

Cutaneous Calciphylaxis: A Retrospective Histopathologic Evaluation

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Abstract: Calciphylaxis is a rare and life-threatening disease characterized by cutaneous necrosis and vascular calcification. Often, skin biopsy specimens are not diagnostic because of the limited depth of the specimen, biopsy site, and clinical stage. To better understand the utility of various histologic features in rendering the diagnosis of calciphylaxis and to compare von Kossa versus Alizarin red stains in the detection of calcium deposits, we retrospectively analyzed the histologic features and histochemical stain findings of 56 skin biopsies from 27 consecutive patients seen at Massachusetts General Hospital from October 2002 to April 2012, with confirmed diagnosis of calciphylaxis and compared with that of 19 skin biopsies from 17 patients with other disease processes. All forms of vascular calcification and vascular thrombosis were significantly associated with cutaneous calciphylaxis. Perieccrine calcium deposition, highly specific to calciphylaxis, was the only form of calcium deposition noted in 4 (7%) skin biopsies from patients with calciphylaxis. Although the staining appears to be comparable, the deposits seen on Alizarin red appeared larger and were birefringent. Although subtle, perieccrine calcification may aid in the diagnosis of calciphylaxis in settings where typical vascular and extravascular calcification are not identified. Performing both von Kossa and Alizarin red stains might increase the detection of calcium deposit.

Key Words: calciphylaxis, von Kossa, Alizarin red, vascular calcification, perieccrine calcification

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INTRODUCTION

Calciphylaxis is a rare life-threatening disease usually observed in the setting of renal failure. Previously estimated to occur in 1% of patients with end-stage renal disease per year, calciphylaxis occurred in 4.1% of patients in one retrospective review of 242 patients undergoing hemodialysis.^{1,2} However, calciphylaxis has also been described rarely in clinical settings other than renal failure. Some of the risk factors associated with the development of calciphylaxis in nontraditional patients include chronic inflammation, obesity, liver disease, malignancies, systemic corticosteroid use, and diabetes mellitus.³ In a population-based study, the incidence of calciphylaxis was

estimated to be 4.5 per 1 million person-years.⁴ Calciphylaxis characteristically presents with painful erythema that progresses to retiform purpura, violaceous plaques, and subcutaneous induration that often progress to bulla formation, cutaneous necrosis, and ulceration. It most often occurs on the extremities.^{5–7} It is a condition with significant associated morbidity and mortality, with one study estimating the 1-year survival rate to be 45.8% with sepsis as the leading cause of death.⁸ Proposed treatment modalities included parathyroidectomy, hyperbaric oxygen therapy, wound management, and pharmacotherapy with sodium thiosulfate, bisphosphonates, and cinacalcet, among others.^{9,10}

Clinical pathologic correlation is required to render the diagnosis of calciphylaxis. Early diagnosis is crucial for initiating prompt treatment, which may prevent the progression of the disease. Laboratory findings often include elevations of calcium phosphorus product and parathyroid hormone levels, although these can be normal.^{5,10} Radiographic studies, including plain radiograph, mammographic technique radiographs, and 3-phase bone scan, may show evidence of vascular and soft tissue calcification.^{11–14} However, these imaging techniques can be costly and may have low sensitivity and specificity. A skin biopsy has the advantage of identifying the microscopic features of calciphylaxis and excluding other entities in the differential diagnosis. There are multiple histologic features that are known to be associated with this condition, yet only the presence of calcium deposition within subcutaneous vessels is considered to be a diagnostic histologic feature.^{6,15} Often skin biopsy specimens are not diagnostic because of the limited depth of the specimen, the biopsy site, and the clinical stage of the process. Larger wedge biopsies may induce new lesion formation or create slow healing wounds. In some instances, the von Kossa stains performed on these biopsies are negative even in the setting of proven calciphylaxis clinically.

To better understand the utility of various histologic features in rendering the diagnosis of calciphylaxis, we retrospectively analyzed the biopsy material of a cohort of consecutive patients with clinically and pathologically confirmed diagnoses of calciphylaxis. We further compared 2 histochemical methods, von Kossa and Alizarin red, to assess which stain would better detect small calcium deposits.

