Understanding Premature Atherosclerosis in Pediatric SLE: Risk Factors of Increased Carotid Intima Medial Thickness (CIMT) in the Atherosclerosis Prevention in Pediatric Lupus Erythematosus (APPLE) Cohort

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Abstract

Objective—To evaluate risk factors of sub-clinical atherosclerosis in a pediatric SLE population.

Methods—A prospective multicenter cohort of 221 patients underwent baseline measurements of carotid intima medial thickening (CIMT) as part of the Atherosclerosis Prevention in Pediatric Lupus Erythematosus (APPLE) trial. SLE disease measures, medications, and traditional risk factors for atherosclerosis were assessed. A standardized protocol was used to assess thickness of the bilateral common carotids and mean maximal IMT of 12 segments. Univariable analysis identified potential associations with CIMT that were examined in multivariable linear regression modeling.

Results—Based on mean-mean common or mean-max CIMT as the dependent variable, univariable analysis showed significant associations with increased CIMT: increasing age, longer SLE duration, minority status, higher BMI, male sex, increased creatinine clearance, higher Lp(a), proteinuria, azathioprine use, and prednisone dose. Azathioprine use (P=0.005 for mean-mean common; P=0.102 for mean-max model) and male sex (P< 0.001) were both associated with increases in mean-max CIMT. Moderate dose prednisone (0.15–0.4 mg/kg/day) was associated with decreases in mean-max CIMT (P=0.024) while high or low dose prednisone was associated with mean-mean common CIMT (P=0.021) or mean-max CIMT (P=0.064), respectively. BMI (P<0.001) and creatinine clearance (P=0.031), remained associated with increased mean-mean common CIMT, while increasing age (P<0.001) and increasing Lp(a) (P=0.005) were associated with increased mean-max CIMT.

Conclusion—Traditional as well as non-traditional risk factors are associated with increased CIMT in pediatric SLE patients in this cohort. Azathioprine treatment was associated with increased CIMT. The relationship of CIMT with prednisone dose may not be linear.

Over the last 3 decades, lupus-related mortality has decreased in all categories except cardiovascular disease (1). The lack of improvement in cardiovascular morbidity and

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mortality in systemic lupus erythematosus (SLE) may reflect improved survival as patients with lupus now live long enough to develop cardiovascular disease; however, women under 40 years of age with SLE are at high risk of developing myocardial infarction (MI) with risks ranging up to 50 times higher than control populations (2). The unique convergence of immune and vascular pathology in SLE places young women at unusually high risk of cardiovascular disease (3)—a risk that is not well addressed by current treatment or prevention regimens.

Atherosclerosis begins in childhood even in the absence of SLE. Autopsy studies of trauma victims commonly showed fatty streaks in the arteries of children (4). Children and adolescents with SLE are particularly at risk as they age given their life-long burden of exposure to multisystem inflammatory disease with high atherogenic potential. As a result, prevention of long-term cardiovascular complications in pediatric onset SLE presents a particularly attractive target for intervention with the possibility of significantly improving quality of life and increasing survival over many decades.

Several investigators have demonstrated atherosclerotic heart disease in asymptomatic patients with SLE across the age range using noninvasive imaging techniques such as computerized tomography, carotid ultrasound and arterial flow-mediated dilatation (3,5–8). These studies have identified a variety of factors contributing to the development of subclinical atherosclerosis, including traditional risk factors (obesity, smoking, glucose intolerance, family history), medications, hypertension, increased homocysteine levels, and renal disease (9,10), but also indicate that important additional factors exist in this population. The identity of lupus-specific factors is not well defined but likely relates to the immune pathogenesis of SLE (6,11), implicating SLE itself as a potent independent risk factor. Although few published studies specifically address cardiovascular risk factors in pediatric SLE, the pathogenesis of atherosclerosis in children and adolescents with SLE is likely multifactorial as in adults. Investigators have shown that nephrotic range proteinuria in children with SLE is associated with carotid intima media thickening (CIMT) (5).

Since children and adolescents with SLE have fewer co-morbidities and traditional cardiovascular risk factors than adults with lupus, this population presents a unique opportunity to further understand the role of SLE in the pathogenesis of premature atherosclerosis. Currently there are no standard imaging procedures for assessment of preclinical atherosclerosis in young people; however, non-invasive techniques utilized in ongoing observational studies and clinical trials in a variety of disorders may result in clinically useful technology in the future. CIMT as measured by carotid ultrasound is a well studied surrogate marker of atherosclerosis which has been shown to predict cardiovascular events (strokes and myocardial infarction) (12). It has been used widely as an outcome measure in clinical trials including studies in children with increased risk of atherosclerosis due to familial hypercholesterolemia (13,14). Although CIMT increases with age, the progression rate in healthy children and adolescents is negligible, 0.000 mm/year between 10–24 years of age in females and 10–19 years of age in males (15). In contrast, the progression rate as measured by CIMT in adult women with SLE is 0.004 mm/year (women <30 years of age) to 0.010mm/year (women >30 years of age) (Susan Manzi, personal communication) (16). Population studies show that increases in carotid wall thickening as small as 0.1 mm strongly correlate with increases in coronary and cerebral vascular events (17).

