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Skin tissue cholesterol ($SkinT_c$) is related to angiographically-defined cardiovascular disease

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Abstract

Parallel changes associated with aging found in the vasculature and skin at necropsy, have prompted small preliminary studies to assess the relation between skin tissue cholesterol ($SkinT_c$) and cardiovascular disease. While these studies have been suggestive, no formal investigation is available to test this association. It would, therefore, be valuable to determine whether a relation between $SkinT_c$ and angiographic narrowing actually exists, the latter representing one accepted measurement of coronary atherosclerosis. *Methods:* Patients at three hospitals undergoing coronary angiography and not on lipid altering agents ($n = 649$) were examined for $skinT_c$ using a non-invasive method. Vessels were evaluated manually (number with stenosis $\geq 50\%$). Clinical characteristics, current medication use, and Framingham global risk score were recorded. *Results:* $SkinT_c$ was significantly higher in patients with angiographic disease (124 ± 30 vs. 118 ± 30, $P = 0.02$). After adjustment for traditional coronary artery disease risk factors, $SkinT_c$ provided 7% additional risk (per 10 $SkinT_c$ units) for angiographic disease. *Conclusion:* $SkinT_c$, measured with a non-invasive method, is associated with the presence of coronary artery disease as determined by catheterization. Such an assay may eventually help stratify patient risk and target prevention efforts.

Keywords: Cholesterol; Risk factors; Tissue; Tests

1. Introduction

A novel association between skin tissue cholesterol ($SkinT_c$) and vascular cholesterol deposition has been reported [1]. Specifically, skin biopsies reveal considerably higher total sterols and total lipids in those with aortic atheroma [2] and $skinT_c$ is found elevated in subjects with coronary heart disease [3]. Analyses of pilot data in our own clinic demonstrated $SkinT_c$ measurements as predictive of cardiovascular stress test results [4]. However, these reports have all been small pilot evaluations, leaving no available rigorous data to examine the value of $SkinT_c$ in predicting vascular disease. Prior to proceeding to determine the value of skin-c testing related to cardiac event outcome, we need to corroborate these prior efforts i.e. does $SkinT_c$ independently predict coronary atherosclerosis, herein defined by angiographic vascular narrowing. Previous efforts towards larger studies of $skinT_c$ were hampered by methods that required a skin sample for cholesterol measurement. Many of these concerns have now been resolved by the development of a non-invasive method to determine $skinT_c$ [4].

2. Methods

Six hundred and forty nine patients, not on lipid lowering medications, undergoing diagnostic catheterization had $SkinT_c$ measured and baseline risk data recorded. The patient population was recruited from three separate sites, two in Canada (St. Michaels Hospital (SMH), $n = 323$; Trillium Health Center (THC), $n = 179$) and one in the United States (Cleveland Clinic Foundation (CCF), $n = 147$). The

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major indication for the catheterization was anginal symptoms (>90%), often accompanied by a positive stress test (>60%).

Within 24 h prior to the angiographic procedure, $SkinT_c$ measurement was performed. The non-biopsy based skin-cholesterol assay system (Cholesterol 1,2,3^{TM}) has been recently described [4] and uses a synthetic copolymer conjugated with digitonin and horseradish peroxidase to bind and quantify $SkinT_c$. Cholesterol is assayed only within the stratum corneum. Repetitive readings provide a mean day-to-day coefficient of variation of 3–8% with a between hand average reproducibility of 95% [4]. The entire process requires about 3–4 min.

Medications were noted and incorporated into the database by the nurse coordinator. Technicians at each site were trained in an identical manner to measure $SkinT_c$. All catheterization interpretations were performed manually and independently at each site. Extent of disease was defined by the number of vessels with ≥50% stenosis, and patients were further classified as having angiographic disease if the extent of disease was at least "one". Institutional Review Boards approved the study protocols at each site and all subjects provided informed consent prior to study enrollment.

