Donor-Specific Human Leukocyte Antigen Antibody Formation After Allograft Glenoid Reconstruction Occurs But Does Not Impact Clinicoradiographic Outcomes

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Background: Recurrent shoulder instability is a prevalent condition, with glenoid bone loss as a common cause. Arthroscopic repair using distal tibial allografts provides long-lasting treatment by restoring glenoid surface area and presumably avoids risks of sensitization against donor human leukocyte antigen (HLA). Two case studies have challenged this assumption, suggesting that small bone allografts are able to induce host adaptive immune responses to donor HLA. The incidence of small bone allograft HLA sensitization and its effects on resorption and patient outcomes are unclear.

Purpose/Hypothesis: The purpose was to assess the rate of sensitization against donor HLA after distal tibial allograft procedures for shoulder instability due to glenoid bone loss and to find whether HLA sensitization negatively affects patient-reported and radiographic outcomes. We hypothesized that sensitized patients would have worse radiographic and self-reported outcomes compared with nonsensitized patients.

Study Design: Cohort study; Level of evidence, 3.

Methods: A total of 71 patients with a mean age of 28.85 years (range, 13.58-61.31 years) were enrolled, with 58 patients submitting sufficient pre- and postoperative blood samples for HLA antibody testing. In patients who developed HLA antibodies postoperatively, donor HLA typing was used to confirm donor-specific sensitization. Pre- and postoperative computerized tomography scans (0.9 ± 0.8 years follow-up) were used to grade resorption based on the modified Zhu resorption grade classification (ie, grade 0 = no resorption; grade 1 = less than 25% resorption; grade 2 = between 25% and 50% resorption; and grade 3 = larger than 50% resorption). The Western Ontario Shoulder Instability Index outcome scores were obtained preoperatively and at regular postoperative appointments. Resorption and outcome data were compared between sensitized and nonsensitized patients using the Fisher exact test, independent 2-tailed Student t tests, and the Wilcoxon rank-sum test to determine the effect of HLA sensitization on radiographic and patient-reported outcomes.

Results: A total of 7 (12.1%) patients with sufficient HLA samples were sensitized against donor HLA postoperatively. Sensitized patients did not have significantly higher rates of resorption (21.9% vs 14.3%, 21.9% vs 28.6%, 43.8% vs 28.6%, and 12.5% vs 28.6% for respective resorption grades 0-3; \( P = .67; \alpha = .05 \)). Self-reported outcomes were not statistically significant between sensitized and nonsensitized patients (24.9 ± 27.61 vs 40.16 ± 18.99; \( P = .37; \alpha = .05 \)) and did not differ significantly based on resorption grade (47.4 ± 0.0 vs 55.2 ± 18.8, 30.4 ± 15.8 vs 39.9 ± 20.9, 41.2 ± 0.0 vs 39.1 ± 13.1, and -24.9 ± 0 vs 24.4 ± 19.6 for resorption grades 0-3; \( P > .05; \alpha = .05 \)).

Conclusion: Sensitization against donor HLA after small bone graft allografting was not previously considered but has been brought to light as a possibility. Aside from potential complications for future organ transplants, HLA sensitization does not introduce a risk for adverse outcomes or higher grades of resorption compared with nonsensitized patients after small bone allografting for shoulder instability.

Keywords: human leukocyte antigen; arthroscopic anatomic glenoid reconstruction; distal tibial allografts; patient-reported outcomes; graft resorption

Recurrent shoulder instability is a prevalent condition, particularly affecting young and athletic populations, with traumatic injury and dislocation as well as baseline glenohumeral morphology acting as risk factors for instability.
and recurrence.\textsuperscript{8,9,10,19} Unsurprisingly, traumatic glenohumeral defects and bone loss are associated with a significant increase in recurrent dislocation and thus mandate the use of procedures capable of restoring or augmenting glenohumeral morphology to improve joint stability and reduce recurrence risk.\textsuperscript{2} A recent method developed by Wong and Urquhart\textsuperscript{8} provides an arthroscopic technique to restore glenoid surface area using distal tibial allograft with excellent short-term outcomes.\textsuperscript{4} However, postoperative allograft resorption and nonunion remain predictors of poor outcomes.\textsuperscript{1} A recent case study published by Liwski et al\textsuperscript{5} has suggested sensitization and antibody formation against donor human leukocyte antigens (HLAs) as a possible mechanism of graft resorption after distal tibial allografting and consequently a potential predictor of recurrent glenohumeral instability.

