Persistent postoperative pain and joint stiffness after total knee arthroplasty performed for osteoarthritis (Pain and Stiffness)

FUNDER: Department of Anesthesiology

PROTOCOL NO.: 2015-361

VERSION & DATE: 11/27/2019

TABLE OF CONTENTS

PROTOCOL SYNOPSIS

Protocol Title:	Persistent postoperative pain and joint stiffness after total knee arthroplasty performed for osteoarthritis (Pain and Stiffness)
Protocol Number:	2015_361
Protocol Date:	11/27/2019
Sponsor:	Department of Anesthesiology
Principal Investigator:	Meghan Kirksey, MD PhD
Objective:	1. Determine if there is an association between preoperative joint fluid TNF-alpha, cytokine, chemokine, and adipokine levels and development of persistent postoperative pain and/or joint stiffness after TKA. 2. Determine if there is an association between preoperative plasma TNF-alpha, cytokine, chemokine, and adipokine levels and development of persistent postoperative pain and/or joint stiffness after TKA. 3. Determine the incidence, characteristics, and kinetics of persistent postoperative pain after TKA. 4. Determine the incidence, characteristics, and kinetics of postoperative joint stiffness after TKA. 5. Collect and store blood, joint fluid, and joint tissue for exploration of inflammatory signatures (identified by protein analyses, immunohistochemistry, RNA analysis including nanostring and sequencing analysis [RNAseq] and/or cellular analysis including flow cytometry and mass cytometry [cytoF]) associated with and potentially contributing to development of persistent postoperative pain and/or joint stiffness after TKA. 6. Collect clinical data for examination of interactions between demographic factors, comorbidities, pre- and post-operative pain, joint stiffness, inflammatory profiles, postoperative attainment of physical therapy (PT) goals, and functional outcomes after TKA.
Study Design:	Prospective cohort study
Enrollment:	179
Subject Criteria:	1. Elective primary unilateral total knee arthroplasty 2. OA with radiologic evidence of “severe narrowing” and/or “bone to bone” in the affected joint

	3. Patients of surgeons who have agreed to participate in the study 4. Age > 18 years 5. American society of Anesthesiologists (ASA) physical status 1-3 6. Regional Anesthesia 7. Epidural PCA for postoperative pain 8. Adductor canal block for postoperative pain
Data Collection:	Sources: EPIC, Medical Records, and Patient Reported. Variables: Name, MRN, Age, Race, Ethnicity, Gender, Height, Weight, BMI, Telephone number, Email address, Knee extension (degrees), Knee flexion (degrees), History of depression, history of anxiety, history of diabetes, history hyperlipidemia, history of tobacco use, Widespread pain inventory, Average daily opioid and non-opioid pain medication consumption, duration of preoperative opioid use, Most recent intra-articular steroid injection in affected joint, Statin use, gabapentinoid use, anticonvulsant use, SSRI use, Tricyclic antidepressant use, SNRI use, Presence of osteophytes on X-ray, NRS pain score in operative knee at rest, NRS pain score in operative knee with movement, worst NRS pain score in operative knee in past 24 hours, least NRS pain score in operative knee in past 24 hours, PCS Questionnaire, PainDETECT Questionnaire, KOOS Knee Survey, Stiffness Questionnaire, Pain Questionnaire, Quality of life Questionnaire, KOOS Physical Function Shortform, Tourniquet time in OR, Surgery start time, Surgery end time, Length of Surgery, Surgeon, Anesthesiologist, ASA, Blood Draw completed (Y/N), Blood Draw arterial or venous (Y/N), Blood draw from side with IV (Y/N), Plasma aliquoted and stored (Y/N), Blood hemolyzed (Y/N), Joint fluid aspirated (Y/N), Did the joint fluid contain blood (Y/N), Was midazolam administered (Y/N), Joint fluid aliquoted and stored (Y/N), Was propofol administered (Y/N), was 4mg dexamethasone administered intravenously (Y/N), was fentanyl administered(Y/N), Was ketorolac administered (Y/N), was ketamine administered (Y/N), was dexmedetomide administered (Y/N), Did the patient receive neuraxial anesthesia with an epidural for postoperative pain? (Y/N), Did a patient receive an adductor canal/subsartorial saphenous nerve block containing ONLY local anesthetics (no additives)? (Y/N), Was topical TXA administered? (Y/N; dose), Was IV TXA administered either intraoperatively or postoperatively? (Y/N; dose), Changes from default TKA postop protocol? (Y/N), Was there escalation from the postoperative pain protocol at any point during the inpatient stay? (Protocol: PCEA with standard settings, oxycodone or hydrocodone 510 mg q4h prn, PO or IV acetaminophen standing dose or prn, meloxicam. E.g., needing higher doses, needing extra days of PCEA, addition of a new class of medication such as gabapentinoids, etc.) (Y/N), Epidural PCA

