Evaluating Joint Morbidity After Chondral Harvest for Autologous Chondrocyte Implantation (ACI): a Study of ACI Treated Ankles and Hips with a Knee Chondral Harvest.

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Abstract

Objective: To establish if harvesting cartilage to source chondrocytes for autologous chondrocyte implantation (ACI) results in donor-site morbidity.

Design: Twenty-three patients underwent ACI for chondral defects of either the ankle or the hip. This involved cartilage harvest from the knee (stage I), chondrocyte expansion in the laboratory and implantation surgery (stage II) into the affected joint. Prior to chondral harvest, no patient had sought treatment for their knee. Lysholm knee scores were completed prior to chondral harvest and annually post-ACI. Histological analyses of the donor site were performed at 12.3±1.5 months for 3 additional patients who had previously had ACI of the knee.

Results: The median pre-operative Lysholm score was 100, with no significant differences observed at either 13.7±1.7 months or 4.8±1.8 years post-harvest (median Lysholm scores 91.7 and 87.5, respectively). Patients whose cartilage was harvested from the central or medial trochlea had a significantly higher median Lysholm score at latest follow-up (97.9 and 93.4 respectively), compared with those taken from the intercondylar notch (median Lysholm score 66.7). The mean ICRS II histological score for the biopsies taken from the donor site of three additional knee ACI patients was 117+/-10 (maximum score 140).

Conclusions: This study suggests that the chondral harvest site in ACI is not associated with significant joint morbidity, at least up to 5 years post-harvest. However, one should carefully consider the location for chondral harvest as this has been shown to affect knee function in the longer term.

Keywords: Autologous Chondrocyte Implantation; Cartilage repair; donor-site morbidity; osteoarthritis; histology

Introduction

Chondral and osteochondral lesions in the hyaline cartilage of an articulating joint often require surgical intervention due to the limited ability of cartilage self-repair. Many techniques to restore articular cartilage and joint function have been established over the years, including mosaicplasty, osteochondral autograft transport system (OATS) and autologous chondrocyte implantation (ACI). Mosaicplasty and OATS involve obtaining cylindrical osteochondral grafts of varying sizes from the lesser loaded areas of the articular surface of the donor joint and implanting them into the recipient osteochondral lesion. Traditionally, mosaicplasty requires multiple small osteochondral grafts (typically 3-8mm)[1-3] whereas OATS usually requires one single large defect-sized graft (typically ~10mm)[4] and either procedure can be performed in a single operation. ACI on the other hand is a two-stage procedure, requiring a chondral harvest of macroscopically healthy cartilage, also from a lesser loaded area of the joint (stage I) and subsequent chondrocyte isolation, culture and proliferation. The resulting cells are then implanted into the defect (stage II) approximately 3-4 weeks later,[5] beneath either a periosteal or collagen membrane such as ChondroGide[®].

Originally developed to treat chondral lesions in the knee,[5] ACI has since been adapted for the treatment of chondral defects in other joints such as the ankle[6-9] and less commonly, the hip.[10-13] Whilst encouraging results in terms of cartilage growth and improvement of joint function in the treated joint have been reported, little has been published on the potential morbidity caused by harvesting healthy tissue from the joint in ACI. What has been described regarding donor-site morbidity in the literature to date predominantly relates to osteochondral harvest for mosaicplasty[3] or OATS[4,14] which has obvious differences in potential issues for donor-site morbidity than solely a chondral harvest due to the depth and size of the harvest taken. Donor-site morbidity is an understandable concern as the integrity of the healthy, intact hyaline cartilage is violated.

In this study we aimed to assess the effect of chondral harvest on knee function by looking at a small population of patients who had ACI treatment of either the ankle or the hip but with a chondral harvest obtained from the knee. This group of patients therefore provide a unique opportunity to assess the effect of controlled chondral injury on knee function. We have also analysed the repair tissue formed at the donor site from 3 additional patients who previously underwent ACI for the knee.

Method and Materials

Ankle and hip ACI patients

Patients and surgical technique

Between 1998 and 2009, 23 patients (16 males, 7 females; mean age 38.4±10.1 years, range 17.2-61.3) underwent ACI treatment for chondral defects of the ankle (n=19) and hip (n=4), respectively. The average defect size treated was 2.1±1.6cm^2 (range, 0.5 to 7.8). ACI surgery involved chondrocyte harvest at the knee (stage I) from the ispilateral side in 21 patients and the contralateral side for 1 ankle and 1 hip patient, followed by chondrocyte implantation surgery (stage II). Chondral harvest was performed arthroscopically through standard anterolateral and anteromedial portals under tourniquet control. Specimens of cartilage (mean weight 271.9±97.6mg, range 103 to 520) were taken using a 5mm gouge from a region of the knee with low weight-bearing status. The biopsy site locations were recorded and the biopsy material was transported to our on-site Good Manufacturing Practice (GMP) standard 'Oscell' cell manufacturing facility for chondrocyte culture. Ten

samples were taken from the central trochlea (mean age 36.0±14.0 years, range 17.2 to 61.3), 2 from the lateral trochlea (mean age 44.6±1.6 years, range 43.5 to 45.8), 6 from the medial trochlea (mean age 40.7±5.6 years, range 31.9 to 47.2) and 5 from the intercondylar notch (mean age 38.0±6.1 years, range 28.3 to 44.3). A mean yield of $5.2x10^5±1.3x10^5$ (range $3x10^5$ to $8x10^5$) chondrocytes were obtained from the harvest biopsy. Following cell culture, a mean of $4.9x10^6±1.9x10^6$ chondrocytes (range $1.3x10^6$ to $8x10^6$) were implanted into the treatment site approximately 3 weeks later under either a periosteal (n=20) or ChondroGide® (n=3) patch. One ankle patient required a follow-up knee arthroscopy 1.4 years post-harvest. Patients in this study have been investigated as part of an ethically approved project (REACT 09/H1203/90, granted by West Midlands National Research Ethics Service).

