A screen of the histopathologic database identified 142 biopsy-proven cases and 30 cases had both clinical and dermoscopic images of the biopsy site available. All dermoscopic images were captured using a DermLite II Pro polarized light dermatoscope (3Gen LLC). Dermoscopic images were evaluated by two independent dermatologists. Dermoscopic variables were adopted from previous literature describing the dermoscopic features of inflammatory purpuric lesions.[2-5]

Fifteen cases of true vasculitis and 15 cases of vasculopathy were included in this study. Statistical analysis was performed using Fisher's exact test. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were also obtained for the dermatoscopic diagnosis of vasculitis (Table I). A mottled purpuric pattern was commonly detected in all cases in both groups. Purpuric globules and dots were identified in 40% of the vasculitis group and in 26.7% of the vasculopathy group. An orange-brown background was found in both groups (40% in the vasculitis group and 53.3% in the vasculopathy group). Blue-gray blotches were exclusively found in the vasculitis group, affecting 73.3% of cases ($P < .001$; sensitivity 73.3%, specificity 100%, PPV 100%, NPV 78.9%; Fig 1).

These results demonstrated that blue-gray blotch was the most specific dermoscopic feature of true vasculitis, implying vascular injury with severe inflammation. Mottled purpuric pattern, purpuric globules, or dots and orange-brown background were commonly observed in both groups, mirroring the peculiar histology of this condition involving perivascular inflammatory cell infiltration, red blood cell extravasation, and hemosiderin deposition, respectively.[2] Based on these findings we recommend searching for a biopsy site that shows dermoscopic findings of blue-gray blotch for histologic confirmation of true vasculitis. Further prospective studies are needed to confirm the usefulness of the blue-gray blotch pattern for selection of the biopsy site.

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The proteolytic effect of bromelain on bullous pemphigoid antigen-2

To the Editor: Pineapple, *Ananas comosus*, is a member of the bromeliad family and is a well-known cause of irritant dermatitis and stomatitis.[1] Pineapple extracts contain a collection of proteolytic enzymes known as bromelain, as well as chemical and physical irritants including citric acid and calcium oxalate.[2] Clinically, bromelain has demonstrated utility in post-burn debridement.[3]

Collagen XVII (BPAG2, BP-180) is a 180-kDa transmembrane protein that acts as a structural component of hemidesmosomes, mediating adhesion of epidermal keratinocytes to the basement membrane.[4] Its importance in epithelia is illustrated by several diseases in which it is targeted or abnormal,[4] including bullous pemphigoid and junctional epidermolysis bullosa.[5]

Our inspiration for this project is a patient with longstanding oral cicatricial pemphigoid in whom disease flares were preceded by an inability to tolerate pineapple consumption. In our clinical experience, intolerance of other citric acid-containing fruits has also been noted in patients with mucosal blistering diseases. We hypothesized that pineapple proteases might specifically cleave collagen XVII, a hemidesmosomal protein commonly targeted by autoantibodies in cicatricial pemphigoid,[5] thereby further decreasing the already reduced adhesive function of this protein. For our study, confluent neonatal normal human epidermal keratinocytes were exposed for different times to different concentrations of bromelain. Equal

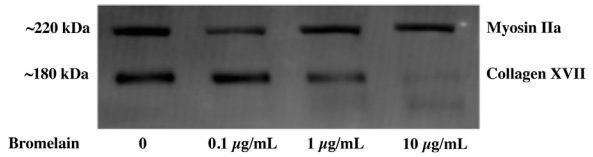

Fig 1. Effects of bromelain on collagen XVII. Cultured neonatal human keratinocytes were treated with the indicated concentrations of bromelain for 3 minutes and the cells harvested for Western analysis. Equal amounts of protein were separated on 10% sodium dodecyl sulfate gels and transferred to Immobilon-P membranes and after blocking incubated with primary antibodies (anti-collagen XVII from Thermo Scientific and rabbit anti-myosin IIA from Sigma-Aldrich). Immunoreactive proteins were visualized using an IRDye 680-conjugated goat anti-rabbit IgG secondary antibody (LI-COR Biosciences) and a LI-COR infrared Odyssey imaging system. Bands represent 180 kDa collagen XVII and the loading control myosin IIa as indicated. There is little effect of bromelain at 0.1 μg/mL. At 1 and 10 μg/mL, cleavage of collagen XVII, but not myosin IIa, becomes apparent (seen as a faint smaller band).

