# Investigation of the Optimal Interval for Staged Total Knee Arthroplasty for the Treatment of Advanced Bilateral Gonarthrosis

Zkoumání optimálního intervalu mezi operacemi pro oboustrannou totální náhradu kolenního kloubu při léčbě pokročilé oboustranné gonartrózy

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### **ABSTRACT**

### PURPOSE OF THE STUDY

This study investigated whether there was an optimal interval between two operations for total knee arthroplasties in patients with advanced bilateral gonarthrosis scheduled to undergo staged total knee arthroplasty (TKA).

### MATERIAL AND METHODS

A prospective cohort of 219 patients (136 females, 83 males) undergoing staged total knee arthroplasty for the treatment of advanced bilateral gonarthrosis were followed for up to 12 months. The mean was 69.51±5.02 (56–80) years. Patients were categorized into five groups based on the time between the first and second operations; Group I (21–90 days), Group II (91–180 days), Group III (181–270 days), Group IV (271–360 days), and Group V (more than 360 days). Patients were evaluated based on time from surgery and were assigned to corresponding groups. The data recorded included age, body mass index (BMI), side of operated knee, complications, and radiological and clinical findings. Visual analog scale (VAS) for non-operated knees was applied. Activities of Daily Living Score (ADLS) was applied to the patients at last follow-up.

### RESULTS

No statistically significant difference was noted in BMI values (p=0.634), range of joint motion (RJM) (p=0.940) and age (p=0.785) distribution between the five groups. In Group I, the mean VAS score increased by 7.83 to 7.98, 7.86 to 8.53 in Group II, by 7.85 to 8.54 in Group III, 7.85 to 8.59 in Group IV, and 7.88 to 8.64 in Group V. There was no statistically significant difference in preoperative ADLS between the groups (p=0.064), but there was a statistically significant difference in postoperative ADLS (p=0.001). Group I patients had significantly lower postoperative ADLS compared to the other groups (p=0.001). The mean increase in postoperative ADLS versus preoperative scores of all groups were statistically significant The most significant improvements occurred in Groups II and III. Similarly, preexisting pain in the non-operated knee started to increase in Group II and continued in all groups.

# DISCUSSION

Given all these findings, we believe that it is reasonable to advise patients to receive their second TKA, 3–6 months after their initial TKA, as this interval will allow for the greatest improvements in functional and daily living activities, and pain in the non-operated knee simultaneously becomes more severe. This recommended interval would minimize both the functional problems with the operated extremity due to pain, and deformity and dysfunction in the non-operated knee and the subsequent overloading.

### CONCLUSIONS

Even though a number of factors influence the optimal interval for staged TKAs in bilateral gonarthrosis, an interval of 91–270 days appears to be the optimal interval between surgeries in terms of minimizing pain and maximizing ADL s and knee scores.

Key words: bilateral total knee arthroplasty, optimal interval, knee society scores, activities of daily living.

## INTRODUCTION

Total knee arthroplasty (TKA) is a surgical technique intended to maximize patients' quality of life by providing pain-free joint motion, maintaining soft-tissue equilibrium between the ligament and muscle structures controlling the knee joint, and by correcting deformity. TKA has been widely used to relieve pain, correct deformity,

and improve knee function in advanced stage gonarthrosis patients who have not responded to conservative treatment options (3).

As the population ages and life expectancy increases, the number of patients who undergo TKA has grown with time. There has been a corresponding increase in the num-

Table 1. Demographical characteristics of the patients

	GROUP-I (N=47)	GROUP-II (N=43)	GROUP-III (N=46)	GROUP-IV (N=41)	GROUP-V (N=42)	р
Age (Mean+SD)	69.94±4.83	70.09±4.6	68.91±5.48	69.37±4.71	69.21±5.51	²0.785
BMI ( <i>Mean+SD</i> )	32.27±3.29	33.04±4.14	32.53±4.39	32.16±3.75	33.15±2.4	<sup>a</sup> 0.634
RJM ( <i>Mean+SD</i> )	106.28±6.47	106.63±5.64	106.52±8.09	107.2±6.62	105.95±6.37	ª0.940

<sup>a</sup>Oneway Anova test SD: standard deviation; BMI: body mass index; RJM: range of joint motion

ber of patients who receive bilateral TKAs (18). Bilateral TKAs can be performed simultaneously during the same hospital stay, staggered within the same hospital stay, or staged over different hospital visits. Previous studies have compared the outcome and complications of staged bilateral TKAs and simultaneous bilateral TKA (7, 23).

