

# **Comparison of Five-Year Clinical Outcomes of 524 Cemented and Cementless Medial Unicompartmental Knee Replacements**

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## 1 Introduction

2 The two main options for knee replacement are total (TKR) and unicompartmental knee  
3 replacement (UKR). UKR provides faster recovery, better function, lower cost, and fewer and  
4 less severe complications<sup>1-3</sup>, albeit with higher revision rates in national registries<sup>4</sup>. Aseptic  
5 loosening is a common reason for such revisions<sup>5, 6</sup>. In an attempt to address this, a cementless  
6 UKR was developed.

7 The cementless medial, mobile bearing Oxford<sup>®</sup> Partial Knee (Zimmer Biomet, UK), which  
8 began clinical assessment about 15 years ago, is identical to the cemented version except for  
9 the fixation surface. The main differences are that the cementless has hydroxyapatite-coated  
10 porous titanium surfaces for bone attachment, a parallel-sided rather than a tapered femoral  
11 peg, and an additional anterior peg to improve primary stability (**Figure 1a,b**). The surgical  
12 technique and instrumentation are identical, except for the final fixation; the slot in the tibia for  
13 the implant keel is narrower in the cementless UKR so as to achieve a press fit, but wider with  
14 a cement mantle in the cemented UKR. The cementless components are impacted until fully  
15 seated and achieve an interference fit<sup>7, 8</sup>. The overall time of procedure varies with surgeon and  
16 patient factors, but cementless UKR is 20% quicker on average<sup>9</sup>.

17 The revision rate of the cementless UKR reported in national registries is about half that of the  
18 cemented UKR<sup>10</sup>. This may partly be due to experienced surgeons disproportionately using the  
19 cementless UKR. These surgeons have lower revision rates because then tend to do larger  
20 numbers, have better surgical techniques, and adhere to recommended indications<sup>11</sup>.  
21 Nonetheless, a matched study based on 10-year national registry data correcting for  
22 confounding factors, such as surgeon caseload, has shown that the revision rate of the  
23 cementless is still 25% less than that of the cemented<sup>6</sup>. This difference was attributed mainly  
24 to a halving of revision for aseptic loosening and pain.

Two small randomised studies, done as part of the early assessment of the cementless UKR, showed that the incidence of radiolucent lines was much lower with cementless compared to cemented components, suggesting that cementless fixation was better<sup>12, 13</sup>. This likely explains the decrease in revisions for aseptic loosening. The decrease in revision for pain is less well understood. In these randomised trials, there was some evidence of improved outcome scores with the cementless components, but the differences were not statistically significant. This may be because these studies, designed for radiographic outcome measures, were underpowered to assess outcome scores.

The aim of this study was to compare clinical outcomes for cementless and cemented medial UKR with an appropriately powered study. In order to ensure any difference was due to the fixation, the Oxford<sup>®</sup> Partial Knee, which otherwise has identical cemented and cementless components, was studied, and the UKR were implanted by high volume surgeons, using identical indications and technique.

## **Materials and Methods**

A longitudinal cohort study comparing the 5-year outcomes of cemented and cementless medial Oxford UKR implanted between 2006 and 2012 by 4 high-volume knee surgeons<sup>14</sup> was performed. During this period, the surgeons transitioned from implanting the cemented to the cementless components. The indications and contraindications<sup>15, 16</sup>, pre-operative care, and post-operative care, were identical for both procedures. Surgical technique was identical<sup>17</sup>, with the exception of method of fixation. Hybrid implants, using both cemented and cementless components, were excluded. Patients were reviewed by independent research physiotherapists pre-operatively, and 5 years post-operatively. Patients that could not be reviewed in clinic were contacted by post or telephone.

Information about post-operative revisions, re-operations and complications occurring within the 5-year follow-up period were obtained from patients and their records. Revision was defined as the removal, exchange, or addition of any component<sup>18</sup>. This includes conversions to TKR, bearing exchange for dislocation or during debridement and washout, and the addition of a lateral UKR for progression of osteoarthritis. Re-operation was defined as any knee-related procedure where the patient required anaesthesia, including '*manipulation under anaesthesia*' and diagnostic/therapeutic arthroscopies. Any complications relating to the knee or the operation were recorded.

Patients were assessed using the following outcome measures: Oxford Knee Score (OKS), American Knee Society Score (AKSS), and EQ-5D-5L.

The OKS is a knee-specific Patient Reported Outcome Measures (PROM) designed to assess the outcome of knee replacement<sup>19</sup>. The OKS includes 2 components encompassing pain (Questions 1,4,5,8,9) and function (Questions 2,3,6,7,10,11,12)<sup>20</sup>. The 12 questions generate a score between 0 and 48, where higher is better. Scores were categorised according to Kalairajah: 'Excellent' (42-48), 'Good' (34-41), 'Fair' (27-33), and 'Poor' (0-26)<sup>21-23</sup>.

