

Keloid scarring, but not Dupuytren's contracture, is associated with unexplained carotid atherosclerosis

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Abstract

Background—Atherosclerosis, a response to injury, may be thought of as scarring in the artery wall. TGF- β and associated signaling molecules have been implicated in the pathophysiology of keloid scarring, Dupuytren's Contracture and atherosclerotic plaques in independent studies.

Purpose—To test the hypothesis that excess cutaneous scarring and Dupuytren's contractures predispose independently to carotid atherosclerosis.

Methods—Among 1,747 patients with plaque measurements and complete data for multivariable regression analysis, 57 Caucasian patients had Dupuytren's contractures and 12 had keloid scars. Carotid total plaque area (TPA) was measured by 2-Dimensional ultrasound.

Results—In linear multivariable regression analysis with coronary risk factors, keloid scars were associated with TPA ($P=0.018$), but Dupuytren's contractures were not. Patients with keloid scarring were younger ($P<0.0001$), and more likely to be diabetic ($P<0.0001$)

Conclusions—Keloid scarring is a clinical clue to excess atherosclerosis not explained by traditional risk factors. Such patients may benefit from therapy directed at targets related to signalling molecules common to both the process of keloid scarring and atherosclerosis. These findings suggest previously unexplored possibilities for the prevention and treatment of atherosclerosis. The differences between Dupuytren's and keloid scars that may identify such targets are discussed.

Traditional risk factors for atherosclerosis include age, sex, blood pressure (BP), cholesterol levels, smoking history, and diabetes. These risk factors are essentially the same in different levels of the arterial tree.^{1–4} It has become increasingly clear, however, that these risk factors do not completely account for the degree of atherosclerosis in many patients.^{5,6} It is thus important to identify previously unrecognized risk factors for atherosclerosis. Doing so

provides insights into the pathophysiology of the disease as well as the potential for new therapeutic approaches.

This study began with the presentation in 1994 of a young man with unusually severe atherosclerosis for his age. At age 31 he had required bilateral femoropopliteal bypass, although he did not have unusually high levels of traditional risk factors. However, he had been treated on seven occasions by plastic surgeons to remove keloid scar tissue. What made this remarkable was that he was Caucasian. As atherosclerosis shares some components with scar tissue – a reaction in the artery wall to injury^{7,8} – JDS wondered if this association may be more than a coincidence. As the result of this experience, excess cutaneous scarring became a routine part of the physical examination and functional inquiry for subsequent patients referred to our vascular prevention clinic. In this paper we present the results of our observations since this young man first presented.

The purpose of this study was to test the hypothesis that keloid scarring is a marker for “unexplained atherosclerosis” (UE); that is, atherosclerosis not explained by traditional coronary risk factors. Specifically, our aim was to determine whether keloid formation and Dupuytren’s contractures are independently associated with carotid plaque burden, after adjusting in multiple regression for traditional risk factors

We began measuring carotid total plaque area (TPA) as a measure of atheroma burden in 1990⁹ and, since 1995, have routinely used this measurement in the management of patients referred for treatment of atherosclerosis.¹⁰ Validation work from our laboratory has shown that both baseline TPA and progression of TPA identify patients at higher risk of cardiovascular events: after adjustment for age, sex, cholesterol, systolic blood pressure, pack-years of smoking, total homocysteine, diabetes and treatment of blood pressure and lipids, patients in the top quartile of plaque area had 3.4 times higher risk of stroke, death or myocardial infarction over 5 years and, those with progression, had twice the risk of those with stable plaque or regression.¹¹ Our findings have been validated in a population-based study of over 6,000 participants in the Tromsø Study.¹² In our earlier studies, we used multivariable linear regression analysis to identify determinants of carotid plaque area (TPA).¹³ We found that traditional risk factors were significant determinants of variation in TPA, accounting for 52% of variance in TPA.⁵ Patients with high residual scores in the regression model (i.e. those far to the right of the regression line) were recognized as having more carotid atherosclerosis than would be predicted by traditional risk factors. Thus, the term “unexplained atherosclerosis” (UE) was introduced to refer to a marked difference between observed and expected TPA⁵. This concept is illustrated in Figure 1.

Methods

Study population and data collection

We analyzed a cohort of 1,747 patients from the Stroke Prevention and Atherosclerosis Research Centre at the Robarts Research Institute, London, Canada. Included in the analysis were all those with complete data for all variables used in the regression model. For these patients, the following information was collected from the initial visit: age, sex, systolic blood pressure (SBP), total cholesterol, pack-years of smoking, presence of diabetes

mellitus, whether the patients were on antihypertensive therapy or lipid-lowering agents, and the baseline TPA.

