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New Paradigms in Rejection Monitoring: Lymphocyte Subsets as Noninvasive Graft Markers in Vascularized Composite Allotransplantation

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Background: In vascularized composite allotransplantation, face transplantation stands as a transformative intervention for patients with severe facial disfigurement. Monitoring of graft rejection, however, remains a critical challenge. This study aimed to investigate the role of lymphocyte subsets in the early detection and monitoring of graft rejection in face transplantation.

Methods: We conducted a retrospective chart review of 3 face transplant recipients who underwent face transplantation at our institution. Peripheral blood samples were analyzed for lymphocyte subsets at multiple time points posttransplantation. A linear mixed-effects model was used, aiming to identify any upregulation associated with episodes of graft rejection.

Results: A statistically significant relationship was found between clinically treated episodes of rejection, ultimately confirmed by histology, and several lymphocytic subsets. $CD3^+$ and $CD3^+CD4^+$ cell lineages were found to be significantly upregulated during times of rejection ($P = 0.0147$ and $P = 0.0153$, respectively). Furthermore, $CD3^+CD8^+$ and $CD16^+CD56^+$ cell lineages were also found to be significantly associated with rejection ($P = 0.0490$ and $P = 0.0019$, respectively). Further stratification with tacrolimus as a fixed effect demonstrated that $CD3^+$, $CD3^+CD4^+$, and $CD15^+CD56^+$ cell lineages remained significantly associated with rejection ($P = 0.0167$, $P = 0.0223$, and $P = 0.0015$, respectively).

Conclusions: Our study demonstrates that monitoring specific lymphocyte subsets offers a promising adjunct for graft surveillance that is less invasive when compared with traditionally used punch biopsies. This approach not only enhances the precision of rejection monitoring but also improves patient comfort and compliance, thereby contributing to better long-term graft outcomes. *(Plast Reconstr Surg Glob Open 2025;13:e6598; doi: 10.1097/GOX.0000000000006598; Published online 6 March 2025.)*

INTRODUCTION

Vascularized composite allotransplantation (VCA) tops the reconstructive ladder and offers patients with severe disfigurement a reconstructive option with the potential of restoring both form and function. The technical feasibility of VCA procedures has been repeatedly demonstrated; however, challenges remain with the short- and long-term management of allograft rejection as well as immunologic monitoring of transplant recipients.[1]

Understanding graft rejection in VCA recipients has been evolving with the expansion of the human recipient pool as well as advancement in translational models focused on understanding immunologic processes responsible for rejection. Acute rejection is often characterized by clinical signs of inflammation and lymphocytic infiltration of the allograft, and is graded by the Banff criteria. Most recently, the 2022 Banff VCA meeting demonstrated the need for an update to the Banff criteria with a discussion on the necessary inclusion criteria for the diagnosis and grading of VCA rejection.[2] The mechanisms involved in chronic VCA rejection are more ambiguous, and recent

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research has demonstrated that chronic graft changes may be due to repeated acute insults that ultimately lead to irreversible damage, increasing the risk of graft dysfunction and failure.[3] Resultantly, there has been increasing research interest focusing on various modalities that may provide physicians with useful clinical tools to allow real-time graft monitoring with the goal of preventing allograft insult while improving long-term graft function and survival.[4–6]

Banff grading of VCA skin biopsies demonstrating inflammatory infiltrates is currently the standard for diagnosis and grading of rejection episodes. The Banff criteria, however, are not capable of being the "gold standard" considering the discordance that is often seen between histological findings and clinical presentation as well as the invasive need to analyze skin samples that are frequently from visible areas of transplanted tissue.[7] Importantly, monitoring of allograft tissue is also taken from allograft skin and does not consider mucosal changes, which may in fact demonstrate differing degrees of rejection at the time of presentation. Furthermore, the development of noninvasive biomarkers for graft rejection has thus far been limited by issues of efficacy, efficiency, and lack of widespread access. To date, no clinical study has yet to analyze the relationship between a state of acute rejection and a patient's concurrent lymphocytic subset distribution at the time of presentation. Elucidating possible trends of blood-borne markers in relation to acute rejection episodes may provide clinical decision support to VCA providers by providing a readily available and cost-effective blood test.

