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Systematic pathological component scores for skin-containing vascularized composite allografts

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ABSTRACT

Clinical management of skin-containing vascularized composite allografts (VCA) requires accurate assessment of the graft status, typically based on skin biopsies. The Banff 2007 Working Classification proposed 4 grades of acute rejection, but did not score individual features or include vascular rejection. Here we report a systematic scoring system developed from MHC-mismatched porcine skin-containing VCA. Biopsies from 20 VCA, 9 autologous skin flaps and 9 normal skin were analyzed to optimize the methodology and set thresholds. The components quantified were: perivascular cells/dermal vessel (pc), perivascular dermal infiltrate area (pa), luminal leukocytes/capillary or venule (c), epidermal infiltrate (ei), epidermal apoptosis or necrosis (e), endarteritis (v), and chronic allograft vasculopathy (cav). To evaluate prognostic value, we scored a separate group of 28 serial biopsies from 8 recipients (4 that were ultimately accepted and 4 that rejected. Parameters on the initial biopsies predicting later graft rejection included pc ($p < 0.02$), pa ($p < 0.03$), ei ($p < 0.0005$), e ($p < 0.003$) and c ($p < 0.005$). Reproducibility between 2 pathologists blinded to clinical data was acceptable, with weighted kappa scores for pc (0.673), pa (0.399), ei (0.464), e (0.663), v (0.766), and c (0.642). This component scoring system can be adapted clinically, since human and porcine skin are highly similar. Vascular lesions in VCA are also highlighted in this system and could impact graft outcome. The component score approach complements Banff 2007 grades and will enable the establishment of clinically significant thresholds.

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Introduction

Assessment of skin-containing VCA status is currently performed by visual inspection and skin biopsies. Clinical and pathologic features have been described in both human and experimental settings[1-3] and these features were integral to the formulation of the Banff 2007 Working Classification for composite tissue allografts.[4,5] Banff 2007 grades are based on combinations of epidermal infiltrates, epidermal apoptosis and dermal/perivascular infiltrates. These are not scored separately and do not include vascular inflammation, nor have been formally tested for reproducibility or clinical significance.

Evaluation of individual pathologic components through systematic semi-quantitative scores offers the opportunity to define grades of the separate pathologic lesions (rather than mild moderate severe), refine criteria of rejection, identify distinctive patterns of rejection, increase reproducibility between centers, and provide a detailed data structure for reporting clinical and experimental research. We have developed a pathologic component scoring system based on experimental studies in porcine skin-containing VCA that is reproducible and predictive of graft loss. It includes the features considered in the Banff 2007 Working Classification

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(plus vascular lesions) and is potentially suitable for assessing human skin-containing VCA.

Materials and methods

Animals

MGH miniature swine were bred in a specific pathogen-free facility with defined MHC while maintaining minor antigen variation.[6] Animals were housed at the Transplantation Biology Research Center in accordance with the Guide for the Care and Use of Laboratory Animals. All experiments were conducted with the approval of the Institutional Animal Care and Use Committee of the MGH.

VCA transplantation

A fasciocutaneous vascularized composite allotransplant model was used. Donor animals were placed under general anesthesia, positioned supine with the hind limbs extended and prepared for surgery. A skin island of approximately 12 × 8 cm was elevated, with the underlying subcutaneous tissue and fascia, on a vascular pedicle comprising the medial saphenous artery and veins to their junctions with the superficial femoral vessels, which were recovered to a length of 2 cm to facilitate anastomosis. Flaps were recovered and flushed with 100 U/mL heparin sulfate in 0.9% normal saline. Defects were prepared in the recipients and donor vessels anastomosed to the carotid artery/internal jugular vein using standard microsurgical techniques. VCA outcome was followed by clinical appearance, protocol biopsy, with additional biopsies at times of clinically suspected acute rejection. The autologous skin flaps were raised from the groin and are perforator flaps based on the medial saphenous artery and vein. Native skin biopsies were also taken from the groin.

Treatment protocols

Group A included 4 animals (20680, 20681, 21556 and 21557; A1–4, respectively) that were haploidentical donor/recipient combinations selected from the MGH inbred miniature swine herd, treated on a mixed-chimerism protocol to induce transplant tolerance.[6] The treatment protocol for Group A animals included CD3-Immunotoxin 50ug/kg twice daily day -4 to -1, 100cGy TBI day -2, haematopoietic cell transplant (HCT) day 0–2 at 15×10^9 cells/kg, followed by VCA on day 3. HCT was with cytokine mobilized pBMC collected from donors by leukapheresis after mobilization with pIL3 and pSCF injected IM at dose of 100ug/kg to 30kg body weight, then 50ug/kg for each additional kg. Group B included 3 animals on related treatment protocols: Animal B1 (22667) was on the same conditioning protocol as Group A, but received a class I MHC mismatched transplant. Animal B2 (22419) received the same conditioning as Group A and received a haploidentical, unmobilized haematopoietic cell transplant (HCT). Animal B3 (21656) also received the same conditioning as Group A and received a haploidentical, bone marrow transplant (BMT). The fourth animal, B4 (22183), was a control animal that received a haploidentical VCA and no treatment.

The VCA of animals from Group A were accepted without clinical evidence of rejection throughout follow up (mean 293 days, range 139–486). The VCA of animals from Group B were ultimately rejected, following modest prolongation of survival by the treatment protocols (mean survival 40 days, range 8–63).

