response directed to self-antigens. These abnormalities in the adaptive immune system play a pivotal role in the pathogenesis of conditions such as SLE. The innate immune response also plays a significant role and the balance between pro-inflammatory and anti-inflammatory cytokines produced by helper T (Th) cells influences the clinical manifestations in many rheumatic diseases.

The three pediatric rheumatic diseases of this study all involve chronic inflammation and it is possible that these diseases (with different associated cytokine profiles(51)) have a differential effect on the lipid profile of these children. It has been well described that both the innate immune response associated with inflammation(52) and treatment with corticosteroids(53,54)
can alter the lipid profile. Although all three disease groups are treated using different regimens, the use of corticosteroids to resolve uncontrolled inflammation is a common thread. The examination of lipid levels at presentation, prior to the introduction of corticosteroid therapy may allow for a better determination of not only the lipid abnormality associated with active disease but also of the role of inflammation in altering the lipid profile of patients. Furthermore, in patients with pediatric rheumatic disease, smoking, diabetes and uncontrolled hypertension are not common and therefore abnormal lipid profiles may be an important risk factor for the development of subclinical atherosclerosis in these children.

The lipid profile of both children and adults with chronic inflammatory disease is the result of a combination of the influences of active disease, therapies and genetics. Although the influence of genetic predisposition is difficult to either alter or accurately determine, the role of disease activity and therapies, in particular corticosteroid treatment can be examined.

1.5 Difficulties in assessing early atherosclerosis and the use of surrogate markers.

The annual incidence of a cardiovascular or cerebrovascular event in children with rheumatic disease is very low and therefore too small to adequately power studies that can be completed in a reasonable timeline. Additionally, we are interested in preventive strategies and therefore need to detect early evidence of atherosclerosis, prior to a clinical event. Therefore, a good way to study premature atherosclerosis is by using surrogate markers that can be measured earlier on in the atherogenic process.

Studies in adults have shown that early atherosclerosis can be detected using surrogate markers of atherosclerosis using non-invasive B-mode ultrasound vascular function measurements: flow-mediated dilatation (FMD), carotid intima-media thickness (CIMT) and pulse-wave velocity (PWV) (55-57). These 3 methods have been used extensively in adults with rheumatic disease with CIMT the most frequently used method(58-64).

1.5.1 Carotid Intima Media Thickness (CIMT).

CIMT is a measure of the thickness between the two inner layers of the carotid artery (the intima and media). The thicker the arterial walls in the carotid artery (or any blood vessel), the smaller the space will be for blood to travel through the vessel. There is an age-dependent
physiologic thickening of arterial walls that begins in childhood. The atherosclerotic process also contributes to an increase in thickness and to plaque formation which is extremely rare in children but often begins in young adults(9,65). A recent systematic review and meta-analysis has shown that CIMT can be used as a predictor of future vascular events in otherwise healthy adults. In particular, it was shown that for an absolute carotid IMT difference of 0.1 mm, the future risk of myocardial infarction increases by 10% to 15%, and the stroke risk increases by 13% to 18%(55). This relationship between CIMT and cardiovascular events/mortality remains to be confirmed in children.

1.5.2 Arterial Stiffness (PWV).

Within the arterial circulation, arterial stiffness (or arterial compliance) relates to the changes in vessel diameter following left ventricular ejection. Increases in vessel stiffness may result from many different factors including dyslipidaemia, hypertension, oxidative stress, and endothelial dysfunction. Several surrogate measures of large artery stiffness have been shown to be predictive of all-cause and cardiovascular mortality. The most commonly measured is pulse wave velocity (PWV) and is a non-invasive, reliable and reproducible way of measuring early changes in arterial wall stiffness and arterial distensibility(66). The elastic modulus, stiffness index, and vascular impedance can also be assessed using similar Echo-Doppler methods. A recent systematic review has found that aortic stiffness expressed as aortic PWV is a strong predictor of future CV events and all-cause mortality. Subjects considered to have an increased aortic PWV were found to be at twice the level of risk for cardiovascular events, cardiovascular mortality, and all-cause mortality. Furthermore, the predictive ability of arterial stiffness is higher in subjects with a higher baseline CVD risk(57). Pediatric studies in patients with systemic inflammation associated with polyarteritis nodosa and Kawasaki disease have shown abnormal distensibility and PWV(67,68). However, the relationship between increased arterial PWV and cardiovascular events/mortality remains to be confirmed in children.

1.5.3 Endothelial Reactivity (FMD).

The vascular endothelium, which lines the blood vessel, is situated at the interface between the blood and tissues and has important regulatory functions in atherosclerosis. In healthy arteries, the endothelium has non-adhesive and antithrombotic properties and provides a barrier to
plasma protein and lipoprotein extravasation. Brachial flow-mediated dilation (FMD) is a measure of vessel dilation resulting from the release of nitric oxide by the endothelium due to a transient flow stimulus. Impaired brachial FMD is widely regarded as an early, and potentially reversible, manifestation of vascular disease. Endothelial injury is considered an important initial event in the development of atherosclerosis. Endothelial dysfunction, as a surrogate marker of atherosclerosis, has been shown to predict future vascular events in low risk adult populations\(^\text{56,69}\). The relationship between impaired FMD and cardiovascular events/mortality remains to be confirmed in children. However, studies in familial hyperlipidemia, a high-risk population, have shown that FMD can be used to effectively measure endothelial function in children\(^\text{70}\).

There are relatively few vascular studies of pediatric rheumatic diseases and therefore it is not clear which method is the most sensitive to detect early atherosclerotic changes in children where their endothelium is relatively healthy prior to the onset of disease\(^\text{71,72}\). To date there has been no study in children measuring all three vascular function markers in three distinct pediatric rheumatic disease populations: JDM, SJIA, and SLE.

### 1.6 Study framework

Significantly increased rates of atherosclerosis causing heart attacks and strokes have been found to occur in adults with chronic inflammatory diseases such as SLE and RA. The process of atherosclerosis begins in childhood. For most children, atherosclerosis is mild and progresses slowly. Atherosclerosis may worsen more rapidly in children with rheumatic disease increasing the risk of heart disease and stroke in early adult life. It is important to identify which children are at risk for early atherosclerosis and to begin making improvements in lifestyle and treatment. Our hypotheses are: 1) Acute and chronic inflammation associated with pediatric rheumatic disease will result in increased traditional and non-traditional risk factors resulting in measurable changes in vascular function in these children; and 2) Different pediatric rheumatic diseases will show differing levels of influence on these outcomes due to their specific etiology and associated treatment.

We are interested in preventive strategies and therefore need to detect early evidence of atherosclerosis, prior to a clinical event. To this end non-invasive ultrasound based surrogate
markers of early atherosclerosis have been developed. The aim of the first project is to systematically review and perform a meta-analysis on studies which examine whether rheumatic diseases are associated with an increased CIMT, the most studied surrogate marker, when compared to healthy controls. We will also provide summary estimates for the effect size for all populations together and then stratified by pre-existing CVD and rheumatic disease type. Our hypothesis is that CIMT represents a valid non-invasive surrogate marker of early atherosclerosis for our population of interest and that rheumatic disease is associated with an increased CIMT.

It has been well established that the risk of developing atherosclerosis increases with age and is worsened by traditional risk factors such as smoking, hypertension, increased body mass index, and the presence of diabetes mellitus. However, the main determinants of atherosclerotic risk in the general population remain the concentrations of serum LDL and HDL cholesterol. The aim of the second project is to determine the frequency and pattern of dyslipoproteinemia at presentation of pediatric SLE, a chronic inflammatory rheumatic disease of childhood, and to study the association between dyslipoproteinemia and markers of disease activity and inflammatory markers. Our hypothesis is that children with pSLE at presentation and before the confounding effects of treatment with corticosteroids will be at higher risk of dyslipidemia. Disease activity and its associated inflammation will play a role in these changes to the lipid profile of these patients.

The third project will build upon the findings of the second project by expanding our study to include two other pediatric rheumatic diseases, JDM and SJIA. The aim is to compare and contrast the lipid profiles of patients with JDM, SJIA and pSLE and to determine the association between dyslipoproteinemia and inflammation, disease activity, and treatment. Our hypothesis is that pediatric diseases of different etiology and treatment regimens will affect the lipid profile of these children differently.

Studies in adults have shown that early atherosclerosis can be detected using surrogate markers of atherosclerosis using non-invasive B-mode ultrasound vascular function measurements. These 3 methods have been used extensively in adults with rheumatic disease. However, to date there have been few studies determining these measures in children with rheumatic disease and
no study measuring all three markers in three distinct pediatric rheumatic disease populations JDM, SJIA, and SLE. The fourth project will assess whether the vascular markers of early atherosclerotic changes, CIMT, PWV, and FMD, are similar in 3 different pediatric rheumatic diseases- JDM, SJIA, and SLE as well as correlate treatment- and disease activity-related factors with the vascular markers of early atherosclerotic changes. Our hypothesis is vascular markers of early atherosclerotic changes will differ between pediatric rheumatic diseases of different etiology and treatment regimens leading to differences in the eventual CVD risk of these children.

The last project will build upon the findings of the fourth project by studying progression of markers of early atherosclerotic changes over a short period of time. The aim of this project is to determine the progression of vascular markers of premature atherosclerosis (CIMT, FMD and PWV) in a prospectively followed pSLE cohort, who we suspect are at risk for vascular changes early in their disease course. We will also correlate disease activity, treatment and markers of inflammation with progression in these vascular markers. Our hypothesis is that children with pSLE will show progression of vascular markers of early atherosclerotic change and that these changes will be associated with inflammation related to disease.

1.7 Study objectives

The specific objectives addressed by this dissertation are the following:

1. Evaluate the risk of atherosclerosis in rheumatic disease compared to healthy controls as measured non-invasively by ultrasound based techniques (Project One);
2. Assess the lipid profile of children with pSLE at presentation before treatment with corticosteroids (Project Two);
3. Assess the lipid profiles of children with JDM, SJIA, and compare with pSLE (Project Three);
4. Evaluate the extent of early atherosclerosis in children with JDM, SJIA, and pSLE as measured by vascular function measures (Project Four);
5. Investigate the progression of early markers of atherosclerosis in children with pSLE (Project Five);
1.8 Study methodology

We first performed a prospective observational study of an inception cohort of children with pSLE. The primary study outcome was the fasting serum lipid profile at the time of presentation. We were not able to include children with JDM or SJIA as the incidence of newly diagnosed patients with either of these two diseases was too low to allow for a sufficient number of patients over the recruitment period. This study was a continuation of previous work by Sarkissian et al.(155). Only 9 of 54 patients were included in both studies. The second lipid project was a prospective observational study of a prevalent cohort of children with JDM, SJIA, and pSLE. The primary study outcome was the fasting serum lipid profile at the time of recruitment. This study included 43 of 54 pSLE patients from the previous inception cohort but the lipids were measured during the disease course and not at diagnosis. The next study was a cross-sectional study evaluating vascular function in the same prevalent cohort in whom lipid levels were measured. The primary study outcome was the vascular function measures of CIMT, FMD, and PWV. The final study was a prospective longitudinal study of pSLE subjects measuring change in vascular function over time. This study serially examined the prevalent cohort described above but was restricted to SLE patients as there were insufficient numbers of children with JDM and SJIA with sufficient follow-up during the study period as a result of the lower number of patients.
Chapter 2. Rheumatic Disease and Carotid Intima Media Thickness: A Systematic Review and Meta-Analysis.

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Rheumatic Disease and Carotid Intima Media Thickness: A Systematic Review and Meta-Analysis

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Key words: Rheumatic disease, carotid intima media thickness, atherosclerosis
Key points: 1- CIMT is increased in rheumatic disease populations.
2- Increase in CIMT is similar to 5-6 year increase in age.
3- Rheumatic disease types have differential effect on CIMT.
4- Protocols need to be standardized for future studies.

Abstract

Background: Accelerated atherosclerosis is a common complication of autoimmune rheumatic diseases with early changes seen even in pediatric patients. Carotid intima-media thickness (CIMT) is increasingly used as a surrogate marker for atherosclerosis. We performed a systematic review and meta-analysis to examine whether rheumatic disease is associated with an increased CIMT when compared to healthy controls.

Methods and Results: A pre-specified search strategy was used to identify relevant studies in MEDLINE and EMBASE databases (January 1986 to December 2008). Methodological quality was assessed using the Newcastle-Ottawa score for observational studies. Sixty-eight controlled comparisons from 60 different studies were reviewed: 37% rheumatoid arthritis; 35% systemic lupus erythematosus; 9% systemic sclerosis; and 19% other rheumatic diseases. Random effects meta-regression analysis was performed. The estimated summary effect size between control and study subject CIMT measurements in comparisons with pre-existing cardiovascular disease excluded was 0.64 (95% CI: 0.46-0.82) representing an overall absolute mean difference of 0.055 mm (95% CI: 0.048-0.063). Pre-existing cardiovascular disease, rheumatic disease type and disease duration contributed to heterogeneity.

Conclusions: CIMT was significantly increased in rheumatic disease populations. Future studies need to use a standardized protocol in order to ensure clinically meaningful results when measuring CIMT as a surrogate for premature atherosclerosis.
2.1 Introduction

Beginning in the early 1970s accelerated atherosclerosis leading to premature cardiovascular disease (CVD) began to be recognized as a significant cause of morbidity and mortality in autoimmune diseases and in particular in rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE)(45,73,74). This was initially felt to be due to traditional risk factors for atherosclerosis and to the use of certain drugs, in particular corticosteroids. More recently however, it is has been shown that traditional risk factors alone cannot explain the extent of observed premature atherosclerosis and that the chronic inflammation seen in patients with autoimmune diseases is an important contributing factor(75). It has also been demonstrated that the atherosclerotic process starts in childhood(8,76) and that chronic inflammation, as demonstrated by an elevated c-reactive protein, leads to early cardiovascular disease(7). A recent systematic review has found that RA is associated with a 60% increase in risk of CV death(77). Furthermore, a recent comparative study between RA and diabetes has suggested that preclinical atherosclerosis appears to be of equal frequency and severity, and that CV risk factors in RA may need to be targeted as aggressively as in diabetes(64). It has become evident that the identification of subclinical atherosclerosis prior to cardiovascular or cerebrovascular events is needed to allow the clinician to design preventive therapy.

To this end non-invasive surrogate markers of early atherosclerosis have been developed to allow early detection of atherosclerosis prior to overt disease. High-resolution ultrasound measurement of the carotid intima-media thickness (CIMT) is one such method. Multiple studies have shown that changes in CIMT can be used as a surrogate end point for determining the success of interventions to decrease the risk of cardiovascular disease. A recent systematic review and meta-analysis has shown that CIMT can be used as a predictor of future vascular events in otherwise healthy individuals. In particular, it was shown that for an absolute carotid IMT difference of 0.1 mm, the future risk of myocardial infarction increases by 10% to 15%, and the stroke risk increases by 13% to 18%(55).

The aim of this systematic review and meta-analysis was to examine whether rheumatic diseases are associated with an increased CIMT when compared to healthy controls and provide summary estimates for the effect size for all populations together and then stratified by pre-existing CVD and rheumatic disease type.
2.2 Methods

2.2.1 Search strategy

A pre-specified search strategy was used to identify from 1986, when CIMT first appeared in the literature, relevant studies in MEDLINE (January 1986 to December 2008) and EMBASE (January 1986 to December 2008) databases. Keywords for the searches were carotid intima media thickness, flow-mediated dilatation and pulse wave velocity and myocardial infarction, stroke, cardiovascular disease, carotid artery disease, or death and rheumatic disease. Variants for all keywords were used in order to increase the number of studies returned by the search. Flow-mediated dilatation and pulse wave velocity were included as keywords in the search as these two modalities are often used along with carotid intima media thickness in study protocols and this helped to minimize the chances of missing a relevant article. The references in the identified or related articles were then manually reviewed in the search for other relevant citations. All resulting articles were then screened for CIMT measures and inclusion of healthy control groups.

2.2.2 Inclusion criteria

Inclusion criteria were (i) cohort or cross-sectional studies with data on both patients with a rheumatic disease and healthy age- and gender-matched controls; and (ii) use of high-resolution ultrasound to measure CIMT. All subjects with rheumatic diseases needed to fulfill the standard accepted diagnostic criteria and healthy controls needed to be clearly described by the authors to be included in our analysis. Final inclusion of the studies was determined by both reviewers independently [PNT and TB]. If a study was reported in more than one publication, we considered all publications for data abstraction, but only one report was included in our study. If a given publication included several groups of patients, the paper yielded a corresponding number of comparisons in our database. Six publications that were not available in English were assessed and data extracted with the help of a translator. In 5 publications that did not contain all information necessary for meta-analysis and/or for quality assessment, missing information was requested directly from the authors.
2.2.3 Data extraction

The quality of the studies was assessed and scored by two authors [PNT and TB] using the Newcastle-Ottawa quality assessment scale (NOS) for observational studies. From each paper, the following data items were extracted: study design, recruitment and number of patients, rheumatic disease studied, disease duration, inclusion or exclusion of patients with pre-existing CVD, sex and age distribution, mean CIMT and standard deviation, and ultrasound methodology including type (B-mode/ M-mode), location of measurements [common carotid artery (CCA), bifurcation (BCA), or internal carotid artery (ICA); right/left; near/far wall], inclusion or exclusion of plaque in CIMT measurement, inter-rater and/or intra-rater variation, and blinding. In case of uncertainty, issues were resolved by consensus with a third author [EDS].

2.2.4 Statistical analysis

The effect size for each study was calculated using the standardized mean difference, Cohen’s D(78): CD=m[e] - m[c] / SD[pooled] with SD[pooled]=SQRT(((n[e] - 1) * SD[e]^2 + (n[c] - 1) * SD[c]^2) / (N)) with e = exposed and c = control. These estimates and 95% confidence intervals were illustrated with a Forest plot and graphed by disease type. Pooled estimates were calculated using a fixed effects model to test and assess the level of heterogeneity and a random effects model(79) in order to obtain an average effect size and to study heterogeneity. Heterogeneity was tested with the chi-square heterogeneity statistic Q and quantified using I^2, which is a measure that describes the percentage of total variation across comparisons that is due to heterogeneity rather than chance(80). We considered I^2 values of 25, 50, and 75% to indicate low, moderate, and high variation, respectively(81). Meta-regression was performed modeling the following independent variables: age, sex (ratio of female to male), disease duration, NOS score, disease type (RA, SLE, Systemic Sclerosis [SSc], Other rheumatic), exclusion of plaque in CIMT measurement or not, exclusion of subjects with pre-existing CVD or not. This last model was tested in a multivariable model adjusting for age, sex ratio, and disease duration. Continuous variables were centered to their respective group mean before being entered into meta-regression. Heterogeneity was then further assessed by between-study variance (Tau^2) obtained from a
mixed effects model with fixed covariate effects and random study effects. Publication bias was assessed graphically using an inverse standard error funnel plot and graphed by disease type. Pooled estimates, meta-regression and other statistical analyses were performed with SAS 9.2 (The SAS Institute, Cary, NC, USA). Plots were obtained using MIX ver 1.7(82).

2.3 Results

2.3.1 Studies and Subjects

The search was performed December, 2008 and yielded a total of 1030 papers (Figure 1). Of the original 1030 papers reviewed: 271 were found to be duplicates; 679 were excluded because: 1) the study did not include patients with a clearly defined rheumatic disease; 2) the study did not include CIMT measurements; or 3) a control group was not included. Eighty articles were retrieved for detailed analysis. Six articles were then found to not contain a comparable healthy control group, 11 articles were found to contain studies based on previously published data, and 5 articles had missing data, of which only 2 of 5 authors responded to our request for information and therefore the 3 other articles with missing data were excluded. Sixty studies were available for assessment comprising 68 comparisons with a total of 6864 subjects (3761 cases and 3093 controls)(61,83-141). Translation was required for 8 papers: 1 German(98), 3 Italian(97,102,119), 2 Polish(92,111), 1 Spanish(136), and 1 Russian(128). Five articles were submitted to review by the third author (EDS) for arbitration.

The median study mean age was 45 years (range 11-79 years) with the majority of subjects being female (median 89% female, range 39-100%). Most comparisons (80%) clearly indicated matching of cases and controls by sex and age by design, while the remainder showed no significant difference between study groups. Eleven of 68 comparisons received a minimum of 5 out of 9 possible stars (median 7, range 5-9) of the Newcastle-Ottawa quality assessment scale. Thirty-two comparisons had a score of 8 of more. The 68 comparisons were divided into: 25 RA (37%), 24 SLE (35%), 6 SSc (9%), and 13 other rheumatic diseases (19%) including 1 ankylosing spondylitis, 1 Behcet’s disease, 1 connective tissue disorders, 2 familial Mediterranean fever, 1 giant cell arteritis, 4 primary antiphospholipid syndrome, 1 psoriatic arthritis, 1 Sjogren’s syndrome, and 1 Wegener’s Granulomatosis. Only 3 comparisons included children, 2 pediatric SLE and 1 of juvenile idiopathic
arthritis(85,128,134). Forty-one comparisons (60%) did not specify exclusion of subjects with pre-existing CVD and 27 comparisons (40%) excluded subjects with pre-existing CVD (Table 1). Twenty of the 27 studies clearly indicated that this exclusion applied to both cases and controls. Seven studies did not indicate whether this exclusion applied to the controls or not. Studies most often defined pre-existing CVD as having developed cardiovascular or cerebrovascular events, or having evidence of cardiovascular disease. Other exclusions included diabetes mellitus, smoking, kidney disease, hypertension and having received drugs affecting the cardiovascular system (antihypertensive or antiaggregant drugs, nitrates, and statins).

2.3.2 CIMT measurement

Fifty-two of the 68 comparisons measured CIMT using B-mode ultrasound (76%), 4 using M-mode (6%) and the remaining did not specify. Fourteen comparisons (21%) clearly specified that they avoided areas of thickening due to atheromatous plaques in the CIMT measurement, 19 (28%) indicated that it was possible their measurements included areas of plaque, but 35 (51%) did not specify. The location of the measurements (i.e. segments: common carotid artery, bifurcation, or internal carotid artery; far wall or near wall; right or left side), measurements used for summary CIMT value, and reproducibility (number of observers, blinding, inter/intra-rater reliability) are also included in Table 1.

In 40/68 (59%) comparisons, the study authors reported a statistically significant greater mean CIMT in patients as compared to the healthy control, including 17 of the 41 comparisons (41%) with CVD included and 23 of the 27 comparisons (85%) with CVD excluded. When considering the presence of plaques, 29 of the 44 comparisons (66%) that reported the prevalence of plaques, found there to be a significant difference between patients and controls. No agreement between the two methods (CIMT measure and plaque prevalence) was found (kappa=-0.0452).

A summary estimate of the absolute mean difference in CIMT between case and control groups was calculated using a fixed effects model (overall mean difference of 0.042 mm, 95% CI: 0.037-0.048) and a random effects model (overall summary estimate of 0.061 mm, 95% CI: 0.045-0.076).
In order to estimate the strength as well as the significance of the relationship of the difference in CIMT, the effect size for all comparisons (Cohen’s D) were calculated and graphically represented in a forest plot by disease type, ordered by author with meta-analysis weight included (Figure 2). The estimated summary effect size calculated by a fixed effect model was 0.36 (95% CI: 0.31-0.41). The overall estimated summary effect size calculated by a random effect model was 0.47 (95% CI: 0.34-0.60) (Table 2). No significant difference between disease types (type 3 test p-value = 0.513) was observed. Summary estimate for SSc comparisons was the largest at 0.58 (95% CI: 0.15-1.00), followed by RA 0.56 (95% CI: 0.36-0.76), Other rheumatic 0.47 (95% CI: 0.19-0.75) and SLE 0.35 (95% CI: 0.14-0.55).