Takeaways

Question: The study aimed to identify whether lymphocytic subset distribution can serve as a reliable noninvasive marker to diagnose acute rejection in vascularized composite allotransplantation (VCA) recipients.

Findings: A retrospective review of VCA recipients showed that upregulation of $CD3^+$, $CD3^+CD4^+$, and $CD16^+CD56^+$ lymphocyte subsets was significantly associated with acute rejection episodes, even after adjusting for tacrolimus levels.

Meaning: Lymphocytic subset distribution may be a useful adjunct for diagnosing acute VCA rejection, potentially reducing the need for invasive biopsies.

METHODS

A retrospective review was conducted of 3 patients, 2 of whom received facial VCAs and 1 who received a facial VCA and bilateral hand VCAs at NYU Langone Health, New York, between August 2015 and June 2023 (IRB No. i19-00621). Record review was performed for encounters where data on lymphocytic subsets were collected. Data extracted from each encounter included patient demographics, lymphocytic subsets ($CD3^+$, $CD3^+CD4^+$, $CD3^+CD8^+$, $CD19^+$, and $CD16^+CD56^+$ absolute counts; CD3%, $CD3^+CD4^+$%, $CD3^+CD8^+$%, $CD19^+$%, and $CD16^+CD56^+$%; $CD4^+/CD8^+$ ratio), immunosuppressive regimen, current medications, Banff grading of available skin biopsies, and if there was presence of clinically suspected or confirmed infection. Skin biopsies were on allograft tissue either on the neck or on the forearm and were taken either for routine monitoring or due to suspicion of acute rejection. Routine monitoring varies, but patients typically present for follow-up every 3–6 months. Lymphocyte subsets were collected and analyzed via standard flow cytometry in either an inpatient or outpatient laboratory setting. Additionally, the percentage of a cell lineage (eg, CD3%) refers to the proportion of a given cell type found in each blood sample. All encounters with suspected or confirmed infection were excluded. Clinical rejection was defined as Banff ≥ 2 + clinical signs of rejection (eg, facial edema, erythema, and ulceration).[6] Diagnosis of allograft rejection was in accordance with the previously published technique, with punch biopsies of allograft skin being obtained during episodes of suspected rejection as well as those performed during protocol-scheduled surveillance. During episodes of rejection, lymphocytic samples were taken at the time of presentation, daily during admission, and before discharge. Following the processing of tissue, histological grading was performed according to the Banff 2007 classification of skin containing VCA, and all tissue was evaluated by an independent dermatopathologist. The 2007 Banff criteria for vascularized composite allografts primarily focus on cellular rejection with a grading system based on histopathologic features such as lymphocytic infiltration and epidermal involvement. In contrast, the 2022 Banff criteria plan to expand and include a greater emphasis on antibody-mediated rejection as well as touch on specific recommendations on grading vascular involvement to improve diagnostic accuracy and patient monitoring. The 2007 guidelines were used throughout this study, as longitudinal monitoring for our patient cohort was processed before the release of the updated guidelines.

Immunosuppression

Induction immunosuppression consisted of corticosteroid taper, antithymocyte globulin, rituximab, and an anti-CD19 monoclonal antibody. The use of our B cell–depleting protocol has demonstrated robust efficacy in suppressing postoperative donor-specific antibody formation with the goal of limiting antibody-mediated rejection. Maintenance immunosuppression for all patients was similar and consisted of corticosteroids, tacrolimus, and mycophenolate with dosing adjustments made for clinical reasons (eg, renal function or neutropenia). Our induction and early maintenance protocol has reduced the early risk and incidence of acute rejection episodes without an increase in infectious events.[8] Standard infectious prophylaxis for transplant recipients was used. Acute rejection episodes were typically treated with the following modalities: pulsed steroid therapy; adjustment of maintenance immunosuppression; and in 1 patient, plasmapheresis. Plasmapheresis was uncommon, occurring only once in our cohort, and was used to combat rejection for a transplant recipient with elevated antibody levels at the time of presentation.

