Long-Term Retrospective Follow-Up of Fresh Osteochondral Allograft Transplantation for Steroid-Associated Osteonecrosis of the Femoral Condyles

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Abstract

Objective. No studies currently exist with long-term follow-up of use of osteochondral allografting (OCA) for treatment of steroid-associated osteonecrosis of femoral condyles in young, active patients who wish to avoid total knee arthroplasty (TKA). We evaluate the extent to which fresh osteochondral allografts can (1) prevent or postpone need for prosthetic arthroplasty and (2) maintain long-term clinically meaningful decrease in pain and improvement in function at mean 11-year follow-up. *Design*. Twenty-five patients (33 knees) who underwent OCA transplantation for osteonecrosis of the knee between 1984 and 2013 were evaluated, including 22 females and 11 males with average age of 25 years (range, 16-48 years). Mean total allograft surface area was 10.6 cm² (range, 4.0-19.0 cm²). Evaluation included International Knee Documentation Committee (IKDC) scores, Knee Society function (KS-F) score, and modified (for the knee) Merle d'Aubigné-Postel (18-point) score. *Results*. OCA survivorship was 90% at 5 years and 82% at 10 years. Twenty-eight of 33 knees (85%) avoided arthroplasty and 25 of 33 knees (73%) avoided other surgical intervention. Mean IKDC pain score improved (P = 0.001) from 7.2 preoperatively to 2.8 at latest follow-up, mean IKDC function score increased (P = 0.005) from 3.3 to 6.5, and mean IKDC total score improved (P = 0.001) from 31.9 to 61.1. Mean KS-F score improved (P = 0.003) from 61.7 to 87.5. Mean modified Merle d'Aubigné-Postel (18-point) score improved (P = 0.001) from 11.4 to 15.1. *Conclusions*. Our findings suggest that OCA transplantation is a reasonable surgical treatment option for steroid-associated osteonecrosis of the femoral condyles, with durable long-term outcomes.

Keywords

osteochondral allografting, steroid-associated osteonecrosis, femoral condyles, survivorship

Introduction

Secondary avascular osteonecrosis of the knee is a rare but serious side effect of systemic high-dose corticosteroid therapy, often seen in young patients following steroid treatment for autoimmune disease or primary malignancy.¹ The femoral condyles are the second most common site to be affected, after the femoral head. Steroid-induced lesions form in the subchondral bone, with eventual fracture and progression to overlying chondrosis, joint collapse, and arthritis.¹ Treatment of steroid-associated osteonecrosis remains controversial, with proposed therapeutic approaches including activity modification and surgical intervention, including arthroscopic debridement, core decompression, osteotomy, osteochondral grafting, and partial and total knee arthroplasty (TKA).²⁻⁸ Regardless of the etiology for osteonecrosis of the femoral condyles, symptomatic, highgrade (modified Ficat/Arlet stages III-IV) osteonecrotic

lesions of the distal femur generally require TKA for definitive treatment. $^{\rm 8}$

Young patients, however, are more likely to continue placing high demands on their replaced joints and are thus more likely to require future revisions of TKA procedures due to aseptic loosening and polyethylene wear, as has been demonstrated in multiple national registry studies.⁹⁻¹¹ Biological repair strategies such as osteo-chondral allograft (OCA) transplantation of the femoral

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condyles have emerged as a durable method to postpone the need for arthroplasty in young active patients.¹ By replacing both osseous and chondral components of juxta-articular necrotic lesions, OCA may provide the most durable support possible to the damaged joint short of TKA, without precluding eventual successful TKA in case of eventual failure.¹² Outcomes at median follow-up of 22 years have previously been obtained in patients with either direct trauma or osteochondritis dessicans as etiology for osteonecrosis of the knee¹³; however, patients with other etiologies such as steroid-associated osteonecrosis have not been characterized for such longterm follow-up. While earlier findings support a continued role for OCA transplantation in steroid-associated osteonecrosis of the femoral condyles, additional followup is necessary to characterize long-term graft performance and clinical outcomes.