MATERIALS AND METHODS

This study has been approved by the Massachusetts General Hospital Institutional Review Board (IRB# P2011-2442). Using a research patient data repository system, we

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identified 56 skin biopsies from 27 patients seen at Massachusetts General Hospital from October 2002 to April 2012 with confirmed diagnosis of calciphylaxis based on clinical findings and laboratory and imaging studies. We also included 19 skin biopsies from 17 patients who presented with possible calciphylaxis, yet ultimately were confirmed to have other disease processes. Archival materials of these cases were retrieved from the pathology files of the Massachusetts General Hospital, Boston, MA. The clinical information was extracted from the patients' medical records. All patient data were de-identified.

Histologic Evaluation

The histologic sections of all cases with available archival materials were reviewed by two of the authors. For each case, the following architectural and cytologic features were graded as either present or absent: process extending to the subcutaneous tissue; dermal–epidermal separation; epidermal necrosis; ulceration; vascular thrombosis; epidermal calcification; dermal calcification; perieccrine calcification; hair follicle calcification; perineural calcification; subcutaneous septal calcification; subcutaneous lobular calcification; septal panniculitis; lobular panniculitis; acute versus chronic panniculitis; fat necrosis; lipomembranous fat necrosis; prominent neutrophilic infiltrate; stippled versus chunky calcium deposit; calcification of any sized vessels including capillaries, small, medium, or large vessel; internal elastic lamina calcification; and intimal thickening.

Special Staining

The von Kossa (Fisher Scientific, New Jersey) and Alizarin red (Aros Organics, New Jersey) staining were performed per protocol provided in the purchased kit. The von Kossa stain was performed on 56 calciphylaxis and 19 control cases, whereas the Alizarin red stain was performed on 43 calciphylaxis cases. The quality and the presence of calcium within vessels and extravascular structures were scored for both stains.

Statistical Analysis

The presence or absence of the architectural and cytologic features in the 2 groups and the statistical association of clinical diagnosis of calciphylaxis and the presence of tissue calcium detected by each special stain were compared using Fisher exact test because of the categorical nature of the data and the sample size. Two-tailed *P* values of <0.05 were considered to be statistically significant.

RESULTS

Clinical Evaluation

We identified 27 patients with calciphylaxis and available archival materials whose ages ranged from 33 to 91 years (median, 66 years). The female to male ratio was 17:10. Twenty-five patients had a history of renal failure, whereas the remaining 2 did not. The sites of these 56 biopsies were from thigh (27), lower leg (22), abdomen (2), chest (2), flank (2), and buttock (1).

Of the 17 patients in the control group, whose ages ranged from 14 to 77 years (median, 63 years), 9 had a history of renal failure. The female to male ratio was 10:7. The histologic diagnoses of the skin specimens included non-specific ulceration (7), stasis dermatitis (4), thrombotic vasculopathy (3), dermal/subcutaneous necrosis (2), traumatic (2), and nephrogenic systemic fibrosis (1). The sites of these 19 biopsies were lower leg (9), thigh (7), forearm (2), and abdomen (1).

Histologic Evaluation

The histologic features of the 56 calciphylaxis cases and 19 control cases are summarized in Table 1. Evaluating with von Kossa stains, the presence of stippled calcification; chunky calcification; calcification of any vessels size, including capillaries and small to medium vessels, and calcification of vascular media; and vascular thrombosis in calciphylaxis cases was significantly different from the control group. Although not significantly different from the control group, 100% specificity was noted for perieccrine and internal elastic lamina calcification. Perieccrine calcium deposition was noted in 6 cases (11%) (Fig. 1). Calcification of the internal elastic laminae of arteries was noted in 10 cases (18%). Extravascular calcification involving the dermis, subcutaneous septae (Fig. 1), and lobules was noted in 11%, 29%, and 16% of our cases, respectively.

Although the von Kossa and Alizarin red stains showed comparable calcium detection in our study, the deposits seen on Alizarin red appeared larger and also demonstrated birefringence (Fig. 1; Table 1). In 7 (12%) biopsies, Alizarin red stain detected focal calcium deposition that was not seen on von Kossa stain. Calcification of epidermis, hair follicle, and perineurium was seen neither in the calciphylaxis or control cases using both von Kossa and Alizarin red stains; thus, these results were not included in Table 1.