The ongoing Atherosclerosis Prevention in Pediatric Lupus Erythematosus (APPLE) Trial is designed to prospectively assess the effect of atorvastatin on the progression of CIMT in children and young adults with SLE. While the final results of the APPLE Trial will not be available until 2010, the purpose of the current analysis is to investigate the association of

traditional and non-traditional risk factors with a surrogate marker of early atherosclerosis (CIMT) in children and adolescents with SLE using APPLE baseline data.

METHODS

Participants

A prospective multicenter cohort of 221 children and adolescents with SLE from 21 sites in North America underwent ultrasound measurement of CIMT at enrollment as part of the APPLE Trial. The APPLE Trial is a double-blind randomized placebo controlled trial designed to determine the efficacy and safety of atorvastatin in preventing the progression of atherosclerosis in children and adolescents with SLE treated for 3 years. The primary endpoint is the rate of progression of mean CIMT in the common carotid artery over 3 years. Subjects are randomized to receive either placebo or atorvastatin (10 or 20 mg daily depending on weight). All participants are treated with folate, hydroxychloroquine, low dose aspirin, dietary recommendations, and risk factor counseling. Enrollment was completed in 2006 with study results expected in 2010.

All subjects met the American College of Rheumatology (ACR) 1997 revised diagnostic criteria for SLE. Additional inclusion requirements included weight ≥25 kg, outpatient status, age between 10 and 21 years, ability to complete self-report questionnaires in English or Spanish, willingness to comply with recommended diet, and willingness to use an approved birth control method. Patients were excluded from the study if they had active nephrotic syndrome, myositis, liver disease, renal insufficiency, or hypercholesterolemia warranting treatment (total cholesterol >350 mg/dL) at baseline. See Table 1 for the full inclusion and exclusion criteria for the APPLE Trial.

Clinical variables of interest

The APPLE baseline assessment included demographics, a history and physical examination performed by a pediatric rheumatologist, chart review, and subject and parent questionnaires. Variables of interest for this analysis were SLE clinical and laboratory parameters, non-lupus and lupus-related medical history, family history, current medication use, traditional risk factors for atherosclerosis, and SLE disease activity and damage. Race and ethnicity were self-reported. For the purposes of this analysis, subjects were categorized as minority if they were either Hispanic or non-Caucasian.

SLE disease activity and damage were assessed using the modified SELENA SLE Disease Activity Index (SLEDAI) (18) and Systemic Lupus International Collaborating Clinics/ACR Damage Index (SLICC) (19). For these analyses, the total SLEDAI score was used as a continuous variable. SLICC scores were analyzed as a dichotomous variable with subjects grouped as score of 0 (no damage) or score ≥1 (damage present). Additionally, SLE-specific laboratory testing was obtained. Proteinuria was defined as a spot urine protein/creatinine ratio > 0.5 or a timed urine with protein > 500 mg/24 hours. Creatinine clearance was calculated using the Schwartz formula (20).

Traditional risk factors for atherosclerosis, including hypertension, cardiovascular family history, smoking, diet, exercise, and body mass index (BMI) were assessed. A history of hypertension referred to reported current or historical hypertension. Similarly a history of nephrotic syndrome and history of nephritis referred to reported current or historical event. A family history of cardiovascular disease was defined as subject-reported cerebrovascular accident, myocardial infarction, angina or atherosclerosis in a parent or grandparent.

Oral glucocorticoid doses were weight-adjusted and recorded as prednisone equivalent mg/kg/day. Twelve hour fasting lipids, homocysteine, lipoprotein A (Lp(a)), and high sensitivity

c-reactive protein (hsCRP) were processed centrally (PPD, Highland Heights, Kentucky). Total cholesterol, triglycerides and high density lipoprotein (HDL) were measured directly and low density lipoprotein (LDL) was calculated by the Friedwald equation (21). All other laboratory assessments were performed locally. HsCRP and Lp(a) were log transformed to achieve approximately normal distributions.

Measurement of carotid intima media thickening (CIMT)

Two CIMTs were obtained at baseline using a standardized ultrasound protocol similar to those from several previous clinical trials (22–24). Carotid ultrasound exams were performed using portable Siemens Cypress systems (Siemens AG, Munich, Germany) shared between clinical centers. All scanners were equipped with 7L3 transducers, a cardiology package with ECG tracing and specific APPLE presets in order to reduce variability across centers. Standardized longitudinal b-mode images were collected for three arterial segments defined relative to the tip of the flow divider (TFD) as the common carotid artery (CCA) (from 10 to 20 mm proximal to the tip of the flow divider), the carotid bifurcation (from the TFD to 10 mm proximal to the TFD) and the proximal 10 mm of the internal carotid artery (ICA). Near and far walls were imaged simultaneously in the CCA, but separately in the carotid bifurcation and ICA to improve the ability to align each wall horizontally in these segments. For each arterial segment, images were selected at 90, 120, 150 and 180 degrees marked on the Meijer's Arc® when the right side was scanned and at 270, 240, 210 and 180 degrees when the left side was scanned. Image selections were saved as 5 second digital clips and written to 640 MB magnetic optical disks for transfer to a central reading center (Ward A. Riley Ultrasound Center, Wake Forest University School of Medicine, Winston-Salem NC). All ultrasound scans were read by a single experienced reader using Image Pro software (Media Cybernetics, Inc. Bethesda, MD). For each image sequence, the single reader selected one frame for measurement when the heart was in systole (ECG tracing is on QRS complex). Leading (far wall) and trailing (near wall) edges of visualized blood-intima and media-adventitia boundaries were traced with a computer mouse-controlled caliper within a region of interest specified by the reader. Quality assurance procedures included central training and certification of all sonographers and the reader as well as regular site visits and performance reviews.

