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Sub-clinical Atherosclerosis in Pediatric Liver Transplant Recipients: Carotid and Aorta Intima-media Thickness and Their Predictors

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Abstract

Objective—To investigate prevalence and predictors of cardiovascular risk in pediatric liver transplant recipients using non-invasive markers of subclinical atherosclerosis: intima-media thickness in the carotid arteries (cIMT) and aorta (aIMT).

Study design—Cross-sectional study of 88 pediatric liver transplant recipients. cIMT, aIMT measured by ultrasound using standardized protocol.

Results—Participants were 15.4 ± 4.8 years, and 11.2 ± 5.6 years post-transplant. cIMT and aIMT were both higher in males than females. In analyses adjusted for sex, age, and height, cIMT was higher in subjects transplanted for chronic/cirrhotic liver disease and lower in subjects on cyclosporine (n=9) than tacrolimus (n=71). cIMT was not associated with rejection history or current corticosteroid use. cIMT increased with increasing diastolic blood pressure and triglycerides. aIMT (n=83) also increased with age, and its rate of increase post-transplant varied

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by age at transplant. In adjusted analyses, aIMT was higher in subjects with glucose intolerance. In analysis of patients ≤20 years for whom blood pressure percentiles could be calculated (n=66), aIMT increased with increasing diastolic blood pressure percentile (0.010 mm per 5-percentile, 95% CI 0.000–0.021, *P*=0.05). Neither cIMT nor aIMT was associated with obesity, systolic hypertension, or other dyslipidemia at study visit.

Conclusion—Measures of long-term cardiovascular risk were associated with conditions that are more common in pediatric liver transplant recipients than non-transplanted peers—diastolic hypertension and glucose intolerance. Larger, longitudinal studies are warranted to investigate whether cIMT could be useful for stratifying these patients' cardiovascular risk—and potential need for proactive intervention—during long-term follow-up.

Keywords

liver transplantation; cardiovascular risk; atherosclerosis; metabolic syndrome; children

In adult liver transplant recipients, cardiovascular disease is the third leading cause of death. (1) A key risk factor for cardiovascular events in adults, ie, myocardial infarction or stroke, is post-transplant metabolic syndrome (PTMS)—a clustering of obesity, hypertension, dyslipidemia, and glucose intolerance. (2, 3) In pediatric liver transplant recipients, we have recently shown that PTMS and its components are common: 28% of children and young adults are overweight or obese, almost 35% have hypertension or pre-hypertension, 44% have pre-diabetes, and 37% have low HDL. (4)

Survival well into adulthood is now the norm for these children. However, the impact of PTMS components on long-term cardiovascular health in these children is not known. Thus, elucidation of cardiovascular disease precursors and risk factors—particularly those that could be treated to prevent later morbidity—is a priority. (5)

In non-transplanted children, carotid intima-media thickness (cIMT) has proven useful as a noninvasive method for predicting later cardiovascular risk. cIMT is measured by ultrasound in the common carotid arteries, and standard measurement protocols have been endorsed by the American Heart Association for assessing subclinical atherosclerosis in pediatric clinical research. (6) cIMT is a direct measure of arterial thickening, so it is appealingly applicable across populations that may have different risk factors for cardiovascular disease. Norms by age, height, and sex have been established. It changes reliably enough with treatment, for example of hypertension or dyslipidemia, that it is used an endpoint in clinical trials. (6, 7) Most importantly, it predicts future cardiovascular events in adults. (6, 7)

In this study, we measured cIMT in pediatric liver transplant recipients with the aim of investigating whether the high prevalence of PTMS components is accompanied by end-organ evidence of early atherosclerosis. (6) We also assessed aortic intimal-medial thickness (aIMT), which may detect earlier subclinical atherosclerosis than cIMT. (8–10) There are no previous reports of aIMT in this population.

METHODS

We performed a cross-sectional study of pediatric liver transplant recipients, aged 8–30 years at study visit, <18 years at first liver transplant. At study visit, all were at least one year from transplant and on stable immunosuppressive regimens for at least three months. This study was approved by University of California San Francisco's Committee on Human Research (UCSF CHR, IRB# 12-10290, 14-13939). After age-appropriate consent and assent were obtained, subjects were evaluated in UCSF's Pediatric Clinical Research Center or during elective inpatient admission for a surveillance liver biopsy, which were done only during clinically stable periods. Ultrasounds were done in UCSF's Pediatric Radiology suite. Visits were completed September 2013 through March 2017. In addition to demographic factors like age and sex, we evaluated disease for which the participants were transplanted as a predictor; we classified patients by major disease categories (Table I) and by acute/non-cirrhotic disease (e.g. acute liver failure, hepatoblastoma, urea cycle disorders) versus chronic/cirrhotic liver disease (e.g. biliary atresia, progressive familial intrahepatic cholestasis (PFIC), Alagille, alpha 1 antitrypsin deficiency).