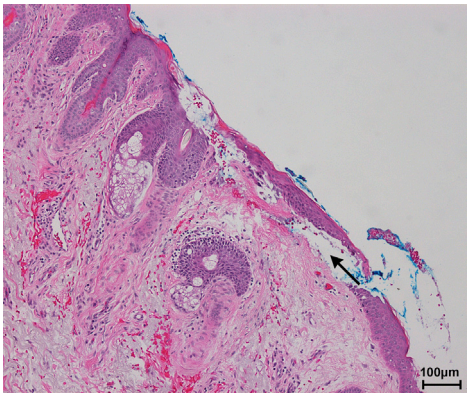

Fig 2. Effects of bromelain on intact adult facial skin. Intact nonlesional adult facial skin removed to facilitate wound closure after Mohs surgery was exposed to bromelain (300 μg/mL) for 30 minutes and fixed with 10% formalin. Focal areas of epidermal separation (arrow), which appear to include the dermal-epidermal junction, can be seen in the treated tissue. (Hematoxylin-eosin stain; original magnification: ×10.) Intact, untreated control tissue was unaffected (data not shown).

amounts of protein were separated on 10% sodium dodecyl sulfate gels, transferred to Immobilon-P membranes, incubated with antibodies recognizing collagen XVII and myosin IIa and secondary antibody (680-conjugated goat anti-rabbit IgG), and visualized using an infrared imaging system. In initial experiments we determined that keratinocytes treated with 10 μg/mL bromelain for 10 minutes exhibited a decrease in the levels of intact type XVII collagen, but not the myosin IIa loading control compared with untreated keratinocytes (data not shown). Fig 1 shows similar effects of incubating keratinocytes for a 3-minute treatment time with concentrations of bromelain ranging from 0.1 to 10 μg/mL. There was minimal effect of bromelain at 0.1 μg/mL, but at 1 and 10 μg/mL, collagen XVII was cleaved. Again, myosin IIa was unaffected.

To determine whether bromelain had an effect on intact skin, redundant adult facial skin removed to facilitate wound closure after Mohs surgery was exposed to bromelain (300 μg/mL) for 30 minutes, fixed with 10% formalin, and sectioned for histologic analysis. Shown in Fig 2 are focal areas of separation, located at the dermal-epidermal junction, present in treated but not control tissue.

To our knowledge, we have demonstrated for the first time the proteolytic effect of bromelain on a dermatologically significant protein; collagen XVII is cleaved by bromelain in vitro even after short treatment times and at low concentrations. Subepidermal separation of intact adult skin is also induced after exposure to bromelain. We hypothesize that exposure to bromelain enhances the epidermal separation experienced by patients with vulnerable oral mucosa such as those with mucous membrane pemphigoid. In highlighting the unique characteristics of pineapple we suggest that caution may be warranted regarding consumption of this fruit in vulnerable patients such as those with autoimmune mucosal blistering diseases. Additional studies are needed before dietary exclusion can be recommended.