To date, there have been few studies to have investigated the optimal interval for staged bilateral TKAs. This study investigated whether there was an optimal interval between the first and second operations for TKA in patients needing bilateral TKA.

### MATERIAL AND METHODS

After our study received approval from the institutional review board, we retrospectively reviewed our prospectively collected electronic total joint arthroplasty database to identify patients who underwent staged bilateral TKA between October 2010 and December 2013. During the study period, TKA was performed in 306 patients and 242 patients were evaluated whom applied two-staged procedure. Of these, 23 patients were lost to follow-up and the study included 219 patients who ranged in the age from 56 to 80 years. This study included patients with a primary diagnosis of advanced stage bilateral gonarthrosis who met the Lawrence criteria (17) and who had been scheduled to undergo staged TKA, underwent TKA on the first knee, attended all follow up examinations, and who had at least one year follow up after the initial TKA procedure and underwent the same surgical protocol. The exclusion criteria were patients without bilateral gonarthrosis, earlier stages of gonarthrosis, incomplete follow-up data, or experienced other pathologies, non-surgical complications, or other diseases that can interfere with the process or cause joint pain.

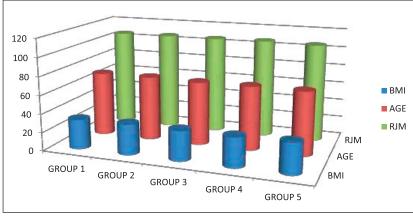


Fig. 1 Demographical characteristics of the patients.

Which knee would be operated upon first was decided by patient preference. A total of 219 patients (83 males, 136 females) with complete follow-up data were categorized into five groups based on time from TKA on one knee: Group I (21–90 days), Group II (91–180 days), Group III (181–270 days), Group IV (271–360 days), and Group V (more than 360 days). Patients were evaluated based on time from surgery and were assigned to corresponding groups. The data recorded included age, body mass index (BMI), side of operated knee, complications, and radiological and clinical findings. There was no statistically significant distribution of additional comorbidities that might compromise patient mobility and clinical outcomes between the groups. The patients had no pathologies that might compromise surgical outcomes.

Changes in the severity of pain, which is the most common symptom of patients with advanced stage gonarthrosis, were measured using the visual analog scale (VAS) for non-operated knees (5, 16). At the final follow up, the ability of the patient to function was evaluated using the Activities of Daily Living Score (ADLS), which measures symptoms and functional limitations in daily life (27). Symptoms include pain, crepitation, tenderness, swelling, instability, and weakness. Functional limitation caused by each symptom is scored on a scale that ranges from no symptoms to total function loss. The ADLS assesses functional limitations (difficulty ascending and descending stairs, standing, kneeling, squatting, sitting, and rising up from sitting position). Each response ranges from no limitation to total limitation. Scores from all 17 items on the ADLS were summed, divided by the number of patients, and multiplied by 100 to give a percentage.

Statistical analyses of the findings obtained in the study were made using IBM SPSS v. 22 (IBM, SPSS) software.

When evaluating the study data, conformity to normal distribution was tested with the Shapiro Wilk test. In the evaluation of the data, descriptive statistical methods were used (mean, standard deviation, frequency). In the comparison of quantitative data, the Oneway Anova test was used and Tukey HDS test was used to define the group which caused difference. Kruskal-Wallis test was used to evaluate between groups which aren't normally distributed. Student t test was used to evaluate between groups which are normally distributed, Mann-Whitney U test was used to evaluate intergroups which are not. Paired simple t test was used to evaluate quantitative datas of intergroups while Wilcoxon signed rank test was used in not normally distributed parameters. A value of p<0.05 was accepted as statistically significant.