Baseline TPA was measured as previously described.¹¹ Briefly, total plaque area was defined as the sum of the cross-sectional areas of all plaques seen in the left and right internal, external, and common carotid arteries. Plaques were defined as localized increases in thickness of the intima-media complex >1 mm in thickness. Each plaque was measured in the longitudinal plane in which it was biggest, by tracing with a track-ball cursor its cross-sectional area. Total plaque area is the sum of these areas for all plaques seen in the left and right common, internal and external carotid.

As we are reporting on the results of clinical practice from our own patients, such reports were exempted from a requirement for explicit signed consent by the UWO ethics review board in 1977.

Statistical analysis

Multivariable linear regression, using a model that we have previously described⁷, was used to assess whether the different forms of cutaneous scarring were independent predictors of TPA. For this model, the distribution of TPA was normalized by a cube-root transformation. The following variables maximally explain the variance in TPA: age, sex, SBP, total cholesterol, smoking history in pack-years, a history of treatment for hypertension and a history of treatment of for hyperlipidemia (each $P < 0.05$). To this model were added either keloid scarring or Dupuytren's contractures.

Chi-square was used to compare non-parametric variables, and ANOVA to compare continuous variables across the three groups of patients: no scarring, Dupuytren's contractures or keloid scarring.

All statistical analyses were performed using SPSS for Windows (Version 16). A two-tailed P value of 0.05 was deemed to be statistically significant.

Results

Among the patients with complete data for all the variables used in the multivariable regression model, 57 had Dupuytren's contractures, and 12 had keloid scars.

The total cholesterol was not available for one patient, who was not on a lipid-lowering agent. For this patient, an imputed baseline total cholesterol value of 5.59 mmol/L was used from the mean level for patients in the database who were not on a lipid-lowering agent at the time of the first visit, but who subsequently received such treatment.

The adjusted R^2 value for the multivariable regression model was 0.51, - 51% of the variance in the transformed TPA variable was explained by age, sex, SBP, total cholesterol, pack-years of smoking and treatment of lipids or hypertension. (The R^2 for untransformed TPA was 0.36.) Figure 2, a cumulative probability plot of residual scores, shows that the regression model is very robust.

Tables 1 and 2 show the results of adding to the regression model keloid scarring or Dupuytren's as independent predictors of TPA. The presence of Dupuytren's contracture was not significant as an independent predictor of TPA ($p = 0.653$), but keloid scarring was significantly associated with TPA ($p = 0.018$).

Baseline values for the variables used in the multivariable regression model are shown in Table 3. The three groups of scarring were different in age, with the keloid scarring patients being younger, and were all more likely to be male; those with Dupuytren's contractures were predominantly male. Patients with keloid scarring were much more likely to be diabetic.

Discussion

Our results suggest that keloid scarring is a physical finding that may identify patients with excess atherosclerosis not explained by traditional risk factors. To our knowledge, this study represents the first association between these two processes. Patients with this form of scarring were shown to have increased TPA when compared to controls with similar traditional risk factors. Furthermore, keloid scarring was an independent predictor of TPA in multivariable regression. The finding of an independent association between keloid scarring and excess TPA despite the small number of patients with this phenomenon suggests a powerful biological link. Plausible links may be found in inflammation and scarring.

A weakness of our study is the small number of patients with keloid scarring. Strengths are the measurement of carotid plaque, as opposed to intima-media thickness, and the adjustment for traditional coronary risk factors in multivariable regression. Carotid total plaque area is biologically and genetically distinct from IMT^{9,14,15}, and total plaque area is a stronger predictor of coronary events than are IMT measurements that do not include plaque thickness.¹⁶ Recently we showed that lipoprotein (a) is a strong predictor of carotid stenosis and occlusion, but not plaque area¹⁷, highlighting the importance of distinguishing between carotid ultrasound phenotypes. Table 3 shows that patients with Dupuytren's had more plaque, but they were older, smoked more, and were predominantly male. Adjustment in multivariable regression makes it clear that the Dupuytren's was not an independent predictor of plaque.