The AKSS is a knee-specific score designed to be completed by an observer to assess the outcome of knee replacement. It includes patient-response questions and objective measurements. It consists of an objective (AKSS-O), and a functional (AKSS-F) component, each scoring between 0 and 100, where higher is better<sup>24</sup>. A subset of the AKSS-O addresses pain, scoring between 0 and 50. The AKSS was created to assess TKR (at a time when optimal alignment was considered to be neutral) and points were deducted for varus and valgus mal-alignment. However, these deductions are not appropriate for the Oxford UKR, where the aim is to restore pre-arthritic alignment of the knee, which is commonly varus<sup>25</sup>. Hence, the measure used in this study excludes these deductions, and is termed 'AKSS-UKR variant'.

The EQ-5D-5L is a generic health status PROM. It has 5 domains (Mobility, Self-Care, Usual Activities, Pain/Discomfort, and Anxiety/Depression), each scored from 0 to 5, where lower is better<sup>26</sup>. An overall index score is calculated as a weighted average of the scores from the 5 domains scored from -0.285 to 1, using a reference UK population of 996 healthy individuals<sup>27</sup>. In addition there is a Visual Analogue Scale (VAS), scored from 0 to 100, where higher is better.

545 knees (267 cemented, 278 cementless) in 441 patients were consented and recruited to this study. These knees were assessed for revisions, re-operations, and complications. 4 knees (0.7%) were lost to follow up so we do not know their outcome; however they were not recorded as revisions by the NJR. At 5 years, an additional 14 knees could not be reviewed due to death from unrelated reasons (n=6) or serious illness (n=8). During the 5 years, 3 UKR had been converted to TKR. Therefore, the final cemented and cementless UKR cohorts that were assessed clinically at 5 years consisted of 524 knees (262 cemented, 262 cementless). Although these knees were all assessed with the primary outcome measure, the OKS, at 5 years, all scores were not available from all knees at all time points, with the numbers available being shown in **Tables 1-3**.

The change in implantation of cemented UKRs to cementless UKRs was done progressively across the middle of the study period, with the cemented generally implanted before the cementless. Differences between cohorts may hence be affected by other factors that progressively changed across the study period, such as improvements to surgical technique. To assess the likelihood of this occurrence, both cemented and cementless cohorts were split into ‘early’ and ‘late’ halves. If differences are found between the ‘*early-cemented*’ and ‘*late-cemented*’, or ‘*early-cementless*’ and ‘*late-cementless*’ groups, then the differences are likely to be due to confounding factors that change with time. In contrast, if differences are found

between the '*late-cemented*' and '*early-cementless*' groups, which were implanted at a similar time, then the difference are likely to be due to fixation.

## Statistics

All data is classified by 'knee'. 98 patients consented to the study of both their knees (196 knees), of which 38 patients (76 knees) had one cemented and one cementless UKR. In these bilateral cases, each knee was assessed independently. Continuous values were tested using the unpaired Student *t*-test for parametric (Shapiro-Wilk  $p > 0.05$ ), and the Mann-Whitney U-test for non-parametric (Shapiro-Wilk  $p < 0.05$ ) distributions. Discrete categories were assessed with the chi-square test or Fisher's exact test, as appropriate. Statistical significance is defined by p-values of  $< 0.05$  for all tests. Data was analysed and visualised using GraphPad Prism (Version 8.3.0 – © 1992-2019 GraphPad Software LLC.) and Microsoft Excel with the Real Statistics Resource Pack (Release 5.4) Zaiontz C. (2018).

A power calculation was performed. Data from a randomised controlled trial of cementless versus cemented UKRs provided baseline OKS data: at one year the cemented arm had OKS of 39 (SD 9.2) and the cementless arm had OKS 41.7 (SD 5.3)<sup>28</sup>. With a standardised difference 0.3, a power of 0.9, and a significance level of 0.05, a minimum of 500 patients (250 cementless, 250 cemented) would be required for this study (following Altman's Nomogram<sup>29</sup>).

## Results

### Pre-operative

Pre-operatively, there was no statistically significant difference between cemented and cementless groups in age, gender, height, weight, and Body Mass Index (BMI) (**Table 1**). There was also no significant difference in pre-operative OKS and AKSS.

### Revisions, Re-operations, Complications

There were 2 revisions in the cemented cohort (1 insertion of a new bearing for bearing dislocation and 1 conversion to TKR for lateral compartment arthritis) and 2 revisions in the cementless cohort (2 conversions to TKR for lateral compartment arthritis) (**Table 2**). The knee with revision for bearing insertion remained a UKR and was included in further analysis, while the knees with revisions to TKR were excluded from further analysis. The 5-year survival was 99.3% in both cemented and cementless cohorts.

There were 4 (1.4%) non-revision re-operations in both cohorts, consisting of arthroscopies, washouts, and manipulation-under-anaesthetic (**Table 2**). There were 12 recorded medical complications in the cemented cohort and 13 in the cementless cohort. No patients died for reasons directly or indirectly relating to their knee replacement.