For subjects younger than 18 years at study visit, BMI percentile for age and sex was calculated based on 2000 CDC growth chart data. (11) Subjects were classified as overweight for BMI percentile 85^{th}–94^{th} percentile and obese for BMI percentile ≥ 95^{th} percentile. (12) Elevated waist circumference was considered ≥90^{th} percentile for age and sex. (13, 14) Systolic and diastolic hypertension were defined as use of anti-hypertensives or blood pressure ≥95^{th} percentile for sex, age, and height; pre-hypertension included those with blood pressure percentiles 90–94^{th} percentile. (13, 15)

Subjects 18 years or older were classified according to adult guidelines. Overweight was considered BMI 25–29.9kg/m^2 and obese BMI ≥30 kg/m^2. Elevated waist circumference was ≥88cm for females and ≥102cm for males. (14) Hypertension was defined as use of anti-hypertensives or systolic blood pressure ≥140 mmHg; diastolic ≥90mmHg. Pre-hypertension included those with systolic ≥120 mmHg; diastolic ≥80mmHg. (13)

Elevated lipids for all subjects represented values at or above the 75^{th} percentile for children and young adults. (13) Cutoffs were: triglycerides ≥ 75mg/dL for children 9 or younger and ≥ 90 mg/dL for those 10 or older; low-density lipoprotein (LDL) >110 mg/dL, and total cholesterol ≥ 170mg/dL. Low HDL was ≤ 40 mg/dL, which is ≤10^{th} percentile. (13) Oral glucose tolerance testing (OGTT) was done with a weight-based glucose load; elevated fasting glucose was ≥100 mg/dL and impaired glucose tolerance (IGT) ≥140mg/dL at two hours, following American Diabetes Association definitions. (16) HOMA-IR, a measure of insulin resistance, was calculated as (fasting glucose × fasting insulin)/4.05. (17)

We defined post-transplant metabolic syndrome as the presence of three or more of the following: (1) elevated waist circumference, (2) systolic or diastolic hypertension, (3) elevated triglycerides, (4) low HDL, (5) elevated fasting glucose or IGT. (13, 18)

cIMT was measured following American Heart Association (AHA) pediatric guidelines for non-invasive assessment of subclinical atherosclerosis in clinical research. (6) Patients were positioned supine, with neck in slight extension and head turned 45 degrees away from the

side being measured. Using a General Electric Logiq E9 ultrasound machine, with linear 9MHz transducer, the intimal plus medial layer thickness of the common carotid artery far wall was measured 1–2cm proximal to its bifurcation in two planes (anterior/oblique and lateral), on both the right and left carotids, by a trained sonographer. Mean cIMT represents the average of 4 measurements: far-wall thickness on the right and left sides, in true coronal and oblique coronal planes. (6) All measurements were verified at a radiology workstation (with manual adjustment of calipers if needed) by a single pediatric radiologist (AP) (Figure 1).

cIMT z-scores were calculated utilizing sex-specific LMS tables normalized for age in children 6–18, which is the range included in the LMS tables. LMS tables for the same cohort of >1000, non-obese, normotensive children are available normalized for height. (19) cIMT measurements used in the generation of LMS tables were averaged far-wall thickness of the common carotid artery, matching our measurement protocol. (20)

To consider predictors of relatively “elevated” cIMT within our cohort, we calculated cIMT z-scores, normalized for age and sex. Z-scores were calculated only for participants ≤ 18 years of age, to mirror the healthy children from which the norms were derived (19) (Figure 2; available at www.jpeds.com) We divided our cohort into quartiles by cIMT z-score, and compared our top quartile to our lower 3 because there is no established cutoff for an “elevated” cIMT and we did not have a local control group against which to validate our z-scores.

aIMT was measured in the distal abdominal aorta far wall, within 15mm of the aortic bifurcation. (21) Z-scores for aIMT were not calculated given the lack of published LMS tables to allow for calculation. (21)

Statistical analyses

Differences in mean IMT between groups were first assessed using t-tests allowing for unequal variances. When median, interquartile ranges were reported for sample subsets or skewed groups, p-values were derived from Kruskal-Wallis non-parametric testing. Associations between mean IMT and clinical predictors—demographics, medications, metabolic syndrome components—were then investigated using linear regression, with all models adjusted for sex and age, and cIMT additionally adjusted for height. (19, 22) For each association with cIMT, we also tested an age × predictor interaction term for significance, to evaluate whether the association magnitude varies by age—as has been reported in previous studies of pediatric cohorts that span childhood and adolescence (23) (TABLE 2). aIMT values were predicted from the fully-adjusted linear regression model.

RESULTS

This cross-sectional study included a diverse group of 88 pediatric liver transplant recipients, at a mean age of 15.4 years and 11.2 years after first liver transplant (TABLE 1). Four patients were transplanted for PFIC (PFIC1=2, PFIC2=2). Two had Alagille syndrome, and none were transplanted for familial hypercholesterolemia or cholesterol ester storage disease. Most patients were receiving calcineurin-inhibitor monotherapy (TABLE 1); only 4

were receiving corticosteroids (2 on ≤5 mg daily, 2 receiving 20mg daily). None were receiving sirolimus. All 6 participants that were re-transplanted had it done within 6 months of initial transplant—five for hepatic artery thrombosis and 1 for cholangitis.

Overweight/obesity, systolic hypertension, and low HDL were each present in more than 25% of the cohort, as has been reported previously (TABLE 1).(4) 37% of participants had at least one component of PTMS, 20% had 2, and 16% had 3 or more. Only 1 patient was on anti-hypertensives at study visit, with controlled hypertension. None were on lipid-lowering or anti-hyperglycemic medications.

Mean cIMT in the cohort was slightly higher in males than females. It increased with age, after controlling for sex and height (TABLE 2). When age at visit was divided into years prior to and after liver transplant, cIMT increased for each year since transplant (0.005 mm, 95% CI 0.002 – 0.009, p=0.006) and remained associated with age at transplant (0.005 mm per year of age, 0.001–0.009, p=0.02), with no significant interaction term between the two predictors ($P = .55$). Thus, the rate of cIMT increase per year thus appeared to be the same before and after transplant. All subsequent cIMT analyses were adjusted for sex, age at visit, and height.

cIMT was significantly higher in patients transplanted for chronic/cirrhotic liver disease (TABLE 2) but did not differ significantly by Hispanic ethnicity, type of transplant (whole vs split/partial) or donor (living vs. deceased) in these adjusted models (data not shown). cIMT was slightly lower in multi-racial subjects (−0.044 mm, 95% CI −0.077 – −0.008, p=0.02) compared with white subjects with no other significant differences by race (data not shown for other categories). cIMT was not associated with corticosteroid use at study visit (n=4), history of any acute rejection, number of acute rejection episodes, and diagnosis of chronic rejection (n=5) or kidney/liver laboratory tests at study visit (serum creatinine, estimated glomerular filtration rate by Schwarz equation, AST, ALT, or total bilirubin at study visit; data not shown).