The combination of 3 arterial segments, two walls and two sides of the neck provides a set of 12 CIMT measurement sites, each imaged from 4 angles. For each measurement site, a maximum CIMT value defined as the largest of the 4 angle specific maximum CIMTs was calculated. The 12 maximum CIMT values were then averaged to determine the mean-max CIMT over near and far walls of the right and left CCA, carotid bifurcation and ICA. For each of the 4 measurement sites in the CCA, a mean CIMT value defined as the average of the 4 angle specific mean CIMTs was also calculated. The 4 mean CIMT values were then averaged to determine the mean-mean common CIMT. Per protocol, duplicate ultrasound exams were performed within a 4 week interval at baseline and prior to randomization for each participant. The values of mean-mean common CIMT and mean-max CIMT were averaged for these two exams to obtain stable baseline measures of CIMT. Both CIMT summary measures are recognized as noninvasive surrogate predictors of future cardiovascular events and widely used in clinical trials testing the efficacy of drugs in slowing or reversing atherosclerosis (25–28).

Statistical approaches

The characteristics of the study sample were summarized using descriptive statistics with dichotomous or ordinal data presented as percentages and continuous data as means, standard deviations (SD), and medians. Differences between groups were assessed with either the chi-squared test or the non-parametric wilcoxon test.

Using cross sectional baseline data from the APPLE Trial, we first investigated potential risk factors and their univariable relationship with each of the two baseline CIMT outcomes: 1) mean-mean common CIMT (mm) and 2) mean-max CIMT (mm). The potential risk factors included demographics, physical characteristics, renal function, markers of inflammation, medical history, lipid levels, current or recent medications, and disease indices. Linear regression models were fitted using one potential risk factor of CIMT at a time to select candidate variables for multivariable modeling. Residual plots of the regression models were used to assess the suitability of the linear model. Scatter plots with overlay of LOESS (locally weighted scatterplot smoothing) curves (29) were produced to examine the descriptive relationship between continuous risk factors and CIMT outcome variables. Histograms of CIMT values by levels of the categorical risk factors were used to examine the relationship between categorical variables and CIMT outcomes. For non-linear relationships between the continuous risk factors and the CIMT outcome, transformations or piecewise linear regression models were used to better characterize the univariable relationship.

Variables with p-value ≤0.2 in the univariable model were included in multivariable linear regression modeling. Several model building procedures were used including forward, backward, and stepwise regression to produce two final models, one for each of the two CIMT outcomes. Several methods were used to assess colinearity and stability of the final models, including assessment of the association between independent variables, colinearity diagnostics within regression framework, and Mallow's Cp values. All statistical inference was done within a linear regression model framework and a p-value ≤ 0.1 is considered statistically significant due to exploratory nature of the analyses. All calculations were performed using SAS version 8.2 (30).

RESULTS

Subject characteristics

A summary of baseline demographic and clinical SLE characteristics are shown in Table 2. Of the 221 enrolled subjects, the mean age was 15.7 years (range 10.1–21.7 years), 83% were female, and 65% were minority (Hispanic or non-Caucasian). Subjects had mean disease duration of 31 months (median 25 months, interquartile range 8 to 46 months) and had mildly active disease with an average SLEDAI score of 4.6. Most subjects had no damage as measured by the SLICC. Of the 178 subjects on prednisone at study baseline, the mean weight-adjusted prednisone dose is 0.19 mg/kg/day (median 015 mg/kg/day, and interquartile range 0.05 to 0.26 mg/kg/day). The two baseline (prerandomization) CIMTs were performed a median of 9 days apart (interquartile range 2, 21).

Univariable analysis

The average of the two baseline CIMT measurements rather than a single baseline measurement was used for analysis to increase precision. The two baseline scans were strongly correlated with Pearson correlation coefficients of 0.75 for mean-mean common CIMT and 0.76 for mean-max CIMT. Based on either mean-mean common or mean-max CIMT as the dependent variable, univariable analysis suggested the following risk factors to be significantly associated with increased CIMT: increasing age, longer duration of SLE, minority status, higher BMI, male sex, higher creatinine clearance, higher Lp(a), presence of proteinuria, current azathioprine use, and current weight-adjusted prednisone dose. The complete univariable modeling results are shown in Table 3.