2.3.3 Assessment of Heterogeneity and Confounding

In order to assess publication bias, a funnel plot was produced with comparisons sorted by the inverse standard error of the calculated effect size. A slightly positively skewed distribution with larger samples showing smaller effect sizes was observed similarly for all disease types (Figure 3). In order to further assess this bias we used the trim-and-fill procedure that resulted in an adjusted fixed effects summary estimate for all comparisons that remained statistically significant and clinically relevant, with an effect size of 0.13 (95% CI 0.08-0.17). The degree of inconsistency in the comparisons’ results was tested using the Q statistic and measured using $I^2$. A significant Q statistic of 225 (p<0.0001) and a high level of variation with an $I^2$ of 84% (95%CI: 81-87%) were found for the overall fixed model (all comparisons). Having found such a high degree of heterogeneity, we progressed to a random effects model to further define this heterogeneity. Inconsistency in the comparisons’ results was estimated by Tau^2 from the random effects model. A significant level of heterogeneity was found for the crude model Tau^2 = 0.22 (95% CI: 0.15-0.35) and was used as the reference level for further modeling (Table 2). In order to explore possible sources of heterogeneity, we first investigated age, sex ratio, disease duration, and NOS score independently as possible contributors to between study variance. None of these 4 variables were found to remain significant in the model. However, disease duration was found to reduce heterogeneity by 22% as compared to the crude model. Age, sex ratio and NOS score reduced heterogeneity by very little (1-3%).
We then considered methodological factors that could be contributing to heterogeneity. Considering the coronary segment where the IMT was measured, a sensitivity analysis of the 41 comparisons that measured the CIMT in the CCA segment exclusively (i.e. excluding the 27 comparisons that did not clearly indicate the measured segment or included multiple measured segments), resulted in a small increase in the summary estimate of effect size (2%) and no change in residual heterogeneity (data not shown). Considering the ultrasound imaging mode used, a sensitivity analysis of the 52 comparisons that specifically performed B-mode (i.e. excluding the 4 comparisons that used M-mode and the 12 comparisons that did not clearly indicate the method used), resulted in a small increase in summary effect size (6%), but a 14% reduction in residual heterogeneity (data not shown). Considering the 23 comparisons that clearly indicated areas of plaque were excluded from the CIMT measurement relative to the 45 comparisons that did not, the summary effect size was no different in the model (0.43 vs. 0.55, p=0.394) and little change in residual heterogeneity (Table 2). Finally, considering the 27 comparisons with pre-existing CVD excluded relative to the 41 comparisons that did not, the summary effect size was increased by 91% (0.65 vs. 0.34, p=0.011) with an 11% reduction in residual heterogeneity (Table 2). When stratifying pre-existing CVD excluded by disease type the 27 comparisons were 16/25 RA, 4/24 SLE, 2/6 SSc and 5/13 other rheumatic and the ratios between disease types differed significantly (p=0.009). When considering both factors (pre-existing CVD excluded and disease type) in the model together, pre-existing CVD excluded remained significant (p=0.032) whereas disease type was not (p=0.892) and residual heterogeneity was reduced by 16% (data not shown).

We then considered a multivariable model which included pre-existing CVD excluded as the factor of interest and adjusted for age, sex ratio and disease duration (Table 2). The difference between summary estimates with pre-existing CVD excluded vs. not, remained significant (0.64 vs. 0.27, p=0.004) and residual heterogeneity was reduced by 43%. In order to obtain an absolute mean difference representative of the studies included in our analysis, we performed a fixed effect meta-analysis of the mean difference of comparisons with pre-existing CVD excluded and obtained a summary estimate of 0.055 mm (95% CI: 0.048-0.063). This absolute difference suggests that the effect of rheumatic disease on CIMT with pre-existing CVD excluded can be estimated to be a 7-8 year increase in age when assuming
a linear relationship between age and CIMT thickening where with every 10 year increase one can expect a thickening of 0.079 mm(142).

It is of interest to note that when the analysis was performed with only the pre-existing CVD excluded comparisons that the resulting summary estimates were markedly increased. As, when considering pediatric populations that because of the young age of the subjects are inherently pre-existing CVD excluded, the effect sizes were large and similar for both studied diseases: pediatric SLE Falaschi et al., 2000: 0.73 (95%CI: 0.17-1.30) and Bowser et al., 2008: 0.66 (95%CI: 0.03-1.30) and juvenile idiopathic arthritis Pietrewicz et al., 2007: 0.74 (95%CI: 0.19-1.30).

2.4 Discussion

In this paper, we reviewed data from 68 comparisons of rheumatic disease populations with controls, including a total of 6864 subjects who had CIMT measurements reported. Although CIMT is a known strong predictor of cardiovascular events in the general population(55), and has been predominantly reported in the literature on patients with rheumatic diseases, it has been suggested that detection of carotid plaques by ultrasonography is a more reliable predictor of cardiovascular events(143). We observed in 59% of the 68 reviewed comparisons that the study authors reported a statistically significant greater mean CIMT in patients with rheumatic diseases compared with healthy controls and similarly increased prevalence of plaque in 66% of the 44 reviewed comparisons that reported the presence of carotid plaque. Interestingly no agreement between the two methods was observed, but this likely reflects the considerable differences in methodological approach to inclusion or exclusion of plaque in the CIMT measurement between different study authors. Furthermore, as each rheumatic disease may differ in the amount and type of inflammation, active inflammatory plaques could be less in number but potentially larger and/or more destructive. Patients in this case would potentially have an increased CIMT but a lesser number of plaques.

A significant amount of heterogeneity was found to exist between comparisons and therefore a random effects model was chosen in order to best model and explore sources of this heterogeneity. The best meta- regression analysis model of the effect size between CIMT of patients with rheumatic diseases compared to healthy controls, adjusted for age, sex ratio,
and disease duration and excluded patients with pre-existing CVD. The resulting significant summary effect size estimate of 0.64 (95% CI: 0.46-0.82) represented an overall mean difference of 0.055 mm (95% CI: 0.048-0.063). The effect of rheumatic disease on CIMT when excluding pre-existing CVD can be estimated to be a 7 year increase in age.

When considering the effect of including subjects with pre-existing CVD, whereas in total 59% of reviewed comparisons reported a greater mean CIMT in patients with rheumatic diseases, this was in 85% of comparisons with CVD excluded and in only 41% with CVD included. This was similarly reflected in our random effects model, with an increase in effect size and decrease in heterogeneity for the comparisons with CVD excluded. This would suggest that the inclusion of subjects with pre-existing CVD might significantly confound the interpretation of the independent effect of rheumatic diseases on CIMT. Although there are too few pediatric comparisons for any conclusive results, this would be consistent with the generally larger effect sizes seen in the 3 pediatric comparisons included with the lowest possible risk for premature atherosclerosis due to Framingham risk factors(8).

The three most frequently included rheumatic diseases of RA, SLE, and SSc had different overall effect sizes, but these were not found to be significantly different as between comparison variability was found to be high and the number of comparisons per group differed substantially. However, the multiple rheumatic diseases included did contribute to heterogeneity overall. This may be due to the effect of chronic inflammation not being uniform across the rheumatic diseases and subgroups included and the potential for overlap in the diagnostic criteria of some of the rheumatic diseases included. Also, there are many possible reasons to expect differences(75) between disease types as each disease has a different amount and/or type of chronic inflammation which has been shown to be important factors leading to premature atherosclerosis. Although all 3 diseases may involve blood vessel inflammation, the type and degree of inflammation differs among the disease. Given that SSc, unlike RA or SLE, always involves some degree of vascular involvement, it may not be surprising that it had one of the largest effect sizes. Finally, although the estimate for SLE was the smallest, this may have been again confounded by the fact that too few comparisons excluded subjects with pre-existing CVD as compared with the other diseases.
Other possible sources of variability include the carotid ultrasound methods. Comparisons differed by carotid segment(s) investigated, whether both or single sides were measured, whether the far wall, near wall, or both were measured, whether the final result was expressed as a mean or maximal IMT, as well as which ultrasound imaging mode was used (B-mode vs. M-mode). Reproducibility also has improved steadily since the initial development of the techniques, but is infrequently reported (34% of comparisons in this review). We only considered comparisons that included healthy age- and gender-matched controls and most measured the CCA segment and calculated the mean CIMT with a minimum of 3 measures. However, while it is beyond the scope of this review to assess differences between ultrasound measurement techniques, it is of interest that most of these technical considerations were found to make little difference and only B-mode vs. M-mode ultrasound was found to contribute to the observed heterogeneity of the model. We support the suggestion by others of the need to use a standardized protocol for future studies in order to ensure clinically meaningful results (55, 72, 144).

Measuring CIMT in areas of thickening due to atheromatous plaques is of definite concern. Although our analysis showed little difference in the summary effect size between healthy and rheumatic disease populations when plaque was included or excluded in the measure, the absolute CIMT measures were found to be somewhat inflated when areas of plaques were potentially included. In future studies, this again stresses the need for a standardized protocol and approach to distinguishing early atherosclerotic plaque formation from thickening of intima-media thickness.

2.4.1 Limitations

This systematic review and meta-analysis clearly demonstrated that there was a significant increase in CIMT in rheumatic disease populations as compared to age- and gender-matched healthy controls. However, some limitations are apparent. First, the choice of ‘healthy controls’ as the comparator group comes with challenges that need to be taken into consideration. Most healthy controls were volunteers and were generally not described with the same rigour as the cases. This can be problematic when the study design has as purpose to elicit differences between these groups. Population controls have also served as
comparator group, especially for studying the effects of plaque, and should be considered as a viable alternative. Second, some publication bias was present. This bias is often accentuated when the disease of interest is relatively rare (often the case in rheumatic diseases) as studies in these situations are often of small sample size and with increased variability leading to not significant comparisons that ultimately remain unpublished (145,146). With the large potential sources of heterogeneity that existed between comparisons, sampling bias could become a factor if the reason for the heterogeneity (rheumatic disease population, exclusion of pre-existing CVD, disease duration) could influence the authors’ decision on how to report (i.e. mean or maximal IMT) the results. We are not overly concerned with this potential problem as most comparisons reported a mean CIMT or both. Finally, while most studies were assessed to be of acceptable quality it was the comparability of the methodological considerations (i.e. independent blind assessment), that mostly affected the NOS score. As previously suggested, a standardized methodology would lead to less variation and, therefore, better summary effect size estimates.

In conclusion, CIMT was found to be significantly increased in rheumatic disease populations compared to age- and gender-matched healthy populations in our systematic review and meta-analysis of 68 comparisons. The type of rheumatic disease, pre-existing atherogenic risk factors, and disease duration were important in considering the utility of CIMT as a surrogate for premature atherosclerosis. As cardiovascular events are rare in children, CIMT measurements may be particularly useful in younger populations as an endpoint for epidemiological and treatment studies.

Acknowledgments
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Disclosure

No authors have any conflict(s) of interest to disclose.

Tables

Table 1. Characteristics of Abstracted Studies
<table>
<thead>
<tr>
<th>Population</th>
<th>Reference</th>
<th>Case</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ankylosing spondylitis</td>
<td>Sari, 2006</td>
<td>N = 54 (46)</td>
<td>Age Mean(SD) or Median(Range) = 37 ±11</td>
</tr>
<tr>
<td>Behect disease</td>
<td>Keser, 2005</td>
<td>N = 114 (40)</td>
<td>Age Mean(SD) or Median(Range) = 38.15 ±9.44</td>
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<tr>
<td>Connective tissue disorders</td>
<td>Dropinski, 2003</td>
<td>N = 74 (85)</td>
<td>Age Mean(SD) or Median(Range) = 34 ±9</td>
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<tr>
<td>Connective tissue disorders</td>
<td>Akdogan, 2006</td>
<td>N = 43 (42)</td>
<td>Age Mean(SD) or Median(Range) = 27.5 ±7.2</td>
</tr>
<tr>
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<td>Sari, 2007</td>
<td>N = 61 (51)</td>
<td>Age Mean(SD) or Median(Range) = 31.5 (18-54)</td>
</tr>
<tr>
<td>Juvenile idiopathic arthritis</td>
<td>Pietrewicz, 2007</td>
<td>N = 40 (50)</td>
<td>Age Mean(SD) or Median(Range) = 11 (4-16)</td>
</tr>
<tr>
<td>Pediatric SLE</td>
<td>Bowers, 2008</td>
<td>N = 20 (95)</td>
<td>Age Mean(SD) or Median(Range) = 16.90 ±2.27</td>
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<tr>
<td>Pediatric SLE</td>
<td>Falsaschi, 2000</td>
<td>N = 26 (81)</td>
<td>Age Mean(SD) or Median(Range) = 17.1 (6.2-25.4)</td>
</tr>
<tr>
<td>Primary antiphospholipid syndrome</td>
<td>Ames, 2005</td>
<td>N = 20 (65)</td>
<td>Age Mean(SD) or Median(Range) = 35 ±12</td>
</tr>
<tr>
<td>Primary antiphospholipid syndrome</td>
<td>Der, 2007</td>
<td>N = 44 (57)</td>
<td>Age Mean(SD) or Median(Range) = 52 ±15</td>
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<tr>
<td>Primary antiphospholipid syndrome</td>
<td>Roch, 2004</td>
<td>N = 20 (80)</td>
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<td>Primary antiphospholipid syndrome</td>
<td>Vlachoyiannopoulos, 2003</td>
<td>N = 33 (100)</td>
<td>Age Mean(SD) or Median(Range) = 33.9 ±7.4</td>
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<td>Psoriatic arthritis</td>
<td>Gonzalez, Juaneaty, 2007</td>
<td>N = 75 (47)</td>
<td>Age Mean(SD) or Median(Range) = 48.8 ±12.4</td>
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<td>Abu-Shakra, 2005</td>
<td>N = 57 (82)</td>
<td>Age Mean(SD) or Median(Range) = 52.1 ±14.6</td>
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<tr>
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<td>Alkabbi, 2003</td>
<td>N = 40 (50)</td>
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<tr>
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<td>Bocci, 2005</td>
<td>N = 32 (88)</td>
<td>Age Mean(SD) or Median(Range) = 50 ±7</td>
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<tr>
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<td>Carotti, 2007</td>
<td>N = 40 (68)</td>
<td>Age Mean(SD) or Median(Range) = 59.9 ±11.9</td>
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<tr>
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<table>
<thead>
<tr>
<th>Disease Duration (yrs)</th>
<th>NOS</th>
<th>Manifest CVD excluded</th>
<th>Segments</th>
<th>Plaque Excluded</th>
<th>Type of Ultrasound</th>
<th>IMT Definition</th>
<th># of readers (Blinded Y/N)</th>
<th>Reproducibility</th>
</tr>
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<tr>
<td>12.4</td>
<td>8</td>
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<td>CCA</td>
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<td>NA</td>
<td>Both sides</td>
<td>1 (Yes)</td>
<td>NA</td>
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<tr>
<td>10.1</td>
<td>9</td>
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<td>CCA</td>
<td>Yes</td>
<td>B-Mode</td>
<td>Far Wall, both sides</td>
<td>1 (Yes)</td>
<td>Pearson r=0.816</td>
</tr>
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<td>NA</td>
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<td>NA</td>
<td>Far Wall</td>
<td>NA</td>
<td>NA</td>
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<tr>
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<td>NA</td>
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<td>NA</td>
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<td>3.3</td>
<td>7</td>
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<td>No</td>
<td>B-Mode</td>
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<tr>
<td>5.5</td>
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<td>NA</td>
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<td>1 (No)</td>
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<td>No</td>
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<td>Far Wall, both sides</td>
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<td>NA</td>
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<td>7</td>
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<tr>
<td>7.4</td>
<td>6</td>
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<td>NA</td>
<td>NA</td>
<td>B-Mode</td>
<td>Far Wall, both sides</td>
<td>NA</td>
<td>Coefficient of variation =10.4%</td>
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<td>NA</td>
<td>Right side only</td>
<td>1 (Yes)</td>
<td>0.98 correlation coefficient for</td>
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<td>CCA</td>
<td>NA</td>
<td>B-Mode</td>
<td>Far Wall, both sides</td>
<td>1 (Yes)</td>
<td>CV-inter= 8.3% CV-intra= 0.7%</td>
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<tr>
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<td>B-Mode</td>
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<tr>
<td>11.0</td>
<td>8</td>
<td>Yes</td>
<td>CCA, BIF, ICA</td>
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<td>B-Mode</td>
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<td>NA</td>
<td>NA</td>
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<td>7</td>
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<td>B-Mode</td>
<td>Far Wall</td>
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<td>NA</td>
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<tr>
<td>9.5</td>
<td>8</td>
<td>Yes</td>
<td>CCA, BIF</td>
<td>Yes</td>
<td>NA</td>
<td>Far Wall, right side only</td>
<td>1 (Yes)</td>
<td>Coefficient of variation =1.4%</td>
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<td>8</td>
<td>Yes</td>
<td>CCA, ICA, ECA</td>
<td>No</td>
<td>NA</td>
<td>Far Wall, both sides</td>
<td>2 (Yes)</td>
<td>NA</td>
</tr>
</tbody>
</table>

**Note:** The table includes additional columns for reproducibility, but the exact values are not provided in the given text.
Table 1b (continued). Characteristics of abstracted studies

<table>
<thead>
<tr>
<th>Population</th>
<th>Reference</th>
<th>Case</th>
<th>Control</th>
<th>N (%Female)</th>
<th>Age Mean(SD) or Median(Range)</th>
<th>N (%Female)</th>
<th>Age Mean(SD) or Median(Range)</th>
<th>Disease Duration (yrs)</th>
<th>NOS</th>
<th>Manifest CVD excluded</th>
<th>Segments Excluded</th>
<th>Type of Ultrasound</th>
<th>IMT Definition</th>
<th># of readers (Blinded Y/N)</th>
<th>Reproducibility</th>
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</thead>
<tbody>
<tr>
<td>RA</td>
<td>Cuomo, 2004</td>
<td>48 (73)</td>
<td>55 (26-69)</td>
<td>22 (73)</td>
<td>50 (28-66)</td>
<td>8.7</td>
<td>9</td>
<td>Yes</td>
<td>CCA</td>
<td>No</td>
<td>B-Mode</td>
<td>Far Wall</td>
<td>1 (Yes)</td>
<td>NA</td>
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<tr>
<td>RA</td>
<td>Daza, 2007</td>
<td>55 (100)</td>
<td>43.6 ±8.39</td>
<td>20 (100)</td>
<td>40.7 ±6.94</td>
<td>11.5</td>
<td>8</td>
<td>Yes</td>
<td>CCA</td>
<td>NA</td>
<td>B-Mode</td>
<td>Far Wall, right side only</td>
<td>2 (Yes)</td>
<td>ICC = 0.94</td>
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<tr>
<td>RA</td>
<td>Del Rincon, 2003</td>
<td>204 (89)</td>
<td>59.6 (40-83)</td>
<td>102 (88)</td>
<td>59.7 (40.81)</td>
<td>NA</td>
<td>8</td>
<td>No</td>
<td>CCA, ICA, combined</td>
<td>NA</td>
<td>B-Mode</td>
<td>Near and Far Wall</td>
<td>1 (Yes)</td>
<td>Intra-ICC = 0.99 Inter-ICC=0.94</td>
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<tr>
<td>RA</td>
<td>Georgiadis, 2008</td>
<td>40 (75)</td>
<td>53.1 ±13.4</td>
<td>45 (67)</td>
<td>52.2 ±11.7</td>
<td>&lt;1</td>
<td>9</td>
<td>Yes</td>
<td>CCA</td>
<td>NA</td>
<td>B-Mode</td>
<td>Far Wall</td>
<td>1 (Yes)</td>
<td>NA</td>
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</tr>
<tr>
<td>RA</td>
<td>Gonzalez-Juanatey, 2003</td>
<td>47 (77)</td>
<td>59.2 ±12.5</td>
<td>47 (74)</td>
<td>60.5 ±12.5</td>
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<td>9</td>
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<td>Far Wall, right side only</td>
<td>2 (Yes)</td>
<td>ICC = 0.986</td>
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<tr>
<td>RA</td>
<td>Grover, 2006</td>
<td>57 (91)</td>
<td>41.5 ±7.53</td>
<td>45 (89)</td>
<td>39.7 ±6.6</td>
<td>8.0</td>
<td>8</td>
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<td>B-Mode</td>
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<td>1 (Yes)</td>
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<td>Hannawel, 2007</td>
<td>40 (68)</td>
<td>53 (22-78)</td>
<td>40 (68)</td>
<td>53 (21-80)</td>
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<td>B-Mode</td>
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<tr>
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<td>Kamatas, 2008</td>
<td>52 (77)</td>
<td>51.2 ±12.3</td>
<td>40 (78)</td>
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<td>7</td>
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<td>No</td>
<td>B-Mode</td>
<td>Far Wall, both sides</td>
<td>1 (No)</td>
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<tr>
<td>RA</td>
<td>Kumada, 2002</td>
<td>138 (88)</td>
<td>55 ±10.7</td>
<td>94 (90)</td>
<td>52 ±14.5</td>
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<td>Yes</td>
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<td>Coefficient of variation = 2.8%</td>
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<td>RA</td>
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<td>70 (100)</td>
<td>42 ±5.5</td>
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<tr>
<td>RA</td>
<td>Park, 2002</td>
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<td>98 (98)</td>
<td>47 ±13</td>
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<td>30 (93)</td>
<td>60.4 ±12.9</td>
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<td>8</td>
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<td>Yes</td>
<td>B-Mode</td>
<td>Both sides</td>
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<td>Wallberg Jonsson, 2001</td>
<td>39 (77)</td>
<td>51.6 (36-65)</td>
<td>39 (77)</td>
<td>51.6 (37-65)</td>
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<td>Vaudo, 2005</td>
<td>37 (100)</td>
<td>48 ±14</td>
<td>35 (100)</td>
<td>51 ±16</td>
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<td>2 (Yes)</td>
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<td>SLE</td>
<td>Bhatt, 2006</td>
<td>50 (94)</td>
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<td>50 (94)</td>
<td>31.3 ±9.78</td>
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<tr>
<td>SLE</td>
<td>De Leeuw, 2006</td>
<td>72 (88)</td>
<td>41 ±12</td>
<td>36 (92)</td>
<td>41 ±12</td>
<td>8.7</td>
<td>6</td>
<td>No</td>
<td>NA</td>
<td>NA</td>
<td>B-Mode</td>
<td>Far Wall, right side only</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>SLE</td>
<td>De Leeuw, 2007</td>
<td>55 (85)</td>
<td>43 ±12</td>
<td>55 (85)</td>
<td>43 ±13</td>
<td>12.1</td>
<td>7</td>
<td>No</td>
<td>NA</td>
<td>No</td>
<td>B-Mode</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>SLE</td>
<td>Estévez Del Toro, 2008</td>
<td>51 (90)</td>
<td>37.9 (11.0)</td>
<td>51 (90)</td>
<td>37.8 (11.9)</td>
<td>10.1</td>
<td>7</td>
<td>No</td>
<td>CCA, BIF, ICA</td>
<td>NA</td>
<td>B-Mode</td>
<td>NA</td>
<td>1 (Yes)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>SLE</td>
<td>Fischer, 2006</td>
<td>103 (87)</td>
<td>44.5 (19-76)</td>
<td>30 (80)</td>
<td>42.9 (20-74)</td>
<td>NA</td>
<td>5</td>
<td>No</td>
<td>NA</td>
<td>NA</td>
<td>B-Mode</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
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</table>
Table 1c (continued), Characteristics of abstracted studies