Atherosclerosis may be thought of as an inflammatory process.^{18–20} The molecular mechanisms of this inflammatory process are complex and involve cytokines with pro-inflammatory and anti-inflammatory activity.^{20,21} Of the many cytokines involved, recent studies suggest that transforming growth factor-beta (TGF-beta) and vascular endothelial growth factor (VEGF) may play key roles in atherosclerosis, including the formation of carotid atherosclerotic plaques.^{22–27} It is interesting that these same cytokines have been implicated in the pathogenesis of abnormal cutaneous scarring^{28–34} and suggested as components of Dupuytren's contracture.^{35,36}

Atherosclerosis, a response to injury^{7,8}, includes a major component of scarring in the artery wall. Dustan³⁷ hypothesized that keloid formation may be associated with excess risk of

hypertension and vascular disease among blacks, and Dupuytren's contracture has been associated with diabetes mellitus and its microvascular complications.^{38,39}

Mechanisms involved in such associations include effects of transforming growth factor beta (TGF β). Wang et al. demonstrated that cells derived from abnormal cutaneous scar tissue produce more TGF β than normal skin cells.²⁸ Similarly, exogenous TGF β -1 causes a greater increase in collagen synthesis in keloid-derived fibroblasts compared to fibroblasts derived from normal dermis.³⁰ Both Wu et al.³¹ and Gira et al.³² have shown increased levels of VEGF in keloids. Dupuytren's Contracture cord and wound granulation tissue have similar histological features, leading to the view that DD may be a dysregulated, fibrotic wound healing response.⁴⁰ TGF β -1 has not only been implicated in promoting myofibroblast development in Dupuytren's Contracture^{33,41}, but primary cells derived from Dupuytren's Contracture cord are reported to have an enhanced sensitivity to its effects.²⁹

TGF β signalling triggers long-term responses through a variety of signalling intermediates⁴² and some of these molecules have been demonstrated to be components of atherosclerosis, keloid scarring and DD. One of these is β -catenin, a signalling molecule regulated by a variety of growth factors including TGF β : β -catenin accumulation has been implicated in atherosclerosis⁴³, abnormal cutaneous scarring and wound healing^{44,45} and Dupuytren's Contracture⁴⁶ in independent studies. Thus, it is quite possible that the changes in gene expression initiated by β -catenin accumulation and transcription factor transactivation may underlie some of the molecular mechanisms leading to the formation of keloid scar tissue, Dupuytren's contracture cords and atherosclerosis. Other TGF β signalling intermediates, however, may not be common components of these conditions. Connective Tissue Growth Factor (CTGF) is a TGF β -inducible signalling component of keloid scar formation⁴⁷ and atherosclerotic plaques that is not evident in microarray analyses of gene expression in DD tissues or primary DD cells.⁴⁸ Thus, there is molecular evidence that atherosclerosis, keloid scarring and DD share some, but not all, of the TGF β -mediated intermediates associated with the wound healing response.

Accumulation of β -catenin and CTGF up-regulation are both associated with the differentiation of fibroblasts into myofibroblasts.⁴⁹ One intriguing possibility is that myofibroblasts in atherosclerotic plaques and keloid scars may be derived from similar cellular precursors while the myofibroblasts in DD cord may be derived from different precursor cells via overlapping, but different signalling pathways. This idea is suggested by a recent report suggesting that, while myofibroblasts in lung fibrosis are largely derived from resident fibroblasts, the phenotypically identical cell type is contributed by hepatic stellate cells in liver fibrosis and media smooth muscle cells in atheromatous plaques.⁵⁰ Consistent with this speculation, therapies that inhibit the mammalian target of rapamycin (mTOR) have been shown to prevent both atherosclerotic plaque formation in an animal model⁵¹ and myofibroblast development by keloid fibroblasts⁵² in independent studies. Research focused on identifying the molecular pathways that promote myofibroblast differentiation in patients prone to keloid scar development and atherosclerotic plaque formation may therefore reveal additional potential targets for therapeutic interventions.

Conclusion

Keloid scarring, but not Dupuytren's contractures, was associated with excess atherosclerosis not accounted for by traditional risk factors. This discrepancy suggests that factors differentiating these two types of excess scarring may point to factors linking scarring to atherosclerosis, and thus to new potential therapeutic targets for atherosclerosis. Keloid scarring may be a clinical clue to excessive atherosclerosis not explained by traditional coronary risk factors.

