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REVIEW

Vascularized composite allotransplantation: a closer look at the banff working classification

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SUMMARY

The first Banff vascularized composite allotransplantation meeting was held in 2007 to standardize criteria for the characterization and reporting of severity and types of rejection. As a result, the 2007 Banff VCA working classification for skin allograft pathology was formalized and now serves as the standard for diagnosis of VCA rejection. Similar to other working classification systems, strengths and limitations have been identified including the adequacy of the specimen, the definition of severity between grades, the reproducibility, the adequacy of the specimens, the types of rejection, and the integration of newer technologies such as molecular and genomic approaches. Although a relatively few number of cases have been performed and followed up to date, additional phenotypes such as antibody-mediated rejection, fibrosis, atrophy, and vascular changes are being reported and characterized based on accumulated experience in the field of VCA and parallels with other solid organs. This study aims to consider strengths and limitations of the Banff VCA working system and highlights ongoing challenges and opportunities available related to histopathology in this emerging field of transplantation.

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Introduction

Vascularized composite allotransplantation (VCA) has emerged as an option to correct limb or other multilayered tissue defects that are not salvageable by autologous reconstruction. Vascularized composite allografts are composed of heterogeneous tissues from different embryological origins including skin, muscle, nerves, vasculature, subcutaneous tissue, tendon, and bone. The biologic complexity of these grafts may pose specific immunologic challenges and it has not been determined as to whether there is an immune hierarchy or differential susceptibility of rejection among the included tissues.Skin-containing vascularized composite allografts are distinct from solid organ transplants in several aspects including the ability of visual monitoring of rejection through the evaluation of its dermatologic manifestations. Due to the accessibility, skin biopsy has proven to be the mainstay for histologic assessment of rejection in VCA. In 2007, the Banff working classification for the evaluation of rejection in VCA was developed and it became the standard tool in evaluating rejection. As an emerging field and similar to other scoring systems, the Banff VCA working classification is modified as data become available. Currently, the Banff VCA working group is working on the first revision of the classification. At this stage, the classification focuses on acute cellular rejection. Nonetheless, even though a relatively few number of cases and long follow-up have been performed to date, additional phenotypes such as

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antibody-mediated rejection, and vascular and chronic changes are being reported in VCA and are being discussed for inclusion in the system. This study aims to review strengths and limitations of the Banff VCA working system and highlight ongoing challenges and opportunities available related to histopathology in this emerging field of transplantation.

Banff Working Classification

The Banff meetings remain a forum by which international transplant pathology classification systems are developed through a consensus process. The first meeting was convened in 1991 to address the need to develop a universally recognized schema for the pathologic assessment of rejection in kidney transplants [1]. Until then, multiple classification systems had been proposed, but none were in general use. It has since progressed to encompass evaluation of other solid organ transplants (SOT). Of its many benefits, the standardization of the language of rejection afforded by Banff enabled objective histopathologic endpoints for international communication, clinical reporting, and preclinical and clinical studies. The concerted effort to standardize the language of rejection and implement evidence-based medicine compiled from several international centers has led to continued and responsive evolution of the schemata and enhanced patient care.

Following the first successful hand transplant in 1998, the field of VCA has expanded in the clinical arena. As anticipated, essentially all recipients of a VCA experienced skin rejection with 85% of patients diagnosed within the first year [2]. By 2006, four classification systems for the assessment of VCA rejection had been proposed [3–6]. Recognizing the importance of standardization, a group of clinicians and investigators from different institutions worldwide met at the Ninth Banff Conference on Allograft Pathology in 2007. At the time of the meeting, 41 patients had received skin-containing VCA (twenty-eight had received hands, nine abdominal walls, three faces, and one knee with a skin island). As the field was in its relative infancy with the longest clinical follow-up of 8 years (one patient), evidence-based assessment of chronic rejection and antibody-mediated rejection was precluded. Consequently, focus was turned to evaluation of acute rejection through assessment of skin biopsies. The consensus schema is outlined in Table 1 and Fig. 1.

Strengths and limitations of the 2007 Banff VCA Working Classification

Across multiple subspecialties, the Banff working classification remains a standard in transplant pathology as it provides a platform for standardization, interpretation, reporting, and development of clinical treatment algorithms. As a living document, it allows for revisions as new data become available.