Prior to chondral harvest, no patient had sought treatment for their knee. Modified Lysholm scores, a measure of knee function,[15] were completed pre-operatively and at yearly intervals post-cell implantation. Lysholm scores were categorized as excellent (95–100), good (84–94), fair (65–83) or poor (≤64).[16]

Statistical analysis

A post-hoc power analysis demonstrated a power of 0.8, at a significance level of 5% (p=0.05) to identify a Lysholm score difference of 13 points[19], indicating that this study would require a paired sample of at least 10 patients. Data were tested for normality using the Shapiro-Wilk normality test. Non-parametric paired data (Lysholm scores) were analysed for statistical differences with the Wilcoxon signed-rank test. Non-parametric un-paired data were analysed for statistical differences using the Mann-Whitney U Test. Correlations were tested for using a Spearman's Rank correlation. Statistical differences between grouped

frequency data of the Lysholm Score parameters were tested for using a Chi-squared test of independence. A p-value of <0.05 was deemed significant. All statistical analyses were performed using the software programme Analyse-it Software Ltd, Leeds, UK.

Additional Donor Site Study from Three Knee ACI Patients

Histology

Three additional patients, who had received ACI treatment for chondral defects in their knees, underwent a follow-up arthroscopy at 12.3±1.5 months, with full informed consent. The donor site was examined and core biopsies of the repair tissue formed were obtained. These were snap-frozen in liquid nitrogen-cooled hexane prior to sectioning and 7µm thick cryosections were collected onto poly-L-lysine coated slides. Sections were stained with either haematoxylin and eosin (H&E) or toluidine blue (TB) and viewed with bright light microscopy to assess the general morphology and proteoglycan content of the repair tissue, respectively. Sections were also viewed under polarised light to determine collagen fibril orientation to distinguish between hyaline cartilage and fibrocartilage morphologies. Sections were scored using both the ICRS II[17] (maximum score 140) and the OsScore[18] (maximum score 10) scoring systems, where a higher score for either systems represents a better quality of repair tissue.

Immunohistochemistry

In addition, immunohistochemistry for collagen types I and II was undertaken; for this cryosections were incubated with hyaluronidase prior to fixing in 4% formaldehyde.

Antibodies against collagen type I (1:500, clone I-8H5, MP Biomedicals, Cambridge) or type II (1:10, clone CIIC1, Developmental Studies Hybridoma Bank, Iowa) were incubated for 60 minutes prior to the secondary goat anti-mouse biotinylated antibody for 60 minuntes (Vectastain Elite ABC kit, Vector Laboratories, Peterborough). Adjacent sections were stained with an isotype-matched murine IgG1 (Dako, Cambridge) as a negative control. Non-specific binding and endogenous peroxidase activity were blocked using normal goat serum in 3% bovine serum albumin and 0.3% hydrogen peroxide in methanol respectively. Sections were washed three times with PBS between steps and all steps were performed at room temperature. Labelling was enhanced with streptavidin-peroxidase (Vectastain Elite ABC kit, Vector Laboratories, Peterborough) and visualised with diaminobenzadine (DAB).

Results

Ankle and hip ACI patients

At a mean of 5.1±8.5 days pre-operatively (range 0-31 days), the median Lysholm score for all patients was 100 (IQR 8.7). Median Lysholm scores at the first annual review (13.7±1.7 months post-harvest, range 12 to 18.3 months) and at 4.8±1.8 years post-harvest (range 1.2 to 7.8, termed "latest follow-up" from here on) were 91.7 (IQR 12.5) and 87.5 (IQR 22.2), respectively. Neither the first annual review nor the latest follow-up scores resulted in significantly different scores to the pre-operative scores (Figure 1A). The majority of patients were classified as having either an excellent or good Lysholm score at all three time points (Table 1). At latest follow-up, the median Lysholm score of the opposite knee was 100 (IQR 5.3) and significantly higher than the harvest knee (p=0.0046). Women had a greater change in median score from the pre-operative score compared to men at both the first annual follow up (median change -4.2 and 0, respectively) and the latest follow-up (median

change -16.7 and 0, respectively), but neither were significant, either between the time points or between the sexes.