# **RESULTS**

Mean age of patients were 69.51±5.02 years. None of the patients developed any complications that might compromise study results either during or after surgery. No statistically significant difference was noted in BMI values (p=0.634), range of joint motion (RJM) (p=0.940) and age (p=0.785) distribution between the five groups (Table 1) (Fig. 1). No patients enrolled in this study had early loosening of the component at final follow-up.

Postoperative knee scores were significantly lower in Group I than those in the other groups. On the other hand, the improvement in knee scores of Groups IV and V was significantly lower when

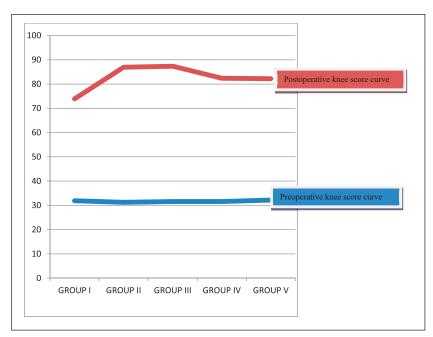


Fig. 2. Increase rates in the American Knee Society knee scores.

compared to that in Groups II and III (Fig. 2). In other words, Groups II and III showed the most significant improvement in knee scores (Table 2).

Table 2. Pre- and postoperative scores of the groups on the American Knee Society Scoring System

		GROUP I	GROUP II	GROUP III	GROUP IV	GROUP V	<sup>а</sup> р
Pain; <i>Mean+SD</i>	Preop	7.87±4.14 (10)	7.67±4.27 (10)	6.74±4.74 (10)	8.05±4.01 (10)	8.1±3.97 (10)	0.545
	Postop	37.87±6.06 (40)	45.93±2.5 (45)	46.3±2.22 (45)	44.27±1.79 (45)	45.24±1.9 (45)	0.001**
<i>b</i> р		0.001**	0.001**	0.001**	0.001**	0.001**	
Mobility;	Preop	19.32±1.14 (20)	19.16±1.07 (20)	18.78±1.35 (18)	18.73±1.05 (18)	18.62±1.08 (18)	0.008**
Mean+SD	Postop	20.51±1.02 (20)	21.21±1.19 (22)	21.26±1.67 (22)	21.32±1.33 (22)	21.12±1.55 (21.5)	0.012*
<i>b</i> <b>p</b>		0.001**	0.001**	0.001**	0.001**	0.001**	
Stability;	Preop	17.23±2.51 (15)	16.4±2.27 (15)	17.83±2.51 (20)	16.22±2.17 (15)	16.67±2.39 (15)	0.011*
Mean+SD	Postop	24.57±1.41 (25)	24.53±1.47 (25)	24.78±1.03 (25)	24.02±2.01 (25)	23.33±2.39 (25)	0.001**
<i>b</i> <b>р</b>		0.001**	0.001**	0.001**	0.001**	0.001**	
Flexion contracture Mean+SD	Preop	3.09±1.46 (2)	3.12±1.47 (2)	3.13±1.75 (2)	2.59±1.2 (2)	2.57±1.19 (2)	0.142
	Postop	3.47±1.52 (2)	2.05±1.43 (2)	1.57±0.83 (2)	2.34±1.2 (2)	2.05±1.45 (2)	0.001**
<sup>b</sup> p		0.239	0.003*	0.001**	0.537	0.259	
Extension loss; Mean+SD	Preop	9.04±1.99 (10)	8.6±2.27 (10)	8.26±2.41 (10)	8.54±2.3 (10)	8.33±2.39 (10)	0.498
	Postop	5.21±1.02 (5)	2.56±2.53 (5)	3.26±2.41 (5)	4.27±1.79 (5)	3.93±2.08 (5)	0.001**
<sup>ь</sup> р		0.001**	0.001**	0.001**	0.001**	0.001**	
Alignment;	Preop	0.38±1.01 (0)	0.28±0.88 (0)	0.39±1.02 (0)	0.29±0.9 (0)	0.29±0.89 (0)	0.960
Mean+SD	Postop	0.38±1.01 (0)	0.42±1.05 (0)	0.39±1.02 (0)	0.59±1.2 (0)	1.07±1.45 (0)	0.031*
<sup>ь</sup> р		1.000	0.527	1.000	0.157	0.005**	
Knee score;	Preop	31.91±4.42(31)	31.23±3.38(30)	31.57±4.41(32)	31.59±3.19(31)	32.19±2.93(32)	0.723
Mean+SD	Postop	73.89±6.83(78)	86.93±4.97(90)	87.35±5.13(87)	82.41±4.63(83)	82.21±6.21(83)	0.001**
Difference; Mean+	SD	41.98±6.39(42)	55.7±3.59 (57)	55.78±6.06(56)	50.83±3.43(52)	50.02±6.21(52)	0.001**
<sup>ь</sup> р		0.001**	0.001**	0.001**	0.001**	0.001**	