Osteochondral allografting for the reconstruction of bone defects has been shown to induce antibodies directed against donor HLA, albeit in the context of massive allografts for reconstruction of lesions arising as a result of degenerative disorders and neoplasms.\textsuperscript{15} While further studies have shown immunological response after large bone allografting,\textsuperscript{16,17} small bone allografts, such as those currently used in glenoid anatomic reconstruction, are thought to be immunologically inert. This is due to implementation of freeze-drying and washing procedures to reduce the overall immunogenicity of small bone allografts by removing antigenic material.\textsuperscript{5,6,7,12,14,16,17} However, 2 recent case studies have provided evidence of development of donor-specific HLA antibodies after small bone allografting procedures.\textsuperscript{5,7} Both cases highlight the potential risks of HLA sensitization after elective surgery, particularly in the case presented by O’Sullivan et al\textsuperscript{8} regarding a patient who was placed on a deceased donor waitlist for renal transplant after the sensitization event. Importantly, the case study presented by Liwski et al\textsuperscript{5} suggests a possible relationship between postoperative sensitization against donor HLA and resorption. Our study investigated the incidence of HLA antibody formation in a cohort of 71 patients undergoing arthroscopic glenoid reconstruction using distal tibial allografts for the treatment of shoulder instability. Also, our study examined the association of HLA antibody formation with the degree of graft resorption and postoperative patient outcomes to expand upon the findings of these 2 case studies\textsuperscript{5,7} and their possible implications for highly effective allografting procedures. We hypothesized that patients who were sensitized against donor HLA postoperatively would experience higher rates of graft resorption and poor self-reported outcomes compared with nonsensitized patients.

METHODS

Study Design and Data Collection

A retrospective chart review of the consecutive patients undergoing arthroscopic anatomic glenoid reconstruction with distal tibial allografts performed at the Halifax Infirmary between November 2013 and December 2018 was conducted (surgical images are depicted in Figure 1). This technique was originally developed by Wong and Urquhart,\textsuperscript{18} and in February 2016, this procedure was slightly modified upon receiving a new irrigator at our institution and the introduction of a secondary allograft washing step for all surgical procedures from this point onward.

This study was approved by the Nova Scotia Health Authority Research Ethics Board. Pre- and postoperative computerized tomography (CT) scans and radiographs (0.9 ± 0.8 years follow-up), the Western Ontario Shoulder Instability Index (WOSI) outcome scores, HLA antibody testing, and patient pregnancy and transplant histories were collected, along with basic patient characteristics (age at surgery and patient sex) and donor identification cards, when available. Patients were required to have a minimum of 6-month follow-up data to be included in analyses of self-reported outcomes. Patients were excluded if postoperative HLA antibody test results were unavailable, if both pre- and postoperative HLA antibody test results were unavailable, if preoperative HLA antibody testing was unavailable with no donor HLA typing for correlation, or if donor HLA typing was unavailable to confirm possible postoperative sensitization event in the context of preoperative HLA antibodies. Additionally, patients were excluded from the analysis involving outcome data if their preoperative WOSI scores were <25 to ensure that baseline self-reported status was not overly inflated.