Methods

Retrospective review of our institution's OCA registry identified 26 patients (34 knees) who underwent OCA transplantation for knee osteonecrosis between 1984 and 2013, all at least 2 years postoperative. One patient (1 knee) was deceased attributable to his underlying condition and the status of his knee was not ascertained. The remaining 25 patients (33 knees) comprise the current study population (**Table 1**), including 22 knees in females and 11 in males with average age of 25 years (range, 16-48 years) and mean body mass index of 21.8 kg/m² (range, 17.1-28.1 kg/m²). All patients provided informed consent and were entered prospectively in an institutional review board–approved clinical database.

Patients' underlying diagnoses were primarily related to an autoimmune disorder (44% of patients with underlying diagnosis of systemic lupus erythematosus, ulcerative colitis, Crohn's disease or myositis) or malignancy (32% of patients with underlying diagnosis of leukemia or Hodgkin's lymphoma), with the remainder of underlying diagnoses being less common causes to receive high-dose corticosteroid therapy—one each of sickle cell disease, closed head injury, renal transplant, transient allergies, renal infection, and heart transplant. Steroid use at the time of OCA was reported in 2 out of 11 (18%) of patients with underlying autoimmune disorder diagnosis, in 0 out of 8 (0%) with underlying malignancy, and in 1 out of 6 (17%) with other underlying diagnosis.

All patients underwent OCA transplantation for osteoarticular stage III-IV (modified Ficat/Arlet stage) lesions sustained secondary to steroid-associated osteonecrosis of the femoral condyles. History was notable for a medical diagnosis requiring prednisone use exceeding 20 mg per day, with 3 patients in the study continuing to receive corticosteroid therapy at time of surgery (**Table 1**). All patients were younger than 50 years and had symptoms that did not respond to other treatment modalities. Patients were candidates for arthroplasty but had declined due to young age and were referred to allografting as an alternative treatment option. Patients were evaluated preoperatively with 54-inch standing radiographs for limb malalignment to rule out realignment osteotomy before consideration of OCA. Meniscus status and ligament stability were normal in all patients preoperatively. Fifteen of 33 (45.5%) knees had an average of 1.5 previous surgeries (range, 1-5 surgeries), including arthroscopic debridement (10), loose body removal (5), drilling (4), bone grafting (3), bone cement packing (1), and distal femoral osteotomy (1), but never OCA transplantation. No previous surgeries had been performed in the remaining 18 knees.

Thirteen surgeries involved only the left knee, 4 involved only the right, and 16 were bilateral. Twenty-five knees had unicondylar lesions (13 lateral, 12 medial), whereas 8 knees had bicondylar involvement (medial and lateral femoral condyles in the same knee) and received allografts to both condyles. Mean total allograft surface area was 10.6 cm² (range, 4.0-19.0 cm²). Seventeen of 33 (51.5%) knees had multiple grafts; these included cases of bicondylar involvement, large lesions using dowel technique, or additional nonstructural particulate bone allografting of necrotic areas beneath the grafts. Overall, patients required an average of 1.7 osteochondral allografts per knee (range 1-4).

Standard AP radiographs, corrected for magnification, were used to measure mediolateral tibial plateau dimensions for recipients, and matched to donors by direct measurement of donor dimensions. Blood typing, tissue typing and immunosuppression were not used. Donor tissue was recovered within 24 hours of donor death, and grafts implanted between 5 and 28 days of donor death. Processing of fresh allograft tissue involved storage at 4°C in tissue culture media from a commercial tissue bank. All allografts were obtained from healthy donors, aged 15 to 40 years, and who met the criteria of the American Association of Tissue Banks.

Patients were placed in supine position for surgery under tourniquet control, using a full arthrotomy through midline incision as described previously,¹ with key considerations to technique summarized as follows: The location, size, and shape of debrided lesions determined feasibility of dowel versus shell technique for allograft placement. Particulate bone graft was used to fill any lesions requiring curettage to depth >12 mm, and a host bed of 50% or more viable bleeding bone was considered acceptable for graft placement. Copious intraoperative lavage was employed to remove debris and reduce immunogenicity of graft material. Need for supplemental screw fixation was assessed on a case-by-case basis, generally when geometry of the allograft prevented a press fit. With increased operator experience, exclusive use of shell

Table I. Patient Data.