### 5-year Scores

OKS was significantly better in the cementless cohort than cemented (43.3 vs 41.2,  $p=0.008$ ), and this stemmed mainly from higher pain scores (18.2 vs 17.0,  $p<0.0001$ ) rather than function scores (25.1 vs 24.2,  $p=0.01$ ) (**Table 3, Figure 2**). OKS were categorised into 'Poor', 'Fair', 'Good', and 'Excellent', based on Kalairajah classification (**Figure 3**)<sup>22</sup>. The cementless cohort had significantly more 'Excellent' outcomes than the cemented cohort (75.1% vs 64.9%,

p=0.01), and less than half the number of 'Poor' (2.67% vs 6.49%) or 'Fair' outcomes (5.34% vs 10.3%).

AKSS-O was significantly better for the cementless cohort (93.5 vs 90.4, p=0.049). The pain subscale which contributes to the AKSS-O was also significantly better (46.2 vs 43.1, p=0.0046), whereas there was no significant difference in the non-pain related part of the AKSS-O (47.4 vs 47.3, p=0.98) (**Table 3, Figure 2**). AKSS-F was not significantly different (p>0.05).

The cementless cohort had a significantly better EQ-5D-5L Index (0.87 vs 0.81, p=0.0001) (**Figure 2**). This difference stems primarily from a significantly better mean score in the 'Pain/Discomfort' domain (0.49 vs 0.79, p=0.0001, lower is better). There were also significantly better scores in the 'Usual Activities' (0.55 vs 0.42, p=0.017) and 'Mobility' (0.57 vs 0.43, p=0.025) domains, but the differences were smaller. There were no significant differences in the 'Self-Care' and 'Anxiety/Depression' domains. (**Figure 4**). There was no significant difference in the VAS (p=0.90).

#### Impact of non-contemporaneity

The cemented procedures were performed on average 3 years earlier than the cementless procedures, albeit with substantial overlap (**Figure 5**). To assess if differences were due to non-contemporaneity, each cohort was divided into early and late subgroups by splitting at their respective medians (cemented: 22/01/2008, cementless: 24/07/2011). There was no significant improvement in any scores between the *early* and *late cemented* subgroups or the *early* and *late cementless* subgroups (**Figure 6, Table 4**), demonstrating that there was no significant improvement in the scores with time. In contrast, compared to the *late-cemented*, the contemporaneous *early-cementless* subgroup had significantly better OKS (total, pain, and



function), AKSS Pain, and EQ-5D-5L (Index and ‘Pain/Discomfort’) (**Figure 6, Table 4**), consistent with the differences found between the overall cemented and cementless cohorts. AKSS-O was not significantly different between *late-cemented* and *early-cementless* subgroups. However, the magnitude of its difference between *late-cemented* and *early-cementless* subgroups was similar to the whole cohorts; the lack of significance is likely due to having half the numbers in the comparison. Furthermore, AKSS-O’s pain scores in these subgroups were significantly different, and thus consistent with the whole cohorts (**Table 4**).

## Discussion

This study has found that 5-year outcome scores, both generic and knee-specific, are significantly better with the cementless UKR rather than cemented UKR. These differences were particularly marked for scores assessing pain. The revision, re-operation and complication rates were similar and very low for both cohorts. The study therefore suggests that although both operations are safe, the cementless provides better clinical outcomes, at least for high volume surgeons.

The primary outcome measure, the knee-specific PROM was the OKS, which was 2.1 points (out of 48,  $p=0.008$ ) higher for the cementless group. When categorised, cementless achieved significantly more ‘Excellent’(75% vs 65%), and half the number of ‘Poor’ (3% vs 6%) or ‘Fair’ (6% vs 10%) results. Both the OKS ‘Pain’ and ‘Function’ were significantly better for cementless, but the difference as a proportion of the scale was greater in the OKS ‘Pain’, rather than ‘Function’ subscale. Similarly, the significantly higher AKSS-O score for the cementless cohort stems from its significantly better constituent ‘Pain’ sub-score, as there was no significant difference in the AKSS-O score between the cohorts when pain was excluded.

The general quality-of-life PROM used was the EQ-5D-5L. The EQ-5D-5L Index score was significantly ( $p=0.0001$ ) better for patients with cementless UKR. Of the domains, 'Pain/Discomfort', 'Mobility', and 'Usual Activities', were significantly better for cementless cohort (difference in score of 30%  $p=0.0001$ , 14%  $p=0.025$ , 12.6%  $p=0.017$  respectively). The 'Pain/Discomfort' had the largest, as a proportion of the scale, and the most significant difference. It is perhaps not surprising that there was no difference in the 'Self-Care' and 'Anxiety/Depression' domains.

The finding that the PROMs were significantly better with the cementless was unexpected. The improvement consistently stemmed from differences in the amount of pain across the different measures. Other differences (relating to function, mobility and usual activities), may be a manifestation of this decrease in pain<sup>30</sup>. Decreased pain with the cementless UKR is consistent with registry findings that the cementless UKR have less revisions for pain<sup>6</sup>. The finding that there was no difference in AKSS-O, when excluding its 'Pain' sub-score, suggests that there is no difference in the surgical aspect of the operation. Cemented and cementless implants are nearly identical, differing primarily in fixation surface; the difference in pain is thus likely to be a manifestation of improved fixation in the cementless implant. Further study is needed to understand this in more detail.