<table>
<thead>
<tr>
<th>Population</th>
<th>Reference</th>
<th>Case</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (% Female)</td>
<td>Age Mean(SD) or Median(Range)</td>
<td>N (% Female)</td>
</tr>
<tr>
<td>SLE/ secondary antiphospholipid syndrome</td>
<td>Belizna, 2008</td>
<td>58 (62)</td>
<td>29.10 ±10</td>
</tr>
<tr>
<td>SLE/ secondary antiphospholipid syndrome</td>
<td>Jimenez, 2005</td>
<td>25 (100)</td>
<td>38.6 ±11.4</td>
</tr>
<tr>
<td>SLE/ secondary antiphospholipid syndrome</td>
<td>Roch, 2004</td>
<td>14 (93)</td>
<td>43 ±16</td>
</tr>
<tr>
<td>SSc</td>
<td>Bartoli, 2007</td>
<td>53 (89)</td>
<td>60.4 ±10.68</td>
</tr>
<tr>
<td>SSc</td>
<td>Hettema, 2008</td>
<td>49 (84)</td>
<td>55.40 ±11.6</td>
</tr>
<tr>
<td>SSc</td>
<td>Lekakis, 1998</td>
<td>12 (100)</td>
<td>49 ±14</td>
</tr>
<tr>
<td>SSc</td>
<td>Roustit, 2008</td>
<td>42 (90)</td>
<td>51.00 ±13</td>
</tr>
<tr>
<td>SSc</td>
<td>Szucs, 2007</td>
<td>29 (86)</td>
<td>51.8 ±10</td>
</tr>
<tr>
<td>Wegener’s Granulomatosis</td>
<td>Nienhuis, 2007</td>
<td>28 (39)</td>
<td>49 ± 9</td>
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</tbody>
</table>
Table 2. Meta-Regression Analysis Summary

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>$\beta$ (95% CI, p-value)</th>
<th>Mean effect size (95% CI)</th>
<th>$\tau^2$ (95% CI, p-value)</th>
<th>$\Delta \tau^2$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept only</td>
<td>0.47 (0.34 to 0.60, &lt;.001)</td>
<td>0.47 (0.34 to 0.60)</td>
<td>0.22 (0.15 to 0.35, &lt;.001)</td>
<td>Reference</td>
</tr>
<tr>
<td>+ age</td>
<td>-0.006 (-0.017 to 0.005, 0.316)</td>
<td>0.22 (0.15 to 0.34, &lt;.001)</td>
<td>-2</td>
<td></td>
</tr>
<tr>
<td>+ sex</td>
<td>-0.409 (-1.124 to 0.306, 0.262)</td>
<td>0.21 (0.15 to 0.34, &lt;.001)</td>
<td>-3</td>
<td></td>
</tr>
<tr>
<td>+ disease duration (n=56)</td>
<td>-0.012 (-0.041 to 0.017, 0.420)</td>
<td>0.17 (0.11 to 0.30, &lt;.001)</td>
<td>-22</td>
<td></td>
</tr>
<tr>
<td>+ NOS, quality score</td>
<td>0.067 (-0.056 to 0.190, 0.284)</td>
<td>0.22 (0.15 to 0.34, &lt;.001)</td>
<td>-2</td>
<td></td>
</tr>
<tr>
<td>+ Disease type: RA</td>
<td>-0.022 (-0.491 to 0.448, 0.928)</td>
<td>0.56 (0.36 to 0.76)</td>
<td>0.21 (0.14 to 0.34, &lt;.001)</td>
<td>-4</td>
</tr>
<tr>
<td>SLE</td>
<td>-0.300 (-0.702 to 0.243, 0.340)</td>
<td>0.35 (0.14 to 0.55)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other Rheumatic</td>
<td>-0.104 (-0.614 to 0.405, 0.688)</td>
<td>0.47 (0.19 to 0.75)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SSc</td>
<td>Reference</td>
<td>0.58 (0.15 to 1.00)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ area of plaque: excluded from measure</td>
<td>-0.026 (-0.329 to 0.151, 0.866)</td>
<td>0.45 (0.18 to 0.72)</td>
<td>0.22 (0.15 to 0.35, &lt;.001)</td>
<td>0</td>
</tr>
<tr>
<td>included in measure</td>
<td>Reference</td>
<td>0.47 (0.33 to 0.62)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ pre-existing CVD: excluded from study</td>
<td>-0.314 (-0.557 to -0.071, 0.011)</td>
<td>0.65 (0.47 to 0.85)</td>
<td>0.19 (0.13 to 0.31, &lt;.001)</td>
<td>-11</td>
</tr>
<tr>
<td>not excluded from study</td>
<td>Reference</td>
<td>0.34 (0.19 to 0.50)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multivariable model (all variables below, n=56): + pre-existing CVD: excluded from study</td>
<td>-0.371 (-0.620 to -0.122, 0.004)</td>
<td>0.64 (0.46 to 0.82)</td>
<td>0.13 (0.08 to 0.24, &lt;.001)</td>
<td>-43</td>
</tr>
<tr>
<td>not excluded from study</td>
<td>Reference</td>
<td>0.27 (0.11 to 0.42)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ age</td>
<td>0.009 (-0.003 to 0.022, 0.144)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ sex</td>
<td>0.201 (-0.517 to 0.919, 0.583)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ disease duration</td>
<td>-0.016 (-0.041 to 0.017, 0.261)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Figure legends**

**Figure 1.** Flowchart of selected articles.

**Figure 2.** Meta-analysis showing the effect size (Cohen’s D) of the difference in carotid artery intima-media thickness (CIMT) between rheumatic disease patients and control subjects. Plots are separated into major rheumatic disease populations: rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), systemic sclerosis (SSc), and other rheumatic disease (Other). The random effects weight (%) of each included comparison is listed to the right of each plot.

**Figure 3.** Funnel plot of the effect size (Cohen’s D) of the difference in carotid artery intima-media thickness (CIMT) between rheumatic patients and control subjects by inverse standard error. Lines represent meta-analysis summary estimate and 95% confidence interval. Plots are separated into major rheumatic disease populations: rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), systemic sclerosis (SSc), and other rheumatic disease (Other).
Figures
Figure 1

- Search
  - Medline (n=438)
  - EMBASE (n=592)

  Potentially relevant articles identified and screened for retrieval (n=1030)
  - Exclusions (total=950)
    - Duplicates (n=271)
    - No relevant outcome measure or Not rheumatic disease or No control group (n=679)

  Articles retrieved for more detailed evaluation (n=80)
  - Exclusions (total=20)
    - Not comparable healthy controls (n=6)
    - Missing information (n=3)
    - Studies previously published (n=11)

  Articles included in the systematic review (n=60)
  - Representing 68 comparisons
Figure 2
Figure 3

The image shows a scatter plot with data points distributed across different categories labeled RA, SLE, SSc, and Other. The x-axis represents Cohen's D, and the y-axis represents Inverse Standard Error.
Chapter 3. Predictors of lipid abnormalities in children with new onset systemic lupus erythmatosus.

Publication


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Predictors of lipid abnormalities in children with new onset systemic lupus erythmatosus.

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Running title: Dyslipoproteinemia in pediatric SLE

Key words: Lupus, pediatrics, autoimmune disease, lipids, atherosclerosis

Institutions: Divisions of Rheumatology and Child Health Evaluative Sciences, The Hospital for Sick Children, Departments of Pediatrics, Immunology, and Health Policy, Management and Evaluation, and Public Health Sciences, University of Toronto, Toronto, Canada.

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Abstract

Background: Lipid abnormalities in patients with Systemic Lupus Erythematosus (SLE) are common and likely are one of the causes of premature atherosclerosis in these patients.

Objective: 1) Determine the frequency and pattern of dyslipoproteinemia at presentation of pediatric SLE; and 2) Determine the association between dyslipoproteinemia and markers of disease activity and inflammatory markers at presentation of pediatric SLE.

Methods: Serum lipid measurements were obtained at diagnosis before corticosteroid treatment for an inception cohort of 54 patients. Total cholesterol, triglyceride, LDL-C, HDL-C levels were regressed on measures of inflammation, disease activity and disease symptoms.
**Results:** At least one lipid abnormality was present in the majority of patients (63%) with an elevated triglyceride level being the most common lipid abnormality (62%). Triglycerides were best predicted by fibrinogen, nephritis and pleuritis (model $R^2=0.6$). Albumin, C4, and WBC were found to predict HDL-C (model $R^2=0.6$). ESR, CNS involvement, nasal ulcers, and nephritis were found as predictors for LDL-C:HDL-C (model $R^2=0.5$). No significant predictors were found for CHOL or LDL-C. The ECLAM disease activity score best predicted abnormal TG and HDL-C levels (OR=1.7 95% CI: 1.2 – 2.3).

**Conclusions:** Newly diagnosed children with SLE exhibited the distinct pattern of dyslipoproteinemia of increased TG and depressed HDL-C that was twice as common with the presence of kidney disease. This lipid profile puts them at risk for premature atherosclerosis. Good disease control and individualizing lipid-lowering agents based on the observed pattern of lipid abnormalities may lower the risk of premature atherosclerosis.

### 3.1 Introduction

Pediatric Systemic Lupus Erythematosus (pSLE) accounts for 20% of all SLE cases(147). Premature atherosclerosis has been recognized as an important issue for patients with Systemic Lupus Erythematosus (SLE) since the mid 1970’s(45,148). The identification of risk factors leading to premature atherosclerosis is an ongoing active area of research in SLE and has lead to the discovery of a role for both traditional risk and non-traditional risk factors(86,149,150). Among the traditional risk factors of atherosclerosis a history of smoking, diabetes, hypertension and abnormal lipid profile have been shown to be important factors(12). In patients with pediatric SLE smoking, diabetes and uncontrolled hypertension are not common and therefore abnormal lipid profiles may be the most important risk factor.

The lipid profile of both children and adults with SLE is the result of a combination of the influences of active disease, therapies and genetics. Although the influence of genetic predisposition is difficult to either alter or accurately determine, the role of disease activity and therapies, in particular corticosteroid treatment can be examined. The best way to determine the maximal potential effect of disease activity itself would be to examine patients at the time of presentation of SLE when patients are likely to have high disease activity but without any effect of corticosteroid therapy. Previous studies in patients with active SLE have suggested that there is a distinct pattern of lipid abnormalities of increased very low density lipoprotein...
(VLDL) and triglycerides and decreased high-density lipoprotein (HDL-C), cholesterol and apolipoprotein A1 levels (‘active SLE pattern’)(53,151-156). The examination of lipid levels at presentation, prior to the introduction of corticosteroid therapy would allow for a better determination of not only the lipid abnormality associated with active SLE but also of the role of inflammation in altering the lipid profile of SLE patients. Few studies to date have addressed lipid levels at the time of SLE diagnosis in children and even fewer before corticosteroid treatment(155,157-159).

The aims of this study were to: 1) Determine the frequency and pattern of dyslipoproteinemia at presentation of pediatric SLE; and 2) Determine the association between dyslipoproteinemia and markers of disease activity and inflammatory markers at presentation of pSLE.

3.2 Patients and methods

3.2.1 Study design and patient population

A single center observational study of pSLE patients was performed. The inception cohort consisted of all 54 of the 190 patients followed at SickKids Pediatric Lupus Clinic seen between May 1996 to December 2005 who meet the following inclusion criteria: 1) Fulfilled at least four of eleven ACR classification criteria for the diagnosis of pSLE (160); 2) Onset of disease prior to their 18th birthday; 3) Fasting lipid profile (Cholesterol (CHOL), Low Density Lipoprotein (LDL-C), High Density Lipoprotein (HDL-C), and Triglycerides (TG) levels performed within 6 months of diagnosis; and 4) Lipid testing was performed prior to the onset of treatment with corticosteroids, or a minimum of 30 days after cessation of, all possible corticosteroid drug use for medical conditions other than pSLE (1 patient met this condition). Ethics approval was obtained from the Research Ethics Board at SickKids.

3.2.2 Data collection

3.2.2.1 Lipid profile

Measurements for CHOL, TG, LDL-C, and HDL-C were obtained following an overnight fast. Lipids were analyzed in the biochemistry laboratory at SickKids using an automated analyzer (Vitros; Ortho-Clinical Diagnostics, Rochester, NY, USA), which uses dry slide chemistry technology. LDL-C levels were calculated according to the following formula: LDL-C
(mmoles/liter) = CHOL – HDL-C –\((TG/2.2)\). LDL-C estimation is not available for triglycerides exceeding 4.00 mmoles/liter but none of the triglyceride levels measured in our cohort exceeded this value.

For CHOL, TG, and LDL-C, the percentage of patients with values above the age and gender specific normal range were considered abnormal, while for HDL-C, percentages below the normal range were abnormal. As normal serum lipid values are age and gender dependent, z-scores were calculated.

### 3.2.2.2 Clinical and laboratory measurements

All data were prospectively collected and recorded using a standard protocol. Independent disease symptom variables were dichotomous (present/absent) and included the following: Alopecia, photosensitivity, arthritis, central nervous system involvement, diffuse lymphadenopathy, digital ulcer, headache, malar rash, other rashes, myositis, oral and nasal ulcers, kidney involvement (includes nephritis diagnosed by biopsy or persistently abnormal urinalysis and nephrotic syndrome diagnosed by proteinuria (>50 mg/kg/day) and hypoalbuminemia (<30 gm/l), pericarditis, pleuritis, Raynaud’s Phenomenon, and the presence of anti DNA antibodies and antiphospholipid antibodies (aPL) (positive for either or both of IgG anticardiolipin antibodies and Lupus Anticoagulant). The continuous variables examined were: the disease activity scores, Systemic lupus erythematosus disease activity index (SLEDAI or SLEDAI-2k(161)) and European consensus lupus activity measure (ECLAM) which were prospectively obtained at the first visit(162-164) and laboratory inflammation markers: Albumin, complement level C3 and C4, c-reactive protein (CRP), erythrocyte sedimentation rate (ESR), fibrinogen, hemoglobin level, proteinuria (total grams protein per 24 hrs urine collection), and white blood cell count (WBC). All patients were assessed for disease symptoms at presentation. Not all patients had disease activity scores and inflammatory markers recorded within 7 days of the lipid profile.

### 3.2.3 Statistical analysis

Univariate regression analysis was used to determine the association of serum lipids with measurements of disease activity and inflammation and to screen continuous variables for co-
linearity and Chi-square analysis was used for assessing categorical variables for extremely high agreement. A single patient was found to have significantly different values (outlier) for CHOL and LDL-C from the inception cohort. All analyses were performed with and without this patient’s data. No significant differences were found and we chose to report all analysis results having excluded this patient. The ratio of LDL-C to HDL-C was found to be positively skewed and, therefore, data were log transformed for analysis. Each lipid (continuous dependent variable) was tested against each independent variable (disease activity scores, inflammation measures, disease symptoms) separately to determine the significance of the association. Only associations where $r \geq 0.3$ with p-values $< 0.05$ were considered statistically significant and were retained for analysis. In each case the magnitude of the effect ($R^2$) was considered. Following variable selection, multiple regression models (or logistic models for categorical outcomes) were used to determine which variables were significant predictors of each lipid. Models were first developed using a stepwise regression method. The threshold for entry of variables into the model for the stepwise procedure was $p \leq 0.10$ and $p > 0.05$ for removal of variables from the model. Adjustments were also made by excluding variables from the model that were suspected of being clinically similar, found to be statistically interrelated and added little information to explain additional variance. In each model, standardized regression coefficients ($\beta$) were reported to show the magnitude of the contribution of each predictor to the regression equation. All statistical test results were considered significant at the 0.05 level and multiple comparisons were corrected using Tukey’s studentized range test. SAS 9.1 for windows (SAS Institute Inc., NC, USA) was used for all analyses.

### 3.3 Results

All 4 lipid levels were evaluated in 54 patients at the time of diagnosis of pSLE prior to therapy with corticosteroids. Four patients had received anti-malarial treatment and six patients, NSAIDS, before or at the time of lipid measurement. Mean time from diagnosis to testing was 8.7 days (SD=49.4). Fifty patients (93%) were female and the mean age at time of diagnosis for the cohort was 13.4 years (SD=3.1 years). There was no statistically significant difference in any of the clinical characteristics at time of diagnosis of the inception cohort and total SickKids pSLE cohort (Table 1). The mean BMI for 41 of 54 patients was 20.8 (SD=5.1) and was found
to be positively correlated with HDL-C \((r=0.43\ p=0.0047)\) and total cholesterol \((r=0.31\ p=0.0465)\).

3.3.1 a) Lipid abnormalities

The mean serum lipid levels and disease measures at diagnosis for the inception cohort are shown in Table 2. At least one lipid abnormality was present in the majority of patients (63%) with an elevated triglyceride level being the most common lipid abnormality (62%) and elevated LDL-C the least common (4%). Abnormally low levels of HDL-C and abnormally high CHOL levels were found in 24% and 20% of patients, respectively. Only 1 patient (2%) had abnormal levels in all 4 lipids and 13 patients (24%) had the ‘active’ lupus lipid profile of abnormally low HDL-C and elevated triglyceride levels\(^{11}\). Nine of these patients had kidney involvement and 4 no kidney involvement. By grouping the cohort by patients’ level of kidney involvement (no kidney involvement, nephritis but not nephrotic, and nephrotic syndrome) we show in figure 1 for CHOL, TG, and LDL-C levels, the percentage of patients with values above the normal range are shown, while for HDL-C, percentages below the normal range are shown. Very little difference exists between the groups for CHOL or LDL-C. Half of patients with kidney involvement (nephritis or nephrotic syndrome), have abnormal low HDL-C levels and most have abnormally high TG levels. This is close to twice or more than what was observed in patients with no kidney involvement.

Interestingly, 43% of patients had abnormally low CHOL and 31% abnormally low LDL-C levels while none of the patients had an abnormally low TG or abnormally high HDL-C level. Mean z-score values were significantly different from 0 for all 4 lipids although only the mean value of the z-scores for triglyceride levels was found to be outside the normal range of 2 standard deviations (TG level: 3.35 SD±3.32 \(p<0.0001\)). The mean z-scores for the other lipids demonstrated that both the mean CHOL level at \(-0.93\ SD±2.90\ (p=0.0235)\) and LDL-C level at \(-1.33\ SD±1.52\ (p<0.0001)\) were significantly lower than expected as was the mean HDL-C level at \(-1.43\ SD±1.00\ (p<0.0001)\) (Figure 2).

3.3.2 b) Correlation of lipids with measures of disease
We determined the association of individual disease manifestations, laboratory measures and
disease activity measures listed in table 2 with the individual lipid levels. We chose to only
include “Kidney involvement” as Nephritis and Nephrotic Syndrome are closely associated.
Only statistically significant associations (p<0.05) with $R^2 \geq 0.09$ were considered. Odds ratios
were calculated for binary independent variables (Table 3).

Triglyceride levels were significantly associated with both of the disease activity measures
(SLEDAI and ECLAM), fibrinogen, albumin, C3 and C4 levels (the latter 3 were negative
associations), serositis (pleuritis, pericarditis), nephritis, and non-malar rash. The only
statistically significant associations for cholesterol were with WBC and headache. LDL-C
levels were associated with CNS involvement, headache, and myositis but none of the
laboratory or disease activity measures. HDL-C levels were negatively correlated with both
disease activity measures, ESR, nephritis, nasal ulcers, and CNS involvement and positively
associated with albumin, C3, C4, hemoglobin, WBC. The LDL-C:HDL-C ratio was positively
associated with both disease activity scores, ESR nephritis, and nasal ulcers, and negatively
with albumin, hemoglobin and C3, C4 levels, and CNS involvement (Table 3).

3.3.3 c) Multiple regression analysis of serum lipid levels: Statistically significant (p<0.05
and r>0.3) variables were entered into multiple regression analysis (Table 4). Only models with
an $R^2$ of 30% or greater were considered. Stepwise multiple regression revealed albumin, C4,
and WBC as the significant predictors of HDL-C (model $R^2=0.6$). Triglycerides were best
predicted by fibrinogen, nephritis and pleuritis (model $R^2=0.6$). ESR, CNS involvement, nasal
ulcers, and nephritis were found as significant predictors for LDL-C:HDL-C-C (model $R^2=0.5$).
None of the variables we investigated were found to be significant predictors of CHOL or
LDL-C.

3.3.4 d) Multiple logistic regression analysis of combined abnormality of increased TG
and decreased HDL-C: Variables which were statistically significantly associated with the
active lupus lipid profile(154) (also the most common combined lipid abnormality) were
entered into stepwise logistic regression analysis. The ECLAM disease activity score was found
to be the best statistically significant predictor with an odds ratio of 1.7 (95%CL: 1.2 – 2.3)
while the SLEDAI had an odds ratio of 1.1 (95%CL: 1.0 – 1.2). None of the other variables remained in the model.

### 3.3.5 e) Association of organ disease with abnormal triglyceride and HDL-C levels:

When we examined the association of active nephritis and abnormal lipid levels we found that 86% (6/7) of patients with nephritis but not nephrotic and 100% (12/12) of patients with nephrotic syndrome were also found to have abnormal TG levels while only 42% (4/7) of patients with nephritis but not nephrotic and 47% (5/12) of patients with nephrotic syndrome had abnormally low HDL-C levels. Having both abnormal levels of TG and HDL-C was found in 57% (5/7) of nephritis but not nephrotic and 42% (5/12) of nephrotic syndrome patients. In order to determine the association of CNS involvement and abnormal TG and HDL levels, we eliminated the 3 patients who had both CNS and kidney involvement which left only 4 patients with CNS involvement without kidney disease. Only 25% of these patients had abnormal TG levels and none had abnormally low HDL-C levels.

In order to further explore the role of nephritis and proteinuria in determining abnormal TG levels we categorized patients with kidney involvement into: nephrotic syndrome, nephritis but not nephrotic, and no kidney involvement. Measures of proteinuria as total grams protein per 24 hrs urine collection were obtained for 26 patients (median=0.31 0.04-7.4). Those patients that were not tested were assumed to have values <0.02 g/ 24hrs as there was no indication of nephritis. Post hoc ANOVA analysis indicated that low serum albumin levels were associated with kidney involvement ($R^2= 0.55 p<0.0001$), TG levels ($R^2= 0.35 p<0.0001$) and disease activity as measured by the SLEDAI ($R^2= 0.52 p<0.0001$). After adjusting for serum albumin levels using ANCOVA, there was still a statistically significant association of a high TG level and the presence of kidney disease ($p=0.0119$). We found that the mean TG level was significantly higher in patients with nephrotic syndrome as compared to nephritis but not nephrotic and no kidney involvement, however, there was no significant difference found between nephritis but not nephrotic and no kidney involvement. The adjusted mean (least square mean) TG levels were significantly higher in nephrotic syndrome (2.24 mmol/L) as compared to nephritis but not nephrotic (1.63 mmol/L) and no kidney involvement (1.40 mmol/L).
In comparison we did not find any association of HDL with kidney involvement categorized into having nephrotic syndrome, nephritis but not nephrotic, or no kidney involvement using the same methodology.

3.4 Discussion
SLE is an autoimmune disease characterized by chronic inflammation and frequently requires treatment with prolonged courses of high dose corticosteroids. Both of these factors have been associated with the development of an abnormal lipid profile and premature atherosclerosis. This association has been confirmed by studies in SLE which have demonstrated that both cardiovascular and cerebral vascular events are significantly more common in young adults with SLE than the general population(45,148). Although multiple studies have demonstrated that patients with SLE have abnormal lipid profiles, it has been difficult to differentiate the precise role of the inflammation and of the therapy on patients’ lipid profiles(155). The best way to determine the maximal role of inflammation on lipid profiles in SLE is to study lipid levels at diagnosis of SLE prior to therapy. The study presented here is a continuation of previous work by Sarkissian et al(155) and 9 of our 54 patients were included in both studies. In our current study, we found that in a large inception cohort of patients with pSLE, the majority of patients had at least one lipid abnormality. An elevated triglyceride level was the most common lipid abnormality seen in the majority of patients (61%) while a high LDL-C level was the least common (seen in only 4%).