	Placement Time, Epidural PCA Removal Time, Time Epidural PCA Was in Place, Did the patient require IV PCA? (Y/N), Time to ambulation of 40 feet (with or without assistance), Time to independent transfer (sitstand), Postoperative joint stiffness? (Y/N), Was manipulation performed for joint stiffness?, Postoperative persistent pain? (Y/N).
Statistical Analysis:	• Proposed analysis (e.g., student's ttest, ANOVA, chi-square, regression, etc.): Firth's penalized likelihood logistic regression • Alpha level: 0.05 • Beta or Power level: 0.8 • Primary outcome variable estimate (mean +/ s.d. for continuous outcome, frequency/percentage for categorical variable): An estimated 10% of TKR patients will develop persistent postoperative pain (defined as NRS pain ≥ 4 with activity in the operated joint at 6 months postoperatively) • Number of groups being compared (use 1 for paired analysis within the same subjects): 1 • Effect size or change expected between groups: It is estimated that a onestandard deviation increase (an estimated 2.6 pg/ml [1]) in intraoperative joint fluid TNF-alpha level will be associated with 2.49 times the odds of developing persistent postoperative pain. • Resulting number per group: Estimating an R 2 of 0.3 if TNFalpha were regressed on model covariates (i.e., model covariates would explain 30% of the variability in TNFalpha level), a total of 150 patients will need to be enrolled to detect an odds ratio of 2.49. • Total sample size required: To account for 10% postoperative exclusions, we will enroll a total of 165 patients. 179 patients enrolled

1.0 INTRODUCTION

The primary condition being studied is persistent pain following total knee arthroplasty (TKA) performed for knee osteoarthritis (OA). Published literature suggests that persistent postarthroplasty pain occurs in 1040% and joint stiffness requiring intervention occurs in 1040% of such patients and can adversely impact function and quality of life. This study is designed to test whether there is an association between joint fluid TNFalpha levels and development of persistent postoperative pain. The secondary condition being studied is joint stiffness following TKA performed for OA. Persistent pain can interfere with participation in physical therapy and may predispose patients to joint stiffness. Reciprocally, inflammation contributing to joint fibrosis and stiffness may also cause persistent postoperative pain. This study has the potential to shed light on the interaction between clinical, demographic and biological factors contributing to persistent postarthroplasty pain and joint stiffness in patients with knee OA.

Approximately 4 million Americans have undergone total knee arthroplasty (Weinstein AM et al., Estimating the burden of total knee replacement in the United States. J Bone Joint Surg Am 2013;95:38592), and persistent postoperative pain and joint stiffness may cause significant morbidity in up to 40% of these patients. This project aims to identify inflammatory pathways that contribute to and predict development of these complications. Identification of such pathways may allow us to target preventative measures to the patients at highest risk of persistent postoperative pain and joint stiffness.

While little is known about the contributions of inflammation and oxidative stress to the pathology of OA, even less is known about their contribution to adverse outcomes after arthroplasty. This study aims to define the incidence and characteristics of persistent postoperative pain and joint stiffness after TKA in patients with severe OA. This study also aims to explore demographic and medical factors as well as inflammatory pathways associated with the development of persistent postoperative pain and joint stiffness. Persistent postoperative pain may occur in as many as 2040% of patients (1), and clinical risk factors may include severity of preoperative pain (2), severe early postoperative pain and psychological factors, such as depression, anxiety, and pain catastrophizing (3). There is growing evidence that cytokines and adipokines are associated with OA severity, progression, and associated pain severity. Rodent studies have associated a number of cytokines, including tumor necrosis factor (TNF)alpha, interleukin (IL)1β, IL6, IL10, nerve growth factor and calcitonin generelated peptide, to OAassociated pain syndromes, including inflammatory pain and central sensitization (4,5). IL1β and IL18 levels in joint fluid have been associated with OA severity and progression in humans (6), and serum IL15 levels have been shown to correlate with pain severity in patients with OA (7). In patients with knee OA, TNFalpha in synovial fluid is associated with total Western Ontario and McMaster Osteoarthritis Index (WOMAC) scores and each of its three subscales (8). In patients with OA scheduled to undergo knee arthroplasty, low levels of adiponectin, high levels of leptin, and low adiponectin/leptin ratios in synovial fluid have been associated with