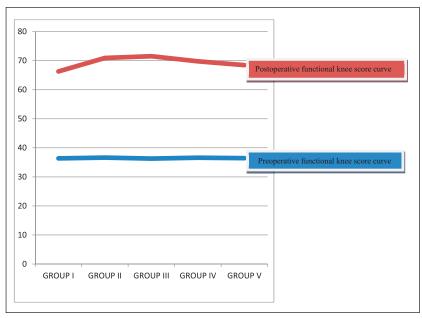


Fig. 3. Increase rates in the American Knee Society functional scores.

The difference in preoperative functional knee score as compared to postoperative functional total score was significantly lower in Group 1 than that in Groups II, III, IV, and V. This score change was significantly higher in Group II and Group III as compared to the score

change observed in Groups IV and V (Fig. 3, Table 3).

The baseline Visual Analog Scale (VAS) scores of the patient's non-operated knees were compared with their VAS scores at final follow-up. In Group I, the mean VAS score increased by 7.83 to 7.98, 7.86 to 8.53 in Group II, by 7.85 to 8.54 in Group III, 7.85 to 8.59 in Group IV, and 7.88 to 8.64 in Group V. The mean VAS score increase in Group I patients was significantly lower than the mean VAS score increase in the other groups. When presented in graphic form, Group II (i.e., four to six month interval) showed the first significant increase, followed by gradually raising increases in the other groups (Fig. 4, Table 4).

There was no statistically significant difference in preoperative ADLS between the groups (p=0.064), but there was a statistically significant difference

in postoperative ADLS (p=0.001). Group I patients had significantly lower postoperative ADLS compared to the other groups (p=0.001). The mean increase in postoperative ADLS versus preoperative scores of all groups were statistically significant (Table 5). When presented

Table 3. Pre- and postoperative scores of the groups on the American Knee Society functional scoring system