All patients with nephritis and/or nephrotic syndrome were found to have abnormally high TG levels which were associated with abnormally low HDL-C levels in approximately 50%. Post hoc analysis suggested TG levels were highest in patients with active kidney disease even after adjustment for the potential confounding effects of albumin. These findings suggest that there may be another process contributing to an elevated TG level intrinsic to SLE nephritis other than the effects of a reduced albumin level that is typical of nephrotic syndrome (which itself has been associated with elevated triglyceride levels(165,166). A previous pediatric study demonstrated that elevated triglycerides and depressed HDL-C levels were associated with kidney disease although they did not attempt to differentiate the effect of nephrotic syndrome from the potential confounding effect of nephrotic range proteinuria(157). In contrast we did not find any association of HDL levels and kidney disease suggesting that the processes(s) driving low albumin levels may also be responsible for decreased HDL-C levels. Furthermore,
though only 4 patients had only CNS without kidney involvement, the majority of these patients (75%) did not have abnormal TG or HDL-C levels despite having active major organ involvement and high disease activity scores. This observation suggests that disease activity resulting from kidney or CNS involvement may differ in their effects on serum lipids. The ‘active lupus pattern’ of elevated triglyceride and decreased HDL-C was initially described in 1988 in pediatric patients and later in adult patients(151,154). The main limitations to the previous studies were the relatively small number of patients, the fact that not all patients were seen at presentation, and serum lipid measurements were not made exclusively before treatment. In our cohort of 54 patients, 24% of patients had the combination of an abnormal triglyceride and HDL-C levels. This lipid profile was the result of active SLE, and in particular active nephritis, as it was seen prior to initiation of prednisone therapy and at presentation which is generally associated with significantly active disease as seen in our patients who had a median SLEDAI level of 9.5. These findings expand previous results in adults that also suggested that lipid profile abnormalities in lupus are aggravated by disease activity(151).

Previous studies have also suggested that dyslipoproteinemia of active SLE may be secondary to the effects of TNFα and/or autoantibodies(156,167). Furthermore, disease activity (ECLAM or SLEDAI) was found to best predict ‘lupus dyslipoproteinemia’. The active lupus pattern of dyslipoproteinemia is not restricted to SLE patients as it is also seen in other instances associated with acute inflammation including sarcoidosis and macrophage activation syndrome(168,169). We suggest that the best way to alleviate these lipid abnormalities and decrease the risk of cardiovascular disease is by good control of the patient’s SLE.

As may be expected in the absence of corticosteroid therapy measures of inflammation were found to be related to most lipid levels. Elevated inflammatory markers including, fibrinogen, CRP, ESR and albumin levels have been shown to be predictors for risk of cardiovascular disease(170,171). Interestingly, global measures of disease activity did not correlate with all lipids and the best predictors for lipid levels were frequently measures of organ-specific disease. It therefore appears that in the absence of corticosteroid therapy, an abnormal lipid profile was likely secondary to active SLE with major organ involvement. This was supported by the association of LDL-C:HDL-C ratio with markers of both kidney and neuropsychiatric diseases.
Both the LDL-C and total cholesterol levels were most often either normal or depressed but not increased in these patients. This also contributed in a LDL-C:HDL-C ratio in the normal range, a finding which is generally associated with a low risk of coronary artery disease (172). However, because elevated TG levels, a risk factor for cardiovascular disease (reviewed (173)) were found in the majority of patients (62%), early recognition of this lipid abnormality may be important in decreasing the risk of cardiovascular disease.

We acknowledge the limitations imposed by the relatively small sample size of this study. The possible effects of diet and physical activity on our lipid prediction models were not considered in this study. Though there was some missing data for some of the laboratory measurements which contributed to a larger standard error, we are confident that our results reflect the true relationship that exists between the ‘active lupus pattern’ and clinical and laboratory measures.

In conclusion, newly diagnosed children with SLE exhibited the distinct pattern of dyslipoproteinemia of increased TG and depressed HDL-C that was twice as common with the presence of kidney disease. This lipid profile puts them at risk for premature atherosclerosis. We suggest that disease control will correct these lipid abnormalities and that the individual patient’s observed pattern of lipid levels should be determined to correctly select a lipid-lowering strategy. It is likely that good disease control is the optimum way to prevent premature atherosclerosis in pediatric SLE.
## Tables

### Table 1: Patient characteristics at presentation

<table>
<thead>
<tr>
<th></th>
<th>Inception cohort</th>
<th>All Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>54</td>
<td>190</td>
</tr>
<tr>
<td>Gender M:F (%Female)</td>
<td>4:50 (92.6)</td>
<td>33:157 (82.6)</td>
</tr>
<tr>
<td>Mean age (Years ±SD)</td>
<td>13.4 ±3.1</td>
<td>13.2 ±3.3</td>
</tr>
<tr>
<td>Mean BMI (Units ±SD)</td>
<td>20.7 ±5.0</td>
<td>20.1 ±4.4</td>
</tr>
</tbody>
</table>

Symptoms at Diagnosis (%Present)*:

**Mucocutaneous:**
- Malar Rash: 79.6 68.4
- Non-Malar Rash: 35.2 33.7
- Alopecia: 29.6 22.6
- Photosensitivity: 22.2 22.6
- Oral Ulcers: 20.4 24.7
- Nasal Ulcers: 7.4 7.4
- Digital Ulcer: 3.7 3.2
- Raynauds: 14.8 15.3
- Arthritis: 59.3 63.7
- Kidney involvement: 35.2 39.1
  - Nephritis but not nephrotic: 13.0 12.0
  - Nephrotic syndrome: 22.2 27.1
- Diffuse Lymphadenopathy: 16.7 23.2
- Central nervous system: 13.0 17.9
  - Headache: 9.3 14.7
- Pleuritis: 3.7 12.1
- Pericarditis: 3.7 7.9
- Myositis: 3.7 4.2
- Anti-DNA: 57.4 62.0
- Antiphospholipid: 59.3 55.7

*within 6 months of Diagnosis*
### Table 2: Clinical and laboratory parameters

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean (Median)</th>
<th>SD (min-max)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHOL</td>
<td>3.5</td>
<td>0.9</td>
<td>53</td>
</tr>
<tr>
<td>TG</td>
<td>1.6</td>
<td>0.7</td>
<td>53</td>
</tr>
<tr>
<td>LDL-C</td>
<td>2.0</td>
<td>0.7</td>
<td>53</td>
</tr>
<tr>
<td>HDL-C</td>
<td>0.8</td>
<td>0.4</td>
<td>53</td>
</tr>
<tr>
<td>LDL-C:HDL-C</td>
<td>3.0</td>
<td>1.8</td>
<td>53</td>
</tr>
<tr>
<td>Disease</td>
<td>SLEDAI</td>
<td>(9.5)</td>
<td>(0-34)</td>
</tr>
<tr>
<td>Activity</td>
<td>ECLAM</td>
<td>(5.0)</td>
<td>(0-10)</td>
</tr>
<tr>
<td>Inflammation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin</td>
<td>37.3</td>
<td>7.3</td>
<td>53</td>
</tr>
<tr>
<td>C3</td>
<td>0.8</td>
<td>0.4</td>
<td>51</td>
</tr>
<tr>
<td>C4</td>
<td>0.1</td>
<td>0.1</td>
<td>51</td>
</tr>
<tr>
<td>CRP</td>
<td>4.9</td>
<td>14.7</td>
<td>40</td>
</tr>
<tr>
<td>ESR</td>
<td>62.1</td>
<td>39.2</td>
<td>51</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>3.2</td>
<td>0.8</td>
<td>32</td>
</tr>
<tr>
<td>HGB</td>
<td>111.3</td>
<td>19.4</td>
<td>53</td>
</tr>
<tr>
<td>WBC</td>
<td>5.6</td>
<td>3.2</td>
<td>53</td>
</tr>
</tbody>
</table>

ALB = Albumin (g/L)
C3, C4 = Complement Components 3 and 4 (g/L)
CHOL = Total Cholesterol (mmol/L)
CRP = C-Reactive Protein
ECLAM = European Consensus Lupus Activity Measure
ESR = Erythrocyte Sedimentation Rate (mm/hr)
FIB = Fibrinogen
HDL-C = High density lipoprotein (mmol/L)
HGB = Hemoglobin (g/L)
LDL-C = Low density lipoprotein (mmol/L)
SLEDAI = Systemic Lupus Erythematosus Disease Activity Index
TG = Triglyceride (mmol/L)
WBC = White Blood Cell Count (x10⁹/L)
Table 3: Univariate analysis of variance of serum lipid levels at diagnosis with inflammation measures and disease symptoms

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Symptom</th>
<th>$R^2$ (OR)</th>
<th>P-Value (95% CL)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHOL</td>
<td>WBC</td>
<td>0.12</td>
<td>0.0096</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>Headache</td>
<td>(6.1)</td>
<td>(1.2 - 31.2)</td>
<td>53</td>
</tr>
<tr>
<td>LDL-C</td>
<td>Myositis*</td>
<td>(0.03)</td>
<td>(0.001 – 0.9)</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>Headache</td>
<td>(5.3)</td>
<td>(1.1 – 26.2)</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>CNS*</td>
<td>(0.2)</td>
<td>(0.03 – 0.9)</td>
<td>53</td>
</tr>
<tr>
<td>HDL-C</td>
<td>Albumin</td>
<td>0.42</td>
<td>&lt;0.0001</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>ECLAM*</td>
<td>0.41</td>
<td>&lt;0.0001</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>C3</td>
<td>0.36</td>
<td>&lt;0.0001</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>Kidney involvement*</td>
<td>(0.001)</td>
<td>(&lt;0.001 – 0.05)</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>SLEDAI*</td>
<td>0.30</td>
<td>&lt;0.0001</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>C4</td>
<td>0.27</td>
<td>&lt;0.0001</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>ESR*</td>
<td>0.27</td>
<td>0.0001</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>HGB</td>
<td>0.26</td>
<td>0.0001</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>WBC</td>
<td>0.16</td>
<td>0.0028</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>Nasal Ulcers*</td>
<td>(&lt;0.001)</td>
<td>(&lt;0.001 – 0.3)</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>Other Rash*</td>
<td>(0.1)</td>
<td>(0.02 – 0.9)</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>aPL*</td>
<td>(0.1)</td>
<td>(0.02 – 0.7)</td>
<td>53</td>
</tr>
<tr>
<td>TG</td>
<td>Albumin*</td>
<td>0.35</td>
<td>&lt;0.0001</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>Kidney involvement</td>
<td>(9.8)</td>
<td>(2.8 – 35.0)</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>SLEDAI</td>
<td>0.29</td>
<td>&lt;0.0001</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>ECLAM</td>
<td>0.27</td>
<td>&lt;0.0001</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>Fibrinogen</td>
<td>0.14</td>
<td>0.0319</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>C3*</td>
<td>0.14</td>
<td>0.0071</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>Other Rash</td>
<td>(3.2)</td>
<td>(1.3 – 7.6)</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>Pleuritis</td>
<td>(22.2)</td>
<td>(1.1 – 468.7)</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>C4*</td>
<td>0.13</td>
<td>0.0095</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>Anti-DNA</td>
<td>(2.5)</td>
<td>(1.1 – 5.8)</td>
<td>53</td>
</tr>
<tr>
<td>Lipid</td>
<td>Variable</td>
<td>Regression Coefficient</td>
<td>P-value</td>
<td>R²</td>
</tr>
<tr>
<td>-----------------</td>
<td>-------------------</td>
<td>------------------------</td>
<td>---------</td>
<td>------</td>
</tr>
<tr>
<td>HDL-C</td>
<td>Albumin</td>
<td>0.027</td>
<td>&lt;0.0001</td>
<td>0.577</td>
</tr>
<tr>
<td></td>
<td>C4</td>
<td>1.139</td>
<td>0.0187</td>
<td></td>
</tr>
<tr>
<td></td>
<td>WBC</td>
<td>0.029</td>
<td>0.0127</td>
<td></td>
</tr>
<tr>
<td>TG</td>
<td>FIB</td>
<td>0.308</td>
<td>0.0129</td>
<td>0.629</td>
</tr>
<tr>
<td></td>
<td>Kidney involvement</td>
<td>0.879</td>
<td>0.0002</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pleuritis</td>
<td>1.392</td>
<td>0.0010</td>
<td></td>
</tr>
<tr>
<td>LDL-C:HDL-C</td>
<td>CNS</td>
<td>-0.420</td>
<td>0.0197</td>
<td>0.499</td>
</tr>
<tr>
<td></td>
<td>ESR</td>
<td>0.004</td>
<td>0.0231</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nasal Ulcers</td>
<td>0.633</td>
<td>0.0088</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kidney involvement</td>
<td>0.292</td>
<td>0.0498</td>
<td></td>
</tr>
</tbody>
</table>

* Negative association
Figures

Figure 1

![Bar chart showing the percentage of abnormal lipids by kidney involvement.](chart_image)
Figure 2
Figure legends

Figure 1: Percent abnormal serum lipid measurements for patients with no kidney involvement, nephritis but not nephrotic, and nephrotic syndrome. On the x-axis are the different serum lipid measures as indicated. On the y-axis is the percentage of patients with an abnormal lipid value adjusted for age and gender. Actual percentages are listed above the respective bars.

Figure 2: Z-score values for serum lipid measurements. On the x-axis are the different serum lipid measures as indicated. On the y-axis is the z-score with standard deviations as units. The mean z-score value is indicated by a star and labeled with the numeric value. Two horizontal lines represent +2 and -2 standard deviations from a z-score of 0.
Chapter 4. Lipid Profiles of Children with Rheumatic Disease

Lipid Profiles of Children with Rheumatic Disease

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Key words: Lupus, pediatrics, autoimmune disease, vascular function, atherosclerosis

Word count: Abstract = 273  Body = 3385

Short running title: Lipids in pediatric rheumatic disease.

Abstract

Purpose: Rheumatic disease and atherosclerosis are both inflammatory conditions. The atherogenic process can start as early as childhood and worsens with age and by traditional risk factors. The aims of this study were to assess the lipid profile and the role of treatment and
disease activity related factors in children with SLE, systemic juvenile idiopathic arthritis (SJIA) and juvenile dermatomyositis (JDM).

**Methods:** Subjects were enrolled into a prospective longitudinal study and assessed following the first visit. Drug therapy and disease activity were recorded. Fasting lipid and glycemic profiles were performed. These indices were converted to z-scores. Between group comparisons were made using parametric methods.

**Results:** Of the 137 children tested, SLE patients were older and more predominantly female (n = 88, mean age: 15.4± 2.5, 83% female) than JDM (n = 28, 13.9± 2.3, 50% female) and SJIA patients (n = 21, 13.9 ± 2.4, 57% female). Most children had a relatively healthy BMI (mean 22.4 ± 4.6) and a normal lipid profile of total cholesterol, LDL and HDL cholesterol, and triglyceride levels. However, one quarter of all patients were found to have insulin resistance. Children with JDM exhibited the highest prevalence of dyslipidemia (54%) followed by SLE (40%) and SJIA (33%). At the time of testing, subjects had been followed for a mean of 3.1± 3.0 years and 91% had been treated with corticosteroids (mean cumulative dose/kg: 0.2± 0.3g).

**Conclusion:** We found that most children with rheumatic disease had a normal lipid profile. The most common abnormalities were found for total cholesterol and triglyceride levels. 25% of all patients had insulin resistance that could not be solely attributed to traditional CVD risk factors.

**4.1 Introduction**

The atherogenic process can start early in childhood, as studies of the aorta of children and young adults have shown(9,174).

The risk of developing atherosclerosis increases with age and traditional cardiovascular disease (CVD) risk factors: blood levels of total and high-density lipoprotein (HDL) cholesterol, blood pressure, smoking status, diabetes mellitus and left ventricular hypertrophy(12). One of the main, potentially alterable, determinants of atherosclerotic risk in the general population is the concentration of serum low-density lipoprotein (LDL) and HDL cholesterol(19,175). Current evidence strongly suggests that chronic inflammation is an important factor leading to atherosclerosis, which may partly be due to the effect of abnormal circulating lipids on the endothelium(7).
Lipid abnormalities are common in chronic inflammatory diseases and are likely the result of chronic inflammation that may be modified by immunosuppressive therapy (153, 176, 177). Dyslipidemia resulting from chronic inflammation is not limited to adults as we and others have previously shown that pediatric patients with the rheumatic disease, systemic lupus erythematosus (pSLE), even at initial presentation and before treatment with corticosteroids, have elevated triglycerides but depressed HDL, LDL, and total cholesterol levels (178, 179). The lipid profiles were modified by treatment with corticosteroids with normalization of triglycerides and a rise in HDL, LDL, and total cholesterol levels, likely as a result of controlling the inflammation (155).

PSLE, Juvenile dermatomyositis (JDM) and systemic juvenile idiopathic arthritis (SJIA) are three pediatric rheumatic diseases characterized by chronic inflammation that are treated with corticosteroids. To date, few studies have assessed lipids in children with rheumatic disease (154, 159, 178, 180-182) and to date there has not been any study that compared lipid profiles from these three childhood rheumatic diseases. It is not known whether the burden of chronic inflammation and/or anti-rheumatic treatment from these different diseases has similar influence on lipid metabolism and, therefore, atherosclerotic risk.

The objectives of this study were: 1) To determine lipid profiles in patients with JDM and SJIA and to compare and contrast the lipid profiles of patients with JDM, SJIA and pSLE; 2) Determine the association between dyslipoproteinemia and inflammation, disease activity, and treatment.

4.2 Patients and methods

4.2.1 Study design and patient population

A prospective, prevalence cohort of patients attending the rheumatology clinic at The Hospital for Sick Children (SickKids), Toronto was collected between September 2002 and June 2009. Consecutive patients between the ages of 8 to 18 years and who fulfilled the American College of Rheumatology (ACR) classification criteria for SLE (183), Bohan and Peter Criteria for JDM (184), and International League Against Rheumatism Criteria for SJIA (185) were approached for inclusion in the study. Written, informed consent was obtained from either the
participant or their parent(s). The study was approved by the SickKids Research Ethics Board (REB# 2002-168).

### 4.2.2 Laboratory measurements

Laboratory parameters were measured after a 12 hour fast.

1) Lipids
   Total cholesterol, LDL-C, HDL-C and tryglycerides were analyzed in the biochemistry laboratory at SickKids Hospital using an automated analyzer (Vitros; Ortho-Clinical Diagnostics, Rochester, NY, USA), which uses dry slide chemistry technology. LDL-C levels were calculated according to the following formula: LDL-C (mmoles/liter) = CHOL – HDL-C – (TG/2.2). LDL-C estimation was not available for triglycerides exceeding 4.00 mmoles/liter. For cholesterol, triglycerides, and LDL-C, the percentage of patients with values above the age and gender specific normal range were considered abnormal, while for HDL-C, percentages below the normal range were abnormal. As normal serum lipid values are age- and sex-dependent, z-scores were calculated. Apoprotein A1 (APO A1) (APOAT in vitro diagnostic reagent system, Cobas Integra Tina-Quant, Roche Diagnostics, Indianapolis, IN, USA), apoprotein B (APO B) (Cobas Integra Tina-Quant APOBT in vitro diagnostic reagent system, Roche Diagnostics, Indianapolis, IN, USA); and lipoprotein A (Lp(a)) (Cobas Integra Tina-Quant LPALX in vitro diagnostic reagent system, Roche Diagnostics, Indianapolis, IN, USA) were measured in the Lipid Research Laboratory of Dr. Adeli at SickKids; Anti-oxidizedLDL (ox-LDL) antibodies were measured using a commercially available direct ELISA assay according to instructions outlined by manufacturer (ELISA Kit, APLCO Diagnostics, New Hampshire, USA) in the Research Laboratory of Dr. Silverman at SickKids.

2) Glucose control
   Insulin, C-peptide, glucose, hemoglobin_{A1c} (Hb_{A1c}) and insulin resistance were measured. Insulin resistance was measured using the Homeostasis Model-Insulin Resistance (HOMA-IR) which relates fasting glucose (FG; mmol/L) and insulin levels (FI; μU/mL) using the following formula: \( HOMA-IR = FI \times FG/22.5 \) (Radziuk 2000). This has been shown to correlate well in children to the hyperinsulinemic euglycemic clamp measure of insulin resistance measurement(186).

3) Markers of inflammation and non-traditional CVD
Fibrinogen, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), albumin, complement levels C3 and C4, white blood cell count (WBC) and homocysteine were measured in the clinical laboratory at SickKids.

4) Medication
Corticosteroid usage (cumulative dose was assessed from time of diagnosis to time of measure), hydroxychloroquine and immunosuppressant therapy including cyclophosphamide, azathioprine, cyclosporine, and methotrexate (considered if used within one month of time of measure) were obtained on all patients.

5) Disease-related risk factors
Disease activity was measured using the physician global assessment scale (100 mm visual analog scale where 0 represents no activity) for all patients. The modified systemic lupus erythematosus disease activity index (SLEDAI-2K)(187) and the European Consensus Lupus Activity Measure (ECLAM)(188) were also recorded for SLE patients only. Creatine phosphokinase (CPK) and nailfold capillaroscopy abnormalities were recorded for JDM patients only and number of active joints was recorded for SJIA patients only.

4.2.3 Statistical analysis

Descriptive statistics were reported with mean ± SD or median (minimum-maximum) for continuous variables and as percentages for categorical variables. Univariable analysis (Pearson’s correlation) was used to determine the relationship of responses (lipid measures and other CVD risk factors) to explanatory variables (measurements of disease activity and inflammation) and to screen continuous variables for co-linearity. Only statistically significant associations (p < 0.05) with $r \geq 0.3$ or $r < -0.3$ were considered. A Bonferroni correction was applied to alpha (0.05) in order to correct for the multiple correlations. Chi-square analysis was used for assessing categorical variables for extremely high agreement. Comparative linear regression analysis was performed with lipid measures as the response and disease type as the group variable. For predictive modeling, statistically significant (p < 0.05 and $r > 0.3$) correlations of lipid measures with markers of disease and inflammation were entered into multivariable regression analysis. Only models with an $R^2$ of 30% or greater were considered. Highly positively skewed variables were log-transformed when appropriate before analysis. Variable comparisons were performed using chi-square or Fisher’s exact test for categorical variables and ANOVA, Student’s t-test or Wilcoxon’s sign rank test for continuous variables,
where appropriate. All statistical test results were considered significant at the 0.05 level. SAS 9.2 for windows (SAS Institute Inc., NC, USA) was used for all analyses.

4.3 Results

The cohort consisted of a total of 137 patients, 99 (72%) were female (Table 1). The overall mean age at diagnosis was 11.8 ± 3.9 years at the time of study. SLE patients were significantly older and more often female (Table 1). The overall mean disease duration was 3.1 ± 3.0 years at the time of study with SLE patients having statistically significantly shorter disease duration as compared to both JDM and SJIA patients. Medications at the time of the study are shown in Table 1.