At the first annual review, Lysholm scores had decreased from pre-operative scores in 44% of patients, increased in 25% and remained the same in 31% of patients. The median change in Lysholm score from pre-operative to first annual review was 0. By the latest follow-up, Lysholm scores had decreased from pre-operative scores in 56% of patients, increased in 13% and remained the same in 31% of patients. The median change in Lysholm score from pre-operative to latest follow-up was -4.2 (Figure 1B). From the Lysholm score, a significantly increased occurrence of pain was reported by patients at the latest follow-up compared to pre-operatively ($p=0.05$, Figure 2). In addition, significantly more catching and locking sensations were reported by patients at both the first annual review and latest follow-up ($p=0.004$ and $p=0.019$ respectively, Figure 2). Only 3 patients reported swelling of their knee. One patient reported a constantly swollen knee at the first annual review, but no swelling 6.3 years post-harvest at latest follow-up. Two patients reported swelling only upon severe exertion at 7.8 and 6.1 years follow-up.

The influence of the location of the chondral harvest was considered; there was no significant difference between the mean ages of each harvest location group. The pre-operative Lysholm score for harvests obtained from the central trochlea was 97.6 and 100 for both the medial trochlea and the intercondylar notch (Figure 3A), with no significant differences in scores between the different harvest locations. At the first annual review, median Lysholm scores were lower, but not significantly, than pre-operative scores for all regions; 87.5, 95.8 and 91.7 for the central trochlea, medial trochlea and intercondylar notch, respectively (Figure 3B). By the latest follow-up, Lysholm scores for the central trochlea had returned to pre-operative levels (median 97.9, mean follow-up 5.5±0.9 years, range 4 to 6.5 years). Lysholm scores for the medial trochlea group at the latest follow-up were maintained

at 93.4 with a mean follow-up of 4.9±1.6 years (range 3.4 to 7.8 years), whilst for the intercondylar notch group, the median Lysholm score was 66.7 with a mean follow up of 2.9±2.1 years (range 1.2 to 6.1 years). These were significantly lower than for both the central and medial trochlea groups (p=0.01 and 0.02, respectively, Figure 3C). The two patients who had a chondral harvest from the lateral trochlea, had pre-operative Lysholm scores of 87.5 and 100, first annual review scores of 70.8 and 100 and latest follow-up scores of 79.2 and 75.

There was no significant relationship between the site of chondral harvest and the weight of harvest taken (p=0.33) or the Lysholm score at either first annual review or latest follow-up (p=0.996 and p=0.148 respectively). Patient's age at ACI was also found not to correlate with either the pre-operative or latest follow-up Lysholm scores (p=0.59 and p=0.12 respectively), nor were there any significant differences in either the first annual review or follow-up Lysholm scores between the sexes.

One patient in the series required a further knee arthroscopy 1.4 years post-harvest after complaining of clicking and grinding behind the patella and reporting a Lysholm score of 68. At the stage I arthroscopy, this patient was noted by the surgeon to have a small Grade III chondral defect on the trochlea; at this follow-up arthroscopy this defect was observed to be larger and was subsequently debrided. The chondral harvest site on the lateral trochlea was found to have healed with a good level of fill and with good integration with the surrounding native articular cartilage, but had a softer consistency. There were also some fronds present here, which were debrided. Five years post-arthroscopy, this patient now reports a Lysholm score of 91.

Additional Donor Site Study

All 3 donor sites of the patients who underwent a follow-up arthroscopy following ACI of the knee, were seen by the surgeon to be well healed with smooth, white cartilage that appeared to be well integrated into the surrounding native hyaline cartilage (Figure 4). The core biopsies obtained were full depth (Figure 5A) with generally good matrix metachromasia (Figure 5B). Under polarised light, one biopsy was observed to be hyaline cartilage and the other 2 biopsies were a mixture of both hyaline cartilage and fibrocartilage, the hyaline cartilage being at the base of the biopsy in both cases and well integrated into both the underlying subchondral bone and the upper fibrocartilaginous portion (Figure 5C-D). All 3 biopsies had moderately good surface architecture (Figure 5E) and generally good cell morphology with most cells being rounded and of a chondrocytic appearance. The mean ICRS II and OsScore histological scores for the biopsies taken from the donor site were 116.8+/-10 and 8.7±0.8 respectively. Immunohistochemical staining for collagen type I was detected throughout the full depth of the biopsies (Fig 5F), whereas collagen type II was predominantly restricted to the hyaline portions of the biopsies, but could also be detected to a lesser amount in the fibrocartilaginous portions adjacent to and above this (Fig 5G).

Discussion

ACI has been used since 1994[5] as treatment for cartilage defects with good long-term results.[20,21] Little is known, however, of the effect of the chondral injury needed to source the chondrocytes for ACI on knee function. There are many reports on the use of osteochondral grafting for locations such as the ankle, where the donor site has been the knee; a few of these studies make reference to donor-site morbidity,[1-4,6,8,14,22-25] but often is not a focussed outcome of the study. Chondral harvest from the knee for the treatment of the ankle is rarely performed nowadays as most chondral harvests are taken from the joint to be treated[7,9,26] for

various reasons, including restricting surgical trauma to a second joint and both anatomical and biomechanical differences between knee and ankle cartilage.[27] A recent study has also demonstrated that chondrocytes extracted from the margins of both grade III and IV osteochondral lesions in the knee are comparable to those from non-weight bearing areas of the joint currently used for chondral harvest.[28] Adopting this type of approach would eliminate any potential for donor-site morbidity, but there are no long-term clinical data on the outcome of the use of debrided edge cartilage as a source of autologous chondrocytes.