increased preoperative pain severity. Finally, using the novel technology of mass cytometry, a recent study of total hip arthroplasty patients identified a correlation between specific signaling responses in CD14+ monocytes and rates of postoperative recovery of function and resolution of pain (9). Postoperative joint stiffness, which is defined as range of motion (ROM) ≤95 degrees (10), and persistent pain can cause significant morbidity following total knee arthroplasty (TKA). Clinically, risk of postoperative joint stiffness has been associated with factors such as preoperative ROM, younger age, nonwhite race, smoking, high cholesterol, improperly sized components, malalignment, retained osteophytes, and severity of postoperative pain (11,12,13). There is evidence of increased fibroblasts, T cells, mast cells, oxidative stress and activation of the TGFbeta pathway in arthrofibrotic tissue from patients who have undergone total knee arthroplasty (14,15,16). Information is lacking about which inflammatory pathways are active in the perioperative period that may cause persistent postoperative pain and joint stiffness and/or serve as predictors for those patients at highest risk of developing these complications. This study aims to help fill this knowledge gap using targeted tissue analysis as well as unbiased screening methods. By gathering longitudinal clinical data as well as blood, synovial fluid, and tissue samples from OA patients undergoing TKA, we can begin to elucidate risk factors for and inflammatory pathways contributing to persistent pain and/or stiffness compared to those who do not. While it is well known that deregulation of the immune response plays a major role in many autoimmune diseases like RA, the contribution of inflammation to the pathogenesis of OA, disarrayed postarthroplasty healing, and persistent postoperative pain has not been extensively explored. We hypothesize, based on prior studies noted above, that TNFalpha will be elevated in the synovial fluid of patients who develop persistent pain after TKA. In addition, we hypothesize that specific immune signatures predispose patients to develop persistent pain and joint stiffness. Using an unbiased informatics approach to survey complex pathways potentially contributing to adverse outcomes after TKA, we will perform cytokine protein arrays on joint fluid from all patients. In addition, a subset of 30 enrolled patients will be coenrolled as part of a control cohort for the rheumatoid arthritis FLARE study (IRB #13146). Blood and tissue from these patients will be prepared and stored to undergo cellbased analyses (flow cytometry and cytoF), histological examinations (immunohistochemistry), and RNA analysis (nanostring, reverse transcription polymerase chain reaction and RNAseq). Analysis of clinical and biological profiles from OA patients will enable us to better characterize risk factors for and inflammatory pathways contributing to persistent postoperative pain and restricted ROM after TKA. Ultimately, enhanced understanding of such associations has the potential to enable targeting of appropriate preventative interventions to those at highest risk of developing these complications.

References

1. Searle R, Simpson K. Chronic post-surgical pain. Continuing Education in Anaesthesia, Critical Care & Pain 2010;10:12-14.
2. Pinto PR, McIntyre T, Ferrero R, Almeida A, Araujo-Soares V. Risk factors for moderate and severe persistent pain in patients undergoing total knee and hip arthroplasty: A prospective predictive study. PLoS One 2013;8:e73917.

3. Lewis GN, Rice DA, McNair PJ, Kluger M. Predictors of persistent pain after total knee arthroplasty: A systematic review and meta-analysis. Br J Anaesth 2014.
4. Orita S, Ishikawa T, Miyagi M et al. Pain-related sensory innervation in monoiodoacetate-induced osteoarthritis in rat knees that gradually develops neuronal injury in addition to inflammatory pain. BMC Musculoskelet Disord 2011;12:134.
5. Burston JJ, Sagar DR, Shao P et al. Cannabinoid cb2 receptors regulate central sensitization and pain responses associated with osteoarthritis of the knee joint. PLoS One 2013;8:e80440.
6. Denoble AE, Huffman KM, Stabler TV et al. Uric acid is a danger signal of increasing risk for osteoarthritis through inflammasome activation. Proc Natl Acad Sci U S A 2011;108:2088-93.
7. Sun JM, Sun LZ, Liu J, Su BH, Shi L. Serum interleukin-15 levels are associated with severity of pain in patients with knee osteoarthritis. Dis Markers 2013;35:203-6.
8. Orita S, Koshi T, Mitsuka T et al. Associations between proinflammatory cytokines in the synovial fluid and radiographic grading and pain-related scores in 47 consecutive patients with osteoarthritis of the knee. BMC Musculoskelet Disord 2011;12:144.
9. Gaudilliere B, Fragiadakis GK, Bruggner RV, Nicolau M, Finck R, Tingle M, Silva J, Ganio EA, Yeh CG, Maloney WJ, Huddleston JI, Goodman SB, Davis MM, Bendall SC, Fantl WJ, Angst MS, Nolan GP. Clinical recovery from surgery correlates with single-cell immune signatures. Sci Transl Med 2014;6(255):255.
10. Della Valle A, Leali A, Haas S. Etiology and surgical interventions for stiff total knee replacements. HSS Journal 2007;3:182-189.
11. Pfefferle KJ, Shemory ST, Dilisio MF, Fening SD, Gradisar IM. Risk factors for manipulation after total knee arthroplasty: a pooled electronic health record database study. J Arthroplasty 2014;29(10);2036-2038.
12. Issa K, Rifai A, Boylan MR, Pourtaheri S, McInerney VK, Mont MA. Do various factors affect the frequency of manipulation under anesthesia after primary total knee arthroplasty? Clin Orthop Relat Res 2015;473:143-147.
13. Della Valle AG, Leali A, Haas S. Etiology and surgical interventions for stiff total knee replacements. HSS J. 2007;3(2):182-189.
14. Freeman TA, Parvizi J, Della Valle CJ, Steinbeck MJ. Reactive oxygen and nitrogen species induce protein and DNA modifications driving arthrofibrosis following total knee arthroplasty. Fibrogenesis and Tissue Repair 2009;2:5.
15. Bosch U, Zeichen J, Skutek M, Haeder L, van Griensven M. Arthrofibrosis is the result of a T cell mediated immune response. Knee Surg, Sports Traumatol, Arthrosc 2001;9:282-289.
16. Freeman TA, Parvizi J, Della Valle CJ, Steinbeck MJ. Mast cells and hypoxia drive tissue metaplasia and heterotopic ossification in idiopathic arthrofibrosis after total knee arthroplasty. Fibrogenesis and Tissue Repair 2010;3:17.