HLA Antibody Testing and HLA Typing

Blood samples were requested at scheduled preoperative appointments. HLA antibody testing was performed using the LABScreen single antigen bead assay kits according to the manufacturer’s instructions (One Lambda). Postoperative samples were requested 3 months after the procedure for postoperative HLA antibody testing. Postoperative blood samples from patients who developed new HLA antibodies were used for HLA typing. Recipient and donor HLA typing was performed using the LABType RSSO kits according to the manufacturer’s instructions (One Lambda). Pre- and postoperative antibody profiles were

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compared to determine HLA sensitization as a result of surgery, and graft donor HLA typing was used to determine donor specificity when available. In cases where preoperative antibodies were detected (due to pregnancy or previous transplant), donor HLA typing was used to determine the donor specificity of postoperative antibodies.

Donor Graft Resorption Measurement

Preoperative radiographs and CT scans taken at least 1 week before surgery were compared with postoperative plain radiographs taken at 2-week follow-up appointments and CT scans taken between the 6-month and 1-year follow-up (mean, 0.9 ± 0.8 years) to determine the degree of graft resorption. Graft resorption was described and quantified according to the classification system outlined by Zhu et al.20 In summary, graft resorption was described as either grade 0 (screw is embedded in graft with no graft resorption), grade 1 (screw head is exposed with less than 25% graft resorption), grade 2 (partial screw shaft exposure with between 25% and 50% graft resorption), or grade 3 (full screw shaft exposure with larger than 50% graft resorption).20 Radiographs and CT scans were rated by independent blinded observers.

Statistical Analysis

Statistical significance was calculated at α = .05 and all statistical analyses were conducted using R Version 3.6.0 (CRANProject).11 The Fisher exact test was used to compare the frequency of each resorption grade between patients sensitized against HLA and those who were not sensitized to determine whether HLA sensitization was associated with higher rate of graft resorption postoperatively. Mean pre- and postoperative WOSI scores were compared with respect to each resorption grade using a 2-tailed Student t test to establish a trend between the degree of postoperative graft resorption and patient-reported outcomes. The change in pre- and postoperative WOSI scores (termed the delta WOSI score) was calculated for all patients if they had a baseline WOSI score and a postoperative WOSI score at least 6 months after surgery. The mean of these changes in WOSI scores was calculated for each resorption grade irrespective of HLA sensitization status and compared using an unpaired ANOVA and paired 2-tailed Student t tests to represent the relative improvement in patient outcomes with respect to the degree of postoperative graft resorption. Mean delta WOSI scores, irrespective of the resorption grade, were compared with respect to HLA sensitization status using an unpaired 2-tailed Student t test to compare overall changes in patient postoperative outcomes between sensitized and nonsensitized patients. Last, mean delta WOSI scores, with respect to both resorption grade and HLA sensitization status, were compared using a Wilcoxon rank-sum test (unpaired) due to the small number of patients in respective groups. This test was used to determine if the change in patient outcome associated with a particular degree of resorption was significantly different between HLA sensitized and nonsensitized patients.

RESULTS

Patient Groups and Exclusion From Analysis

Initially, a total of 71 patients were included in the study (mean age, 28.85 years [range, 13.58-61.31 years]). A total of 13 patients from the original cohort were excluded from the analysis because of unavailable postoperative HLA antibody test results (n = 8), unavailable pre- and postoperative HLA antibody testing (n = 1), unavailable preoperative HLA antibody testing with no donor HLA typing for correlation (n = 3), and unavailable donor HLA typing for confirmation of a postoperative sensitization event in the context of
preoperative HLA antibodies (n = 1). The remaining 58 patients with known sensitization status (7 sensitized and 51 nonsensitized patients) were evaluated for the frequency of postoperative HLA sensitization. The basic characteristics of the sensitized and nonsensitized populations are summarized in Table 1.

All patients with known HLA sensitization status (n = 58) were included in an assessment of the rate of postoperative sensitization in this cohort. However, patients were excluded from the analysis of resorption rate, self-reported outcome data, or both because of missing data or anomalous results as outlined above. A summary of the study design and the inclusion and exclusion of patients from downstream analyses is presented in Figure 2.