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The cutaneous expression of vitamin K-dependent and other osteogenic proteins in calciphylaxis stratified by clinical features and warfarin use: A case control study

To the Editor: Calciphylaxis is a vasculopathy with a poorly understood pathogenesis. Some risk factors include liver disease and warfarin use.[1,2] Warfarin down-regulates the function of vitamin K-dependent proteins (VKDPs) by inhibiting gamma carboxylation.[3] We attempted to examine whether warfarin exerts its association with calciphylaxis through interactions with matrix Gla protein (MGP), a vitamin K-dependent inhibitor of extraosseous calcification and related proteins.[3,4] Bone morphogenic protein-2 (BMP-2) induces de-differentiation of smooth muscle cells into a phenotype that promotes vascular calcification and is inhibited by MGP.[2] MGP and BMP-2 are highly expressed in diseases of vessel calcification including atherosclerosis.[1] Fetuin-B is a liver-derived plasma protein that inhibits vascular calcification. We compared expression of BMP-2, MGP, and fetuin-B in biopsy-proven calciphylaxis specimens from warfarin-treated patients against nontreated controls.

We queried the Ohio State University Wexner Medical Center database from 2004-2014 and identified 21 patients with biopsy-proven calciphylaxis and 17 controls with renal disease and skin ulcers without calciphylaxis. After optimization, MGP, BMP-2, and fetuin-B expression were graded into high versus low levels by a masked, board-certified dermatopathologist, and correlated with patient demographics and outcomes. Univariate logistic regression was performed to analyze for calciphylaxis association. Continuous data were dichotomized based on the median.

Consistent with previous reports, thrombophilia ($P < .05$), liver disease ($P < .05$), and warfarin use (OR = 8.3, $P < .05$) were positively associated with calciphylaxis (Table I).[1,2] High alkaline phosphatase (OR = 7.5, $P < .05$) and low platelet count (OR = 0.14, $P < .05$) were also associated after dichotomization. On histology, MGP (OR = 7.3, $P = .02$) and BMP-2 expression (OR = 5.0, $P = .03$) were positively associated with calciphylaxis; but fetuin-B was not (Fig 1).

MGP, BMP-2, and fetuin B expression were evaluated in patients with calciphylaxis to elucidate the modifying role of warfarin. Warfarin inhibits clotting cascade factors,[3] but less is known about peripheral activation of the resultant vasculature effects of VKDP. We hypothesized that MGP levels would be lower in warfarin users; the OR was in the expected direction but insignificant (OR = 0.56, $P = .70$). BMP-2 activation induces MGP, thereby providing negative feedback regulation, which may have confounded interpretation of data.[5] Although high BMP-2 expression was expected in warfarin users, this small sample found no such association, perhaps confounded by MGP levels.

Study limitations included the rarity of calciphylaxis. Warfarin was not definitively linked to the protein expression that we examined, although BMP-2 expression tended to be lower in warfarin users but did not reach statistical significance. This could be due to small sample size and potential

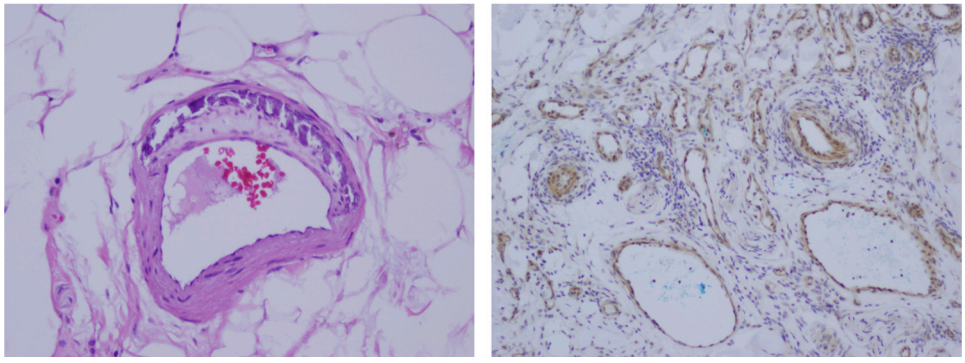

Fig 1. Calciphylaxis. BMP-2 Immunohistochemical staining of endothelial cells. (Original magnification: ×200.)