2.0 OBJECTIVE(S) OF CLINICAL STUDY

This study is novel in taking a systematic approach to identifying and characterizing persistent pain and joint stiffness following TKA and analyzing activity of inflammatory pathways that may contribute to the development of these complications at a cellular and molecular level. This study has unique potential to fill in gaps in our understanding of how molecular and cellular pathology are associated with demographic factors, psychological factors, and medical comorbidities that contribute to postoperative pain and stiffness.

This study is designed to give us a better understanding of the inflammatory milieu associated with and potentially contributing to the development of persistent pain and/or joint stiffness after TKA. A better understanding of inflammatory contributors and clinical risk factors may allow us to recognize patients at high risk of these complications and may enable targeted interventions to reduce their incidence.

1. Determine if there is an association between preoperative joint fluid TNF-alpha, cytokine, chemokine, and adipokine levels and development of persistent postoperative pain and/or joint stiffness after TKA.
2. Determine if there is an association between preoperative plasma TNF-alpha, cytokine, chemokine, and adipokine levels and development of persistent postoperative pain and/or joint stiffness after TKA.
3. Determine the incidence, characteristics, and kinetics of persistent postoperative pain after TKA.
4. Determine the incidence, characteristics, and kinetics of postoperative joint stiffness after TKA.
5. Collect and store blood, joint fluid, and joint tissue for exploration of inflammatory signatures (identified by protein analyses, immunohistochemistry, RNA analysis including nanostring and sequencing analysis [RNAseq] and/or cellular analysis including flow cytometry and mass cytometry [cytoF]) associated with and potentially contributing to development of persistent postoperative pain and/or joint stiffness after TKA.
6. Collect clinical data for examination of interactions between demographic factors, comorbidities, pre- and post-operative pain, joint stiffness, inflammatory profiles, postoperative attainment of physical therapy (PT) goals, and functional outcomes after TKA.

3.0 STUDY HYPOTHESES

1. Elevated levels of TNF-alpha in joint fluid of patients with OA is associated with the risk of development of persistent postoperative pain after TKA.

2. Persistent pain (defined as NRS≥4 with activity in the operated joint at 6 months postoperatively) occurs in 10% of patients undergoing TKA for OA.

3. Joint stiffness (defined as range of motion [ROM]≤95 degrees measured at 6 ± 2 weeks postoperatively) occurs in 7% of patients undergoing TKA for OA.

4. Distinct immune signatures (identified via protein analyses, immunohistochemistry, nanostring, RNA-Seq, fluorescence-activated cell sorting (FACS), and/or mass cytometry [CytoF]) are associated with the risk of developing persistent postoperative pain following TKA in OA patients.

5. Distinct immune signatures (identified via protein analyses, immunohistochemistry, nanostring, RNA-seq, FACS, and/or CytoF) are associated with the risk of developing joint stiffness following TKA.

6. Demographic profile, comorbidities, psychological factors, pain profiles, pre and postoperative stiffness and inflammatory profiles interact to determine attainment of PT goals and functional outcomes following TKA.

4.0 STUDY DESIGN

4.1 Endpoints

4.1.1 Primary Endpoint

- The presence of persistent pain (defined as NRS≥4 with activity in the operated joint) at 6 months postoperatively (measured at 6+ months).