		GROUP I	GROUP II	GROUP III	GROUP IV	GROUP V	ap
Walking ability; Mean <i>+SD</i>	Preop	18.09±7.7 (20)	17.44±9.02(10)	15.22±6.58(10)	17.56±5.38(20)	17.62±6.92(20)	0.299
	Postop	39.15±2.82(40)	40±0 (40)	40.43±2.06(40)	39.51±2.18(40)	39.29±3.42(40)	0.066
<sup>b</sup> p		0.001**	0.001**	0.001**	0.001**	0.001**	
Stair climbing;	Preop	24.57±7.29(30)	23.72±7.49(30)	27.39±5.75(30)	27.07±6.02(30)	26.07±6.68(30)	0.047*
Mean+SD	Postop	29.79±3.75(30)	33.26±4.74(30)	33.04±4.65(30)	32.93±4.61(30)	31.19±3.28(30)	0.001**
<sup>ь</sup> р		0.001**	0.001**	0.001**	0.001**	0.001**	
Walking aids;	Preop	6.28±3.03 (5)	4.53±3.59 (5)	6.3±2.67 (5)	8.05±2.47 (10)	7.26±4.31 (5)	0.001**
Mean+SD	Postop	2.87±2.5 (5)	2.33±2.52 (0)	1.96±2.47 (0)	2.68±2.52 (5)	2.02±2.48 (0)	0.330
<sup>b</sup> р		0.001**	0.004**	0.001**	0.001**	0.001**	
Function score;	Preop	36.38±5.87(35)	36.63±6.05(35)	36.3±4.53 (35)	36.59±5.96(40)	36.43±5.77(35)	0.936
Mean+SD	Postop	66.28±4.94(65)	70.93±3.97(70)	71.52±5.86(70)	69.76±4.6 (70)	68.45±4.75(70)	0.001**
Difference; <i>Meai</i>	Oifference; Mean+SD		34.3±7.53 (40)	35.22±4.34(35)	33.17±5.56(35)	32.02±5.07(35)	0.001**
<sup>b</sup> р		0.001**	0.001**	0.001**	0.001**	0.001**	

<sup>&</sup>lt;sup>a</sup>Kruskal-Wallis test

\*\*p<0.01

Table 4. Patients' VAS scores

VAS	GROUP I (Mean+SD)	GROUP II (Mean+SD)	GROUP III (Mean+SD)	GROUP IV (Mean+SD)	GROUP V (Mean+SD)	<sup>a</sup> p
PRE-OPERATIVE	7.83±0.99 (8)	7.86±0.56 (8)	7.85±0.67 (8)	7.85±0.48 (8)	7.88±0.59 (8)	0.888
POST-OPERATIVE	7.98±0.87 (8)	8.53±0.59 (9)	8.54±0.91 (9)	8.59±0.59 (9)	8.64±0.48 (9)	0.001**
Difference; Mean+SD	0.15±0.72 (0)	0.67±0.64 (1)	0.7±0.7 (1)	0.73±0.74 (1)	0.76±0.79(1)	0.001**
р	0.162	0.001**	0.001**	0.001**	0.001**	

<sup>&</sup>lt;sup>b</sup>Wilcoxon signed rank test

<sup>\*</sup>p<0.05

in graphic form, it is apparent that Group II (i.e., 4–6 month interval) showed the first significant increase in ADLS change ADLS, followed by gradual increases in the other groups (Fig. 5).

### DISCUSSION

Gonarthrosis, is a common degenerative disease that usually affects both knees, and TKA is generally acknowledged as a successful surgical technique to treat it. Bilateral TKAs can be performed simultaneously, in a staggered fashion during the same hospital stay, or in a staged manner during different hospital stays. Each procedure has its advantages and disadvantages, and it has not yet been determined which procedure yields the best outcomes (9). Most studies that compare simultaneous bilateral TKA with staged TKA procedures have suggested that simultaneous bilateral TKA is associated with shorter hospital stay, reduced exposure to anesthesia during surgery, shorter postoperative rehabilitation period, and decreased wound

problems, all of which result in reduced hospital costs (7, 29). However, some studies have suggested that simultaneous bilateral TKA is associated with a longer and more difficult rehabilitation course, prolonged physical therapy, extended hospital stay, increased postoperative pain, increased need for blood transfusions, and increased hospital costs (2, 6, 8, 21, 29). As compared to unilateral TKA, simultaneous bilateral TKA is associated with higher early postoperative mortality rates, and higher rates of severe medical complications associated with cardiovascular system and fat embolism (1, 19, 20, 24).

The morbidity and clinical success of any TKA technique has been found to be related to a number of factors such as preoperative comorbidities (i.e., cardiovascular diseases), patient age, surgical technique, and postoperative rehabilitation process (11, 12, 14, 15).