There were no significant differences between the cemented and cementless UKR in revision, re-operation and complication rates, demonstrating that the procedures are equally safe. Registry studies have shown that the revision rate of the cementless Oxford UKR is lower than the cemented<sup>6</sup>, but is not necessarily inconsistent with this study; with 2 revisions in each cohort, this study is too underpowered to show a difference. The survival rate of both cemented and cementless UKR was 99% at 5 years, which is similar to that of the best TKR, but the revisions are more minor (being conversions to primary TKR or bearing exchange).

Furthermore, the re-operation and complication rates are substantially lower than those of TKR.

The main limitation of the study is that it is not randomised. Although the cohorts are well matched for demographics and pre-operative scores (**Table 1**), the cementless cases were implanted on average 3 years after the cemented, introducing the possibility of confounding due to differences associated with the date of operation, such as progressive improvements in surgical technique with time. However, when comparing the *early* and *late* sub-groups within the *cemented* and *cementless* cohorts, there was no significant improvement in outcomes. In contrast, when comparing the *late-cemented* and *early-cementless* sub-groups, which were approximately contemporaneous, there were significant differences in scores and they were similar to those in the overall comparison (**Figure 5**). As the *early-cementless* cases were done at about the same time as the *late-cemented* cases, this suggests the differences are due to fixation and not improvements in technique with time. Furthermore, differences in outcome scores in this study were similar to those found in previous randomised trials that were underpowered for clinical outcomes<sup>12, 13, 28</sup>, further supporting these findings.

The procedures in this study were performed by high volume surgeons, which limits its generalisability. However, evidence suggests that if surgeons adhere to the recommended indications and surgical techniques, they get similar results<sup>31-33</sup>. Therefore the conclusions of this study should relate to all surgeons using the recommended indications and techniques. Most of the differences in the scores were similar to, or less than, the published Minimum Clinically Important Differences (MCID)<sup>34</sup>. However, the scores in both cohorts are high, and they therefore having marked ceiling effects, causing MCIDs to have limited value. A further limitation was that particularly for scores that could not be obtained by phone there was incomplete data. Finally the Oxford UKR, used in this study, is ideally suited to cementless

229 fixation as, due to the mobile bearing, loads at the implant-bone interface tend to be  
230 compressive with minimal shear or tensile forces. Hence, these findings may not apply to other  
231 devices.

## 232 **Conclusion**

233 This study demonstrates that at 5 years, both cemented and cementless Oxford UKR are safe  
234 with implant survival of 99.2%, non-revision re-operation rates of 2%, and complication rates  
235 of 5%. Although both devices had excellent PROMs, the cementless had significantly better  
236 PROMs than the cemented. The differences were most marked in the pain scores, and the other  
237 functional advantages seen may be due to decreased pain. However, whatever the reason, this  
238 study suggests that surgeons using the recommended indications and techniques should  
239 consider using cementless rather than cemented fixation.

## 240 **Ethics and Declaration of Interests**

241 This study received Institutional Review Board approval (REC 11/SC/0480). This research  
242 did not receive any specific grant from funding agencies in the public, commercial, or not-  
243 for-profit sectors. Institutional funding and personal fees for consulting work, unrelated to  
244 submitted work, were received from commercial parties by one or more of the authors.

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**Figure 1a (left):** Oxford® Cemented Partial Knee (Phase 3).

**Figure 1b (right):** Oxford® Cementless Partial Knee.

*Photos from Zimmer Biomet. Used with permission.*

**Table 1: Pre-operative characteristics of cemented and cementless cohorts.**

	Cemented (n=267)		Cementless (n=278)		p-value
	No of values	Mean (SEM)	No of values	Mean (SEM)	
<b>Age (years)</b>	267	65.6 (0.596)	278	65.50 (0.704)	0.935 <sup>1</sup>
<b>Height (cm)</b>		169 (0.630)		171 (0.580)	0.118 <sup>1</sup>
<b>Weight (kg)</b>		85.4 (1.03)		85.76 (0.931)	0.787 <sup>1</sup>
<b>BMI</b>		29.8 (0.320)		29.43 (0.259)	0.379 <sup>1</sup>
<b>Sex</b>		57.7% male 42.3% female		62.7% male 37.1% female	0.209 <sup>2</sup>
<b>OKS</b>	175	26.1 (0.680)	191	26.4 (0.597)	0.871 <sup>1</sup>
<b>AKSS-F<sup>3</sup></b>	165	71.3 (1.494)	178	71.4 (1.211)	0.564 <sup>1</sup>
<b>AKSS-O<sup>3</sup></b>	165	45.5 (1.702)	178	44.9 (1.800)	0.800 <sup>1</sup>

All distributions were determined to be non-parametric (Shapiro-Wilk  $p < 0.05$ ).

<sup>1</sup>Mann-Whitney U Test, <sup>2</sup>Chi-Square Test.

<sup>3</sup>AKSS-UKA Variant used.