Pathologic evaluation

A total of 66 biopsy samples were analyzed (Fig. 1). Nine autologous skin flaps and 9 native skin biopsies

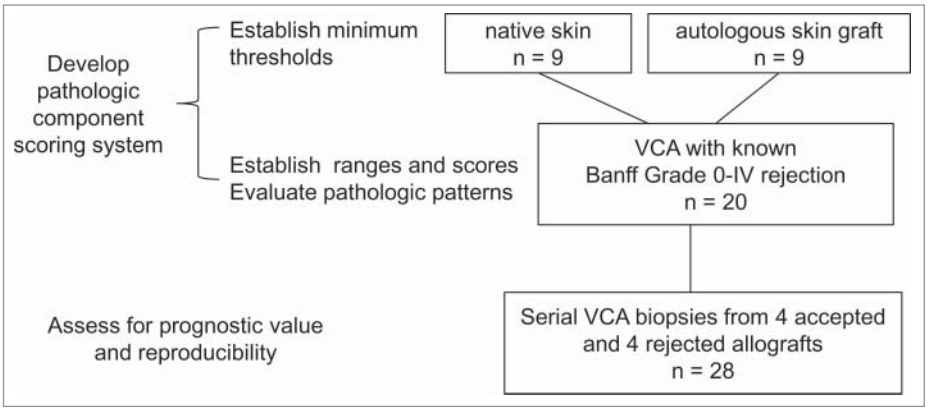

Figure 1. Development of the pathologic component scoring system and evaluation for prognostic value and reproducibility.

were used to develop minimum thresholds and 20 VCA biopsies with known rejection were used to further establish other pathologic components and corresponding ranges and scores. To evaluate for prognosis and reproducibility, 28 serial VCA biopsies from 8 animals were reviewed and scored. Six mm punch biopsies were placed in formalin and processed by routine histologic methods, followed by hematoxylin and eosin staining. Quantification of cells was done at 20x and 40x magnification using a BX53 microscope (Olympus, Center Valley, PA). The field diameter of the 20x field was 1.325 mm and the 40x was 0.6625 mm. C4d staining was not done due to lack of a suitable antibody for porcine C4d.

Development of the scoring system

Threshold values and pathologic patterns of cell infiltration (Table 1)

To establish threshold values for normal skin and nonimmunologic graft inflammation for each of the pathologic components, skin punch biopsies from 9 porcine autologous skin flaps and 9 porcine native skin were reviewed and assessed for amount of mononuclear cells and patterns of cell infiltration.

The perivascular infiltrate was assessed in 2 different ways. First, the mononuclear cells surrounding dermal vessels (arterioles, venules and capillaries) were counted at 40x magnification in at least 4 random areas (pc). The mean number of perivascular mononuclear cells was calculated as (n1 + n2 + n3 + n4)/4, where n represents total number of perivascular mononuclear cells per vessel in a 40x field. In the second method, the perivascular infiltrate area (pa) was expressed as the percentage occupied by the perivascular infiltrate at 40x magnification in a given discrete collection of dermal vessels and was scored in the most involved area.

The score for ei was determined by counting the number of mononuclear cells within the full length of the epidermis at 20x magnification. The number of mononuclear cells in the epidermis was calculated as N = n1 + n2 + n3 + n4…, where n represents number of mononuclear cells per 20x field and N is the total number of cells in all fields spanning the epidermis, expressed as the number of cells per 4 20x fields (typical of a 6 mm punch biopsy).

The number of leukocytes inside capillary lumina or venules (c) was counted in at least 4 fields at 40x magnification and the mean numbers calculated. This is similar to the capillaritis (ptc or microvascular inflammation) score in the Banff kidney classification. This is not the same as the "capillaritis" used by dermatopathologists that requires changes in the capillary wall. The c score was evaluated by assessing all capillaries in the sample and taking the value of the most involved capillary.

Evaluation for any epidermal injury (e), endothelialitis (v) and chronic allograft vasculopathy (cav) was also done. The scores for epidermal injury (e) were determined by the presence of single keratinocyte

Table 1. Definitions of pathological components and scores.

Component	Definition and Scoring
pc	perivascular cells – number of cells surrounding dermal vessels (capillaries, venules and arterioles) in the superficial and deep dermis; scored on the most involved vessels
	pc0 <10 cells/vessel
	pc1 10–25 cells/vessel
	pc2 26–50 cells/vessel
	pc3 >50 cells/vessel
pa	perivascular dermal infiltrate area – expressed as percent area occupied by the most involved dermal vessels at 40x magnification (example on Fig. 4D)
	pa0 <25%
	pa1 25–50%
	pa2 50–75%
	pa3 >75%
ei	epidermal infiltrate – total number of mononuclear cells per 4 20x fields
	ei0 <10 cells
	ei1 10–20 cells
	ei2 >20 cells
	ei3 transepidermal infiltrate
	eix no epidermis
v	endarteritis – mononuclear cells underneath arterial endothelium; scored on the most involved artery, arterioles not scored
	v0 none
	v1 <25% of lumen/vessel
	v2 >25% of lumen/vessel
	v3 fibrinoid necrosis/transmural involvement
	vx no arteries
e	epidermal injury and necrosis – presence of keratinocyte apoptosis and necrosis
	e0 none
	e1 apoptosis
	e2 focal necrosis
	e3 sloughed
cav	chronic allograft vasculopathy – intimal thickening with luminal reduction; scored as percent luminal reduction
	cav0 none
	cav1 <25% luminal reduction
	cav2 25–50% luminal reduction
	cav3 >50% luminal reduction
	cavx no arteries
c	capillaritis – maximum number of cells per capillary cross section; scored on most involved capillaries
	c0 0–1/capillary
	c1 2–4/capillary
	c2 5–10/capillary
	c3 >10/capillary
	ct thrombi

apoptosis or extent of necrosis. Endarteritis (v) and chronic allograft vasculopathy (cav) scores were adapted from the v and cav scores of the Banff 2013 Classification for kidney allografts.[7]

Optimization of the scoring system

To optimize the schema and to establish ranges for the component scores, similar methods were used to evaluate for pc, pa, ei, e, v and cav on 20 porcine skin-containing VCA indication punch biopsies with a spectrum of grades of rejection by Banff 2007 Working Classification. The 20 VCA samples included 16 with Grade I - IV rejection and 4 with Grade 0 rejection. Pathologic patterns and other features were also noted. Five of the 16 biopsies had Grade I rejection, 7 with Grade II rejection, 3 with Grade III rejection and 1 with Grade IV rejection.

The mean values obtained from the native skin biopsies, autologous skin flap biopsies and VCA biopsies with known Banff Grade 0 - IV rejection were used to establish the pathologic components and to assign cutoff values and ranges for the scores.

Reproducibility

To test for reproducibility, 28 serial biopsies from 8 animals (Group A and Group B) were coded and scored by 2 pathologists blinded to the various treatment protocols, gross features and outcome.