4.3.1 Traditional CVD risk factors

Most children had a BMI (mean 22.4 ± 4.6) within the ‘healthy’ range with 31 (23%) patients overweight and 17 (12%) obese [JDM: 7 (25%) and 2 (7%); SJIA: 6 (29%) and 2 (10%); SLE: 18 (20%) and 13 (15%), respectively]. Fourteen (10% of cohort) patients required antihypertensive medication at the time of study (12/14 were SLE patients) but all had well controlled blood pressure. Of the 104 patients who provided their family history there was no significant difference for this risk factor among the groups. One of the patients 12 years of age or older admitted to smoking (patients less than 12 years old were assumed to be non-smokers) and none of the patients had diabetes mellitus (Table 2). We then considered the number of abnormal traditional risk factors (lipids, hypertension, BMI, diabetes, smoking, family history of CVD) per subject. We found that of the 99 patients, for whom we had complete data, 63% of patients had one or more risk (median of 1 range of 0 to 4) and 23% had 2 or more risks. The most prevalent risks were abnormal lipids (37%) and BMI (36%). No significant difference was found between disease groups (p = 0.640).

4.3.2 Lipid levels

No significance differences were observed for mean lipid values between disease groups (Table 2) Children with JDM exhibited the highest prevalence of dyslipidemia at more than half of
patients followed by SJIA and then SLE (Figure 1a). Total cholesterol and triglycerides were the lipids most frequently abnormal (Figure 1b).

Although all of the mean z-score values for all 4 of the traditional lipid values were within the normal range of 2 SD for the 3 disease groups, differences from predicted (mean z-score of 0) existed (mean z-score, p-value): total cholesterol [all: 0.74, p=0.004; JDM: 1.19, p=0.015; SJIA 0.61, p=0.394; SLE 0.63, p=0.061], LDL cholesterol [all: -0.68, p<0.001; JDM -0.52, p=0.051; SJIA -0.72, p=0.075; SLE -0.73, p<0.001], HDL cholesterol [all: -0.14, p=0.096; JDM -0.20, p=0.323; SJIA -0.36, p=0.057; SLE -0.06, p=0.551] and triglyceride levels [all: 0.97, p<0.001; JDM 1.81, p=0.004; SJIA 0.95, p=0.211; SLE 0.71 p=0.011].

No significant differences among the groups for other lipid values (apolipoproteins, ApoB:ApoA1 and L(p)a) and for anti-ox-LDL antibodies were observed (Table 3).

4.3.3 Glucose control

The mean insulin, glucose and HbA1c were all within the normal range for all 3 groups of patients (Table 2). All 137 patients had HbA1c levels below 6.5%. Although the mean HOMA-IR was in the normal range at 2.5 ± 1.8, 23/91 (25%) patients (5/28 JDM, 6/21 SJIA, 12/88 SLE, p = 0.577) had a value above the cutoff point of 3.16 for children and adolescents suggested insulin resistance (189). Most patients with insulin resistance (65%) were considered overweight or obese. Prednisone treatment at the time of measurement did not affect this association (on-prednisone: 67% vs. off-prednisone: 64%).

4.3.4 Non-traditional risk factors

When compared to the other disease groups, pSLE patients were found to have significantly lower mean complement C3 levels and albumin levels and an elevated mean ESR while SJIA patients had elevated mean complement C4 and CRP levels (Table 3). Mean fibrinogen and homocysteine levels were within the normal range for all 3 groups but mean homocysteine levels were significantly higher in SLE (8.4 micromol/L) patients than in JDM and SJIA (6.6 and 6.9 micromol/L, respectively; p 0.003). Homocysteine levels were elevated (> 10 micromol/L) in only 21 of 115 patients (18 %) [JDM 15%, SJIA 12%, SLE 21%] and fibrinogen (> 4.3 g/L) was elevated in 33 of 129 (26%) [JDM 23%, SJIA 25%, SLE 27%].
4.3.5 Treatment

Treatments differed significantly between disease groups (Table 1). When considering prednisone treatment, we found that total cholesterol (4.3 vs. 3.9 mmol/L, \( p = 0.049 \)) and homocysteine (8.8 vs. 7.4 micromole/L, \( p = 0.026 \)) was increased for patients on prednisone as compared to those not on prednisone at the time of measurement. The majority of patients on prednisone at the time of measure had SLE (94%). The median cumulative prednisone dose for all patients at the time of testing was 0.16 g/kg (range 0 – 1.70). The cumulative prednisone dose was not correlated with any of the risk factors. When considering other medications, patients on other immunosuppressants [cyclophosphamide, cyclosporine, mycophenolate mofetil (MMF)] were found to have increased triglyceride levels (1.4 vs. 1.0 mmol/L, \( p = 0.049 \)) and patients on anti-hypertensive medication were found to have increased total (4.7 vs. 4.0 mmol/L, \( p = 0.043 \)) and LDL cholesterol (2.7 vs. 2.2 mmol/L, \( p = 0.023 \)). Eighty-six percent of patients on anti-hypertensive medication were SLE. No other significant differences were found for other medications.

4.3.6 Correlation of risk factors for atherosclerosis with disease, laboratory measures and treatment

We determined the correlation between lipids and the all the measured risk factors for atherosclerosis with treatment and laboratory values by disease type (Table 4a and 4b). Only statistically significant associations (\( p < 0.05 \), uncorrected) with \( r \geq 0.3 \) or \( \leq -0.3 \)are shown in Tables 4a and b.

4.3.7 Multivariable modeling of risk factors for atherosclerosis

Variables that were found to be statistically significantly associated with lipids or other known CVD risk factors (\( p < 0.05 \) and \( r \geq 0.3 \)) were entered into predictive modeling multivariable regression analysis. Only models with an \( R^2 \) of 30% or greater were considered. None of the individual variables we investigated were found to be significant predictors of lipids or other known CVD risk factors in a multivariable model.
There was no correlation of APO B:APO A ratio with prednisone dose at the time of measurement and patients who were on or off prednisone showed no significant difference in ratio. However, patients who were on prednisone had shorter disease duration (2.0 vs. 3.4 years) and a greater cumulative dose/kg prednisone from diagnosis (1.4 vs. 1.0 g/kg). Furthermore, the ESR differed between patients on (26.4 ± 25.7) and off (14.4 ± 18.5) prednisone. A post-hoc analysis adjusting for disease duration (β = 0.009, SE = 0.009, p = 0.795) and the surrogate for inflammation, ESR (β= 0.080 SE= 0.026, p = 0.003), showed for patients on prednisone at the time of measure that a lower APO B:APO A ratio (better) was associated with higher cumulative dose prednisone/kg (β= -0.157, SE= 0.074, p = 0.037). In contrast, the APO B:APO A ratio was not found to be associated (β= 0.008, SE= 0.015, p = 0.602) with cumulative dose prednisone for patients who were not taking prednisone at the time of measurement. When we performed the same analysis using the LDL-C:HDL-C ratio, we obtained very similar but not statistically significant results. This in spite of the fact that we found both ratios to be strongly correlated (r = 0.9, p < 0.001).

4.4 Discussion

Lipid abnormalities are common to chronic inflammatory diseases and are likely the result of chronic inflammation. These determinants of atherosclerotic risk, if left unaddressed, have been associated with an increased risk of cardiovascular disease(50). Dyslipidemia resulting from chronic inflammation is present in both adults and children and so is the associated risk of developing early cardiovascular disease(12,190). This study is the first to compare the lipid profiles and to study the role of treatment and disease activity-related factors in children with JDM, SJIA, and SLE. The majority of patients in all groups were found to have a normal lipid profile. The most common abnormalities were found for total cholesterol and triglyceride levels and most often in children with JDM. One quarter of all patients had insulin resistance.

When comparing the serum lipid levels of our study cohort with normal control levels (z-score), we found that a little more than 1/3 of all patients had at least one lipid abnormality abnormalities of total cholesterol and triglyceride levels most frequently observed. Children with JDM exhibited the highest prevalence of dyslipidemia and SJIA the least. These findings are of potential significance as the presence of these traditional risk factors during childhood
and adolescence has been associated with an increased risk of cardiovascular disease (53). All 3 of the pediatric rheumatic diseases studied are characterized by chronic inflammation and by the presence of different inflammatory cytokines which have been shown to have differing effects on lipid levels (179,191). However, we did not observe any statistically significant differences for any of the serum lipid levels measured among the 3 different pediatric rheumatic diseases. This finding suggests that either the effects of inflammation and treatment on lipid metabolism were similar among the three diseases or that our sample size was not large enough to detect the differences. Importantly, the majority of patients were measured during a quiescent state when disease activity was low and therefore it is possible that differences would have been present at times of high disease activity. It would be of interest to measure patients at presentation of disease when disease activity is high and the confounding effects of treatment on the lipid profile are eliminated or significantly minimized.

Lipid profiles are altered by inflammation and medications used to control the inflammation and in particular corticosteroids and anti-malarials (53). Although all 3 disease groups were treated with different regimens, the use of corticosteroids to resolve the inflammation was a common thread but different doses and duration of therapy were found among the groups. In this study, we found association of total cholesterol and prednisone therapy at the time of lipid measurement. However, the effect of prednisone is complex as it may be pro-atherogenic by increasing total cholesterol and triglycerides but it may also be protective by increasing HDL cholesterol (53,192). It has been shown that the APOB:APOA ratio may be more important than total cholesterol in determining atherosclerotic risk with higher ratios associated with greater risk (193). We found that patients who were on prednisone at the time of study had a slightly higher ratio APOB:APOA ratio (worse) at the time of measurement but that the ratio improved with increasing cumulative dose prednisone/kg from diagnosis. Similar to what has been observed when examining HDL:LDL ratios, it is possible the elevated APOB:APOA ratio is the result of active disease (inflammation) and that treatment with corticosteroids improved this ratio by reducing the effects of inflammation on lipid metabolism (155). Therefore, aggressive treatment of disease-associated inflammation will likely decrease the deleterious effects of inflammation on atherosclerosis as suggested in previous studies (94,137,194,195).
Traditional Framingham risk factors have been shown to be important risks factors for the development of cardiovascular disease(12). The majority of patients in our study had one or more traditional risk factors (lipids, hypertension, BMI, diabetes, smoking, family history of CVD) and just under one quarter had 2 or more risks. Importantly, one quarter of patients had an elevated HOMA-IR, a measure of insulin resistance that when associated with obesity are considered key components of metabolic syndrome and an early sign of type 2 diabetes(196). Fat tissue has been associated with a chronic inflammatory response(197) and has more recently been suggested to be a low-grade inflammatory state(198). It is therefore important to consider the possibility that increased adiposity may also be contributing to the systemic inflammation in these children. Anti-inflammatory treatment in combination with adequate control of metabolic syndrome will lead to better reduction of CVD risk.

When considering non-traditional risk factors, observational studies suggest that elevated homocysteine is a modest independent predictor of cardiovascular disease and stroke risk in healthy populations(36). In this study we show for the first time that children with JDM and SJIA have relatively normal homocysteine levels compared to healthy populations. Active disease may be contributing to elevated homocysteine levels we observed in our SLE patients compared to JDM and SJIA. However, this difference may have also been attributed to the fact that approximately 50% of JDM and SJIA patients were treated with methotrexate and supplemented with folic acid (known to reduce homocysteine levels (199)) to reduce side effects. Supplementary dietary folic acid for all children with rheumatic disease may be warranted and should be further investigated.

The main limitation of this study was the small sample size of the JDM and SJIA disease groups that resulted in an unbalanced comparison with a relatively large SLE group. These small sample sizes also significantly affected our ability to perform sub-group analysis. However, despite these limitations, we were able to show differences in the lipid profile between the disease groups.

In conclusion, this study was the first to assess the lipid profiles in children with JDM and SJIA, and to compare these profiles to each other and to patients with pSLE. Patients with JDM differed from SJIA with a little over half compared to one third having an abnormal lipid
profile. Patients with SLE were found to be a little worse than SJIA but better than JDM. The most common abnormalities were found for total cholesterol and triglyceride levels and most often in children with JDM. The three disease groups were not found to differ with respect to other CVD risk factors other than a significantly higher homocysteine level in SLE. Additionally, the presence of insulin resistance and obesity suggests a need for closer monitoring of the development of metabolic syndrome in these children in order to minimize related CVD risk. Future longitudinal analysis of this cohort will allow for a better understanding of the effects of sustained disease activity and immunosuppressive medications on lipid metabolism and atherosclerotic risk profile in children with chronic rheumatic diseases.

Acknowledgements
This work was supported by a grant from The Heart and Stroke Foundation of Ontario to Drs. Silverman, Bradley, and Beyene. Mr. Tyrrell was supported by a doctoral award from The Heart and Stroke Foundation of Canada. Dr. Feldman is supported by a Canada Research Chair in Childhood Arthritis.
Figure legends

Figure 1a,b.

a: Percent abnormal serum lipid measurements for patients with JDM, SJIA, or SLE. On the x-axis are the different serum lipid measures as indicated. On the y-axis is the percentage of patients with an abnormal lipid value adjusted for age and gender. Actual percentages are listed above the respective bars.

b: Number of abnormal lipid values for patients with JDM, SJIA, or SLE. On the x-axis are the increasing number of abnormal lipid measures. On the y-axis is the percentage of patients with an abnormal lipid. Actual percentages are listed above the respective bars.
Figures

Figure 1a

Abnormal lipid values:
cross-sectional

Lipid

% Patients

CHOL  LDL C  HDLC  TG

JDM  SJIA  SLE

Figure 1b

# of abnormal lipid values in profile
(CHOL, LDLC, HDLC, TG)

% Patients

None  ≥1  ≥2  ≥3

JDM  SJIA  SLE

# of abnormal lipid values
### Table 1. Demographics

<table>
<thead>
<tr>
<th>Variable†</th>
<th>JDM n = 28</th>
<th>SJIA n = 21</th>
<th>SLE n = 88</th>
<th>Group comparison (p-value)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender M:F (%Female)</td>
<td>14:14 (50)</td>
<td>9:12 (57)</td>
<td>15:73 (83)</td>
<td>0.001</td>
</tr>
<tr>
<td>Age at Diagnosis, Years</td>
<td>8.5 ± 4.1</td>
<td>9.4 ± 2.9</td>
<td>13.4 ± 3.0</td>
<td>&lt; .0001</td>
</tr>
<tr>
<td>Age at time of measure, Years</td>
<td>13.9 ± 2.3</td>
<td>13.9 ± 2.4</td>
<td>15.4 ± 2.5</td>
<td>0.003</td>
</tr>
<tr>
<td>Disease duration, years</td>
<td>5.5 ± 3.6</td>
<td>4.5 ± 3.1</td>
<td>2.0 ± 2.2</td>
<td>&lt; .0001</td>
</tr>
</tbody>
</table>

### Treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>JDM 0 (0)</th>
<th>SJIA 2 (10)</th>
<th>SLE 31 (35)</th>
<th>Group comparison (p-value)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prednisone at time of measurement</td>
<td>0 (0)</td>
<td>2 (10)</td>
<td>31 (35)</td>
<td>0.001</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>14 (50)</td>
<td>10 (48)</td>
<td>3 (3)</td>
<td>&lt; .0001</td>
</tr>
<tr>
<td>Intravenous immunoglobulin (IVIG)</td>
<td>5 (18)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>&lt; .0001</td>
</tr>
<tr>
<td>Hydroxychloroquine</td>
<td>1 (4)</td>
<td>0 (0)</td>
<td>17 (20)</td>
<td>0.015</td>
</tr>
<tr>
<td>Non-steroidal anti-inflammatory drug</td>
<td>1 (4)</td>
<td>10 (48)</td>
<td>3 (3)</td>
<td>&lt; .0001</td>
</tr>
<tr>
<td>Other immunosuppressants¥</td>
<td>9 (32)</td>
<td>8 (38)</td>
<td>15 (17)</td>
<td>0.058</td>
</tr>
<tr>
<td>Biologics</td>
<td>0 (0)</td>
<td>6 (28)</td>
<td>0 (0)</td>
<td>&lt; .0001</td>
</tr>
<tr>
<td>Cumulative steroid dose, g/kg</td>
<td>0.3 ± 0.3</td>
<td>0.3 ± 0.5</td>
<td>0.2 ± 0.2</td>
<td>0.152</td>
</tr>
</tbody>
</table>

†Mean ± SD or n (%)  
¥Cyclophosphamide, cyclosporin, mycophenolate mofetil  
*ANOVA or X² or Fisher’s exact
Table 2. Comparison of Traditional Risk factors

<table>
<thead>
<tr>
<th>Variable†</th>
<th>JDM n = 28</th>
<th>SJIA n = 21</th>
<th>SLE n = 88</th>
<th>Group comparison (p-value)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family history of cardiovascular disease</td>
<td>2/26</td>
<td>0/17</td>
<td>4/61</td>
<td>0.778</td>
</tr>
<tr>
<td>Smoking</td>
<td>0/26</td>
<td>1/17</td>
<td>0/71</td>
<td>0.149</td>
</tr>
<tr>
<td>Treated for hypertension</td>
<td>1</td>
<td>1</td>
<td>12</td>
<td>0.025</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>21.5 ± 5.4</td>
<td>21.5 ± 3.2</td>
<td>22.9 ± 4.5</td>
<td>0.137</td>
</tr>
</tbody>
</table>

**Lipids**

<table>
<thead>
<tr>
<th></th>
<th>JDM</th>
<th>SJIA</th>
<th>SLE</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.2 ± 0.7</td>
<td>4.0 ± 1.0</td>
<td>4.0 ± 0.9</td>
<td>0.712</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>2.3 ± 0.6</td>
<td>2.2 ± 0.8</td>
<td>2.2 ± 0.7</td>
<td>0.748</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.3 ± 0.4</td>
<td>1.2 ± 0.3</td>
<td>1.3 ± 0.3</td>
<td>0.600</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.3 ± 0.7</td>
<td>1.1 ± 0.8</td>
<td>1.1 ± 0.6</td>
<td>0.315</td>
</tr>
</tbody>
</table>

**z-score Lipids**

<table>
<thead>
<tr>
<th></th>
<th>JDM</th>
<th>SJIA</th>
<th>SLE</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>1.2 ± 2.4</td>
<td>0.6 ± 3.2</td>
<td>0.6 ± 3.0</td>
<td>0.676</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>-0.5 ± 1.3</td>
<td>-0.7 ± 1.7</td>
<td>-0.7 ± 1.6</td>
<td>0.837</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>-0.2 ± 1.0</td>
<td>-0.4 ± 0.8</td>
<td>-0.1 ± 0.9</td>
<td>0.416</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.8 ± 2.9</td>
<td>1.0 ± 3.4</td>
<td>0.7 ± 2.5</td>
<td>0.194</td>
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</table>

**Glucose Control**

<table>
<thead>
<tr>
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<th>JDM</th>
<th>SJIA</th>
<th>SLE</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mg/L</td>
<td>4.6 ± 0.4</td>
<td>4.4 ± 0.3</td>
<td>4.5 ± 0.5</td>
<td>0.321</td>
</tr>
<tr>
<td>Hemoglobin A1C, %</td>
<td>5.2 ± 0.4</td>
<td>5.1 ± 0.4</td>
<td>5.3 ± 0.3</td>
<td>0.119</td>
</tr>
<tr>
<td></td>
<td>77.0 ±</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin, picomol/L</td>
<td>54.2</td>
<td>73.7 ± 58.1</td>
<td>89.5 ± 54.6</td>
<td>0.162</td>
</tr>
<tr>
<td>C-peptide, picomol/L</td>
<td>607 ± 388</td>
<td>612 ± 461</td>
<td>563 ± 562</td>
<td>0.287</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.3 ± 1.6</td>
<td>2.1 ± 1.5</td>
<td>2.8 ± 2.0</td>
<td>0.219</td>
</tr>
</tbody>
</table>

†Mean ± SD or n (%)

*ANOVA or X²
Table 3. Comparison of Non-Traditional Risk factors

<table>
<thead>
<tr>
<th>Variable†</th>
<th>JDM n = 28</th>
<th>SJIA n = 21</th>
<th>SLE n = 88</th>
<th>Group comparison (p-value)*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Other Lipids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>APO A1, mmol/L</td>
<td>1.3 ± 0.2</td>
<td>1.3 ± 0.2</td>
<td>1.3 ± 0.2</td>
<td>0.819</td>
</tr>
<tr>
<td>APO B, mmol/L</td>
<td>0.7 ± 0.2</td>
<td>0.6 ± 0.2</td>
<td>0.7 ± 0.3</td>
<td>0.875</td>
</tr>
<tr>
<td>APO B: APO A1 ratio</td>
<td>0.5 ± 0.2</td>
<td>0.5 ± 0.2</td>
<td>0.5 ± 0.2</td>
<td>0.884</td>
</tr>
<tr>
<td>Lp(a), g/L</td>
<td>0.4 ± 0.4</td>
<td>0.3 ± 0.6</td>
<td>0.2 ± 0.2</td>
<td>0.199</td>
</tr>
<tr>
<td></td>
<td>897.0 ± 509.1</td>
<td>888.9 ± 310.3</td>
<td>924.4 ± 452.3</td>
<td></td>
</tr>
<tr>
<td>α-oxLDLC, counts</td>
<td>509.1</td>
<td>310.3</td>
<td>452.3</td>
<td>0.926</td>
</tr>
<tr>
<td>Free fatty acids, mmol/L (n = 74)</td>
<td>0.58 ± 0.3</td>
<td>0.74 ± 0.3</td>
<td>0.69 ± 0.3</td>
<td>0.331</td>
</tr>
<tr>
<td><strong>Disease Activity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physician Global Assessment (mm)</td>
<td>10 ± 13</td>
<td>8 ± 16</td>
<td>14 ± 20</td>
<td>0.087</td>
</tr>
<tr>
<td><strong>Laboratory Markers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin, g/L</td>
<td>44.3 ± 2.7</td>
<td>43.8 ± 4.1</td>
<td>41.7 ± 4.0</td>
<td>0.002</td>
</tr>
<tr>
<td>C3, g/L</td>
<td>1.2 ± 0.2</td>
<td>1.2 ± 0.2</td>
<td>1.0 ± 0.2</td>
<td>&lt; .0001</td>
</tr>
<tr>
<td>C4, g/L</td>
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†Mean ± SD or n (%)

*ANOVA or X²
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Chapter 5. Comparison of Multiple Measures of Early Atherosclerotic Changes in Patients with Pediatric Systemic Lupus Erythematosus, Juvenile Dermatomyositis and Systemic Juvenile Idiopathic Arthritis

Comparison of Multiple Measures of Early Atherosclerotic Changes in Patients with Pediatric Systemic Lupus Erythematosus, Juvenile Dermatomyositis and Systemic Juvenile Idiopathic Arthritis

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Key words: Lupus, pediatrics, autoimmune disease, vascular function, atherosclerosis
Word count: Abstract = 270 Body = 3511

Short running title: Early atherosclerosis in pediatric rheumatic disease
Abstract

**Purpose:** Adults with systemic lupus erythematosus (SLE) and rheumatoid arthritis are at increased risk of premature atherosclerosis irrespective of the presence of traditional cardiovascular risk factors. The aims of this study were to compare vascular markers of early atherosclerosis and the role of treatment and disease activity-related factors on these markers in children with SLE, systemic juvenile idiopathic arthritis (SJIA) and juvenile dermatomyositis (JDM).

**Methods:** Children were enrolled into a prospective longitudinal study of early atherosclerosis in pediatric rheumatic diseases. Drug therapy and disease activity were recorded. Vascular assessments comprising carotid intima-media thickness (CIMT), flow-mediated dilatation (FMD), pulse wave velocity (PWV) were tested after a mean disease duration of 3.1 ± 3.0 years. These indices were compared to normal population data from the same center.