2.1. Statistics

Spearman's rank correlation coefficient was used to evaluate the relationship between $SkinT_c$ and extent of disease. Unpaired t-tests were used to assess the difference in mean $SkinT_c$ values between patients with and without angiographic disease. Bootstrapping with 10 000 samples was used to assess the robustness of the observed differences between those with and without angiographic disease. Briefly, the unpaired t-test compares the observed value of the test statistic to the values in a table of its theoretical distribution. The bootstrap further allows the comparison of the observed value to the many possible distributions (e.g. 10 000 in this case) based on resampling the original data. Logistic regression procedures were used to estimate the relative risk associated with each 10-unit increase in $SkinT_c$. In order to ascertain potential relationships between skin cholesterol and traditional risk factors with regard to angiographic disease, logistic regression models were constructed with skin cholesterol and each individual Framingham risk factor. Finally, adjustment for aggregate traditional risk burden was made using the Framingham global risk score.

3. Results

Fig. 1 shows that $SkinT_c$ was progressively higher as extent of disease (i.e. number of vessels with ≥50% stenosis) increased ($P = 0.003$). $SkinT_c$ was significantly higher in patients with angiographic disease, defined as at least one effected vessel (124 ± 30 vs. 118 ± 30, $P = 0.02$). Table 1 details presentation characteristics by disease status. The unadjusted odds ratio for each 10-unit increase in $SkinT_c$ was 1.07 (95% CI = 1.01–1.13, $P = 0.02$). Table 2 shows that skin cholesterol remains significantly associated

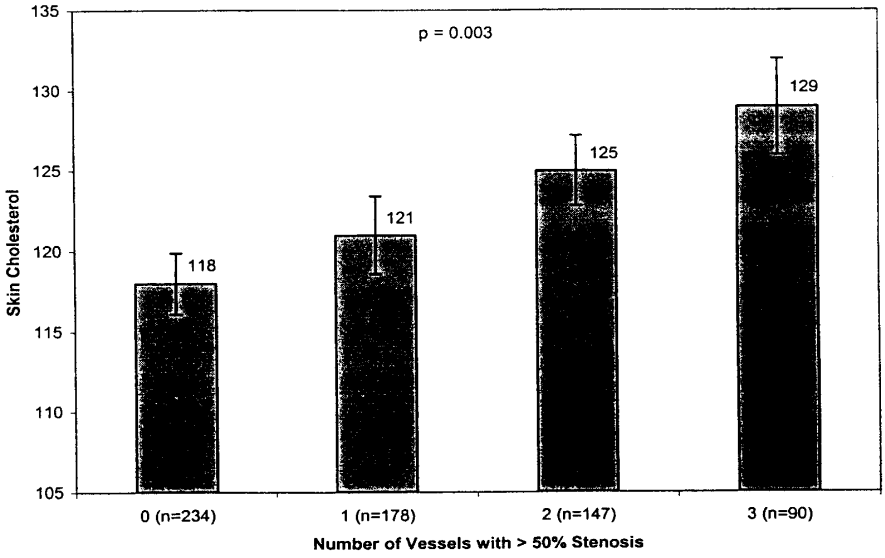

Fig. 1. Mean ± S.E.M. SkinTc values by extent of disease defined as the number of vessels with ≥50% stenosis.

Table 1
Traditional risk factors (i.e. Framingham) at presentation by angiographic disease status

	No angiographic disease	Angiographic disease	*P*-value
N	234 (36%)	415 (64%)	–
Age (years)	60 ± 12	66 ± 11	< 0.001
Women	111 (47%)	103 (25%)	< 0.001
LDL-cholesterol (mg/dl)	116 + 32	118 ± 31	0.47
HDL-cholesterol (mg/dl)	46 ± 15	40 ± 12	< 0.001
Hypertension	120 (51%)	244 (59%)	0.06
Diabetes	18 (8%)	97 (23%)	< 0.001
Smoker (past year)	58 (25%)	147 (35%)	0.005
Framingham	7.5 ± 3.5	9.3 ± 3.3	< 0.001
$SkinT_c$	118 ± 30	124 ± 30	0.02

Continuous measures are shown and mean ± standard deviation (S.D.) and categorical measures are shown as number and percent with factor present.