Mean cIMT was significantly lower in the 9 patients on cyclosporine than those on tacrolimus (TABLE 2). Those on cyclosporine were of similar age at visit (median 16.9 vs. 15.8 years, p=0.38) but significantly farther from transplant (median 16.3 years, IQR 12.4 – 18.4) and younger at time of transplant (median 0.64 years, IQR 0.44 – 1.36) than those on tacrolimus (median 10.2 years from transplant, IQR 5.8 – 14.4; median 2.49 years of age at transplant, IQR 0.77 – 7.53, p=0.007 for both comparisons). There was no difference in number of previous acute rejection episodes (median 1 for each group, p=0.93). In sensitivity analysis excluding the 4 patients on corticosteroids, those on cyclosporine still had significantly lower cIMT at study visit than those on tacrolimus (−0.184 mm, 95% CI −0.347 – −0.020, p=0.03), and there was still a significant interaction with age (p=0.03).

In examining associations between PTMS components and cIMT, we considered interactions between each cardiovascular risk factor and age. (23) cIMT was significantly higher in participants with diastolic hypertension and elevated triglycerides, as categorized by age-specific criteria, in univariate analysis. (TABLE 2)

cIMT increased in tandem with diastolic blood pressure and triglycerides in fully adjusted models. (TABLE 2) cIMT was also associated with HDL, although unexpectedly increases in HDL were associated with increases in cIMT. In models considering PTMS components categorically as “normal” vs. “abnormal,” patients with elevated triglycerides for age had higher cIMT. (TABLE 3; available at www.jpeds.com) Sensitivity analysis excluding re-transplanted patients did not impact variable significance or coefficient magnitude.

In our subjects ≤18 years of age at study visit (n=67), median cIMT z-score was 1.94, IQR 0.88–2.58. Of interest, 57% of patients transplanted for chronic/cirrhotic liver disease had a c-IMT z-score ≥2, compared with 32% of those transplanted for acute/non-cirrhotic disease. Comparing the patients with cIMT z-scores in the highest quartile (≥2.59) with those in the lower 3 revealed no significant differences in age at or years since transplant, race/ethnicity, transplant for chronic/cirrhotic liver disease, years of immunosuppression, or number of previous acute rejection episodes (data not shown). Across cIMT z-score quartiles, there were no significant differences in the prevalence of overweight/obesity, systolic or diastolic hypertension, dyslipidemias, glucose intolerance, or PTMS (data not shown).

All 4 of the ≤18-year-old subjects with a history of chronic rejection had a cIMT z-score in the highest quartile, compared with 20% of those without chronic rejection ($p<0.001$). These ranged in age from 8.9–13.4 years of age at study visit, and were 6.4–11.5 years from transplant. None were on corticosteroids or sirolimus. One had borderline high triglycerides and systolic pre-hypertension, but none had diastolic hypertension.

Paralleling one previous study, both PFIC1 patients in our cohort had cIMT z-scores in the highest quartile despite being normotensive, not obese, with normal lipids and on tacrolimus monotherapy. (24) None of the PFIC3 or Alagille syndrome patients had cIMT z-score in the highest quartile (n=4, z-score range 0.89–1.80).

As a sensitivity analysis, we also calculated cIMT z-scores normalized for height and sex. (19) The age and height-normalized z-scores correlated tightly ($r=0.99$, $p<0.001$). Repeating the above analyses using height-normalized z-scores did not change any of the reported relationships or quartile categorization.

Analysis of aortic intima media (aIMT) thickness included 83 of the 88 participants with technically adequate ultrasounds. aIMT and cIMT were not significantly correlated in linear regression ($r=0.15$, $p=0.18$). aIMT was significantly higher than cIMT in paired analysis in both males (mean aIMT 0.671 mm, 95%CI 0.605–0.737 vs. mean cIMT 0.480 mm, 95% CI 0.462–0.498, $p<0.001$) and females (mean aIMT 0.587 mm, 95%CI 0.534–0.639 vs. mean cIMT 0.456 mm, 95%CI 0.438–0.474, $p<0.001$).

In univariate analysis, aIMT increased significantly with age (0.009 mm per 1 year, 95% CI 0.000–0.018, $p=0.05$), height (0.013 mm per 5 cm height, 95% CI 0.000–0.025, $p=0.05$), and sex (0.008 mm higher in males than females, CI −0.001–0.169, $p=0.053$). Of interest, both age at transplant and years since transplant were highly associated with increasing aIMT, (TABLE 4) and there was a significant statistical interaction between the two variables ($p=0.03$). This suggests that aIMT may increase at a different rate post-transplant, depending on the age at transplant. (FIGURE 3; available at www.jpeds.com) All subsequent

analyses were adjusted for sex, age at transplant, years since transplant, and an interaction between the latter two.