Evaluating for prognostic value of pathologic components

To evaluate the prognostic value of this schema, we compared the scores of the first biopsies of 4 allografts from Group A that were subsequently accepted (mean follow-up of 293 days) and 4 allografts from Group B that rejected (mean graft survival of 40 days) (Table 2).

Statistical analysis was performed using GraphPad Prism 7 software (Graphpad Software Inc., La Jolla, CA). Mann-Whitney U test was used for comparisons. A p value of less than 0.05 was considered statistically significant.

Results

The pathological component scoring system

Thresholds and pathologic patterns of cell infiltration

Native skin biopsies typically showed epidermal mononuclear infiltrates ranging from 1 - 9 cells counted at 20x magnification over the full length of the epidermis or an average of 4 cells per 4 20x fields.

Seven autologous skin flap biopsies taken at >15 d post-transplant showed a higher range of 5 – 15 cells over the full length of the epidermis or an average of 10 cells per four 20x fields, while 2 other autologous skin flap biopsies taken at earlier time points showed an average of 22 cells per four 20x fields (Fig. 2). In autologous skin flap biopsies, epidermal inflammatory cells are seen in greater numbers in focal distribution in comparison to native skin where they are usually more uniform along the length of the epidermis. Taking the means of the total number of epidermal mononuclear cells in both native skin and autologous skin flaps, the number of epidermal mononuclear cells for a score of ei0 was established at <10 cells over the full length of the epidermis or per 4 medium power (20x) fields. Capillaries showed a mean of 1 intracapillary cell in both native and autologous skin flap biopsies. None of the native skin or autologous skin flap biopsies showed endothelialitis. None had apoptotic cells.

Table 2. Pathologic component scores of first biopsies between 2 groups.

Animal number	Gross Appearance	Days post-transplant	Pathologic Component Scores							Graft survival (number of days)
			pc	pa	ei	e	v	c	cav	
Group A										
A1	Indistinguishable from surrounding skin	POD 56	0	0	0	0	0	0	0	486 days*
A2	Indistinguishable from surrounding skin	POD 58	0	0	0	0	0	0	0	139 days*
A3	Mild edema	POD 13	0	1	0	0	0	0	0	275 days*
A4	Mild edema	POD 13	1	1	0	0	0	0	0	275 days*
Group B										
B1	Edema	POD 14	1	1	1	2	0	2	0	40 days
B2	Edema	POD 15	2	3	2	1	0	2	0	50 days
B3	Erythema, induration	POD 42	3	3	2	1	0	2	0	63 days
B4	Erythema	POD 2	2	2	2	1	2	2	0	8 days

*No evidence of rejection at end of experimental follow up

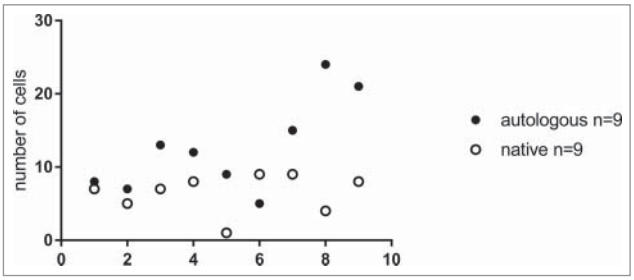

Figure 2. Number of epidermal mononuclear cells per 4 medium power (20x) fields in native and autologous skin flaps

In the 20 VCA indication biopsies, 4 did not show evidence of rejection and were classified as Banff Grade 0. The perivascular infiltrate (pc) had a mean of 2 cells/vessel and the biopsies did not show edema. The epidermal infiltrate (ei) was an average of 3 cells per four 20x fields. Capillaries had 0 - 1 intraluminal cells.

Five of the 20 VCA indication biopsies had Banff 2007 Grade I rejection, all of which showing superficial dermal edema. The amount of perivascular infiltrate ranged from 8–15 cells/vessel with a mean of 10 cells/vessel. The perivascular dermal infiltrate area (pa) in the most involved vessels showed a range of 20–70% of a 40x field with a mean of 46%. The epidermal infiltrate had an average of 11 cells per four 20x fields. One biopsy had 25 cells in the epidermis but showed only a mild perivascular infiltrate and therefore did not meet criteria for Banff Grade 2 rejection. Eosinophils were occasionally seen in the perivascular infiltrate. None of the biopsies had endothelialitis. Capillaries showed a mean of 4 intracapillary cells in the most involved capillary. Two biopsies showed neutrophils in capillaries.

Seven of the 20 VCA indication biopsies had Grade II rejection and showed different patterns. All the biopsies showed edema, which was prominent in the superficial dermis in 5 biopsies and was localized to the subcutaneous region in 2 biopsies. All showed increased mononuclear infiltrates around the dermal vessels, the most involved regions showing a range of 20–80% of a 40x field with a mean of 52%. Mast cells and occasional eosinophils were also present in the perivascular infiltrate. Four showed discrete and moderate epidermal inflammation, which were evident at 10x magnification. In these biopsies, the mean number of cells in the epidermal infiltrate was 20 cells per 4 20x fields. In 2 biopsies, the infiltrate was focal but transepidermal. Two biopsies which were diagnosed as Banff 2007 Grade II or III rejection had a single apoptotic keratinocyte. In general, these patterns demonstrate that perivascular or epidermal inflammation can be dominant with one being more pronounced than the other. The global grades of rejection in the Banff 2007 Working Classification required degrees of perivascular inflammation to be present, with or without epidermal inflammation, but these did not always occur together. Five of the 7 biopsies had endarteritis, one of which also showed early chronic allograft vasculopathy (cav). Intracapillary cell counts ranged from 4–6 cells in the most involved capillaries.

Three biopsies with Banff 2007 Grade III rejection and one with Grade IV rejection were reviewed. All showed severe and diffuse epidermal infiltration and full thickness epidermal involvement. Two had multiple foci of epidermal apoptosis. Perivascular inflammation was also extensive and severe. The biopsy with Grade IV rejection showed frank epidermal necrosis and mononuclear cell infiltration of hair follicles and adnexal glands. Transmural endarteritis was seen in 2 of the 3 biopsies with Grade III rejection and in the biopsy that showed Grade IV rejection. Chronic allograft vasculopathy was present in the biopsy with Grade IV rejection. Capillaritis was diffuse in both Grade III and IV rejection, with a maximum of 8 cells in the most involved capillary. Capillary thrombosis was present in the biopsy with Grade IV rejection. Spongiosis was seen in some of the biopsies with Grade II-IV rejection but not consistently throughout the samples that showed rejection of the VCA. The component scores for the biopsies with different grades of rejection are summarized in Fig. 3.