To our knowledge, our study is the first to look at the effects of chondral harvest alone on knee function. We found that following a biopsy of approximately 270mg of cartilage from the knee, the majority of patients maintained a good or excellent Lysholm score. Scores at 13.7 months post-harvest were not significantly different to pre-operative scores and were maintained at 4.8 years follow up. Although our study did not assess the area of chondral harvest, we did record the mass of the tissue harvested and we did not find a significant correlation between it and change in knee function. In addition, we found no correlation between either the patient's age or sex and knee function.

It is also worth noting that whilst none of the patients in our study had previously sought treatment for their knees prior to harvest, the pre-operative Lysholm scores were not all 100, with some as low as 62.5 (where scores less than 83 are considered "fair" and scores less than 64 are considered "poor").[16] Two patients who received ACI for chondral defects in the hip, reported low pre-operative Lysholm scores, which could be attributed to referred pain from the hip,[29] particularly as the Lysholm score increased dramatically at the first annual review. This demonstrates how patient perception and clinical observation differ and do not always correlate; it also highlights the importance of comparing the difference in knee scores from pre- to post-operative rather than the post-operative score in isolation. Likewise, a reduced Lysholm score at the latest follow-up could possibly be due to either existing or

newly developed degenerative changes within the joint and not actually related to the donor-site. However, our results demonstrate that the median Lysholm score at latest follow-up for the opposite knee to which the harvest was taken was significantly greater than the harvest knee, despite both knees having a median Lysholm score of 100 pre-operatively. This, we believe, is a reflection on the spread of the data and the limited number of patients in this study. Indeed, some studies have refrained from using the Lysholm score as a measure of knee function when analysing the effect of an osteochondral harvest for ankle mosaicplasty, as they believe that the score may be biased by the painful and functionally reduced ipsilateral ankle joint and hence mask the true knee score.[25] We respect that the Lysholm score is not, in everyone's opinion, the best score to use and there are several other scores that are also validated which are frequently used. However, the scores used in the present study are historical, some being collected up to 17 years ago when only Lysholm scores were collected. Despite this, the Lysholm score is recognised as a reliable measure for use in patients with chondral injuries.[30]

The first ACI procedures reported harvesting cartilage from "a minor load-bearing area of the upper medial femoral condyle".[5] One would assume this was to limit potential donor-site morbidity, although this particular rationale was not mentioned. A study using a biomechanical cadaveric model demonstrated the lowest contact pressures in the knee to be on the medial trochlea and thus recommended this location for future procedures requiring an osteochondral harvest to minimise donor site morbidity.[31] Garretson et al.,[31] also suggest the "worse" place for an osteochondral harvest to be the central trochlea, due to having the highest contact pressures. Interestingly, in our study, we found patients who had a harvest obtained from either the central or the medial trochlea had significantly better Lysholm scores at latest follow-up than those where the harvest was taken from the intercondylar notch. Garretson's study did not include the intercondylar notch. Unfortunately, we were unable to statistically compare the effect of a chondral harvest from the lateral trochlea with

the other locations included in this study due to lack of patients within this category, but, at the latest follow-up, both patients only had a "fair" Lysholm score indicating the lateral trochlea to be a less than optimal harvest location. However, the small number of patients involved in the study (n=25) and even fewer numbers for individual locations (n=2-10) is obviously a limiting factor in being able to make any statistically supported recommendations for an optimal location for chondral harvest.

It is difficult to make a direct comparison of donor-site morbidity between osteochondral and chondral harvests due to the difference in the size of harvest, the types of tissue removed and the resulting defect created. One could speculate that perhaps an osteochondral harvest may in fact have an improved healing potential since they extend beyond the subchondral bone and thus encourage bleeding and entry of bone-marrow stromal cells into the donor-site, which may contribute to the repair process, much in the way that a standard microfracture procedure works. A chondral harvest, however, does not extend into the subchondral bone and so there is no such response stimulated. On the other hand, only one donor site is required for a chondral harvest for ACI, whereas procedures like mosaicplasty often result in multiple donor sites and have a greater overall donor area. Large areas of OATS harvests have previously been reported to correlate with a poorer Lysholm score at final follow-up.[4]

Macroscopically, all 4 donor-sites (1 from knee harvest for ankle ACI and 3 from knee ACI patients) observed by arthroscopy in our study were found to have healed with good integration into the surrounding cartilage. One donor-site in particular, that was noted to be of a softer consistency than the surrounding cartilage, could indicate the presence of a fibrocartilaginous-like tissue rather than a hyaline-like tissue. Microscopic examination of 3 repair tissue biopsies revealed 2 were a mixture of both hyaline cartilage and fibrocartilage whilst the other was solely hyaline cartilage. Previous studies have reported that following an

osteochondral harvest, the donor site fills with a fibrocartilaginous tissue.[1] It is possible that

the hyaline cartilage observed microscopically, or at least some of it, could be residual native

hyaline cartilage due to the nature of the gouge instrument used to harvest the initial

cartilage for Stage 1 and the "U-shape" of the defect left behind. However, there is evidence

that the repair tissue formed at the treatment site following ACI matures with time[32,33] and so

maturation of the tissue formed in the donor-site may also occur.