Patient	Knee	Gender	Age (Years)	Underlying Diagnosis	Steroid Use at Time of OCA	No. of Grafts	Condyle	Total Graft Area (cm ²)	Graft Technique	Bone Grafting	Follow- up (Years)	Modified Merle d'Aubigné-Postel Score (Category) or Outcome
01	01	Female	19	Sickle cell anemia	No	I	Medial		Shell	No	3.9	Deceased
02	02	Male	17	Leukemia	No	I	Lateral	9.9	Shell	Yes	13.7 ^a	ТКА
	03	Male	17	Leukemia	No	I	Lateral	11.6	Shell	Yes	13.7 ^a	ТКА
03	04	Female	16	Leukemia	No	I	Lateral	11.8	Shell	Yes	3.8ª	Revision allograft
	05	Female	17	Leukemia	No	2	Both	11.0	Shell	No	11.4	I7 (good)
04	06	Female	25	SLE	No	2	Both	9.25	Shell	No	10.6 ^a	ТКА
05	07	Female	47	Crohn's disease	No	2	Both	17.2	Shell	No	16.1	l2 (poor)
	08	Female	48	Crohn's disease	No	I	Lateral	9.2	Shell	No	15.5	II (poor)
06	09	Male	25	SLE	No	I	Medial	17.5	Shell	Yes	22.6	14 (fair)/deceased
07	10	Male	29	Leukemia	No	2	Lateral	6.3	Plug	Yes	11.3	<pre>18 (excellent)</pre>
08	11	Female	25	SLE	Yes	I	Lateral	5.25	Shell	Yes	3.1	17 (good)/deceased
09	12	Male	32	Ulcerative colitis	No	I	Medial	7.4	Shell	Yes	1.6 ^a	Revision allograft
	13	Male	35	Ulcerative colitis	No	2	Both	9.0	Plug	Yes	8.9 ^a	ТКА
10	14	Female	19	Leukemia	No	I	Medial	10.0	Shell	No	14.9	l 6 (good)
11	15	Female	37	Closed head injury	No	I	Lateral	7.8	Shell	No	29	<pre>18 (excellent)</pre>
12	16	Female	17	Hodgkin's lymphoma	No	3	Medial	11.2	Plug	Yes	10.7	13 (fair)
	17	Female	17	Hodgkin's lymphoma	No	3	Medial	12.0	Plug	Yes	10.7	13 (fair)
13	18	Female	21	Leukemia	No	3	Both	19.0	Plug	Yes	5.3	l 6 (good)
14	19	Female	18	Renal transplant	No	I	Lateral	11.0	Shell	Yes	18.4	l 4 (fair)
15	20	Female	30	Transient allergies	No	2	Both	13.75	Shell	Yes	6.5 ^a	ТКА
16	21	Female	23	SLE	No	2	Lateral	10.5	Shell	Yes	22.6	II (poor)
17	22	Female	18	Leukemia	No	I	Lateral	5.0	Plug	No	10.5	17 (good)
	23	Female	18	Leukemia	No	I	Medial	9.7	Shell	Yes	10.5	17 (good)
18	24	Female	20	Ulcerative colitis	No	2	Medial	6.3	Plug	Yes	11.2	17 (good)
19	25	Female	18	SLE	No	3	Both	19.0	Plug	Yes	3.3ª	Revision allograft
20	26	Female	44	Renal infection	No	I	Lateral	10	Shell	No	10.1	II (poor)
21	27	Male	27	Ulcerative colitis	No	2	Medial	8.5	Plug	Yes	11.6	<pre>18 (excellent)</pre>
22	28	Male	16	Myositis	Yes	I	Lateral	12.6	Shell	No	10.7	l 6 (good)
23	29	Male	24	Leukemia	No	4	Both	16.0	Plug	Yes	2.9	l 4 (fair)
	30	Male	24	Leukemia	No	2	Medial	12.5	Plug	No	3.5	l 4 (fair)
24	31	Male	23	Heart transplant	Yes	2	Lateral	9.0	Plug	No	3.0	l 6 (good)
25	32	Female	22	Ulcerative colitis	No	I	Medial	4.0	Plug	No	3.3	17 (good)
	33	Female	22	Ulcerative colitis	No	2	Medial	5.5	Plug	No	3.3	17 (good)

OCA = osteochondral allografting; TKA = total knee arthroplasty; SLE = systemic lupus erythematosus. ^aTime to revision allograft/conversion to TKA.

technique in earlier patients eventually progressed to the technically simpler and more easily reproducible dowel technique.