In adjusted analyses, aIMT increased with stimulated glucose (2-hour glucose during oral glucose tolerance test) and HOMA-IR (TABLE 4). aIMT was significantly higher in subjects that had glucose intolerance (2-hour glucose≥140mg/dL). aIMT's increase with increasing diastolic blood pressure had borderline statistical significance. In a model limited to patients ≤20 years of age for whom blood pressure percentiles could be calculated (n=66), aIMT did significantly increase with increasing DBP percentile (0.010 mm per 5-percentile DBP increase, 95% CI 0.000–0.021, p=0.05) but not SBP percentile.

aIMT retained a significant association with increasing stimulated glucose and increasing HOMA-IR in fully adjusted models. In models considering PTMS components categorically as "normal" vs. "abnormal," patients with glucose intolerance had significantly higher aIMT than those with normal glucose tolerance (TABLE 3). Sensitivity analysis excluding re-transplanted patients did not impact variable significance or coefficient magnitude.

DISCUSSION

This cohort of pediatric liver transplant recipients is the largest in which cIMT has been measured and the first to report on aIMT. In this cohort, increasing diastolic blood pressure, triglycerides, and transplant for chronic/cirrhotic liver disease were associated with increasing cIMT. (25) Higher aIMT was associated with glucose intolerance, and with increasing diastolic blood pressure percentile in recipients ≤20 years of age, but not with transplant indication. We have recently shown that pediatric liver transplant recipients are at increased risk for diastolic hypertension and glucose intolerance compared with non-transplanted peers; (4) this analysis connects those morbidities to a potential long-term risk of cardiovascular disease.

Previous research, and published guidelines, have not included pediatric liver transplant recipients as an at-risk group for long-term cardiovascular disease, (13, 26, 27) but these conclusions were not based on robust evidence. The two previous studies of cIMT in pediatric liver transplant recipient failed to demonstrate any correlation between cIMT and any metabolic syndrome components. But both were very small (n=9, n=31), and likely did not have adequate power to detect significant correlations. (26, 27)

Although our sample size is still limited, we did detect correlations between some PTMS components—notably diastolic hypertension, hypertriglyceridemia, and glucose intolerance—and markers of later cardiovascular risk. Our analysis does suggest that larger, longitudinal studies, are warranted to determine whether pediatric liver transplant recipients—or potentially subsets depending on transplant indication—are at-risk for elevated cIMT or aIMT and early cardiovascular events. Prospective studies will be key for developing evidence-based screening protocols and preventive strategies to ameliorate long-term cardiovascular risk in these children and young adults. (28)

Also important is that obesity was not a reliable predictor of cIMT or aIMT in our cohort. It is possible that something related to the liver disease pre-transplant, the transplant itself, or

the immunosuppression exposure following it has a stronger impact on cIMT and aIMT than current obesity or full-blown metabolic syndrome in our patients. Our data suggested, for example, that aIMT increased at a different rate post-transplant depending on the age at transplant. Patients with chronic liver disease are often have hyperdynamic cardiovascular function and relatively low blood pressure—which may impact vascular stress and thickness pre-transplant. Post-transplant, those requiring higher levels of calcineurin-inhibitors to suppress rejection may have more hypertension—again impacting vascular stress and thickness. However, repeated measures in patients from the time of transplant are needed to confirm this and understand its significance. Medications used routinely post-transplant—especially calcineurin-inhibitors and corticosteroids—can cause transient conditions that may accelerate cIMT increase: hypertension, dyslipidemia, and glucose intolerance. Patients with chronic rejection had relatively elevated cIMT scores compared with others in the cohort, which may represent an impact of previous or intermittent hypertension or dyslipidemia related to immunosuppression. A recent systematic review found that pediatric kidney transplant recipients are have higher cIMT than healthy controls after adjusting for hypertension—again supporting the hypothesis that immunosuppression medications, which are the same as those used in liver transplant recipients—may be contributing to risk. (29)

More detailed, prospective tracking of medication exposure will be helpful to evaluate whether arterial thickness might increase with high levels—and concurrent hypertension, dyslipidemia, or other risk factors—and then regress if exposure is low and stable. Our finding that patients on cyclosporine had lower cIMT than those on tacrolimus suggests this pattern. Although these patients were relatively far from transplant, they remained on cyclosporine because they had been clinically stable at low doses; none were hypertensive at study visit, only one of 9 was overweight with elevated triglycerides.

The contribution of genetic diseases that affect both the liver and the cardiovascular system also warrants further study. One previous case series identified the same pattern we observed: higher-than-expected cIMTs in PFIC1 patients, but normal range cIMT in Alagille patients. (24) Nagasaka et al found that children with PFIC1 and 2 had high oxidized LDL, dense LDL particles enriched in triglycerides, and low HDL—an atherogenic profile. In contrast, children with Alagille syndrome had high levels of lipoprotein X and HDL with minimally elevated oxidized LDL—a pattern protective against atherosclerosis. (24)

PFIC1 has been associated with decreased farnesoid X receptor (FXR) activity in intestinal cells. (30) Interestingly, FXR is also expressed in vascular endothelium, some macrophages, and other organs; a role in atherosclerosis has been posited through mechanisms including changes in lipid trafficking, vasoconstriction, and inflammation. (31) Liver transplant would presumably ameliorate FXR dysfunction in the livers of PFIC1 patients, but its persistent dysfunction in other tissues is one possible explanation for elevated cIMT in these patients.