**Frequency of Postoperative HLA Sensitization Is Independent of Resorption Rate**

Of the 58 patients with known postoperative HLA sensitization status, 7 (12.07%) were found to have been sensitized against donor HLA antigens. Of the 7 HLA sensitized patients, 3 developed antibodies against both class I and II HLA, 1 developed antibodies to class I HLA only, and 3 developed antibodies to class II HLA only (Table 2).

To determine if postoperative HLA sensitization was associated with differential rates of donor graft resorption, the number of HLA sensitized and nonsensitized patients (7 and 32, respectively, with sufficient resorption data) was compared with each respective grade of donor graft resorption to determine if there was a significant difference in resorption rates between the 2 groups. Figure 3 summarizes the frequency of each resorption grade (21.9% vs 14.3%, 21.9% vs 28.6%, 43.8% vs 28.6%, 12.5% vs 28.6% for respective resorption grades 0-3) and indicates whether a patient was considered HLA sensitized or nonsensitized. A Fisher exact test was used to determine whether differences in resorption grade frequency were due to postoperative HLA sensitization. The results of the test indicated that the observed distribution was due to chance alone ($P = .67$).

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**TABLE 1**

| Sensitization Status | Total Patients | Mean Age, Years | Sex (Male) | Surgical Side (Right) | Follow-up, Months |
|----------------------|---------------|----------------|------------|-----------------------|------------------|
| Sensitized           | 7             | 25.94 ± 8.24   | 3          | 3                     | 18.86 ± 10.84    |
| Nonsensitized        | 51            | 29.25 ± 12.89  | 35         | 21                    | 13.53 ± 7.40     |

*Values are presented as mean ± SD.*
Patient Outcomes Are Independent of Donor HLA Sensitization Status

To determine the trend between postoperative graft resorption and patient-reported outcomes, delta WOSI scores were compared between patients with each degree of graft resorption. A total of 28 patients (5 sensitized and 23 nonsensitized patients) had sufficient outcomes and resorption data for comparison. Delta WOSI scores were used in place of pre- and postoperative scores to better quantify patient-reported improvement. Figure 4 summarizes these results and indicates the general descending trend in patient-reported outcomes with respect to increasingly severe grades of resorption. The results of the 1-way analysis of variance (ANOVA) showed a statistically significant difference between the 4 groups ($P = .033$). However, the results of the independent 2-tailed Student $t$ tests revealed no statistically significant differences between mean delta WOSI scores of the resorption groups (Table 3).

Last, the delta WOSI scores of sensitized patients were compared with those of nonsensitized patients of the same resorption grade to determine whether patient outcomes differed between the 2 groups of patients at different rates of resorption (5 sensitized and 23 nonsensitized patients). The results showed that patient outcomes as measured by delta WOSI scores decreased as the extent of graft resorption increased (Figure 6). The delta WOSI scores did not differ significantly based on resorption grade ($47.4 \pm 24.9$ vs $39.1 \pm 0.0$ for resorption grades 0-3). The results of a Wilcoxon rank-sum test indicated no statistically significant difference between sensitized and nonsensitized patients with equivalent grades of graft resorption ($P = .05$) (Figure 6).

DISCUSSION

This study has served to provide evidence that sensitization against donor HLA antigens is not only plausible but

| Patient  | Class I HLA          | Class II HLA          |
|----------|-----------------------|-----------------------|
| Patient 1 | Aw4/Bw4               | DR4;DR7;DR9           |
| Patient 2 | A2;A68;A69            | DR1;DR4;DR14;DR53;DR103 |
| Patient 3 | A2;A24;A68;A6         | DR7;DR8;DR9           |
| Patient 4 | C9;C10                | DR4;DR11;DR12;DR13;DR14;DR17;DR18 |
| Patient 5 | —                     | DR7;DR9;DR12;DR52;DR52 | DR7;DR9;DR12;DR52;DR52 |
| Patient 6 | —                     | DR4;DP1;DP3;DP4;DP6;DP9;DP10;DP11;DP13;DP14;DP17;DP19;DP20 |
| Patient 7 | —                     | DP4;DP11;DP15;DP18;DP28 |

*Donor HLA molecules against which sensitized patients formed antibodies. Donor HLA molecules are arranged according to their designation as either Class I (A, B, C) or Class II (DP, DQ, DR) HLA, with allele groups indicated. Aw4/Bw4 refer to antibodies generated against related immunogenic HLA epitopes of the corresponding classes. HLA, human leukocyte antigen.