Table 1. Patient Demographics and Characteristics

	Patient 1	Patient 2	Patient 3
Date, y	2015	2018	2020
Recipient			
Age	41	25	21
Sex	M	M	M
Initial injury	Total scalp and full facial burn while working as a firefighter, 2001	Self-inflicted GSW to the face, 2016	80% total body surface area burn including the face, neck, trunk, and upper and lower extremities
Extent of facial injury	Scalp, forehead, eyelids, nose, cheeks, lower face, ears, lips, and neck	Eyelids, nose, cheek, lips, maxilla, mandible, zygoma, and right orbital floor	Eyelid, ear, nose, lip, and neck
Previous reconstructive procedures (no.)	>70	>10	>30
Procedure			
Graft type	Full face	Partial face	Full face and bilateral hands
Bones included in allograft	Nasal, genial, and orbitozygomatic skeletal segments	Maxilla, zygoma, and mandible	Calvaria, nasal, mandible, bilateral zygoma, distal radius, and ulna
Ischemia time	3 h 15 min	4 h 35 min	2 h 52 min
Total operative time	25 h 41 min	25 h	23 h 3 min
Immunosuppression			
Induction	MP/ATG/RT	MP/ATG/RT	MP/ATG/RT
Maintenance	TAC/MM/P	TAC/MM/P	TAC/MM/P
Sensitization			
PRA	—	—	94%
HLA mismatch	2 A, 2 B, 2 DR	1 A, 1 B, 2 DR	1 A, 2 C, 1 DR, 1 DQ, 2 DPB1
Pretransplant DSA	<2000	<2000	<2000
Outcomes			
Follow-up (mo)	93.83	24.29	34.45
Allograft loss	No	No	No
First rejection (POD)	537	1466	282
Mortality	No	No	No

ATG, antithymocyte globulin rabbit; DSA, donor specific antibody; GSW, gunshot wound; HLA, human leukocyte antigen; MM, mycophenolate mofetil; MP, methylprednisolone; P, prednisone; POD, postoperative day; PRA, panel reactive antibody; RT, rituximab; TAC, tacrolimus.

Statistical Analysis

Relevant markers were determined, and mean values as well as SDs were calculated and stratified based on clinical rejection status. To fully investigate the relationship between laboratory values and clinical rejection (Banff grade ≥ 2 + clinical signs of rejection), linear mixed-effects (LME) models were used fit to the relevant data. Utilization of LME models allowed control of the non-independent nature of the data, as all data were collected from the same subjects longitudinally.[6] Furthermore, the use of the LME model allows us to process and analyze the relatively small sample size used in this study in a statistically powerful manner. Importantly, tacrolimus has been documented as being a known confounder of immunologically active cells within the bloodstream and, as a result, required special consideration due to its potential impact on all relevant variables.[9] Bayesian information criterion (BIC) was applied to all models to compare fit among models with and without the inclusion of tacrolimus as a fixed effect. When comparing different LME models, a lower BIC demonstrates a better fit of the model with the included data. Tacrolimus 12-hour trough levels, collected during chart review, were included in the various models as a fixed effect to control for the confounding effect it has on lymphocytic cell lineages. We expect tacrolimus to have a consistent pharmacological effect across all subjects in the study and, therefore, treating it as a fixed effect allowed us to consistently and systemically assess its impact across the different subjects in this study. All statistical tests were conducted to a significance level of P equal to 0.05 and were 2-sided. All statistics were conducted in R version 4.3.1.

RESULTS

Characteristics

All patients were men and White, with ages ranging from 21 to 45 years. The mechanism of initial injury was thermal burn in 2 patients and ballistic trauma in 1 patient. Follow-up was an average of 50.85 months. No patients have experienced allograft loss or mortality during the review period of this study. Further demographics and patient characteristics can be found in Table 1, including patient-specific immunogenic factors. Mean values of hematologic variables, stratified by clinical rejection status, can be found in Table 2. Figure 1 depicts mean values of hematologic variables, and associated SDs, in a standard box plot.