4.1.2 Secondary Endpoints

- 1. The incidence of postoperative joint stiffness (defined as ROM≤95 degrees) measured at the 6week (±2 weeks) postoperative surgical visit
- 2. Postoperative NRS pain scores measured on POD 1, POD 2, at 4 weeks, 3 months, and 6 months postoperatively
- 3. Postoperative knee ROM measured at 6 weeks (±2 weeks) postoperatively
- 4. Postoperative KOOS and PainDETECT (neuropathic pain assessment) scores measured at 4 weeks, 3 months, and 6 months postoperatively
- 5. Time to attainment of physical therapy goals (Ambulation 40 feet, independent transfer) measured during inpatient stay
- 6. New diagnoses and postoperative medications (recorded during hospital admission, during followup visits, and from postoperative phone followups and chart reviews)

4.2 Study Sites

This study will take place at the main campus of the Hospital for Special Surgery (HSS).

5.0 STUDY POPULATION

5.1 Number of Subjects

179

5.2 Inclusion Criteria

Subjects of either gender will be included if:

- Elective primary unilateral total knee arthroplasty
- OA with radiologic evidence of “severe narrowing” and/or “bone on bone” in the affected joint
- Patients of surgeons who have agreed to participate in the study
- Age > 18 years
- American Society of Anesthesiologists (ASA) physical status 1-3
- Regional anesthesia
- Epidural PCA for postoperative pain
- Adductor canal block for postoperative pain

5.3 Exclusion Criteria

Subjects will be excluded from the study if:

- Contraindication to regional anesthesia, NSAIDs, dexamethasone or acetaminophen
- Use of general anesthesia
- History of >6 weeks of daily opioid use and/or any use of non-prescribed opioids
- Preoperative systemic steroid use in the past 6 months
- Intra-articular steroid injection within one month of scheduled surgery in affected joint
- Non-English speakers
- Pre-existing diagnosis of rheumatic disease, autoimmune disease or immunodeficiency (e.g., rheumatoid arthritis, psoriatic arthritis, inflammatory bowel disease, multiple sclerosis, Type 1 diabetes)
- Peri-articular injections or infusions for postoperative pain
- Diagnosis of crystalline arthropathy
- Diagnosis of osteonecrosis
- Active infection or use of antibiotics
- Pregnant women
- Previous study participants

6.0 PROCEDURES

6.1 Intraoperative Protocol

Anesthesia and Analgesia:

The intraoperative anesthesia regimen will be standardized to include neuraxial anesthesia with an epidural. Patients must receive 4mg dexamethasone for nausea prophylaxis. Propofol, midazolam, up to 100mcg fentanyl and up to 30mg ketorolac can be administered intraoperatively at the discretion of the anesthesiologist. Ketamine and dexmedetomidine will not be administered. Violations of intraoperative anesthesia guidelines will be grounds for exclusion at the discretion of the PI in consultation with the anesthesia provider.

The postoperative pain regimen will include: adductor canal nerve block (local anesthetic with no additives) and the default TKA orderset (postoperative epidural patient controlled analgesia with standard settings, prn oxycodone or hydrocodone 5mg10mg q4h, PO or IV acetaminophen standing dose or prn, and meloxicam). Postoperative pain management regimens will be adjusted by the pain management team as required to achieve adequate analgesia. Chronic pain patients will be maintained on standing and prn pain medications prescribed at the discretion of the treating chronic pain physician. Epidural PCA failure requiring use of IV PCA will be ground for exclusion after enrollment.

Sample Collection:

During hospital admission, baseline (prior to surgical incision), early postoperative (drawn on arrival to PACU), POD 1 (24±2 h), and POD 2 (48±2 h) blood samples will be collected for each enrolled patient from a preexisting arterial line or by venous or arterial puncture if an arterial line is not in place. In the operating room, participating surgeons will draw joint fluid aspirates (up to 5mL) prior to surgical arthrotomy and will collect intraoperative tissue samples that will be sent to pathology for processing.