**Results:** Of the 137 children tested, SLE patients were older and more predominantly female (n = 88, mean age: 15.4 ± 2.5, 83% female) than JDM (n = 28, 13.9 ± 2.3, 50% female) and SJIA patients (n = 21, 13.9 ± 2.4, 57% female). No significant difference between the three disease groups was found for CIMT, FMD or PWV. When comparing vascular function of patients to healthy controls from the same center, we found that CIMT was significantly lower (z-score: -0.33 mm, p = 0.001) and that PWV (z-score: 0.54 m/sec, p < 0.001) was greater than controls. FMD was not found to be significantly different.

**Conclusion:** Vascular markers of early atherosclerosis were found in children with rheumatic diseases. Though CIMT and FMD were found to be no worse, PWV, a measure of arterial stiffness, was found to be increased compared to healthy controls.

5.1 Introduction

Chronic rheumatic diseases, including rheumatoid arthritis, systemic lupus erythematosus (SLE) and pediatric rheumatic diseases, are the result of abnormal immune regulation leading to chronic inflammation. This unregulated inflammation leads to the hallmark joint and tissue damage and contributes to the development of atherosclerosis (200). A systematic review in 2009 found that adult patients (> 50 yrs of age) with rheumatoid arthritis had a 60% increased
risk of cardiovascular related death compare to the normal population (77). It has long been reported in SLE that although early patient mortality is more frequently due to active uncontrolled disease, death later in the disease course is frequently the result of complications of atherosclerosis (45,143).

This risk of developing atherosclerosis and its complications increases with age and is exacerbated by the presence of the traditional risk factors for atherosclerosis (50). It also has been suggested that other risk factors such as treatment with corticosteroids, disease activity and chronic inflammation are important for the increased risk observed in patients with rheumatic disease. Pediatric rheumatic diseases, similar to adult rheumatic diseases, are characterized by the chronic inflammation and the use of corticosteroids and therefore it is reasonable to suggest that these patients are at risk to develop atherosclerotic changes more frequently than age-matched controls. However, to date, there have been few studies of early atherosclerotic changes in these patients (128,201,202).

One of the challenges of studying the influences of rheumatic disease and its treatment on the early development of early atherosclerotic changes in pediatric patients is how to capture these changes. The identification of subclinical atherosclerosis is needed to allow the clinician to design preventive therapy for high-risk patients prior to a cardiovascular or cerebrovascular event. Studies in adults have shown that early atherosclerosis can be detected using surrogate markers of atherosclerosis using non-invasive B-mode ultrasound vascular function measurements (55). The most frequently used modalities are flow-mediated dilatation (FMD), carotid intima-media thickness (CIMT) and pulse-wave velocity (PWV). These 3 methods have been used extensively in adults with rheumatic with CIMT the most frequently used methods(58-64). However, in pediatric rheumatic there is much less data and it is not clear which method is the most sensitive to detect early atherosclerotic changes (which may occur on an endothelium which is relatively pristine prior to the onset of the disease) (72).

To date there have been few studies determining these measures in children with rheumatic disease and no study measuring all three markers in three distinct pediatric rheumatic disease populations: juvenile dermatomyositis (JDM), systemic juvenile idiopathic arthritis (SJIA), and systemic lupus erythematosus (SLE). The objectives of this study were: 1) To assess whether endothelial function, subclinical atherosclerosis and stiffness patterns as measured by FMD,
CIMT, and PWV, respectively, are similar in 3 different pediatric rheumatic disease- JDM, SJIA, and SLE; and 2) To correlate treatment and disease activity related factors with the vascular markers of early atherosclerotic changes.

5.2 Patients and methods

5.2.1 Study design and patient population

A prospective cohort study of patients attending the SLE clinic at The Hospital for Sick Children (Sick Kids), Toronto was undertaken between September 2002 and June 2009. Consecutive patients between the ages of 8 to 18 years and who fulfilled American College of Rheumatology (ACR) classification criteria for SLE, Bohan and Peter Criteria for JDM(184), and International League Against Rheumatism Criteria for SJIA(185) were approached for inclusion in the study. Written, informed consent was obtained from either the participant or their parent(s). The study was approved by The Hospital for Sick Children (SickKids) Research Ethics Board (REB# 2002-168).

5.2.2 Vascular measurements

All vascular tests were performed by 2 experienced vascular sonographers in a quiet room after an overnight fast and 10 minutes of supine rest, using standardized protocols(203,204). CIMT was assessed with high resolution B-mode imaging of the right and left carotid arteries using a Vivid 7 high-definition ultrasound machine and a 12 MHz linear-array transducer (GE/Vingmed, Milwaukee, WI). Using antero-oblique insonation, imaging of each common carotid artery was optimized in the longitudinal plane and three sets of end-diastolic images were recorded. CIMT was measured offline using electronic calipers as the average of three right and left far wall measurements 10 to 20 mm proximal the bifurcation. FMD was assessed similarly with high-resolution B-mode imaging and using an automatic edge-detection algorithm (Vascular Tools, Medical Imaging Applications, Coralville, IA) to acquire longitudinal, ECG-gated, end-diastolic images of the right brachial artery just above the antecubital fossa every 3 seconds. Images were recorded for 1 minute baseline before and after 5 minutes pressure cuff inflation to > 200 mmHg around the forearm distal to the elbow for 5 minutes after cuff deflation. FMD was calculated as the maximal percentage change in brachial artery diameter after reactive hyperemia. PWV was assessed by sequentially recording ECG-
gated right carotid and femoral artery waveforms with a high-fidelity micromanometer (SPC-301, Millar Instruments). The wave transit time was determined and the PWV calculated by the system software using the measured surface distance between the two recording sites (SphygmoCor, AtCor Medical Systems Inc., Sydney, Australia).

Vascular data in our patients was compared with normal control vascular data from 96 healthy volunteers, tested under the same conditions and using the same standardized protocols. All volunteers were healthy children aged 6 to 18 years old, who were not on any vasoactive medications and did not have familial hyperlipidemia, diabetes, obesity, hypertension, or any other significant cardiac, renal or systemic disease.

Repeated measures performed by our 2 experienced vascular sonographers (CS and HW) of the different vascular methods in healthy volunteers demonstrated similar results to those previously reported in the literature for intra-observer, inter-observer and test-retest (29±27 days) variability: (1) CIMT \( (n = 20) \) intra-observer mean difference (mean\( \Delta \)) -0.001 mm, proportion of mean (%mean) -0.1 %, \( r = 0.884 \), coefficient of variation (CV) 3.0%; inter-observer mean\( \Delta \) -0.014 mm, %mean -3.0 %, \( r = 0.740 \), CV 7.4 %; and test-retest mean\( \Delta \) -0.003 mm, %mean -0.7 %, \( r = 0.708 \), CV 4.6%; (2) FMD baseline \( (n = 19) \) intra-observer mean\( \Delta \) -0.014 mm, %mean -0.3 %, \( r = 0.960 \), CV 1.4%; inter-observer mean\( \Delta \) -0.003 mm, %mean -0.1 %, \( r = 0.870 \), CV 2.9%; and test-retest \( (n = 9 \) only) mean\( \Delta \) -0.104 mm, %mean -2.6 %, \( r = 0.350 \), CV 5.1%; (3) FMD percent change \( (n = 19) \) intra-observer mean\( \Delta \) 0.1 %, %mean 1.7 %, \( r = 0.864 \), CV 18.3%; inter-observer mean\( \Delta \) 0.7 %, %mean 11.7 %, \( r = 0.689 \), CV 25.4%; and test-retest \( (n = 9 \) only) mean\( \Delta \) 1.2 %, %mean 19.0 %, \( r = 0.517 \), CV 29.8%; and (4) PWV \( (n = 20) \) intra-observer mean\( \Delta \) 0.32 m/s, %mean 4.0 %, \( r = 0.837 \), CV 5.1%; inter-observer mean\( \Delta \) 0.18 m/s, %mean 2.3 %, \( r = 0.818 \), CV 6.0%; and test-retest mean\( \Delta \) 0.06 m/s, %mean 0.3 %, \( r = 0.740 \), CV 8.8%.

### 5.2.3 Laboratory measurements

A standard lipid profile was measured after a 12 hour fast: Triglycerides, total cholesterol, low density lipoprotein cholesterol (LDL-C), and high density lipoprotein cholesterol (HDL-C). Lipids were analyzed in the biochemistry laboratory at SickKids using an automated analyzer (Vitros; Ortho-Clinical Diagnostics, Rochester, NY, USA), which uses dry slide chemistry technology. LDL-C levels were calculated according to the following formula: LDL-C
(mmoles/liter) = CHOL – HDL-C – (TG/2.2). LDL-C estimation was not available for triglycerides exceeding 4.00 mmoles/liter. For CHOL, TG, and LDL-C, patients with values above the age and gender specific normal range were considered abnormal, while for HDL-C, values below the normal range were abnormal. As normal serum lipid values are age and gender dependent, normalized values (z-scores) were calculated.

Corticosteroid usage (cumulative dose was assessed from time of diagnosis to time of measure), hydroxychloroquine, immunosuppressant therapy including cyclophosphamide, azathioprine, cyclosporine, and methotrexate (considered if used within one month of time of measure), and biologic therapy were recorded. Disease activity was measured using the physician global assessment scale (10 cm visual analog scale where 0 represents no activity).

5.2.4 Statistical analysis

Descriptive statistics were reported with mean ± SD or median (minimum-maximum) for continuous variables and as percentages for categorical variables. Patients were classified into quartiles according to vascular function measures: CIMT, PWV, and FMD. Variable comparisons were performed using chi-square or Fisher’s exact test for categorical variables and ANOVA, Student’s t-test or Wilcoxon’s sign rank test for continuous variables, where appropriate. Univariable analysis (Pearson’s correlation) was used to determine the relationship of responses (vascular function testing measures) to explanatory variables (disease type, measurements of disease activity and inflammation) and to screen continuous variables for co-linearity. Only statistically significant associations (p < 0.05) with \( r \geq 0.3 \) or \( r \leq -0.3 \) were considered. A Bonferroni correction was applied to alpha (0.05) in order to correct for the multiple correlations. Chi-square analysis was used for assessing categorical variables for extremely high agreement. Comparative linear regression analysis was performed with vascular measures as the response and disease type as the group variable. For predictive modeling, statistically significant (p < 0.05 and \( r > 0.3 \)) correlations of vascular function measures with markers of disease and inflammation were entered into multivariable regression analysis. Only models with an \( R^2 \) of 30% or greater were considered. Highly positively skewed variables were log-transformed when appropriate before analysis. All statistical test results were considered significant at the 0.05 level. SAS 9.2 for windows (SAS Institute Inc., NC, USA) was used for all analyses.
5.3 Results

5.3.1 Baseline demographic characteristics and traditional CVD risk factors

The cohort consisted of a total of 137 patients, 99 (72%) were female. SLE patients were older and more predominantly female (n = 88, mean age: 15.4 ± 2.5, 83% female) than JDM (n = 28, 13.9 ± 2.3, 50% female) and SJIA patients (n = 21, 13.9 ± 2.4, 57% female) (Table 1).

5.3.2 Traditional cardiovascular risk factors

Most children had a relatively healthy BMI (mean 22.4 ± 4.6) with 31 (23%) patients overweight and 17 (12%) obese [JDM: 7 (25%) and 2 (7%); SJIA: 6 (29%) and 2 (10%); SLE: 18 (20%) and 13 (15%), respectively]. Although the mean z-score lipid values were within the normal range of 2 SD, the mean cholesterol (0.7± 2.9, p = 0.004), and triglycerides (1.0 ± 3.8, p < 0.001) were statistically significantly elevated as compared to control values while the mean LDL-C (-0.7± 1.5, p < 0.001) was significantly decreased and the mean HDL-C (-0.1 ± 0.9, p = 0.096) was not significantly different. No significant differences among disease groups for lipid values were found. Of the 104 patients who provided their family history of CVD there was no significant difference for this risk factor among the groups. One of the patients who were 12 years of age or older admitted to smoking (patients less than 12 years old were assumed to be non-smokers) and none of the patients suffered from diabetes mellitus. We then considered the number of abnormal traditional risk factors (lipids, hypertension, BMI, diabetes, smoking, family history of CVD) per subject. We found that of the 69 patients, for whom we had complete data, the median risk factors was 1 with a range of 0 to 4. No significant difference in was found between disease groups. Sixty-seven percent of patients had one or more risk and 28% had 2 or more risks. The most prevalent risks were lipids (37%) and BMI (36%).

5.3.3 Disease activity and treatment

SLE patients had a shorter disease duration than the other 2 diseases of our study (table 1). At the time of measurement, most patients were found to have little disease activity as measured by the physician global assessment (median PGA: 5 range: 0-80). Medications at the time of testing are listed in Table 1. The majority of patients (57% overall; SJIA 71%; SLE 56%; JDM
54%) were receiving anti-inflammatory treatment at the time of measurement. All patients had received prednisone before the start of the study. At the time of measure, a little over a third of SLE children were receiving prednisone, a little less than a third of SJIA were receiving biologics, and half of JDM and SJIA were receiving methotrexate. Twenty percent of SLE children were receiving hydroxychloroquine.

5.3.4 Vascular function measures

The mean vascular function measures for the cohort by disease group are shown in Table 2. CIMT, FMD and PWV measures were found to follow a normal distribution (all $p > 0.1$: Shapiro-Wilk test for normality). No significant differences in the vascular function measures CIMT, FMD, PWV, and peripheral PWV among the disease groups were found. We divided all patients into quartiles by vascular function as measured by CIMT, PWV, and FMD. When considering the expected equal distribution of patients by disease type across quartiles (25% per quartile for each disease) for the 3 vascular function measures, there were fewer SJIA patients in the upper 2 quartiles (worse) for both CIMT and PWV (25% and 38% of an expected 50%, respectively). When considering other vascular function measures, regression analysis revealed that JDM was associated with a higher elastic modulus than SLE (400 vs. 324, $p = 0.015$); JDM and SJIA patients had significantly lower carotid AIX than SLE patients (-1.6 vs. 9.3  $p < 0.001$ and -1.0 vs. 9.3  $p = 0.004$, respectively); lastly, JDM was associated with a higher stiffness index than SLE (4.9 vs. 3.8, $p = 0.004$). When we performed a post hoc analysis where we adjusted for disease duration, age at time of measure, and gender, these differences remained statistically significant.

When we considered vascular function of all patients compared to healthy controls from the same center, we found that in an unadjusted analysis CIMT was significantly lower (controls: 0.42 mm, patients: 0.40 mm; $p = 0.001$) and that PWV (controls: 4.9 m/sec, patients: 5.5 m/sec; $p < 0.001$) was greater than controls. FMD was not found to be significantly different. These significant differences were maintained when we adjusted for the potential confounding effects of gender, age, height, weight, heart rate and systolic BP [CIMT (controls: 0.42 mm, patients: 0.40 mm; $p = 0.0013$) and PWV (controls: 4.9 m/sec, patients: 5.5 m/sec; $p = 0.004$)]. When considering the disease groups separately (Tukey adjusted pairwise comparisons) in the
adjusted model, the mean CIMT for only SJIA patients (controls: 0.42 mm, SJIA: 0.39 mm; p = 0.0212) was found to be significantly less compared to controls and PWV that was higher in only JDM (controls: 4.9 m/sec, JDM: 5.8 m/sec; p = 0.0019).

We found no significant differences in vascular function measures when we compared patients receiving prednisone, biologics, methotrexate, or hydroxychloroquine to patients who were not.

5.3.5 Correlation of vascular function measures.

We examined the correlation between the vascular function measures CIMT, FMD, PWV and pPWV with: 1) themselves and 2) traditional risk factors for atherosclerosis. Only statistically significant associations (p < 0.05) with |r| ≥ 0.3 were considered. As we considered multiple pairwise comparisons all patients as well as for individual disease groups, we chose to provide statistically appropriate Bonferroni corrected p-values as well as the uncorrected p-values for hypothesis generating purposes.

No significant (Bonferoni corrected) correlations were found between the vascular function measures.

1) CIMT was found to be correlated with the APO B: APO A1 ratio for all patients (r = +0.3, p = 0.002, corrected-p = 0.052) and when individual patient groups were examined in JDM but not pSLE or SJIA (r = +0.6, p < 0.001, corrected-p = 0.050) as well as with HDL cholesterol (r = -0.5, p = 0.013, corrected-p = 0.063) for JDM patients only.

2) FMD was found to be correlated with CRP (r = -0.4, p = 0.045, corrected-p = 0.094) in JDM and fibrinogen (r = 0.3, p = 0.016, corrected-p = 0.066) in SLE patients only.

3) PWV was found to be correlated with triglyceride levels (r = +0.4, p = 0.032, corrected-p = 0.082) in JDM patients only.

4) pPWV was found to be positively correlated with HOMA-IR levels for all patients (r = +0.3, p = 0.016, corrected-p = 0.066) and by individual groups in JDM but not pSLE of SJIA patients (r = +0.5, p = 0.004, corrected-p = 0.054).

5.4 Discussion

The aim of this study was to determine the prevalence of early vascular markers of atherosclerosis and the role of treatment and disease activity related factors on early atherosclerosis in children with JDM, SJIA, and SLE. Observed differences as compared to
controls from the same center were small but support the hypothesis that early changes of atherosclerosis can be found in pediatric rheumatic diseases characterized by chronic inflammation. Our data however, suggest that different levels of disease activity and different mechanisms leading to chronic inflammation coupled with resultant treatment regimes may have different effects on chronic inflammation. Specifically, we found that there were a higher percentage of SJIA patients than expected were in the 2 quartiles associated with better CIMT and PWV.

This is the first study to compare vascular measurements across different pediatric rheumatic disease. We found that there was an uneven distribution of patients across the CIMT quartiles with a higher percentage of patients with SJIA in the lower 2 quartiles, lower CIMT, than patients with JDM or pSLE. A systematic review and meta-analysis has shown that CIMT can be used as a predictor of future vascular events in otherwise healthy adults (55). In our recent systematic review and meta-analysis we showed that patients with rheumatic diseases had a higher CIMT measurement as compared to controls and that the CIMT increase differed among the diseases (205). In addition, although we did not find difference in the mean CIMT for all children with rheumatic diseases as compared to controls, the mean CIMT for SJIA patients was lower than age- and sex-matched controls and again suggests that CIMT differed among the 3 groups.

Findings from our study suggest that JDM may be associated with more early atherosclerotic changes than the other two diseases. Though, we found no difference of FMD among the groups and that PWV did not differ among the diseases, a higher elastic modulus and stiffness index (other measures of vascular compliance) was seen in JDM as compared to the other patient groups while SLE patients in our study had the worse augmentation index measures. Elastic modulus and stiffness index have been associated with early signs of and/or risk factors for atherosclerosis (206,207). These findings support similar comparative findings in adults (208). Furthermore, when we compared our patients to healthy controls from the same center, we found PWV to be higher than controls in JDM and SLE whereas CIMT and FMD were no worse. Though the observed differences with controls were small, they support the hypothesis of early vascular function changes in diseases with chronic inflammation and suggest that the mechanisms responsible for systemic inflammation coupled with anti-rheumatic and
immunosuppressive treatment may have significant vascular effects. Control of systemic inflammation may have a protective effect by reducing the vascular disease burden. However, prolonged treatment could result in negative effects on vascular function, dyslipidemia, and insulin sensitivity.

There was some indication that important CVD risk factors, such as lipid levels, CRP, fibrinogen, and insulin resistance were of negative influence on all vascular markers of early atherosclerosis investigated (due to the high number of comparisons in this study, these correlations will need to be confirmed in the future). Systemic inflammation and the underlying rheumatic disease may be primarily responsible for the increased risk of early atherosclerosis. However, other associated risk factors such as dyslipidemia, thrombogenic factors, and insulin resistance due to disease and/or to treatment should be closely monitored as they contribute to the overall risk to vascular disease.

The three principal vascular function measures of this study, CIMT, FMD, and PWV were not found to be correlated with each other contrary to results from a large adult study(209). The differences between the 2 studies may be a result of the difference in the vasculature of children and adults and/or the relatively small sample size of our study as compared to the adult study. We acknowledge the limitations imposed by the relatively small sample size and cross-sectional methodology of this study. The sample size may not have sufficient power to identify all meaningful clinical associations of risk factors with vascular measures. However, potential relationships reported here are hypothesis generating and can be confirmed by future studies. Longitudinal analysis of a larger cohort will allow for a better understanding of the effects of sustained disease activity and inflammation as well as immunosuppressive therapy on vascular structure and function.

In conclusion, this study was the first to investigate the presence of early atherosclerosis in children with one of three rheumatic diseases: JDM, SJIA, and SLE. We found that patients with all 3 rheumatic diseases have both traditional and non-traditional risk factors for atherosclerosis and that there was some indication that these factors influenced vascular function. Furthermore, there were differences among the groups in these measures of early atherosclerosis with SJIA patients having the best profile of measurements. These results
suggest that the degree or type of chronic inflammation or differing inflammatory mechanisms and associated anti-rheumatic/inflammatory treatment among the diseases may lead to different risk of atherosclerosis in pediatric rheumatic diseases. In addition there were some small but significant differences between patients and controls supporting that hypothesis of early atherosclerosis in pediatric rheumatic diseases.