In conclusion, our study demonstrates that at a mean of 4.8 years following chondral harvest

for ACI treatment, there was no significant change in median Lysholm knee scores

compared to pre-operative scores. These results suggest that there is no significant

harvest-site morbidity of the knee associated with harvesting cells for use in ACI, particularly

when taken from the central or medial trochlea. Some patients, however, did report greater

pain, catching and locking at their final follow-up, though whether this was due to donor-site

morbidity *per se* or injury or degeneration during the average 4.8 year post-harvest follow-up

time, is not possible to differentiate. However, one should carefully consider the location for

chondral harvest as this has been shown to affect knee function long-term.   It is

recommended that patients should be made aware that they may experience some

functional deficit, which is likely to be transient. It is also vital that pre-operative knee scores

are measured, as patients who rate their knees as asymptomatic may have lower knee

scores than the surgeon assumes. Whilst this study demonstrates no significant donor-site

morbidity and good healing of the chondral harvest site both macroscopically and

microscopically, more in-depth studies with a larger cohort of patients are required to fully

assess the effect of harvest location on knee function and both the *in-vivo* appearance and

the histological quality of the repair tissue formed within the donor site to fully assess donor-

site morbidity and understand the processes involved in what appears to be spontaneous

cartilage regeneration.

Acknowledgements

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Figure Legends

Figure 1

Box and whisker plots displaying A) Lysholm scores for the following time points: pre-operative, 13.7±1.7 months post-harvest (first annual review) and at 4.8±1.8 years post-harvest (latest follow-up) and B) median difference in Lysholm scores at the first annual review and latest follow-up compared with pre-operative scores. Lysholm scores at the first annual review (median 91.7) and at latest follow up (median 87.5) were not significantly different to pre-operative scores and neither was the difference in Lysholm score. The box and the horizontal line represent the interquartile range (IQR) and the median respectively. Outliers are represented as a small triangle.

Figure 2

Histograms depicting the 7 different scoring parameters of the modified Lysholm score[13] and the percentage of patients within each category pre-operatively (dark grey bar), at the first annual review (light grey bar) and the latest follow-up (white bar). A significantly higher occurance of pain was reported by patients at the latest follow-up compared to pre-operatively (p=0.05) and significantly more catching and locking sensations were reported at both the first annual review and latest follow-up (p=0.004 and p=0.019 respectively).

Figure 3

Box and whisker plots comparing Lysholm scores for different donor sites pre-operatively (A), at first annual review (B) and at latest follow-up (C). Lysholm scores were not significantly different from each other pre-operatively or at first annual review, but at the latest follow-up, the Lysholm score of patients who had chondral harvest from the

intercondylar notch, had significantly lower scores than patients with harvests taken from the central trochlea (**p=0.01) and the medial trochlea (*p=0.02). Actual Lysholm scores for the lateral condyle at the three time points are represented as small crosses. The box and the horizontal line represent the interquartile range (IQR) and the median respectively. Outliers are represented as a small triangle.

Figure 4

Representative arthroscopic image of the donor site in a patient 11 months post-cell implantation for ACI of the knee. The white portion in the centre of the image (black arrow) is the repaired cartilage in the centre of the trochlea, observed to be smooth and well integrated into the surrounding cartilage.