DISCUSSION

The diagnosis of calciphylaxis is confirmed by tissue biopsy, yet there are few published articles analyzing the cutaneous histologic features of calciphylaxis.^{6,15–20} Histopathologic features of calciphylaxis include vascular calcification of small and medium sized vessels, intimal hyperplasia, microthrombi, extravascular soft tissue calcification, septal and lobular panniculitis, dermal–epidermal separation, epidermal ulceration, and dermal and subdermal necrosis. The histopathologic differential diagnosis of calciphylaxis encompasses a spectrum of calcifying panniculitides, such as lupus panniculitis and pancreatic panniculitis; processes of vascular calcification, including atherosclerotic peripheral vascular disease and Mönckeberg sclerosis; and thrombotic diseases, such as warfarin skin necrosis and proteins C and S deficiencies.^{15,20}

Two histochemical stains for calcium, von Kossa and Alizarin red, are available; however, comparison of their usefulness in the setting of cutaneous calciphylaxis has not been reported.^{15,21} von Kossa staining, the most often used laboratory techniques to detect calcium deposition in tissue, is a silver reduction method that exposes phosphates and

TABLE 1. Histologic Features of Calciophylaxis Versus Noncalciophylaxis Controls

	Histologic Features	Calciophylaxis*	Control	P	Sensitivity*	Specificity
Calcium quality	Stippled calcification	36/56 (33/43)	0/19	<0.0001	0.64 (0.77)	1.00
	Chunky calcification	37/56 (25/43)	5/19	0.0034	0.66 (0.58)	0.74
Vascular calcification	Capillaries	28/56 (26/43)	0/19	<0.0001	0.50 (0.60)	1.00
	Small to medium vessels	39/56 (27/43)	4/19	0.0003	0.70 (0.63)	0.79
	Any vessel size	43/56 (32/43)	4/19	<0.0001	0.77 (0.74)	0.79
	Vascular media	33/56 (20/43)	3/19	0.00130	0.59 (0.47)	0.84
	Internal elastic lamina	10/56 (7/43)	0/19	0.057	0.18 (0.16)	1.00
Vascular changes	Intimal hyperplasia	18/56	2/19	0.078	0.32	0.89
	Vascular thrombosis	37/56	7/19	0.033	0.66	0.63
Extra-vascular calcification	Dermal collagen	6/54 (7/42)	2/19	1	0.11 (0.17)	0.89
	Periecrine	6/55 (6/43)	0/19	0.33	0.11 (0.14)	1.00
	Septal panniculus	16/56 (15/43)	3/19	0.37	0.29 (0.35)	0.84
	Lobular panniculus	9/55 (9/43)	2/19	0.72	0.16 (0.21)	0.89
Subdermal changes	Panniculitis	24/56	3/19	0.052	0.43	0.84
	Septal panniculitis	18/56	2/19	0.078	0.32	0.89
	Lobular panniculitis	15/56	3/19	0.53	0.27	0.84
	Fat necrosis	39/56	9/19	0.10	0.70	0.53
	Lipomembranous fat necrosis	13/39	4/9	0.70	0.33	0.56
Dermal and epidermal changes	Dermal epidermal separation	12/48	1/19	0.090	0.25	0.95
	Epidermal necrosis	33/49	8/19	0.096	0.67	0.58
	Ulceration	29/50	8/19	0.29	0.58	0.58

*Evaluation with Alizarin red stain rather than von Kossa stain. Significant *p*-values, < 0.05, are shown in bold.

carbonates that are usually present along with calcium. Alizarin red, an anthraquinone derivative, forms an orange-red lake with calcium at a pH of 4.2, creating an end product that is also birefringent. Although the 2 stains seem to be

comparable in our study, the deposits seen on Alizarin red appear larger and also demonstrate birefringence. This facilitates screening slides at low magnification and minimizes failure to detect the microscopic deposits. In 7 (12%)