Acknowledgements
This work was supported by a grant from The Heart and Stroke Foundation of Ontario to Drs. Silverman, Bradley, and Beyene. Mr. Tyrrell was supported by a doctoral award from The Heart and Stroke Foundation of Canada. Brian Feldman is supported by a Canada Research Chair in Childhood Arthritis.
# Tables

## Table 1. Cohort Demographics as Mean ± SD or n (%)

<table>
<thead>
<tr>
<th>Variable</th>
<th>JDM n = 28</th>
<th>SJIA n = 21</th>
<th>SLE n = 88</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender M:F (%Female)</td>
<td>14:14 (50)</td>
<td>9:12 (57)</td>
<td>15.73 (83)</td>
<td>0.001</td>
</tr>
<tr>
<td>Age at Diagnosis, Years</td>
<td>8.5 ± 4.1</td>
<td>9.4 ± 2.9</td>
<td>13.4 ± 3.0</td>
<td>&lt; .0001</td>
</tr>
<tr>
<td>Age at time of measure, Years</td>
<td>13.9 ± 2.3</td>
<td>13.9 ± 2.4</td>
<td>15.4 ± 2.5</td>
<td>0.003</td>
</tr>
<tr>
<td>Disease duration, years</td>
<td>5.5 ± 3.6</td>
<td>4.5 ± 3.1</td>
<td>2.0 ± 2.2</td>
<td>&lt; .0001</td>
</tr>
<tr>
<td>Family history of CVD</td>
<td>2/26</td>
<td>0/17</td>
<td>4/61</td>
<td>0.778</td>
</tr>
<tr>
<td>Smoking</td>
<td>0/26</td>
<td>1/17</td>
<td>0/71</td>
<td>0.066</td>
</tr>
<tr>
<td>Treated for hypertension</td>
<td>1</td>
<td>1</td>
<td>12</td>
<td>0.267</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>21.5 ± 5.4</td>
<td>21.5 ± 3.2</td>
<td>22.9 ± 4.5</td>
<td>0.137</td>
</tr>
</tbody>
</table>

### Treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>JDM n = 28</th>
<th>SJIA n = 21</th>
<th>SLE n = 88</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prednisone at time of measure</td>
<td>0 (0)</td>
<td>2 (10)</td>
<td>31 (35)</td>
<td>0.001</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>14 (50)</td>
<td>10 (48)</td>
<td>3 (3)</td>
<td>&lt; .0001</td>
</tr>
<tr>
<td>Immunoglobulin (IVIG)</td>
<td>5 (18)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>&lt; .0001</td>
</tr>
<tr>
<td>Hydroxychloroquine</td>
<td>1 (4)</td>
<td>0 (0)</td>
<td>17 (20)</td>
<td>0.015</td>
</tr>
<tr>
<td>Non-steroidal anti-inflammatory drug</td>
<td>1 (4)</td>
<td>10 (48)</td>
<td>3 (3)</td>
<td>&lt; .0001</td>
</tr>
<tr>
<td>Other immunosuppressants†</td>
<td>9 (32)</td>
<td>8 (38)</td>
<td>15 (17)</td>
<td>0.058</td>
</tr>
<tr>
<td>Biologics</td>
<td>0 (0)</td>
<td>6 (28)</td>
<td>0 (0)</td>
<td>&lt; .0001</td>
</tr>
<tr>
<td>Cumulative steroid dose, g/kg</td>
<td>0.3 ± 0.3</td>
<td>0.3 ± 0.5</td>
<td>0.2 ± 0.2</td>
<td>0.152</td>
</tr>
</tbody>
</table>

### Lipids

<table>
<thead>
<tr>
<th>Lipid</th>
<th>JDM n = 28</th>
<th>SJIA n = 21</th>
<th>SLE n = 88</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.2 ± 0.7</td>
<td>4.0 ± 1.0</td>
<td>4.0 ± 0.9</td>
<td>0.712</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>2.3 ± 0.6</td>
<td>2.2 ± 0.8</td>
<td>2.2 ± 0.7</td>
<td>0.748</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.3 ± 0.4</td>
<td>1.2 ± 0.3</td>
<td>1.3 ± 0.3</td>
<td>0.600</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.3 ± 0.7</td>
<td>1.1 ± 0.8</td>
<td>1.1 ± 0.6</td>
<td>0.315</td>
</tr>
</tbody>
</table>

†cyclophosphamide, cyclosporin, mycophenolate mofetil

*ANOVA or X² or Fisher’s exact
Table 2. Ultrasound vascular function testing data as Mean ± SD

<table>
<thead>
<tr>
<th>Vascular measure</th>
<th>Controls</th>
<th>JDM</th>
<th>SJIA</th>
<th>SLE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 96</td>
<td>n = 28</td>
<td>n = 21</td>
<td>n = 88</td>
</tr>
<tr>
<td><strong>Resting heart rate (BPM)</strong></td>
<td>69.0 ± 12.7</td>
<td>75.2 ± 13.9</td>
<td>76.3 ± 18.8</td>
<td>72.4 ± 11.6</td>
</tr>
<tr>
<td><strong>Structure</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CIMT (mm)</td>
<td>0.42 ± 0.04</td>
<td>0.40 ± 0.07</td>
<td>0.39 ± 0.06</td>
<td>0.41 ± 0.05</td>
</tr>
<tr>
<td><strong>Reactivity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FMD (%)</td>
<td>8.3 ± 3.9</td>
<td>8.3 ± 4.1</td>
<td>7.8 ± 3.3</td>
<td>8.0 ± 4.2</td>
</tr>
<tr>
<td><strong>Stiffness</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central-femoral PWV (m/sec)</td>
<td>4.9 ± 1.0</td>
<td>5.8 ± 1.0</td>
<td>5.3 ± 0.8</td>
<td>5.5 ± 1.0</td>
</tr>
<tr>
<td>Peripheral PWV (m/sec)</td>
<td>na</td>
<td>7.4 ± 2.1</td>
<td>6.9 ± 1.1</td>
<td>7.7 ± 1.7</td>
</tr>
<tr>
<td>PWA (carotid AIX)</td>
<td>na</td>
<td>-1.6 ± 12.3</td>
<td>-1.0 ± 11.6</td>
<td>9.3 ± 13.5</td>
</tr>
<tr>
<td><strong>Pulse Pressures</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>55.5 ± 7.1</td>
<td>59.4 ± 6.2</td>
<td>58.0 ± 7.7</td>
<td>64.6 ± 8.3</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>106.3 ± 10.0</td>
<td>111.6 ± 11.1</td>
<td>109.0 ± 9.6</td>
<td>113.3 ± 10.9</td>
</tr>
<tr>
<td><strong>Impedance</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Characteristic (dyne•s/cm$^5$)</td>
<td>na</td>
<td>146.7 ± 39.4</td>
<td>183.4 ± 62.8</td>
<td>161.5 ± 54.8</td>
</tr>
<tr>
<td>Vascular input (dyne•s/cm$^5$)</td>
<td>na</td>
<td>224.7 ± 53.6</td>
<td>234.5 ± 52.3</td>
<td>224.6 ± 72.1</td>
</tr>
</tbody>
</table>
Chapter 6. Longitudinal Study of Vascular Markers of Premature Atherosclerosis in Pediatric Systemic Lupus Erythematosus

Longitudinal Study of Vascular Markers of Premature Atherosclerosis in Pediatric Systemic Lupus Erythematosus

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Key words: pediatric, systemic lupus erythematosus, vascular markers, atherosclerosis

Word count: Abstract =224/250 Entire manuscript =4863/5000 #Tables: 3

Short running title: Premature atherosclerosis in pediatric SLE
Abstract

**Background:** Patients with pediatric Systemic Lupus Erythematosus (pSLE) are at increased risk of premature atherosclerosis irrespective of traditional cardiovascular risk factors. The goals of this study were to determine progression of vascular markers of premature atherosclerosis in a prospectively followed pSLE cohort.

**Methods:** Cardiovascular risk profile and anthropometric measures were recorded. Carotid intima-media thickness (CIMT), flow-mediated dilatation (FMD) and pulse wave velocity (PWV) were measured at baseline and follow-up. Vascular measures were compared with normal control vascular data at baseline. Linear regression analysis was performed to assess the association between baseline risk factors and the change in vascular measures.

**Results:** Fifty-one SLE patients [age 14.3±2.6 years (mean±SD), 84% female] were studied. Median follow-up time was 1.5 (0.9-3.0) years. At baseline pSLE patients showed no difference in CIMT, FMD or PWV when compared with normal control vascular data adjusting for the potential confounding effects of gender, age, height, weight, heart rate and systolic blood pressure. At follow-up there was no change in the mean CIMT or FMD, but a trend toward increased PWV (5.4 vs 5.6 m/s, p=0.055). Higher cumulative corticosteroid dose from the study period correlated with an improvement in CIMT (p=0.006).

**Conclusions:** In pSLE, we did not find evidence of progression in vascular function. However, we found that an improvement in CIMT was associated with an increased corticosteroid use suggesting that aggressive immunosuppression may reduce the atherogenic burden of chronic inflammation.
6.1 Introduction

Adults with Systemic Lupus Erythematosus (SLE), an autoimmune disease that primarily affects women, are at increased risk of premature atherosclerosis that cannot be explained by traditional Framingham cardiovascular risk factors for atherosclerosis alone. It has been suggested that the increased burden of cardiovascular disease in SLE can be at least partially attributed to chronic inflammation and SLE-specific factors such as disease activity, corticosteroid and immunosuppressant use and antiphospholipid antibodies.(50)

Approximately twenty percent of patients with SLE have their diagnosis during childhood or adolescence. Examination of early atherosclerosis in pediatric SLE (pSLE) is an important area of study, as these patients tend to have less traditional risk factors for atherosclerosis, including lipid abnormalities, than their adult counterparts and so should have a relatively atherosclerotic free endothelium at the time of diagnosis of SLE(4,210). Therefore, the influence of SLE-specific factors, including chronic inflammation, on the development of premature atherosclerosis can be investigated in pSLE patients with fewer confounding factors.

The identification of subclinical atherosclerosis prior to cardiovascular or cerebrovascular events is needed to allow the clinician to institute preventive therapy. Studies in patients with adult SLE have shown that abnormalities of flow-mediated dilatation (FMD) carotid intima-media thickness (CIMT) and pulse-wave velocity (PWV) correlate with atherosclerotic burden and vascular measurements can be serially followed to measure change(59,61). To date there has not been any study in pSLE longitudinally examining and correlating multiple vascular measures with multiple traditional and non-traditional risk factors for atherosclerosis(85,159,202,210,211).

The objectives of this study were: 1) to determine the progression of vascular markers of premature atherosclerosis (CIMT, FMD and PWV) in a prospectively followed pSLE cohort; and 2) to correlate disease activity, treatment and markers of inflammation with progression in these vascular markers.

6.2 Patients and methods

6.2.1 Study design and patient population
A prospective cohort study of patients 6 to 18 years old, who fulfilled the American College of Rheumatology (ACR) classification criteria for SLE, and who attended the SLE clinic at The Hospital for Sick Children (SickKids), Toronto, was undertaken between September 2002 and March 2008. The study was approved by the Sick Kids Research Ethics Board (REB# 2002-168). Clinical assessments, laboratory and vascular measurements were performed in pSLE at baseline and follow-up.

6.2.2 Clinical assessment

All patients had a detailed history taken including SLE presentation and manifestations, plus a family history for traditional Framingham cardiovascular risk factors. Weight and height were recorded, and body mass index (BMI) was calculated. Blood pressure was measured using an age-appropriate cuff as the average of 3 readings with an automated DINAMAP sphygmomanometer (Critikon, Tampa, FL) and recorded along with resting heart rate.

6.2.3 Laboratory measurements

Laboratory parameters were measured after a 12 hour fast.

a) Lipids: Triglycerides, total cholesterol, low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), apoprotein A1 (APO A1), and apoprotein B (APO B) and lipoprotein A (Lp(a)) were measured. Lipids were analyzed using an automated analyzer (Vitros; Ortho-Clinical Diagnostics, Rochester, NY, USA), which uses dry slide chemistry technology. LDL-C levels were calculated according to the following formula: LDL-C (mmol/liter) = total cholesterol – HDL-C – (triglycerides/2.2). LDL-C estimation was not possible when serum triglycerides exceeded 4.0 mmol/liter. Age- and sex-matched control values were provided by the manufacturer (Ortho-Clinical Diagnostics).

b) Glucose control: Glucose, HbA1c, C-peptide and insulin were measured. Insulin resistance was assessed using the Homeostasis Model-Insulin Resistance (HOMA-IR) which relates fasting glucose (FG; mmol/L) and insulin levels (FI; µU/mL) using the following formula: 
\[
HOMA-IR = FI \times FG / 22.5
\]
(186,212).

SLE-specific factors

a) Disease activity: Modified systemic lupus erythematosus disease activity index (SLEDAI-2K)(187) and the European Consensus Lupus Activity Measure (ECLAM)(162-164) were recorded and complement level C3 and C4, lupus anticoagulant, anticardiolipin antibodies,
double-stranded DNA antibodies (by ELISA), and white blood cell count (WBC) were measured.

b) **Drug therapy:** Corticosteroid usage (cumulative dose was assessed from time of diagnosis to baseline measure and again from baseline to follow-up measure), hydroxychloroquine and all immunosuppressant therapy were recorded.

c) **Inflammatory markers:** Fibrinogen, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), albumin, and homocysteine were measured.

### 6.2.4 Vascular measurements

All vascular tests were performed by 2 experienced vascular sonographers in a quiet room after an overnight fast and 10 minutes of supine rest, using standardized protocols (72,203). CIMT was assessed with high resolution B-mode imaging of the right and left carotid arteries using a Vivid 7 high-definition ultrasound machine and a 12 MHz linear-array transducer (GE/Vingmed, Milwaukee, WI). Using antero-oblique insonation, imaging of each common carotid artery was optimized in the longitudinal plane and three sets of end-diastolic images or three consecutive cardiac cycles were recorded. CIMT was measured offline using an automatic edge-detection algorithm Carotid Analyzer for Research (Vascular Tools, Medical Imaging Applications LLC, Coralville, IA) and the mean CIMT was calculated. FMD was assessed similarly with high-resolution B-mode imaging and using an automatic edge-detection algorithm Brachial Analyzer (Vascular Tools, MIA LLC, Coralville, IA) to acquire longitudinal, ECG-gated, end-diastolic images of the right brachial artery just above the antecubital fossa every 3 seconds. Images were recorded for 1 minute baseline before and after 5 minutes pressure cuff inflation to > 200 mmHg around the forearm distal to the elbow for 5 minutes after cuff deflation. FMD was calculated as the maximal percentage change in brachial artery diameter after reactive hyperemia. PWV was assessed by sequentially recording ECG-gated right carotid and femoral artery waveforms with a high-fidelity micromanometer (SPC-301, Millar Instruments). The wave transit time was determined and the PWV calculated by the system software using the measured surface distance between the two recording sites (SphygmoCor, AtCor Medical Systems Inc., Sydney, Australia).

Vascular data in our pSLE patients at baseline was compared with normal control vascular data from 96 healthy volunteers, tested under the same conditions and using the same standardized protocols. All volunteers were healthy children aged 6 to 18 years old, who were not on any
Repetitive measures performed by our 2 experienced vascular sonographers (CS and HW) of the different vascular methods in healthy volunteers demonstrated similar results to those previously reported in the literature for intra-observer, inter-observer and test-retest (29±27 days) variability: (1) CIMT (n = 20) intra-observer mean difference (meanΔ) -0.001 mm, proportion of mean (%mean) -0.1 %, r = 0.884, coefficient of variation (CV) 3.0%; inter-observer meanΔ -0.014 mm, %mean -3.0 %, r = 0.740, CV 7.4 %; and test-retest meanΔ -0.003 mm, %mean -0.7 %, r = 0.708, CV 4.6%; (2) FMD baseline (n = 19) intra-observer meanΔ -0.014 mm, %mean -0.3 %, r = 0.960, CV 1.4%; inter-observer meanΔ -0.003 mm, %mean -0.1 %, r = 0.870, CV 2.9%; and test-retest (n = 9 only) meanΔ -0.104 mm, %mean -2.6 %, r = 0.350, CV 5.1%; (3) FMD percent change (n = 19) intra-observer meanΔ 0.1 %, %mean 1.7%, r = 0.864, CV 18.3%; inter-observer meanΔ 0.7 %, %mean 11.7%, r= 0.689, CV 25.4%; and test-retest (n = 9 only) meanΔ 1.2 %, %mean 19.0%, r = 0.517, CV 29.8%; and (4) PWV (n = 20) intra-observer meanΔ 0.32 m/s, %mean 4.0%, r = 0.837, CV 5.1%; inter-observer meanΔ 0.18 m/s, %mean 2.3%, r = 0.818, CV 6.0%; and test-retest meanΔ 0.06 m/s, %mean 0.3%, r = 0.740, CV 8.8%.

6.2.5 Statistical analysis

Descriptive statistics were reported with mean ±SD or median (minimum-maximum) for continuous variables and as percentages for categorical variables. Highly positively skewed variables were log-transformed when appropriate before analysis. Single variable comparisons between baseline and follow-up were performed using chi-square or Fisher’s exact test for categorical variables and paired Student’s t-test or Wilcoxon’s sign rank test for continuous variables, where appropriate. Linear regression analysis was performed to assess the association between baseline risk factors and the follow-up vascular measure as the outcome, adjusted for the baseline vascular measure and follow-up time. The study sample size estimate was powered to a detect difference in the primary outcome of a five year longitudinal study of the progression rate of PWV. All statistical test results were considered significant at the <0.05 level. SAS 9.2 for Windows (SAS Institute Inc., NC, USA) was used for all analyses.
6.3 Results

6.3.1 Study population

The 51 SLE patients had a mean age at the baseline assessment of 14.3 years and 43 (84%) were female (Table 1).

6.3.2 Traditional risk factors for cardiovascular disease

Eighteen were overweight (35%) and 2 obese (4%). Nine (18%) required antihypertensive medication at the time of study. Of the 35 patients who provided the information, 2 had an immediate family history of cardio/cerebrovascular disease. None of the patients admitted to smoking. At baseline the mean z-score lipid values were elevated significantly for total cholesterol (1.1 ±3.2, p=0.021) and triglycerides (1.7 ±3.4, p=0.001) with 29% and 12% respectively having a high z-score > 2 SD compared with the age- and sex-matched control values. Mean z-score LDL-C was significantly lower than control values (-0.7 ±1.3, p=0.001) with only 1 patient having a high z-score. Mean z-score HDL-C (-0.2 ±0.9, p=0.115) was similar to control values with none of the patients having a low z-score < 2 SD.

6.3.3 Other lipid levels

Five patients had an abnormally low APO A1 level, 1 an abnormally high APO B level and 2 abnormally high Lp(a) levels.

6.3.4 Markers of glucose control

At baseline mean z-score glucose (-0.4 ±0.8, p=0.012) was not elevated and HbA1c level was within the normal range of <6.5% for all patients measured. Mean z-score C-peptide (-0.3±1.5, p=0.156) was also not elevated, but mean insulin z-score (1.1±1.9, p=<0.001) was significantly elevated with 29% of patients having hyperinsulinemia. Nine patients had HOMA-IR that was above the cutoff point of 3.16 for children and adolescents demonstrating insulin resistance measured (189).

6.3.5 SLE manifestations

The median SLE disease duration at baseline was 1.1 years (range 0.1-13.2 years) (Table 2). Many patients (47%) had a history of renal disease while almost one third (29%) had a history of neuropsychiatric manifestations. Most patients had no or low disease activity with a median
SLEDAI score of 2 (0-15) and a median ECLAM score of 1 (0-5) at the time of measure. Most patients were anti-ds-DNA antibody (71%) and antiphospholipid antibody positive (53%) with 45% having anticardiolipin antibodies and 14% the lupus anticoagulant. All patients had received prednisone during the course of their illness with a median cumulative prednisone dose of 0.2 g/kg (range 0-1.2 g/kg) and 48 patients (94%) were taking prednisone at the time of the initial vascular testing. Patients received prednisone during the course of the study with a median cumulative dose of 0.1 g/kg (range 0-0.5 g/kg). Other medications at study entry were: hydroxychloroquine in 36 (71%, with 5 additional patients started treatment during the study period) and immunosuppressive drugs in 26 (51%) patients. Mean complement, C3 and C4, WBC and albumin levels were in the normal range but the mean ESR was elevated at 23.6±24.4 mm/first hr (67% of patients were >10 mm/hr). Mean CRP, fibrinogen, and homocysteine were all found to be within normal ranges (Table 2). CRP levels were low in 72%, average in 16%, and high in 12% according to AHA guidelines(213), homocysteine levels were elevated (>10 µ/L) in 20% of the patients measured, while all fibrinogen levels were found to be in the normal range.

6.3.6 Vascular markers

SLE patients were measured at baseline and then again after a median follow-up time of 1.5 (0.9-3.0) years. CIMT, FMD and PWV measures were found to follow a normal distribution (all p>0.1: Shapiro-Wilk test for normality). At baseline SLE patients compared with our normal control vascular data (Table 3), were older (14.3 vs 13.0 yrs, p=0.007), more predominantly female (84% vs 50%, p<0.001), heavier (57 vs 49 kg, p=0.012) and had higher BMI (23 vs 20 kg/m², p<0.001), systolic (111 vs 106 mmHg, p=0.003) and diastolic (65 vs 56 mmHg, p<0.001) blood pressures. Unadjusted, SLE patients had similar CIMT (0.425 vs 0.423 mm, p=0.843) and FMD (7.4 vs 8.3%, p=0.185), but increased (worse) PWV (5.4 vs 4.9 m/s, p=0.005). However, this significant difference was lost when adjusted for the potential confounding effects of gender, age, height, weight, heart rate and systolic BP [CIMT (p=0.832), FMD (p=0.095) and PWV (p=0.636)].

Between baseline and follow-up (Table 3), SLE patients grew larger. No statistically significant changes in the mean CIMT or FMD were found, but there was a trend toward increased PWV (worse) in the SLE patients over the follow-up period (5.4 vs 5.6 m/s, p=0.055). When we examined individual patients, in only 54% the CIMT was classified as no significant change
(change of $\leq 0.5$ standard deviation between measurements) while in 29% there was worsening and in 17% there was improvement (changes of $\geq 0.5$ standard deviation). Forty-seven percent of the patients had no significant change in FMD while 26% improved (largest percentage of patients with improvement of vascular measure) and 26% significantly worsened. In contrast to the other measurements, only a small number of patients did not have a significant change in PWV (18%) while 26% significantly improved and the majority had a significant worsening (55%). Follow-up time was not found to be correlated with the change in any vascular function measures over the study period. When we performed sensitivity analysis excluding those patients with a follow up time of $>2$ years (9 patients) or $>2.5$ years (3 patients) we found no significant difference with the findings presented.

Interestingly in a post hoc analysis we also found that the SLE patients with lower PWV at baseline worsened over the follow-up period (difference of 0.7, $p<0.001$) whereas those with higher PWV at baseline did not (difference of -0.3, $p=0.217$). At baseline there was no difference in age, gender ratio, BMI, or systolic BP between patients lower or higher PWV. When comparing the results of all 3 vascular measurements of patients with renal disease and/or neuropsychiatric manifestations to the rest of the study cohort no significant differences were observed.

### 6.3.7 Effect of baseline disease activity, treatment and inflammatory markers on progression in vascular markers.

Linear regression analyses of follow-up vascular function measures with baseline disease activity, treatment and inflammatory markers, when adjusted for baseline measure and follow-up time, showed the following associations: a lower (better) CIMT was associated with a higher cumulative dose prednisone per kg ($R^2=25\%, p=0.006$); a lower (worse) FMD was associated with a longer disease duration ($R^2=25\%, p=0.022$) and associated with a lower HDL-C ($R^2=24\%, p=0.028$); and a higher (worse) PWV was associated with a higher LDL-C ($R^2=35\%, p=0.014$) and a higher homocysteine ($R^2=34\%, p=0.008$).

No significant multivariable models were found.

### 6.4 Discussion

Atherosclerosis has become an important cause of late death in SLE. Cross-sectional studies in adults with SLE have demonstrated abnormalities in CIMT, FMD and PWV(61,105,214) while
longitudinal studies have shown progression in plaque development but not CIMT(61,215). The purpose of our study was to determine if there was any evidence of early atherosclerosis and/or progression of atherosclerosis in patients with pSLE using multiple vascular markers of early atherosclerotic burden. Although we did not find evidence of statistically significant progression in vascular function, we found that an improvement in CIMT was associated with a higher corticosteroid use.

When we investigated CIMT we found no difference in pSLE at baseline compared to our normal control vascular data when adjusting for the potential confounding effects of any differences in gender, age, height, weight, resting heart rate and systolic blood pressure(216). Our finding of no significant increase in CIMT over a relatively short follow-up period is consistent with findings in the only previously published study in pediatric SLE(211). However, we found that patients could show improvement in CIMT and that this improvement was correlated with a higher cumulative dose prednisone over the study period. The correlation of improvement of CIMT with cumulative prednisone dose was independent of age, duration of disease, gender, BMI, disease activity as measured by SLEDAI, and lipid measures. This finding adds to the previous observation in the cross-sectional APPLE study in pediatric SLE that a moderate dose of prednisone was associated with a better CIMT than low or high doses of prednisone(202). These findings suggest that prednisone, likely by decreasing inflammation, may lead to less atherosclerosis while the effect of other immunosuppressive agents may depend on the indications for their use and the doses used. These results support the findings of studies in adult SLE that control of disease activity may be the most important factor to reduce the risk of atherosclerosis in SLE patients (94).