The relationship between weight-adjusted prednisone dose and both mean-mean common or mean-max CIMT was found to be non-monotonic with three distinct slopes. We identified

three different slopes upon examining the relationship between prednisone and CIMT and therefore performed 3 piece-wise linear regressions using the following weight-adjusted prednisone dose ranges − 0.0 to 0.15 mg/kg/day, 0.15 to 0.4 mg/kg/day, and > 0.4 mg/kg/day. Descriptive relationships are shown in Figure 1 with LOESS curves overlaying the scatter plots of mean-mean common or mean-max CIMT vs. weight-adjusted prednisone dose.

Multivariable analysis

Different model building procedures yielded the same two final models. The results of the multivariable analysis for each mean-mean common CIMT and mean-max CIMT are shown in Table 4 including the adjusted slope estimates from the final linear regression models of the potential risk factors with the CIMT variable, corresponding standard errors, and p-values. Duration of SLE, minority status and presence of proteinuria are not present in the final models due to non-significant associations after adjustment of the other variables. Azathioprine use (P=0.005 for mean-mean common model and P=0.102 for mean-max model) and male sex (P< 0.001) both were associated with increases in mean-max CIMT. Moderate dose prednisone (0.15–0.4 mg/kg/day) was only associated with decreases in mean max CIMT (P=0.024) while high (>0.4 mg/kg/day) or low (<0.15 mg/kg/day) dose prednisone was associated with mean-mean common CIMT (P=0.021) or mean-max CIMT (P=0.064), respectively. BMI (P<0.001) and creatinine clearance (P=0.031), presumably related to hyperfiltration (31), remained associated only with increased mean-mean common CIMT, while increasing age (P<0.001) and increasing Lp(a) (P=0.005) were associated only with increased mean-max CIMT in the multivariable models. For the mean-mean common CIMT model, $R^2 = 0.22$ and for the mean-max model, $R^2 = 0.20$. Thus, the potential risk factors explained approximately 21% of variation in the CIMT variables. The multivariable models were stable and no important collinearity of variables was noted using regression diagnostics, including the Mallow's Cp values.

DISCUSSION

Analysis of the baseline data from the APPLE Trial, a racially and ethnically diverse multicenter prospective cohort of pediatric SLE patients, reveals that both traditional and non-traditional risk factors are associated with increased CIMT in children with SLE. Traditional risk factors predictive of increased CIMT in this cohort include increased BMI, male sex and increasing age. Perhaps more intriguing is the identification of nontraditional risk factors including treatment with azathioprine and weight-adjusted prednisone dose.

The frequent and chronic use of glucocorticoids in children and adolescents with SLE likely plays a role in the prevalence of dyslipidemia and atherosclerosis, but this has not been well characterized to date. Some studies in adult SLE suggest an association between prednisone use and both increased total cholesterol levels and CIMT (32,33). In contrast, other studies in adults with SLE suggest that those with carotid plaque received less aggressive immunosuppressive therapy (including less prednisone and cyclophosphamide) than those without plaque, suggesting a potentially protective effect of immunosuppressive therapy on the development of atherosclerosis, possibly due to better control of underlying inflammatory disease (6,10,34). In the current study, moderate dose prednisone is associated with decreased mean-max CIMT, while higher or lower dose prednisone may predict increased mean-mean or mean-max CIMT, respectively. The demonstration of different relationships between prednisone dose and CIMT may suggest steroids are affecting more than one pathway in atherogenesis. At high doses, prednisone may increase CIMT by increasing traditional risk factors, such as cholesterol, LDL, and BMI. In contrast, with current understanding of the importance of inflammatory mechanisms in the pathogenesis of atherosclerosis (35), lower dose prednisone may be associated with higher CIMT due the

presence of ongoing active inflammatory disease. Given the cross-sectional nature of the data, it was not possible to assess the association between cumulative steroid dose and CIMT. However, this will be assessed in longitudinal analysis of the APPLE Trial. The differences in CIMT based on steroid dose may provide insight into previous conflicting results concerning the impact of oral prednisone on cardiovascular risk and suggest that there may be a "therapeutic window" concerning cardiovascular toxicity in this population.

Other medications used in the treatment of SLE may impact the development of atherosclerosis. For example, cyclosporine contributes to dyslipidemia (36,37), while hydroxychloroquine improves lipid profiles and is associated with decreased cardiovascular events (38). Azathioprine, mycophenolate mofetil, and cyclophosphamide have not previously been shown to have significant impact on lipid profiles or atherogenesis (38). A surprising finding in this study was that azathioprine was associated with increased CIMT, unlike the other immunosuppressants. This appears to be an effect independent of other variables including SLE disease activity and history of nephritis and not explained by collinearity with other variables. To further understand the relationship between azathioprine and CIMT, the 30 subjects taking azathioprine were compared to the 190 patients not taking azathioprine. As noted in Table 5, subjects on azathioprine were on average one year older (mean age 16.7 years vs. 15.6 years; p=0.027), had slightly longer disease duration (median duration 31.5 months vs. 21.5 months; p=0.005), and were more like to have a SLICC score > 0 (43.3% vs. 24.1%; p =0.027). Upon removing azathioprine from multivariable modeling, duration of SLE became statistically significant but SLICC score did not, suggesting a possible confounding effect due to relationship between azathioprine use and disease duration. However, when both variables (azathioprine and disease duration) were included in the multivariable modeling, only azathioprine remained significant suggesting azathioprine has a stronger association with CIMT. In contrast, age was a significant risk factor for higher CIMT even when azathioprine was included in the multivariable model for mean-max CIMT, suggesting both age and azathioprine have independent effects on CIMT. Given the relative affordability of azathioprine, additional analyses to explore whether azathioprine use reflected socioeconomic status revealed no correlation between the use of azathioprine and household income or parental education (data not shown).