For all enrolled patients:
Inpatient whole blood samples: maximum 80mL
· Baseline: 30mL + 1 PAXgene tubes (2.5mL)
· Early postoperative: 5mL + 1 PAXgene tubes (2.5mL)
· POD 1 (24 h): 30mL + 1 PAXgene tubes (2.5mL)
· POD 2 (48 h): 5mL + 1 PAXgene tubes (2.5mL)

Possible 6 week follow up visit whole blood sample: · Max 60mL

After samples are collected, aliquots of blood, plasma and joint fluid will be frozen and stored for future analyses. PAXgene tubes will be stored for future RNA analyses. Sample analysis: Once enrollment is complete, joint fluid and plasma samples will be analyzed for TNFalpha and other cytokine and chemokine levels by the CTSC at Cornell using 30plex panels from Mesoscale. Adiponectin and leptin levels will also be assessed using Mesoscale assays. In addition, fresh blood samples will be processed for cellular and molecular analyses of inflammatory and related pathways using methods potentially including FACS and/or CytoF, RNA analyses, and protein analyses (e.g., assessment of Rho associated protein kinase (ROCK) activity) to be conducted at HSS in the lab of Dr. Pernis, the precision medicine core facility at HSS and/or affiliated core facilities at WCMC/RU/MSKCC). In the event that blood work cannot be collected at the 6week visit (±2

weeks), blood work may be collected at the 3month postoperative visit. Sample Analyses which may be performed (on deidentified samples) by our coinvestigators, the precision medicine laboratory at HSS, affiliated core facilities at WCMC/RU/MSKCC, and/or outside facilities include: · Blood Cells: After blood is collected, composition of white blood cell differential (PMN, monocyte, lymphocytes) may be documented by flow cytometer and/or CytoF. Samples may then be sorted into granulocytes, PBMC, and platelet poor plasma, and cells viably frozen for future validation of RNAseq data. An aliquot of the PBMCs will be directly assayed for ROCK activation, immune cell subsets and T cell function. Another aliquot will be cultured to assess ROCK activation and T cell function after stimulation by measuring molecules such asIRF4, IRF5, IRF8, IBP/Def6, SWAP70, ROCK1, ROCK2, and signaling molecules involved in responsiveness to activation stimuli. Samples may be then subjected to QPCR, Western blotting, immunofluorescence / immunohistochemical analysis, ELISA, FACS or other laboratory analysis to investigate the expression/function of the signaling components that might be implicated in OA and poor postarthroplasty outcomes. If deemed necessary CD4+ T cell purification will be performed, after which the cells will be either left unstimulated or stimulated.

· PAX tube (2.5 mL or 0.5 tsp): RNA samples extracted from PaxGene tubes may be analyzed using RNA sequencing technology, RNA microarray, quantitative PCR, RNA nanostring or other appropriate RNA analysis platforms. Unique reads will be mapped to a reference human genome.

· Blue, green and red top tubes: Plasma will be collected and may be used for measurement of cytokines and other proteins potentially contributing to OA and post-arthroplasty outcomes.

In keeping with the protocols of the AMP studies, intraoperative tissue samples may be collected with special attention to obtaining samples of tissues from standardized anatomic locations including tissue samples of the patella cartilage, distal femur and proximal tibia, and synovium from the suprapatellar pouch.

• Tissue will be processed first in pathology: sample saved in OCT and 2 slides stained with H&E. Slides will be scored according to a modified Scanzello scoring system. Synovial tissue and PBMCs will be processed according the standard operating procedures established by the AMP network. Cellular samples will be analyzed at HSS, and may be shared with the AMP network or other collaborating institutions (in a deidentified manner) for additional specialized technical analytics to explore markers and pathways expressed in tissue and cells, using (but not limited to) those being explored in the AMP network such as RNASeq, ATACSeq, CyTOF, FACS

• Analysis may also include photography of specimens using the Pathology Lab equipment. The resulting photographs will be deidentified before use for research purposes. We may also capture deidentified microscopy images of H&E slides prepared from these samples.

Research specimens will be divided into four aliquots. As discussed with Dr. DiCarlo, all processing in Pathology will be performed by the Rheumatology Research Assistant under the direct supervision of the pathologist or representative.

· The first aliquot will be frozen and sectioned for staining with hematoxylin and eosin for tissue identification and for any later processing that might require fresh frozen tissue. The frozen tissue block (minimum 0.5 cm in diameter) will be preserved in the cryomold and taken to the Crow lab for storage in the 80degree Celsius freezer.

· The second aliquot will be stored fresh in a tube with RNAlater and will be brought by a research assistant to a research assistant to be used for RNA sequencing and/or nanostring or qPCR. The samples will be frozen and subsequently thawed, and RNA will be extracted using an approved methodology (e.g., the TRIzol Plus RNA purification system (Life Technologies)). RNA will be reverse transcribed and a cDNA library will be generated and sequenced on a HiSeq 2500. Data will be processed by filtering, genome mapping with STAR, and raw read counts will then be processed with edgeR for differential gene expression comparing synovial samples from patients who flared to those who did not flare in 6 weeks of followup.

· The third aliquot will be processed in the HSS pathology laboratory for fixation, paraffin embedding and sectioning, and standard of care hematoxylin and eosin staining.

· The fourth aliquot will be processed in the Ivashkiv and Pernis laboratories to isolate live synoviocyte cells.