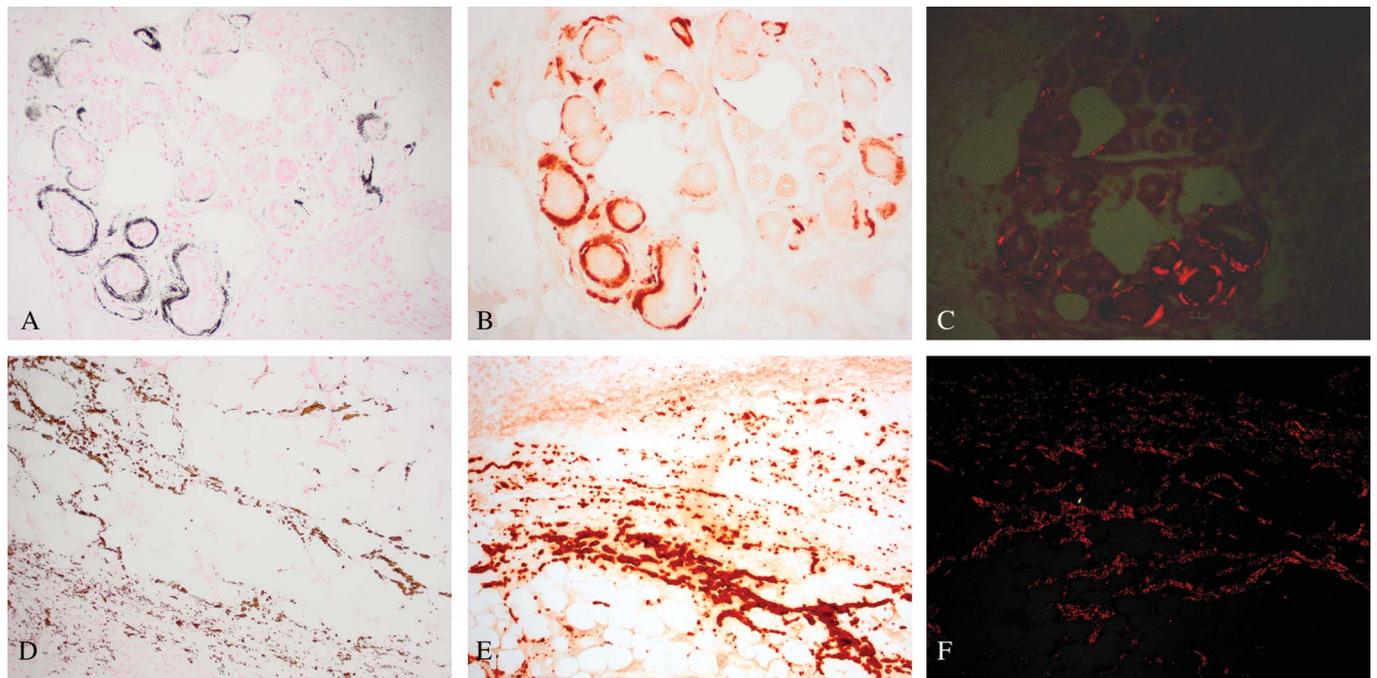


FIGURE 1. Subtle and stippled calcium deposits are noted in the basement membrane surrounding the eccrine coils highlighted by von Kossa stain (A) and Alizarin red stain (B), which exhibited birefringence (C). Prominent extravascular calcium deposition is noted within subcutaneous septa on von Kossa stain (D) and Alizarin red stain (E), which exhibited birefringence (F).

biopsies, Alizarin red stain detected focal calcium deposition that was not seen on von Kossa stain. In 3 (5%) of these biopsies that were diagnosed as negative for calcium deposition with von Kossa stains, a positive diagnosis could have been rendered with Alizarin red (in capillaries, small vessels, and subcutaneous septae). However, the possibility of cutting deeper into the blocks could account for the additional positive staining.

