ORIGINAL ARTICLE

Graft vasculopathy of vascularized composite allografts in humans: a literature review and retrospective study

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SUMMARY

Mechanisms of chronic rejection of vascularized composite allografts (VCA) remain poorly understood and likely present along a spectrum of highly varied clinicopathological findings. Across both animal and human VCA however, graft vasculopathy (GV) has been the most consistent pathological finding resulting clinically in irreversible allograft dysfunction and eventual loss. A literature review of all reported clinical VCA cases with documented GV up to December 2018 was thus performed to elucidate the possible mechanisms involved. Relevant data extracted include C4d deposition, donor-specific antibody (DSA) formation, extent of human leukocyte antigen (HLA) mismatch, pretransplant panel reactive antibody levels, induction and maintenance immunosuppression used, the number of preceding acute rejection episodes, and time to histological confirmation of GV. Approximately 6% (13 of 205) of all VCA patients reported to date developed GV at a mean of 6 years post-transplantation. 46% of these patients have either lost or had their VCAs removed. Neither C4d nor DSA alone was predictive of GV development; however, when both are present, VCA loss appears inevitable due to progressive GV. Of utmost concern, GV in VCA does not appear to be abrogated by currently available immunosuppressive treatment and is essentially irreversible by the time of diagnosis with allograft loss a likely eventuality.

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Key words
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Introduction

Reconstructive transplantation through vascularized composite allografts (VCAs) has revolutionized the treatment of complex soft tissue absence, loss and injuries by providing an anatomically exact tissue unit enabling like-for-like restoration. In recent years, there has been rapid growth in the application of VCA with upper extremity [1], face [2], penis [3], abdominal wall [4], larynx [5], uterus [6] and even knee transplants [7] performed.

Much variation in inclusion/exclusion criteria and induction, maintenance and rescue immunosuppression regimens exists between different VCA centers [8]. As a result, long-term immunological consequences remain poorly understood and are difficult to compare between centers. For instance, the extent of HLA matching has long been known to impact upon allograft rejection and

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longevity in solid organ transplantation (SOT) [9]; in VCA however, matching of skin color and allograft size between donor and recipient takes priority instead due to the need for optimizing functional and aesthetic outcomes [10].

Despite early enthusiasm in the field, like any transplanted allograft, VCAs are not spared from chronic rejection with recent clinical reports confirming as such [11]. While the mechanisms of chronic rejection in VCA remain to be formally defined, reports have suggested a highly varied range of associated clinical and pathological findings, all of which likely represent manifestation of the underlying disease process along a spectrum [11–13]. Of note, experimental (from small and large animal models) and clinical VCA subjects have both developed a common pathological feature in graft vasculopathy (GV) whereby myointimal proliferation and gradual luminal narrowing lead to progressive graft ischemia, necrosis and eventual loss [11–13]. The roles of antibody-mediated rejection (AMR), donor-specific antibody (DSA) formation and C4d deposition (or not) with consequent GV in the context of VCA remain equivocal, however. Thus, this study aims to review the current clinical evidence on GV as the main component of chronic vascular rejection in VCA and associated outcomes including allograft loss.

Patients and methods

A PubMed search for English language articles on confirmed cases of chronic VCA rejection (based on histological confirmation of GV development) was performed up to December 2018. Search terms include: "graft vasculopathy vascularized composite allotransplantation", "graft vasculopathy vascularized composite allograft", "graft vasculopathy composite tissue allotransplantation", "graft vasculopathy composite tissue allograft". References of individual articles were reviewed to minimize missing out on potentially relevant reports.

Relevant data extracted include the type of VCA (e.g., osteomyocutaneous, myocutaneous), the extent of HLA matching between donor and recipient, pretransplant panel reactive antibody (PRA) status, induction agent, whether donor bone marrow cells (BMCs) were infused, maintenance immunosuppression regimen, prior number of acute rejection episodes before histological confirmation of GV (including time to diagnosis, site and type of biopsy performed), DSA and C4d statuses, length of follow-up before development of GV, and final outcome of the VCA after developing GV. Reported cases of chronic rejection without GV development were excluded.