As is seen in most settings, all classification systems have strengths and limitations. The current approach to grading of histopathologic lesions remains semiquantitative [7,8]. There is inevitable interobserver variability dependent on the population of the group tested reflecting different levels of experience as well Banff Working Classification as biologic variability of tissue. Although an aim of some working groups is to incorporate molecular and genomics information into the working classification where relevant, the disparity between resource availability internationally may prove to be an obstacle [9–13]. Nonetheless, these newer technologies can overcome limitations seen with conventional histopathology studies. The integration of these technologies is a future challenge.

Histologic Commonalities in VCA

As is seen with other SOT, there could be overlap of clinical and histologic features of rejection in VCA with

Table 1. The Banff VCA working classification system [14].

Grade	Inflammatory infiltrate	Involvement of epithelium (epidermis or adnexal)
0 (no rejection)	None/rare	None
I (mild rejection)	Mild perivascular	None
II (moderate rejection)	Moderate to severe perivascular	Mild (limited to spongiosis or lymphocytic exocytosis)
III (severe rejection)	Dense	Apoptosis, dyskeratosis, and/or keratinolysis
IV (acute necrotizing rejection)	Frank necrosis of the epidermis or its structures	

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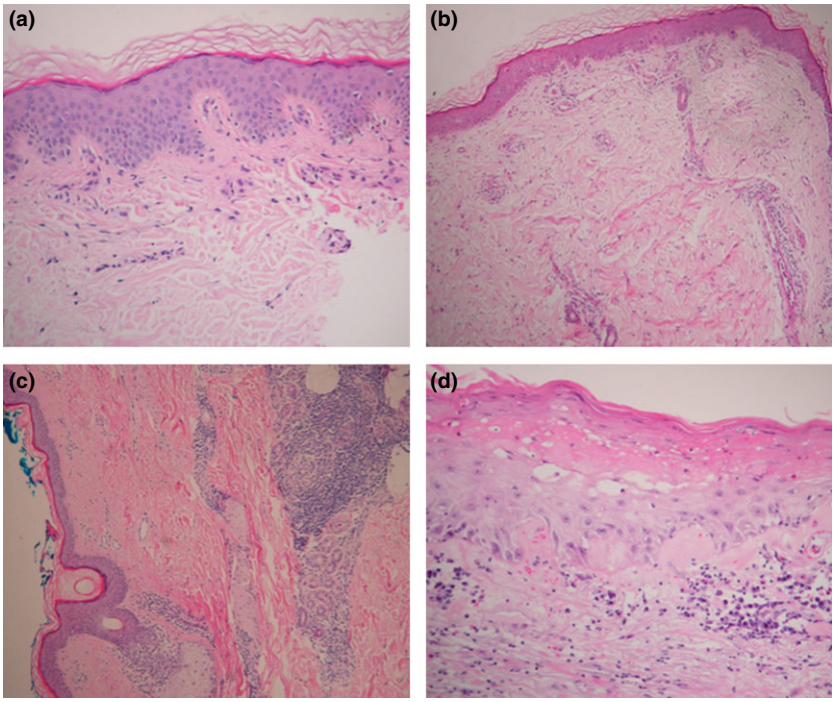

Figure 1 The Banff VCA working classification system. a. Grade I, b. Grade II, c. Grade III, d. Grade IV.

other pathologic processes. Indeed, it is recognized that skin changes in a VCA are not limited to alloimmune-related changes. Based on the grade of rejection, a list of differential diagnoses could be considered, and as the severity of rejection increases, the list of histologic differentials lengthens. Grade I, or mild lesions may have features that overlap with viral eruptions. Grade II, or moderate lesions may share features of viral or drug eruptions, contact dermatitis (particularly if eosinophils compose part of the infiltrate), arthropod assault, or dermatophyte infection. For Grade III or severe lesions, cutaneous pseudolymphoma, cutaneous B-cell lymphoma, and lichenoid dermatoses are in the differential diagnosis. In addition to the above considerations, in Grade IV rejection, which has the most severe and necrotizing lesions, severe drug reactions such as toxic epidermal necrolysis are a consideration [14,15]. Similar to other transplant settings, additional studies such as immunohistochemical stains may aid in the delineation between inflammatory processes and rejection although there is currently no reliable marker of rejection. The histologic overlap underscores the critical importance of clinicopathologic correlation to ensure medical intervention in a timely and rational manner.