**Table 2: Post-operative revision, re-operation, and complication rates.**

		Cemented (n=267)		Cementless (n=278)		p-value <sup>1</sup>
		Total	% of cohort	Total	% of cohort	
<b>Revision Surgeries</b>	Conversion to TKR	1	0.37%	2	0.72%	0.59
	Bearing insertion	1	0.37%	0	0%	0.31
	<b>Subtotal</b>	<b>2</b>	<b>0.75%</b>	<b>2</b>	<b>0.72%</b>	0.97
<b>Re-operations</b>	Arthroscopy	3	1.12%	2	0.72%	0.62
	Washout	1	0.37%	1	0.36%	0.98
	Manipulation Under Anaesthetic	0	0%	1	0.36%	0.32
	<b>Subtotal</b>	<b>4</b>	<b>1.50%</b>	<b>4</b>	<b>1.44%</b>	0.95
<b>Complications</b>	Heart attack	1	0.37%	3	1.08%	0.34
	PE	1	0.37%	2	0.72%	0.59
	DVT	0	0%	2	0.72%	0.17
	Stroke	0	0%	2	0.72%	0.17
	Transfusion	1	0.37%	0	0%	0.31
	Superficial wound infection	9	3.37%	3	1.08%	0.07
	Deep infection	0	0%	1	0.36%	0.33
	Chest infection	0	0%	0	0%	>0.99
	<b>Subtotal</b>	<b>12</b>	<b>4.49%</b>	<b>13</b>	<b>4.68%</b>	<b>0.92</b>
	<b>Total</b>	<b>18</b>	<b>6.74%</b>	<b>19</b>	<b>6.83%</b>	<b>0.97</b>

Incidence of revision surgeries, re-operations and post-operative complications within the cemented and cementless cohorts.

<sup>1</sup>Chi-square tests were used in comparing the incidence between cohorts.

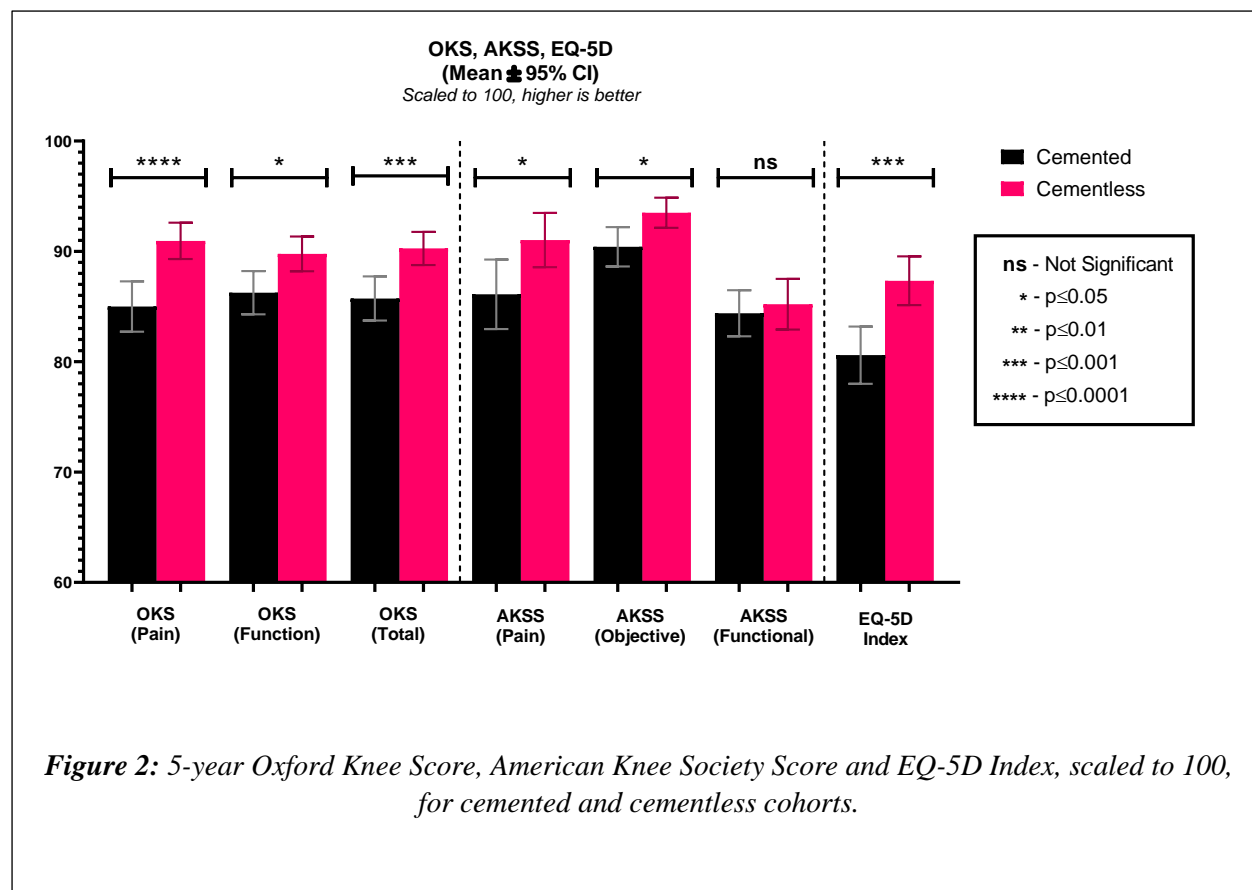
**Table 3: 5-year response rates and responses for Oxford Knee Score and American Knee Society Score.**