Results

Our literature search yielded a total of 121 articles. After accounting for duplicate data, exclusion of reports on chronic rejection without GV, and initially missed articles located from reference lists, there were a total of 13 individual reports of VCA patients who had developed GV as confirmed by histopathology.

Patient demographics

Overall, approximately 205 cases of all types of VCA have been performed worldwide and at the time of this study, 13 have reported histological confirmation of GV development (overall incidence of 13/205 = 6%). Diagnosis was confirmed by histopathology (through for-cause or terminal biopsies) at a mean of 5.9 years post-transplantation (range, 0.5–13 years). Seven patients have had their allograft removed for an overall VCA survival of 46% from the time of GV diagnosis; of the remaining 6 patients with GV, the mean reported follow-up without subsequent loss of VCA was 5.6 years (range, 0.75–10).

Potential contributory factors to graft vasculopathy development

Due to limited data available, there was no observable association between the extent of HLA mismatch (from 0/6 to 6/6), PRA status, type of VCA performed (with and without vascularized bone marrow as part of the composite unit), immunosuppressive regimen and number of prior acute rejection episodes (mean = 3.5; range 1–8) before GV was diagnosed.

Of note, induction with alemtuzumab does not appear to result in DSA formation ($n = 5$; 1 was positive only *after* VCA removal [14]) within the reported follow-up period compared to basiliximab and anti-thymocyte globulin (ATG), where 3 of 5 patients developed DSA (given the available data). In terms of maintenance immunosuppression, steroid-sparing regimens ($n = 4$) appear to result in earlier development of GV (2.6 vs. 7.4 years in protocols with steroids).

Potential mechanisms of graft vasculopathy in VCA

Evidence for AMR (based on DSA positivity alone) was suggested in five VCA patients who developed GV (Patients #3, 5, 9, 10, 13; Table 1) but corresponding C4d

Transplant International 2019; 32: 831–838

deposition (tested for in all protocol and for-cause biopsies) was not always associated with circulating DSA.

In patient #3, multiple (seven) prior episodes of acute rejection appears to have contributed towards DSA+/C4d− GV at 8 years post-transplantation although the VCA remained viable at 10 years follow-up. Similarly, patient #9 developed DSA+/C4d− GV at 6 years post-transplantation in spite of few (three) prior acute rejection episodes and eventually required VCA amputation at 11 years post-transplantation due to progressive ischemia and necrosis of skin lesions. In contrast, patient #5 also had few (two) prior acute rejection episodes but developed ischemia of the VCA that ultimately necessitated amputation at only 9 months post-transplantation; DSA+/C4d− GV was detected only on terminal histology analysis.

With regard to C4d deposition, this was seen in five VCA patients who developed GV (Patients #4, 7, 8, 10,13; Table 1). Patients #4 and #7 developed DSA−/C4d+ GV after few (two to three) prior episodes of acute rejection and relatively early at only 2 years and 6 months post-transplantation respectively although VCA loss was not reported within the available follow-up data. Patient #8 developed DSA−/C4d+ GV at 13 years post-transplantation after five prior episodes of acute rejection and the VCA was eventually removed 1 month later due to gradual, self-discontinuation of immunosuppression over the preceding year. Of note, C4d was detected only in deep vessels of the VCA in these patients.

Patients #10 and #13 both developed DSA+/C4d+ AMR-mediated GV and eventually lost the VCA. In patient #10, there were two episodes of acute rejection prior to detection of high DSA levels from 3 years post-transplantation onwards and two further episodes of acute AMR before GV and C4d were detected after VCA amputation at 7 years post-transplantation due to recalcitrant rejection episodes that were likely due to chronic AMR. Similarly, patient #13 only had two prior episodes of acute rejection before DSA levels became detectable after 7.5 years post-transplantation and eventually, resulted in progressive rejection, necrosis and loss of the VCA at 10 years post-transplantation that was likely also due to underlying, chronic AMR with corresponding detection of GV and C4d.