Acute Rejection

Currently, Grade I, or mild rejection, is the most commonly reported degree of rejection following a VCA. However, its clinical impact and effect on graft sustainability is not yet clearly understood [2]. There are a number of inflammatory mimics, and as such, the importance of clinical correlation cannot be overemphasized. Information, such as localization of the visual changes, type and tempo of changes, and/or injury, may be useful although these visual changes may also be subtle and nonspecific.

Histology is the gold standard for the diagnosis of rejection. Nonetheless, its interpretation is not free from a degree of intra- and interobserver variability. This variability appears to be particularly noted in the differentiation between Grade I and Grade II rejection. In the current 2007 Banff schema, the major difference between these two categories is between "mild perivascular inflammation" and "moderate perivascular inflammation" and possible involvement of the epidermis characterized by spongiosis or exocytosis. However, the terms "mild" and "moderate" are not defined by an objective set of parameters. In addition, the correlation between Banff grade and treatment has not been established [16–19]. Comparison between Banff VCA Grade I could be drawn to borderline histologic changes encountered in renal transplant pathology with the subsequent clinical challenge that they pose. The significance of the findings and their treatment remain undefined. It has been proposed to interpret borderline results as part of an algorithm and not the only criterion to commence treatment [20].

At the time that this review is written, there has been no determination as to whether there is immune hierarchy or differential susceptibility of rejection among the included tissues in vascularized composite allograft. This has implications as to the utility of skin as a diagnostic sentinel of rejection and to the design of rescue strategies for rejection or graft loss. Studies were performed by our group in collaboration with 2 other different centers making use of a new multicenter collaborative initiative in VCA (VCAci), and we demonstrated in preclinical models of VCA that animals diagnosed with Banff VCA IV and Banff VCA III rejection showed signs of rejection in all underlying soft tissues included in the transplant at the same time point. One animal showed Banff VCA Grade 0 in the skin with simultaneous endothelialitis around the tendon (data not shown) [21]. Thus, in the majority of cases the skin acts as a forerunner of rejection. Additional systematic studies are required to define the immune hierarchy of the different tissues.

Another challenge in VCA is when histologic rejection is diagnosed without visual changes in the skin. Although a considerable number of VCA recipients do not report dysfunction or clinical signs at the time of acute rejection, this setting could be an analog to a condition referred as subclinical rejection in other organ transplants [22]. Thus, VCA is also similar to other organ transplants in that the management of rejection is guided by imperfect diagnostic techniques. Our group performs protocol and 'for-cause' skin biopsies and treats subclinical rejection.

Immunohistochemical studies have been performed in an attempt to identify a specific marker of rejection. However, parallels between acute skin rejection and inflammation also exist on molecular and cellular levels. Several groups have demonstrated that the infiltrate in mild rejection is composed predominantly of CD3+/CD4+ T lymphocytes, a smaller component of CD8+ TIA-1+ cytotoxic T lymphocytes (with a tendency toward CD8+ T cells as severity increases), and FoxP3+ T-regulatory lymphocytes. CD20+ B lymphocytes are usually sparse, and CD68+ macrophages often compose <50% of the infiltrate. This immunophenotype is not distinct [15,17,23,24]. Adhesion molecules like LFA-1, ICAM-1, and E-selectin have been reported to be upregulated during acute rejection [25]. But upregulation of these molecules has also been reported in inflammatory conditions such as psoriasis [26]. Currently, there is no reproducible marker that demarcates the difference between rejection and inflammation in VCA.

To date, no laboratory tests that can act as a systematic surrogate marker of rejection in VCA have been reported. In our institution, we consider the skin in a VCA as the analog of creatinine in renal allografts and do not treat rejection with local immunosuppression. Extensive research is ongoing in the field of transplantation at large to develop alternative means of rejection detection such as genetic markers, cellular analysis with biomarker quantification, and proteomic analysis [27–30]. Similar to other solid organs, integrating the clinical with the pathologic features and resampling as needed is crucial to diagnose rejection. This underscores the importance of continued open communication between clinicians and pathologists to optimize patient care.