Patients underwent formal physical therapy after the procedure, including supervised range-of-motion exercises and quadriceps strengthening. Patients progressed to cycling or other closed-chain exercises at one month postoperatively. Only limited (toe touch) weightbearing was permitted for the first 6 weeks, after which patients were progressed gradually to full weightbearing at 3 months if radiographs demonstrated full osseous integration of the allograft at that time. Patients were permitted to resume unrestricted physical activity at 6 months postoperatively.

Patients returned for clinical evaluation at standard follow-up intervals as described in the previous report, at 6 weeks, 3 months, 6 months, and then annually.¹ Patients who did not live locally were sent a questionnaire via mail. Further surgery after OCA transplantation was documented. Allograft failure was defined as revision OCA transplantation or conversion to arthroplasty. Pain and function were assessed preoperatively and postoperatively (for patients with the allograft remaining *in situ*) using International Knee Documentation Committee (IKDC) scores, Knee Society function (KS-F) score, and the modified Merle d'Aubigné-Postel (18-point) score. Patient satisfaction was captured at each follow-up interval using a 5-item categorical scale. The most recently available postoperative outcomes scores were analyzed for the study.



Figure 1. Kaplan Meier curve showing graft survivorship of 90% at 5 years and 82% at10 years.

Statistics

Preoperative and postoperative IKDC, KS-F, and modified Merle d'Aubigné-Postel (18-point) scores were compared using Wilcoxon signed-rank tests. Allograft survivorship was determined using Kaplan-Meier method with failure as the endpoint (revision OCA or arthroplasty conversion). SPSS version 13.0 was used for all analyses. All analyses were performed on a per-knee basis. Patient sample sizes were too small for formal consideration of t test or chisquare analysis to compare subgroup performances.

Results

Nine of 33 knees (27%) had further surgery following the OCA transplantation. Of these, 1 knee underwent 2 arthroscopic debridements and a loose body removal within 5 years after OCA, but had no further surgery after that, and is now 18 years post-OCA with graft in situ. The remaining eight knees (24% of entire cohort) underwent further surgery that involved graft removal and were classified as OCA failures (3 revision OCA transplantations and 5 conversions to TKA). All 3 patients undergoing repeat allografting had recurrent pain and radiographic evidence of allograft resorption, collapse, and fragmentation. One had bicondylar involvement and had multiple grafts revised at 40 months. The second patient underwent distal femoral varus osteotomy to correct residual valgus deformity at 26 months and subsequently underwent revision allografting of the lateral femoral condyle at 45 months from the initial allograft procedure. The third patient underwent revision allografting 1.6 years after primary OCA. The 5 TKA conversions occurred at 6.5, 8.9, 10.6, and 13.7 (2 knees) years after OCA

transplantation. Mean time to OCA failure (including OCA revisions and conversions to TKA) was 7.8 years (range 1.6-13.7 years). Graft survivorship was 90% at 5 years and 82% at 10 years (**Fig. 1**).

Among the 25 knees that had the allograft in situ, the mean follow-up duration was 11.0 years (range, 2.9-29 years). Pain and function scores decreased from early follow-up to long-term follow-up (Table 2), but all scores were statistically better at latest follow-up than preoperatively. Of the 23 knees with postoperative IKDC and KS-F scores available, mean IKDC pain score improved from 7.2 preoperatively to 2.8 at latest follow-up (P =0.001) and mean IKDC function score increased from 3.3 to 6.5 (P = 0.005). Mean KS-F score improved from 61.7 to 87.5 (P = 0.003). Mean modified Merle d'Aubigné-Postel (18-point) score improved from 11.4 to 15.1 (P <0.001); of the 22 knees that had postoperative modified Merle d'Aubigné-Postel (18-point) scores available, 12 (54%) scored 15 or greater (representing a score of "good" or "excellent"), 7 (32%) were classified as "fair," and 3 (14%) as "poor." At latest follow-up among 23 knees that had data regarding satisfaction, 10 (43%) reported being "extremely satisfied" with the results of the OCA transplantation, 8 (35%) were "satisfied," 3 (13%) were "somewhat satisfied," and 2 (9%) were "somewhat dissatisfied."

Statistical analysis (*t* test and chi-square) for subgroups to evaluate if any difference in outcomes could be found based on patients' demographics, underlying diagnosis, previous surgeries, amount and duration of steroid use, and continued steroid use were limited by the small patient sample and did not yield significant differences between any of the above patient subgroups.