Figure 5

Representative histological images of a full depth repair tissue biopsy from the donor site in a patient 11 months post-cell implantation for ACI of the knee, stained with H&E (A) and TB (B). Histological analysis of the repair cartilage demonstrated good matrix metachromasia (B) and good integration into the subchondral bone (C, higher powered image of dashed-line box region in A). Polarised light revealed a mixture of hyaline cartilage and fibrocartilage (D, polarised image of C, f=fibrocartilage, h=hyaline cartilage, b=bone) also well integrated with each other. All biopsies demonstrated a good surface (E, higher power image of the solid line box region in A). Immunohistochemistry of sections demonstrate the widespread presence of type I collagen (F) but more restricted type II collagen in the lower region (G). Scale bars represent 500μm (A-B, F-G) and 250μm (C-E).
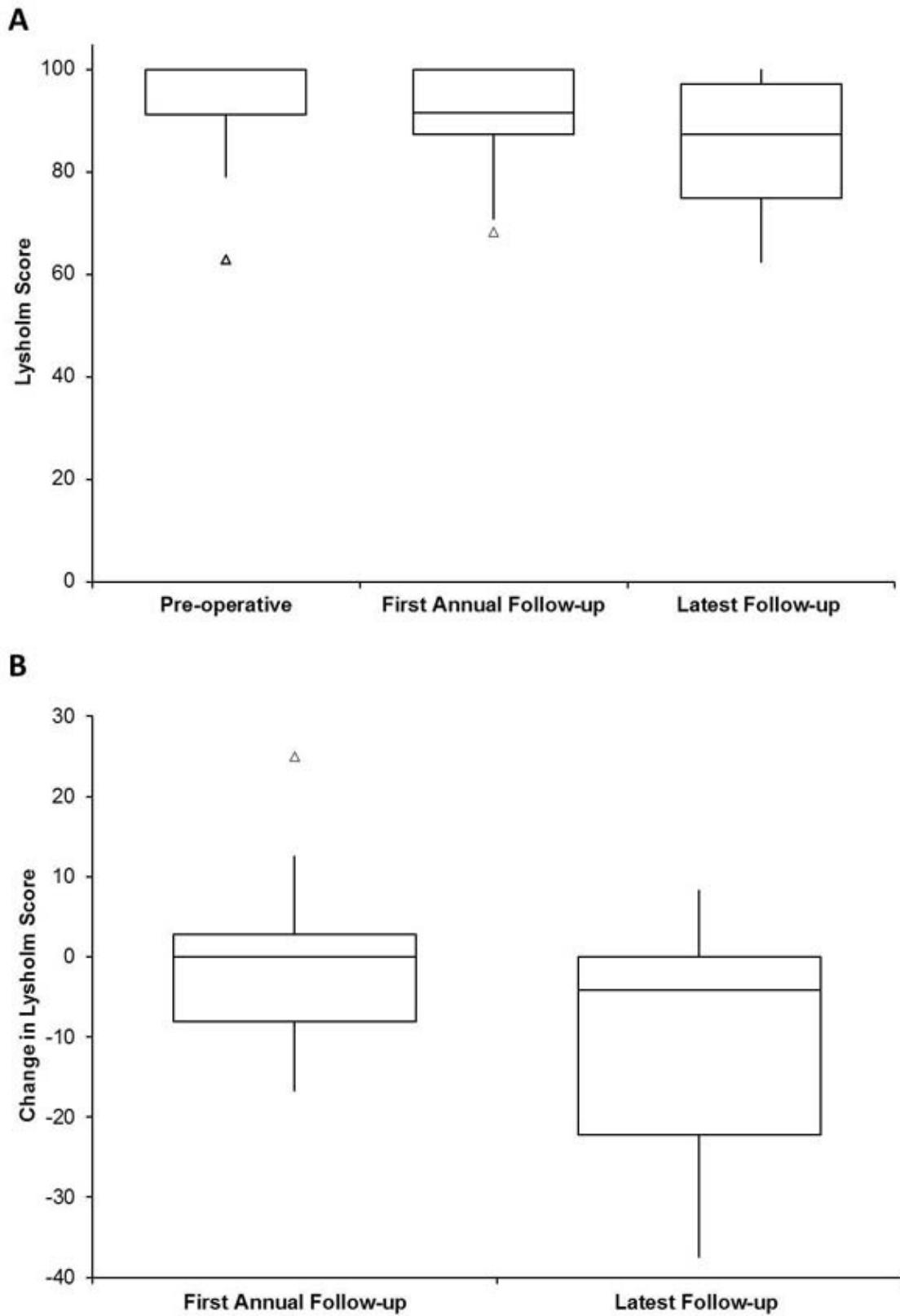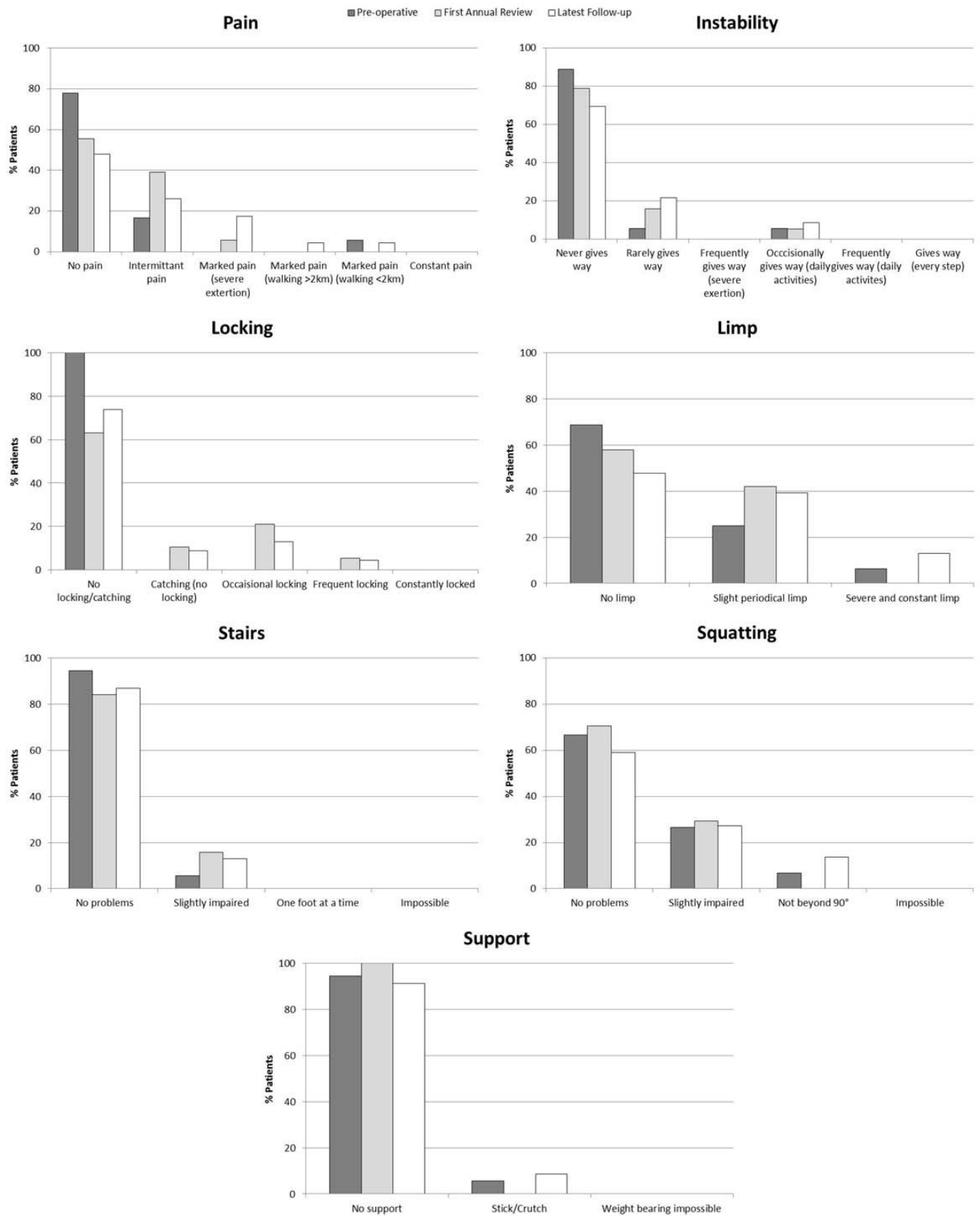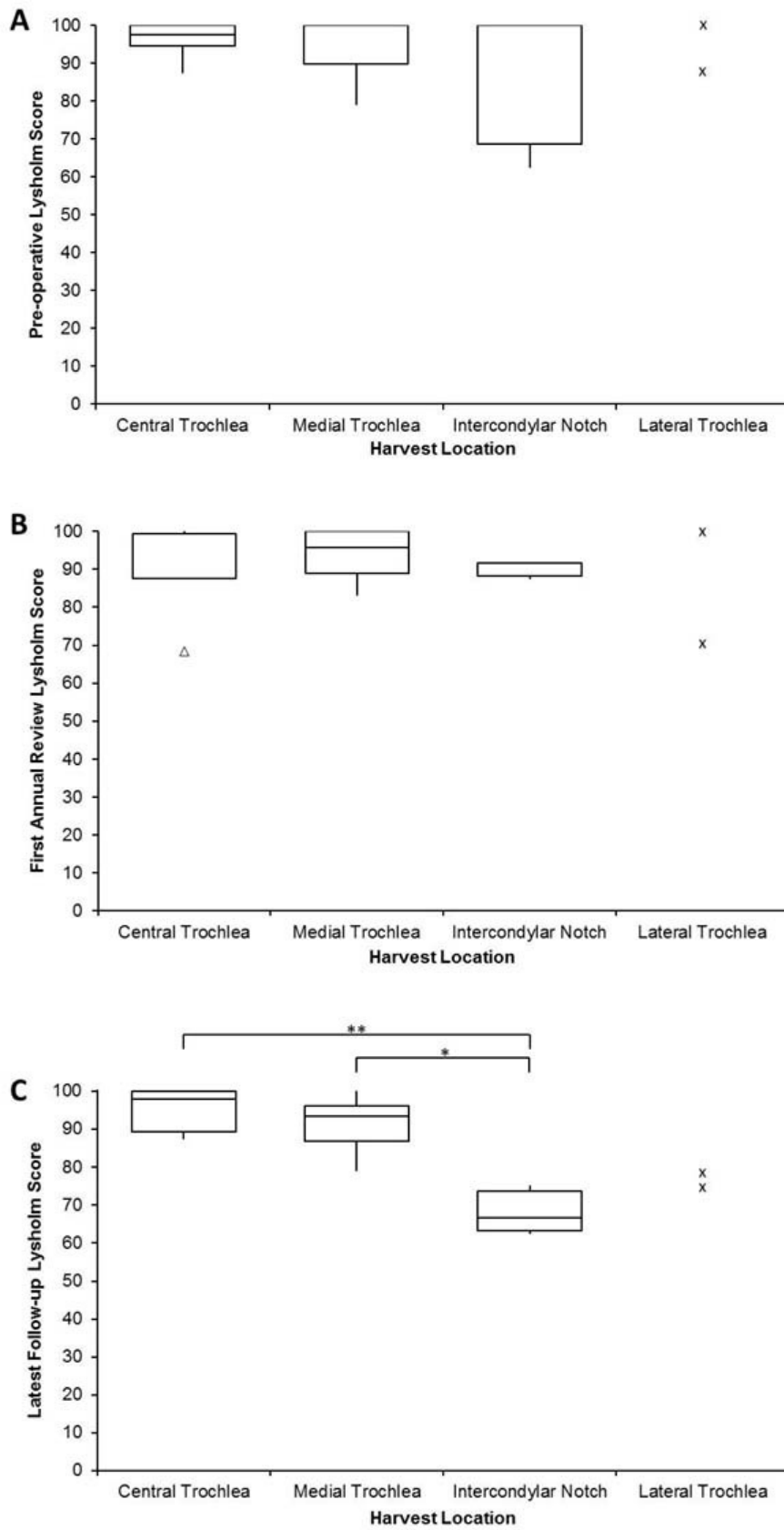
Figure 1