We found FMD and PWV in pSLE at baseline to be similar to our normal control vascular data when again adjusting for the potential confounding effects as reported in the literature(217). However, we did find some evidence, although not statistically significant, for worsening of PWV during follow-up of pSLE. Furthermore, it was the pSLE patients with better PWV at baseline, who were more likely to worsen. Arterial stiffness, as measured by PWV, is likely one of the earliest changes seen in atherosclerosis seen in children and adolescents(218). These results are consistent with follow-up studies of pediatric patients with Kawasaki disease that have shown abnormal PWV with normal CIMT(68).
It has also become apparent that although traditional cardiovascular risk factors cannot completely explain the accelerated atherosclerosis seen in adults with SLE, they likely have some role (50). Examination of traditional risk factors for atherosclerosis in our cohort showed:

i) no overt diabetes mellitus, but hyperinsulinemia and elevated HOMA-IR (risk-factors for the development of type 2 diabetes) in some; ii) almost half were overweight or obese; iii) a minority were on anti-hypertensive therapy but none were persistently hypertensive; iv) a minority with a positive family history of cardio/cerebrovascular disease; and v) none admitted to smoking. At baseline we found an abnormal serum lipid profile consisting of elevated triglyceride, elevated cholesterol while mean HDL-C levels were not significantly depressed, LDL-C levels were low, and APO A1, APO B and Lp(a) levels were normal. Overall, of the traditional risk factors, increased BMI and a mildly pro-atherogenic lipid profile were the most common findings, both of which may be susceptible to intervention. Non-traditional and SLE-associated risk factors likely also adversely affect vascular function. At baseline the majority of patients were antiphospholipid antibody positive, had an elevated ESR (usually only mildly elevated) and a minority had high CRP levels (according to American Heart Association guidelines) and/or elevated homocysteine levels with no significant change throughout the study period. The majority of patients were on hydroxychloroquine, a medication associated with an improved lipid profile(219). Therefore it is possible that the normal values for and lack of progression of most markers of atherosclerotic burden is the result of good disease control over the short time between measurements and possible improvement of lipid profiles by hydroxychloroquine.

The main limitations of this study were the relatively small sample size and short follow-up time of this study. At this point in the study, the sample size may not have sufficient power to identify all meaningful clinical associations of risk factors with vascular measures. However, potential relationships reported here are hypothesis generating and can be confirmed by future studies. It also remains unclear if regression or slowed progression of measures of vascular function, such as CIMT, reflects reduction in cardiovascular events as found in studies of adults(220). No pediatric data is available to our knowledge at the time of publication. These limitations should be recognized as potentially important factors that may falsely reassure clinicians of the lack of progression of atherosclerosis in pSLE.
In conclusion, although we did not find evidence of statistically significant progression in vascular function, we found that serial vascular testing in children with pSLE showed that improvement in CIMT was possible and it correlated with a higher cumulative dose prednisone over the study period. This suggests that treatment of the underlying rheumatic disease with a resultant reduction of inflammation may reduce the atherogenic burden of chronic inflammation in pSLE. Also, there was some evidence to suggest that the progression in arterial stiffness, as measured by PWV, can occur in pSLE over a short period of time. Continued investigation is required to confirm these findings.

Acknowledgements

Mr. Tyrrell was supported by a doctoral award from the Heart and Stroke Foundation of Canada. This work was supported in part from grants from the Heart and Stroke Foundation of Ontario to Drs. Silverman, Bradley, Beyene, Bargman and Adeli.
## Tables

### Table 1
Demographic and laboratory parameters at baseline

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total n</th>
<th>Mean ±SD n (%)</th>
<th>Median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender M:F (%Female)</td>
<td>51</td>
<td>8:43 (84)</td>
<td></td>
</tr>
<tr>
<td>Age at Baseline, years</td>
<td>51</td>
<td>14.3 ±2.6</td>
<td>15.1 (6.3-17.8)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>51</td>
<td>22.9 ±4.9</td>
<td>21.5 (15.1-38.2)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>51</td>
<td>9 (18)</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>51</td>
<td>4.1 ±1.0</td>
<td>4.0 (2.7-8.0)</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>51</td>
<td>1.2 ±0.8</td>
<td>1.0 (0.4-4.2)</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>49</td>
<td>2.3 ±0.6</td>
<td>2.2 (1.1-3.7)</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>50</td>
<td>1.2 ±0.3</td>
<td>1.2 (0.7-2.3)</td>
</tr>
<tr>
<td>APO A1, mmol/L</td>
<td>49</td>
<td>1.3 ±0.2</td>
<td>1.3 (0.9-1.9)</td>
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<td>APO B, mmol/L</td>
<td>49</td>
<td>0.8 ±0.2</td>
<td>0.7 (0.4-1.9)</td>
</tr>
<tr>
<td>Lp(a), g/L</td>
<td>47</td>
<td>0.2 ±0.2</td>
<td>0.1 (0.001-1.3)</td>
</tr>
<tr>
<td>Glucose, mg/L</td>
<td>31</td>
<td>4.4 ±0.6</td>
<td>4.3 (3.3-5.8)</td>
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<tr>
<td>Hemoglobin A1C, %</td>
<td>26</td>
<td>5.3 ±0.4</td>
<td>5.4 (4.1-6.0)</td>
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<tr>
<td>C-peptide, picomol/L</td>
<td>49</td>
<td>907 ±499</td>
<td>837 (290-2498)</td>
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<tr>
<td>Insulin, picomol/L</td>
<td>49</td>
<td>91 ±55</td>
<td>78 (14-216)</td>
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<tr>
<td>HOMA-IR*</td>
<td>31</td>
<td>2.7 ±1.8</td>
<td>2.4 (0.4-7.0)</td>
</tr>
</tbody>
</table>

*HOMA-IR: Homeostasis Model-Insulin Resistance
Table 2
SLE manifestations at baseline

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total n</th>
<th>Mean ±SD</th>
<th>Median (range)</th>
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</thead>
<tbody>
<tr>
<td>SLE duration, years</td>
<td>51</td>
<td>2.0 ±2.4</td>
<td>1.1 (0.1-13.3)</td>
</tr>
<tr>
<td>SLEDAI</td>
<td>51</td>
<td>3.4 ±4.0</td>
<td>2.0 (0-15)</td>
</tr>
<tr>
<td>ECLAM</td>
<td>51</td>
<td>1.5 ±1.5</td>
<td>1.0 (0-5)</td>
</tr>
<tr>
<td>Anticardiolipin antibodies</td>
<td>51</td>
<td>23 (45)</td>
<td></td>
</tr>
<tr>
<td>Lupus anticoagulant</td>
<td>51</td>
<td>7 (14)</td>
<td></td>
</tr>
<tr>
<td>Anti-dsDNA antibodies</td>
<td>51</td>
<td>36 (71)</td>
<td></td>
</tr>
<tr>
<td>Cumulative steroid dose§, g/kg</td>
<td>51</td>
<td>0.2 ±0.2</td>
<td>0.1 (0.0-1.2)</td>
</tr>
<tr>
<td>Hydroxychloroquine</td>
<td>51</td>
<td>36 (71)</td>
<td></td>
</tr>
<tr>
<td>NSAID</td>
<td>51</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Immunosuppressant</td>
<td>51</td>
<td>26 (51)</td>
<td></td>
</tr>
<tr>
<td>Albumin, g/L</td>
<td>51</td>
<td>42.0 ±3.7</td>
<td>43.0 (23.0-47.0)</td>
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<td>C3, g/L</td>
<td>51</td>
<td>1.0 ±0.3</td>
<td>1.0 (0.3-1.6)</td>
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<td>C4, g/L</td>
<td>51</td>
<td>0.2 ±0.2</td>
<td>0.2 (0.04-1.4)</td>
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<td>C-reactive protein, mg/L</td>
<td>45</td>
<td>1.7 ±1.1</td>
<td>0.6 (0.2-26.3)</td>
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<td>ESR, mm/hr</td>
<td>51</td>
<td>23.6 ±24.4</td>
<td>16.0 (1.0-105.0)</td>
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<tr>
<td>Fibrinogen, g/L</td>
<td>46</td>
<td>3.6 ±1.1</td>
<td>3.4 (2.2-6.8)</td>
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<tr>
<td>Homocysteine, micromol/L</td>
<td>49</td>
<td>8.7 ±2.7</td>
<td>8.6 (4.4-19.9)</td>
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<tr>
<td>White blood cell count x10⁹/L</td>
<td>49</td>
<td>6.5 ±3.2</td>
<td>5.3 (1.7-14.1)</td>
</tr>
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</table>

§From diagnosis to baseline
ECLAM = European Consensus Lupus Activity Measure
SLEDAI = Systemic Lupus Erythematosus Disease Activity Index
NSAID= non-steroidal anti-inflammatory drug
<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls (n=96)</th>
<th>SLE (n=51)</th>
<th>Follow-up</th>
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</thead>
<tbody>
<tr>
<td>Gender M:F (%Female)</td>
<td>48:48 (50)</td>
<td>8:43 (84)</td>
<td>8:43 (84)</td>
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<tr>
<td>Age (years)</td>
<td>13.0 ±3.2</td>
<td>14.3 ±2.6</td>
<td>15.9 ±2.6</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>155.5 ±16.4</td>
<td>156.5 ±13.7</td>
<td>159.4 ±12.9</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>49.4 ±17.4</td>
<td>56.9 ±16.5</td>
<td>60.1 ±15.6</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>19.8 ±4.1</td>
<td>22.9 ±4.9</td>
<td>23.5 ±4.7</td>
</tr>
<tr>
<td>Heart rate (BPM)</td>
<td>69.0 ±12.7</td>
<td>72.4 ±11.2</td>
<td>74.1 ±15.0</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>106.3 ±10.0</td>
<td>111.4 ±9.1</td>
<td>114.3 ±9.1</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>55.5 ±7.1</td>
<td>64.9 ±7.7</td>
<td>64.3 ±9.2</td>
</tr>
<tr>
<td>CIMT (mm)</td>
<td>0.423 ±0.042</td>
<td>0.425 ±0.038</td>
<td>0.423 ±0.040</td>
</tr>
<tr>
<td>FMD (%)</td>
<td>8.3 ±4.0</td>
<td>7.4 ±3.3</td>
<td>8.8 ±4.5</td>
</tr>
<tr>
<td>PWV (m/s)</td>
<td>4.9 ±1.0</td>
<td>5.4 ±1.1</td>
<td>5.6 ±1.0</td>
</tr>
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Chapter 7. Summary, Conclusions and Significance

This thesis project demonstrated that there are early markers of atherosclerosis present in pediatric rheumatic disease and explores the contribution of chronic inflammation in the development of premature atherosclerosis. Our study was the first to investigate and compare the presence of sub-clinical atherosclerosis in children with one of three major rheumatic diseases: juvenile dermatomyositis (JDM), systemic juvenile idiopathic arthritis (SJIA), and systemic lupus erythematosus (SLE). Our findings showed that although the differences when compared to healthy controls from the same center were small, these findings support the hypothesis that the risk for early atherosclerosis is increased in pediatric rheumatology patients (diseases characterized by chronic inflammation). We found that pulse wave velocity, a measure of arterial stiffness often associated with premature atherosclerosis, was worse than healthy controls. Furthermore, one third to one half of children had at least one abnormal serum lipid, most frequently triglyceride levels. Although treatment with corticosteroids contributed to the increased total cholesterol level, it reduced disease activity and inflammation. Increased arterial stiffness and an abnormal lipid profile, whether resulting from disease and/or treatment, puts these children at risk for premature atherosclerosis. However, the net effect of the judicious use of corticosteroids may be to minimize the risk of atherosclerosis as the beneficial effect of decreasing inflammation may outweigh the deleterious effect of altering lipid profiles.

7.1 Synthesis

7.1.1 CIMT is increased in rheumatic disease populations as compared to healthy controls.

7.1.1.1. Rheumatic Disease and Carotid Intima Media Thickness: a Systematic Review and Meta-Analysis.

The systematic review and meta-analysis from our first project found that CIMT, a surrogate marker of early atherosclerosis, was found to be significantly increased in rheumatic disease populations compared to age- and gender-matched healthy populations(221). It has been well established that atherosclerosis and rheumatic diseases have inflammation in common. Rheumatic diseases such as SLE and RA have been shown to have an associated increased risk of CVD. It has been suggested that detection of carotid plaques by ultrasonography, a non-invasive method, is a predictor of cardiovascular events and should be considered the gold standard for determining atherosclerosis(94,222). Evidence from both clinical and pathological
studies suggests that increases in necrotic core size and lesion vulnerability best predict clinical events\(^{(223,224)}\). Interestingly, we found no agreement between studies’ findings of the difference between cases and controls in CIMT and plaque number. This may reflect the considerable differences in methodological approach to inclusion or exclusion of plaque in the CIMT measurement between different studies. More study is required to define the relative importance of plaque number compared with plaque morphology. Intimal thickening is considered one of the first manifestations of atherosclerosis. However, age-related thickening of intimal and medial layers of common carotid arteries also occurs in the absence of overt atherosclerosis. CIMT may accurately measure the burden of atherosclerosis per se while the presence of plaque and plaque morphology may be a better measure of cardiovascular events. Once plaques have formed, however, treatment is more difficult as much of the damage may be irreversible. Measuring CIMT may therefore be a better tool for monitoring early development of atherosclerosis and its response to treatment. Furthermore, as each rheumatic disease may differ in the amount and type of inflammation, active inflammatory plaques could be less in number but potentially larger and/or more destructive. Patients in this case would potentially have an increased CIMT but a lesser number of plaques.

Plaques and cardiovascular events are extremely rare in children. CIMT measurements and other non-invasive vascular function measures may, therefore, be particularly useful in younger populations as an end-point for epidemiological and treatment studies as suggested by us and more recently by Lamotte et. al.\(^{(225)}\). However, to date there is little evidence to support a link between progression of CIMT and coronary and cerebral events\(^{(226,227)}\). It is important then to consider the type of disease, pre-existing atherogenic risk factors, and disease duration when considering the management of early atherosclerosis risk in children with rheumatic disease (see Figure 1).

7.1.2 Increased traditional risk factors of children with rheumatic diseases.

7.1.2.1 Predictors of lipid abnormalities in children with new onset systemic lupus erythematosus.

Newly diagnosed children with pSLE before corticosteroi d treatment exhibited the distinct pattern of dyslipoproteinemia of increased triglycerides and depressed HDL-cholesterol. Although multiple studies have demonstrated that patients with the widely studied rheumatic
disease of SLE have abnormal lipid profiles, it has been difficult to differentiate the precise role of the inflammation and of the therapy on patients’ lipid profiles(155). Our second project sought to resolve this dilemma by studying lipid levels at diagnosis of pSLE prior to therapy(178). As few children smoke, are hypertensive, or have diabetes, by studying children with a rheumatic disease we were also able to reduce the influence of these factors which may alter lipid levels.

The lipid abnormalities found in our study are similar to the changes in lipid and lipoprotein metabolism during infection and inflammation(52). These cytokine-induced changes in the structure and function of lipoproteins are generally thought to provide immediate protection from infection and inflammation. However, they become deleterious when prolonged, as found in chronic inflammation, and may contribute to the development of atherosclerosis.

7.1.2.2. Lipid Profiles of Children with Rheumatic Disease

Our third project compared traditional risk factors, including lipid profiles, and studied the role of treatment and disease activity related factors in children with JDM, SJIA, and pSLE. Our study was the first to compare the lipid profiles in children with one of these three rheumatic diseases. One third of children from our study had at least one abnormal lipid value. The most common abnormalities were found for total cholesterol and triglyceride levels and most often in children with JDM. Observed frequency of lipid abnormalities was influenced not only by disease type and treatment but also by successful control of disease activity, often requiring treatment with prolonged courses of high dose corticosteroids. Chronic inflammation represents an additional risk to the traditional risk factors elucidated over the years from population based studies such as Framingham(22). Both inflammation and corticosteroid use have been associated with the development of an abnormal lipid profile. Managing these non-traditional risks as well as established traditional risks in patients is important in order to prevent early onset of CVD (see Figure 1).

One quarter of all patients were found to have insulin resistance that could not be solely attributed to traditional CVD risk factors. The presence of insulin resistance in some patients is of concern as it may contribute to the development of metabolic syndrome when associated with obesity (half of patients with insulin resistance were also overweight) and the development
of dyslipidemia, hypertension, and glucose intolerance(196). When we considered the number of abnormal traditional risk factors (lipids, hypertension, BMI, diabetes, smoking, family history of CVD) per subject, we found that 63% of patients had one or more risk and 23% had 2 or more risks. Higher Framingham risk scores, which includes age, gender, total and HDL cholesterol, blood pressure, diabetes, and smoking to derive an estimated risk of developing coronary heart disease within 10 years, have been shown to be associated with coronary artery atherosclerosis in adults with RA(228). It is not unreasonable to suggest then that the accumulation of Framingham risks increases the overall risk for developing CVD in children with rheumatic diseases (see Figure 1). Lifestyle changes to minimize the risk of metabolic syndrome and aggressive treatment to control inflammation should prove to be a sound approach to reducing the risk of early atherosclerosis in these children.

7.1.3 Markers of early atherosclerosis in children with rheumatic disease.

7.1.3.1 Comparison of Multiple Measures of Early Atherosclerotic Changes in Patients with pSLE, JDM and SJIA.

Our fourth project was the first to investigate the presence of sub-clinical atherosclerosis as measured by the three vascular markers CIMT, PWV, and FMD in children with one of the three rheumatic diseases: JDM, SJIA, and SLE. No significant differences in the vascular function measures among the disease groups were found. However, our findings suggest that different amounts or different mechanisms leading to chronic inflammation may have different effects on chronic inflammation. For example, a higher percentage of SJIA patients than expected were observed in the two quartiles associated with better CIMT and PWV. In patients with JDM, PWV was found to be higher (marker of poorer compliance and early atherosclerosis) than controls from the same center whereas CIMT and FMD were similar. Although the difference in PWV was small, it is important to consider that small changes in these children at this stage of their disease may very well translate into significant negative changes in their vasculature by early adulthood.

Previous studies have demonstrated the importance of serum lipid levels, CRP, fibrinogen, and insulin resistance in altering vascular markers of early atherosclerosis. We found that serum lipid levels and other modifiable risk factors for atherosclerosis were correlated with markers of
inflammation and disease activity. One must consider that though all of these risk factors have been shown to be independently associated with CVD to varying degrees, it is unreasonable to ignore the complex interactions that invariably exist. It would be more prudent to consider a model where risk factors have varying levels of influence but that ultimately all lead to the promotion of the atherogenic process (Figure 1). Once early signs of atherosclerosis have been determined, the next important question to be answered is whether or not these vascular markers will continue to worsen over time.

7.1.3.2. Longitudinal Study of Vascular Markers of Premature Atherosclerosis in pSLE

In our fifth and final project, we sought to determine if there was any evidence of progression of atherosclerosis in children with pSLE using multiple vascular markers of early atherosclerotic burden. Some evidence for progression in arterial stiffness, as measured by PWV, was observed in our pSLE cohort over a short period of time supporting the hypothesis that early atherogenic changes can progress in children with rheumatic disease. Non-traditional and SLE-associated risk factors were present but did not change significantly over the study period. These included: antiphospholipid antibody positivity, an elevated ESR, high CRP levels (according to American Heart Association guidelines) and/or elevated homocysteine levels. When we considered the effects of treatment, we found that improvement in CIMT was possible and it correlated with a higher cumulative dose prednisone over the study period, suggesting that treatment of the disease with a resultant reduction of inflammation may reduce the atherogenic burden of chronic inflammation in pSLE and early changes of atherosclerosis may be reversible with better disease control in patients with pediatric rheumatic diseases.
Figure 1. Early atherosclerotic change in pediatric rheumatic disease

Traditional and non-traditional CVD risk factors contribute to the progression of vascular disease starting with endothelial dysfunction. Later vascular disease stages including vessel stiffness and re-modeling can lead to plaque development and eventual cardiovascular events/mortality. Purple arrows from anti-inflammatory medication lead to decreases in non-traditional risk factors including inflammatory markers, improvement in skeletal muscle inflammation and decreased adipose and liver production of inflammatory cytokines/adipokines. Reduction of traditional CVD risk factors with lifestyle changes coupled with the control of systemic inflammation with appropriate treatment will minimize progression of vascular disease as well as development of co-morbidities such as insulin resistance (vessel wall schema reprinted with permission from Elsevier Inc. The American Journal of Medicine (2008) 121, S21–S3).

7.2 Significance

Our systematic review and meta-analysis has demonstrated that it is indeed possible to detect earlier atherosclerosis in the rheumatic disease populations by measuring CIMT. The ability to assess atherosclerosis before the development of irreversible atheroma and plaque is required in order to prevent or possibly reverse the process. The demonstration that CIMT assesses early atherosclerotic is important in the assessment of children who typically are free of plaque. With the risk of earlier CVD in patients with rheumatic disease, it is important to determine the contributing factors as early as possible in order to best develop and implement an appropriate treatment plan. We can therefore develop strategies to prevent atherosclerosis in these high-risk
patients leading to a healthier population of patients with rheumatic disease and reduce healthcare costs.

Our research has found that dyslipidemia is a problem in children with a rheumatic disease due to both inflammation and treatment with corticosteroids. Pro-inflammatory cytokines, present as a result of the chronic inflammation in these children with rheumatic disease, leads to dyslipidemia and insulin resistance. However, the fact that aggressive therapy was found to be related to a healthier CIMT in children with chronic inflammation supports the hypothesis that the control of inflammation may outweigh the deleterious side-effects of corticosteroid therapy (see Figure 2). These pro-inflammatory cytokines as well as other inflammatory mediators also directly alter vessel wall structure leading to the development of atherosclerosis. By reducing inflammation, both negative effects on lipid metabolism and on the arterial wall are reduced. This is an important message for children with rheumatic disease and their parents as often compliance to therapy is an issue due to the fear of treatment side-effects.

It has been well established that reducing our modifiable risks is the best way to prevent future development of CVD. All three measures we used in our study were relatively non-invasive, fast and reproducible. However, it is not clear which of endothelial dysfunction (FMD), arterial stiffness (PWV), and structural preclinical atherosclerotic changes (CIMT) are the best measures of early atherosclerosis in patients with pediatric rheumatic diseases. FMD and CIMT assessments require more special technical expertise. As arterial stiffness as measured by PWV has been found to correlate with both FMD and CIMT in rheumatic populations (208), PWV may be the simpler and more cost effective choice for early screening of children at high risk for cardiovascular disease as a preventive measure but further studies are still required.

As this study has shown, some children with rheumatic disease have insulin resistance. When associated with obesity, these children are considered to have metabolic syndrome and to be at risk of developing type 2 diabetes. Furthermore, the increased adiposity associated with this condition may also be contributing to the systemic inflammation in these children. Lifestyle changes are the first recommended steps and can often be the only required interventions. However, anti-inflammatory treatment in combination with adequate control of metabolic syndrome will lead to better reduction of CVD risk (see Figure 2).
Individuals at risk for CVD include those with an elevated LDL-C, the metabolic syndrome, or both. Treatment with corticosteroids can reduce CVD risk by controlling systemic inflammation but may also contribute to it by increasing LDL-C levels and promoting insulin resistance. Anti-inflammatory treatment in combination with adequate control of metabolic syndrome will lead to better reduction of CVD risk.

7.3 Future research directions

In conclusion, our study findings suggest that early markers of atherosclerosis are present in pediatric rheumatic disease. Chronic inflammation plays a significant role and should be considered an important predictor of premature atherosclerosis. However, the precise roles and interaction of inflammation with other associated risk factors such as dyslipidemia and insulin resistance remains to be determined. One must also consider that the genetic make-up of an individual influences, at least in part, the success of treatment. Although our understanding of the role of mutations in specific genes causing simple disorders has improved considerably, our understanding of genetic determinants in complex diseases, such as CVD, is still lacking. Studying how genetic differences affect variation in response to therapy will lead to individualized treatment regimens which, in turn, may lower the risk of atherosclerosis. Furthermore, longitudinal studies that will study long-term outcome of children with pediatric
rheumatic diseases will help determine whether inflammation from rheumatic disease is causally related to the development of early CVD. The study of the relationship between early vascular changes in children and later plaque formation as adults will enable clinicians to better plan an appropriate preventative treatment regimen.
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