Specifically, a fragment of synovial lining (~2 cm3†or greater) will be diced into smaller fragments and cells disloged from the matrix by mechanical force and/or enzymatic digestion. The resulting singlecell suspensions will be stained for celltype specific surface markers by FACS and/or CytoF and sorted by flow cytometry. In particular, T and B cell, monocyte and fibroblast populations will be collected. Downstream analyses include various nextgeneration sequencing protocols, which assay for gene expression levels and epigenetic features. The goal of this procedure is to identify cell populations or intracellular pathways found directly within the diseased tissue that associate with other disease parameters.

· From the remaining tissue, HSS pathology will conduct fixation, paraffin embedding, sectioning, and standard of care histopathologic examination.

At least 2 formalin fixed, paraffin embedded tissue blocks will be taken and kept in the pathology department. (Rheumatology research assistant should record Pathology Accession.)

Additional processing of aliquot #1 will include staining for a panel of cytokines known to characterize the presence/recruitment of specific immune cell subsets, particularly Th17 and Th1 cells (IL17, IL21, IFNgamma, and CCL20) as well as the activity state of the ROCK pathway (e.g., by staining for phosphorylated ERM, a well established target of ROCK activity and by staining with newly developed antibodies against phosphorylated ROCK2, IRF4, and IRF8). The expression of these molecules will be further confirmed in the second aliquot after extracting RNA and subjecting the samples to qPCR analysis and/or nanostring. Staining for the expression/activity of these molecules in fresh frozen tissues will only be performed if staining in the formalin fixed, paraffin embedded tissues (aliquot #3) is not successful.

Deidentified samples will then be brought to the Pernis lab or other collaborating lab/core facility and subjected to immunofluorescence / immunohistochemical analysis to investigate the expression/function of the signaling components that might be implicated in OA and poor postarthroplasty outcomes. In particular these studies may assess the composition of the inflammatory infiltrates, the activation state of distinct cellular subsets, the activity of the ROCK pathway (e.g. by staining for pERM a target of Rock activity), and other pathways associated with inflammation, fibrosis, and oxidative stress.

6.2 Postoperative Protocol

Pain and Function:

Depression, anxiety, pain at multiple sites, age, and sex have been shown to be associated with pain severity. Demographic information, psychological and medical history, and assessment of pain at multiple sites (using the Widespread Pain Inventory) will be collected or performed at enrollment. Because pain catastrophizing scale (PCS) scores are also associated with pain severity, the PCS will be administered on postoperative day (POD) 2. Furthermore, the following scales will be used to assess pain type and severity at enrollment and at 4 weeks (±3 days), 3 months (±1 week) and 6 months (±4 weeks) postoperatively. If patients initiate rescheduling of the postoperative phone calls, the surveys can be administered outside of the time window. If necessary, patients will be texted with their permission to schedule phone calls or surveys may be emailed to them via REDCap.

1. Numerical rating scale (NRS) pain scores (at rest, with movement, worst pain, least pain)

2. The PainDETECT scale – a validated measure of neuropathic pain

3. The KOOS questionnaire (Symptoms, Pain, Stiffness and Quality of Life subscales) and KOOS Physical Function Shortform – a commonly utilized measure of pain and function in patients with knee OA NRS pain scores will also be collected on POD 1 and 2. Attainment of physical therapy goals will be recorded by research assistant on each inpatient day. In the OR, knee flexion and extension measurements will be made via visual assessments with the aid of a goniometer by the surgical team after anesthesia induction. At 6 months following enrollment, patients who developed postoperative joint stiffness and/or persistent chronic postoperative pain will be identified. Postoperative joint stiffness will be defined as ROM≤95 degrees at the 6week (±2 weeks) surgical followup in the postoperative clinic. Persistent postoperative pain will be defined as NRS≥4 with activity in the operated joint at 6 months postoperatively.

6.3 Data Collection

The following data will be collected:

Pre-operative/Baseline

- Name
- MRN
- Age
- Race
- Ethnicity
- Gender
- Height
- Weight
- BMI
- Telephone number
- Email address
- Knee Extension (degrees)
- Knee flexion (degrees)
- History of depression
- History of anxiety
- History of diabetes
- History hyperlipidemia
- History of tobacco use
- Widespread pain inventory
- Duration of preoperative opioid use
- Most recent intra-articular steroid injection in affected joint
- Statin use
- Gabapentinoid use
- Anticonvulsant use
- SSRI use
- Tricyclic antidepressant use
- SNRI use
- Presence of osteophytes on x-ray
- NRS pain score in operative knee at rest
- NRS pain score in operative knee with movement
- Worst NRS pain score in operative knee in past 24 hours
- Least NRS pain score in operative knee in past 24 hours
- PainDETECT questionnaire
- KOOS Knee survey
- Pain Questionnaire
- Quality of Life Questionnaire
- KOOS Physical Function Shortform