Discussion

This study has shown that the severity of GV in VCA is highly variable with differing clinical outcomes and temporality from the point of diagnosis. Most concerning, however, is the likelihood for its true incidence to be underestimated, and the pace of GV progression unpredictable due to the inherent challenges of timely detection and accurate diagnosis in clinical VCA [15]. To further compound the problem, diagnostic and treatment options for acute rejection episodes in VCA remain particularly challenging due in part to the ongoing revision and evolution of the Banff 2007 pathological classification of acute VCA rejection [15], and a possible association with eventual GV development [16]. Detection of *de novo* DSA formation, staining and reporting of C4d status in VCA is also not uniformly performed or reported and unfortunately, their significance in GV remains poorly understood thus far [17]. Furthermore, VCA patients with allograft loss have not always been reported in the literature, which only serves to further compound our already limited understanding of the contribution of GV, if any, towards chronic rejection and loss of VCAs.

While the risk factors for GV in VCA remain poorly understood, experimental rodent studies by Unadkat *et al.* [16] have suggested that the accrual of multiple acute rejection episodes may culminate in GV and similar observations have been made in this study. To illustrate: in patient #12, the development of post-transplant lymphoproliferative disorder [18] necessitated the lowering of maintenance immunosuppression, contributed towards eight acute rejection episodes with eventual sclerosis and dyschromia of the facial allograft at 4 years post-transplantation with GV seen in the facial arteries on MRI [18,19]; in patient #8 who had experienced five prior episodes of acute rejection, gradual self-tapering of immunosuppression led to upper extremity VCA removal at 13 years post-transplantation with GV found in the radial artery and perineural vessels of the radial nerve on terminal histopathological analysis [20]. Interestingly, patient #3, who was converted from mycophenolate mofetil (MMF) to rapamycin in the early post-transplant period to allow lowering of maintenance tacrolimus levels, developed seven episodes of acute rejection before DSA formation and GV was detected at 7 and 8 years post-transplantation respectively although the VCA remained viable at 10 years follow-up [14]. However, GV may also develop insidiously following episodes of acute rejection that might have been missed altogether or deemed mild and unnecessary for treatment [5,7,11] as demonstrated in nonhuman primate studies by Mundinger *et al.* [21]. Again, similar observations have been made clinically in patients #5, 9, 10 and 13 with only 2–3 episodes of acute rejection before GV development and eventually, necrosis of the VCA necessitating removal. These findings support our observation that GV in VCA is highly variable in both severity and clinical

Table 1. Reported cases of chronic rejection in VCA based on the development of graft vasculopathy.

Patient	VCA type	HLA mismatch	Pre-VCA PRA	Induction	Maintenance	Donor BMC infusion	Prior TCMR episodes	C4d DSA	GV diagnosis (time, site, biopsy type) final outcome
1 [7]	Knee	N/A	N/A	ATG	Tacrolimus MMF Steroids	No	X 1	N/A N/A	36 months, SSG & VCA synovial and deep tissues, for-cause biopsy Infection, above-knee amputation at 56 months
2 [30]	Upper extremity	N/A	N/A	ATG	Tacrolimus MMF Steroids	No	Yearly	N/A N/A	5 years, VCA skin, for-cause biopsy VCA intact (at 9 years follow-up)
3 [14]	Upper extremity	N/A	0%	Basiliximab	Tacrolimus MMF → Rapamycin (early post-transplant) Steroids (weaned then restarted)	No	X 7	– + (from year 7)	8 years, VCA deep tissue and inter-osseous arteries, for-cause biopsy VCA intact (at 10 years follow-up)
4 [14,31]	Upper extremity	N/A	0%	Alemtuzumab	Tacrolimus Rapamycin Steroids (after GV)	No	X 3	+ (deep vessels) –	2 years, VCA deep tissue and inter-osseous arteries, for-cause biopsy VCA intact (at 6 years follow-up)
5 [14]	Upper extremity	N/A	0%	Alemtuzumab	Tacrolimus MMF	No	X 2	– + (only after amputation)	9 months, VCA arteries & veins, terminal biopsy Ischemia, VCA amputated at 9 months
6 [14]	Upper extremity	N/A	0%	Alemtuzumab	Tacrolimus MMF (after 1st ACR) → rapamycin (after GV) Steroids (after GV)	No	X 3	– –	6 months, VCA deep tissue and inter-osseous arteries, for-cause biopsy VCA intact (at 2 years follow-up)
7 [14]	Upper extremity	N/A	0%	Alemtuzumab	Tacrolimus MMF → rapamycin (after GV) Steroids	No	X 2	+ (adnexae and vessels in adipose, deep vessels in skin) –	6 months, VCA deep tissues, for-cause biopsy VCA intact (at 9 months follow-up)
8 [20]	Upper extremity	6/6	N/A	Basiliximab	Tacrolimus MMF Steroids	No	X 5	+ (perineural vessels only) –	13 years, VCA skin & radial artery, terminal biopsy VCA amputated at 13 years (1 month after stopping Rx)