Lymphocyte Subsets

$CD3^+$, $CD3^+CD4^+$, $CD3^+CD8^+$, $CD19^+$, and $CD16^+CD56^+$ absolute counts; CD3%, $CD3^+CD4^+$%, $CD3^+CD8^+$%, $CD19^+$%, and $CD16^+CD56^+$%; and $CD4^+/CD8^+$ ratio were analyzed and introduced into several LME models. Results stratified against clinical rejection status are

Table 2. Summary of Lymphocytic Sublineages

	Clinical Rejection	
	No (n = 196)	Yes (n = 19)
$CD3^+$ Abs count	345.0 ± 281.9	482.6 ± 281.2
$CD3^+CD4^+$ Abs count	96.3 ± 107.9	157.8 ± 109.0
$CD3^+CD8^+$ Abs count	194.0 ± 192.4	292.6 ± 179.2
$CD19^+$ Abs count	49.2 ± 132.6	81.3 ± 99.0
$CD16^+CD56^+$ Abs count	55.1 ± 48.1	74.1 ± 64.2
CD3%	72.3 ± 21.5	74.3 ± 14.0
$CD3^+CD8^+$%	47.1 ± 18.8	48.6 ± 15.5
$CD3^+CD4^+$%	20.5 ± 10.6	22.5 ± 5.6
$CD19^+$%	8.8 ± 17.9	11.7 ± 9.0
$CD16^+CD56^+$%	13.7 ± 9.3	12.4 ± 7.8
CD4/CD8 ratio	0.5 ± 0.3	0.5 ± 0.3

Values presented as means with SDs unless otherwise specified.
Abs, absolute.

presented in Table 3. Results adjusted with trough tacrolimus blood concentration as a fixed effect are presented in Table 4.

A statistically significant relationship was found between clinically identified episodes of rejection, ultimately confirmed by histology, and several lymphocytic subsets. $CD3^+$, $CD3^+CD4^+$, $CD3^+CD8^+$, and $CD16^+CD56^+$ cell lineages were found to be significantly upregulated during times of rejection ($P = 0.0113$, $P = 0.0058$, $P = 0.0490$, and $P = 0.0019$, respectively). Further stratification by tacrolimus concentration demonstrated that clinical rejection was still significantly associated with elevated levels of $CD3^+$, $CD3^+CD4^+$, and $CD16^+CD56^+$ cell lineages ($P = 0.0167$, $P = 0.0223$, and $P = 0.0015$, respectively). $CD3^+CD8^+$ cells were no longer significantly associated with rejection when adjusted for tacrolimus levels ($P = 0.0533$). Following stratification, analysis revealed that $CD3^+$, $CD3^+CD4^+$, and $CD16^+CD56^+$ cell lineages increased by 158.52, 56.19, and 38.40, respectively, during times of allograft rejection. Furthermore, BIC was on average lower in models that included tacrolimus as a fixed effect (Tables 3, 4).

DISCUSSION

Although the technical feasibility of VCA has been demonstrated with the clinical success of more than 140 transplants worldwide, successful immunologic management both in the acute postsurgical period and longitudinally remains a challenge.[10,11] Currently, biopsy grading by the Banff 2007 criteria and clinical exam remain the gold standard for the diagnosis of VCA rejection.[7] Of note, the Banff criteria were recently updated to include vascular inflammation scoring.[2] The invasive nature of skin sampling as well as lag time for specimen processing, however, act as barriers to safe, effective, and timely diagnosis of allograft rejection. Furthermore, recent research has called into question the validity of using tools such as the Banff criteria, particularly for the diagnosis of low-level VCA rejection.[12,13] As a result, recent VCA research has focused on identifying real-time biomarkers that could aid clinician judgment. A review of current monitoring techniques for VCA rejection highlights the advantages of noninvasive methods. Studies have demonstrated that real-time, in vivo imaging can effectively correlate histopathologic findings with clinical presentation and thereby potentially reduce the need for invasive skin biopsies. Additionally, recent studies demonstrating the integration of molecular diagnostics and blood-based biomarkers, alongside traditional monitoring methods, underscore the importance of early detection and continuous monitoring to manage acute rejection effectively.[14,15] Issues surrounding specificity, cost efficacy, and access, however, currently limit the clinical applicability of these molecular markers, novel or otherwise.[4]

Lymphocytes, and specifically T lymphocytes, and their role in the acute rejection of solid organ transplants (SOT) as well as vascularized composite allografts have been widely studied and are regarded to have largely conserved mechanisms between both cohorts.[16,17] Furthermore, rejection management in VCA recipients has a historical basis in the immunologic management of SOT recipients. Although the early immunologic VCA