Surgical procedure (Intra-operative)

- Knee Extension (degrees)
- Knee flexion (degrees)
- Tourniquet time in OR

- Surgery start time
- Surgery end time
- Length of surgery
- Surgeon
- Anesthesiologist
- ASA
- Blood draw completed? (Y/N; Why)
- Blood draw arterial or venous?
- Blood drawn from side with IV? (Y/N; Why)
- Plasma aliquoted and stored? (Y/N)
- Blood hemolyzed (red plasma)? (Y/N)
- Joint fluid aspirated? (Y/N; Why)
- Did the joint fluid contain blood? (Y/N)
- Joint fluid aliquoted and stored? (Y/N)
- Was midazolam administered? (Y/N; dose)
- Was propofol administered? (Y/N)
- Was 4 mg dexamethasone administered intravenously? (Y/N)
- Was fentanyl administered? (Y/N; dose)
- Was ketorolac administered? (Y/N; dose)
- Was ketamine administered? (Y/N)
- Was dexmedetomidine administered? (Y/N)
- Did the patient receive neuraxial anesthesia with an epidural for postoperative pain? (Y/N)
- Did a patient receive an adductor canal/subsartorial saphenous nerve block containing ONLY local anesthetics (no additives)? (Y/N)
- Was topical TXA administered? (Y/N; dose)
- Was IV TXA administered either intraoperatively or postoperatively? (Y/N; dose)

Post-Operative

- Knee Extension (degrees)
- Knee flexion (degrees)
- Average daily opioid and non-opioid pain medication consumption
- Statin use
- Gabapentinoid use
- Anticonvulsant use
- SSRI use
- Tricyclic antidepressant use
- SNRI use
- NRS pain score in operative knee at rest
- NRS pain score in operative knee with movement
- Worst NRS pain score in operative knee in past 24 hours
- Least NRS pain score in operative knee in past 24 hours
- PCS questionnaire
- PainDETECT questionnaire
- KOOS Knee survey
- Pain Questionnaire

- Quality of Life Questionnaire
- KOOS Physical Function Shortform
- Blood draw completed? (Y/N; Why)
- Blood draw arterial or venous?
- Blood drawn from side with IV? (Y/N; Why)
- Plasma aliquoted and stored? (Y/N)
- Blood hemolyzed (red plasma)? (Y/N)
- Was IV TXA administered either intraoperatively or postoperatively? (Y/N; dose)
- Changes from default TKA postop protocol? (Y/N)
- Was there escalation from the postoperative pain protocol at any point during the inpatient stay? (Protocol: PCEA with standard settings, oxycodone or hydrocodone 510 mg q4h prn, PO or IV acetaminophen standing dose or prn, meloxicam. E.g., needing higher doses, needing extra days of PCEA, addition of a new class of medication such as gabapentinoids, etc.) (Y/N)
- Epidural PCA Placement Time
- Epidural PCA Removal Time
- Time Epidural PCA Was in Place
- Did the patient require IV PCA? (Y/N)
- Time to ambulation of 40 feet (with or without assistance)
- Time to independent transfer (sitstand)
- Postoperative joint stiffness? (Y/N)
- Was manipulation performed for joint stiffness?
- Postoperative persistent pain? (Y/N)

7.0 STATISTICAL ANALYSIS

- Proposed analysis: Firth's penalized likelihood logistic regression
- Interim analysis planned: No
- Alpha level: 0.05
- Beta or power level: 0.8
- Primary outcome variable estimate (mean +/- s.d. for continuous outcome, frequency/percentage for categorical variable): An estimated 10% of TKR patients will develop persistent postoperative pain (defined as NRS pain ≥ 4 with activity in the operated joint at 6 months postoperatively)
- Number of groups being compared: 1
- Effect size or change expected between groups: It is estimated that a one-standard deviation increase (an estimated 2.6 pg/ml [1]) in intraoperative joint fluid TNFalpha level will be associated with 2.49 times the odds of developing persistent postoperative pain.
- Resulting number per group: : Estimating an R 2 of 0.3 if TNFalpha were regressed on model covariates (i.e., model covariates would explain 30% of

the variability in TNFalpha level), a total of 150 patients will need to be enrolled to detect an odds ratio of 2.49.

- Total sample size required: To account for 10% postoperative exclusions, we will enroll a total of 165 patients. 179 patients enrolled

8.0 ADVERSE EVENT ASSESSMENT

All Adverse Events (AEs) will be reported in the final study report.