While the potential pro-atherogenic effects of azathioprine are not known, an interesting *in vitro* observation using human umbilical vein endothelial cells showed that azathioprine and its active metabolite, 6-mercaptopurine, caused dose-dependent decreases in endothelial cell proliferation and altered endothelial nucleotide balance by reducing intracellular ATP and GTP (39). It is possible that azathioprine has a direct deleterious impact on endothelial cell function and the capacity for endothelial repair in SLE. In addition, *in vitro* studies suggest that azathioprine may increase homocysteine levels; however, we did not find a significant relationship between CIMT and homocysteine levels in this analysis (40). It is interesting to note that azathioprine was found to be a contributing predictor of vascular events in a racially/ethnically diverse cohort of adults with SLE in the multicenter LUMINA (Lupus in Minorities: Nature Versus Nurture) cohort (41). Additional study is necessary to further explore this potentially significant association.

Creatinine clearance may be increased in settings of glomerular hyperfiltration, such as proteinuria or hypertension (31,42). Although patients with significant renal insufficiency or nephrotic syndrome were excluded from the APPLE Trial, many participants had proteinuria and hypertension. With univariable analysis, both proteinuria and creatinine clearance were associated with CIMT, but only creatinine clearance remained significant in the multivariable model. It is possible that creatinine clearance and proteinuria are correlated; however, longitudinal study of this cohort will be necessary to further evaluate this effect.

In this study, CIMT was assessed using two different approaches—mean-mean common CIMT and mean-max CIMT with a correlation of 0.78 between the two outcomes, suggesting that the two outcomes are correlated but are not perfectly linearly related. The two approaches differ in the segments of the carotid artery measured, as well as how the thickness is assessed (average vs. maximal thickness of the measured segments). Both have been used in prior clinical trials and review of the literature reveals no clear superiority of one method over the other in predicting atherosclerotic risk (43,44). In multiple regression models, we identified factors (sex and medication use) that were significantly associated with both outcomes, and others that were significantly associated with only one outcome (age and Lp(a) were related to mean-max CIMT only, while BMI and creatinine clearance were related to mean-mean common CIMT only). Segment-specific risk factor associations have been reported previously in IMT studies and are generally considered to reflect the influence on focal thickening of local phenomenon such as sheer stress and arterial remodeling (45,46). Interestingly, Espeland et al. (47) reported segment-specific risk factor associations in coronary artery disease cases, but not in controls of similar age. The observation that obesity is more closely related to common CIMT than to bifurcation or internal CIMT is consistent with prior reports in adults (46,47). Associations between estimated creatinine clearance and common CIMT have previously been reported in children with chronic renal failure (48) and in young adults with myocardial infarction (49). Reports from children and adolescents with growth hormone deficiency (50) and from adult women with SLE and cardiovascular disease (51) have also documented significant increases for both Lp(a) and common CIMT relative to normal controls. Segment-specific differences in associations between CIMT and either creatinine clearance or Lp(a) have not been reported previously.

There are several limitations to this study. The sample size was only powered to detect difference in the primary outcome of the APPLE Trial – progression rate of CIMT. Thus it may not have sufficient power to identify all meaningful clinical associations of risk factors with baseline CIMT. However, this is the largest cohort of pediatric SLE patients ever examined for cardiovascular risk and the potential relationships reported here are hypothesis generating and can be confirmed by future studies. In addition, as in any clinic trial, there is an inherent selection bias such that the APPLE cohort may not be completely representative of pediatric lupus patients as a whole. For example, obese patients were more likely to be excluded due the inability to obtain high quality CIMT images. Patients with nephrotic range proteinuria, very elevated cholesterol or renal insufficiency, also excluded from the APPLE study, are known to have increased CIMT (5), These subsets of pediatric SLE patients may have increased CIMT and increased lipid abnormalities and different relationships between the risk factors and CIMT. Thus, their exclusion may affect the generalizability of the reported findings.

The presented models only explain approximately 21% of the variation in CIMT suggesting other measured or unmeasured risk factors not identified in the current study. Cross sectional analyses of the baseline data also limits the ability to draw conclusions about causation between risk factors and CIMT outcomes. Once the APPLE Trial is complete, longitudinal analysis will provide more meaningful assessment of the impact of glucocorticoids, azathioprine and other factors on CIMT. Unfortunately, the APPLE study does not include matched normal controls. Because of the inherent variation in CIMT measurement techniques, the absolute thickness can not be compared between studies. Therefore, there is no potential to compare the CIMT results in this cohort with a healthy population.