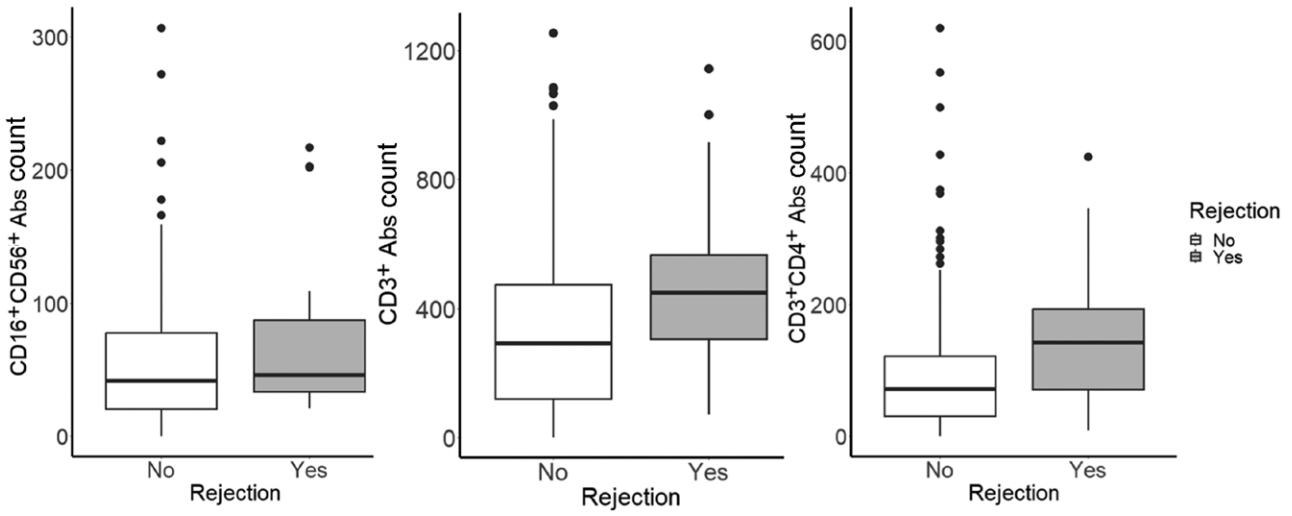

Fig. 1. The mean values of lymphocyte sublineages that remained significant following stratification with tacrolimus as a mixed effect.

Table 3. LME Model Without Inclusion of Tacrolimus as a Fixed Effect

Subset	Estimate	Std. Error	BIC	*t*	*P*
$CD3^+$ Abs count	163.22	64.40	2986.403	2.53	0.0113*
$CD3^+CD4^+$ Abs count	66.14	23.96	2574.108	2.76	0.0058*
$CD3^+CD8^+$ Abs count	88.15	44.77	2832.424	1.97	0.0490*
$CD19^+$ Abs count	55.44	31.11	2680.892	1.77	0.0769
$CD16^+CD56^+$ Abs count	36.24	11.65	2261.386	3.11	0.0019*
$CD3^+$%	0.42	5.15	1921.355	0.08	0.9351
$CD3^+CD8^+$%	1.24	4.56	1866.655	0.27	0.7861
$CD3^+CD4^+$%	−1.02	2.30	1574.328	−0.44	0.6591
$CD19^+$%	2.86	4.17	1841.546	0.69	0.4928
$CD16^+CD56^+$%	0.92	2.25	1564.368	0.41	0.6825
CD4/CD8 ratio	−0.09	0.10	239.8751	−0.95	0.3398

*$P<0.05$.
Abs, absolute; Std. Error, standard error.