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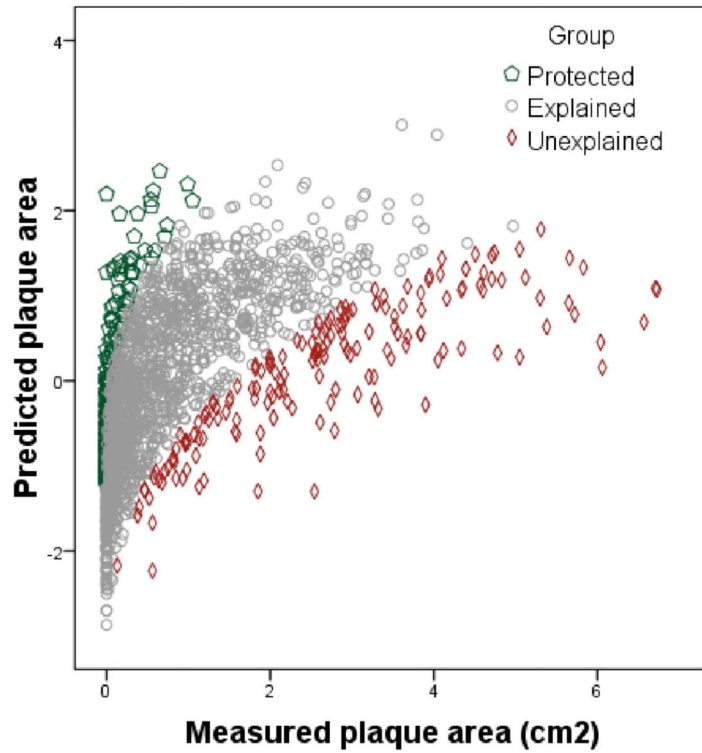


FIGURE 1.

Unexplained atherosclerosis. Predicted plaque area (transformed) on the vertical axis, vs measured plaque area on the horizontal axis. The red diamonds represent patients with residual scores in the top 10% (≥ 1.281); i.e. those with the most atherosclerosis not explained by traditional risk factors (“Unexplained atherosclerosis”); the green pentagons represent those with residual scores in the lowest 10% (< -1.25); i.e. those with protection from atherosclerosis; the grey circles represent the 80% of patients with residual scores nearest the regression line (“Explained atherosclerosis”).