In summary, traditional as well as non-traditional risk factors predict increased CIMT in pediatric patients with SLE. Of interest, treatment with azathioprine was associated with

increased CIMT and the relationship with weight-adjusted oral prednisone dose may not be linear. Future longitudinal analysis of data from the APPLE Trial will further clarify the role of medications such as azathioprine and corticosteroids, as well as other potential risk factors of CIMT progression. Understanding risk factors in the development of atherosclerosis in children and adolescents with SLE will inform evidence-based treatment recommendations to avoid irreversible cardiovascular damage.

Acknowledgments

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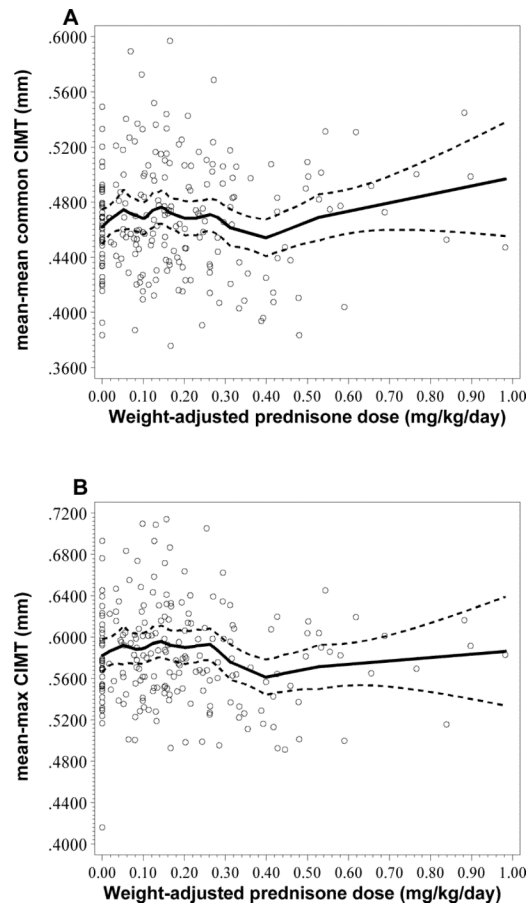
Figure 1.
A. LOESS Curve showing mean-mean common CIMT vs. weight-adjusted prednisone dose
(mg/kg/day) **B.** LOESS curve showing mean-max CIMT vs. Weight-adjusted prednisone
dose (mg/kg/day).

Table 1

Inclusion and exclusion criteria for the APPLE trial

Inclusion Criteria	Exclusion Criteria
• SLE diagnosed based on 1997 Revised ACR criteria	• drug-induced lupus
• weight ≥25 kg	• liver disease (ALT or AST >2x ULN)
• outpatient status	• myositis (CK >3x ULN)
• age between ≥10 and ≤21 years	• nephrotic syndrome (urinary protein >3 g/24 h or protein/creatinine ratio of >3.0 and serum albumin <2.3 g/dL)
• ability to complete self report questionnaires in English or Spanish	• hypercholesterolemia (total cholesterol >350 mg/dL)
• willingness to comply with recommended diet	• presence of xanthoma
• willingness to use approved birth control method	• familial hypercholesterolemia
	• renal insufficiency (dialysis or creatinine > 2.5 mg/dL)
	• current use of cyclosporine or tacrolimus
	• pregnant or lactating
	• current use of oral contraceptives containing ≥50 mcg estradiol
	• life threatening non-SLE illness
	• current drug or alcohol abuse
	• inability to obtain adequate-quality CIMT images

ULN – upper limit of normal

Table 2

Baseline characteristics of the APPLE subjects.

Variable	Mean (S.D.) or n (%)	Median	Total N
Age (years)	15.7 (2.6)	15.5	221
Female	184 (83.3%)		221
Weight (kg)	62.0 (17.2)	58.7	221
Body Mass Index	24.4 (5.3)	23.4	221
Duration of Lupus (months)	31.2 (28.5)	25.0	220
Hispanic	54 (24.4%)		221
Race[***]			
Caucasian	114 (51.6%)		221
African American	59 (26.7%)		221
Asian	23 (10.4%)		221
American Indian	6 (2.7%)		221
Native Hawaiian	5 (2.3%)		221
Other	30 (13.6%)		221
Minority (Hispanic Ethnicity or Non-Caucasian Race)	144 (65.2%)		221
Hx. Smoking (self-report)	7 (3.2%)		221
Prednisone Dose (mg/kg/day)	0.19 (0.19)	0.15	218
Fam. Hx. Cardiovascular disease	79 (37.4%)		211
Creatinine Clearance (mL/min/m2)	139.4 (33.0)	134.1	216
Proteinuria	56 (25.5%)		220
Hx. Renal Abnormalities			
Hx. Hypertension	73 (34.1%)		214
Hx. Nephrotic syndrome	38 (17.4%)		219
Hx. Nephritis	79 (36.1%)		219
Other	20 (10.5%)		190
Current Medications			
Hydroxychloroquine	213 (96.4%)		221
Corticosteroids	181 (81.9%)		221
Cyclophosphamide	26 (11.8%)		221
Mycophenolate	53 (24.0%)		221
Azathioprine	30 (13.6%)		221
Methotrexate	29 (13.1%)		221
ACE[#] inhibitors	54 (24.4%)		221
NSAIDs	68 (30.8%)		221
ASA	147 (66.5%)		221
Total Cholesterol (mg/dL)	155.1 (38.0)	148.0	211
HDL Cholesterol (mg/dL)	46.3 (12.8)	44.0	211
LDL Cholesterol (mg/dL)	86.4 (31.4)	78.5	210
Triglycerides (mg/dL)	114.0 (66.4)	101.0	211
Homocysteine (mcmol/L)	7.5 (3.1)	6.8	207