Although we did identify diastolic blood pressure, triglycerides, and glucose intolerance as correlates of subclinical atherosclerosis in our cohort, we did not identify associations of cIMT and aIMT with other cardiovascular risk factors that have been reported in non-transplanted children —including BMI, systolic blood pressure, and LDL. (10) A larger cohort may be required to detect significant correlates. Because cIMT and aIMT

measurements approach the limits of precision for ultrasound, the range of potential measurements is limited—limiting power to detect correlations in our relatively small sample. One radiologist manually adjusted calipers on all measurements to enhance consistency, although the best method for determining carotid boundaries—manual vs. automatic using computer algorithms—remains a topic of debate.(32)

We found that aIMT was more technically difficult to measure because of bowel gas, and manual calibration often led to significantly different measurements. (FIGURE 1). There are not established norms for aIMT and less specific guidelines on standardizing measures, making validation of our own protocol and measures more difficult. A lack of cIMT and aIMT correlation has also been reported in larger studies of healthy pediatric patients. (10, 21) Previous investigators have posited that this poor correlation may be because cIMT and aIMT change at different rates at different stages of life, and that hemodynamics or even local metabolic milieu may play a role. We found that rate of aIMT change was different pre- and post-transplant—which could reflect a transplant-mediated change in hemodynamics, unknown circulating serum factors, or the impact of immunosuppression. In short, aIMT remains intriguing, but determining its utility as a marker of cardiovascular risk—both in the general pediatric population and for transplant recipients—would require longitudinal and larger studies.

Our assessment of renal function was limited to indirect measures, including creatinine and calculated GFR; measured GFR would more accurately reflect kidney function in these children, and should be included in future studies as feasible. Finally, larger studies have shown that the relationships between cIMT, metabolic syndrome components, and cardiovascular risk strengthen in young adulthood. (19, 33) As our cohort ages, these associations may emerge more robustly.

This analysis demonstrateD an association between diastolic hypertension, triglycerides, and glucose intolerance and measures of subclinical atherosclerosis in pediatric liver transplant recipients. Longitudinal studies in a larger group of patients, ideally with control comparisons, will be needed to determine the utility of cIMT for predicting and following the evolution of cardiovascular risk in pediatric liver transplant recipients. cIMT would be just one tool that we could use to more accurately stratify these patients' cardiovascular risk—and potential need for proactive intervention—during long-term follow-up. (34)

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Abbreviations

aIMT Aorta intima-media thickness

ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BMI	Body mass index
BP	Blood pressure
BPAR	Biopsy-proven acute rejection
CDC	Centers for Disease Control
cIMT	Carotid intima-media thickness
FXR	Farnesoid X receptor
GGT	Gamma-glutamyl transpeptidase
HDL	High-density lipoprotein
HOMA-IR	Homeostatic model assessment – Insulin Resistance
IQR	Interquartile range
LDL	Low-density lipoprotein
OGTT	Oral glucose tolerance test
PFIC	Progressive familial intrahepatic cholestasis
PTMS	Post-transplant metabolic syndrome

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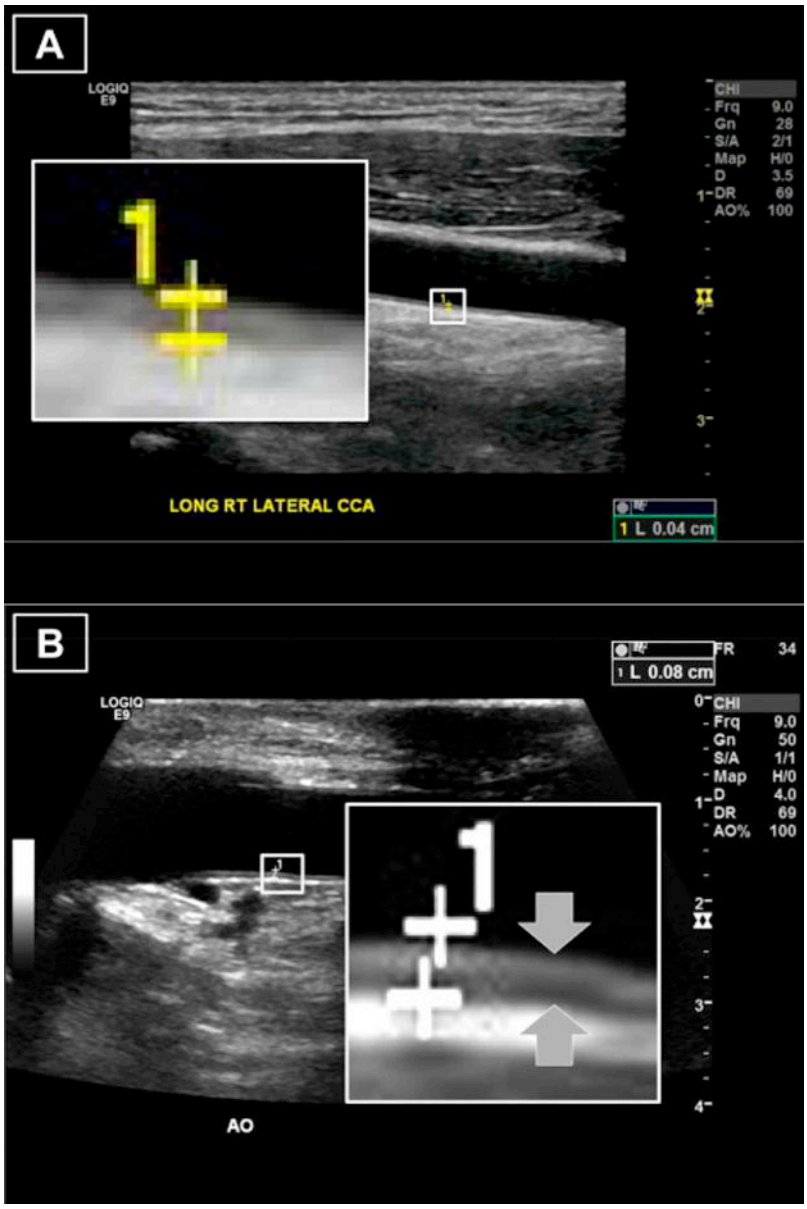

FIGURE 1.