Figure 3. Distribution in the frequency of resorption grades observed in 7 patients sensitized against donor human leukocyte antigen (HLA) and 32 nonsensitized patients. The observations were not found to be statistically significant based on the results of the Fisher exact test ($P = .67$).
also occurs relatively frequently: at a rate of approximately 12% in this particular group of patients. While this has implications for future transplants and procedures performed for patients requiring more immediate organ transplant, sensitization was not found to have a statistically significant effect on the degree of donor graft resorption or patient-reported outcomes as measured by the WOSI system. These findings are reassuring in that they do not implicate sensitization to donor HLA as a predictor of poor postoperative outcomes from both a clinical and patient perspective.

Our results elaborate on the findings presented by Liwski et al and O’Sullivan et al regarding postoperative sensitization to donor HLA in the context of small bone allografts. Notably, the case presented by Liwski et al showed evidence of sensitization against donor Class I and Class II HLA. Similarly, 3 of the 7 patients found to be sensitized in this study demonstrated HLA antibodies against both Class I and Class II HLA antigens, suggesting that this widespread sensitization is not uncommon when it occurs. However, we did not find any suggestion of worse clinical outcomes in those patients who were sensitized against both donor Class I and Class II HLA.

While previous studies have observed sensitization in the context of bone allografting, this is the first study to our knowledge that has assessed the relationship of postoperative sensitization to clinical and patient outcomes. While no significant association was found between donor HLA sensitization and outcomes, questions remain regarding how immunogenic materials are introduced during surgery, the effect sensitization has on subsequent allograft procedures with donors expressing cross-reactive HLA antigens, and the persistence of the HLA antibodies formed after bone allografting. Current methods of donor graft preparation are used partially to reduce the antigenicity of the allograft. Interestingly, a secondary wash procedure before introducing the graft to the patient was implemented at our institution in February 2016. Proportionally,
organ transplant, the rate of sensitization does not detract from the excellent safety and outcomes associated with this procedure for a common and potentially disabling condition.1

CONCLUSION

While sensitization against donor HLA is relatively common among patients undergoing distal tibial allografting for recurrent glenohumeral instability, it does not influence the degree of allograft resorption or postoperative clinical outcomes. The results of this retrospective case series elaborate on recent studies documenting case reports of sensitization events5,7 and suggest that such sensitization events do not adversely affect patient-reported postoperative outcomes or radiographic measures of graft resorption, despite occurring at an observed rate of 12.1% in our patient population. The effect of sensitization against donor HLA after such procedures may not be insignificant for a specific subgroup of patients who are candidates for organ transplant, but it appears to be benign for the majority of patients who undergo small bone allograft procedures. Further study and follow-up are warranted to determine if additional intraoperative tissue irrigation techniques are capable of further reducing sensitization rates associated with small bone allografts of this nature, in addition to determining any antigenic contaminants contained within such grafts that may be eliminated before use.

ACKNOWLEDGMENT

The authors acknowledge Sara Sparavalo and Ryland Murphy for assistance in preparing the study protocol, obtaining approval from the research ethics board, and collecting data and Jie Ma for assistance in manuscript submission.

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Figure 6. Delta WOSI scores of 28 patients (5 sensitized against donor HLA and 23 nonsensitized) plotted with respect to both HLA sensitization status and resorption grade. A Wilcoxon rank-sum test was performed and found no statistically significant differences between the mean delta WOSI scores of HLA sensitized or nonsensitized patients with equivalent resorption grade (P > .05). HLA, human leukocyte antigen; WOSI, Western Ontario Shoulder Instability Index.
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