Variable	Mean (S.D.) or n (%)	Median	Total N
Lp(a) (mg/dL)	23.1 (26.8)	13.5	206
Log Lp(a)	2.5 (1.2)	2.6	206
Mean-Maximal CIMT (mm)	0.585 (0.052)	0.579	221
Mean-Mean Common CIMT (mm)	0.468 (0.041)	0.466	221
HsCRP (mg/L)	3.6 (13.9)	0.7	202
Log hsCRP	−0.27 (1.52)	−0.36	202
ESR	23.1 (22.9)	16.0	148
Anti-DS DNA antibody positive	106 (48.0%)		221
SLEDAI Total	4.6 (4.2)	4.0	221
SLICC Damage Index >0	59 (26.7%)		221

* Creatinine clearance calculated using Schwartz Formula as in methods

** Proteinuria defined as >500 mg/24 hours or spot protein:creatinine ratio of 0.5.

***
Some subjects self-reported more than one race.

#
Angiotensin converting enzyme

Table 3

Univariable relationships between clinical risk factors and CIMTs

Variable	Mean-Mean Common CIMT (mm)		Mean-Max CIMT (mm)	
	Slope (S.E.)	P-Value	Slope (S.E.)	P-Value
Age (Years)	0.001 (0.001)	0.175	0.005 (0.001)	< 0.001
Log hsCRP	0.002 (0.002)	0.237	0.0008 (0.002)	0.752
BMI	0.001 (0.0005)	0.005	0.0004 (0.0007)	0.504
SLEDAI Total	0.0007 (0.0006)	0.302	−0.0002 (0.0008)	0.849
Positive anti-DS DNA antibody	0.005 (0.005)	0.368	0.002 (0.007)	0.738
Duration of Lupus (Months)	0.0002 (0.00010)	0.015	0.0004 (0.0001)	0.001
Creatinine Clearance[*] (mL/min/m2)	0.004 (0.002)	0.008	0.003 (0.002)	0.134
Proteinuria[**]	0.016 (0.006)	0.010	0.010 (0.008)	0.236
Hx. Nephrotic syndrome/Nephritis	0.006 (0.006)	0.276	0.007 (0.007)	0.312
Hx. Hypertension	0.002 (0.006)	0.681	−0.002 (0.008)	0.794
Fam. Hx. of Cardiovascular Disease	−0.003 (0.006)	0.568	−0.002 (0.007)	0.771
LDL	−0.00006 (0.00009)	0.508	−0.00007 (0.0001)	0.547
HDL	−0.0002 (0.0002)	0.372	0.0002 (0.0003)	0.431
Log Lp(a)	0.004 (0.002)	0.092	0.009 (0.003)	0.002
Homocysteine (mcmol/L)	0.0010 (0.0009)	0.281	0.00004 (0.001)	0.975
Female	−0.036 (0.007)	< 0.001	−0.039 (0.009)	< 0.001
SLICC Damage Index > 0	0.006 (0.006)	0.325	0.012 (0.008)	0.131
Minority (Hispanic or non-Caucasian)	0.011 (0.006)	0.055	0.013 (0.007)	0.072
NSAIDs Current	0.007 (0.006)	0.235	0.003 (0.008)	0.731
Cyclophosphamide/Mycophenolate Current	0.001 (0.006)	0.800	0.001 (0.007)	0.847
Azathioprine Current	0.017 (0.008)	0.031	0.023 (0.010)	0.022
Methotrexate Current	0.010 (0.008)	0.209	0.005 (0.010)	0.659
ASA Current	−0.004 (0.006)	0.492	0.007 (0.007)	0.328
ACE[#] Inhibitor Current	0.010 (0.006)	0.125	0.003 (0.008)	0.724
Prednisone (<0.15 mg/kg/day)	0.086 (0.056)	0.129	0.109 (0.072)	0.132
Prednisone (0.15–0.40 mg/kg/day)	−0.096 (0.043)	0.027	−0.150 (0.055)	0.007
Prednisone (>0.40 mg/kg/day)	0.091 (0.041)	0.027	0.052 (0.052)	0.318

[*] Creatinine clearance calculated using Schwartz Formula as in methods

[**] Proteinuria defined as >500 mg/24 hours or spot protein:creatinine ratio of 0.5.