Chronic Rejection

At the time this study is written, it is difficult to measure long-term survival in VCA due to limited of reported long-term follow-up. However, our group anticipates that VCA will demonstrate a trend similar to kidney, liver, lung, and intestine [31,32]. Although not yet included in the Banff VCA classification due to the paucity of data, chronic rejection has been reported both preclinically and clinically in VCA [33,34]. Similar to organ transplants, the prolonged viability of vascularized composite allografts will lead to graft loss due to chronic alloimmune injury.

In a preclinical model, Mundinger *et al.* [35] evaluated 186 skin biopsies from five long-surviving face allografts in nonhuman primates treated with FK506 and either MMF or anti-CD28 therapy. All five grafts demonstrated neointimal hyperplasia and arterial luminal narrowing in large vessels, sometimes progressing to occlusion, compatible with chronic rejection. Similar changes were observed in smaller more distal vessels. These findings were not secondary to anastomotic intimal damage as confirmed by serial histologic examination of the vessels. Although not statistically significant, arteriopathy, intimal hyperplasia, and vessel wall fibrosis were found more frequently in these five long-surviving grafts in comparison with other animals. Furthermore, perivascular tertiary lymphoid follicles composed of a mixture of CD3+ and CD20+ lymphocytes were also appreciated more frequently. Both the vascular changes and follicle formation were demonstrated in the deeper tissues of grafts despite the absence of histologic features of rejection in the skin, suggesting an ongoing background chronic immune response and raising the question of sample procurement. In this

report, chronic rejection did not correlate with IgG or IgM alloantibody production. If observed, C4d deposition was limited to small capillaries and was seen only after cessation of immunosuppressive therapy. In a separate preclinical model, similar chronic changes were illustrated to occur following multiple episodes of acute rejection with intermittent immunosuppression [36].

In the clinical arena, Pei *et al.* [37] described the chronic findings observed over a period of one to 10 years in a report of twelve patients with a total of fifteen hand transplants. One patient who had experienced episodes of acute rejection yearly manifesting as an erythematous rash localized to the transplant skin, progressively developed skin atrophy and decreased graft function. A biopsy obtained 5 years postoperatively demonstrated hyperkeratosis, epidermal atrophy, loss of adnexae, perivascular and vascular inflammation, and thickening and occlusion of blood vessels consistent with chronic rejection. At 9 years of follow-up, his graft was reportedly viable. Other patients exhibited similar clinical phenotypes, however declined histopathologic assessment.

A recently published case report outlines the changes encountered in a patient with a partial face transplant on a decreased immunosuppressive regimen secondary to EBV-associated B-cell lymphoma and smooth muscle tumors of the liver. From the second post-transplant year onwards, the patient clinically developed progressive sclerosis, pigmentary alteration, telangiectasias, and loss of beard hair with associated graft dysfunction. The pathologic correlate showed a transition between an acanthotic epidermis with interface alteration and edema to an atrophic epidermis with dermal sclerosis and hyalinization with loss of adnexal structures. The vessels showed thickened walls, eventually with decreased lumina. The authors noted that the deep aspect of the biopsy showed unremarkable subcutaneous tissue without lymphoid collections. Of note, immunohistochemical staining for vascular C4d deposits was negative throughout his clinical course. An MRI 4 years post-transplant demonstrated focal irregularities in grafted arteries. [34]. These clinical observations provide important information as we consider developments in the guidelines for chronic changes in the skin of a VCA.

Clinically, in a patient with documented episodes of untreated acute rejection, an acute arterial thrombosis secondary to myointimal proliferation was reported 275 days after transplant. [38] On assessment of four bilateral hand transplants and one face transplant by histology, magnetic resonance imaging, ultrasonography, and high resolution peripheral quantitative computed tomography scan, one team found no evidence of chronic rejection over a period of more than one year [38,39]. Similarly, in a 10-year update of three patients with hand transplants by the Innsbruck group, there were no signs of chronic rejection and all grafts had good function [40]. Kaufman *et al.* [33] described early onset of severe and aggressive transplant vasculopathy in two hand-transplant patients. An additional four patients in their study had some degree of vasculopathy. Recently, Kanitakis *et al.* [41] reported graft vasculopathy that affected both large and smaller cutaneous vessels in a noncompliant hand-transplant recipient. This observation demonstrated that vasculopathy can be diagnosed in a skin biopsy and could be a sign of chronic rejection.