Thus, consolidating the means and ranges obtained, including pathologic patterns, the components for the scoring system were established as follows: perivascular cells/dermal vessel (pc), perivascular dermal infiltrate area (pa), luminal leukocytes/capillary or venule (c), epidermal infiltrate (ei), epidermal apoptosis or necrosis (e), endarteritis (v), and chronic allograft vasculopathy (cav). The definition of these components, the scores and corresponding ranges are listed in Table 1. Representative images pathologic components and corresponding scores are in Figs. 4, 5 and 6.

Reproducibility and ease of use

The weighted kappa scores showed moderate to substantial agreement for pc (0.673), pa (0.399), ei (0.464), e (0.663), v (0.766), and c (0.642). With

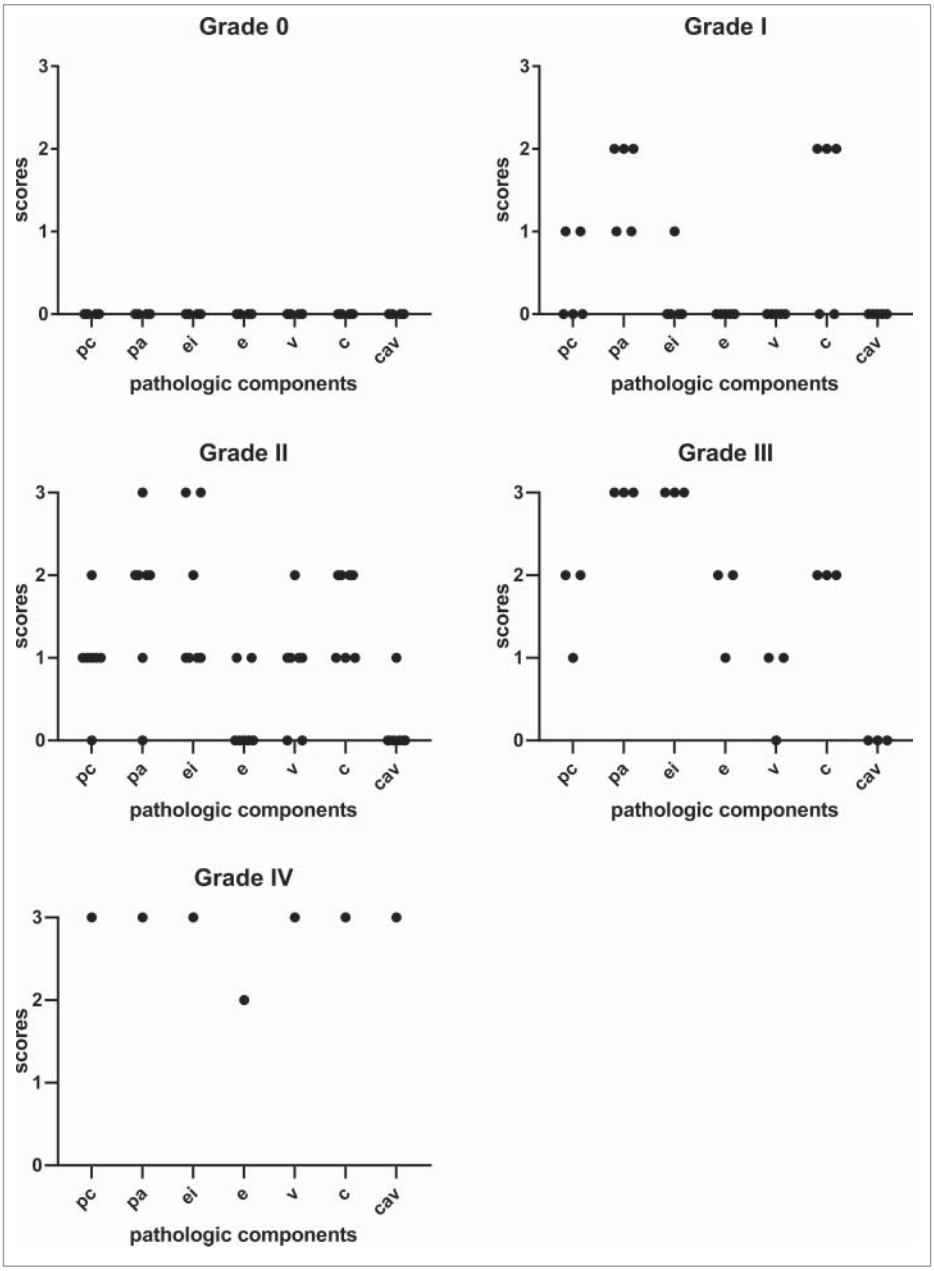

Figure 3. Pathologic component scores across 20 VCA biopsies with different Banff grades of rejection.

experience, the scoring could be done easily and efficiently, which for the study pathologists was less than 10 minutes.

Evolution of pathologic lesions

To follow the evolution of the pathological lesions, serial biopsies were reviewed from grafts that rejected or were accepted for >100 d. Protocol biopsies from allografts from Group A that rejected in a mean of 40 d showed higher pathologic component scores than allografts from Group B that were accepted and survived for more than 100 d. Perivascular inflammation (pc, pa) and epidermal involvement (ei, e) scores showed an increasing trend over time as the grafts rejected. Vascular inflammation scores (c, v) also showed the same pattern and appeared to show higher scores even at early time points (Figs. 7 and 8). None of the biopsies in this cohort showed chronic allograft vasculopathy.