[#] Angiotensin converting enzyme

Table 4

Multivariable adjusted relationships between clinical risk factors and CIMTs[*]

Variable Name	Mean-Mean Common CIMT (mm)		Mean-Max CIMT (mm)	
	Slope (S.E.)	P-Value	Slope (S.E.)	P-Value
Age (Years)	NI		0.004 (0.001)	< 0.001
BMI	0.002 (0.0005)	< 0.001	NI	
Creatinine Clearance (mL/min/m2)	0.003 (0.002)	0.031	NI	
log of Lp(a)	NI		0.008 (0.003)	0.005
Female	−0.032 (0.007)	< 0.001	−0.034 (0.009)	< 0.001
Azathioprine Current	0.021 (0.007)	0.005	0.016 (0.010)	0.102
Prednisone (0.00–0.15 mg/kg/day)	0.057 (0.051)	0.262	0.128 (0.069)	0.064
Prednisone (0.15–0.40 mg/kg/day)	−0.022 (0.041)	0.587	−0.119 (0.052)	0.024
Prednisone (≥0.40 mg/kg/day)	0.086 (0.037)	0.021	0.049 (0.048)	0.308

NI – not included in the final models due to non-significant associations.

[*] Only associations with p-value <= 0.1 in the final models are shown **above**.

Table 5

Comparison of subjects taking azathioprine (N=30) and not taking azathioprine (N=190).

Variable	No Azathioprine Mean (S.D.) or n (%)	Median	Azathioprine Mean (S.D.) or n (%)	Median	P-Value
Age (years)	15.6 (2.7)	15.5	16.7 (2.4)	17.1	0.027
Female	158/191 (82.7%)		26/30 (86.7%)		0.591
Weight (kg)	61.7 (16.9)	59.5	63.9 (19.0)	58.0	0.914
Body Mass Index	24.3 (5.3)	23.3	24.9 (5.8)	24.1	0.690
Duration of Lupus (Months)	28.7 (25.9)	21.5	47.0 (38.3)	31.5	0.005
Total Minority (Hispanic Ethnicity or Non-Caucasian Race)	124/191 (64.9%)		20/30 (66.7%)		0.852
Fam. Hx. CVD	69/182 (37.9%)		10/29 (34.5%)		0.723
History of Smoking	6/191 (3.1%)		1/30 (3.3%)		1.000
Prednisone Dose (mg/kg/day)	0.19 (0.19)	0.14	0.18 (0.17)	0.15	0.870
Creatinine Clearance (mL/min/m2) [*]	140.0 (34.0)	134.4	134.9 (25.4)	133.2	0.604
Proteinuria [**]	45/191 (23.6%)		11/29 (37.9%)		0.098
Renal abnormalities					
Hypertension	65/184 (35.3%)		8/30 (26.7%)		0.354
Nephrotic/Nephritic	83/189 (43.9%)		14/30 (46.7%)		0.778
Current Medications					
Hydroxychloroquine	183/191 (95.8%)		30/30 (100%)		0.602
Corticosteroids	154/191 (80.6%)		27/30 (90.0%)		0.215
Cyclophosphamide	26/191 (13.6%)		0/30 (0.0%)		0.030
Mycophenolate	52/191 (27.2%)		1/30 (3.3%)		0.004
Methotrexate	27/191 (14.1%)		2/30 (6.7%)		0.385
ACE[#] Inhibitor	47/191 (24.6%)		7/30 (23.3%)		0.880
ASA	128/191 (67.0%)		19/30 (63.3%)		0.691
NSAIDs	59/191 (30.9%)		9/30 (30.0%)		0.922
SLICC Damage Index > 0	46/191 (24.1%)		13/30 (43.3%)		0.027
SLEDAI Positive anti-DS DNA	94/191 (49.2%)		12/30 (40.0%)		0.348
SLEDAI Total	4.6 (4.2)	4.0	4.7 (4.6) 4.0		0.985
ESR	22.8 (23.3)	15.0	24.9 (20.9)	18.0	0.297

Variable	No Azathioprine		Azathioprine		
	Mean (S.D.) or n (%)	Median	Mean (S.D.) or n (%)	Median	P-Value
Total Cholesterol (mg/dL)	155.1 (38.4)	148.0	155.6 (35.4)	153.0	0.865
Triglycerides (mg/dL)	115.3 (69.3)	102.0	106.1 (44.9)	97.0	0.888
HDL Cholesterol (mg/dL)	46.0 (12.4)	44.0	48.2 (14.8)	49.0	0.318
LDL Cholesterol (mg/dL)	86.4 (31.3)	79.0	86.2 (32.3)	78.0	0.924
Homocysteine (mcmol/L)	7.4 (3.2)	6.8	7.6 (2.8)	6.5	0.734
Lp(a) (mg/dL)	23.1 (27.6)	14.0	23.0 (21.8)	13.0	0.516
log of Lp(a)	2.5 (1.2)	2.6	2.6 (1.1)	2.6	0.516
HS CRP (mg/L)	3.35 (14.40)	0.60	4.88 (10.83)	0.80	0.165
log of HS CRP	−0.34 (1.47)	−0.51	0.11 (1.77)	−0.22	0.165
Mean-Maximal CIMT (mm)	0.466 (0.040)	0.462	0.483 (0.041)	0.475	0.036
Mean-Mean Common CIMT (mm)	0.582 (0.050)	0.578	0.605 (0.058)	0.611	0.034

*
Creatinine clearance calculated using Schwartz Formula as in methods

**
Proteinuria defined as >500 mg/24 hours or spot protein:creatinine ratio of 0.5.

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Angiotensin converting enzyme