Normal P-P Plot of Regression Standardized Residual

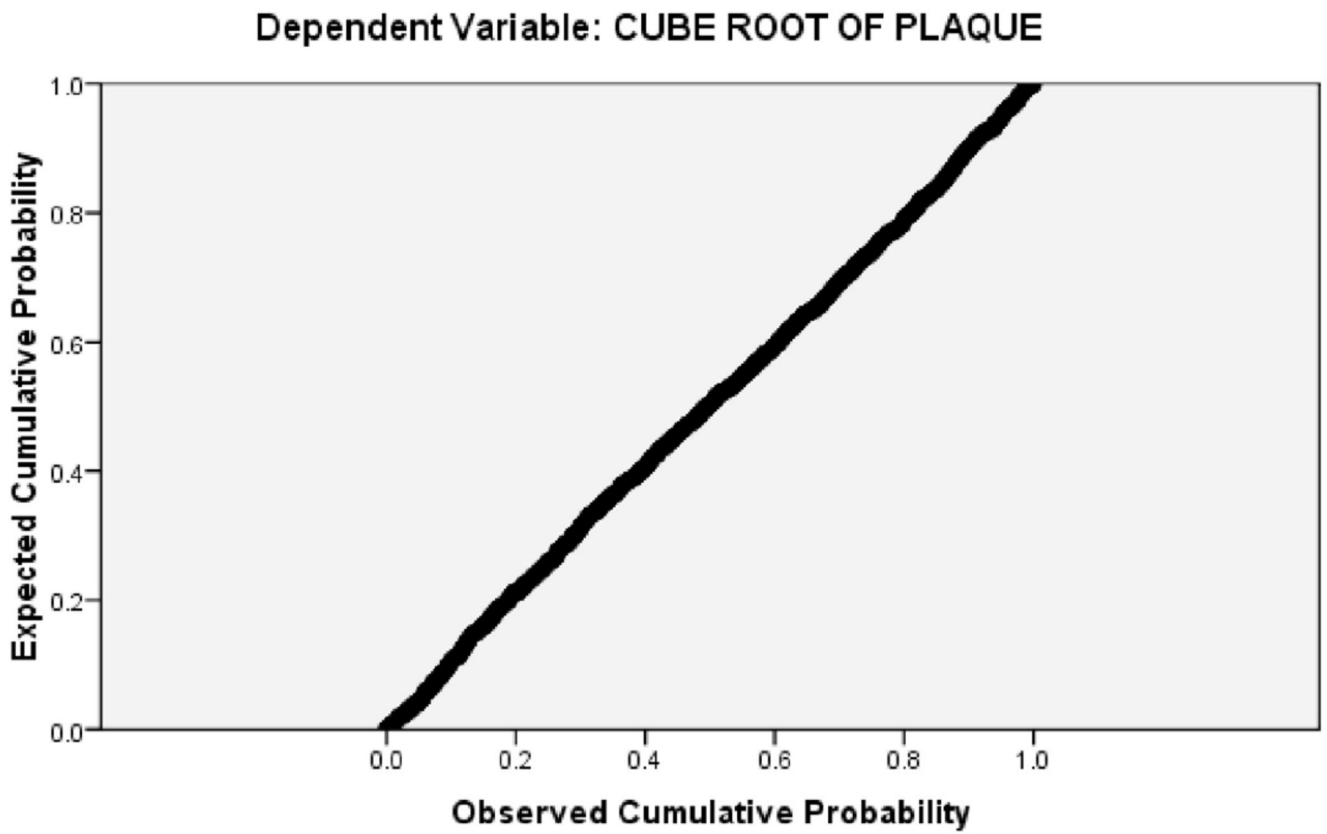


FIGURE 2. Cumulative probability plot of residual scores in the regression model for keloid scarring (Table 1).

TABLE 1

Multivariable linear regression for carotid total plaque area with Keloid scarring added to the model

Model		Coefficients ^a			t	P
		Unstandardized Coefficients		Standardized Coefficients		
		B	Std. Error	Beta		
1	(Constant)	-0.405	0.050		-8.166	0.000
	Age (yr)	0.017	0.001	0.572	29.798	0.000
	Sex (female =1, male=0)	-0.121	0.015	-0.140	-8.198	0.000
	Cholesterol (mmol/L)	0.024	0.007	0.062	3.583	0.000
	On hypertensive meds	0.054	0.016	0.063	3.480	0.001
	On lipid lowering meds	0.107	0.017	0.124	6.496	0.000
	Smoking (pack-years)	0.004	0.000	0.187	10.919	0.000
	Keloid scarring	0.209	0.088	0.040	2.377	0.018

Dependent Variable: Cube root transformation of total plaque area

R²= 0.510; n=1747

TABLE 2

Multivariable linear regression for carotid total plaque area with Dupuytren's contractures added to the model

Model		Coefficients ^a			t	P
		Unstandardized Coefficients		Standardized Coefficients		
		B	Std. Error	Beta		
1	(Constant)	-0.399	0.050		-8.028	0.000
	Age (years)	0.017	0.001	0.571	29.409	0.000
	Sex (female=1, male=0)	-0.122	0.015	-0.141	-8.202	0.000
	Cholesterol (mmol/L)	0.024	0.007	0.061	3.515	0.000
	On hypertensive meds	0.055	0.016	0.063	3.474	0.001
	On lipid lowering meds	0.109	0.017	0.126	6.568	0.000
	Smoking (pack-years)	0.004	0.000	0.186	10.841	0.000
	Dupuytren's	-0.018	0.040	-0.008	-0.450	0.653

Dependent Variable: Cube root transformation of total plaque area

Adjusted R²= 0.509 n=1747

TABLE 3

Baseline values of the variables used in the regression model, for the three groups of patients

	No scarring	Dupuytren's	Keloid	P
Number	1679	57	12	
Age (yr)	56.94 (1.04)	66.11 (9.85)	50.00 (14.81)	<0.0001
Male %	53%	80.7%	58.3%	<0.0001
Diabetic %	8.9%	5.3%	16.7%	0.0001
Smoking Pack-yr	12.51 (18.08)	16.92 (20.03)	5.58 (7.39)	0.08
Total cholesterol (mmol/L)	5.16 (1.10)	4.99 (1.16)	4.82 (0.87)	0.31
Systolic blood pressure (mmHg)	139.74 (21.47)	139.30 (21.47)	149.08 (19.29)	0.32
Antihypertensive therapy %	56.8%	42.1%	66.7%	0.069
Lipid-lowering therapy	47.65	42.1%	66.7%	0.30
Carotid plaque area (cm ²)	0.85 (1.04)	1.35 (1.51)	1.03 (1.03)	0.002

* Chi-square for percentages, ANOVA for continuous variables