Evaluation for prognostic value of pathologic components

Component scores of the initial protocol biopsies were analyzed in relation to outcome in groups A and B

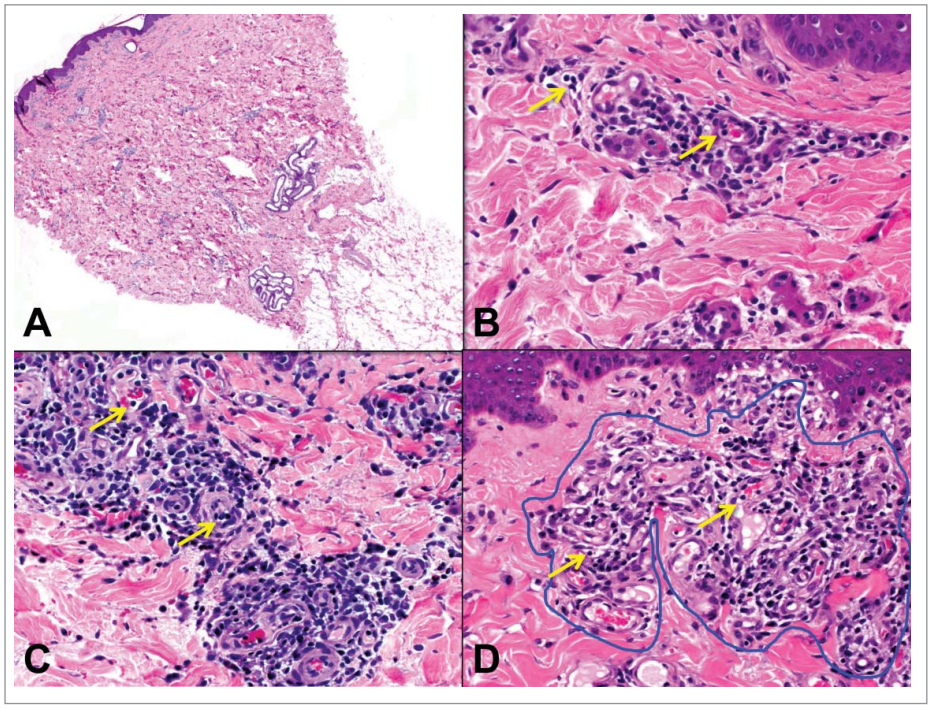

Figure 4. Histologic features of porcine skin and skin-containing VCA and pathologic component scores. A) Normal pig skin, punch biopsy. B) Minimal perivascular infiltrate (pc0) with capillaritis (c1) (arrows) in the superficial dermis. C) Moderately dense perivascular inflammation (pc2) with more evident capillaritis (c2) (arrows) at 40x magnification. D) The perivascular infiltrate surrounds dilated superficial dermal capillaries (pc1) occupying more than half of the field at 40x magnification (pa2) (outlined in blue). Dilated capillaries show occasional intraluminal mononuclear cells (c1) (arrows).

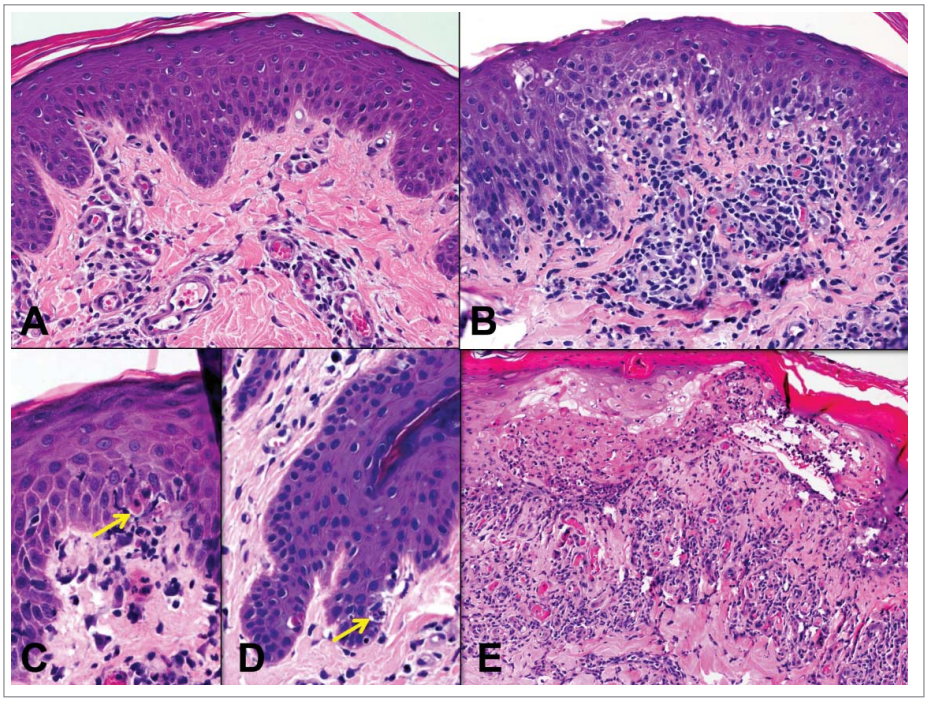

Figure 5. Epidermal inflammation (ei) and injury (e) in skin-containing VCA. A) Epidermis at 40x magnification with no epidermal infiltrate (ei0). Dilated superficial dermal capillaries with minimal perivascular inflammation are also present. B) One focus of prominent epidermal involvement without apoptosis (ei3, e0). C and D) Focal keratinocyte apoptosis (e1) (arrows). E) Frank epidermal necrosis. Moderate perivascular inflammation in the superficial dermis is also present.