We noted a subtle histologic feature, periecrine calcification, highlighted by both von Kossa and Alizarin red stains, not previously reported to the best of our knowledge, which may aid in the diagnosis of calciphylaxis. This feature was highly specific to calciphylaxis in the present study and, as such, may be useful in the routine evaluation of biopsies for calciphylaxis in cases where less subtle features such as frank small vessel calcification are not present. Periecrine calcium deposition was the only form of calcium deposition noted in 4 skin biopsies (2 extending to dermis and 2 to subcutaneous adipose tissue). In retrospect, the diagnosis of calciphylaxis could have been made based on periecrine calcification in these 4 biopsies.

One must also consider that calcification of medium-sized vessels is a well-recognized phenomenon in patients with renal failure, particularly in patients undergoing hemodialysis.^{22,23} Cutaneous vascular calcification can be seen in association with vasculitis, sclerosing panniculitis, and cutaneous tumors.⁶ And indeed, in 2 of our cases, the medium-sized vessel calcification was regarded to be secondary to atherosclerosis. We found stippling calcification of the internal elastic lamina of muscular arteries in 10 (18%) cases. Although found to be specific in our study, calcification of the internal elastic lamina has been reported to be a feature of Mönckeberg sclerosis.²⁴ Our series is small; thus, more cases are needed to determine the usefulness of stippled calcification of the internal elastic lamina in distinguishing calciphylaxis from atherosclerosis.

Confirming the reported literature, all forms of vascular calcification and vascular thrombosis were significantly associated with cutaneous calciphylaxis (Table 1). Greater statistical significance was noted with the smaller vessel size calcification with the highest specificity noted with calcification of capillaries. Although extravascular calcification is thought to be more commonly associated with metastatic calcification than calciphylaxis, as demonstrated here, extravascular calcification of the dermis and subcutis can often be seen in cutaneous calciphylaxis. We did not observe the previously reported rare findings of epidermal and hair follicle calcification,²⁵ perineural calcium deposits,²⁶ and pseudoxanthoma elasticum-like features.²⁷ Of interest, in our study, we noted extravascular calcification, including septal, dermal, and periecrine basement membrane calcification. Similar to argyria, the silver granules are present in greatest numbers in the basement membrane zone, elastic fibers, and wall of capillaries.²⁸

The pathogenesis of calciphylaxis remains controversial and is likely multifactorial. One model attributes calciphylaxis to an elevated calcium/phosphate product because of a dysregulation of calcium and phosphate metabolism often from renal failure and secondary hyperparathyroidism, however, only a fraction of patients demonstrate these

abnormalities on presentation.^{7,29,30} Others have proposed that in a subset of calciphylaxis cases, a hypercoagulable state (quantitative reductions of protein C and S) results in thrombosis in vessels already narrowed by calcification, however, this can be seen in many patients with renal disease who do not develop calciphylaxis.^{31–33} Vasculopathy results from the pathologic narrowing of the dermal and subcutaneous vasculature in combination with superimposed acute thrombotic occlusion, leading to pan-cutaneous ischemic necrosis. The narrowing or stenosis of vasculature is caused by medial arteriolar calcification and intimal hyperplasia, which are in response to a “sensitizing” agent. Sensitizing agents activate several molecular pathways, most of them converging on the activation of nuclear factor kappa B. Nuclear factor kappa B is a transcription factor for growth factors, inflammatory mediators, adhesion molecules, and cytokines that can lead to the transdifferentiation of vascular smooth muscle cells into osteoblastic cells, resulting in medial calcification of vessels.³⁴ Studies on vascular disease, including calciphylaxis, have demonstrated bone glycoproteins, such as matrix Gla protein, osteopontin, and bone morphogenic protein-4, in calcified arteries.^{35,36} Finally, it has been demonstrated that patients with chronic renal failure have decreased anticalcification protein levels in circulation.³¹ Clearly, there are many existing factors that may play a role in calciphylaxis and likely, several to still be elucidated.

In summary, we confirmed that all forms vascular calcification and vascular thrombosis are significantly associated with cutaneous calciphylaxis. A subtle histologic feature, periecrine calcification, was found to be highly specific to calciphylaxis and may aid in the diagnosis of calciphylaxis in setting where vascular and extravascular calcification are not identified. As the von Kossa and Alizarin red methods each demonstrated examples of calcium detection that the other stain missed, performing both stains may increase the detection of calcium deposits.

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