Actually, once TKA is performed, pain relief and functional improvement become evident over time, however,

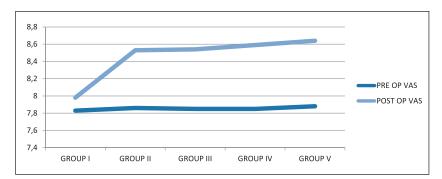


Fig. 4. Changes in VAS scores.

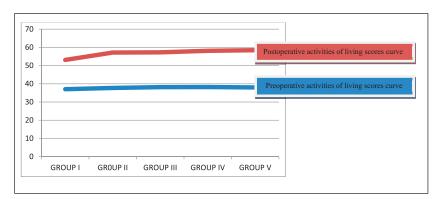


Fig. 5. Changes in activities of daily living scores.

this requires a different amount of time for each patient. For a patient to achieve full knee functionality and attain targeted capabilities, it is important that patients show complete commitment to the rehabilitation process. During this time, they may have difficulty using the operated extremity due to pain and they may have difficulty using the non-operated extremity due to deformity and dysfunction. Either scenario will result in delayed satisfaction due to overloading and other early or late complications. This, coupled with the fear that results from the first operation, might induce patients to avoid the second surgery in the other extremity, resulting in dissatisfaction with the operated knee, progression of arthrosis, increased pain, and loss of function (6, 14, 26, 28). In our study, the interval between the first and second TKA operation for patients with bilateral gonarthrosis who are advised to undergo staged unilateral TKA was chosen based on patient choice, patient rehabilitation status, and surgical recommendation, and the second TKA opera-

Table 5. Patients' postoperative scores on the activities of daily living scale

ADLS	GROUP I (Mean+SD)	GROUP II (Mean+SD)	GROUP III (Mean+SD)	GROUP IV (Mean+SD)	GROUP V (Mean+SD)	<sup>a</sup> p
PRE-OPERATIVE ACTIVITIES OF DAILY LIVING SCALE	37.06±3.91	37.7±2.44	38.17±2.61	38.2±2.68	37.98±1.97	0.288
POST-OPERATIVE ACTIVITIES OF DAILY LIVING SCALE	53.13±2.05	57.16±2.86	57.3±2.87	58.1±3.12	58.48±2.83	0.001**
Difference; Mean+SD	16.06±3.88	19.47±4.03	19.13±4.57	19.9±3.99	20.5±3.86	0.001**
<sup>d</sup> p	0.001**	0.001**	0.001**	0.001**	0.001**	

tion was always scheduled no less than 3 months after the first. In accordance with this process, our literature review revealed no clear consensus on the optimal timing for staged bilateral TKA. One study found changes in balance among patients with bilateral gonarthrosis undergoing staged TKAs, where the two TKA operations were separated by an interval of no more than 12 months and the need for a second TKA operation was determined based on improvement in balance (10). Even though this study emphasized the need for a second TKA, the optimal timing within the 12-month window for the second operation was not clarified. Thus, the study was limited to addressing the need for a second TKA, which was recommended to be performed within 12 months. Similarly, a separate study concluded that the median interval between the first and second operations for staged TKAs was 12.5 months, with half of the patients undergoing the second TKA within 12 months, and a wide range of intervals that ranged from two to 113 months. The results of this study may help patients and physicians plan effective treatment strategies for patients undergoing staged TKAs who have equal symptoms on both sides (11).

A retrospective cohort study of 668 staged bilateral TKA patients reported that patients who benefited from first-side TKA had a significant chance of benefiting from the second-side TKA (30), but this study also failed to clarify the optimal timing for staged operations. In another study that evaluated patient's functional outcomes between the first and second knee replacement in patients undergoing staged bilateral researchers reported reduced length of hospital stay (LOS) after the second procedure in terms of walking ability, use of walking aids and psychological well-being (13); however, this study also did not address the ideal interval between the two procedures. On the other hand, an initial TKA that results in good functioning may neither facilitate an earlier second rehabilitation nor decrease LOS. After controlling for factors such economics and local conditions that largely influence LOS, it appears that simultaneous bilateral TKAs might decrease LOS for certain patients with bilateral knee arthritis (10).