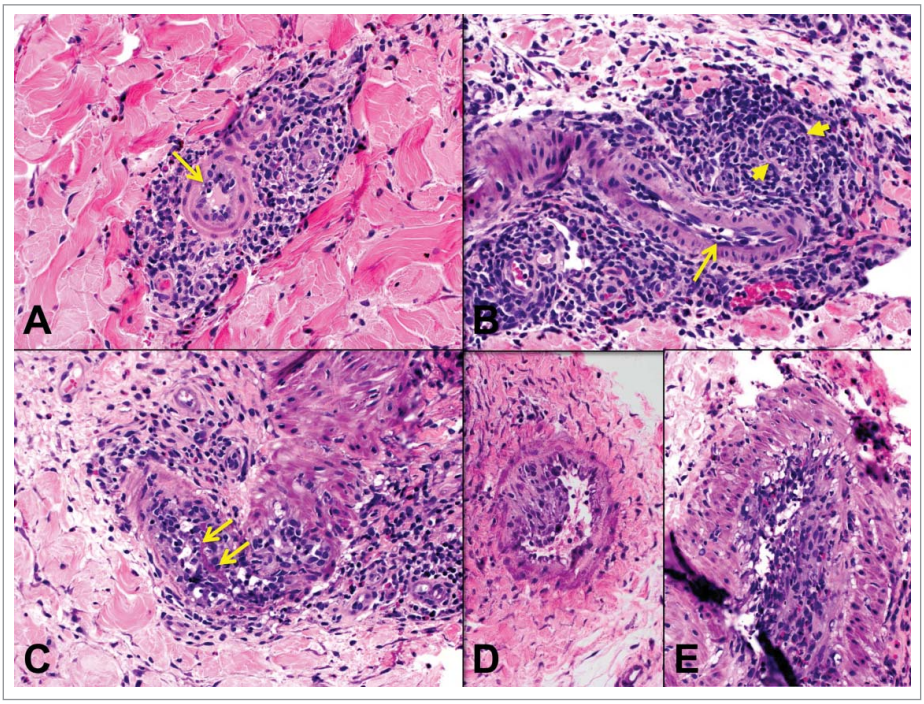

Figure 6. Endothelialitis (v) and chronic allograft vasculopathy (cav). A) Small artery with endothelialitis (v1) (arrow). B) Artery with endothelialitis (arrow) and transmural inflammation in an arteriole (arrowheads) (v3). C) Transmural arteritis (v3). Fibrin (arrows) can be seen in v lesions. D) Early chronic allograft vasculopathy (cav1) in a small artery. E) Severe chronic allograft vasculopathy with luminal reduction (cav3).

(clinical acceptance versus rejection). The following parameters were identified as predictive of graft rejection: pc ($p < 0.02$), pa ($p < 0.03$), ei ($p < 0.0005$), e ($p < 0.003$) and c ($p < 0.005$) in biopsies taken at a mean of 18 d for rejected grafts and 35 d for accepted grafts. Vascular v and cav lesions were not seen in any of the serial biopsy samples. Similar trends, although not statistically significant, were seen in the initial biopsies taken at the same time (13–15 days).

Discussion

Experimental studies on skin have long used pig skin because of its striking similarities to human skin. This applies to its general structure, thickness, hair follicle content, pigmentation, collagen and lipid composition.[8] Porcine epidermal thickness, for example, has been shown to approximate human epidermis, and range from 30 to 140 μm, which is comparable to human skin thickness of 50–120 μm.[8] It is thus optimal to use porcine skin-containing VCA as a platform for establishing pathologic components for scoring systems.

The Banff 2007 Working Classification for Vascularized Composite Tissue Allografts was developed based on histologic profiles of acute rejection from human skin-containing VCA.[5,9-11] The classification has been clinically useful in the diagnosis of rejection, however, the need for characterization of the pathologic features and a better understanding of mechanisms remain.[12,13] The findings in this study highlight the potential usefulness of a systematic approach to evaluation of rejection in allograft biopsies where pathologic patterns may overlap or not meet qualitative characteristics. The richness of this data allows for better clinical or research correlation and can give insights into the varied pathogenesis of rejection in VCA (e.g., epidermal vs. vascular).

The main features of the global grades of rejection of the Banff Classification are perivascular inflammation and epidermal involvement or injury. Similar to the reports in human VCA, initial biopsies of rejected porcine skin-containing VCA showed early histologic lesions which include mild and focal perivascular inflammation, often by a mononuclear infiltrate, edema, as well as intraluminal cells in dermal capillaries or venules. In higher grades of rejection, an increase in perivascular inflammation can be observed, associated with a lobular expansion of dermal vessels and its surrounding stroma. The infiltrate