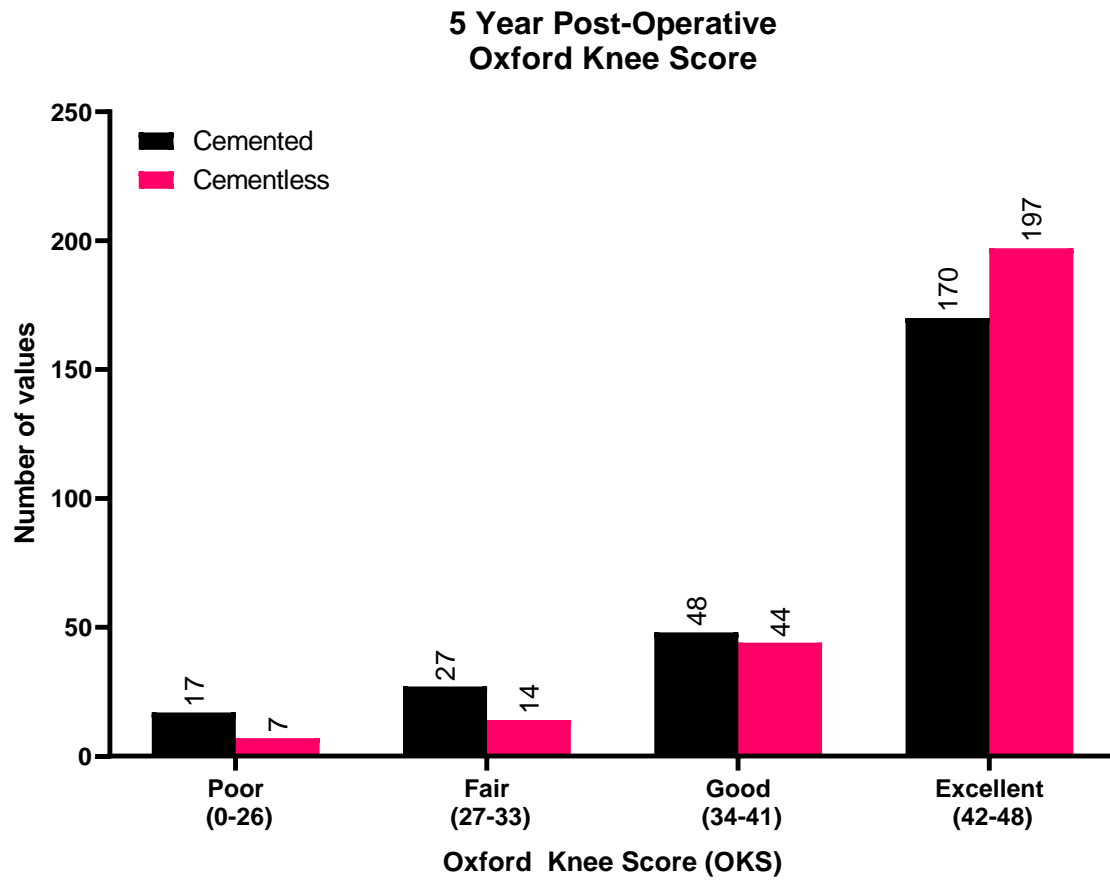
		Cemented		Cementless		p-value <sup>1</sup>
		No of values	Mean (SEM)	No of values	Mean (SEM)	
<b>Oxford Knee Score (OKS)</b>	<b>Total Score</b>	262	41.2 (0.487)	262	43.3 (0.365)	<b>0.008</b>
	<i>of which Pain</i>		17.0 (0.232)		18.2 (0.168)	<b>&lt;0.0001</b>
	<i>of which Function</i>		24.2 (0.278)		25.1 (0.224)	<b>0.010</b>
<b>American Knee Society Score (AKSS)<sup>2</sup></b>	<b>Objective Knee Score (AKSS-O)</b>	224	90.4 (0.903)	195	93.5 (0.689)	<b>0.049</b>
	<i>of which Pain</i>		43.1 (0.798)		46.2 (0.589)	<b>0.005</b>
	<i>of which non-pain</i>		47.4 (0.243)		47.3 (0.251)	0.98
	<b>Functional Knee Score (AKSS-F)</b>	245	84.4 (1.06)	209	85.2 (1.17)	0.45

All distributions were determined to be non-parametric (Shapiro-Wilk  $p < 0.05$ ).