(A) Longitudinal ultrasound of the right common carotid artery. After magnifying the IMT (inset boxes), it is seen that the sonographer's calipers (crosses) are accurately placed at the edges of the intima and media. **(B)** Longitudinal ultrasound of inferior abdominal aorta. After magnifying the IMT (inset box), it is seen that the sonographer's calipers (crosses) are not accurately placed, with one of the calipers being slightly too far into the lumen of the vessel. Therefore, the IMT was manually re-measured by the radiologist between the arrow tips.

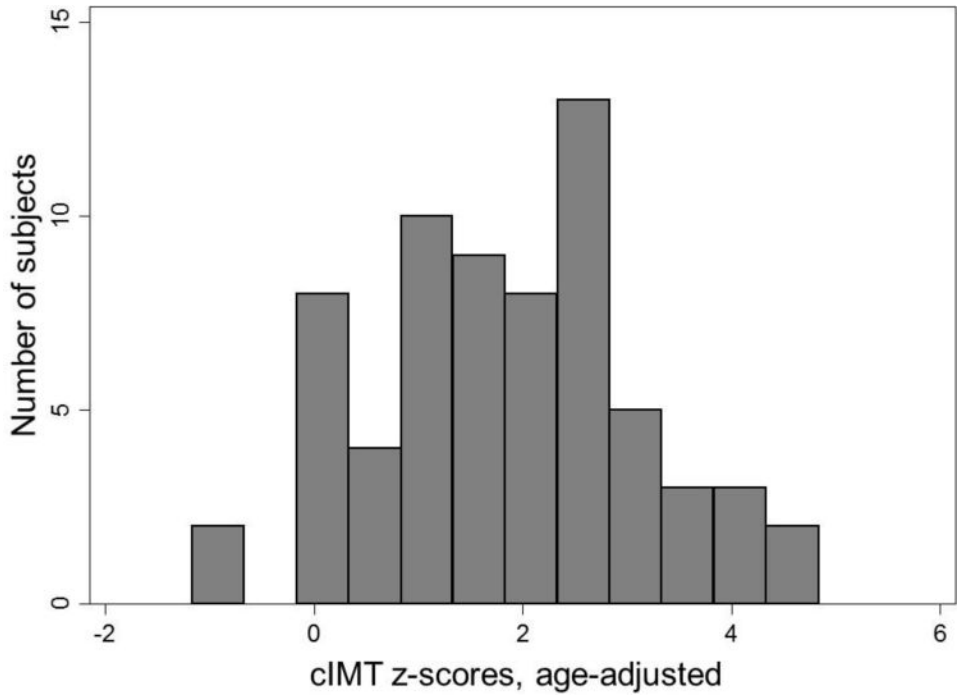

FIGURE 2.

ONLINE ONLY, Distribution of cIMT z-scores for subjects 18 years or younger at study visit, normalized for subject age and sex. (19)

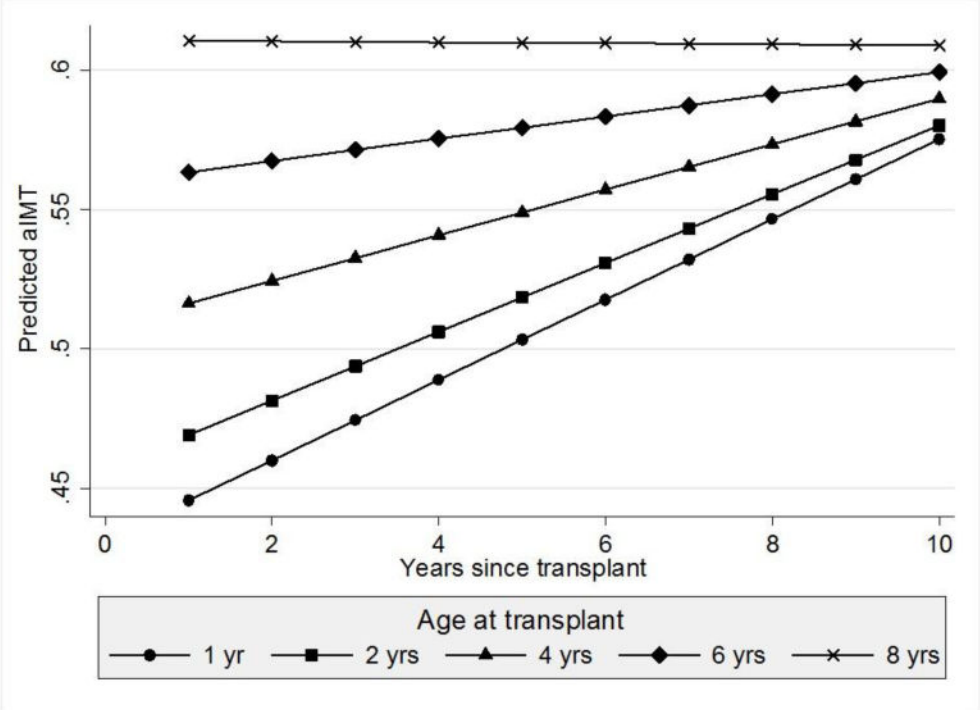

FIGURE 3.

ONLINE ONLY, Predicted aIMT over time after liver transplant varies by the age at transplant. Predicted values derived from multivariate linear regression model adjusted for age at transplant, years at transplant, interaction between these 2 variables, sex and glucose intolerance. See TABLE 3 (available at www.jpeds.coM) for coefficients.