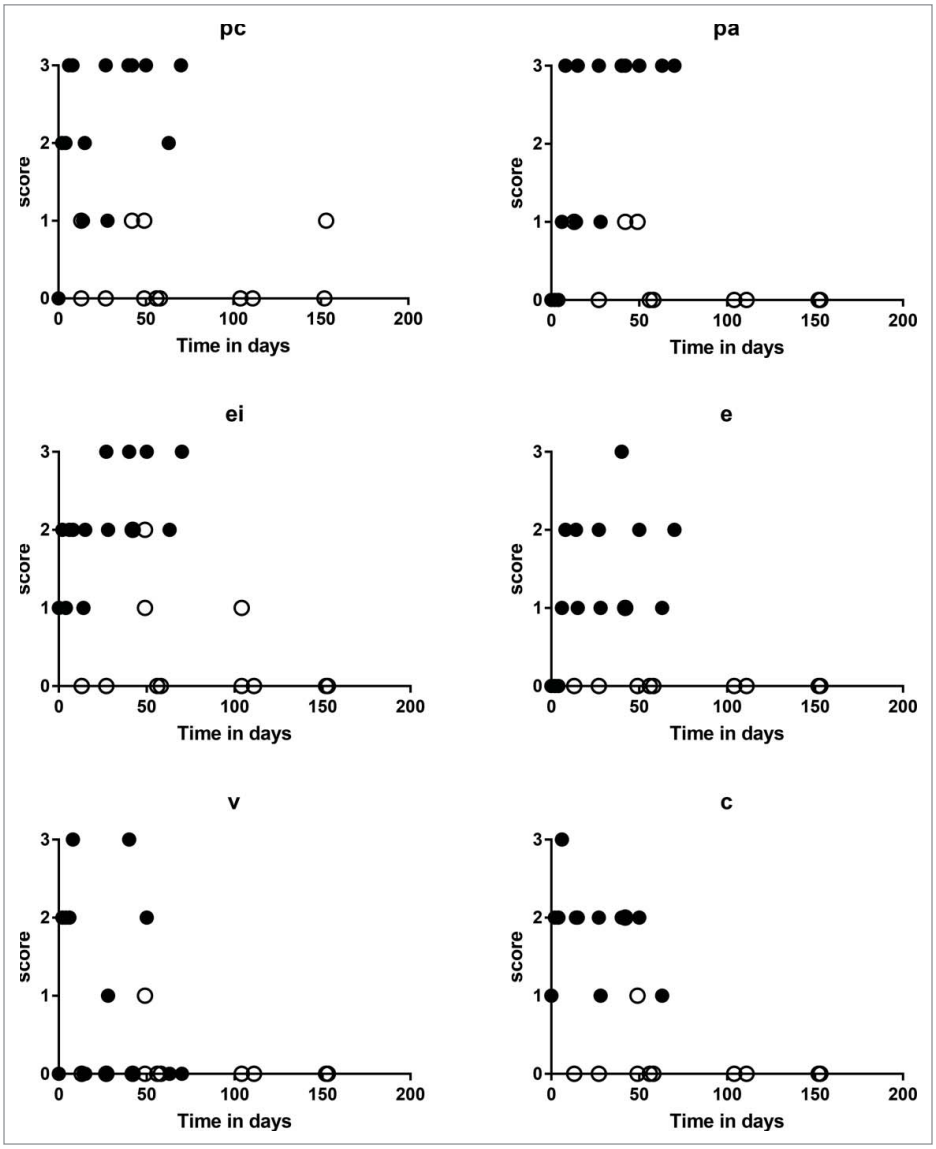

Figure 7. Pathologic component scores across accepted and rejected allografts over time (in days). Grafts that rejected and survived for less than 100 d (black circles) have higher pathologic component scores than grafts that were accepted (white circles).

may also include neutrophils and eosinophils. Epidermal involvement typically begins with lymphocytic infiltration, which may or may not be associated with spongiosis and basal vacuolation. Hyperkeratosis and parakeratosis may also be present. The hallmark of epidermal injury is typically individual cell apoptosis or dyskeratosis. Frank necrosis and a sloughed epidermis are noted in high grade or late stage rejection. Inflammation of adnexal structures can also be seen (Fig. 9C & D). These lesions are highlighted and described in the Banff 2007 Working Classification.

Vascular lesions in VCA, i.e. endarteritis, intracapillary cells, thrombosis and chronic allograft vasculopathy, are not part of the Banff current classification. This scoring system has included vascular lesions based on observations not only across different grades of VCA rejection but also because of its prognostic value in solid organ allografts. In porcine skin-containing VCA, endarteritis can be seen in Grade II to Grade IV rejection (Fig. 6). Higher c scores are seen in higher grades of rejection. In the samples analyzed for predictive value, there was insufficient data to evaluate v lesions as a predictive factor, however, this parameter has been shown to be predictive of graft outcome in kidney allografts. In the recent first penile transplantation done in the United States, the second of 2 allograft biopsies showed mild superficial perivascular inflammation and focal keratinocyte apoptosis, associated with deep dermal transmural arteriolitis and panniculitis. This was diagnosed as Banff Grade III

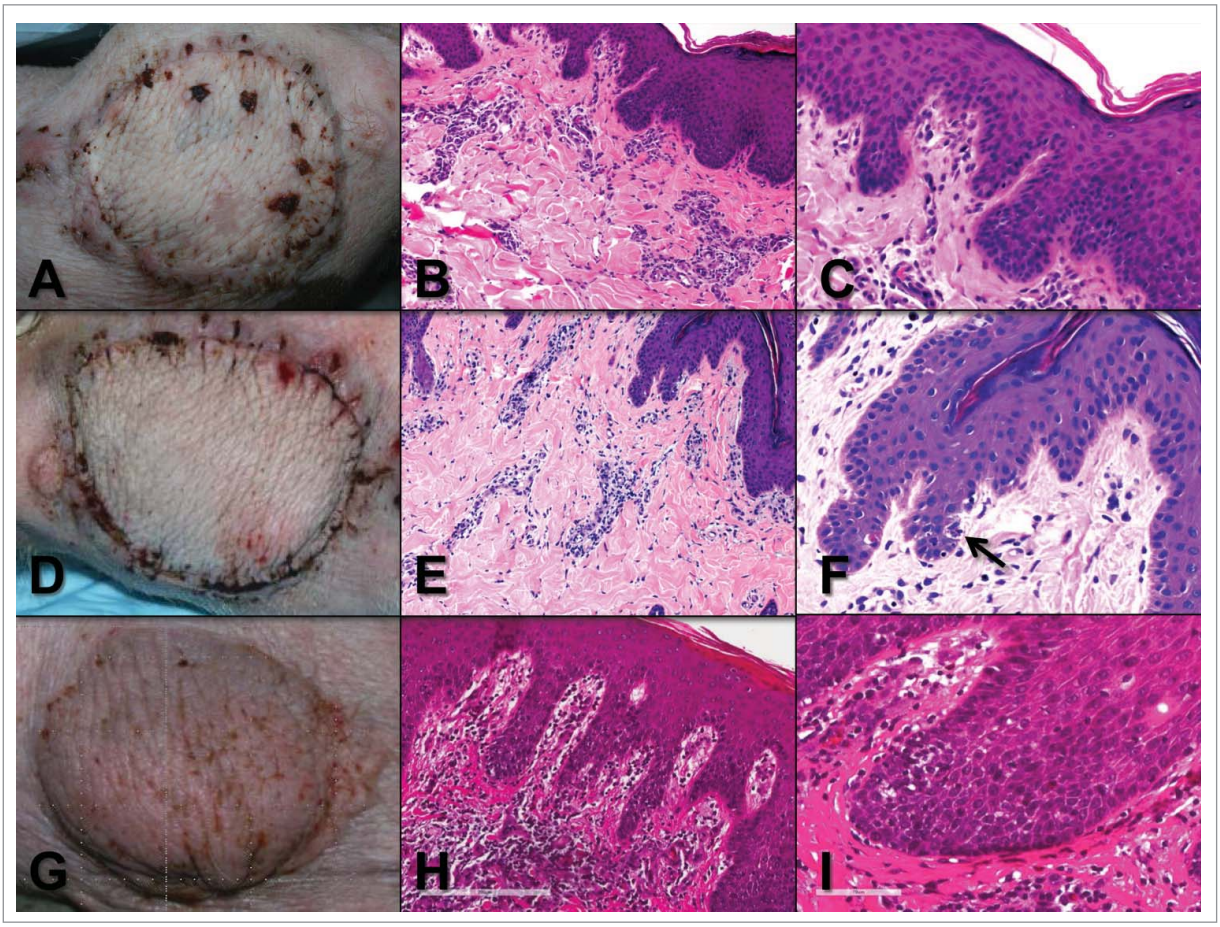

Figure 8. A) Animal A3 VCA post-surgical day 13 showing mild edema. B) Animal A3 POD 13 skin biopsy showing minimal perivascular inflammation (pc1, pa1) and C) no epidermal involvement (ei0, e0). D) Animal B3 POD 14 showing mild edema. E) Animal B3 POD 14 skin punch biopsy showing mild perivascular inflammation (pc1, pa1) but with F) epidermal apoptosis (arrow) and focal necrosis (not shown) (ei1, e2). G) Animal B1 POD 42 showing erythema. H) Animal B1 POD 42 skin biopsy showing severe perivascular inflammation (pc3, pa3) and I) lymphocytic infiltration and focal apoptosis (not shown) (ei2, e1).