Table 2
Logistic regression results adjusting skin cholesterol effect for each Framingham risk variable individually and in aggregate (i.e. Framingham global risk score)

Traditional risk factor	Odds ratio[a] for skin cholesterol (10 unit increment)	Odds ratio for traditional risk factor
Age (10 years)	1.06 (1.01–1.13) $P = 0.03$	1.57 (1.36–1.82) $P < 0.001$
Female	1.06 (1.00–1.12) $P = 0.04$	0.37 (0.27–0.52) $P < 0.001$
Total Cholesterol (10 mg/dl increment)	1.07 (1.01–1.13) $P = 0.01$	1.00 (0.96–1.04) $P = 0.99$
LDL Cholesterol (10 mg/dl increment)	1.07 (1.01–1.13) $P = 0.02$	1.02 (0.97–1.07) $P = 0.44$
HDL Cholesterol	1.06 (1.00–1.12) $P = 0.05$	0.97 (0.95–0.98) $P < 0.001$
Diabetes	1.07 (1.01–1.13) $P = 0.02$	3.66 (2.15–6.24) $P < 0.001$
Hypertension	1.06 (1.01–1.12) $P = 0.03$	1.31 (0.94–1.81) $P = 0.11$
Smoking	1.07 (1.01–1.13) $P = 0.02$	1.69 (1.18–2.42) $P = 0.005$
Framingham score	1.07 (1.01–1.13) $P = 0.03$	1.17 (1.11–1.24) $P < 0.001$

[a] With 95% confidence interval and *P*-value.

with angiographic disease after adjustment for each Framingham risk variable individually and in aggregate. We saw virtually no change for bootstrap *P*-values for either of the primary associations we observed for mean $SkinT_c$ differences between those with and without angiographic disease ($P_{original} = 0.02$, $P_{bootstrap} = 0.03$).

$SkinT_c$ was not significantly correlated with total cholesterol ($r = -0.02, P = 0.67$) or LDLc ($r = -0.02, P = 0.54$), but did show modest correlations to HDL-cholesterol ($r = -0.07$, $P = 0.07$), triglycerides ($r = 0.10$, $P = 0.02$), and a history of hypertension (125 ± 30 with hypertension vs. 118 ± 29, $P = 0.004$). Neither blood pressure medication use, nor number of medications corresponded to $SkinT_c$, or to the latter's prediction of angiographic risk.

4. Discussion

In parallel to Bouissou's [2] observation of parallel aging between the vasculature and skin, and skin-cholesterol differences between healthy and coronary bypass patients [5] our current data indicates that $SkinT_c$ increases with extent of disease. We found that for every 10 units of $SkinT_c$, an additional 7% risk was observed among subjects referred for coronary characterization. The consistency of results after adjustment for traditional risk factors (either individually or in aggregate) indicates that skin cholesterol is providing new information with respect to risk assessment for CAD.

It is noteworthy that $SkinT_c$ and serum levels of LDL-cholesterol (or total cholesterol) were not correlated. In previous studies, serum LDL-cholesterol concentrations have not been associated with skin-cholesterol measures [5] nor have they been particularly valuable in predicting extent of angiographic disease [6,7], a finding consistent with ours. The previously reported association between skin-cholesterol and various cytokines and/or adhesion molecules may provide one explanation for our observed relationship between tissue lipid and vascular disease [8]. The modest correlation noted between $SkinT_c$ and HDL-cholesterol could be the result of cholesterol transport mediated through the scavenger receptor B1 (SRB1) found in basal skin cells [9].

In summary, this is the first major clinical test of the $SkinT_c$:vascular disease theory. $SkinT_c$ measurement correlates with angiographic disease in coronary arteries. This relationship remains significant after adjusting for traditional coronary artery disease risk burden.

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