Table 1. Continued.

Patient	VCA type	HLA mismatch	Pre-VCA PRA	Induction	Maintenance	Donor BMC infusion	Prior TCMR episodes	C4d DSA	GV diagnosis (time, site, biopsy type) final outcome
9 [11]	Upper extremity	4/6	N/A	ATG	Tacrolimus MMF Steroids	No	X 3	N/A + (from year 6)	11 years, VCA skin, for-cause biopsy Amputation of fingers in VCA and eventually whole allograft at 11 years
10 [32]	Upper extremity	6/6	0%	Alemtuzumab	Tacrolimus → Belatacept MMF Steroids (stopped after POD 21)	No	X 4 (had 2 episodes of AMR prior to starting belatacept)	+ (only after amputation) + (from year 3)	7 years, VCA (not specified), terminal biopsy Amputation of affected finger with poor wound healing; VCA amputated at 7 years
11 [5]	Larynx, Pharynx, Trachea, Thyroid, Parathyroid	0/6	N/A	Muromonab-CD3	Cyclosporine → Tacrolimus (after 1st ACR) MMF Steroids	No	X 1	N/A N/A	12 years, VCA (not specified), terminal biopsy VCA explanted at 14 years
12[18]	OMC Face	5/6	0%	ATG	Tacrolimus MMF Steroids	Yes (on day 4)	X 8	– –	4 years, VCA skin, for-cause biopsy VCA intact (at 6 years follow-up)
13 [11,19]	MC Face	1/6	N/A	ATG	Tacrolimus → rapamycin (after 11 months) MMF Steroids	Yes (on days 4 & 11)	X 2	+ (VCA dermal vessels) + (from year 7.5)	10 years, SSG nutrient vessel & VCA facial arteries, for-cause biopsy Necrosis, VCA removed at 10 years; autologous reconstruction

ACR, acute cellular rejection; AMR, antibody-mediated rejection; ATG, anti-thymocyte globulin; BMC, bone marrow cell; DSA, donor-specific antibody; GV, graft vasculopathy; MMF, mycophenolate mofetil; OMC, osteomyocutaneous; PRA, panel reactive antibody; SSG, sentinel skin graft.

presentation that may potentially be followed by irreversible allograft loss, regardless of the various rescue and salvage immunosuppression therapies attempted.