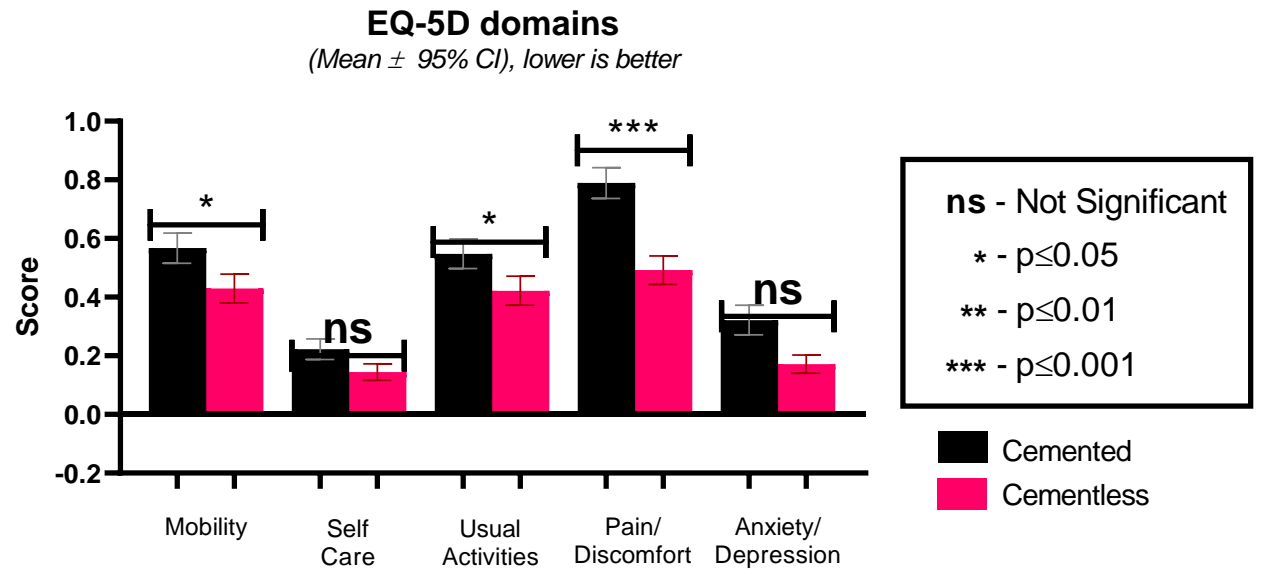
<sup>1</sup>Mann-Whitney U Test used in comparing scores between cohorts.

<sup>2</sup>AKSS-UKR variant used.