rejection and resolved with antithymocyte globulin and methylprednisolone pulse.[14] This further illustrates 1) that discordant features may be present, with relative mild perivascular inflammation in the superficial dermis but with epidermal apoptosis, and that 2) endarteritis may suggest a higher grade of rejection and deep graft inflammation.

In the 2016 International Workshop on VCA Histopathology, the need for a systematic approach to pathologic evaluation of VCA in the clinical setting was raised. Special attention to potential pathologic features pertaining to antibody-mediated rejection (AMR) were emphasized. One of the features that has been clinically reported is capillary thrombosis. Two clinical cases showed capillary thromboses, one of which had deep arteriolar thrombosis and relative sparing of the overlying upper dermis and epidermis.[15] Capillary thrombosis was seen in early VCA biopsies and arteries with chronic allograft vaculopathy (cav) was observed in later biopsies.[15] C4d deposition and (donor-specific antibody) (DSA) were also present in one case. [15] These reports underline the need for evaluation for capillary inflammatory lesions and correlation with C4d and DSA, which offer direction in understanding antibody-mediated or chronic rejection pathways in VCA. Moreover, the prognostic value of these pathologic elements have been described in solid organ allografts and as such will likely prove useful in VCA.[16] For clinical application, C4d should be added as a vascular component, but we did not have a suitable antibody for the pig.

Although there is insufficient data available to define the specific changes of chronic rejection in VCA, chronic allograft vasculopathy can be seen in higher grades of rejection (Fig. 6D, E) and has been reported in experimental[17-19] and human[20–21] VCA

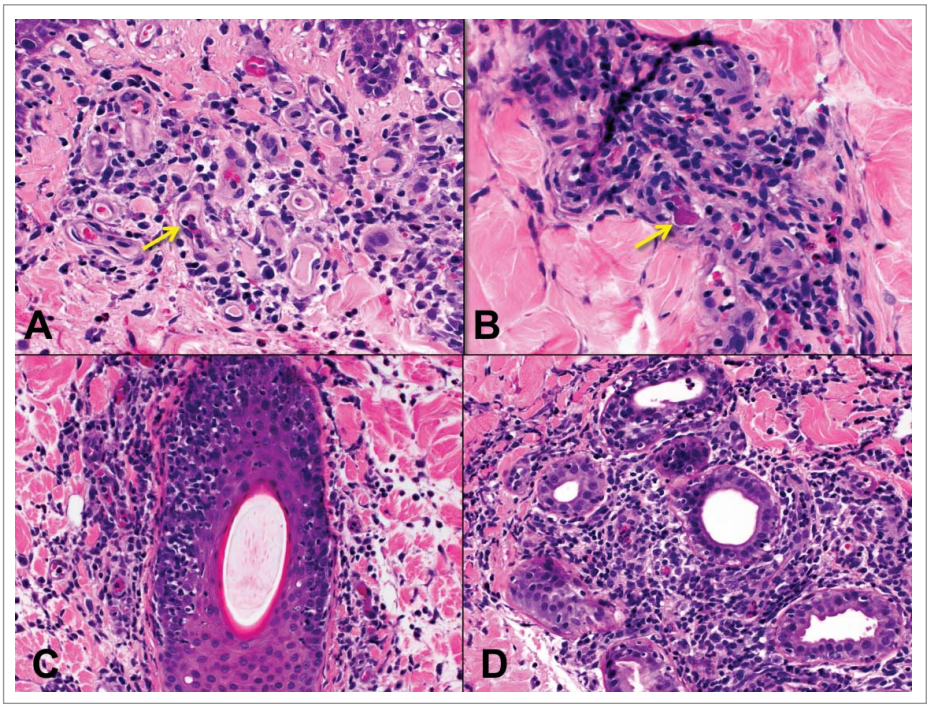

Figure 9. Other pathologic features in rejection. A) Intracapillary mononuclear cells and neutrophils (arrow) in superficial dermal capillaries and a B) capillary thrombus (arrow) in high grade rejection. Mononuclear cell infiltrates along C) a hair follicle and D) sweat glands.

studies. The inclusion of cav in the assessment of VCA will be essential to the understanding of mechanistic pathways of chronic rejection. Features that have already been highlighted in the Banff 2007 Working Classification as indicative of chronic injury include vascular narrowing, loss of adnexa, skin and muscle atrophy, fibrosis of deep tissue, myointimal proliferation and nail changes.[5] Experience with long-term or tolerant VCA[22] may be needed to further describe lesions other than cav that may be of prognostic significance.

In the design and optimization of this VCA scoring system and in the test cohort, vascular lesions were common in high-grade rejection and also show some evidence of their predictive value. Of note, the depth of sampling in routine skin biopsies does not always allow for evaluation of arteries, and early arterial lesions may be missed. Nonetheless, early recognition of vascular lesions when vessels are adequate will be valuable and can expand therapeutic and research direction.

This paper highlights the value of a pathologic component scoring system because it subdivides the pathologic features and identifies other pathologic components which could elucidate different patterns and pathways of rejection. Even in this limited cohort, vascular lesions, for example, are instructive and merit attention and inclusion in evaluating VCA biopsies. The findings in this study offer an opportunity to assess and validate individual pathologic components for prognostic value. This system is potentially a helpful tool for clinical research and should enable the prospective establishment of clinically important thresholds of the pathologic components.

Disclosure of potential conflicts of interest

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