Multiple immunomodulatory approaches have thus been attempted in VCA to reduce the incidence of acute rejection episodes. Such modalities include variations in T ± B cell depletion, steroid-based and steroid-sparing maintenance regimens, switching from calcineurin-based inhibition to rapamycin and/or belatacept, and donor BMC infusion. DSA formation was detected in 3 of 5 VCA patients (#3, 8, 9, 12, 13) who had undergone basiliximab or ATG induction followed by steroid-based maintenance immunosuppression but only in 1 of 5 (patients #4, 5, 6, 7, 10) who had undergone alemtuzumab induction and steroid-sparing maintenance protocols (albeit initially). Perhaps the earlier detection of GV in the latter group and subsequent introduction of additional maintenance steroids halted further progression. If so, it raises doubts about the safety of steroid-sparing regimens that have only had limited follow-up reported so far with further rejection episodes necessitating the re-introduction of, or additional steroid treatment [22,23]. Additionally, it appears that rapamycin may halt the progression of GV (as seen in patients #6 and 7), once detected, with VCA survival of up to 2 years post-transplantation thereafter. The use of novel agents such as belatacept does not appear to have any beneficial effect on DSA+ GV (as seen in patient #10) despite its purported benefits in reducing preexisting DSAs more effectively [24], unlike the antiproliferative agents rapamycin and MMF. Two patients (#12, 13) in this study underwent donor BMC infusion without recipient conditioning, did not develop detectable levels of mixed chimerism, and had no observable effect on the number of acute rejection episodes or time to development of GV, when compared with the other 11 patients who did not receive BMCs. It appears then that currently, standard triple immunosuppression based on tacrolimus, MMF and steroids would provide the best chance of ensuring longevity of the VCA, with switching to or addition of rapamycin being potentially beneficial in slowing the progression of GV once detected.

Fundamentally, the exact mechanisms behind GV in transplantation are poorly understood although AMR remains the most commonly accepted explanation with corresponding detection of DSA and C4d [25]. However, as with experimental animal data, the detection or absence of C4d in clinical VCA subjects did not portend the subsequent development of GV. Also, similar to AMR in SOT, VCA biopsies that are DSA−/C4d+, DSA+/C4d− and DSA+/C4d+ have all been reported in this study. It is possible that this phenomenon may be caused by non-HLA antibodies [26] but regardless, GV appears to be the common end point. Of note, in this study, there were 3, 3 and 2 VCA patients that were DSA−/C4d+, DSA+/C4d− and DSA+/C4d+ respectively. Only one patient (#8) that was DSA−/C4d+ lost the VCA but this was due to medication noncompliance whereas two of three patients (#5, #9) that were DSA+/C4d− required VCA amputation due to progressive and irreversible ischemia and rejection. Of note, both patients (#10, #13) that were DSA+/C4d+ experienced VCA loss. Based on these observations, C4d may be less accurate in predicting the development of GV in VCA as it tends to be localized to deep tissues thereby necessitating deep, open biopsies (which is still subject to sampling errors) whereas DSA, which is detected in peripheral blood, may in fact be a better predictor of GV and eventual allograft loss.

Overall, the role of induction therapy, steroid withdrawal and the respective roles of DSA and C4d in the development of GV are difficult to assess due to the retrospective nature of the study and the small number of patients reported. No definitive treatment exists for GV in VCA currently but this study has shown that early diagnosis and management with maintenance steroids and/or anti-proliferative agents such as rapamycin may potentially halt or slow the progression of an essentially irreversible process [14]. Novel, noninvasive monitoring technologies such as ultrasound biomicroscopy have also been described for earlier detection of GV [14], as well as genomic interrogation and immunohistochemistry of VCA biopsy samples [27]. Nevertheless, by the time such changes are detectable or clinically significant, it may be a case of *too little too late*. Ultimately, we believe that mixed chimerism represents the most likely strategy that will be successful in mitigating GV and chronic VCA loss, as demonstrated in previous swine studies from our laboratory [28,29]. Further studies on reliable methods for the induction of stable, long-term mixed chimerism are required. In the mean time, there is now an urgent need for describing clinical strategies to mitigate the challenges of impending VCA loss due to GV. Such options may include re-transplantation of the face (https://edition.cnn.com/2018/04/17/health/second-face-transplant-bn/index.html; accessed on 31 October 2018) or involve a combination of autologous reconstruction and/or prosthetic fitting in hand VCA recipients.

Authorship

ZYN, AGL participated in research design, performance of the research, data analysis and writing of the paper. IAR and RBC participated in data analysis and writing

Transplant International 2019; 32: 831–838

of the paper. LG and AG participated in performance of the research and writing of the paper. LAL and MAR participated in data analysis. CLC participated in research design, data analysis and writing of the paper.

Conflict of interest

The authors declare no conflicts of interest that might bias this work.

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