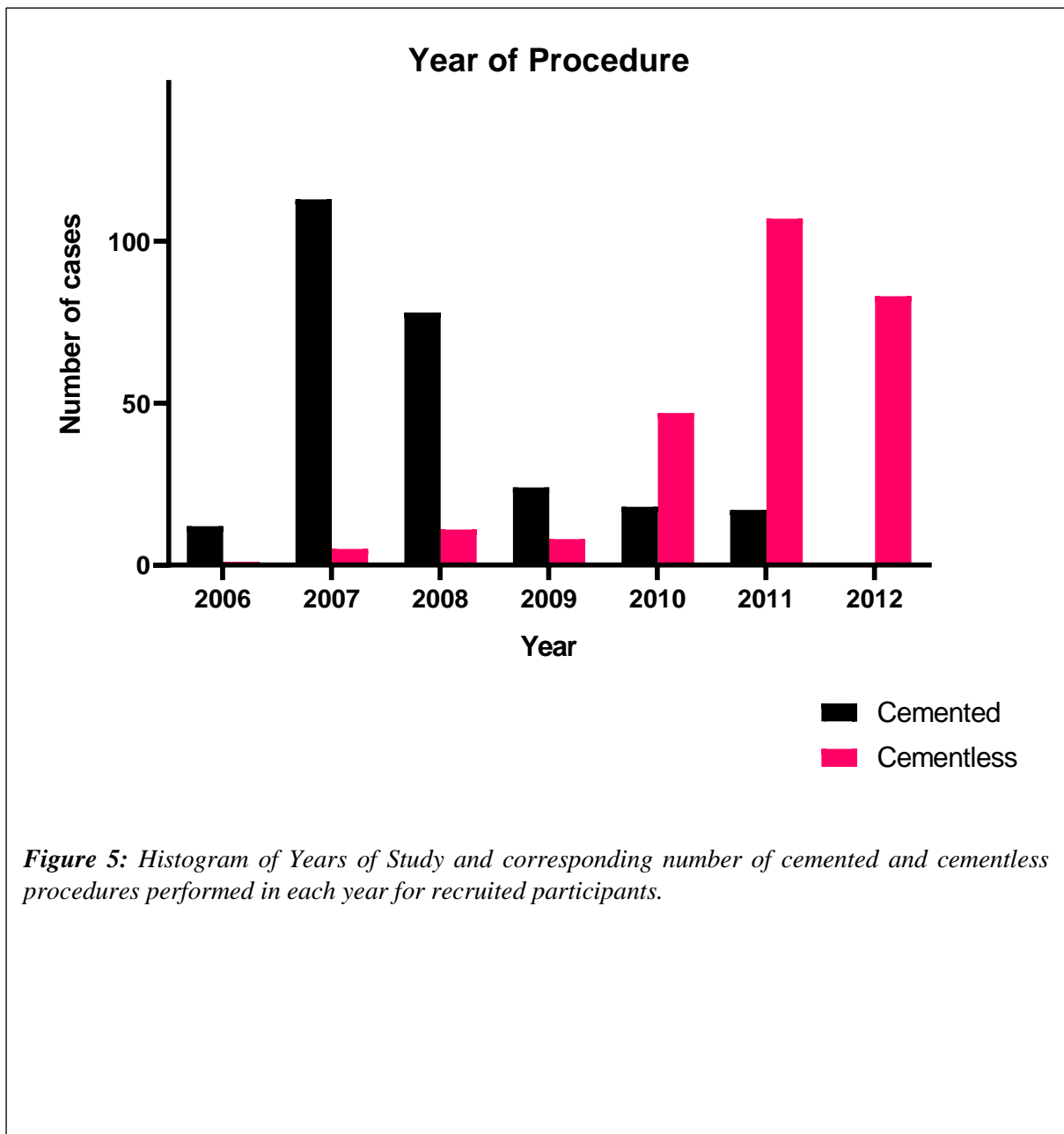




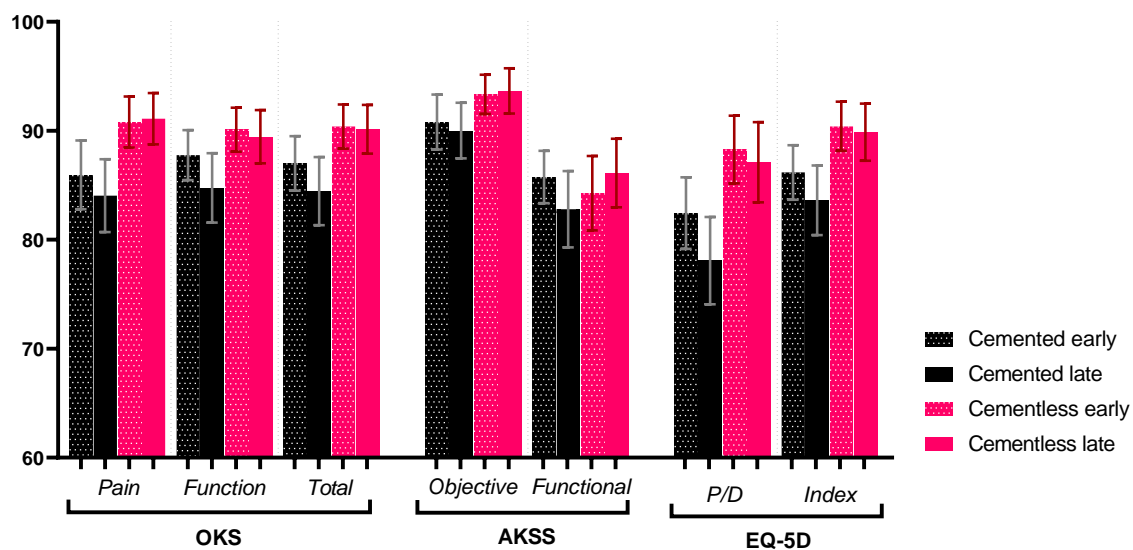
*Figure 3: 5-year OKS for cemented and cementless cohorts, categorised into Kalairajah categories.*



**Figure 4:** 5-year EQ-5D-5L domain scores for cemented and cementless cohorts.



**5-year OKS, AKSS, EQ-5D**  
 Split by cemented and cementless, early and late. Scores scaled and inverted (as needed) to 0-100, where higher is better.  
*Mean  $\pm$  95% CI*



**Figure 6:** Mean  $\pm$  95% CI values for OKS, AKSS (UKR Variant), and EQ-5D (Pain/Discomfort domain and Index score), for cemented and cementless cohorts, further classified into early and late subgroups.

**Table 4:** Comparison early and late cemented and cementless cohorts.

p-values for groups compared	Early Cemented	Early Cementless	Late Cemented	Cemented
	Late Cemented	Late Cementless	Early Cementless	Cementless
OKS Pain (mean scores)	0.35	0.89	<b>0.0012</b>	<b>&lt;0.0001</b>
OKS Function (mean scores)	0.57	0.88	<b>0.048</b>	<b>0.010</b>
OKS Total (mean scores)	0.52	0.99	<b>0.0098</b>	<b>0.0080</b>
AKSS Objective (mean scores) <sup>1</sup>	0.90	0.53	0.27	<b>0.049</b>
<i>of which pain (mean scores)</i> <sup>1</sup>	0.69	0.91	<b>0.033</b>	<b>0.0046</b>
<i>of which non-pain (mean scores)</i> <sup>1</sup>	0.45	0.81	0.81	0.98
AKSS Functional (mean scores) <sup>1</sup>	0.55	0.46	0.65	0.45
EQ-5D Pain/Discomfort Domain (mean scores)	0.19	0.96	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>
EQ-5D Index (mean scores)	0.51	0.96	<b>0.0008</b>	<b>0.0001</b>

All distributions were non-parametric (Shapiro-Wilk  $p < 0.05$ ), hence Mann-Whitney U test performed for all comparisons.

<sup>1</sup>AKSS-UKR variant used. See **Methods** for details.