Measure	Early-Term Follow-up: Median (Range) or %	Long-Term Follow-up: Median (Range) or %	P ^a
IKDC pain	-4 (-7 to 2)	-5 (-9 to 3)	0.001
IKDC function	5 (-1 to 7)	3 (-2 to 7)	0.005
IKDC total	28 (6-34)	23 (-3 to 70)	0.001
KS-F	30 (10-50)	30 (0-50)	0.003
Modified Merle d'Aubigné-Postel	5 (1-16)	4 (-4 to 7)	< 0.001
Excellent (18 points)	27.8	13.6	_
Good (15-17 points)	50.0	40.9	_
Fair (12-14 points)	11.1	31.8	
Poor (<12 points)	11.1	13.6	_
Satisfaction			
Extremely satisfied	57.9	43.5	
Satisfied	26.3	34.8	
Somewhat satisfied	15.8	13.0	
Somewhat dissatisfied	_	8.7	
Dissatisfied	—	—	_

 Table 2.
 Subjective Outcome Difference Scores (Change From Preoperative to Early- and Long-Term Follow-up) and Satisfaction

 Rates Among Patients With Grafts Remaining In Situ.

IKDC = International Knee Documentation Committee; KS-F = Knee Society function.

^aP value for comparison of scores from preoperative to long-term follow-up.

Discussion

In this study, we report a case series of OCA transplantation for steroid-associated osteonecrosis of femoral condyles with a mean follow-up of 11.0 years, the longest follow-up available in patients undergoing OCA for this indication.¹ We note an increase in the rate of new arthroplasties (15%) or other surgical intervention (27%) on affected knees in the present study compared with previous findings at mean 5.6 years' follow-up in 2010 (4% and 15%, respectively). Of the 8 knees requiring additional surgical intervention, 4 involved bicondylar lesions, 4 involved above-average necrotic area (range 11.6-19.0 vs. 10.6 cm²), and 6 were performed using shell allograft technique.

Previous study results in patients undergoing OCA for more favorable etiologies leading to osteochondral defects of the femoral condyles, such as for unipolar posttraumatic osteochondral or osteochondritis dissecans defects in the distal aspect of the femur, have demonstrated survivorship at 10 years of 91%.¹³ In comparison, patients in the current study demonstrate a graft survivorship of only 82% at 10 years. Patients in our study additionally are more likely to have large lesions requiring multiple grafts, or to require allografting of both femoral condyles. Reduced graft survivorship in this study population likely reflects the higher level of lesion complexity in patients with steroid-associated etiology, combined with underlying disease burden from patients' primary diagnoses. Comparing results of this study only to other patient populations with osteonecrosis as an included etiology for defects in the femoral condyles, outcomes compare favorably to meta-analyses on OCA transplantation in the broader population.¹⁴ While outcomes for patients with steroid-associated osteonecrosis of the femoral condyles are not as durable as for patients of a similar age with more benign etiologies; however, the overall graft survivorship is still helpful at preventing progression to TKA in a strong majority of patients.¹³

The second major endpoint for this case series was to evaluate the impact of OCA for long-term maintenance of a clinically meaningful decrease in pain and improvement in function. We noted a persistently high level of satisfaction with the procedure among patients, even among those whose pain and function scores have declined compared with previously reported interval follow-up at median 5.6 years.1 Compared with previous results, clinical performance of surviving allografts has declined at latest followup-of the 19 patients who had not required surgical intervention either in our original case series publication or in latest follow-up, 10 demonstrated a decline in the modified Merle-d'Aubigné-Postel (18-point) scale score compared to prior follow-up and 9 patients scores remained unchanged or improved. Nonetheless, all patient scores remained improved compared with preoperative scores. Postoperative IKDC pain and function scores were slightly lower in the latest follow-up compared to our previous interval report, while remaining significantly above preoperative scores. KS-F scores do not differ significantly from previous interval follow-up.