The information regarding chronic rejection in VCA is growing, but remains limited. Obtaining deeper biopsies and/or obtaining imaging have been proposed as a method to evaluate larger vessels and for the significance of deep inflammatory infiltrates in the absence of visual changes of rejection. In VCA, the use of noninvasive vascular imaging techniques for perfusion is being investigated [42]. The role of noninvasive vascular imaging in monitoring for or evaluating rejection in VCA is undefined, although it may have some promise in other areas of transplantation. A rationale behind current imaging modalities includes detecting tissue viability or perfusion of the transplanted organ. As an example, Doppler imaging of the mitral annulus can be a sensitive, but not specific technique for detecting severe heart transplant rejection [43]. Newer MRI technologies that allow for diffusion weighted imaging can noninvasively evaluate tissue viability and acute kidney transplant rejection [44,45] and postpancreatic transplant complications [46]. The utility of these in VCA and cutaneous evaluation, however, may be more tenuous. Techniques such as laser Doppler flowmetry were initially thought to be a promising diagnostic tool in detecting active scleroderma [47] but have not gained ground in clinical use because it could not distinguish between increased perfusion from active inflammation, and increased signal due to atrophy of surrounding soft tissue and postinflammatory scarring. In the field of graft-versus-host disease, the lack of an objective tool for measuring inflammation and cutaneous sclerosis remains a major obstacle in research and clinical care [48]. There are newer optical technologies such as optical coherence tomography that are useful in imaging retinal vasculature, but their use in vascular imaging through opaque structures such as skin and deeper tissues is not yet developed [49,50]. Our practice does not

include imaging as a monitoring tool to detect rejection in VCA.

Antibody-mediated Rejection (AMR)

Similar to chronic rejection, limited information regarding AMR was available to Banff reviewers in 2007 and continues to be true until today. Most of the current data are limited to case reports of vascularized composite allografts or extrapolation from SOT. It appears that heart and kidney grafts are more susceptible to presensitization [51]. Histologic findings associated with hyperacute rejection in renal allografts include neutrophil and platelet margination in capillaries, stasis of red blood cells, fibrin deposition, thrombosis of small vessels, acute tubular injury, and differing degrees of cortical necrosis [52]. Although once considered a consistent marker of AMR in SOT, reports described C4d-negative AMR in renal allografts, prompting modification of Banff criteria at the 2013 meeting [53].

AMR has been reported after a VCA. Described by Chandraker *et al.*, a 45-year-old female patient with numerous risk factors for presensitzation had confirmed presence of donor-specific antibodies (DSA) and a positive crossmatch on the day of her facial transplant surgery. The team meticulously coordinated the clinical picture, DSA levels, and allograft biopsy results to guide an individualized immunosuppressive regimen. Graft erythema and swelling were noted early in the postoperative period. There was a corresponding increase in DSA levels, which occurred prior to histologic changes of acute rejection. By POD15, the patient had Banff Grade II rejection and strong perivascular C4d deposition. On POD19, biopsies showed progression to Banff Grade III rejection and similar C4d deposition, while concurrent DSA levels were trending upwards. This prompted modification in her immunosuppression regimen, which she remained on until POD51. Biopsy findings at that time showed mild Banff Grade I rejection with persistence of C4d staining. Approximately four months postoperatively (POD 116), there was no active cellular or antibody-mediated rejection [54]. This case of a positive crossmatch and considerable amount of immunosuppression provides important information as we gather data for developments in the guidelines for antibody-mediated rejection in VCA.