Table 4. LME Model With Inclusion of Tacrolimus as a Fixed Effect

Subset	Estimate	Std. Error	BIC	*t*	*P*
$CD3^+$ Abs count	158.52	66.24	2864.332	2.39	0.0167*
$CD3^+CD4^+$ Abs count	56.19	24.58	2468.541	2.29	0.0223*
$CD3^+CD8^+$ Abs count	89.02	46.07	2717.644	1.93	0.0533
$CD19^+$ Abs count	56.75	32.51	2575.725	1.75	0.0809
$CD16^+CD56^+$ Abs count	38.40	12.11	2174.56	3.17	0.0015*
$CD3^+$%	1.03	5.19	1842.474	0.20	0.8431
$CD3^+CD8^+$%	2.22	4.66	1791.658	0.48	0.6346
$CD3^+CD4^+$%	−1.78	2.40	1517.044	−0.07	0.4581
$CD19^+$%	2.36	4.31	1771.335	0.55	0.5837
$CD16^+CD56^+$%	1.67	2.26	1493.404	0.74	0.4612
CD4/CD8 ratio	−0.12	0.10	239.2461	−1.15	0.2513

*$P<0.05$.
Abs, absolute; Std. Error, standard error.

outcomes are acceptable, the incidence of rejection and long-term graft and patient survival must be a focus to improve outcomes, including extending allograft aesthetics and function.[18,19] This is demonstrated by a markedly high 1-year acute rejection rate in VCA when compared with SOT.[16,20] That being said, in our cohort, we have not noticed an elevated rate of rejection within the first postsurgical year, a finding that stands in opposition to common VCA literature. There is, however, frequent discordance between clinical signs of rejection and histological findings. Allograft erythema and edema have a limited positive predictive value in predicting acute rejection episodes, as nonrejection etiologies such as rosacea, sunburn, and allergic reactions can easily yield the same visual findings. Currently, this discordance can only be confirmed by biopsy where Banff grade does not suggest acute rejection.[7,16] Some of the challenges in accurate and reliable diagnosis of allograft rejection may be due to unique rejection properties, such as T-cell clustering around key dermal landmarks in allografts, or differences in functional immune pathways as they relate to the various tissue compartments in VCA.[21,22] Our analysis identified significant upregulation in blood levels of $CD3^+$ cells, which is found in the membrane of all mature T cells. This finding is expected, as previous studies that have utilized the Banff classification system show high levels of T-cell lymphocytic infiltration into donor allografts during acute rejection episodes.[22,23] Our study confirms that this phenomenon is not only seen locally but also that the systemic response to acute rejection involves detectable proliferation of $CD3^+$ T cells. Although gross upregulation of T cells gives us valuable insights into the systemic immune response to a state of rejection, adding granularity allows us to further parse the mechanisms underlying rejection, identifies targets for future translational research, and adds to the utility of lymphocytic subsets as a marker of clinical rejection.

$CD4^+$ T helper cells have been extensively studied within the SOT literature for their role in cell-based rejection. T helper cells are key modulators of the inflammatory cascade and have been noted to mediate activation of cytotoxic T cells and play a role in lymphocytic graft infiltration and orchestration of acute VCA rejection through expression of locoregional inflammatory cytokines such as IFNγ.[24,25] Furthermore, T helper cells will often differentiate into various T-cell subsets, such as TH1, TH2, TH17, and T regulatory cells, all with the ultimate effect of propagating and supporting transplant rejection.[16,20,26] Our analysis demonstrates that systemic levels of $CD3^+CD4^+$ T helper cells were significantly upregulated during times of graft rejection in our VCA recipient cohort. This finding is consistent with previous immunohistochemical studies that have identified $CD4^+$ cells as being the predominant T cell infiltrating the mucosa, dermis, and epidermis of allografts, during rejection.[19,27] Locally, these cells have been shown to form perivascular cuffs that ultimately lead to chronic allograft changes, such as epidermal

dyskeratosis.[28] Although these histopathologic studies are important for identifying longitudinal variables that affect graft performance, the presence of $CD4^+$ T helper cells in the systemic circulation during acute rejection further supplants the role that these cells play during VCA rejection. Additionally, both immunohistochemical analysis and experimental models have highlighted the importance of $CD8^+$ T cells and their role in carrying out cell-based immune rejection in VCA patients.[29] We noted that this trend also occurred in our patients, with $CD8^+$ cells being significantly associated with rejection. However, following stratification by tacrolimus level, the relationship between $CD8^+$ cells was no longer statistically significant. Recent research has demonstrated a higher baseline expression of $CD8^+$ T cells in both hand and kidney transplant recipients, which not only points toward conserved mechanisms of rejection across the 2 cohorts but also may explain why we do not see a significant increase in this sublineage during acute rejection.[30] Cytotoxic T cell–mediated rejection, in these specific scenarios, could be related to a disruption in the equilibrium of regulatory immune markers rather than a gross change in cell quantity in the systemic circulation.[31] Further research will be required to delineate the exact role these lymphocytes play, both locally and systemically, in VCA rejection.