Table 1

Demographics, transplant, and post-transplant characteristics of pediatric liver transplant recipients

	All (n=88)*
Age at visit (years)	15.4 ± 4.8
Female	48%
Hispanic	41%
Race	
White	36%
Black	6%
Asian	14%
Other†	26%
Multi–racial	18%
Indication for transplant‡	
Biliary atresia	33%
Metabolic disease	17%
Cholestatic disease	7%
Acute liver failure, tumor, other	43%
Transplanted for chronic liver disease/cirrhosis	65%
Years since first liver transplant	11.2 ± 5.6
Re-transplant	7%
Biopsy-proven acute rejection episodes	
0	40%
1–2	47%
≥ 3	13%
Chronic rejection	6%
Calcineurin-inhibitor	
Tacrolimus	80%
Cyclosporine	10%
None	10%
Tacrolimus trough at visit (μg/L, n=71)	4.2 (2.7–6.2)
Mean recent tacrolimus trough (μg/L, n=71)¶	4.7 (3.3–6.1)
Cyclosporine trough at visit (μg/L, n=9)	53 (41–70)
Mean recent cyclosporine trough¶ **(μg/L, n=9)**	89 (43–157)
AST (IU/L)	33 (24–45)
ALT (IU/L)	29 (21–46)
GGT	22 (13–51)
Total bilirubin	0.8 (0.6–1.2)
Creatinine (mg/dL)	0.57 (0.47–0.78)
Overweight/obese by BMI percentile/BMI	26%

	All (n=88)*
Systolic hypertension/pre-hypertension	33%
Diastolic hypertension/pre-hypertension	10%
Elevated fasting glucose	20%
Glucose intolerance (n=81)	27%
Hypertriglyceridemia	17%
Low HDL	39%
Hypercholesterolemia	7%
Elevated LDL	3%
Post-transplant metabolic syndrome	16%
Family history of stroke/myocardial infarction	51%
Family history of obesity	59%
Family history of diabetes (1st or 2nd degree)	68%

* Data represents proportion or median (interquartile range) except for age and years since transplant, which are listed as mean ± standard deviation.

† Race and ethnicity self-reported. Other ethnicity includes Native American, Alaskan, Pacific Islander, Hawaiian, Unknown.

‡ Metabolic liver disease includes alpha-1-antitrypsin deficiency, Crigler-Najjar syndrome, cystic fibrosis, glycogen storage disease, inborn errors in bile acid metabolism, neonatal hemochromatosis, primary hyperoxaluria, tyrosinemia, urea cycle defects, Wilson's disease. Cholestatic conditions include Alagille syndrome, Byler disease, progressive intrahepatic cholestatic syndromes, total parenteral nutrition cholestasis, sclerosing cholangitis, and idiopathic cholestasis. Other liver disease includes congenital hepatic fibrosis, Budd-Chiari syndrome, autoimmune hepatitis cirrhosis, drug toxicity, hepatitis C cirrhosis, and unknown cirrhosis.

¶ Mean of 3 most recent trough levels prior to study visit.

TABLE 2

Demographics and metabolic syndrome components associated with carotid intima-medial thickness (cIMT, n=88) *

	Adjusted for sex, age, height		Adjusted for all predictors with p<0.15	
	Increase in cIMT (mm)	**p**	**Increase in cIMT (mm)**	**p**
Male (vs. female)	**0.024 (−0.002 – 0.049)**	**0.07**	0.030 (0.006–0.054)	0.02
Height (per 10 cm)	**−0.012 (−0.023 – −0.001)**	**0.03**	−0.016 (−0.028– −0.004)	0.01
Age at visit (years)	**0.005 (0.002–0.009)**	**0.006**	0.044 (0.017 – 0.072)	0.002
Calcineurin-inhibitor				
Tacrolimus (n=70)	REF		REF	
Cyclosporine (n=9)	**−0.181 (−0.340 – −0.022)** †	**0.03**	−0.192 (−0.346 – −0.039) †	0.02
Off CNI (n=9)	0.008 (−0.125 – 0.140)	0.91	−0.053 (−0.182–0.076)	0.42
Transplanted for chronic liver disease	0.033 (0.009–0.058)	0.009	0.026 (0.001–0.051)	0.04
Continuous predictors				
BMI at visit	0.000 (−0.014 – 0.014)	1.00		
Waist circumference	−0.004 (−0.015 – 0.008)	0.53		
Mean systolic BP (per 10mmHg)	−0.001 (−0.013 – 0.012)	0.91		
Mean diastolic BP (per 10mmHg)	**0.007 (0.001–0.014)** †	**0.03**	0.079 (0.018–0.141) †	0.01
HDL (per 5mg/dL)	0.004 (−0.001 – 0.009)	0.09	0.005 (0.001–0.010)	0.03
Triglycerides (per 10 mg/dL)	0.012 (−0.003–0.027) ‡	0.12	0.018 (0.003–0.033) †	0.02
LDL (per 10 mg/dL)	−0.000 (−0.007 – 0.006)	0.93		
Total cholesterol (per 10 mg/dL)	0.002 (−0.003 – 0.007)	0.49		
Fasting glucose (per 10 mg/dL)	−0.006 (−0.023 – 0.011)	0.49		
Stimulated glucose (per 10 mg/dL)	−0.001 (−0.006–0.003)	0.49		
HOMA-IR	−0.001 (−0.004 – 0.003)	0.76		
Categorical predictors				
Overweight/obese	0.005 (−0.024 – 0.034)	0.75	§	
Systolic hypertension/pre-hypertension	−0.014 (−0.041 – 0.014)	0.33		
Diastolic hypertension/pre-hypertension	**0.234 (0.005–0.463)** ¶	**0.05**		
Low HDL	−0.023 (−0.049 – 0.003)	0.08		
Elevated triglycerides	**0.117 (0.001–0.233)** †	**0.05**		
Elevated LDL	−0.027 (−0.111 – 0.058)	0.53		
Elevated total cholesterol	0.011 (−0.040 – 0.061)	0.67		
Glucose intolerance	−0.008 (−0.037 – 0.022)	0.60		
Post-transplant metabolic syndrome	−0.023 (−0.058 – 0.011)	0.18		

* Interactions with age tested for all predictors. For variables that had a significant interaction with age (p<0.10), the reported coefficient, 95% CI, and p-value accounts for that interaction.