Limited data are available regarding C4d as a systematic marker for AMR in VCA. In non-human models, weak, nonspecific capillary C4d staining in allografts undergoing rejection as well as in native skin and the skin of autografts has been reported. [25,35,54–57] In a study assessing four patients with VCA over a range of 7 days to 7 years, C4d was not detected in numerous biopsies obtained from both skin and mucosa in histologic findings consistent with rejection [58]. Further complicating the picture, C4d deposition has been reported in inflammatory dermatoses without rejection [39]. Following our last 2015 Banff VCA session, systematic data regarding C4d are being collected for future inclusion in the classification system.

Mucosal Biopsy Assessment

At the time of the Ninth Banff Meeting in 2007, only three face transplants had been performed and little data existed regarding evaluation of mucosal biopsies. Since then, several reports have been published. In a 4-year review of a near total face transplant, signs of chronic rejection were not observed; however, histologic features consistent with acute rejection were frequent. Comparing biopsies from the skin and mucosa, many were discordant with more severe changes observed in mucosal biopsies. In particular, the mucosal biopsies showed interface change characterized by vacuolization of the basal layer and the presence of dyskeratotic cells. According to the Banff criteria, the presence of dyskeratotic cells rendered a diagnosis of Grade III rejection. Often adding to the uncertainty were the conflicting simultaneous skin biopsies. [59] Similar findings had previously been reported by Kanitakis *et al.* [60] Given the pathologic findings between sites, lack of clinical features of rejection, overlap with pathologic changes seen secondary to immunosuppression therapy, but the presence of dyskeratosis technically equating to Grade III rejection, the authors found it difficult to decide when treatment was the appropriate recourse. Ultimately, the group treated for acute rejection when there were clinical signs of rejection and when the skin and mucosal samples had shared histologic features. Of note, interface mucositis is also the histologic finding seen in a mycophenolate mofetil-induced drug reaction. In addition, the mucosa is exposed to more insult than the skin, and thus, inflammation may be a more common finding in certain settings. Nonetheless, the role of mucosa biopsies as a diagnostic tool to decide treatment of rejection in VCA independent from the skin remains to be defined.

Summary

In its relative infancy, the field of VCA has emerged as a life-enhancing therapy for a group of carefully selected patients.

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The Banff VCA system is a common language in the field. The formation of classification and diagnostic criteria via consensus conference has proven to be useful in multiple settings. Although imperfect, the Banff VCA system is an international effort that lays the groundwork to advance the understanding of VCA pathology, enhances the communication among investigators, and contributes to clinical analysis. Currently, the working group is gathering data for the first revision of the classification.

The 2007 Banff working classification is the standardized method for the diagnosis of skin rejection in VCA. Similar to other scoring systems, strengths and limitations have been identified. To address some of the limitations, the working group developed a biopsy form to collect parameters in a standardized manner for future analysis and to utilize for the first revision of the classification. Data point includes information related to cellular and antibody-mediated rejection, vascular involvement, atrophy, and difficulties between Banff VCA grades. It is anticipated that the results will provide data toward the first revision of the Banff VCA scoring system. It is also anticipated that the standardized collection of information among groups worldwide will aid the study of the controversies and limitations of the classification.

Although a relatively few number of cases have been performed and followed up to date, the basic biology of vascularized composite allografts is sufficiently similar to that of other organ transplants that phenotypes such as antibody-mediated rejection, chronic fibrosis, atrophy, and vascular and chronic changes are being characterized based on accumulated experience in VCA and parallels with other solid organs. This experience is providing information to the first revision of the classification system.

There are more unknowns than knowns in VCA rejection. Challenges and controversies include subclinical rejection, the treatment of rejection, the role of the sentinel flap as a monitoring tool for rejection, and if the site of the skin (e.g. hand vs. face) show different features at the time of rejection.

Currently, there is no correlation between the scoring system and treatment of rejection. Systematic studies are necessary to review how implementation of the schema in clinical practice guides treatment and affects outcome of the graft. In addition, results from studies with additional technology such as molecular and genomic approaches will need to be integrated with the established conventional histopathology.

Continued emphasis must be placed on the importance of ongoing communication between the clinical and pathologic aspects of a transplant patient. Despite increasing investigation of the cellular, genetic, and molecular level of VCA, at this time, clinic–pathologic correlation remains a reliable tool in the detection and reversal of rejection.

Conflicts of interest

The authors of this manuscript have no conflict of interest to disclose.

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