The role of natural killer (NK) cells during acute rejection of vascularized composite allografts is still unclear. Animal models have identified key chemotactic factors, such as CX3CL-1, that are not only upregulated during VCA rejection, but are also directly implicated in driving cells such as macrophages and NK cells into areas of inflammation.[32,33] Furthermore, research has identified resident memory T cells as having a potentiating effect on NK cells, leading to increased secretion of granzyme B from NK cells during graft rejection.[34] Interestingly, granzyme B secretion not only orchestrates cellular damage and apoptosis, but is also associated with an increase in mesenchymal stem cell populations, which can suppress NK activity.[35,36] This is further complicated by translational and immunohistochemical research that has identified $CD3^+$ T cells as being the main driver of cytotoxic activity and granzyme B production within VCA grafts during acute rejection (71% of cytotoxic events were attributed to $CD3^+$ T cells, whereas 29% were attributed to NK cell activity).[37] Taking the relatively unclear role of NK cells into account, our study consistently demonstrated that upregulation of these cells was the most closely linked with the state of rejection. This remained true with or without stratification by tacrolimus level. The reason behind this pronounced reaction is most likely multifactorial but may be due to our institutional immunosuppressive protocol. Our use of targeted B-cell agents during induction results in suppression of donor-specific antibodies, through rejection, and may limit the sequestration and infiltration of NK cells into rejecting tissue, as these cells are directly responsible for carrying antibody-mediated cytotoxicity. Furthermore, our inductive protocol may lead to a changing phenotype of chronic rejection in which we see relatively unaffected vasculature, histologically, with many cells clustering in near dermal landmarks. The discordance demonstrated by these findings highlights the need for animal and human studies focused on evaluating the role of NK cells in both acute and chronic VCA rejection.

A notable limitation of our study was not assessing the involvement of the mucosa during rejection episodes. Although standard VCA monitoring is typically done via skin biopsy, our lack of mucosal tissue assessment limited our ability to assess correlation between rejection in these tissues and systemic upregulation of lymphocyte subsets. That being said, we have not observed overt mucositis in our cohort. Taking this into account alongside issues surrounding wound healing and infection in VCA patients, the risk profile was deemed too great to justify direct mucosal analysis. Additionally, the cessation of rejection is often difficult to quantify without repeated biopsy, making the delineation of complete disease resolution difficult. Finally, due to varying clinical presentation and distribution of sample collection, making a true statistical commentary about the timing of lymphocytic peaking remained impossible. The strengths of this study, on the other hand, rely on our established institutional VCA monitoring protocol, robust longitudinal data that allowed for advanced statistical analysis, and consistent parameters around the detection and diagnosis of clinical VCA rejection. Furthermore, although we do not aim to eliminate routine surveillance biopsy from current VCA monitoring protocols, we hope these findings will allow for more accurate detection of rejection either during, or before, adverse events. By leveraging lymphocytic data, providers may also choose to initiate early rescue doses of immunosuppression while waiting for final biopsy analysis and interpretation.

CONCLUSIONS

Overall, this study indicates that quantitative lymphocyte subsets can be a useful adjunct for providers in the diagnosis of acute VCA rejection by providing an additional tool to increase or decrease clinical suspicion of rejection at the time of patient presentation. Particularly, significant upregulation in the $CD3^+$, $CD4^+$, and $CD16^+CD56^+$ cell lineages acted as strong markers for the delineation of rejection in our cohort. Further research, however, is required to delineate the exact role these immune cells play in the rejection cascade and how they fluctuate during and after treatment. Ultimately, we hope this marker can help optimize management, reduce biopsy burden, and decrease longitudinal risk incurred upon VCA recipients.

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DISCLOSURES

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DECLARATION OF HELSINKI

This article conforms to the Declaration of Helsinki.

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