Clinical outcomes in our study continue to outperform previous results by Bayne *et al.*,⁶ where 4 out of 4 patients undergoing OCA for steroid-associated osteonecrosis had poor outcomes, and this difference in results may be due to low frequency of continued steroid use in our patient population postoperatively. These results confirm the utility of fresh OCA transplantation as a treatment option for steroid induced osteonecrosis of the knee. This longer term follow-up period demonstrated further failures and decrease in clinical outcome scores in some patients. Steroid-associated osteonecrosis is a multifocal disease process¹⁵⁻¹⁷ and at the time of OCA transplantation, only lesions impacting the chondral surface were treated. Continued degeneration from untreated lesions covered by healthy cartilage may have contributed to decline in graft performance over time.¹⁸ Collectively, these findings support that OCA transplantation continues to provide a long-term advantage compared with preoperative function and can contribute to postponing the need for future arthroplasty or other surgical intervention.

Our study has several limitations, including small study population, lack of long-term radiographic data, and lack of validation for one of the scoring instruments (modified Merle d'Aubigné-Postel scale) in the knee. Radiographic outcomes might improve prognostication for continued OCA performance, including evidence of impending failure, which cannot be assessed clinically. The majority of procedures were performed before the current explosion in the literature on improved OCA transplant indications and techniques, in particular during a time when shell grafts were more commonly indicated. Based on relative ease of alignment and securing tight fit with dowel technique, our preference for all OCA patients is currently for the dowel technique where possible. Given the small patient population, however, we cannot definitively identify these criteria as risk factors for failure. In particular when considering the effect of shell vs. dowel technique on need for additional surgical intervention, we note that the average follow-up time for shell technique grafts is longer than that for dowel technique grafts, and when corrected for years at risk, any differences in risk of additional surgery based on graft technique disappear. Patients undergoing OCA today may experience even greater benefit in ability to postpone TKA with broader dissemination of successful techniques, although longer term follow-up in knees receiving the shell technique would be necessary to confirm this.¹⁹⁻ ²¹ Our study does not address performance of OCA for steroid-associated osteonecrosis of femoral condyles in pediatric or adolescent populations, which account for a substantial portion of candidate patients.²² To fully assess long-term performance of OCA in our patient population, mean age 25

years, compared with performance of TKA, we would ideally be able to compare outcomes with those of similarlyaged patients undergoing TKA. Indications for TKA in such a young patient population are few, however, and to our knowledge, the long-term outcomes for TKA in such a young population have not been reported.^{1,2}

The small patient population in our study is further complicated by the heterogeneity of primary diagnoses contributing to primary steroid use, leading to many small patient subgroups that are even more difficult to analyze and compare against each other. Of particular interest for further analysis would be the possible impact of underlying diagnosis on long-term outcomes following fresh OCA transplantation, as this may shed light on the underlying mechanisms of response to OCA transplantation. While all patients in this study had a primary diagnosis requiring use of high-dose steroids, in 12 out of 25 cases (48%) with either autoimmune or sickle cell disease, the primary diagnosis itself is associated with an increased risk of joint disease, either through chronic joint inflammation or increased vaso-occlusive risk respectively. Although we did not find a difference in long-term outcomes between patients based on primary diagnosis, this would be highly valuable to investigate in the future with a larger patient population. Concerning patients with underlying diagnosis of malignancy, we hesitate to generalize our findings beyond the limited range of diagnoses included here, namely leukemia. The high representation of leukemia within our sample we believe reflects primarily on the relatively common and survivable nature of this disease compared with other malignancies in the younger patient population, leading to increased manifestations of steroid-associated complications in the following decades of life.

This is the largest reported series of OCA for steroidassociated osteonecrosis of the femoral condyles. Patients undergoing the procedure were younger than 50 years, presented with symptoms that did not respond to other treatment modalities, and were seeking an alternative treatment to TKA. Our findings support that fresh OCA transplantation can produce excellent long-term results in this patient population. Overall survivorship, clinical scores and satisfaction continued to be excellent at longterm follow-up. We conclude that for patients with a history of high-dose steroid use and osteonecrosis of the femoral condyles, fresh OCA transplantation can be a durable long-term alternative to TKA in a majority of patients, with similar performance to OCA treatment for other etiologies of osteonecrosis. Longer term follow-up and larger numbers of patients will be necessary to further characterize procedure indications and outcomes, and to determine the relative impact of other patient characteristics such as demographics, duration or amount of steroid use, and primary diagnosis to require steroid use.

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Declaration of Conflicting Interests

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Ethical Approval

Ethical approval for this study was obtained from the Scripps Institutional Review Board.

Informed Consent

Written informed consent was obtained from all subjects before the study.

Trial Registration

Not applicable.

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