81% of participants in the "Consensus Conference on Bilateral Total Knee Arthroplasty Group" agreed that for patients found to be candidates for other-day bilateral TKAs, a second TKA should be scheduled no sooner than three months after the first surgery (25). On the other hand, there is no scientific data in the literature on the optimal interval between the first and second TKAs. However, a study about a safe time frame for performing the second TKA in staged TKAs reported that a second TKA performed between 90 and 270 days is produces fewer complications, particularly periprosthetic joint infections (4). The purpose of this study was to determine if there was a safe time frame for performing the second TKA in staged bilateral TKAs. Patients were included if they underwent bilateral staged TKA within 21 to 90, 91 to 180, 181 to 270, and 271 to 360 days after the first TKA. There were 29 postoperative complication events (4.9%) and there was no difference between time groups and complications. Although the highest rates of periprosthetic joint infection (PJI) occurred when the second TKA was performed between 271 and 360 days (3.6%), followed by the earliest postoperative period (i.e., 21 to 90 days) (2.7%), this finding cannot indicate a certain time point to reduce PJI complications.

Even though a number of factors have been proposed to influence the optimal interval for bilateral TKAs in bilateral gonarthrosis, our findings suggest that the optimal interval between the two TKA procedures is 91–270 days (i.e., Group II and Group III). The all groups' data gave better result after total knee arthroplasties. Groups 2 and 3 showed better knee scores than all other groups. When VAS scores were evaluated, Group II showed the most significant results. Taken together, these results indicate that the best interval between two knee arthroplasties is between 90 and 270 days. All patients enrolled in this study were homogeneously assigned to the study groups based on age, BMI, comorbidities, complications, preoperative pain, ADLS, preoperative knee and functional scores, surgical technique, rehabilitation protocol, and prosthetic type. An evaluation of postoperative knee and functional scores revealed a significant improvement in all groups as compared to preoperative values; however, Groups II and III showed the most significant downward trend, as compared to the other groups (Fig. 2, 3).

An evaluation of pain using the VAS revealed that the pain increase in the non-operated knee was most significant in Group II, showing a downward trend in the other groups (Fig. 4). Finally, an evaluation of ADLS scores showed that Group II (3–6 months) had the most significant increase, with a downward increase trend in the other groups. Given that the issues assessed in ADLS are similar to those assessed by AKS and the VAS, this convergence of results is not surprising (Fig. 5).

# **CONCLUSIONS**

There are few limitations of our study. First, our study is retrospective and carried out in a single institution. Also, the number of patients is not so high to give a certain idea for surgical time of second TKA.

Given all these findings, we believe that it is reasonable to advise patients to receive their second TKA, 3–6 months after their initial TKA, as this interval will allow for the greatest improvements in functional and daily living activities, and pain in the non-operated knee simultaneously becomes more severe. This recommended interval would minimize both the functional problems with the operated extremity due to pain, and deformity and dysfunction in the non-operated knee and the subsequent overloading. This would maximize patient satisfaction with the operated knee, and also avoid progression of arthrosis and the associated pain and loss of function in the non-operated knee.

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The undersigned authors transfer the ownership of copyright to Acta Chirurgiae Orthopaedicae et Traumatologiae Cechoslovaca should their work be published in this journal. They state that the article is original, has not been submitted for publication in other journals and has not yet been published either wholly or in part. They state that they are responsible for the research that they have designed and carried out; that they have participated in drafting and revising the manuscript submitted, whose contents they approve.

In the case of studies carried out on human beings, the authors confirm that the study was approved by the ethics committee and that the patients gave their informed consent.

They also state that the research reported in the paper was undertaken in compliance with the Helsinki Declaration and the International Principles governing research on animals.

**Compliance with ethical standards:** after approval by the institutional review board.

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