Figure 2

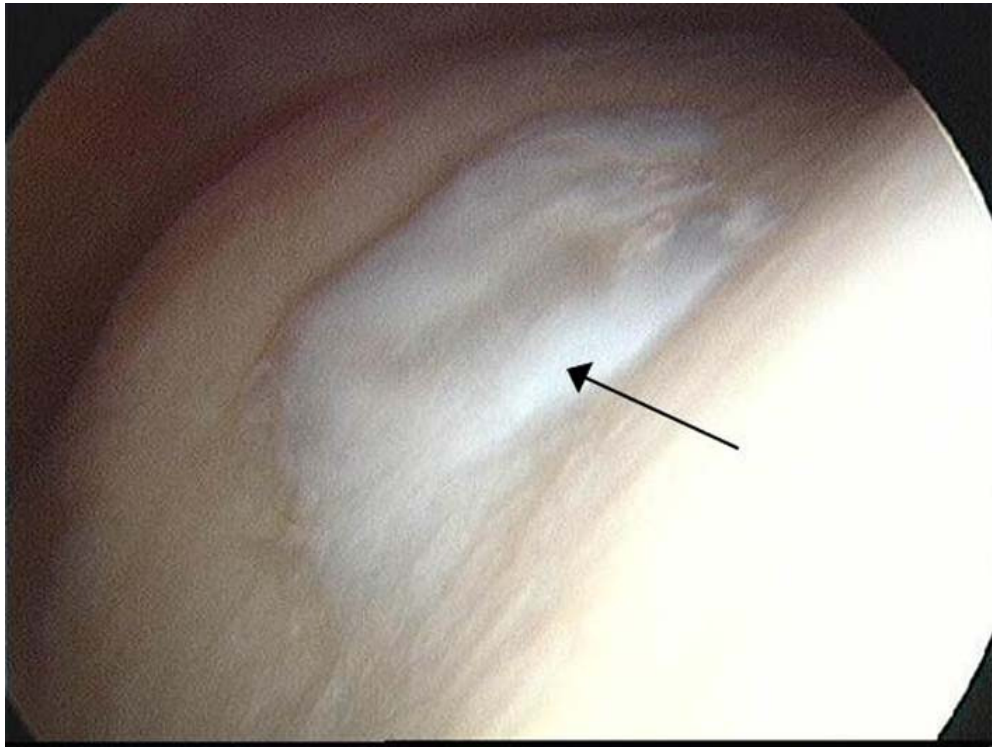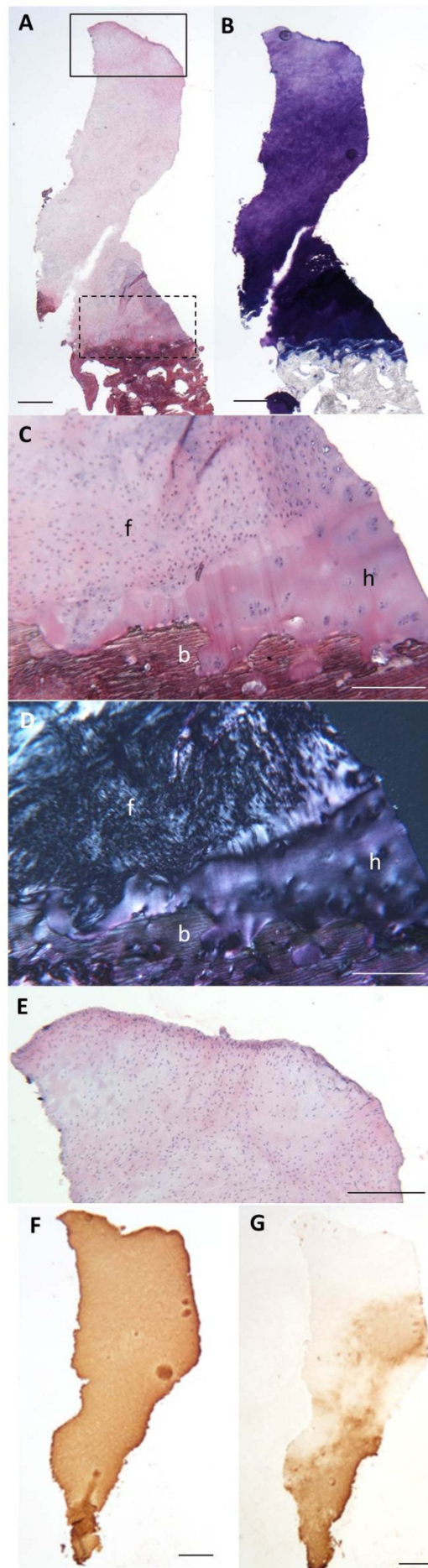**Figure 3**

Figure 4

Figure 5

Table 1

Classification of Lysholm scores pre-operatively, at first annual review and latest follow-up.

	Excellent (95-100)	Good (84-94)	Fair (65-83)	Poor (<64)
Pre-op	72%	17%	5.5%	5.5%
First annual review	42%	37%	21%	-
Latest follow-up	33%	22%	39%	5.5%

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