† p<0.05 for interaction with age

‡ p=0.07 for interaction with age

¶ p=0.06 for interaction with age

§ See TABLE 3 ONLINE ONLY for fully adjusted model, incorporating categorical predictors instead of continuous.

TABLE 3

ONLINE ONLY: Significant predictors of cIMT and aIMT, in multivariable models with categorical predictors

Predictor	Increase in cIMT (mm, n=87)	p	Increase in aIMT (mm, n=77)	p
Male (vs. female)	0.033 (0.008–0.059)	0.01	0.118 (0.036–0.199)	0.005
Height (per 10 cm)	−0.005 (−0.016 – 0.006)	0.36		
Age at visit (years)	0.005 (0.001 – 0.009)	0.02		
Age at transplant (years)			0.026 (0.010–0.042) **	0.002
Years since transplant, at study visit			0.016 (0.005–0.028) **	0.006
Calcineurin-inhibitor				
Tacrolimus (n=70)	REF			
Cyclosporine (n=9)	−0.215 (−0.368 – 0.063) *	0.006		
No calcineurin-inhibitor (n=9)	0.021 (−0.104–0.147)	0.74		
Low HDL	−0.029 (−0.056 – −0.003) *	0.03		
Elevated triglycerides	0.172 (0.056–0.289)	0.004		
Glucose intolerance			0.091 (0.000–0.181)	0.05

* Adjusted for interaction with age at visit, $p<0.05$ for all interaction terms.

** Adjusted for interaction, $p=0.06$ for the interaction term.

TABLE 4

Demographics and metabolic syndrome components associated with aorta intima-medial thickness (aIMT, n=83)

	Adjusted for sex, age at transplant, years since transplant		Adjusted for all predictors with p<0.15 (n=77)	
	Increase in aIMT (mm)	**p**	**Increase in aIMT (mm)**	**p**
Male (vs. female)	**0.086 (0.003–0.170)**	**0.04**	0.091 (0.014–0.167)	0.02
Height (per 5 cm)	0.003 (−0.015 – 0.021)	0.74		
Age at visit (years)	0.008 (−0.005 – 0.020)	0.22		
Age at transplant (years)	**0.024 (0.009–0.040)** *	**0.003**	0.024 (0.009–0.039) **	0.002
Years since transplant, at study visit	**0.018 (0.006–0.029)** *	**0.003**	0.017 (0.007–0.028) **	0.002
Calcineurin-inhibitor				
Tacrolimus (n=70)	REF			
Cyclosporine (n=9)	−0.050 (−0.191– 0.091)	0.48		
Off CNI (n=9)	−0.006 (−0.143–0.131)	0.93		
Transplanted for chronic liver disease	0.004 (−0.087 – 0.094)	0.94		
Continuous predictors				
BMI at visit	0.006 (−0.003–0.016)	0.22		
Waist circumference	0.002 (−0.002–0.006)	0.25		
Mean systolic BP (per 10mmHg)	0.014 (−0.028–0.056)	0.50		
Mean diastolic BP (per 10mmHg)	0.046 (−0.009–0.099)	0.09		
HDL (per 5 mg/dL)	0.000 (−0.003–0.003)	0.99		
Triglycerides (per 10 mg/dL)	−0.002 (−0.016–0.013)	0.83		
LDL (per 10 mg/dL)	0.001 (−0.001–0.003)	0.27		
Total cholesterol (per 10 mg/dL)	0.006 (−0.010–0.022)	0.45		
Fasting glucose (per 10 mg/dL)	−0.036 (−0.94–0.021)	0.21		
Stimulated glucose (per 10 mg/dL, n=77)	**0.012 (0.000–0.025)**	**0.06**	0.012 (0.000–0.024)	0.04
HOMA-IR	**0.017 (0.006–0.028)**	**0.003**	0.017 (0.006–0.027)	0.002
Categorical predictors				
Overweight/obese	0.071 (−0.027–0.168)	0.15	†	
Systolic hypertension/pre-hypertension	0.039 (−0.055–0.132)	0.42		
Diastolic hypertension/pre-hypertension	−0.035 (−0.189–0.118)	0.65		
Low HDL	0.026 (−0.062–0.114)	0.56		
Elevated triglycerides	−0.040 (−0.156–0.075)	0.49		
Elevated LDL	0.045 (−0.235–0.325)	0.75		
Elevated total cholesterol	−0.066 (−0.229–0.097)	0.42		
Glucose intolerance (n=77)	**0.091 (0.000–0.181)**	**0.05**		
Post-transplant metabolic syndrome	0.072 (−0.043–0.186)	0.22		

* Statistically significant interaction between these two variables, p=0.02 in sex-adjusted analysis. All p-values from linear regression models.

** Adjusted for interaction, p=0.06 for the interaction term.

† See Supplemental Table 1 for fully adjusted model, incorporating categorical predictors instead of continuous.